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M. Inoue-Murayama · S. Kawamura · A. Weiss *Editors*

From Genes to Animal Behavior

Social Structures, Personalities, Communication by Color

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Front cover: *Upper*: An adult femle spider monkey, with dichromatic color vision, foraging on the fruit of *Guettarda macrosperma* in Sector Santa Rosa, Área de Conservación Guanacaste, Costa Rica. Photo by Norberto Asensio Herrero. *Center left*: Four-year-old twins playing with a pet hunting dog (Hungarian Vizsla). Photo by Katalin Farkas. *Center middle*: An adult kea, the New Zealand mountain parrot, investigates a hiker's boots. Photo by Andrew Walmsley. *Center right*: An adult blue-black male of *Neochromis greenwoodi* from Mwanza Gulf, Lake Victoria. Photo by Mitsuto Aibara.

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Preface

These individual differences are of the highest importance for us, for they are often inherited, as must be familiar to every one; and they thus afford materials for natural selection to act on and accumulate, in the same manner as man accumulates in any given direction individual differences in his domesticated productions.

Charles Darwin, *On the Origin of Species*

When one looks at the natural world, one is immediately struck by the diversity within humans as well as other species, including primates, dogs, birds, and even squid. This diversity did not escape the notice of early naturalists, including Charles Darwin, and the biological and genetic bases of variation in behavioral diversity have been subjects of broad interest since the early days of ethology, sociobiology, comparative psychology, and many other fields. Research on behavioral diversity is challenging, and thus many approaches have been devised to address its natural origins. Only relatively recently have attempts been made to bring these different approaches together.

We firmly believe that to truly illuminate the pathways from genotypes to behavioral phenotypes requires such an interdisciplinary research program. However, we are aware of certain realities, namely, that behavioral research comprises a seemingly vast number of disciplines and subdisciplines. This fact can be seen by the wide range of subject areas from which the authors of the chapters within this volume hail. A quick glance at the author affiliations shows that they study animal behavior from the perspectives of, among others, evolutionary biology, genetics, anthropology, agriculture, and behavioral ecology. One unfortunate consequence of this diversity is that it makes attempts to unify the study of behavior particularly difficult, as the various disciplines and subdisciplines each include several variables, whether they are proximity behaviors, coloration, or personality, that are not well-understood by those in other areas. Another complicating factor arises from the fact that, given the specialization of many journals, it can be difficult for members of one discipline or even subdiscipline to keep up with the literature in other areas. We therefore felt that there was a need for a volume to describe the methods and findings of researchers in several subdisciplines. It is our sincere hope that this volume represents an advance in this direction and will help researchers studying the pathways from the genotypes to behavioral phenotypes learn from and even collaborate

with one another. To these ends we divided the book into five sections: kin and sexual selection; personality research; the genetic bases of personality; coloration and color vision; and other biochemical measures. The chapters in each of these sections show how researchers in different areas study a particular question or set of questions about animal behavior. While these chapters do not encompass all possible research questions or methods, they have in common the fact that they all demonstrate how by bridging different research areas one can better understand the complex nature of behavioral diversity.

The first section concerns kin and sexual selection, perhaps two of the oldest research areas in modern evolutionary biology. These research areas are essential to understanding the evolution of social behaviors and therefore this section summarizes research on kinship analyses using genetic markers and the analyses of genes related to reproductive behavior, which have provided various insights into sexual and kin selection. In summarizing this research area, this section covers diverse taxa, including primates, rodents, birds, and eusocial insects, and its chapters focus on topics that reflect taxonomic characters. The first chapter in this section is a review by Jörns Fickel and Alexandra Weyrich which examines female mate choice in rodents. Their chapter summarizes recent thinking about sexual selection and highlights several factors that may influence female mate choice, including the major histocompatibility complex, oxytocin, and olfactory receptor genes. In the second chapter, Emmi Schlicht and Bart Kempenaers review the effect of extra-pair paternity on sexual selection (mainly) in birds. They first point out the problems in the calculations used to quantify the effect of extra-pair paternity and how these effects are interpreted. They then suggest that multiple matings appear to drive sexual selection in several monogamous avian species. The third chapter in this section is a review from Eiji Inoue which examines male reproductive success and cooperative and affiliative behaviors among paternal relatives in nonhuman primates. The review shows that paternal kin-biased behavior is observed in some conditions, but that the particular conditions and mechanisms underlying paternal kin recognition are unclear. The final chapter in this section is a review from Koji Tsuchida which examines conflict resolution among eusocial wasps in light of kin selection frameworks with particular regard to foundress groups, sex ratio, and male parentage. He shows that kin selection theory is limited in its ability to explain adaptive conflict resolution among nestmates.

The second section focuses on animal personality research, an area that is just gaining traction in evolutionary biology and behavioral ecology, sometimes under labels such as temperament or behavioral syndromes. Although it has a well-established pedigree in human psychology research, much of this rich knowledge is unfamiliar to researchers in these other areas. Similarly, much of the recent work on personality coming from evolutionary biology and behavioral ecology is unfamiliar to psychologists. We hope that this section introduces members of these disciplines to new methods and concepts, which will enable them to better study substantive questions about personality evolution in humans as well as nonhuman animals. To these ends this section begins with a chapter by Sonja Koski, who reviews the methods used by and some findings of psychologists and evolutionary biologists who

study animal personality. Her chapter synthesizes these areas and should help those who study animal personality in psychology and evolutionary biology better advance their common goals. The second chapter in this section is a theoretical chapter by Mark James Adams which highlights how evolutionary and quantitative genetics can inform the study of personality in nonhuman primates in psychology as well as evolutionary biology, with particular attention being paid to possible evolutionary mechanisms that might maintain variation. In particular, this chapter focuses on illustrating quantitative methods that can be used to study wild populations of nonhuman primates and other animals, which do not lend themselves to more experimental approaches. The third chapter is a review of behavioral genetics research on animal personality by Kees van Oers and David Sinn. Their review focuses on the "phenotypic gambit," an assumption that the phenotypic structure of personality reflects the genetic structure of personality. They conclude that the literature supports this assumption, although they warn of possible dangers, given that little is known about how well genetic influences generalize across age, sex, and environments. They then point out several useful directions for future researchers who wish to address these unanswered questions. The fourth and fifth chapters in this section highlight how animal personality research can be applied to practical problems. The research described in these chapters can lead to discoveries regarding the role personality plays in social interactions and mating in different species, including those that are difficult to study in the wild or in laboratory conditions. Similarly, these chapters highlight how a genetically influenced phenotype such as personality interacts with the environment to bring about positive affect or well-being. In the first of these chapters, Marieke Gartner and David Powell highlight how personality can be applied to improve conservation of zoo-housed animals and maximize their well-being. They then describe in detail how personality data can be used when introducing new animals into existing exhibits, to find pairs that will readily mate and produce offspring, and to devise enrichment programs that respect and cater to the differences among individuals within each exhibit. In the second of these chapters, Simon Turner, Jenny Gibbons, and Marie Haskell describe how they and others have used methods from psychology and biology to develop and validate measures of temperament for agriculturally important species, such as pigs and cattle. Moreover, this chapter highlights the possible practical uses of these measures to improve the conditions of these animals and the safety of their handlers by reducing levels of aggression and fear by selective breeding based on genetic indices.

The third section follows closely from the prior section in that it, too, focuses on studies of personality. However, this section highlights molecular genetic research in the area, and, in particular, attempts to relate personality traits to genetic loci. It begins with a summary by Kouta Kanno and Shoichi Ishiura, who review their attempts to find genetic markers for personality traits in humans, and especially their work on the in vitro expression of the dopamine transporter (*DAT1*) gene. As will become clear to readers, while several candidate genes exist, findings from association studies have been mixed. The next three chapters in this section describe studies of nonhuman animal personality. In the second chapter, Miho Inoue-Murayama, Alexander Weiss, Naruki Morimura, Masayuki Tanaka, Juichi Yamagiwa, and Gen'ichi Idani

describe studies of the genetics of personality in great apes. The chapter summarizes recent research on candidate genes believed to underlie human personality variation in aggression and affiliation. Their research has demonstrated that differences in the allelic distribution of genes such as the monoamine oxidase and vasopressin receptor genes may explain trait differences at the within- and between-species levels of analyses. This chapter is followed by an exciting chapter from Enikő Kubinyi, Mária Sasvári-Székely, and Ádám Miklósi on the genetics of personality in dogs. We think the authors make a convincing case that, because dogs co-evolved with humans and have had their behaviors and other traits shaped by humans via selective breeding, they are an excellent model species for studying the genetics of personality. Their chapter summarizes research in multiple areas, including the means used to measure personality in dogs, the results of genetic association studies, and studies that reveal how gene function differs across breeds. Andrew Fidler's chapter concludes this section. He describes his work combining classical ethological studies and modern molecular genetic approaches to reveal how neuroendocrine mechanisms and evolution have yielded persistent individual differences in avian behavior. Moreover, he shows how the results of his studies can lead to practical interventions to improve poultry breeding and to protecting endangered avian species such as the kea (*Nestor notabilis*).

The fourth section focuses on the evolutionary and genetic bases of vertebrate body coloration and color vision. Sensory organs and adaptations are fundamental determinants of behavior across the animal kingdom. Animals sense external stimuli through sensory organs and react to stimuli with a variety of behaviors, including foraging for food, escaping predators, navigating their environment, and mating. Of the senses, vision is best understood with respect to its genetic mechanisms and variation. In response to environmental conditions, different species evolved several types of color vision, and animal coloration has evolved and diversified as a signal. The first chapter in this section is by Nicholas Mundy, who discusses research on the evolutionary genetics of coloration in vertebrates. The study of coloration provides an excellent opportunity to illuminate the mechanisms of phenotypic evolution from genetics to behavioral adaptation. Thus, this section introduces the proximate bases of coloration and the types of color variation followed by a discussion on progress in identifying the molecular basis of color variation in wild vertebrates. This progress includes discoveries that suggest considerable convergence in the genetic mechanisms underlying color variation across broad phylogenetic scales. However, genes underlying color variation in many nonhuman primates are still waiting to be uncovered. In the second chapter of this section, Yohey Terai and Norihiro Okada review their exciting work on speciation in the cichlid fish in Lake Victoria. They describe how coloration and sensory adaptations to different light environments led to speciation and how such a study system helps to understand the molecular bases of evolution. In the final chapter of this section Shoji Kawamura reviews recent research on the selective pressures leading to the evolution and diversity of vertebrate color vision and aspects of the visual sensory system. The chapter therefore summarizes recent exciting findings on the relationship between opsin genes and behavior. Because patchy and spectrally varying illumination is more common in shallow water and in forests, the review focuses on fish and primate species that are highly polymorphic with respect to color vision.

The fifth section focuses on the biochemical and neurological underpinnings of mind, behavior, and social interactions in humans and nonhuman primates. In doing so, the section describes several new technologies that can be used to study behavior. These physiological measures are advantageous in that, as phenotypes, they are closer to the actual genetic influences underlying behavioral traits than responses to a questionnaire or even behavior. In the first chapter, Takamasa Koyama and Akiko Nakagami, review their experimental research showing how endocrine-disrupting chemicals may influence socialization in macaques (*Macaca mulatta* and *Macaca fascicularis*). This work is important as it highlights the subtle, although potentially serious, effects that environmental pollutants may have on humans and other species. The next two chapters focus on the use of neuroimaging technologies to better understand mechanisms underlying genetically based individual differences in behavior. In the first of these chapters, Hideki Ohira reviews his group's studies on the neural basis of individual differences in positive and negative emotional reactivity and postulate hypotheses regarding the genetic regulation of these relationships in humans. Using positron emissions technology (PET) scanning as well as cardiovascular and neuroendocrine indices, they found that positive and negative emotions lead to coordinated physiological responses, although these responses can be regulated by cognitive appraisal of the situation. In the final chapter, Chihiro Yokoyama and Hirotaka Onoe describe the development of a method that can be used to conduct PET scans on conscious nonhuman primates. They then describe how measures derived via this approach helped to better understand the molecular bases of social behavior and the sensory system in a New World Monkey, the common marmoset (*Callithrix jacchus*). While the use of these methods in studies of nonhuman primate behavior is not widespread at present, we expect they might be important in the near future.

Although this volume is not comprehensive, we hope that by focusing on a selection of topics it will convey the most cutting-edge research in the study of animal behavior, perception, genetics, and evolution. It is our express wish that researchers in various disciplines will benefit from the chapters herein and possibly be inspired to expand their existing research programs with new technologies, measures, or collaborations with researchers in other areas. For example, PET imaging studies on conscious nonhuman primates could be used to better understand the neural mechanisms underlying personality or kin recognition; studies examining whether coloration is linked to behavioral consistencies or personality may enable researchers to identify personality-related genes; and quantitative genetic studies in wild populations may highlight the genetic bases of phenotypes such as female preference for mates. Such interdisciplinarity will be central to gaining insights into animal and human behavior. We look forward to this new body of research and hope it will be the basis of a new volume at some point in the near future.

As should be clear, the authors spent a great deal of time and effort in writing their respective chapters. For this, we offer our most humble gratitude. We would also like to thank Eiji Inoue, who contributed to the book by editing and reviewing chapters, especially those in the first section. In addition, we are also grateful to Nick Mundy and Yohei Terai, who helped review the chapters in the fourth section.

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Part I
Sexual and Kin Selection in Animals

Chapter 1
Female Mate Choice in Rodents

Jörns Fickel and Alexandra Weyrich

1.1 Introduction

Sexual selection theory (Darwin 1871) predicts that in each species members of the two sexes employ different mating tactics – depending on the constraints each sex has on reproduction (Clutton-Brock 1989) – to maximize reproductive success (i.e., Darwinian fitness). These tactics are applied within the mating system of a given species (i.e., monogamy, polyandry, polygyny, promiscuity). This "conflict between the sexes" (reviewed in Chapman et al. 2003) arises because the amount of resources each sex invests in future progeny may differ significantly, depending on the species investigated (Alonzo and Warner 2000). Among mammals, it is usually the female that is the choosier one due to the higher amount of resources allocated to the production of gametes and to the raising of offspring (see Chap. 3). In recent years interest has grown to decipher the way and the reasons how and why the two sexes choose their mates the way they do. As a result, the paradigm on mate choice has shifted from the active (more or less aggressive) male and a passive (more or less coy) female view to a view where both sexes are actively choosing their mating partner from a pool of potential candidates.

Several excellent reviews have been written covering particular topics of reproduction, such as mating systems, mate choice, genetic compatibility, sexual conflict, sperm competition, behavioral genetics, cooperative game theory and sexual selection, and others (Clutton-Brock 1989; Anderson and Iwasa 1996; Zeh and Zeh 1996; Birkhead 2000; Tregenza and Wedell 2000; Paul 2002; Chapman et al. 2003; Stockley 2003; Dall et al. 2006; Roughgarden et al. 2006; Solomon and Keane 2007; Wolff and Sherman 2007; Greenspan 2008). These reviews cover a broad array of issues and provide detailed insights into, and theoretical background of their particular topic. For readers particularly interested in such topics, these reviews

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provide an excellent body of knowledge (more reviews are mentioned in the text). This and the fact that growing interest in these topics has led to a large and continuously growing number of newly published studies in recent years led us to focus on only certain aspects of rodent behavior (those to which genes could be linked) and in particular female mate choice.

In the near future, the application of genomic information retrieved from whole genomes (e.g., mouse and rat; http://www.ncbi.nlm.nih.gov/sites/genome) and techniques such as detailed brain receptor mapping will hopefully help us to gain new insights into genes and pathways involved in the development of behavior (e.g., by knockout genes/animals).

1.2 Mate Choice by Males (Short)

Because sexual selection theory also predicts that the more abundant sex competes for access to the less available sex, research in the past has focused on the mating strategies of males. Their "maximizing of progeny" is embraced between the two extremes of (1) copulation with the largest possible number of females and no parental care and (2) copulation with a single female and securing offspring survival by providing parental care (Clutton-Brock 1989). Successful mating for males requires access to receptive females whose spatial distribution may vary over time on one hand and who may be socially organized in many different ways on the other (e.g., solitary, dispersed, clustered, and matrilinear groups). However, even the presence of receptive females is no guarantee for males to reproduce successfully because other factors, such as environmental ones (e.g., competition with other males, habitat structure), and/or female selective behavior (see below), might compromise the access to females. Thus, males may have to employ a broad array of strategies to gain that access, including defeat of competitors in open fights, competitive mate searching, coercion of females, mate guarding, scent marking, adjustments to ejaculate volume, and sperm competition (Thornhill 1983; Schwagmeyer and Wootner 1986; Schwagmeyer 1990; DelBarco-Trillo and Ferkin 2004; Dean et al. 2006; reviewed in Waterman 2007; Firman and Simmons 2010). Further aspects, in particular with respect to sperm competition, are discussed below in response to female mate choice mechanisms.

1.3 Mate Choice by Females

Female mate choice (or, better, the choice of which potential candidate fertilizes the egg) may be exerted at various stages: before and/or during copulation, after copulation but before fertilization, after fertilization (Birkhead and Møller 1993). Females also use a broad array of traits in mate choice (reviewed in

Solomon and Keane 2007). One of the criteria females apply to their selection approach is the apparent provision of beneficial services by a male (e.g., provision of food, shelter, defense against harassment by other males, or even parental care). The latter, however, is rare in rodents, because most species are polygynous and promiscuous (Solomon and Keane 2007). Two of those rare examples are the California mouse (*Peromyscus californicus*) and the Djungarian hamster (*Phodopus campbelli*). Females of both species need male support for the successful upbringing of their progeny (Wynne-Edwards 1987; Gubernick and Teferi 2000). It is unknown, however, if females apply a "parental care" criterion in their preference for a certain male. Another choice criterion may be the male's genetic background because mating with a particular male may confer greater fitness on the offspring of the choosy female (increased viability, higher mating success, higher fecundity). This is due to the fact that selection acts on the variation in heritable traits; thus, a new combination of genes may increase the fitness of the offspring (Møller and Alatalo 1999). Other criteria employed are the following: mating status, because multiple-mating males tend to have reduced sperm counts and lower fertility in later ejaculates (Austin and Dewsbury 1986); infection status (Klein et al. 1999); dominance status (Shapiro and Dewsbury 1986) (see Chap. 3); body size (Solomon 1993); spatial ability, such as orientation and ability to locate mates or nests (Okasanen et al. 1999; Spritzer et al. 2005); relatedness (Keane 1990); and familiarity (reviewed in Anderson 1994). The latter criterion, for instance, is used by the highly inbred naked mole rat (*Heterocephalus glaber*), where reproductively active females prefer to associate with unfamiliar males, a mechanism that is interpreted as inbreeding avoidance (Clarke and Faulkes 1999) or at least to keep inbreeding below a critical threshold. Even if females are not very selective about their mating partners, they may still have postmating selection mechanisms implemented that allow them to choose between the sperm of several donors and/or to differentiate in their energy allocation toward offspring sired by different mates (Thornhill 1983; Eberhard 1996; Ben-Ari 2000).

However, either way of female choice (before/after mating) based on heritable traits bears a theoretical dilemma. The preference of females for a particular trait (i.e., directional selection) causes genetic variability in this trait to diminish quickly until it becomes fixed (Anderson 1994). This, in turn, reduces choice until it ceases to exist because there is no variability left in the trait. Unfortunately, a heterozygote (fitness) advantage (Brown 1995; Falconer and Mackay 1995) does not provide a remedy to that dilemma because after a population has reached homozygote/heterozygote equilibrium, females cannot increase their offspring's fitness any further by solely mating with a heterozygous male (Partridge 1983; Tregenza and Wedell 2000). However, during the last few decades, evidence has accumulated suggesting that heritability can be extended from fitness-related traits to life-history traits (Mousseau and Roff 1987; reviewed in Roff 1997). Furthermore, as is pointed out later in the chapter, in nature females base their choice on the weighting of multiple traits.

1.4 Polyandry, Fitness, and Genetic Compatibility in Rodents

As mentioned above, in mammals it is usually the female that is the choosier sex because females bear much higher costs in offspring production and upbringing due to the production of larger gametes and higher parental investment. Thus, one would expect that females increase their reproductive success by choosing and mating with one "high-quality" male, whereby only as many copulations should be performed as are needed to fertilize the egg to reduce the costs of mating. In contrast to these expectations, however, polyandry – the mating with more than one male during a single reproduction cycle – appears to be a common reproductive strategy among females of many species (Birkhead and Møller 1998). The intraspecific frequency of such multiple-male matings, however, varies greatly among rodent species. In red squirrels (*Sciurus vulgaris*) the frequency is only ~12% (Wauters et al. 1990), whereas in meadow voles (*Microtus pennsylvanicus*) it may reach 79% (Berteaux et al. 1999). In addition, the percentage of multiple paternities also varies greatly among rodents. Whereas in Columbian ground squirrels (*Spermophilus columbianus*) the proportion of litters sired by more than one male is ~16% (Murie 1995), in a promiscuous wild guinea pig (the yellow-toothed cavy, or *Galea musteloides*) it may go up to 90% (Hohoff et al. 2003). However, these numbers can vary in the same population across different years as shown for the 13-line ground squirrel (*Spermophilus tridecemlineatus*), where the proportion of multiple paternities found in litters ranged from 0 to 50% in different years (Schwagmeyer and Brown 1983). In addition to these varying percentages in multiple-male matings and multiple paternities across rodent species, there is also no clear picture regarding the effects of multi-male mating on the likelihood of conception and producing a litter. Whereas that probability was increased in Gunnisin's prairie dog (*Cynomys gunnisoni*) (Hoogland 1998), it appeared to be reduced in the Djungarian hamster (*P. campbelli*) and the deermouse (*Peromyscus maniculatus*) (Dewsbury 1982; Wynne-Edwards and Lisk 1984). In some species, there were no differences in conception and the birth rates between monoandrously and polyandrously behaving females: e.g., black-tailed prairie dogs (*Cynomys ludovicianus*) (Hoogland 1995), Columbian ground squirrels (*Spermophilus columbianus*) (Murie 1995), prairie voles (*Microtus ochrogaster*) (Wolff and Dunlap 2002), and 13-lined ground squirrels (*Spermophilus tridecemlineatus*) (Schwagmeyer 1986, reviewed in Solomon and Keane 2007).

1.4.1 Polyandry Versus Monoandry

A simple and convincing test for whether polyandry is indeed an advantageous reproduction strategy for females to gain genetic benefits is to compare reproductive success of polyandrous versus monoandrous (mated more than once with the same male) females. A study on female bank voles (*Clethrionomys glareolus*) demonstrated

that offspring of polyandrous females performed significantly better at reproduction than those of monoandrous females, although other fitness parameters (e.g., offspring body mass or winter survival) showed no differences between the two offspring groups (Klemme et al. 2008). Interestingly, there was a sex bias in offspring reproduction performance because the better performance was mainly due to sons of polyandrous females producing more offspring than those of monoandrous females (Klemme et al. 2008). Similar results were obtained in a promiscuous South American rodent, the common yellow-toothed cavy (*Galea musteloides*). Polyandrous females that mated successfully with four males weaned more surviving offspring than monoandrous females, although the litter sizes did not differ between the two groups (Keil and Sachser 1998). Similar results were found in a semelparous (dies after reproduction) marsupial, the brown Antechinus (*Antechinus stuartii*, also called Stuart's Antechinus or Macleay's marsupial mouse, not a rodent but occupying a rodent-like niche), where polyandry greatly increased offspring survival (Fisher et al. 2006). When female house mice (*Mus musculus domesticus*) were experimentally bred with a sibling and a nonsibling, microsatellite data revealed that paternity was biased toward nonsiblings (Firman and Simmons 2008a, b). These data support the hypothesis that polyandrous females copulate with several males to induce sperm competition and/or to enforce cryptic female choice, thereby facilitating postcopulatory inbreeding avoidance and increasing the viability of their offspring (Yasui 1997; Keil and Sachser 1998; Firman and Simmons 2008a).

The question then arises regarding the requirements for a male to be designated "high-quality". Does it have to be a good provider of material resources (e.g., *Mus musculus* females mate more frequently with males that defend high-quality territories) (Wolff 1985), a good provider of genetic resources (see below), or both? It is easily comprehensible that multiple matings require higher fitness costs than do single matings; thus, material benefits may be a good compensation for these additional costs (Hosken and Stokley 2003; Klemme 2006). However, such obvious material benefits appear to be absent in many polyandrous species; thus, the alternative explanation is that polyandrous behavior is driven by genetic benefits (reviewed in Jennions and Petri 2000).

Monoandrous females also have to consider that if they mate with a male that has already mated multiple times in succession, his sperm count and sperm fertility may be reduced (Austin and Dewsbury 1986), temporarily limiting his fertilizing capacity. Therefore, monogamous females should mate with males that have not yet mated with other females (Salo and Dewsbury 1995). Among the socially monogamous prairie voles (*Microtus ochrogaster*), females indeed tend to choose unmated males (Pierce and Dewsbury 1991). Moreover, females of species that mate multiply, such as rats (*Rattus norvegicus*) and golden hamsters (*Mesocricetus auratus*), prefer to mate with previously unmated males (Krames and Mastromatteo 1973; Huck et al. 1986). However, this is not a general rule, as two multiple-mating species of voles, montane voles (*Microtus montanus*) and meadow voles (*M. pennsylvanicus*), do not display preferences for unmated males (Pierce and Dewsbury 1991; Salo and Dewsbury 1995). Unfortunately, the fitness consequences of these preferences (litter sizes, offspring viability) have not yet been explored, nor have studies investigated

if males of these various species have evolutionarily different responses (in terms of sperm production) to the various female choice strategies. Such an evolutionary response of males to monoandrous or polyandrous females, however, was shown in an experiment with house mice (Firman and Simmons 2010). A population of mice that had been held for a long time under enforced monogamy was divided into two groups to create a polygamous line (strong selection for sperm competition among males) and a monogamous line (continuing relaxed selection). It took only eight generations of selection for the epididymal sperm count and sperm motility to be significantly increased in the polygamous line compared with that of the monogamous line still under relaxed selection (Firman and Simmons 2010).

A comparison of rates of early reproductive failure and litter size variation among promiscuous, monogamous, and polygynous (but still relatively monoandrous) mammals found (after controlling for phylogeny) that promiscuous species had significantly lower rates of early reproductive failure (measured as ova produced but wasted between ovulation and early postnatal development) than monogamous and polygynous species (Stockley 2003). The pairwise comparisons included, besides other mammals, 15 high-multiple-mating rodent species and 7 low-multiple-mating rodent species. Monoandrous females compensated for higher early reproductive failure with increased ova production and thus produced average litter sizes similar to those of the average litter sizes produced by more promiscuous females. The results are consistent with predictions of the genetic incompatibility avoidance hypothesis (see below), although alternative explanations may apply as well (e.g., adoption of an insurance strategy of offspring overproduction and subsequent reduction according to local resource availability) (Stockley 2003).

Another interesting aspect is the occurrence of extra-pair copulations (EPCs) and extra-pair paternity (EPP) in monogamous species (see Chap. 2). A study in the European alpine marmot (*Marmota marmota*: $n = 98$ genotyped at 12 microsatellite loci and $n = 499$ genotyped at 5 loci) revealed that females actively sought EPPs (Cohas et al. 2006). The number of occurrences increased with the number of subordinate males present (rendering it more difficult for the dominant pair-male to guard the female); and extra-pair mates were more heterozygous than within-pair mates. However, the occurrence of EPP did not depend on male heterozygosity, indicating that the closer related within-pair males were also successful sires (Cohas et al. 2006). The study concluded that female choice for genetic benefits may be a mechanism driving EPP in monogamous species (Cohas et al. 2006). Interestingly, both the number and the proportion of extra-pair young increased with both high similarity and dissimilarity between the social pair. This is best explained by the genetic compatibility hypothesis (see below), a mechanism to avoid both inbreeding and outbreeding depression (Cohas et al. 2008).

1.4.2 Intrinsic Male Quality Versus Genetic Incompatibility

The potential gain of genetic benefits by polyandrous females leads to the "intrinsic male quality hypothesis" and the "genetic incompatibility hypothesis" (Zeh and

Zeh 1996; Jennions and Petri 2000; Colegrave et al. 2002; Roberts and Gosling 2003). The first one states that "high-quality" males carry "good genes" (Møller and Alatalo 1999; Colegrave et al. 2002; Neff and Pitcher 2005), and sexual selection for them is based on the assumption that good genes (good alleles) in males are equally good for all females because that is their intrinsic virtue (Iwasa et al. 1991; Anderson 1994; Rowe and Houle 1996). A good allele is defined as an allele that increases fitness independent of the architecture of the remaining genome, which in diploid organisms includes the homologue to the particular "good allele." Across the genome, good genes show additive genetic variation (Neff and Pitcher 2005). The genetic incompatibility hypothesis, however, states that selection for genetic compatibility (or avoidance of incompatibility) arises because interactions between male and female genotypes determine offspring viability; therefore, a male that may be a genetically suitable mating partner for one female may not be suited for another (Brown 1995; Zeh and Zeh 1996, 2001; Tregenza and Wedell 2000).

Under the good genes model, variation in genetic quality of males is of interest for females (to recognize the good alleles), but may not be directly assessable (Iwasa et al. 1991; Anderson 1994); hence, secondary but recognizable (indirect) "indicators of quality" have to be employed. Such indicators are useful for assessing quality prior to mating, thereby avoiding resource investment in low quality offspring. However, the model leads to directional selection in males because females prefer males whose indicators promise good genes (Colegrave et al. 2002). The latter become a fixed trait in the population, and choice ceases to exist. Under the genetic incompatibility hypothesis the existence of intrinsically superior alleles is not necessary because each male is assessed separately and the male's quality is determined by each female individually. Hence, quality assessment of males by females depends on the individual genotypes of the mating partners (parental genetic compatibility) rather than on the presence of male "good for all females" alleles (Brown 1995; Zeh and Zeh 1996, 2001; Tregenza and Wedell 2000; Colegrave et al. 2002).

Thus, a working definition of a *compatible allele* is that it is an allele that increases fitness when in a specific genotype – i.e., when paired with a specific homologue (overdominance) or allele at another gene locus (epistasis). Across the genome, compatible alleles then show *nonadditive* genetic variation. Thus, when variation in fitness exists because of compatible alleles, the population does not respond to directional selection, but the mechanisms of acquiring compatible alleles (e.g., preference alleles) respond to directional selection (Neff and Pitcher 2005). The interaction of genotypes, however, can only occur after mating. Females therefore need to have cytological and/or biochemical mechanisms in place by which male quality (=suitability of the male's genotype) can be assessed directly without the use of prior secondary indicators. Such assessment could happen in at least two ways: (1) sperm of genetically better suited males would be given a greater chance to fertilize the egg than the sperm from a less compatible competitor or (2) females could distinguish between the offspring sired by different males. In the latter case, females could allocate more resources to offspring sired by males more genetically compatible with them, potentially leading to differential

viability of offspring sired by males that differ in their degree of genetic compatibility with the particular female (Tregenza and Wedell 2000). The model's advantage lies in the fact that this process does not lead to directional selection because compatibility needs to be assessed separately and anew for any new combination of mating partners, whereby both sexes maintain polymorphisms in their genotypes (Birkhead 1998). The avoidance of inbreeding, and thus the avoidance of accumulating deleterious alleles, can therefore be seen as the most widespread behavior under the genetic incompatibility hypothesis (Tregenza and Wedell 2000; Klemme 2006; Klemme et al. 2008). Theoretical models have shown that as soon as there are costs of mating involved some form of compatibility based sperm selection is necessary for the evolution of "polyandry for compatibility" (Colegrave et al. 2002).

Another aspect, which may not immediately come to mind, is the conservation of species via freezing of gametes (Fickel et al. 2007). The usual practice of freezing only sperm or oocytes (often sperm only) does not take into account the compatibility of genotypes as an important fitness trait of a species. Thus, it should be supplemented (whenever possible) by freezing compatible gametes (wherever compatible individuals are known) to improve the chances of fertilization after thawing (Fickel et al. 2007). This practice would better serve the purpose of conservation, although its feasibility might be limited.

1.5 Influence of the *t*-Complex on Behavior in Mice

1.5.1 Organization and Impact

The *t*-complex in mice, detected during the first third of the last century (reviewed in Bennett 1980), is a very large chromosome segment on chromosome 17 of the mouse genome (Bennett 1975; Lenington et al. 1992) with several of its genes already annotated (*t*-complex proteins: TCP1, TCPs 10a–c) [for chromosomal localization see the National Center for Biotechnology Information (NCBI) GenBank]. It consists of a number of tightly linked loci connected by at least four inversions (Artzt et al. 1982b) that all are inherited as a single genetic unit (haplotypes) due to the suppression of genetic recombination between them (Artzt et al. 1982a, b; Delarbre et al. 1988). Loci of the *t*-complex influence embryonic development, tail length, male sperm transmission ratio (Fraser and Dudley 1999), male fertility, and other traits. Among the tightly linked loci of the *t*-complex is also the major histocompatibility complex (MHC) (Artzt et al. 1982b), and some studies indicate interactions between the latter and other regions of the complex affecting mating behavior (Lenington et al. 1988; Lenington and Egid 1989). In total, the complex comprises about 1% of the total mouse genome, and the variants found in several species of mice may have evolved from a common ancestor (Delarbre et al. 1988).

1.5.2 Natural Occurrence and Distribution of the t-Complex

Wild mice are polymorphic for a recessive mutation that occurs within the *t*-complex, and heterozygous individuals can be found in most house mice populations (*M. m. domesticus*) as well in populations of at least three other species of the genus *Mus* (*M. m. musculus*, *M. cervicolor*, *M. spretus*) (Delarbre et al. 1988). Interestingly, in heterozygotes, carrying two different *t*-haplotypes, *t*-complex recombination is not suppressed, indicating that *t*-chromosomes may be mismatched only in the combination of a *t*- and a wild-type haplotype and not in the combination of two different *t*-haplotypes, because crossing over is permitted between the latter (Silver et al. 1980; Artzt et al. 1982a). To date, more than 25 *t*-haplotypes have been characterized, most of them being lethal in respective homozygotes (Silver 1985). So far, eight lethal classes and one semi-lethal class have been classified by genetic complementation tests (Bennett 1980). Homozygotes carrying the *t*-lethal allele die prenatally, male *t*-semi-lethal homozygotes are sterile, as are males carrying two complementing *t*-lethal haplotypes (Baker 2008).

1.5.3 What Maintains Deleterious Genes?

Without a particular mechanism maintaining the deleterious genes, one would expect *t*-lethals and *t*-semi-lethals to be quickly eliminated by selection. However, despite a strong selection against *t*-complex haplotypes (often also called alleles), they persist (as mentioned above) in a population in proportions of up to 25% (Lenington et al. 1992). The reason for that imbalance is distorted sperm segregation in males, also called transmission ratio distortion (TRD) (Bruck 1957; Bennett and Dunn 1971; Lyttle 1991), allowing heterozygous males to pass *t*-alleles on to more than 90% of their offspring [>95% reported by Ben-Schlomo et al. (2007); 80–100% reported by Baker (2008)]. Although the number of *t*-type sperms produced by heterozygous males equals their number of wild-type (+) sperms produced, TRD causes the latter to be damaged in some way, thus reducing their ability to fertilize a female (Fraser and Dudley 1999). Despite TRD, only ~25% of mice in wild populations are heterozygous (*t*/+) (Lenington et al. 1992). This, in light of TRD's much lower than expected frequency of *t*-alleles in natural populations, cannot be explained by chance; in fact, it was demonstrated to be due to avoidance of heterozygous mates by the opposite sex (Lenington 1983, 1991). Examination of preferences of homozygous +/+ females and heterozygous +/*t* females for males of both genotypes revealed that heterozygous +/*t* females but not homozygous +/+ females had a strong preference for homozygous +/+ males (Lenington et al. 1992). Thus, heterozygous females had greater avoidance of heterozygous males than did homozygous females (Lenington 1983, 1991; Williams and Lenington 1993), a finding that was independent of the particular *t*-allele the female was carrying (Williams and Lenington 1993). The usefulness of that strategy is easily comprehensible: From the mating of a heterozygous female with

a heterozygous male, on average 25% of the offspring are homozygously lethal for the *t*-allele, whereas the offspring from the mating of a homozygous female with a heterozygous male does not contain *t*-allele homozygotes. This shows that the female's avoidance of heterozygous males is related to her own genotype, indicating that (1) genes on *t*-haplotypes function as modulators of these preferences and (2) genetic compatibility influences mate choice (Lenington et al. 1992).

1.5.4 t-Complex and Other Female Choice Guiding Traits

Female mate preference is also affected by factors such as parental genotype (Lenington and Egid 1985) and is stronger among females in estrous than among diestrous females (Lenington et al. 1992; Williams and Lenington 1993). In addition, when heterozygous +/*t* females were forced to choose between two heterozygous +/*t* males (one carrying the same *t*-haplotype, the other carrying a different one), they preferred the male with the haplotype differing from their own (Lenington et al. 1992). Interestingly, female partner preference is also affected by the dominance status of the male (traits affecting dominance status are heritable) (Drickamer 1992; Horne and Ylönen 1998). In a restricted situation, female mice give priority to male dominance status over the *t*-complex genotype (Lenington et al. 1992), indicating that there might be additional forces affecting the frequency of *t*-mutations in wild mice.

In addition to female effects, males also show behavioral variation. They are more aggressive toward heterozygous +/*t* females and less likely to mate with them than with homozygous wild-type females (Lenington 1991; Lenington et al. 1992). The example with the *t*-complex and dominance shows that female mate choice is not a simple choice considering a single trait but, rather, a complex behavior influenced by more than just one trait. It also illustrates that the result of a female's mate choice (i.e., which male eventually fertilizes the egg) is the outcome of a relative weighting procedure by which various traits may be weighted against each other. Dominance, for instance, may be outweighed by infection status or spatial ability. Female house mice (*M. m. domesticus*) preferred odors from nonparasitized but subordinate males over those from parasitized dominant males (Mihalcin 2002, cited in Lacey and Solomon 2003; Kavaliers and Colwell 1995). In a laboratory experiment, female meadow voles (*Microtus pennsylvanicus*) – a species that, in contrast to den-living mice, is far more outspread territorially and lives at lower densities – preferred males with good spatial ability and low dominance rank over males with poor spatial ability and high dominance rank (Spritzer 2003).

1.5.5 Recognition of Heterozygotes

The question then arises, how do the sexes recognize the trait "+/*t*-heterozygous" in the opposite sex? About two decades ago, it was discovered that genes within the

t-complex are associated with specific odors (Drickamer and Lenington 1987) and that both males and females can use these smell cues to recognize and to discriminate against the genotypes of the opposite sex (Lenington 1991).

The mouse genome is fully sequenced (for the sequence of chromosome 17 see http://www.ncbi.nlm.nih.gov/mapview/maps.cgi?taxid=10090andchr=17), but to date not all of its genes have already been annotated and/or assigned to chromosomes. According to GenBank, chromosome 17 carries 1,511 genes; but the function of many of them remains to be elucidated. Because the *t*-complex is so deeply involved in mate choice, the gene(s) that influences mating preferences should be closely linked to the *t*-complex itself. Indeed, tests with female mice carrying the partial *t*-haplotype *t*(w18) indicated that the genes controlling mating preferences lie in the region of the *t*-complex distal to the MHC (Lenington 1991; Lenington et al. 1992).

1.6 Influence of the Major Histocompatibility Complex on Behavior

1.6.1 MHC Organization in Rodents

During the 1960s it was discovered that one of the important gene clusters involved in the immune response in vertebrates is the MHC, which in mice is usually referred to as the H-2 complex (McDevitt and Chinitz 1969) and in rats as the RT1 complex (Kelley et al. 2005). MHC genes are important in tissue recognition, acceptance, and rejection (Steinmetz et al. 1982) because they encode ubiquitously expressed cell-surface glycoproteins, so-called transplantation antigens, that serve as recognition structures for cytotoxic T cells (Stroynowski et al. 1987). The MHC is usually highly polymorphic (reviewed in Jordan and Bruford 1998); and, in contrast to that in birds and fish, in mammals it is inherited as a single unit (haplotype). So far, about 100 alleles have been described in mice (Klein 1975, 1986), but their number is certainly much higher. In fact, the number of potential MHC genotypes comprising two sets of MHC alleles in each diploid individual could easily exceed the size of a population of a given species (Yamazaki and Beauchamp 2005). In the mammalian model, the MHC is generally divided into regions with similar function, including classes I, II, and III (Klein 1986) and extended classes I and II (Herberg et al. 1998). The number of genes and the presence and location of each region varies among species (reviewed in Kelley et al. 2005).

The class I region is composed of classic (Ia) and nonclassic (Ib) genes. MHC Ia molecules generally present peptide antigens to CD8 cytotoxic T lymphocytes through T-cell receptors, whereas the functions of MHC Ib genes are diverse (Williams et al. 2002; Holling et al. 2004). Interestingly, Ib molecules such as M10 genes have numerous positions that are (as in Ia molecules) under positive selection (Emes et al. 2004), sparking questions as to whether they are also involved in ligand

binding (Ishii et al. 2003). Members of both categories may act as ligands for receptors on natural killer (NK) cells.

Class II molecules can present antigens to CD4 T lymphocytes (T-helper cells) (Villadangos 2001). The turnover rate of peptides of the class I region is generally higher than that of the class II region (Takahashi et al. 2000). The class III region contains a highly dense selection of diverse immune and nonimmune genes (Aguado et al. 1996; Milner and Campbell 2001; Xie et al. 2003).

1.6.2 Mouse MHC

The H-2 complex is located on chromosome (chr) 17 of the mouse genome (*M. musculus*, $2n=40$). It is organized in a manner similar to that of human MHC, except for an additional classic class I locus (Walter et al. 2002) located centromeric to the class II region (Kumánovics et al. 2003). The number and sequences of class I loci also differ from those of humans (Trowsdale 1995), although there is some homology regarding class I gene location (Amadou 1999). Unlike primate MHCs, the H-2 lacks MIC (MHC class I chain)-related genes. However, the MIC-related MILL gene family (MHC class I-like located near the leukocyte receptor complex) is located near the leukocyte receptor complex on chr 7 (Kasahara et al. 2002). Interestingly, some mouse species deviate in their MHC organization from this general model. The African pigmy mouse (*Nannomys setulosus*), for instance, has thousands of class I genes (Delarbre et al. 1992). Intermingled in the extended class I region (close to the telomere) are also numerous loci for olfactory receptors (ORs) (Kelley et al. 2005).

In contrast to many other mammals, mouse T cells do not express class II molecules on their surface, which indicates differences in genetic regulation of these molecules in comparison to other mammals (Holling et al. 2004).

1.6.3 Rat MHC

The RT1 complex is located on chr 20 of the rat genome (*R. norvegicus*, $2n=42$). The RT1 class I region contains eight gene clusters (HLA has only 4). One of these clusters, RT1-A, is located centromeric to the class II region, similar to H-2K in the mouse (Walter and Günther 2000; Hurt et al. 2004). Like H-2 in mice, the rat RT1 lacks MIC-related genes (Hurt et al. 2004), such as MICA and MICB, which are present in humans, and has M-like class I genes, homologous to mouse H-2M, which are absent in the human MHC. RT1 also exhibits a duplication of C4 (a gene for a complement component) and flanking genes, but these genes are not tandemly duplicated as in the mouse and humans (Walter et al. 2002; Hurt et al. 2004). The rat MHC also differs by the presence of a larger number of BTNL (butyrophilin-like) genes centromeric to RT1-Da and a second and putatively functional HLA-DRB-

related gene, RT1-Db2 (Hurt et al. 2004). As with the exceptions to the mouse H-2 model of MHC complex organization, there are also exceptions to the rat model of MHC organization. For instance, there are differences within the class II regions; the mole rat (*Spalax ehrenbergi*) completely lacks DR genes; however, the multiple α-genes and β-genes in the DP loci assume its functions (Nizetic et al. 1987). As in the mouse H-2, there are numerous ORs in the extended (telomeric) class I region (Kelley et al. 2005).

1.6.4 Polymorphisms and Antigen-Binding Site

Haplotypes of the MHC are determined by the combination of alleles at either locus (Kelley et al. 2005). In addition to these loci, a number of other genes are also known to reside in the H-2 region (Steinmetz 1983; see also GenBank). Whereas MHC class I gene products are expressed in all nucleated cells and are responsible for the defense against intracellular pathogens (e.g., viruses), class II genes are usually involved in defending against extracellular pathogens (e.g., parasites, bacteria). Each of the class I and II MHC proteins is a dimer that consists of two polypeptide chains (Eggert et al. 1999) (the β_2-microglobulin of class I molecules is not coded by the MHC). Some genes of the MHC are among the most polymorphic loci in vertebrates (Klein 1986), whereby the so-called antigen-binding site (ABS) – a domain of the glycoprotein that binds to the antigen – forms the most variable portion of the proteins (Hughes and Hughes 1995; Hughes and Yeager 1998), resulting in the above-mentioned more than 100 alleles in mouse MHC (Klein 1975, 1986). These allele differences among individual MHC complexes provide their carriers with different degrees of (1) resistance against pathogens (Potts and Slev 1995; Fröschke and Sommer 2005) and (2) susceptibility to autoimmune diseases (reviewed in Apanius et al. 1997).

1.6.5 Other Functions of the MHC

In addition to its immunological function of self/nonself discrimination, MHC loci contribute to an individual's odor (Singer et al. 1997; Schaefer et al. 2001): (1) directly, because some genes encode volatile-binding peptides and soluble proteins (classes I and II in mice, only class I in rats (Eggert et al. 1999) and (2) indirectly, because they influence the composition of the intestinal bacterial flora (in rats but not in mice) (Schellinck and Brown 1992). Thus, high variability in MHC alleles may translate into high variability of odors (Yamazaki et al. 1990), thereby providing the means of individual recognition (Eggert et al. 1999) and kin recognition (Schellinck et al. 1993; reviewed in Brown and Eklund 1994). The fact that MHC genes themselves generate a characteristic type of odor, rather than dedicated odor-determining genes, was shown by point mutations in H-2K and HLA transgenic mice, which generated distinct odor profiles in olfactory assays (Bard et al. 2000). Further evidence for a

central, odor-specifying role of MHC genes themselves was given by demonstrating that mice that lacked β_2-microglobulin (B2m), and thus were unable to express their genomic class I MHC genes, were distinguishable by scent from otherwise identical mice that had an intact B2m gene. This odor-type disparity appeared at 9–12 days of gestational age, the period during which the MHC is first detectable in fetal cells of normal mice (Bard et al. 2000). However, even though these experiments clearly demonstrate that individuals can be distinguished based on their MHC condition, they do not provide proof that this trait is also used in mate choice.

Some nonclassic class I genes in mice and rats are expressed in the vomeronasal organ (VNO) – a region that harbors numerous ORs genes – displaying an additional function in pheromone detection (Schaefer et al. 2001; Ishii et al. 2003; Loconto et al. 2003; reviewed in Emes et al. 2004). However, a study carried out in mice to investigate the role of VNO in the recognition of MHC odor types concluded that the VNO is not involved (at least not in Y-maze tests) in MHC odor recognition because surgical removal of the VNO did not disrupt MHC odor-type discrimination (Yamazaki and Beauchamp 2005).

1.6.6 "Balancing Selection" Versus "Rare Allele Advantage"

So far, six models have been suggested to explain the maintenance of MHC variability (Potts and Slev 1995), of which the two most influential are explained here. In the "balancing selection model" (often misleadingly called "overdominance") (for definitions see Takahata et al. 1992), it is assumed that heterozygous animals are able to bind more foreign peptides than are homozygous individuals (Takahata et al. 1992). However, as pointed out above, there might be a trade-off between the heterozygote advantage (Irwin and Taylor 2000) by expressing numerous alleles on the one hand and the consequentially increased chance of autoimmune disease on the other (Tregenza and Wedell 2000). The maintenance of a balance between these two effects, in the end, promotes an optimal rather than a maximal degree of heterozygosity. This has consequences for female mate choice because females should choose males with an intermediate degree (the optimal complementary set of alleles) of dissimilarity (Penn and Potts 1999; Tregenza and Wedell 2000).

The second model, the "rare allele advantage" model, assumes that the pathogens and MHC alleles are under negative frequency-dependent selection. Thus, a female should be able to increase reproductive success by mating with a male that has a different MHC genotype than that of the female (disassortative mating), thus providing the offspring with a greater variety of MHC alleles (thereby increasing offspring viability). Such MHC dissimilarity from its parents would allow the offspring MHC to recognize pathogens that have evaded the parents' immune cell repertoire (reviewed in Apanius et al. 1997; Penn and Potts 1999). Independent from the model, in both cases the progeny have an MHC dissimilar to either of the parents, resulting in improved resistance against pathogens.

1.6.7 Does MHC Influence Mate Choice?

Although the question of the MHC influencing mate choice has recently been addressed in species other than the mouse (Sommer 2005; Schwensow et al. 2007, 2008), most of the work was done in laboratory mice using MHC-congenic (strains that are genetically identical except for their MHC) inbred strains. Apparent MHC-based mate preferences were observed in crosses of strains set up to produce MHC-congenic strains (Yamazaki et al. 1976). However, strain variation in the source strains generated a widespread pattern of results; mate choice was strongest in homozygotes and weak or intermediate in heterozygotes (mice studies are reviewed in Jordan and Bruford 1998). Other studies have even suggested the presence of additional postmating selection mechanisms (Wolgemuth 1983; Wedekind et al. 1996). In vitro fertilization experiments with two inbred H-2 congenic mouse strains yielded nonrandom MHC combinations in the blastocysts, which according to the authors indicated either oocyte choice for the fertilizing sperm or an influence on the outcome of the second meiotic division after the sperm had entered the egg (Wedekind et al. 1996). Another mouse study showed that both MHC dissimilarity and a good gene indicator (e.g., male investment in scent marking) have a role in determining female preference (the balance of selection pressure on each trait depends on how females weight these desirable qualities under different conditions), but that their relative influence varied depending on the degree of variability in each trait among available males (Roberts and Gosling 2003). In some house mouse strains, the scent-marking rate (an indicator of "male quality") superseded MHC dissimilarity as a predictor of female preferences. The latter became important only when differences in the scent-marking rates among males were small. The authors concluded that such interactions between condition-dependent and disassortative mate choice criteria suggest a mechanism by which female choice can contribute to the maintenance of additive genetic variance in both the MHC and the condition-dependent traits, even under consistent directional selection (Roberts and Gosling 2003). As already noted, female mate choice in nature is a complex behavioral pattern influenced by more than just one male trait.

To corroborate the results from experiments on laboratory MHC-congenic inbred strains, mating experiments were also performed with wild mice populations. In one of those studies (Potts et al. 1991), the analysis of progeny resulted in 27% fewer MHC-homozygous individuals than expected from random mating. However, another study (Eklund 1997), also carried out on wild mice, suggested that although females did make MHC-relates choices they did not necessarily prefer mates dissimilar from their own family MHC genotype. They also chose similar genotypes, showing both assortative and disassortative behavior. This contradicts results from primate studies that showed that there is a MHC similarity disadvantage: Mating of individuals who shared the same MHC haplotype resulted in increased fetal loss (Knapp et al. 1996; Ober et al. 1998).

A study on another rodent, the Malagasy giant jumping rat (*Hypogeomys antimena*), also did not find associations of mating patterns with the MHC genotype

(Sommer 2005). It must be noted, however, that for rodents other than mice there exists no repertoire of MHC-congenic strains, rendering analyses regarding MHC-associated mate choice difficult. In these species, usually only parts of the MHC can be (and have been) studied. Often the homologue to the HLA-DRB2 locus is used, which in mice corresponds to H2-Eβ1 and in rats to RT1-Dβ2 (Kelley et al. 2005). So, if a study in nonmice rodents fails to detect associations of mating patterns with the MHC genotype, it may be due to the fact that either there is indeed no such association or there is just no association with the particular locus studied, although associations may exist with other loci of the MHC (HLA contains more than 260 gene loci, and similar numbers are expected for H-2 and RT1) (Kelley et al. 2005).

These and other problems in the field of mate choice and MHC have sparked considerable controversy due to (at least partially) a lack of robustness of results, failure to reproduce results, flaws in experimental design, and interpretation of results. The point at issue in this controversy is the fact that it is difficult to demonstrate that mate choice depends on MHC variability and not on genotypes of loci that are only linked to the MHC rather than being a true part of it (Hughes and Hughes 1995). In the latter case MHC allelic diversity would be unimportant and had no influence on mate choice. The presence of an association between non-MHC loci in the MHC region and mate choice in humans was indeed suggested (Weitkamp and Ober 1998). Another argument that has been brought forward is that differences in patterns of nucleotide polymorphisms between the parts of loci that code for ABSs and those that code for non-ABSs cannot be explained by sexual selection (Hughes and Nei 1989; Hughes and Hughes 1995). However, this can be argued against if it is the ABS that influences mating preference (e.g., by determining odor) (Singer et al. 1997; Zavazava and Eggert 1997; Eggert et al. 1999). In addition, selection acts on single sites rather than on entire peptides; thus, differences in nucleotide polymorphisms between ABS and non-ABS are expected because both elements may well be under different selective pressures owing to their different functions.

A possible explanation for the controversial findings regarding MHC and mate choice is that the expression of MHC genes (at least of some) depends on heterogeneity in the environment (Ewing 1979) (i.e., on the infection status of the individual studied; Wedekind et al. 1996). Thus, in a pathogenic environment increased fitness would be achieved by the presence of particular individual MHC alleles (condition-dependent trait), which in turn could explain MHC-based selection of currently (in that environment) "good genes," whereas in the absence of such pathogenic environment other alleles may be favored. Therefore, sexual selection of condition-dependent traits during mate choice could be used to select successful MHC alleles, thereby providing offspring with a higher relative immunity in their pathogenic environment (Grob et al. 1998; see also Roberts and Gosling 2003). However, it is not the mere presence of a particular individual MHC allele combination that is of relevance but, rather, their expression. However, because of the highly polymorphic structure of MHC, expression studies are difficult and still rare. Recently developed and established techniques to measure expression levels of MHC alleles (Axtner and Sommer 2009; Weyrich et al. 2010) will render such studies possible in the near future.

An additional, yet not explored aspect is that in the arms race between pathogen and MHC the pathogen should aim at manipulating the host's odor in a way that its host becomes attractive to the opposite sex (despite being infected), which in turn would increase the pathogen's chance to infect a new host during mating. Such strategy would balance the "dissimilarity strategy" of the host species.

Another difficulty lies in the comparison of mate-choice results from laboratory strains and wild mice. It stems from the fact that wild mice employ a large repertoire of mating patterns (e.g., multiple mating, EPCs) (Manning et al. 1992) that is lost when inbred strains are mated experimentally. Such differences were seen when mice held under semi-natural conditions were allowed to establish their own mating system (Potts et al. 1991). Under these conditions, male-controlled female settlements deviated from random expectations (in respect to MHC), and ~25% of the observed MHC homozygote deficits were accounted for by within-territory matings, superficially suggesting that males had based their mate choice on MHC. However, a closer look revealed that the main cause for the MHC homozygote deficit lay in extraterritorial matings by females, where they tended to choose males that had a higher degree of MHC dissimilarity than their territorial males (Potts et al. 1991, 1992).

Despite this ongoing debate, MHC loci remain prime candidates (together with the olfactory sensory system, see below) for involvement in mate choice (Jordan and Bruford 1998) simply because the exceptionally high levels of polymorphisms at MHC loci provide the variability required for a genetically based recognition system. In addition, plausible hypotheses exist for the mechanisms by which MHC molecules might generate individual odors (Schellinck et al. 1993; Zavazava and Eggert 1997; Eggert et al. 1999). For the sake of clear argument, it might be necessary to continue to use MHC-congenic strains in future research because it appears to be the only way to demonstrate unequivocally a direct role of MHC in mate choice (Jordan and Bruford 1998).

1.7 Other Genes Known to Influence Mate Choice in Rodents

1.7.1 Oxytocin (Oxt) in Mice

The gene for oxytocin (*Oxt*, also called *OT*) contains three exons (378 bp total length) and is located on chromosome 2 in mice (chr 2 F1|2 73.5 cM; GenBank http://www.ncbi.nlm.nih.gov/mapview/maps; NC_00068.6) and on chr 3 in rats (chr 3q36; GenBank). The mature hormone itself is a nonapeptide, derived by enzymatic cleavage from a larger precursor. It is synthesized in the hypothalamus and released into the blood from the posterior lobe of the pituitary. It is also expressed in corpora lutea (reviewed in Stormshak 2003) and testes (Bathgate and Sernia 1994; Einspanier and Ivell 1997). The hormone has been associated with various

behaviors, including social recognition, anxiety, pair bonding, and maternal behavior (reviewed in Caldwell and Young 2006).

As noted earlier, if a male's trait in which a female is interested is not assessable directly, females have to employ other, indirect indicators on which to base their assessment of male quality (Iwasa et al. 1991; Anderson 1994). However, not only genetic quality can be evaluated this way, social information can also be acquired directly or indirectly from cues inadvertently produced by individuals ("inadvertent social information"). This "public information" can be used by other individuals in the population for their behavioral response (Danchlin et al. 2004). Female rodents use odors (olfactory cues) to adjust their responses to males.

To investigate the role of oxytocin in rodents, *Oxt* gene wild-type (*Oxt*WT) mice were compared with *Oxt* gene knockout mice (*Oxt*KO) in various preference trials (Kavaliers et al. 2006). In these trials, female *Oxt*WT mice distinguished between parasitized males (subclinically infected with a gastrointestinal nematode parasite) and nonparasitized males, displaying aversive responses (analgesia, increased corticosterone) to, and avoidance of, the odors of parasitized males. The response changed when the odors of another estrous female were associated with parasitized males. The presence of the odor of another estrous female together with that of an infected male (indicative of potential mate interests by other females) attenuated the aversive responses and resulted in a choice for the odor of the infected male (independent of the sexual status of the choosing female). Thus, some cues of one *Oxt*WT female's choice influenced the mate choice by another *Oxt*WT female, even leading to decision reversal. In contrast to *Oxt*WT females, the ability of *Oxt*KO females was impaired in terms of using odor to adjust their responses to either uninfected males of differing sexual states or infected males. It appears that oxytocin is required to process inadvertent social information (Kavaliers et al. 2006).

In female prairie voles (*Microtus ochrogaster*), oxytocin concentrations were increased in the nucleus accumbens during unrestricted interactions with a male compared with the absence of a male (Ross et al. 2009). How these concentration do or do not change in a situation where females are given choices between different males remains to be seen, although, as we have seen earlier, no differences in conception and birth rates were observed between monoandrous and polyandrous female prairie voles (Wolff and Dunlap 2002); therefore, strong differences might not be expected.

A different approach was taken in another study on mice, where strains were generated carrying either a null mutation in the oxytocin receptor gene ($Oxtr^{-/-}$) or in the oxytocin gene ($Oxt^{-/-}$) (Takayanagi et al. 2005). $Oxtr^{-/-}$ mice were viable and had no obvious deficits in fertility or reproductive behavior. Receptor-deficient females had normal parturition but displayed defects in lactation and nurturing, whereas adult $Oxtr^{-/-}$ males were deficient in social discrimination and showed elevated aggressive behavior. $Oxt^{-/-}$ sons from $Oxt^{-/-}$ females, but not from heterozygous $Oxt^{+/-}$ females, showed similar high levels of aggression. These data show that the OXT/OXTR system is part of the mechanism that shapes aggressive behavior in adults (Takayanagi et al. 2005), which in turn may influence the dominance status of a male and thus its reproductive fitness.

In addition to the effects on reproductive behavior mentioned above, testicular oxytocin has been shown to influence reproductive fitness directly. It promotes spermiation (removal of unnecessary cytoplasm and organelles from mature spermatozoa in the seminiferous tubules) and sperm transfer in mice (Assinder et al. 2002). A comparison of oxytocin wild-type mice (*Oxt*WT) with both oxytocin knockout mice (*Oxt*KO) and an oxytocin transgenic mouse strain (bOT4.2), which overexpresses testicular oxytocin, showed that both the timing of spermiation and the appearance of epididymal sperm differed significantly among groups (bOT4.2 < OxtWT < OxtKO) (Assinder et al. 2002).

1.7.2 Pkdrej in Mice: Polycystic Kidney Disease (Polycystin) and REJ (Sperm Receptor for Egg Jelly, Sea Urchin Homologue)-Like

The mouse *Pkdrej* gene, which in contrast to all other *Pkd* genes is expressed in the male germ line only, is a homologue to the sea urchin receptor for egg jelly (REJ). It is located on chr 15 (chr 15 E2) and contains only a single exon (GenBank: NC_000081.5). It is a member of the polycystin-1 gene family, a family of integral membrane proteins that includes Pkd1 as well as Pkd1l1, Pkd1l2, Pk1l3, and Pkdrej. Members of the protein family are present in fish, invertebrates, and mammals. Polycystins are composed of multiple domains and are widely expressed in various cell types. In humans, mutations in polycystin-1 (PKD1) and polycystin-2 (PKD2) have been shown to be the cause for the dominant, autosomally inherited polycystic kidney disease (Sandford et al. 1999). Because in echinoderms polycystin-1 proteins are required for the acrosome reaction (Neill and Vacquier 2004), *Pkdrej* was proposed to be a component of the egg-coating zona pellucida glycoprotein 3 (ZP3)-activated signaling pathway (Jewgenow and Fickel 1999), triggering mammalian acrosome reactions (Hamm et al 2007).

The mouse *Pkdrej* precursor is 2,126 amino acids (aa) long and contains several functional domains. In addition to the 11 transmembrane domains, the three most prominent are (1) the 116 aa long PLAT/LH2 lipase/lipogenase motif of polycystin-1-like proteins, (2) the 544 aa long REJ domain in the extracellular N-terminal region, and (3) the 431 aa long polycystin cation channel (PKD channel) (GenBank http://www.ncbi.nlm.nih.gov/mapview/maps).

A recent study investigated the influence *Pkdrej* has on sperm competence in mice by generating a *Pkdrej*-mutated strain via replacement of the first six transmembrane domains by an internal ribosome entry site-LacZ/neomycin-resistance cassette (Sutton et al. 2008). Fertility of male *Pkdrej*$^{tm/tm}$ homozygous mice were unaffected in unrestricted mating trials. However, mutant males exhibited lower reproductive success when they had to compete with either wild-type males in sequential mating trials or in artificial insemination tests with mixed (mutant + wild-type)-sperm populations (Sutton et al. 2008). The study also revealed that sperm from *Pkdrej*$^{tm/tm}$ mice needed more than 2 h longer to become detectable

within the egg–cumulus complex in the oviduct than those of wild-type males. Although sperm from males of both genotypes were able to capacitate in vitro, one of the component processes of capacitation, the ability to undergo a zona pellucida-evoked acrosome reaction, was decelerated in sperm from mutant males compared to sperm from wild-type males. However, no genotypic differences were observed in another component process of capacitation, the transition to hyperactivated flagellar motility. Thus, at least two processes are differentially regulated by *Pkdrej*: exocytotic competence and motility. These findings suggest that *Pkdrej* controls the timing of fertilization in vivo through effects on sperm transport and exocytotic competence. Moreover, it is a factor in sperm-competitive postcopulatory sexual selection (Sutton et al. 2008).

1.7.3 Olfactory Receptors

We have elaborated on examples of how olfactory cues such as body odor or urine scent marks are used by rodents to communicate mate-choice-relevant (and other) traits and how they modulate the mate preference behavior. For instance, mouse urine vapor is composed of more than 80 chemical compounds (Singer et al. 1997) coming from large chemical groups such as alcohols and aldehydes, esters and ethers, ketones, aromatics, and acids (Schaefer et al. 2001). In addition, mouse urine contains major urinary proteins (MUPs) (in the rat they are termed α_{2u}-globulins) that are produced in the liver and released into urine by filtration from blood (Novotny et al. 1999). These olfactory cues, however, are only the emitting (signal) part of the system. The complementary receiving end is comprised of ORs, as signals need to be received and processed properly to induce an adequate response. ORs are clustered in two sensory systems: the main olfactory system (MOS), with its genes belonging to the largest gene family yet identified (Gaillard et al. 2004); and the accessory olfactory system (AOS).

Receptors of the MOS, called olfactory receptors, belong to the seven transmembrane (7TM) G-protein-coupled receptor superfamily (GPCR) (Emes et al. 2004; Gaillard et al. 2004) and are expressed in the olfactory epithelium, which is connected to the main olfactory bulb (MOB) via nerve axons.

The mouse genome contains 913 intact OR genes and 296 pseudogenes (Godfrey et al. 2004). Humans have ~900 ORs (of which about two-thirds are pseudogenes) divided into 17 families and 300 subfamilies (Glusman et al. 2001; Young and Trask 2002; Zhang and Firestein 2002; Quignon et al. 2005). In the rat (*R. norvegicus*) genome, 1,493 intact ORs and ~350 putative pseudogenes have been identified (Quignon et al. 2005). The genes are clustered in 56 loci (including 8 loci with pseudogenes only), which contain 1–265 genes (including pseudogenes) and are distributed across 19 chromosomes (except chr 6, 18, and Y). The largest loci are on chr 3 (218 intact ORs/47 pseudogenes/37 subfamilies), chr 1 (131/18/54), and chr 8 (109/16/8). Subfamilies vary considerably in size and contain 1–61 genes (Quignon et al. 2005). Mouse (*M. musculus*) ORs are distributed over 51 loci

(including 2 loci with pseudogenes only) across 17 chromosomes (none on chr 5, 12, 18, or Y). Loci contain 1–244 genes (including pseudogenes) that were classified in 241 gene subfamilies by sequence comparison (Godfrey et al. 2004). The largest loci are on chr 2 (189 intact ORs/55 pseudogenes/36 subfamilies), chr 7 (107/26/50), and chr 9 (91/22/10).

As in rats, gene numbers per subfamily varied extensively in mice (Godfrey et al. 2004), indicating that some ligands (odor classes) may be more easily detected or discriminated than others, given that functional diversity is associated with OR subfamilies. The latter assumption is supported by the facts that (1) 94 mouse OR gene loci encode only genes of a single subfamily (92 other loci encode only 2–4 subfamilies) and (2) most subfamilies are encoded by just one locus (Godfrey et al. 2004). For example, mouse OR73 and OR74 both recognize aromatic aldehydes (Kajiya et al. 2001) and are members of the same subfamily of five ORs, leading to the assumption that the other three ORs of this subfamily may also detect aromatic aldehydes. So far, odor ligands have been identified for 22 mouse ORs (Krautwurst et al. 1998; Zhao et al. 1998; Kajiya et al. 2001), allowing the 19 subfamilies to which they belong (containing 96 ORs in total) to be examined for hypothetical functional assignments (Godfrey et al. 2004). Thirteen subfamilies, containing 59 ORs, were predicted to recognize aliphatic odorants, and the other six subfamilies (comprising 37 ORs) most likely recognize odorants of other chemical structures. A phylogenetic tree, constructed with sequences of one OR gene from each of the 241 subfamilies together with 25 ORs whose ligands were known for assignment purposes, showed that 9 (all encoded at a single locus on chr 7) of 13 subfamilies containing receptors for *n*-aliphatic acids/alcohols were located on one distinct tree branch, an observation that had also been made by others (Glusman et al. 2001; Zhang and Firestein 2002). OR subfamilies that contained receptors for other chemical classes of odorants were scattered among the other branches (Godfrey et al. 2004).

To study the effects of different odorants on a female (e.g., body odor or scent marks of different males), the response has to be measured either at the receptors directly or in the region of the brain where the signals are processed. Odor-induced neural activity in the MOB can be detected by measuring and mapping changes in *c-fos* mRNA expression (a proto-oncogene belonging to the immediate early gene family of transcription factors) in the glomerular layer of the bulb. Female anestrous BALB/c mice (MHC haplotype H-2^d) exposed to urine odor of age-matched males from either of two H-2 haplotypes (mice strains B6.AKR:H-2^k and C57BL6:H-2^b) could clearly differentiate between the urine odors of the two male haplotypes, as indicated by the spatially different *c-fos* expression patterns evoked in the MOB (Schaefer et al. 2001).

Receptors of the AOS, called pheromone receptors, are expressed together with some nonclassic MHC class I genes (Ib; see above) in the VNO (Dulac and Torello 2003; Emes et al. 2004), which itself is situated in the septum of the nose. AOS family members belong to two types of receptor – vomeronasal receptor type 1 (V1R) and type 2 (V2R) – whose genes are widely distributed across the genome (Table 1.1). Like ORs, both AOS receptor types belong to the 7TM GPCR, but the difference between them is that V2R belongs to the family of C GPCRs (7TM at the C-terminus)

Table 1.1 Number and distribution of vomeronasal receptor genes in the genomes of the mouse and rat

Chromosome no.	Receptor type	No. of receptor genes[a] in *Mus musculus*	*Rattus norvegicus*
3	V1R/V2R	–/3	–/2
4	V1R/V2R	1/–	1/–
5	V1R/V2R	2/3	1/1
6	V1R/V2R	49/3	54/3
7	V1R/V2R	41/13	47/2
10	V1R/V2R	–/3	–/2
13	V1R/V2R	29/–	–/–
14	V1R/V2R	–/1	–/1
17	V1R/V2R	13/6	16/2
X	V1R/V2R	2/6	1/1

V1R, vomeronasal receptor type 1; V2R, vomeronasal receptor type 2
[a]Without pseudogenes, without duplications, and without receptor-like genes. However, there are seven mouse and two rat V1R genes and ten mouse and one rat V2R genes that are not yet assigned to a chromosome and that will likely change those numbers once assigned. The GenBank search was carried out at: <http://www.ncbi.nlm.nih.gov/projects/mapview/map_search.cgi?taxid=10090&qrng=901&query=vomeronasal>

(Matsunami and Buck 1997). In terms of their functions, it is assumed that V1Rs bind to volatile organic compounds, whereas V2Rs bind to proteins (Emes et al. 2004).

The impact of the VNO on social and reproductive behavior in rodents was demonstrated by deletion of a cluster of 16 V1R genes, which resulted in significant changes of male and female behavior. Males showed reduced libido: The percentage of males that mounted a female was significantly lower in V1R-deletion mutant mice than in the wild-type animals (Del Punta et al. 2002), which is consistent with results obtained after surgical removal of the VNO (Clancy et al. 1984). V1R-deletion mutant females showed a reduced level of maternal aggressive behavior (Del Punta et al. 2002). Results from additional tests in V1R-deletion mutant males, however, differed from results obtained after VNO removal. For instance, the emittance of ultrasound vocalizations (70 kHz) by males during the first minutes of exposure to a female was not altered by deletion of the 16 V1Rs, whereas these calls were attenuated when the VNO was removed (Wysocki et al. 1982). In addition, the percentage and degree of aggressive behavior towards other males were likewise not altered in the mutants compared to the wild-type males. The study also identified three chemical compounds (of eight tested) that mutants were not longer able to detect (Del Punta et al. 2002), supporting the view that each receptor molecule binds only to a certain variety of ligand (Krautwurst et al. 1998; Zhao et al. 1998; Kajiya et al. 2001; Godfrey et al. 2004).

Tests to determine the role of the VNO in recognition of MHC odor types in mice revealed that VNO was not involved in these processes (at least not in Y-maze tests): Surgical removal of the VNO did not disrupt MHC odor-type discrimination (Yamazaki and Beauchamp 2005). This leads to the conclusion that it is not the AOS but the MOS that functions as the primary interface for interactions with the

complexity of MHC odor types (Singer et al. 1997; Schaefer et al. 2001; Yamazaki and Beauchamp 2005).

A male-specific, nonvolatile 7-kDa peptide was identified in mice tears by Kimoto et al. (2005). It is produced in the extraorbital lacrimal gland and released in tear fluid during direct contact with a female (Kimoto et al. 2005; Touhara 2007). The peptide has been named "exocrine gland-secreting peptide 1" (ESP1) and is a member of a likewise newly identified multigene family that consists of ~40 homologous genes clustered in proximity to the MHC class I region (Kimoto et al. 2007). ESP1 stimulates the female vomeronasal V2Rp5 receptor and evokes a calcium signal in vivo. Thus, peptides of the ESP family add to the variation in the pattern of communication signals between individuals, sex, strains, or species.

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Chapter 2
Extra-Pair Paternity and Sexual Selection*

Emmi Schlicht and Bart Kempenaers

2.1 How Does Extra-Pair Paternity Influence Sexual Selection?

Parentage analyses can reveal hidden reproductive interactions between individuals that are not social partners. Extra-pair mating is a special case of promiscuity where social pair bonds exist and persist despite copulations with multiple partners by one or both pair members. The relevance of extra-pair interactions in reshaping social mating systems varies among species. In some species or populations, extra-pair matings are no more than exceptional events (e.g., Dearborn et al. 2001; Egger et al. 2006), whereas in others extra-pair paternity (EPP) is a phenomenon that cannot be ignored when describing mating patterns because of a substantial discrepancy between the observable apparent mating system and the actually realized mating system (e.g., Double and Cockburn 2003; Sefc et al. 2008). Extra-pair copulations (EPCs) are of special interest in socially monogamous species where promiscuity is otherwise absent. Pair bonding and social monogamy are relatively rare – except in birds (Lack 1968, p. 148) – yet occur in a wide range of animal taxa (e.g., Caldwell 1997; Kvarnemo et al. 2000; Baeza 2008; Steinauer 2009). However, social monogamy frequently goes hand in hand with multiple mating (e.g., Griffith et al. 2002; Chapple 2003; Lodé and Lesbarrères 2004; Cohas and Allainé 2009).

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2.1.1 Extra-Pair Paternity

Because social monogamy usually coincides with biparental care (e.g., Caldwell 1997; DeWoody et al. 2000; Runcie 2000; Bennett and Owens 2002, p. 79; Tallamy 2009; Wright et al. 2009), EPCs can be seen as reproductive parasitism of some males at the cost of others. Due to anisogamy, a male can fertilize many more ova than those produced by its mate, and in most species male reproductive success is limited by access to fertile females. Thus, male benefits of EPCs appear straightforward: gaining additional offspring cared for by other males. Indeed, strong selection on male EPC behavior may be all that is needed to explain patterns of EPP. However, such male behavior has several implications that make the situation more complicated (Westneat and Stewart 2003).

First, if some males gain extra-pair young (EPY), other males must lose fertilizations. Male pursuit of EPCs should thus increase male–male competition, and this not only before copulation, at the social level, but also in the form of sperm competition after copulation. Increased competition for access to fertilizations should also lead to the evolution of paternity protection behavior, such as mate guarding or frequent copulation (Birkhead and Møller 1992, Chaps. 7–9), which may trade off with the pursuit of EPCs.

Second, there is a connection between the occurrence of paternal care and sexual selection via EPCs because extra-pair success may come at a cost when males have to trade off the pursuit of extra-pair mates with offspring care (Westneat et al. 1990). When extra-pair fertilizations are successful, the payoff from paternal care for the cuckolded male is lowered. Males that perceive a loss of paternity may then reduce their level of care, although it remains a matter of debate when reduced male care is expected and to what degree it actually occurs (Wright 1998; Whittingham and Dunn 2001; Sheldon 2002; Arnqvist and Kirkpatrick 2007; Griffith 2007; Eliassen and Kokko 2008).

Third, females may or may not benefit from copulating with a male that is not their social partner. A female that does not benefit may be expected to resist copulation attempts by extra-pair males, further reducing the benefits and increasing the costs of EPC behavior for males. Such sexual conflict always exists to some extent because the optimal copulation pattern for a female does not coincide with the optimal copulation pattern for the social mate and extra-pair males. However, in certain male–female constellations, the conflict may be reduced or even absent, namely in those where the female also gains from EPCs. How would females benefit from EPCs? First and foremost, females need their ova to be fertilized, and the social partner may be unable to provide her with enough gametes to do so (e.g., Sheldon 1994; Hasson and Stone 2009) – for instance, when he produces too few or low quality sperm. Females could also benefit from EPCs for a variety of other reasons (Westneat et al. 1990; Birkhead and Møller 1992, pp. 198–209). Most controversial among them is the idea that females may gain indirect (genetic) benefits from EPCs (Arnqvist and Kirkpatrick 2005; Akçay and Roughgarden 2007; Eliassen and Kokko 2008; Uller and Olsson 2008). The quality of the offspring genome is the result of

the combination of the paternal and maternal haplotypes, and an extra-pair male may provide alleles that are more adaptive than those from the social mate, either generally ("good genes") or in combination with the female's alleles ("compatible genes"), or both (Neff and Pitcher 2005). Such a situation could be common under social monogamy because many females may have to settle with a suboptimal social mate when their partner of choice is mated to another female (Gowaty 1996; Hasselquist and Sherman 2001). However, it remains unclear (1) to what extent these and other benefits occur in different populations and under different conditions (Friedl and Klump 2005; Schmoll et al. 2005; Garvin et al. 2006; O'Brien and Dawson 2007; Dreiss et al. 2008; Fossøy et al. 2008; Dunn et al. 2009; Kawano et al. 2009; Townsend et al. 2010) and (2) whether they are sufficient to cause selection on female pursuit of extra-pair matings (Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005). In any case, whenever EPCs occur, male–male competition increases, and additional opportunities for female choice arise, either at the precopulatory stage or later in the form of cryptic female choice among male sperm. This provides the link between EPP and sexual selection.

2.1.2 Sexual Selection

Sexual selection is selection acting on differences in reproductive success among individuals caused by variation in their mating success (Andersson 1994). Such variation may be random, but selective effects are then not transferred to the next generation and do not lead to evolutionary change. Sexual selection on phenotypic traits is thus dealing with variation in mating success as the result of nonrandom mating. Sexual selection is a major force in shaping the structure of animal societies and the behavioral repertoire of individuals, for example through its interaction with the mating system and sex roles (Andersson 1994, Chap. 7). Sex differences in the strength of sexual selection are the primary cause of the sex differences in behavior and morphology found in many species (Andersson 1994, Chaps. 11–15). Under strict monogamy, the number of mating events is constrained to one per individual, and differences between the sexes in the strength of sexual selection are limited. Nevertheless, pronounced sexual dimorphism is also found in many socially monogamous species (Fig. 2.1) (e.g., Knolton 1980; Møller 1986; Leutenegger and Lubach 1987; Boonstra et al. 1993; Kokita and Mizota 2002; Mizuta 2005). Is EPP a candidate to explain sexual dimorphism in socially monogamous species?

Clearly, EPP increases the number of mating events and thereby creates additional opportunities for male–male competition and female choice. Hence, at least in theory, EPP has the potential to alter dramatically the strength of sexual selection experienced by males. However, high levels of EPP do not necessarily lead to increased intensity of sexual selection in males. For instance, if females perform EPCs as insurance against the risk of infertility, they may mate at random with respect to male phenotypic traits. Even then, however, it is unlikely that male mating success is entirely stochastic because the extra-pair behavior of females introduces

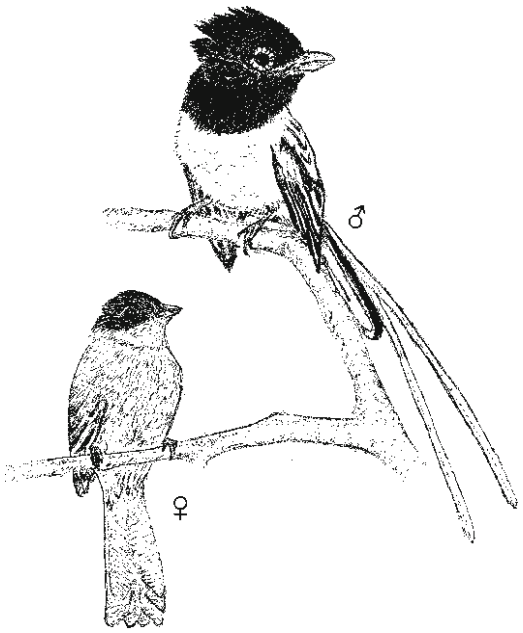

Fig. 2.1 Male and female of the Madagascar paradise flycatcher (*Terpsiphone mutata*). This is a socially monogamous species with biparental care. Can extra-pair paternity play a role in the evolution of the pronounced sexual dimorphism? A study by Raoul Mulder found that in this species 50% of the females had extra-pair young (Eliot 2005)

competition among males to secure EPCs and to fertilize the eggs (sperm competition). Furthermore, male extra-pair behavior may trade off with success with their social mate because males cannot simultaneously pursue EPCs and protect paternity or feed offspring. Variation in mating success may therefore not translate into variation in reproductive success because within-pair and extra-pair success may be negatively correlated. It is even conceivable that EPP leads to reduced male reproductive skew and hence less intense sexual selection. This would be the case if variation in the apparent success among males – unequal success at securing social mates or variation in female fecundity – is reduced via higher extra-pair success of socially less successful males (Webster et al. 1995; Jones et al. 2001; Lawler 2009). In summary, a higher level of EPP does not necessarily cause an increase in sexual selection in males because extra-pair success may be random or uniformly distributed, or it may be negatively associated with within-pair success.

Comparative studies in birds have shown that higher levels of EPP are associated with increased color and size dimorphism, suggesting that EPP does magnify the intensity of sexual selection in males (Møller and Birkhead 1994; Owens and Hartley 1998; Dunn et al. 2001). However, secondary sexual traits (increased size, "weaponry," ornaments) are most exaggerated in males of socially polygynous and lekking species. Indeed, the social mating system remains the best predictor of dimorphism across all birds (Owens and Hartley 1998; Dunn et al. 2001). This is

not surprising because social monogamy tends to equalize selection in males and females unless EPP causes highly skewed male reproductive success. EPP leads to variation in mating systems among species beyond those observable via social pairings and may be especially important for sexual selection through postcopulatory processes such as sperm competition. Interspecific variation in rates of multiple paternity is related to numerous aspects of male reproductive biology, such as testis size or sperm swimming speed (Møller and Briskie 1995; Garamszegi et al. 2005; Ramm et al. 2005; Bryja et al. 2008; Immler et al. 2008; Kleven et al. 2008, 2009; Lüpold et al. 2009; but see Schülke et al. 2004).

An effect of EPP on the strength of sexual selection in males probably varies considerably among species and populations, if not among years. It is thus of interest to quantify the effect of multiple mating on the intensity of sexual selection for specific populations. In this review we discuss studies that have attempted to do this. Because studies of EPP have been conducted overwhelmingly in birds, most of the examples are from avian species. We first introduce several methods that allow quantifying the strength of sexual selection. We then examine how these estimates are affected by EPP, both theoretically and in empirical studies. For each estimate we also discuss problems that arise in the interpretation and comparison of results, which may be of more general importance for research on the strength of sexual selection and male reproductive skew in species with multiple mating (see also Chap. 3).

2.2 Measurements of Sexual Selection

For selection to act, it is necessary that fitness variation among individuals is present. In fact, in an idealized situation where all fitness variance is heritable, standing variation in fitness measures the increase in fitness from one generation to the next (Fisher 1930, p. 35). The variance in relative fitness (absolute fitness divided by the mean absolute fitness) – a measurement referred to as the **opportunity for selection** $\boldsymbol{I}$ – quantifies the maximum rate at which fitness can increase over time (Crow 1958; O'Donald 1970) and thus sets an upper limit to the strength of any form of selection, including sexual selection. Note that I is not a measurement that is specifically concerned with sexual selection alone; rather, it encompasses both natural and sexual selection.

Based on its definition, it is clear that sexual selection can act only if there is variation among individuals regarding their ability to obtain access to mates. This variation can be quantified as the variance in relative mating success, referred to as the **opportunity for sexual selection** $\boldsymbol{I_{mates}}$ (Wade 1979; Wade and Arnold 1980). Molecular parentage assignment often reveals that a male's true success in obtaining mates for fertilization is different than appears from his number of social mates. Here we define realized mating success, based on parentage analysis, as the number of individuals of the opposite sex with which an individual produces genetic offspring. I and I_{mates} are of interest because they set upper limits to parameters that are relevant to the intensity of sexual selection, and they can be estimated independent of the phenotypic traits that are the target of sexual selection. Furthermore, the maximum intensity

of sexual selection that any phenotypic trait could be subjected to is interesting in its own right. Estimating variation in relative reproductive (I) and mating success (I_{mates}) is one of many possibilities for measuring male reproductive and mating skew (Kokko et al. 1999; Nonacs 2003). I and I_{mates} have the advantage that they are closely linked to selection theory, thus allowing direct interpretation regarding the evolutionary process (Jones et al. 2002, 2004). (For applications of reproductive skew theory in primates and social insects, see Chaps. 3 and 4).

The strength of selection on a trait depends on how variation in the trait relates to variation in fitness. This relationship can be measured as the partial regression of relative fitness on the trait while all other traits are held constant. The corresponding regression coefficient is called a selection gradient β (Lande 1979). In the context of sexual selection, the trait of interest is the "ability to obtain mates." The selection gradient for this trait is called the **Bateman gradient** β_{ss} (Andersson and Iwasa 1996). The Bateman gradient thus measures the slope of the least-squares regression of relative fitness on mating success, which is the direct expression of sexual selection resulting from differences in the ability to obtain access to mates (Arnold and Duvall 1994). Including this measurement in quantitative analyses of sexual selection may provide more reliable information than variance-based estimates alone (Jones et al. 2002, 2004, 2005; Mills et al. 2007; see also Bjork and Pitnick 2006). One disadvantage of selection gradients is that they may not be easily comparable among studies because they are affected by the particular choice of phenotypic traits that are included in the multivariate regression analysis (Arnold and Wade 1984; Andersson 1994, pp. 91–94). Furthermore, they need to be standardized for comparisons, which may not be straightforward (Hereford et al. 2004; Jones 2009).

Bateman (1948) illustrated that sex differences in all three of the above estimates (I, I_{mates}, β_{ss}) go hand in hand with a sex difference in the strength of sexual selection. This is known as Bateman's principles (Arnold 1994). As a consequence of anisogamy, males are typically subject to stronger sexual selection than females. In other words, in species with "typical" sex roles, males exhibit higher variance in fitness (I) and higher variance in mating success (I_{mates}) than females because for them there is a greater range of reproductive outcomes as a result of mating competition. Most fundamentally, for males of these species there is a stronger correlation between mating and reproductive success (β_{ss}) than for females.

2.3 Realized and Apparent Reproductive and Mating Success

2.3.1 $I_{realized}/I_{apparent}$ Ratio

Let us now consider how different levels of EPP influence measurements of the strength of sexual selection. The most common approach to quantifying the effect of EPP on sexual selection in a population is to compare the relative variance in apparent and realized male reproductive success (number of young in social nests and number of young sired), i.e., $I_{apparent}$ and $I_{realized}$ (summarized in Table 2.1).

This is based on the idea that EPP increases sexual selection in males when it increases variation in male reproductive success due to sperm competition or non-random mating. Thus, for EPP to increase sexual selection, some males must be consistently more successful at acquiring extra-pair offspring than others because they are more successful at acquiring extra-pair mates or more successful in post-copulatory competition to fertilize the eggs. In that case, offspring are redistributed from the unsuccessful males to the successful sires, and we expect an increase in the opportunity for selection from the apparent to the realized mating system. Conversely, if all males are equally successful extra-pair sires or EPP trades off with within-pair success, extra-pair males should simultaneously gain and lose paternity; and these paternity exchanges should leave their overall reproductive success largely unaffected (e.g., Ketterson et al. 1997). Furthermore, when EPP reduces sexual selection by providing an alternative route to fertilizations for males with small apparent success (e.g., unpaired males), the opportunity for selection decreases from the apparent to the realized mating system. Hence, an $I_{realized}/I_{apparent}$ ratio significantly greater than 1 is considered evidence that EPP increases sexual selection in males. Ratios of $I_{realized}/I_{apparent} > 1$ are frequently reported in studies of socially monogamous birds (mean reported $I_{realized}/I_{apparent} = 3.4$) (Table 2.1). However, these estimates suffer from a number of problems.

2.3.2 *Effects of Sampling Limitations on I*

Measurements of *I* are sensitive to sampling effort and limitations (Downhower et al. 1987). In most studies of EPP, the focal individuals are part of an open population where reproductive interactions extend beyond the sampled nests. Thus, the males that are included in the study usually have sired some offspring in nonregistered nests. Estimated variances may then be too high or too low if males that are unsuccessful on the study site are more or less successful in unsampled nests (Webster et al. 1995; Freeman-Gallant et al. 2005). Also, in an open population, some of the offspring in sampled nests are sired by unknown males (indicated as the assignment rate in Table 2.1) and are excluded from further calculations. This generally causes an increase of $I_{realized}$ over $I_{apparent}$ by lowering the mean realized reproductive success (Møller and Ninni 1998; Freeman-Gallant et al. 2005). It may also bias variance calculations when the unknown sires are a nonrandom subsample of males (e.g., unpaired males) (Jones et al. 2001).

2.3.3 *Effects of Random Mating on I*

Although the use of relative instead of absolute fitness variance appears to remove scaling effects, estimates of *I* are not generally independent of mean fitness and the number of competitors (Downhower et al. 1987; Ruzzante et al. 1996; Kokko et al.

Table 2.1 Overview of studies calculating $I_{realized}/I_{apparent}$ ratios and comparison with $I_{realized}/I_{random}$ ratios from model of random extra-pair mating (bold when $I_{random} > I_{apparent}$)

Species (scientific name)	Common name	N[a]	p (%)[b]	AR (%)[c]	$\bar{c}$[d]	MS[e]	$I_{realized}$[f]	$I_{apparent}$[g]	I_{random}[h]	$\frac{I_{realized}}{I_{apparent}}$	$\frac{I_{realized}}{I_{random}}$	ξ[i]	Ref[j]
Acrocephalus arundinaceus	Great reed warbler	121	3	100	4.6	P	1.02	1.00	0.94	1.02	1.08	*4.60*	*1*
Agelaius phoeniceus 1	Red-winged blackbird	13	37	93	5.8	P	0.39	0.25	0.15	1.56	2.63	1.45	*2*
Agelaius phoeniceus 2	Red-winged blackbird	51	25	57	3.2	P	1.20	1.02	0.67	1.17	1.80	3.27	*3*
Agelaius phoeniceus 3	Red-winged blackbird	103	26	78	4.3	P	0.74	0.64	0.41	1.16	1.78	2.72	*4*
Carpodacus erythrinus	Scarlet rosefinch	46	18	73	4.3	M	0.40	0.12	0.12	3.31	3.31	0.52	*5*
Cyanistes caeruleus 1	Blue tit	32	11	73	9.8	M	0.27	0.16	0.14	1.69	1.98	1.57	*6*
Cyanistes caeruleus 2	Blue tit	47	15	74	11.6	M	0.12	0.04	0.04	3.51	**3.26**	0.46	*7*
Delichon urbica	House martin	17	19	100	3.6	M	0.31	0.06	0.09	5.17	**3.51**	**0.21**	*8*
Dendroica caerulescens	Black-throated blue warbler	67	21	62	4.0	M	0.72	0.51	0.37	1.41	1.95	2.04	*9*
Dendroica pennsylvanica	Chestnut-sided warbler	37	47	87	2.9	M	0.70	0.19	0.21	3.75	**3.35**	0.54	*10*
Dendroica petechia	Yellow warbler	14	37	35	4.0	M	0.10	0.03	0.10	3.25	**1.06**	**0.13**	*11*
Ficedula albicollis	Collared flycatcher	44	15	93	5.7	M	0.14	0.03	0.05	5.11	**3.03**	**0.15**	*12*
Geothlypis trichas	Common yellowthroat	21	26	83	4.4	M	0.48	0.28	0.20	1.71	2.36	1.24	*13*
Hirundo rustica erythrogaster	North American barn swallow	86	31	100	5.2	M	0.53	0.07	0.09	7.96	**5.85**	0.35	*14*
Hirundo rustica rustica	European barn swallow	63	28	–	4.1	M	0.36	0.15	0.14	2.37	2.49	0.63	*15*
Icterus galbula bullockii	Bullock's oriole	31	32	45	4.3	M	0.17	0.07	0.10	2.43	**1.65**	**0.30**	*16*
Junco hyemalis	Dark-eyed junco	50	28	55	3.7	M	0.72	0.55	0.35	1.32	2.06	2.03	*17*
Luscinia svecica svecica	Bluethroat	134	29	52	5.4	M	0.37	0.08	0.09	4.63	**3.96**	0.43	*18*
Malurus splendens	Splendid fairy-wren	204	42	91	–	CB	1.42	1.47	–	0.97	–	–	*19*
Melospiza melodia	Song sparrow	299	8	95	1.6	M	1.33	1.21	1.08	1.10	1.23	1.90	*20*
Passerculus sandwichensis	Savannah sparrow	80	47	92	4.6	P	0.48	0.27	0.17	1.78	2.74	1.24	*21*

Poecile atricapillus	Black-capped chickadee	58	9	47	6.2	M	0.10	0.04	0.05	2.50	**2.11**	**0.25**	*22*
Progne subis	Purple martin	41	19	54	3.4	M	0.33	0.05	0.09	6.69	**3.79**	**0.16**	*23*
Sialia currucoides	Mountain bluebird	59	36	70	5.0	M	0.27	0.04	0.09	7.32	**3.15**	**0.19**	*24*
Tachycineta bicolor	Tree swallow	19	51	66	4.9	M	0.79	0.09	0.12	8.78	**6.72**	0.44	*25*
Troglodytes aedon	House wren	68	10	88	8.6	P	0.22	0.18	0.16	1.22	1.40	1.55	*26*
Tyrannus tyrannus	Eastern kingbird	89	47	56	3.0	M	0.85	0.09	0.18	9.44	**4.69**	**0.27**	*27*
Wilsonia citrina	Hooded warbler	100	27	55	3.4	M	0.46	0.18	0.17	2.56	2.66	0.62	*28*

[a]Sample size (number of males)

[b]Frequency (%) of EPP among young

[c]Assignment rate (AR): percentage of extra-pair young assigned

[d]Mean apparent reproductive success

[e]Social mating system (MS): socially monogamous (M), polygynous (P), or cooperative breeder (CB)

[f]Opportunity for selection based on realized male reproductive success

[g]Opportunity for selection based on apparent male reproductive success

[h]Opportunity for selection generated by random extra-pair mating, calculated from (6) of Online Supplementary Material

[i]Parameter $\xi = I_{\text{apparent}}\,\overline{c}$, calculated from (5) of Online Supplementary Material. Values < 0.4 are in boldface type, values ≥ 4 are in italics

[j]References: see Appendix

1999; Fairbairn and Wilby 2001; Walsh and Lynch 2008; see also Galimberti et al. 2002) because the value of *I* under the null hypothesis of random success changes systematically as a function of these parameters. This is mostly an expression of the fact that chance can create fitness variance that is unrelated to phenotypic traits (Sutherland 1985a, b).

It is generally accepted that selection and response to selection are two separate issues. Indeed, the extent to which the action of selection on a particular trait is transferred to the next generation depends on the trait's heritability (e.g., Wade and Arnold 1980; Lande and Arnold 1983) and on whether selection acts on the trait's genetic component or on its environmental components (Price et al. 1988). Thus, measurements of *I* provide an estimate of the standing variation in fitness, and selection acts on this variation both when it is of random origin and when it is not (Shuster and Wade 2003, pp. 31–34). As long as some trait with marginal fitness effects exists, selection on this trait is stronger when *I* is larger. Still, it is worth noting that an increase in *I* does not necessarily imply an increase in selection on specific phenotypes because fitness variance may be stochastic (i.e., unrelated to phenotypic traits). Because the contribution of chance to fitness variance may vary systematically under different scenarios (Sutherland 1985a, b; Hubbell and Johnson 1987; Gowaty and Hubbell 2005), it is helpful to calculate which value of *I* we would expect under randomness and use this as a reference when comparing estimates of *I* (e.g., Wade 1995; Haydock and Koenig 2003; Nonacs 2003; Cerchio et al. 2005).

This may be particularly relevant when considering the effect of different EPP rates on *I*. In a socially monogamous system, almost any deviation from monogamy may increase variance in male fitness because the apparent mating system should produce a relatively uniform distribution of offspring among males (Jones et al. 2001; Lawler 2009). As an example, for the socially monogamous purple martin (*Progne subis*), the standard deviation in apparent reproductive success among 41 males was only 0.8, whereas the mean apparent reproductive success was 3.4 (Wagner et al. 1996) (Table 2.1). In such cases, the redistribution of offspring among males via EPP can, just by chance, lead to some males being more successful than others, even if all males are equally likely to gain or lose offspring. It is erroneous to assume that random processes lead to equal extra-pair success for all males and thus to paternity exchanges that leave the variance in reproductive success unaffected. Therefore, the correct reference value for I_{realized} can be smaller or larger than I_{apparent}. When variation in apparent success among males is high – e.g., when many males fail to secure a social mate ("floaters") – introducing random extra-pair mating leads to a reduction in *I*, as some of the floaters randomly obtain extra-pair fertilizations. Conversely, when variation in apparent success among males is low (e.g., when there are no unpaired males and the clutch size variation and nest predation are limited) (e.g., Whittingham and Lifjeld 1995; Sheldon and Ellegren 1999; Richardson and Burke 2001; Dolan et al. 2007; Balenger et al. 2009), *I* is expected to increase with the rate of EPP under random mating because some males randomly obtain more extra-pair fertilizations (see also simulations in Webster et al. 1995).

Based on a model of random extra-pair mating, we calculated the expected opportunity for selection, I_{random}, and the associated variance ratio for published

studies and compared them with the reported variance ratios (Table 2.1; see Online Supplementary Material for details of the model). The variance ratio obtained in this way was greater than the reported ratio in roughly half of the studies (13/27). In the other half (14/27 studies) the ratio based on random mating was smaller than the reported ratio (boldface type in Table 2.1). In nine and three studies, respectively, the reductions were >20 and >50%. This indicates that for these studies the role of EPP in generating stronger sexual selection strength (than under the apparent mating system) may not be as important as previously thought because part of the variance increase is expected even under random mating. Another result from this model of random extra-pair mating is that the opportunity for selection increases systematically with the rate of EPP under random mating for certain parameter constellations that may occur naturally (parameter $\xi < 0.4$) (Table 2.1; see Online Supplementary Material for details).

Complete randomness of male extra-pair success is unlikely, of course, because some male trait probably affects extra-pair success to some extent. Because EPP increases the number of mating events, it increases the number of events where sexual selection, if it occurs, comes into action. However, we have seen above that we can imagine biological situations that introduce stochasticity into male extra-pair success, such as random female choice of extra-pair mates as insurance against the risk of infertility. When a significant increase in I_{realized} over I_{apparent} is found, it undoubtedly reflects an increase in the opportunity for selection caused by EPP. How this relates to the strength of selection on traits that are the targets of sexual selection is another question. The effect of selection opportunity on sexually selected traits may vary systematically with the frequency of EPP irrespective of the heritability of these traits.

2.3.4 *Opportunity for Selection in Females*

Some studies have used a similar approach and compared variance in relative female fitness $I_{\female}$ to variance in relative male fitness $I_{\male}$ (realized reproductive success) (Ketterson et al. 1997; Weatherhead and Boag 1997; Webster et al. 2001; Byers et al. 2004; Kraaijeveld et al. 2004; Freeman-Gallant et al. 2005; Whittingham and Dunn 2005; Albrecht et al. 2007). This approach has the advantage that it relates only to actual parentage, which may be more relevant biologically. A sex difference in I is thought to be related to a sex difference in the strength of sexual selection (Bateman 1948). Here too, however, chance may systematically increase fitness variance – in this case, that of males over that of females (Sutherland 1985a, b; Hubbell and Johnson 1987; Gowaty and Hubbell 2005). This is immediately clear when we consider that most of the studies concern socially monogamous species, whereby only breeding males are included; thus, apparent male reproductive success and female reproductive success are identical. Calculating $I_{\female}$ is more interesting with respect to sex differences in the opportunity for selection when unpaired males are included (Ketterson et al. 1997; Whittingham and Dunn 2005;

Albrecht et al. 2007) or when the study species is socially polygynous (Gibbs et al. 1990; Westneat 1993; Hasselquist et al. 1995; Weatherhead and Boag 1997; Freeman-Gallant et al. 2005; Whittingham and Dunn 2005; Westneat 2006), but then it is difficult to quantify the effect of EPP.

2.3.5 Variation in the Number of Mates

The variance in relative mating success I_{mates} has rarely been calculated in studies of EPP (but see Ketterson et al. 1997). Variance in mating success is a necessary prerequisite for sexual selection to occur, and the standard deviation in relative mating success also gives an upper boundary for the effect of any trait on mating success (Jones 2009). In the absence of EPP, variation in mate number among individuals of a socially monogamous species arises only from differences in pairing status (breeding or nonbreeding). Variance in mating success is thus expected to increase dramatically with the EPP rate. However, here it is most obvious that any form of extra-pair mating, even random mating, is bound to increase the variance in mate number. Comparisons between apparent and realized I_{mates} are therefore not very informative. To assess the effect of EPP on the strength of sexual selection in males or females, a reference value for I_{mates} should be defined based on a random mating process (e.g., McLain 1986; see also Online Supplementary Material). Still, it is interesting to compare male and female variation in mate number.

2.4 Fitness Components

The influence of EPP on sexual selection can also be assessed by estimating the magnitude of the fitness components that contribute to fitness variation (Webster et al. 1995). A male's total reproductive success (T) is the product of the number of mates (M) he has, the average clutch size (N) of these mates, and the proportion (P) of young in all these clutches that he sires.

$$T = M\,N\,P$$

These variables reflect variation in male reproductive success due to the number of mates he can acquire, the quality (fecundity) of these mates, and the success at securing fertilizations with these mates. Furthermore, a male's total reproductive success is the sum of the young he sires in his own and in other males' nests (his within-pair and his extra-pair success).

$$T = W + E$$

Male total reproductive success can thus be written as

$$T = M_w N_w P_w + M_e N_e P_e,$$

where the indices w and e refer to the variables for within-pair and extra-pair success, respectively. Each of these six components contributes to variance in male reproductive success. It is thus possible to split up the total variance in reproductive success into terms that correspond to the variation due to each of the six components and to the covariance between components (Webster et al. 1995). For example, when we restrict ourselves to the two variables W and E, without further partitioning, we find the following.

$$\mathrm{Var}(T) = \mathrm{Var}(W) + \mathrm{Var}(E) + 2\,\mathrm{Cov}(W,E).$$

Thus, one can calculate the proportion of the total variance that is attributable to variance in within-pair success and extra-pair success and to the covariance between the two. This type of variance partitioning can be performed for all six components and their associated covariances.

2.4.1 Influence of EPP on Fitness Components

Table 2.2 provides an overview of studies that have used the described method of variance partitioning. Components contributing at least 10–15% of the total variance in reproductive success are usually thought to be important (e.g., Webster et al. 2001; Lawler 2007; but see Whittingham and Dunn 2005), whereas contributions of <5% are considered negligible (Webster et al. 1995). The influence of EPP on the total variance in male reproductive success is indicated in three ways.

1. Var(E)/Var(T) directly indicates which proportion of the total reproductive success of males is due to success with extra-pair mates and would thus generate opportunities for selection and sexual selection. The contribution of Var(E) exceeds 20% in more than two-thirds of the studies; it is less than 10% in only one study (Table 2.2). In eight of nine studies (where it was assessed), most of the variance in extra-pair success was due to the number of acquired extra-pair mates (M_e), which means that variation in extra-pair success directly reflects the opportunity for sexual selection (because sexual selection is caused by variation in reproductive success that arises from variation in mating success).
2. The effect of Var(P_w) indicates the proportion of the total reproductive success of males that is due to variation in paternity loss in the own brood. Typically, the greatest part of the total variance in reproductive success remains with Var(W). In 9 of 11 studies (where it was assessed) variation in P_w contributed substantially (>10% of total variance) to this variance in within-pair success (Table 2.2). This is caused by differences among males in their ability to secure paternity in their own nest(s), which is related to their success in competition over mates via female choice, male–male competition (e.g., territory defense), or sperm competition.
3. A positive covariance between within-pair and extra-pair success indicates that males that are successful with their social mate(s) are also successful at siring extra-pair offspring. Conversely, when the covariance is negative, increased extra-pair success coincides with lower within-pair success, which suggests a trade-off

Table 2.2 Overview of studies that report variance components in male reproductive success

Species (scientific name)	Common name	N[a]	p (%)[b]	$I_{realized}$[c]	% Total variance[d]					MS[e]	Outside[f]	Floaters[g]		Ref[h]
–	–	–	–	–	W	E	Cov	P_w	M_e	–	–	Present	Included	–
Agelaius phoeniceus 2	Red-winged blackbird	21	25	0.49	69.3	9.7	20.1	35.2	10.2	P	R	R	N	*3*
Agelaius phoeniceus 3	Red-winged blackbird	103	26	0.74	72.0	15.0	13.0	–	–	P	R	Y	N	*4*
Agelaius phoeniceus 4	Red-winged blackbird	275	40	1.36	76.0	11.0	20.0	19.0	–	P	Y	?	N	*29*
Carpodacus erythrinus	Scarlet rosefinch	46	18	0.40	67.3	22.9	9.8	28.7	18.9	M	R	Y	Y[i]	*5*
Delichon urbica	House martin	17	19	0.31	41.5	56.6	1.9	–	–	M	N	N	N	*8*
Dendroica caerulescens	Black-throated blue warbler	67	21	0.72	76.3	11.0	12.7	–	–	M	R	N or R	N	*9*
Dendroica pennsylvanica	Chestnut-sided warbler	37	47	0.70	55.8	44.2	2.1	18.9	–	M	R	N or R	N	*10*
Geothlypis trichas 1	Common yellowthroat	21	26	0.48	57.7	20.8	21.5	22.6	22.7	M	R	Y	Y[i]	*13*
Geothlypis trichas 2	Common yellowthroat	101	18	0.71	56.3	22.6	21.1	8.5	16.9	M	N or R	Y	Y	*30*
Hirundo rustica erythrogaster	North American barn swallow	86	31	0.53	33.0	48.0	19.0	–	–	M	N or R	N or R	N	*14*
Malurus splendens	Splendid fairy-wren	204	42	1.42	58.8	42.0	–0.8	12.7	42.4	CB	N	N	N	*19*
Passerculus sandwichensis 2002	Savannah sparrow	57	56	0.58	34.6	65.3	–0.6	29.6	56.6	P	N	N	N	*21*
Passerculus sandwichensis 2003	Savannah sparrow	33	37	0.37	75.1	36.9	–11.9	34.0	23.6	P	N	N	N	*21*
Sialia currucoides	Mountain bluebird	59	36	0.27	60.4	33.6	6.0	9.2	9.5	M	Y	Y	N	*24*
Troglodytes aedon	House wren	68	10	0.22	97.3	10.4	–7.7	22.9	8.3	P	R	R	N	*13*
Tyrannus tyrannus	Eastern kingbird	89	47	0.85	42.0	46.0	12.0	36.5	33.0	M	Y	?	N	*27*
Propithecus verreauxi verreauxi	Verreaux's sifaka (primate)[j]	134	46	2.95	37.7[k]	33.9[k]	10.5[k]	–	–	MF	N	"Y"[l]	"Y"[l]	*31*

[a]Sample size (number of males)
[b]Frequency (%) of EPP among the young
[c]Opportunity for selection based on realized male reproductive success
[d]Proportion (%) of total variance in male reproductive success attributable to variation in within-pair (W) and extra-pair (E) success and the covariance between them [2Cov(W,E)] as well as to variation in the proportion of young sired in social nest(s) (P_w) and variation in the number of extra-pair mates (M_e). Note that components need not sum up because other terms can be negative (Webster et al. 1995). Fields are empty where values are not available
[e]Social mating system (MS): socially monogamous (M), polygynous (P), cooperative breeder (CB), or multi-male multi-female groups (MF)
[f]Opportunities for fertilizations of focal males in nonmonitored nests shown as yes (Y), no (N), or restricted (R)
[g]Presence of floaters in the population shown as yes (Y), no (N), or rare (R); and floaters included in the calculations of reproductive success shown as yes (Y) or no (N)
[h]References: see Appendix
[i]Some floaters may not have been caught
[j]This is the only non-avian species included. E refers to extra-group paternity in this primate
[k]Contribution without remainder terms
[l]Males without reproductive success are not floaters but members of the studied groups

between investing in the own brood or in EPCs. In this case, EPP may decrease the strength of sexual selection by providing an alternative route to reproductive success (e.g., for males that fail to nest). In 11 of 16 studies, the contribution of Cov(*W*,*E*) was nonnegligible (>5%) and positive (Table 2.2). In at least three studies, however, the covariance term was small, suggesting no clear relation between a male's extra-pair and within-pair success. Extra-pair and within-pair reproduction then represent two independent pathways through which sexual selection can act. Overall, we expect this to weaken the strength of sexual selection because reproductive skew among males should decrease with the existence of multiple uncorrelated pathways to mating success (e.g., Candolin 2003). Random mating also leads to zero covariance between extra-pair and within-pair success.

Although the studies listed in Table 2.2 generally seem to support the idea that EPP increases the intensity of sexual selection, there are some caveats to consider. In some studies estimates are highly inconsistent among years (savannah sparrow, *Passerculus sandwichensis* in Table 2.2) (Freeman-Gallant et al. 2005; Webster et al. 2007; see also Weatherhead and Boag 1997; Kleven et al. 2006), which would imply that the intensity of sexual selection may vary among years. However, the inconsistency is probably due to the low level of confidence associated with estimates of fitness components based on small sample sizes (Table 2.2). This uncertainty could be quantified by calculating confidence intervals for all estimates of the intensity of sexual selection (e.g., via bootstrapping), as is shown in Table 2.3 for the contribution of fitness components in the blue tit (*Cyanistes caeruleus*). Note that the contribution of the

Table 2.3 Confidence intervals for fitness components in blue tits

	Percentage of total variance	95% CI
Year 1998 (40 males)		
Var(*W*)	72.6	50.8 to 89.7
Var(*E*)	10.3	4.1 to 19.4
2Cov(*W*,*E*)	17.1	3.8 to 32.8
Year 2001 (28 males)		
Var(*W*)	79.6	46.2 to104.6
Var (*E*)	28.5	1.1 to 104.7
2Cov(*W*,*E*)	–8.2	–50.1 to 12.6
Years 1998–2003 (274 males)		
Var(*W*)	84.9	77.1 to 91.1
Var(*E*)	10.4	7.3 to 15.4
2Cov(*W*,*E*)	4.7	–0.7 to 10.6

The data represent the proportional contribution of fitness components (within-pair and extra-pair success and their covariance) to total variance in male reproductive success (%) for a population of blue tits (*Cyanistes caeruleus*). Data for 1998, 2001, and all 6 years of the study are shown (see Delhey et al. 2003 for details on the study). The 95% confidence intervals (CI) were constructed for the proportional contributions via bootstrapping using the package "boot" (Canty and Ripley 2009) in the software R 2.9.0 (R Development Core Team 2009) based on Davison and Hinkley (1997, Chaps. 5 and 11). For intervals shown here, parameters were set to 10,000 replicates, simulation type "ordinary," and interval type "bca" (adjusted percentile method). Other simulation and interval types led to similar results

covariance term in particular is highly variable among years, but confidence intervals overlap. Annual sample sizes are well within the range of the sample sizes reported in other studies (Table 2.2), and only the collation of data from 6 years allows a more precise assessment. Confidence intervals for *I* can be similarly constructed to provide information on the quality of the estimate. In any case, results from studies based on relatively small sample sizes should be viewed with caution.

2.4.2 *Effects of Sampling Limitations on Fitness Components*

Table 2.2 also contains information concerning the problem of sampling limitation, which may bias not only estimates of *I* but also those of fitness components. As outlined above, extra-pair success may be wrongly assessed when there are abundant opportunities for focal males to sire young in nonmonitored nests. Based on published information, this is a potential problem for only three of the studies listed in Table 2.2. Notably, Dolan et al. (2007) were presumably able to locate all nests; but because of the large distances over which reproductive interactions took place in this population, uncertainty remained about whether reproductive success of focal males was registered completely.

Variation in reproductive success and its components may also be misrepresented if the focal males, which are almost always paired, do not represent a random subsample of the entire male population – that is, when there are unpaired males (floaters, "satellites," or "sneakers") in the population. For example, a recent study of EPP in the common yellowthroat (*Geothlypis trichas*) that did include unpaired males found that differences among inexperienced males in their ability to secure a social mate (M_w) accounted for 70% of the variance in reproductive success in this age group (Freeman-Gallant et al. 2009). In contrast, among experienced males, variation in extra-pair success (*E*) became a major factor, explaining 40% of the total variance in reproductive success, because most of these males obtained a social mate. Thus, if unpaired males are common and even sire EPY (e.g., Kempenaers et al. 2001), estimates based solely on the breeding population may be misleading.

In most of the studies summarized in Table 2.2, floaters are thought to be rare or absent. However, this is often difficult to assess because nonbreeding males may be cryptic and therefore challenging to observe despite intensive study. Consider, for example, that in the ruff (*Philomachus pugnax*), a species that has been studied extensively for years, a "sneaky" male type (the "faeder") was discovered only recently (Jukema and Piersma 2006). Faeders are female mimics that sneak copulations and thus represent an alternative mating strategy (Lank and McRae 2008). Similarly, extra-pair behavior could represent a specialized mating tactic in other species, at least for some males. It is difficult to exclude this possibility as long as the sires of many EPY remain unassigned. The example of the ruff is also illustrative in another respect. The other two well-known alternative reproductive types of male ruffs, the "independent" and "satellite" males, are genetically determined (Lank et al. 1995); and this appears to be the case for faeders as well (McRae et al. 2008). Usually,

extra-pair behavior is viewed as a phenotypically plastic trait so each individual can optimize its mating behavior depending on the situation. It is thus assumed that all individuals engage in extra-pair behavior if it is optimal to do so in a particular environment. However, just as for the mating types among ruffs, individuals may differ in their propensity e.g., to form strong/loose pair bonds, to invest more/less in parental care/in courtship, or to show a low/high sex drive; and this may have a heritable component (Forstmeier 2007; van Oers et al. 2008). If such genetic divergence is common, it is an additional reason why nesting males may not be representative of the entire male population.

2.4.3 Effects of Random Mating on Fitness Components

The last issue to consider in connection with fitness components is the influence of stochastic events. As discussed above, random extra-pair mating can introduce variance in reproductive success and thus be a major contribution to *I*. When partitioning variance, we are making a statement only about the opportunity for selection mediated by EPP without relating it to phenotypic traits and heritability. This is probably less problematic than for $I_{realized}/I_{apparent}$ ratios because we may expect systematic contributions of chance to influence different components roughly equally. However, this assumption may not always hold. For the model of random extra-pair mating mentioned above, the contribution of variance in extra-pair success to total reproductive success increases with the rate of EPP (Fig. 2.2). The opposite is true for the contribution of variance in within-pair success under parameter constellations that prevail in natural systems ($\xi<4$) (Table 2.1; see Online Supplementary Material for details). Comparing results from variance partitioning from different populations may be problematic when there is evidence that stochastic effects may be influential (e.g., when the contribution of covariance is close to zero).

2.5 Bateman Gradient

The Bateman gradient is a direct reflection of the influence of additional mates on reproductive success and should thus provide the most accurate measure of sexual selection strength. Bateman gradients based on parentage analysis are expected to differ strongly between the sexes. For females of most species, additional mates should not lead to additional offspring. In some cases, the relation between the number of surviving offspring (e.g., fledglings) and the number of sires could be negative as a result of sexual conflict or even slightly positive (e.g., if EPY are more likely to survive until they fledge). For males, on the other hand, we expect a strong positive relationship between the total number of sired young and the number of mates unless there is a trade-off between within-pair and extra-pair success.

Calculating the Bateman gradient for males and females based on apparent and realized measurements of reproductive and mating success should thus reveal no differences in the slope for females but an increase for males if EPP increases the

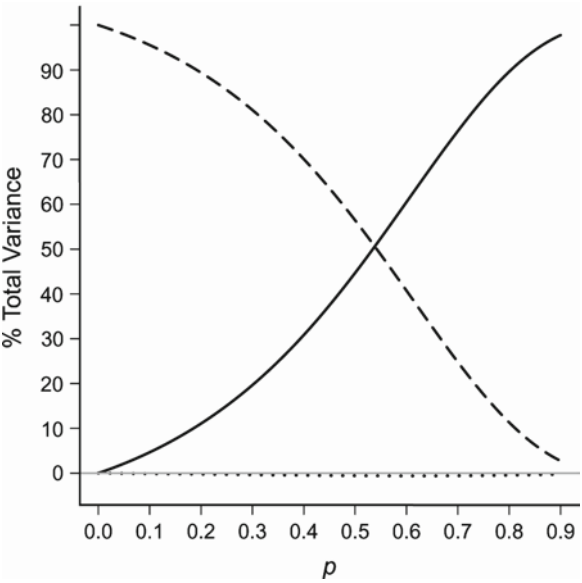

Fig. 2.2 Fitness components in relation to the frequency of extra-pair paternity based on a model of random extra-pair mating. Shown is the contribution (%) of variance in extra-pair success (*solid line*) and within-pair success (*dashed line*) as well as their covariance (*dotted line*) to total variance in reproductive success with changing frequency of extra-pair paternity among offspring (*p*). This example is for model A with parameters $N = 100$, $\bar{c} = 5$, and $I_{\text{apparent}} = 0.5$ (see Online Supplementary Material for details)

strength of sexual selection in males. In socially polygynous species, it is not immediately clear whether variation in reproductive success due to additional social mates (Bateman gradient based on apparent mating system) or additional extra-pair mates (influence of EPP on Bateman gradient based on realized mating system) has a stronger effect on sexual selection. Here, calculating Bateman gradients from measurements of apparent and realized reproductive and mating success may be particularly informative.

The Bateman gradient has been calculated in three studies of EPP in socially monogamous species (Ketterson et al. 1997; Webster et al. 2007; Balenger et al. 2009). All support the view that EPP drives sexual selection in males (Figs. 2.3, 2.4a and 2.5a). Still, the interpretation of these results is less straightforward than it may seem, as explained below.

2.5.1 Bateman Gradient in Females

Figures 2.4b and 2.5b show the Bateman gradient for females from two of the three studies. As expected, reproductive success is independent of mating success for

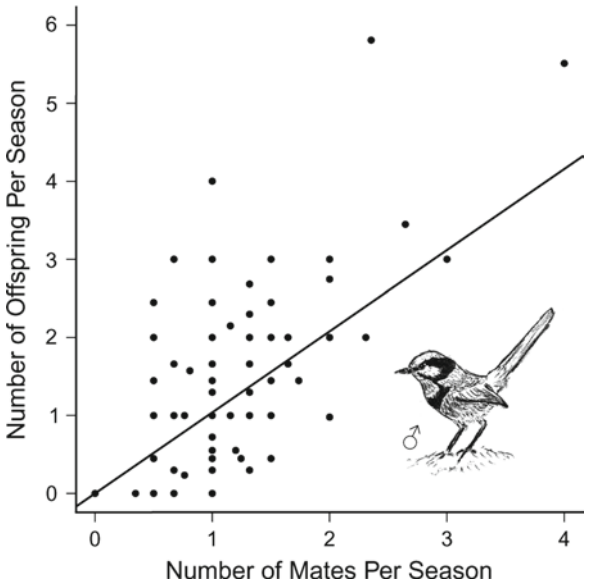

Fig. 2.3 Bateman gradient for male splendid fairy-wrens (*Malurus splendens*). Shown is the relationship between mating success (number of females with which a male sired genetic offspring) and reproductive success (log-transformed data: $N=204$, $R^2=0.68$, $P<0.0001$). This is a cooperatively breeding species, and helper males (auxiliaries) are included here. Results are similar when analysis is restricted to breeding males only. Redrawn with permission from Webster et al. (2007)

female mountain bluebirds (*Sialia currucoides*) (Fig. 2.4b). However, female dark-eyed juncos (*Junco hyemalis*) seem to increase their reproductive success when mating with more males (Fig. 2.5b), and this increase is at least as strong as in males (Fig. 2.5a). This seems to suggest that females obtain substantial benefits from mating with multiple males. However, female mating and reproductive success may covary without a causal relation. When extra-pair fertilization of any one egg is equally likely for all females, we expect a higher number of extra-pair mates in larger clutches (Ketterson et al. 1997; Parker and Tang-Martinez 2005).

The causality between mating and reproductive success may also be reversed for females. For example, when more fecund females are the target of more copulation attempts by extra-pair males, they may end up with a higher number of mates fertilizing their offspring (Ketterson et al. 1997). Hence, females may produce the same number of offspring in the absence of multiple mating, and higher fitness is not necessarily the result of higher mate number (sexual selection) but may be the cause of an increase in mate numbers. This explanation is unlikely to apply for male Bateman gradients because males that are the target of female EPC attempts will not – only because they are more fertile – sire the same number of offspring in the absence of multiple mating.

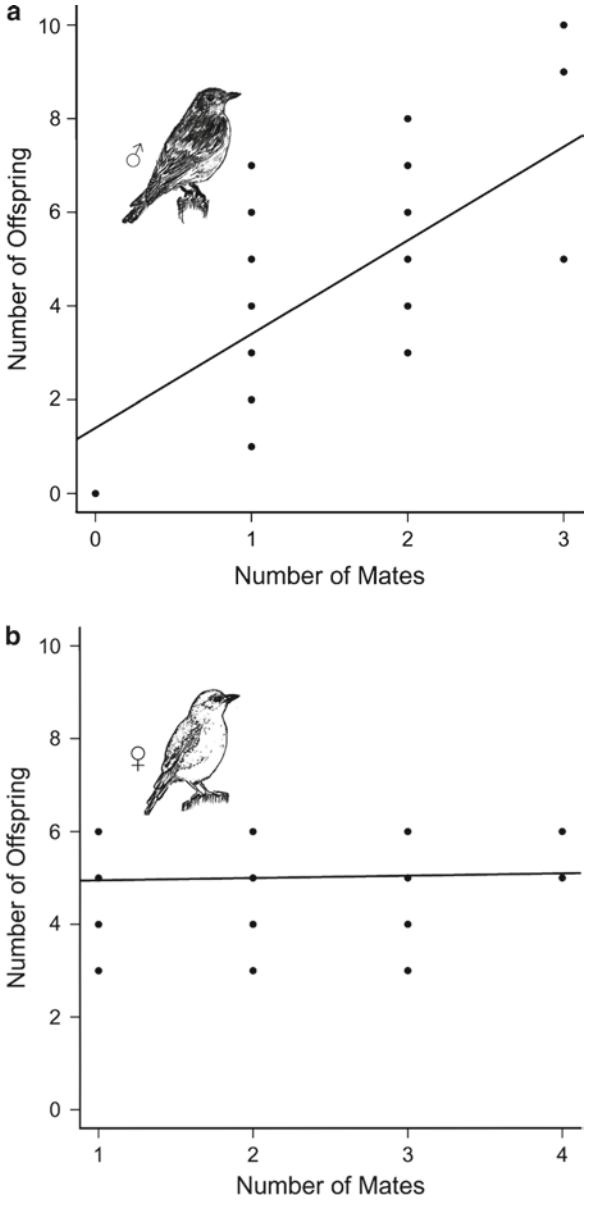

Fig. 2.4 Bateman gradients for males (**a**) and females (**b**) of the socially monogamous mountain bluebird (*Sialia currucoides*). Shown is the relationship between mating success (number of individuals with which genetic offspring are produced) and reproductive success. The Bateman gradient is significant and steep in males ($\beta_{ss} = 2.0$, $N = 59$, $R^2 = 0.42$, $P = 0.003$) and nonsignificant in females ($\beta_{ss} = 0.0$, $N = 59$, $R^2 < 0.01$, $P = 0.75$). Redrawn with permission from Balenger et al. (2009)

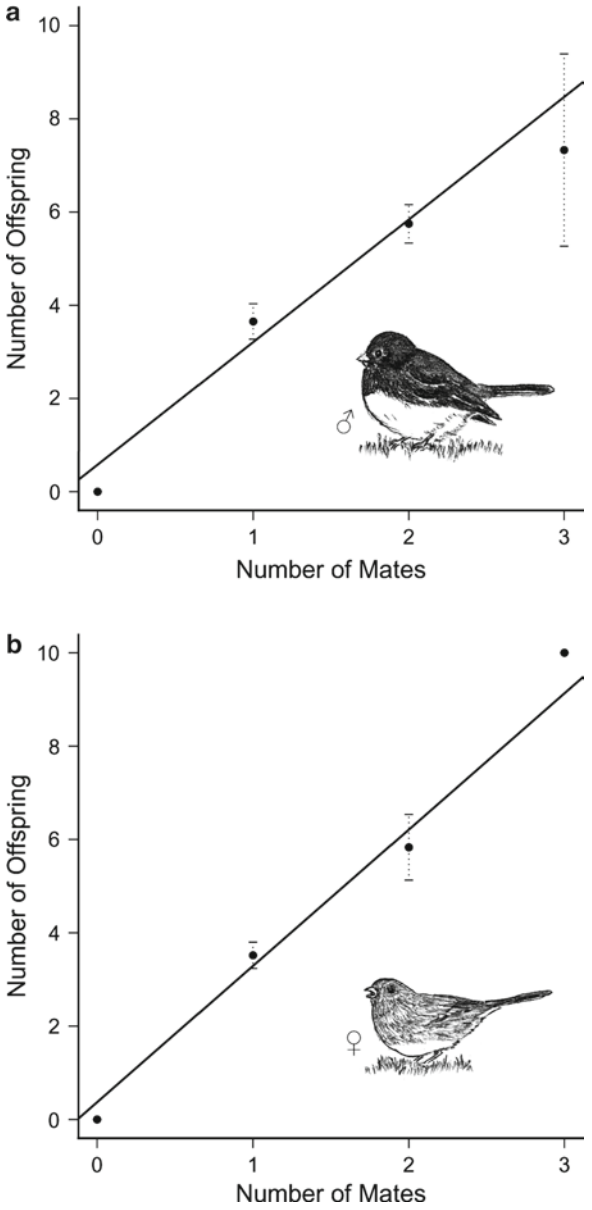

Fig. 2.5 Bateman gradients for males (**a**) and females (**b**) of the socially monogamous dark-eyed junco (*Junco hyemalis*). Shown is the relationship between mating success (number of individuals with which genetic offspring are produced) and reproductive success (mean ± SE). The Bateman gradient is significant and steep in males ($\beta_{ss} = 2.6$, $N = 50$, $R^2 = 0.61$, $P < 0.0001$). In females, it is also significant and even steeper ($\beta_{ss} = 2.9$, $N = 45$, $R^2 = 0.59$, $P < 0.0001$). Redrawn with permission from Ketterson et al. (1997)

2.5.2 *Effects of Sampling Limitations on the Bateman Gradient*

Male Bateman gradients may also be biased due to sampling limitations. When focal males sire EPY in nonmonitored nests, both reproductive and mating success are underestimated. This is unlikely to affect calculations of the Bateman gradient unless male fertilization success per extra-pair mate is different for these nests. However, when unassigned EPY are sired by socially unsuccessful males (e.g., floaters), the effect of EPP on sexual selection may be very different than it appears from the calculations restricted to nesting males.

First, when some males do not secure a social mate there is variation in apparent mating success, and a significant, high apparent Bateman gradient can be expected because the success of floaters is zero, whereas the success of breeders equals the clutch or brood size. Second, when floaters successfully sire EPY, the relation between realized mating and reproductive success may be weakened or even absent. In species with larger clutches and relatively low proportions of EPY within broods, floaters may have to secure several extra-pair mates to sire as many offspring as the average mated male with his social mate.

In the studies on mountain bluebirds and dark-eyed juncos, unmated males may have been present (Ketterson et al. 1997; Balenger et al. 2009). The study on the splendid fairy-wrens (*Malurus splendens*) (Webster et al. 2007) is special in that this species is a cooperative breeder where 17–42% of males are helpers with no apparent reproductive or mating success. Given that 25% of EPY are sired by such males, EPP might reduce the intensity of sexual selection by providing an alternative path to reproductive success for auxiliaries (Webster et al. 2004). [Note that the $I_{realized}/I_{apparent}$ ratio observed in this study is <1 (Table 2.1).] Thus, counterintuitively, similar species with lower EPP rates might experience stronger sexual selection in males, arising from competition among males to enter the breeding pool. Still, in the splendid fairy-wren, EPP drives sexual selection – independent of its effect on absolute strength – because it is the major source of variation in reproductive success among males (Table 2.2).

As for opportunity estimates and fitness components, there may be systematic influences of the EPP rate on the size of the Bateman gradient under random mating. However, this is not further considered here. Comparisons among published studies are hampered by the fact that only unstandardized Bateman gradients are reported.

2.6 Conclusions

We reviewed studies that quantify effects of EPP on the strength of sexual selection and briefly described the methods used to do this. So far, all measurements have been presented as point estimates. Including confidence intervals for these estimates

may be a simple way to add information on their reliability. We emphasized two issues about the interpretation of the measurements of the intensity of sexual selection, which we now briefly discuss further.

The first issue is the sensitivity of the measurements to sampling limitations. For studies that are unable to account for all offspring of focal males, the Bateman gradient is probably the estimate of choice; but all measurements can be strongly affected by the presence of floaters in the population. Attempts to maximize the number of identified sires or to obtain information about nonbreeders through extensive behavioral observations are therefore valuable. Where this is impossible, at least the potential role of EPP in shaping sexual selection for this subset of males can be investigated. To compare different populations or species, it might be useful to concentrate on paternity loss because this can always be recorded completely for the focal males (as long as the brood is genotyped). One can then search for patterns that indicate a reshuffling of paternity in favor of a subset of males as a consequence of EPCs. For example, when extra-pair sires lost less paternity in their own brood compared to other males in the population or compared to the males they cuckolded, EPP probably increases the reproductive skew among nesting males (Stutchbury et al. 1997). Conversely, when reciprocal cuckoldry is common, it indicates a lack of strong directional selection on males through EPP (Freeman-Gallant et al. 2005).

Modern methods of sibship analysis allow estimating the number of males involved in siring unassigned EPY (e.g., Jones 2001; Wang 2004; Croshaw et al. 2009). This can provide an indication of the size of the unmonitored population of reproductively active males. Furthermore, paternity assignment to "virtual" sires allows assessing the reproductive skew for these males. When paternity is spread widely among unknown sires, measurements of sexual selection based on only part of the male population probably suffer less from sampling limitations than when a few unknown sires have fathered a large number of offspring (Westneat 2006).

The second recurring issue is the question how random mating affects measurements of the strength of sexual selection. We do see selection measurements as a sign of current selection strength, even when it does not lead to evolutionary change. Still, systematic stochastic effects may be an important issue for comparisons among populations, and it may be instructive to consider this question in future studies. Specifically, it may be useful to construct reference values for selection measures based on an appropriate random mating process and include the deviation of the realized estimates from these reference values (see Online Supplementary Material for an example).

Although the current evidence is still limited, it suggests that extra-pair matings provide a major path to male reproductive success in some bird species (e.g., Dolan et al. 2007; Webster et al. 2007). This does not necessarily imply a strong increase in the strength of sexual selection with the rate of EPP (Dunn et al. 2001)

because, as we have seen above, sometimes sexual selection might be even stronger in the absence of EPP. How universal the role of EPP is for sexual selection remains debatable (Whittingham and Dunn 2005) even though in several species it appears to be important (Table 2.2). The next step, then, is to examine whether differences in mating and reproductive success mediated by EPP are linked to phenotypic traits, that is, to identify the targets of sexual selection. The quantitative estimates discussed here, particularly fitness components, can be helpful in establishing the main arena of sexual selection and predicting which traits may be important. In some cases, it has been confirmed that among-male variation in sexually dimorphic traits is linked to variation in extra-pair success (e.g., Kleven et al. 2006; Dolan et al. 2007; Albrecht et al. 2009; see also Møller and Ninni 1998), whereas in others there is no such relation (e.g., Westneat 2006; Neuman et al. 2007; see also Akçay and Roughgarden 2007). Variation among species and populations in life-history (Albrecht et al. 2007), geography (Neuman et al. 2007), or habitat (Kingma et al. 2009) is an important determinant of these differences. Molecular techniques are now routinely used and have made it possible to assess reproductive interactions with much greater accuracy (see also Chaps. 1 and 3). This will allow further study on how multiple mating affects sexual selection and through which mechanism.

Acknowledgments We are grateful to Robert Schlicht for extensive help with mathematical issues and to James Dale, Kaspar Delhey, and Wolfgang Forstmeier for valuable comments on this chapter.

2.7 Appendix

References for Tables 1 and 2: 1, Hasselquist et al. (1995); 2, Gibbs et al. (1990); 3, Westneat (1993), fitness components calculated by Webster et al. (1995); 4, Weatherhead and Boag (1997); 5, Albrecht et al. (2007); 6, Kempenaers et al. (1992); 7, Delhey et al. (2003); 8, Whittingham and Lifjeld (1995), fitness components calculated by Whittingham and Dunn (2005); 9, Webster et al. (2001); 10, Byers et al. (2004); 11, Yezerinac et al. (1995), "lower bound estimate"; 12, Sheldon and Ellegren (1999); 13, Whittingham and Dunn (2005); 14, Kleven et al. (2006); 15, Møller and Tegelström (1997) and Møller and Ninni (1998); 16, Richardson and Burke (2001); 17, Ketterson et al. (1997); 18, Johnsen et al. (2001); 19, Webster et al. (2004, 2007); 20, O'Connor et al. (2006), averaged over years; 21, Freeman-Gallant et al. (2005); 22, Otter et al. (1998) and Whittingham and Dunn (2005); 23, Wagner et al. (1996) and Møller and Ninni (1998); 24, Balenger et al. (2009); 25, Kempenaers et al. (2001), among residents; 26, Whittingham and Dunn (2005); 27, Dolan et al. (2007); 28, Stutchbury et al. (1997); 29, Westneat (2006); 30, Freeman-Gallant et al. (2009); 31, Lawler (2007) and Lawler et al. (2003).

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Chapter 3
Male Reproductive Skew and Paternal Kin-Biased Behavior in Primates

Eiji Inoue

3.1 Introduction

Primate societies and behaviors are complex. Behavior may be affected by both the environment and genes. Several approaches are used to investigate primate behavior. One deals with the way to examine the evolution of behavior. Sexual selection and kin selection theories are important for understanding the evolution of complex primate behavior. Data on male reproductive success and kin-biased behaviors are necessary to investigate these issues, and we cannot obtain such data without DNA analyses. Microsatellite markers can provide researchers with paternity and kinship information.

Noninvasive DNA samples are used in genetic studies of wild primates because obtaining these samples is minimally disruptive and therefore preferred (Vigilant and Guschanski 2009). Feces, hair, urine, saliva, and discarded food items are useful for analyzing DNA (Hashimoto et al. 1996; Goldberg and Wrangham 1997; Hayakawa and Takenaka 1999; Vigilant 2002; Inoue et al. 2007) and provide accurate genotyping (Taberlet et al. 1996; Morin et al. 2001). Although funding to obtain genotyping data is needed, researchers can get information on paternity and kinship even in wild primates.

Male reproductive success and paternal kin-biased behaviors are reviewed in this chapter. For male reproductive success, the focus is on male reproductive skew because this parameter affects the number of paternal half-siblings within groups. After a short review of male reproductive skew, the studies on paternal kin-biased behavior are summarized.

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3.2 Male Reproductive Success

Males compete with other males for access to fertile females (Fig. 3.1), whereas females are more selective for reproduction because of their high degree of investment (see Chap. 1). Altmann (1962) proposed the "priority of access" model in which dominant males have a priority of access to fertile males. This model predicts that dominant males can monopolize mating and paternity when the number of females in estrus is small. Ellis (1995) reviewed the effect of male dominance on reproductive success using approximately 700 studies. The review showed that high-ranking nonprimate vertebrate males enjoy higher reproductive success than do subordinate males but that high-ranking primate males do not always attain higher reproductive success.

Many studies have conducted paternity analyses recently. Some comparative studies also revealed the condition in which high-ranking males attain high reproductive success. Those studies and male reproductive skew are discussed next.

3.2.1 Effect of Male Dominance Rank on Reproductive Success

If high-ranking males can monopolize mating with fertile females, their reproductive success should be high. Some early findings were consistent with this hypothesis. For example, dominant male mandrills (*Mandrillus sphinx*), whose faces and sexual skins

Fig. 3.1 Japanese macaques in Iwatayama Monkey Park at Mount Arashiyama. A male (*left*) sits with a female in estrus (*right*)

have bright pigmentation, monopolized most of the paternity (Dixson et al. 1993). Positive correlations between male dominance rank and reproductive success were also found in long-tailed macaques (*Macaca fascicularis*) (de Ruiter and van Hooff 1993), chimpanzees (*Pan troglodytes*) (Takenaka et al. 1993), and red howler monkeys (*Alouatta seniculus*) (Pope 1990). On the other hand, no correlation was found in Japanese macaques (*Macaca fuscata*) (Inoue et al. 1993), rhesus macaques (*Macaca mulatta*) (Berard et al. 1993), or Barbary macaques (*Macaca sylvanus*) (Modolo and Martin 2008). Nonresident males sired offspring in wild patas monkeys (*Erythrocebus patas*) (Ohsawa et al. 1993), Japanese macaques (Hayakawa 2008; Inoue and Takenaka 2008), and Verreaux's sifaka (*Propithecus verreauxi*) (Lawler 2007). Extra-unit paternity was found in hamadryas baboons (*Papio hamadryas*) (Yamane et al. 2003), similar to extra-pair paternity in avian species (see Chap. 2).

Paul (1997) reviewed early findings and showed that positive correlations between male dominance rank and reproductive success were found in nonseasonally breeding primates, whereas no significant correlations were found in seasonally breeding primates. The number of females simultaneously in estrus may be larger in seasonal breeders than in nonseasonal breeders, which may be an important determinant in male reproductive success. Soltis et al. (2001) compared male reproductive success in two Japanese macaque groups and suggested that the number of females mating simultaneously influenced the degree of paternity monopolization by higher-ranking males.

Some studies also reported the effect of the number of males on male reproductive skew. Comparative data on paternity in one group showed that the proportion of offspring sired by alpha males decreased as the number of rivals increased among mandrills (Setchell et al. 2005) and chimpanzees (Boesch et al. 2006). In chimpanzees, paternity analyses have been conducted in four groups: in Bossou, Guinea (Sugiyama et al. 1993); in Gombe, Tanzania (Constable et al. 2001; Wroblewski et al. 2009); in Taï, Côte d'Ivoire (Vigilant et al. 2001; Boesch et al. 2006); in Mahale, Tanzania (Inoue et al. 2008); and in Budongo Forest, Uganda (Newton-Fisher et al. 2010). Using data on paternity from the four groups, I compared male reproductive skew with the number of males and found a negative linear relation (Fig. 3.2). The number

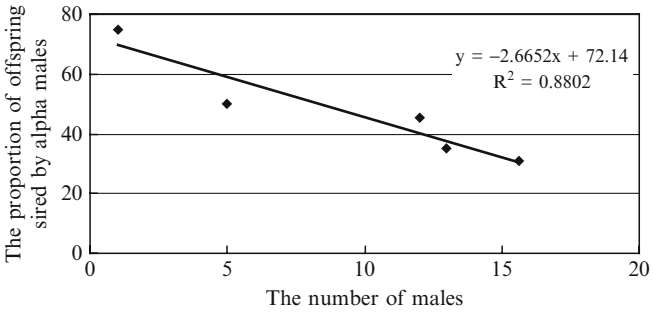

Fig. 3.2 Correlation between the proportion of offspring sired by alpha males and the number of males in groups of chimpanzees

of males is associated with female mating synchrony (Nunn 1999). However, it is difficult to distinguish which effect – the number of males or the number of females in estrus – strongly influences male reproductive skew by comparisons within groups or within species because of the small sample size.

Kutsukake and Nunn (2006) conducted phylogenetic comparative analyses using the male mating skew data of 31 species. They showed that mating skew decreased as the number of males increased; and they found no correlation between female estrous synchrony and male mating skew. Ostner et al. (2008) conducted similar analyses using the male reproductive skew data of 19 primate species. They found that male reproductive skew decreased as female reproductive synchrony and the number of males increased. Of these two factors, female reproductive synchrony explained more variation of male reproductive skew. Ostner et al. (2008) discussed the differences in the results between mating skew (Kutsukake and Nunn 2006) and reproductive skew and suggested that the risk of infanticide may lead females to copulate with multiple males so as to confuse paternity. Consequently, females copulate with more males as the number of males increases, whereas paternity is dominated by alpha males.

3.2.2 Priority of Access Model

Some studies tested whether the priority of access model (Altmann 1962) can predict the paternity. This model predicts that the most dominant male can sire an infant when only one female is in estrus and that several males can share paternity in proportion to dominance rank when several females are simultaneously in estrus. Altmann et al. (1996) found evidence supporting the reproductive priority of dominant males in wild savannah baboons (*Papio cynocephalus*). Similar results were obtained in wild chimpanzees (Boesch et al. 2006; Wroblewski et al. 2009) and in captive mandrills (Setchell et al. 2005). All three studies were conducted on nonseasonal breeding primates. For seasonal breeding primates, however, male dominance rank did not predict male reproductive success; and we should consider other factors such as female mate choice (Inoue and Takenaka 2008).

3.2.3 Reproductive Skew Models

Reproductive skew is the extent to which paternity is monopolized by dominant males. Reproductive skew theory proposes that the degree of skew is caused by several factors, such as the number of individuals and relatedness among groups (Clutton-Brock 1998; Johnstone 2000) (see Chap. 4). The two basic models are the concession model and the limited-control (sometimes called tug-of-war) model.

The concession model holds that dominant males can control mating of subordinates and allow them to sire. Among primates, no study has supported this model. On the other hand, the limited-control model holds that dominant males cannot control the reproduction of subordinates. The limited-control model was supported in rhesus macaques (Widdig et al. 2004) and mountain gorillas (*Gorilla beringei*) (Bradley et al. 2005). The limited-control model predicted that male reproductive skew decreases as the number of males and female mating synchrony increased (Kutsukake and Nunn 2009). Consequently, phylogenetic comparative analyses also supported the limited-control model because the prediction was found as mentioned (Kutsukake and Nunn 2006, 2009; Ostner et al. 2008). Kutsukake and Nunn (2009) also pointed out that the prediction of the priority of access model is similar to that of the tug-of-war model and that skew models can include more factors (Kutsukake and Nunn 2009). So far, only a small number of studies have applied the reproductive skew models, and more studies will apply these models in future.

3.2.4 Summary of Male Reproductive Skew

Among primates, female mating synchrony and the number of males affected male reproductive skew. Using comparative analyses based on several species, Ostner et al. (2008) suggested that female mating synchrony had the stronger effect. Primate societies are diverse. Therefore, researchers need to conduct comparative studies within species to clarify the species-specific effects. Researchers also need to clarify the effect on male reproductive success when female mating synchrony is high. Precopulatory and postcopulatory female choice and heterozygosity of the major histocompatibility complex (MHC) are possible factors (Widdig et al. 2004; Inoue and Takenaka 2008; Wroblewski et al. 2009).

Male reproductive skew may affect the genetic structure within groups. As male reproductive skew increases, the number of paternal relatives in groups becomes larger. The following sections review the effect of paternal kin on behavior.

3.3 Kin-Biased Behavior

Cooperation and affinitive behaviors of primates among maternal relatives have been observed, which may be explained by the kin selection theory. In primates, grooming and infant care behaviors have been frequently observed between maternal relatives (Silk 2002). However, no studies on cooperation and affinitive behaviors among paternal relatives were conducted before the advent of modern molecular genetic analyses. These analyses can tell us which pair is father–son or paternal siblings. Some recent studies showed positive or negative results of paternal kin-biased behaviors. Those studies are reviewed next, and the mechanisms to recognize paternal kin are discussed.

3.3.1 Estimation Method of Pairwise Relatedness

To obtain moderate confidence around a single pairwise relatedness estimate, we need approximately 30–40 genetic markers (Blouin 2003). A study by Langergraber and his colleagues (2007) involved detailed analyses of relatedness using microsatellite markers. They analyzed 44 markers on autosomes, 13 markers on the X chromosome, and 13 markers on the Y chromosome to estimate the relatedness of wild chimpanzees. Their study showed a low error rate (less than 5%) in distinguishing paternal and maternal half-siblings.

Although Langergraber et al. (2007) demonstrated the possibility of accurately estimating single pairwise relatedness using microsatellite loci, conducting such analyses is costly and time-consuming. In contrast, when we know mothers of offspring we can discriminate paternity using only approximately ten microsatellite loci. Therefore, most studies to investigate the effect of paternal relatives on behaviors have been conducted using the results of paternity.

3.3.2 Cooperation and Affinitive Behaviors Between Paternal Half-Siblings

3.3.2.1 Positive Results

Widdig et al. (2001) studied the effect of paternal kinship on behavior in rhesus macaques. They created an affiliation index using data on proximity, grooming, and approaches. The affiliation index among non-kin peers was significantly higher than that among non-kin non-peers. Then they differentiated between peers and non-peers to analyze the effect of parental kinship. They showed that the affiliation index among maternal half-sisters was higher than that among non-kin and paternal half-sisters who were non-peers; and they demonstrated a significantly higher affiliation index among paternal half-sisters than among non-kin, among both peers and non-peers. Furthermore, Widdig et al. (2006) analyzed agonistic interventions of rhesus macaques and showed that females supported maternal half-sisters significantly more often than paternal half-sisters or non-kin. A significant difference between paternal half-sisters and non-kin was observed only when the cost of intervention was low. These two studies suggested that rhesus macaques can discriminate paternal half-sisters.

Smith et al. (2003) studied savannah baboons and reported results similar to those found by Widdig et al. in rhesus macaques. They reported a significantly higher affiliation index among maternal and paternal half-sisters than among non-kin and no difference between the index among maternal half-sisters and among paternal half-sisters. Silk et al. (2006) investigated the social relationships of female baboons using data on 118 individuals obtained over the course of 16 years. In contrast to the results of Smith et al. (2003), Silk et al. found that the sociality index

among paternal half-sisters was higher than that among non-kin but lower than that among maternal half-sisters. Females engaged in a stronger social relationship with paternal sisters when they did not have maternal relatives. The sociality index among unrelated females who were close in ages was also high.

Charpentier et al. (2007) observed a tendency of paternal kin discrimination in juvenile mandrills. When they interacted with adult females, both maternal and paternal half-siblings were more affiliated than non-kin dyads. Affiliation with males was higher among father–offspring and maternal half-siblings than among non-kin. Among juveniles, maternal half-siblings were more affiliated than paternal half-siblings and non-kin dyads. Age differences in juveniles did not affect the affiliation, but differences in maternal rank had an influence; the lowest affiliation indexes were observed among pairs involving at least one high-ranking juvenile.

3.3.2.2 Negative Results

Perry et al. (2008) investigated social behaviors in adult female white-faced capuchins (*Cebus capucinus*) and showed that full sisters, maternal half-sisters, and mother–daughter dyads affiliated more than paternal half-sisters, and paternal half-sisters were not more affiliated than non-kin dyads.

Similarly, Langergraber et al. (2007) investigated paternal kin discrimination of chimpanzees in Kibale, Uganda. Among most mammals, it is the males that typically emigrate from their natal group. Among chimpanzees, however, females are the ones that typically emigrate from their natal group. Therefore, Langergraber et al. (2007) studied the social behaviors of adult males. Cooperation and affiliation (association, grooming, proximity, coalition, meat sharing, patrolling) among maternal brothers were more frequently observed than among non-kin dyads, whereas this was not true of cooperation and affiliation among paternal brothers. Mitani et al. (2002) earlier on investigated the same group of chimpanzees and observed that the frequency of cooperation and affiliation behaviors among males of the same-age cohort was higher than among males of a different-age cohort. Subsequently, Langergraber et al. (2007) investigated the difference in relatedness between males of same-age cohorts and males of different-age cohorts. Relatedness among males of same-age cohorts was not different from that among males of different-age cohorts. The size of their study group was 150 individuals, which was large for chimpanzees. This unusually large group size may reflect no difference in relatedness because male reproductive skew may decrease as the group size increases (Boesch et al. 2006; Kutsukake and Nunn 2006; Ostner et al. 2008). Quite recently, we investigated the relatedness of chimpanzees in Mahale, Tanzania (Inoue et al. 2008). The size of our study group was moderate (approximately 60 individuals). We found high reproductive success of alpha males, but the relatedness among males of same-age cohorts was not significantly higher than among males of different-age cohorts. Therefore, age proximity may not be a reliable cue to discriminate paternal kin in chimpanzees.

3.3.3 Father–Offspring Interactions

Infant care by males was observed among Barbary macaques. Ménard et al. (2001) showed that male-infant caretaking was not related to paternity. Male Barbary macaques did not tend to care for infants of females with whom they frequently mated. They showed that males participating in infant care achieved higher mating frequencies than other males with the mothers of the relevant infants. These results suggest that male Barbary macaques care for infants because they can attain higher reproductive success in the future.

Buchan et al. (2003) analyzed the interventions in agonistic behaviors by adult savannah baboon males on behalf of juveniles and showed that males tended to support their offspring more than other juveniles. These authors defined the juveniles for which the male consorted with the mothers during the period of likely conception as his nongenetic "behavioral" offspring but for which he was not a father determined by genetic analyses. Most males more frequently supported their offspring than their nongenetic "behavioral" offspring. Males did not support their nongenetic "behavioral" offspring more frequently than juveniles that were neither genetic nor "behavioral" offspring. In contrast to the results of Buchan et al. (2003), captive Japanese macaque males did not tend to support their offspring during agonistic behaviors (Machida et al. 1991).

3.3.4 Sexual Behaviors Between Paternal Relatives

Alberts (1999) investigated the difference of sexual behaviors between 669 non-kin female–male dyads and 357 paternal half-sibling dyads in savannah baboons. Consortship cohesiveness, measured by 12 sexual behavioral measures, among paternal half-sibling dyads was significantly lower than that among non-kin dyads. The consortship cohesiveness score among female–male dyads belonging to same-age cohorts was significantly lower than among female–male dyads of different-age cohorts. Based on these results, the author pointed out that age proximity may be a cue used to discriminate the paternal relatives.

Muniz et al. (2006) observed father–daughter inbreeding avoidance in white-faced capuchins. In their study group, the tenure of alpha males was 7 years, which was longer than the age at which females became sexually mature. The alpha male monopolized 79% paternity of females who were not his daughters but sired only 6% of the offspring of his daughters. When discussing the results, these authors suggested that father–daughter pairs actively avoided mating, although the data on copulation was absent. Infants spend more time in proximity to the alpha male, so familiarity may result in mating avoidance. However, Muniz et al. also discussed the possibility that they can avoid mating in the absence of close familiarity early in life.

On the other hand, reproduction between father–daughter and paternal half-sibling pairs was observed in Japanese macaques (Inoue et al. 1990). In this group,

copulation among maternal relatives was not observed. It was probably difficult for them to distinguish paternal relatives. In another group of Japanese macaques, males with shorter residence sired more infants (Inoue and Takenaka 2008). These results suggested social familiarity was important for inbreeding avoidance in Japanese macaques.

3.3.5 Possible Mechanisms of Paternal Kin Recognition

3.3.5.1 Discriminating Paternal Half-Siblings

Age proximity may be a possible cue to discriminate paternal half-siblings in some primates (Alberts 1999; Silk et al. 2006). If male reproductive skew is high, same-age cohorts include many dyads of paternal half-siblings, although same-age cohorts rarely include maternal half-siblings because primate females rarely give birth to twins. In rhesus macaques, savannah baboons, and chimpanzees, individuals of same-age cohorts are more affiliated than individuals of different-age cohorts (Widdig et al. 2001; Silk et al. 2006; Langergraber et al. 2007). Langergraber et al. (2007) summarized the proportion of paternal half-siblings in chimpanzees, rhesus macaques, and savannah baboons and showed that a positive effect of paternal siblings on cooperation and affiliation was found in the species with a relatively high proportion of paternal half-siblings. The estimated percentages of paternal half-siblings among mandrills and white-face capuchins are indicated in Table 3.1. The percentage among the white-faced capuchins is relatively high, although dyads among paternal half-siblings were not more affiliated than dyads among non-kin (Perry et al. 2008). Therefore, a high percentage of paternal half-siblings in same-age cohorts may be a prerequisite for discrimination of paternal half-siblings, but paternal kin-biased behavior was not observed in all the groups with a high percentage of paternal half-siblings.

Widdig et al. (2001) showed that paternal half-siblings were more affiliated than non-kin among same-age cohorts, suggesting that another explanation is needed for

Table 3.1 Paternal half-siblings among dyads of same-age cohorts

Species	Paternal half-siblings in same-age cohort (%)	Reference
Chimpanzee	5.1	Langergraber et al. (2007)
Rhesus macaque	12.7	Langergraber et al. (2007)
Savannah baboon	37.5	Langergraber et al. (2007)
Mandrill	59.1	Dixson et al. (1993)
White-faced capuchin	31.6[a]	Muniz et al. (2006)

[a]The average percentage of offspring sired by alpha males in three groups was 56.2% (Table S2 in Muniz et al. 2006). The percentage of paternal half-siblings were calculated from this value. Therefore, this value is underestimated because it includes only paternal half-sibling dyads whose fathers are alpha males

paternal kin recognition. They proposed that the mechanism may be phenotype matching. This mechanism is discussed below along with other references.

Although the percentage of paternal half-siblings is high (Table 3.1), the effect of age proximity on affiliation was not found in mandrills (Charpentier et al. 2007). The authors proposed two mechanisms. One is related to social apprenticeship, in which mothers mediate affiliation between infants who share paternity. The other is related to mating information transmitted maternally, where mothers are affiliated with the male with whom they have conceived. Those two mechanisms are not mutually exclusive.

3.3.5.2 Discriminating Their Offspring by Males

Buchan et al. (2003) showed the ability of males to discriminate their own offspring from unrelated infants. They clarified that males did not support their "behavioral" offspring like their own offspring. However, they discussed the possibility that behavioral cues were used to discriminate their offspring. Males can use the information on what proportion of the mother's copulation period they monopolized. This value was correlated with the probability of paternity, and the authors showed that males might use this information. Also, female–male relationships after copulation have been observed in Japanese macaques. Some adult male–female pairs developed the peculiar proximate relation after they had mated (Takahata 1982). Mating behavior may affect the affiliation, as Charpentier et al. (2007) also discussed.

3.3.5.3 Discriminating Father–Daughter Relationship

In white-faced capuchins, daughter–father may avoid mating (Muniz et al. 2006). As already discussed above, social familiarity early in life may affect mating avoidance. This tendency is also consistent with the results of Japanese macaques (Inoue and Takenaka 2008).

3.3.5.4 Phenotype Matching

Some studies showed that possible behavioral cues did not explain the paternal kin recognition and suggested the role of phenotype matching (Widdig et al. 2001; Smith et al. 2003). Olfactory cues may be important for human mate choice (Ober et al. 1997). In some animals, vocalization and odor cues can play a role in distinguishing kin (Price 1999; Mateo 2003). In primates, chimpanzees under experimental conditions can discriminate kin relationships through visual cues (Parr and de Waal 1999). Widdig et al. (2001) proposed the hypothesis that macaques can discriminate kin through behavioral traits. Some studies of the association between behavioral traits and genes have been conducted (see Chaps. 10–13). In primates, the proof of phenotype matching is still sparse, and more studies are needed to confirm this phenomenon.

3.3.6 Summary of Paternal Kin Recognition

Paternal kin-biased behaviors have been reported in some species of primates (see summary in Table 3.2). Although some different mechanisms have been proposed in different studies, independent studies showed paternal kin-biased behavior in savannah baboons. Therefore, paternal kin discrimination may exist in this species. There is not sufficient data in other species to conclude that paternal kin discrimination exists in those species. In the future, it will be necessary to accumulate data on paternal kin-biased behavior in more species and more groups.

Social or behavioral cues may be important means of identifying paternal kin. Some studies suggested that the only reasonable mechanism of paternal kin-biased behavior was phenotype matching. If they can obtain the information on accurate relatedness, their behavior with paternal relatives should be similar to that with maternal relatives. However, some studies showed that affiliation among maternal half-siblings was higher than that among paternal half-siblings (Widdig et al. 2001; Silk et al. 2006). These data suggest that phenotype matching may influence the behavior to some extent but that primates only roughly estimate relatedness by this method.

A cue for maternal kin recognition is thought to be close association during early life in primates (Silk 2002). Early association with mother and maternal relatives can lead monkeys to recognize maternal kin. Like maternal kin recognition, some studies suggest that early association with paternal relatives such as age mates may be a cue for paternal kin discrimination (Alberts 1999; Buchan et al. 2003; Muniz et al. 2006).

Table 3.2 Summary of studies on paternal kin-biased behavior

Parameter	Kin recognition	Possible mechanism	References
Affiliation between paternal half-siblings			
Rhesus macaque	Yes	Phenotype matching	Widdig et al. (2001, 2006)
Savannah baboon	Yes	Phenotype matching/age proximity	Smith et al. (2003), Silk et al. (2006)
Mandrill	Yes	Behavioral information	Charpentier et al. (2007)
Chimpanzee	No	–	Langergraber et al. (2007)
White-faced-capuchin	No	–	Perry et al. (2008)
Father behavior to offspring			
Barbary macaque	No	–	Ménard et al. (2001)
Savannah baboon	Yes	Phenotype matching/ behavioral information	Buchan et al. (2003)
Japanese macaque	No	–	Machida et al. (1991)
Sexual interaction			
Savannah baboon	Yes	Age proximity	Alberts (1999)
Inbreeding between Father and daughter			
White-faced-capuchin	Yes	Phenotype matching/ behavioral information	Muniz et al. (2006)
Japanese macaque	No	–	Inoue et al. (1993)

Mating behavior may also affect paternal kin-biased behavior (Buchan et al. 2003; Charpentier et al. 2007). These behavioral and social cues are probably important for paternal kin-biased behavior.

3.4 Future Perspectives

It is still unclear whether primates can discriminate paternal relatives from unrelated individuals. In some situations, paternal kin-biased behavior has been observed, and the possible cues are social and behavioral cues (e.g., age mates) or phenotype matching. In primate societies in which male reproductive skew is high, age proximity may be a reliable cue to discriminate paternal half-siblings from unrelated individuals. The studies of paternity analyses have been accumulated, and so we probably extract the condition in which the effect of age proximity on social interactions is effective if we choose appropriate comparative species or appropriate comparative groups in the same species. Similar approaches can be applied for analyzing other behavioral cues such as the effect of the mother–father relationship on the father–offspring relationship. For example, we should study species that are diverse with respect to their mating behavior and female–male relationships. Experimental studies for kin recognition are also needed for clarifying the phenotype-matching mechanism in primates. More studies will enable us to understand paternal kin recognition in primates.

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Chapter 4
Social Structures and Conflict Resolution in Primitively Eusocial *Polistes* Wasps

Koji Tsuchida

4.1 Introduction

The theory of kin selection (Hamilton 1964a, b) explains the evolution of helping behavior among relatives (see Chap. 3), but it also predicts several conflicts among relatives. First, individuals belonging to the same generation compete over resources. For example, several foundresses can compete with one another upon colony founding. Second, queen(s) and daughter workers belonging to different generations compete over the sex ratio. Third, queen(s) and workers compete over male parentage. The second and third conflicts are expected when the relatedness between interacting providers and recipients of altruism differs among kin groups.

The theory of kin selection hypothesizes that eusociality may have been favored in Hymenoptera because of the genetic system in which females are more closely related to their sisters than they are to their own offspring due to haplodiploidy. Eusociality evolved in hymenopteran insects, most notably in the ants, wasps (Fig. 4.1), and bees. Eusocial wasps primarily include three subfamilies within Vespidae: Stenogastrinae (hover wasps), Polistinae (paper wasps), and Vespinae (hornets and yellowjackets) (e.g., Carpenter 1991).

Figure 4.2 outlines the phylogenetic relations for the subfamilies of Vespidae. In Vespinae, queens of most species found colonies alone, with a few exceptions. The remaining subfamilies, Stenogastrinae and Polistinae, found colonies by the two modes of colony founding: independent-founding and swarm-founding. Colonies of independent-founding species are initiated by one or several inseminated queens, without the help of any workers. In contrast, colonies of swarm-founding species are initiated by a swarm consisting of a large number of workers accompanied by a smaller number of queens (Jeanne 1991). Although the number of queens varies substantially among eusocial wasps, monandry is predominant, with the exception

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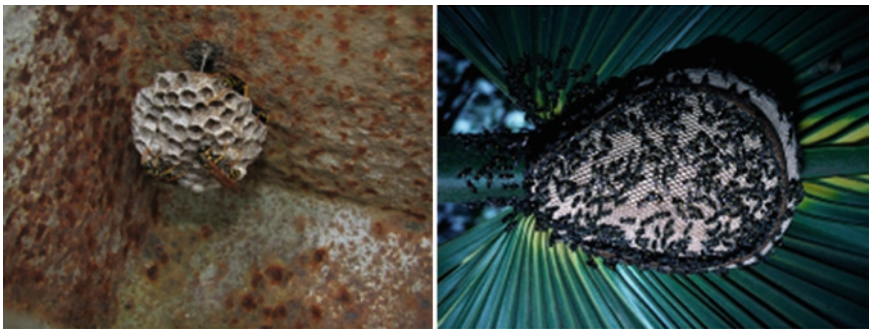

Fig. 4.1 Independent-founding (haplometrosis) by *Polistes chinensis* (*left*) and swarm-founding by *Polybia paulista* (*right*)

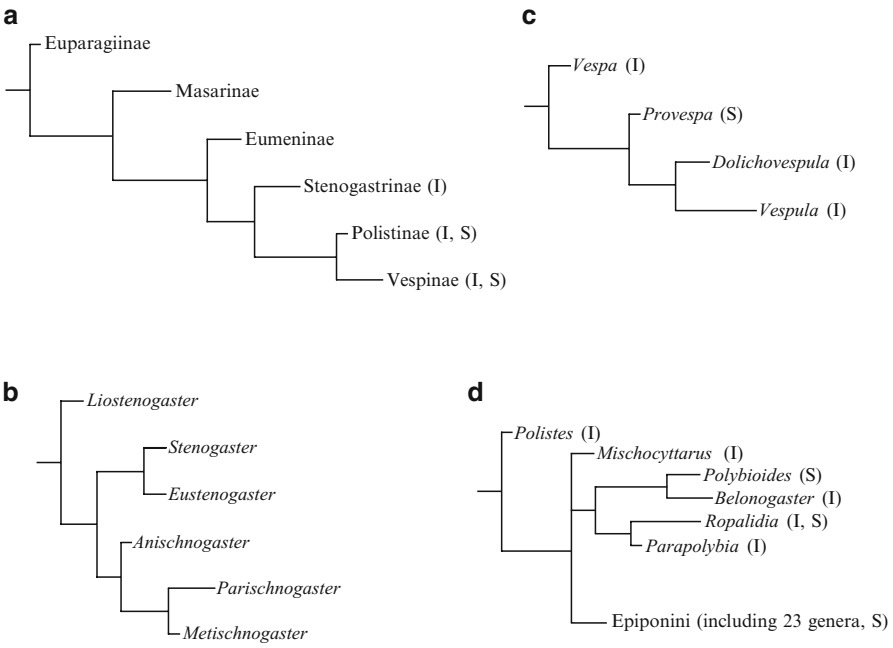

Fig. 4.2 Phylogenetic relationships of the subfamilies of Vespidae (**a**): Stenogastrinae (**b**), Vespinae (**c**), and Polistinae (**d**), Eusociality is observed in these three subfamilies with one exception. I, independent-founding; S, swarm-founding. Modified from Carpenter (1991)

of yellowjackets. A recent phylogenetic study indicated that lifetime monogamy is essential to the evolution of eusociality (Hughes et al. 2008).

In this review, the genetic colony structures of primitively eusocial wasps, mainly of the genus *Polistes*, are described in light of kin selection. *Polistes* belongs to the group of "primitively eusocial" wasps because they lack apparent size differences

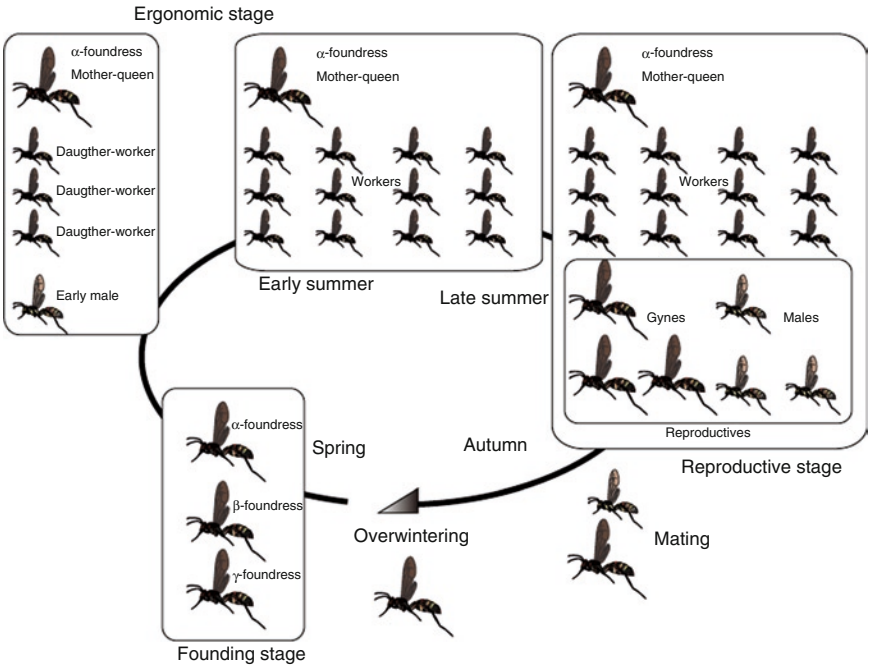

Fig. 4.3 Outline of the life history of wasps. In the spring, either a sole foundress (haplometrosis) or a foundress group (pleometrosis) founds nests, and the most dominant foundress (α-foundress) becomes a queen. The queen produces workers (ergonomic stage), and they cooperatively produce reproductives (new queens and males) in late summer and autumn (reproductive stage). Near the end of summer, the mother-queen dies. Mating takes place between new queens and males in the autumn, and only the resulting mated queens overwinter. Occasionally, the first batch of offspring includes some males, which are called early males. These early males are either the mating partners of workers or a reproductive dead-end due to their diploidy (diploid males). Although colonies are essentially simple families derived from a monogynous and monandrous queen, some factors can cause violations of this simplicity (e.g., reproduction by a subordinate foundress, male production by unmated workers, female production by mated workers, queen replacement)

and reproductive potential between queens and workers. These characteristics render this genus a suitable test species for social evolution via kin selection and/or the benefits of group living.

Figure 4.3 outlines the life history of independent-founding wasps. Nests are initiated by a sole overwintered foundress (haplometrosis) or a group of foundresses (pleometrosis) in the spring. Within the foundress group, the most dominant foundress (α-foundress) becomes a queen and produces daughters (workers and new queens) and most of the males. Occasionally, the first batch of offspring includes some males, which are called early males. These early males are either the mating partners of workers or a reproductive dead-end due to their diploidy (diploid males). However, most mating takes place between new queens and males, and only mated queens overwinter.

4.2 Conflict Among Foundress Associations

4.2.1 Foundress Associations

Pleometrosis usually ends when the first workers emerge. Subsequently, the single most-productive egg-layer (dominant foundress) becomes the sole queen around the time when the first workers emerge. The remaining subordinate foundresses either remain as subordinate workers or disappear from the nests (Reeve 1991). With the exception of *Camponotus yamaokai* (Satoh et al. 1997), the foundresses in pleometrotic ant societies are genetically unrelated to each other (Hagen et al. 1988; Sasaki et al. 1996). The lack of genetic relatedness among ant foundress groups is likely due to their mating flight behavior in that related foundresses are unlikely to meet again after mating (Bernasconi and Strassmann 1999).

In contrast to ant societies, foundresses in pleometrosis of wasps and bees generally exhibit moderate genetic relatedness (but see Schwarz et al. 2007). In an Australian allodapine bee, *Exoneura bicolor*, the genetic relatedness among foundresses was estimated to be around 0.6 (Schwarz 1987). The genetic relatedness among foundresses of several polistine wasps (*Polistes* and *Mischocyttarus*) were found to range from 0.3 to 0.8 (e.g., Queller et al. 1988). However, recent advances in highly variable microsatellite markers have enabled the fine-scale resolution of family structure among colonies. In an Italian population of *Polistes dominulus*, Queller et al. (2000) used several microsatellite markers to estimate that 35% of foundresses were not genetically related. Similarly, Zanette and Field (2008) estimated that the foundress groups of a Spanish population of *P. dominulus* contained many unrelated individuals. In both reports, the estimated values of mean genetic relatedness were moderately high, yet small and significant proportions of foundresses were unrelated.

4.2.2 Dominance Hierarchy in Pleometrosis

In wasp pleometrosis, the order of dominance is generally determined through direct physical aggression, and the resulting most-dominant foundress becomes the sole egg producer (queen) after the first worker emergence. Subsequently, the remaining foundresses become subordinate workers that can rank above daughter workers (e.g., in *Polistes annularis*) (Hughes et al. 1987) or below them (e.g., in *Ropalidia plebeiana*) (Tsuchida, unpublished data). Dominance behaviors are generally believed to be ritualistic, and the foundresses who were subdued by aggression rarely die. Dominance behaviors are common across independently founding polistine wasps (e.g., *P. dominulus*, Pardi 1948; *Polistes metricus*, Gamboa and Dropkin 1979; *Ropalidia fasciata*, Itô 1993; *Polistes fuscatus*, Gamboa and Stump 1996; *Belonogaster petiolata*, Keeping 1992; *Belonogaster juncea juncea*, (Tindo et al. 1997). The most dominant foundress exclusively oviposits eggs, whereas subordinate

foundresses forage more frequently and experience shrinking of their ovaries (Pardi 1948). Dominance behaviors are not only observed among foundresses but also among nest-mate workers, and they appear to control colony activities as well as reproduction (Reeve and Gamboa 1983; Gamboa et al. 1990). A replacement queen, who becomes the new queen when an original queen disappears, is the second most dominant individual just beneath the original queen (e.g., Hughes et al. 1987). Whereas in temperate regions the replacement queen is generally the oldest individual among the daughter generation, a process termed "gerontocracy" (Strassmann and Meyer 1983) takes place in other regions whereby an individual becomes a replacement queen as a function of climate (e.g., Tsuchida and Suzuki 2006). In temperate areas with an annual life cycle, gerontocracy is dominant, whereas in tropical regions with a perennial life cycle the reverse relation (paedocracy) is observed. Tsuji and Tsuji (2005) hypothesized that the relative individual life expectancy compared to the colony life expectancy can generate such an unusual pattern of dominance structure.

The conflict over dominance status among foundresses has attracted the attention of many entomologists, and this conflict has become a main issue in studies of the evolution of cooperative behaviors among social insects. To date, competitive ability, which is positively associated with body size; the order of arrival at nests; physiological condition; body appearance; and genetic relatedness have all been hypothesized to influence the dominance order.

4.2.2.1 Mechanisms Determining the Hierarchy

Subfertility Hypothesis

Helpers who forfeit their own reproduction and devote their energy to other individuals exist in many vertebrate and invertebrate animals. The subfertility hypothesis predicts that helpers originally lack sufficient reproductive abilities and make the "best of a bad job" (West-Eberhard 1975). This hypothesis is based on the assumption that small individuals are less fertile because they did not receive sufficient food during immature stages. Although dominance rank in vertebrates appears to be generally positively associated with body size (e.g., Buston 2003), the effect of body size on dominance rank in wasps has not been clearly documented. Gadagkar et al. (1988, 1991) revealed that some foundresses of *Ropalidia marginata* are physiologically constrained to found a colony independently, as 50% of foundresses failed to found a nest under laboratory conditions. Only a limited number of studies have reported a positive association between body size and dominance rank in wasps (e.g., Cervo et al. 2008). Likewise, a positive association between dominance rank and nest arrival order has rarely been observed (but see Seppä et al. 2002; Zanette and Field 2009). In the study of Zanette and Field (2009), however, this relation only held when the first and second most dominant foundresses within nests were analyzed, and it disappeared when all foundresses were included in the analyses. However, in *Polistes jadwigae*, large foundresses tend to start their nests

earlier in the season (Tsuchida 1991), and such behavior could attract other (smaller) foundresses to their nests.

In terms of physiological activities, the juvenile hormone (JH) titer is positively correlated with dominance rank and ovarian development in polistine wasps. Röseler et al. (1984) reported that the dominant foundresses of *Polistes gallicus* did not have larger body sizes; instead, they exhibited higher hormonal titers, and injections of JH to subordinate foundresses resulted in increases in their dominance rank. However, although ablation of ovaries led to decreases in the ecdysteroid titer in hemolymph, it did not affect dominance rank (Röseler et al. 1985).

The JH titer affects ovarian development, but whether high JH titers can cause high dominance rank or vice versa is difficult to determine. Although an associative relation between JH titer and ovarian development clearly exists, we do not have reliable data to support the subfertility hypothesis for explaining subordinate foundresses among *Polistes* wasps.

Fertility Signal in Cuticular Hydrocarbons

How are such dominant individuals recognized by subordinates and vice versa? The body surface of insects is generally coated with cuticular hydrocarbons (CHC) for waterproofing (Howard and Blomquist 1982). These chemicals play a role as recognition cues at the individual, kin, and nest-mate levels. Sledge et al. (2001) analyzed the differences in CHC profiles between dominant and subordinate foundresses in *P. dominulus* and found that such differences are not clear at the early stage of nest founding but become evident upon worker emergence. At that time, the CHC profiles of the dominants exhibited a greater proportion of distinctive unsaturated alkenes of longer chain length compared to those of subordinates and subsequent worker offspring. In addition, when original queens were removed, the CHC profile of the replacement queen became similar to that of the original queen (Sledge et al. 2001). These results clearly demonstrated that the CHC profile is, at the very least, an associative signal for fertility. In an experiment with *P. dominulus*, an artificial egg-removal treatment induced oviposition by subordinates as well as dominants (Liebig et al. 2005). Using this trait, Dapporto et al. (2007) induced subordinate oviposition and analyzed whether CHC was a signal for fertility or dominance; they found that CHC was a signal for dominance, as subordinate individuals with developed ovaries still exhibited profiles that differed from those of dominant individuals. This result demonstrated that ovarian development does not directly cause CHC differences, but the dominance status itself is independent of ovarian development.

Fertility Signal in Facial Features

With regard to dominance ranks of foundress associations, studies by Tibbetts et al. have provided new insight into insect recognition systems. This group investigated

the possibility that differences in body appearance (face and abdomen) may be linked to individual recognition. For example, Tibbetts (2002) examined whether artificial alteration of yellow portions of the face and abdomen of *P. fuscatus* could cause changes in aggressiveness among nest-mates. The treated wasps were the targets of more frequent aggressive acts from resident nest-mates upon landing on the nests. The effect of the alteration lasted for only 30 min, but it was observed in both foundresses and workers. A subsequent study using the same species revealed that the memory of opponents lasted 7 days under laboratory conditions (Sheehan and Tibbetts 2008), suggesting that this wasp can discriminate individuals and is able to remember opponents for several days. Tibbetts et al. suggested that these visual features could also serve as a signal of individual quality. The hypothesis was tested by artificially altering the visual features of the face (clypeus) of subordinate individuals to resemble those of dominants. The results indicated that dominant-looking individuals, whose visual features had originally been those of a subordinate, underwent an increased number of aggressive acts from nest-mates after the treatment, suggesting that visual features may act as an honest signal (Tibbetts and Dale 2004). Furthermore, individuals reared with more food developed into dominant-looking adults (Tibbetts and Curtis 2007), and the visual feature of the clypeus appeared to be used for evaluating the quality of opponents upon accessing prey (Tibbetts and Lindsay 2008). Each experiment was conducted under laboratory conditions; thus, whether this signal is used under natural conditions warrants investigation. However, a similar study of a natural Italian population of *P. dominulus* did not support the honest signaling hypothesis (Cervo et al. 2008). Zanette and Field (2009) found a positive association between the visual features of the clypeus and dominance in a Spanish population of *P. dominulus*, but the relation was not as clear as that observed by Tibbetts and Dale (2004) because of the low frequency of dominant-featured individuals per nest. The genetic features of the introduced U.S. population could have changed from those of native populations; however, this possibility is unlikely, and the contradiction currently remains unexplained.

In the native Brazilian population of *Polistes satan*, the brown facial features of the wasps serve as a dominant honest signal (Tannure-Nascimento et al. 2008), as the brown facial area and distinct CHC profiles are both associated with dominance. Together, these studies suggest that facial features could reflect the quality of individuals and thus could be used as an honest signal in some *Polistes* species. However, more supporting evidence is needed for additional species.

Selective Advantage of Foundress Associations

The selective advantage of co-founding nests in *Polistes* wasps has been empirically explained using the inclusive fitness framework. Metcalf and Whitt (1977a, b) revealed that subordinates of *P. metricus* enjoyed more inclusive fitness when group nesting than when nesting alone. Similar inclusive fitness advantages for group nesting were confirmed in *P. fuscatus* and *P. annularis* (Noonan 1981; Queller and

Strassmann 1988), for which values of genetic relatedness among foundresses were estimated at around 0.5 using allozyme markers. Other selective advantages of foundress group formation primarily fall under two frameworks. First, the presence of a subordinate could decrease the dominant individual's workload and increase the probability that at least one foundress will survive to leave offspring (survival insurance) (Reeve 1991; Nonacs and Reeve 1995). Second, the presence of a subordinate, even when it survives for a rather short period, could augment the number of surviving immature individuals (assured fitness return, or AFR) (Queller 1989; Gadagkar 1990; Field et al. 2000). Gadagkar (1990) pointed out that AFR secures a selective advantage of group nesting, even if the relatedness between the dominant and subordinate is 0.1. These two hypotheses involve the prerequisite that interacting individuals are genetically related to some extent.

Reproductive Skew Models for Foundress Associations

The above theoretical studies explain the inclusive fitness advantage of group nesting but do not explain the intercolonial variation in reproductive shares and behaviors among foundresses. Reproductive skew (RS) models predict how reproductive partitioning among members of animal groups should be resolved under ecological, genetic, and social constraints (Reeve and Keller 2001).

The RS models can be classified into three main types. First, concession models attempt to explain the degree of skew by predicting the conditions under which the dominant breeder should yield just enough reproduction to a subordinate to make it favorable for the subordinate to stay in the group and cooperate peacefully rather than leave the group and reproduce independently. In contrast, restraint models attempt to explain the degree of skew by predicting conditions under which the subordinate breeder should claim the largest share of reproduction that the dominant breeder will tolerate before ejecting the subordinate. In the former models the dominant breeder is assumed to control both group membership and the distribution of reproduction within the group, whereas in the latter models the dominant breeder is assumed to control group membership, but the subordinate breeder is assumed to control the reproductive share within the group. From the viewpoint of the subordinate, the fraction of its reproduction within the group is placed within either end area by the concession or restraint models. Between these two extremes is the tug-of-war model with incomplete control (see Reeve and Keller 2001 for detailed explanation).

The AFR model handles conditions similarly to the RS models; the former involves situations in which the subordinate does not reproduce directly but aids reproduction indirectly, and the latter involves sharing reproduction within colonies by the dominant and subordinate. Therefore, AFR is an extreme case of RS models that provides no direct reproductive share for the subordinate (Nonacs et al. 2006).

The RS models have been tested to explain the reproductive share among foundresses in several *Polistes* wasps. Some reports have supported these

models (Reeve and Nonacs 1992, 1997; Reeve et al. 2000), whereas others have failed to demonstrate support (Field et al. 1998; Liebert and Starks 2006; Nonacs et al. 2006). Thus, the validity of these models remains controversial. The limited explanatory power of RS models could stem from three factors: (1) the observed dominance behaviors may not reflect real reproductive skew; (2) some foundress groups may lack any kin associations and/or variation of relatedness among colonies; and (3) recognition error could contribute to non-kin foundress group formations (Nonacs et al. 2004, 2006; Liebert and Starks 2006).

In foundress associations, dominance behaviors (i.e., pecking and darting) have been interpreted as suppressing ovarian development in individuals subjected to those behaviors. However, no guaranteed link exists between reproductive skew and the observed dominance behaviors. For example, in *R. marginata*, dominance behaviors functioned to stimulate foraging behaviors but were not associated with reproduction (Bruyndonckx et al. 2006). The queen of this species is a docile individual, and observers could not retrospectively identify the replacement queen when the original queen was artificially removed (Bruyndonckx et al. 2006; Lamba et al. 2007; Gadagkar 2009). The inhibition of reproduction in subordinates appears to be mediated by a primer pheromone (Bhadra and Gadagkar 2008). Similarly, the dominance behaviors of *R. plebeiana* seem to elicit foraging behavior of daughter workers (Tsuchida, personal observation) but do not regulate reproduction. Therefore, dominance behaviors are likely associated with reproduction in *Polistes* wasps, but they could serve another function in some *Ropalidia* wasps. Care must be taken to acknowledge differences in the meaning of "dominance behaviors" among species and genera.

As mentioned previously, Queller et al. (2000) and Zanette and Field (2008) suggested that *P. dominulus* foundress groups were not purely kin-based. Alternatively, if the foundress group is a kin-based trait, other factors (recognition error and/or substantial costs for incorrect recognition) might prohibit explicit nepotistic relations.

Social Queuing of Foundress Groups

In foundress groups, the most dominant individual monopolizes the reproduction among colonies, and the second- and lower-rankers wait for a rise in rank after the disappearance of higher rankers (social queuing, or SQ). As direct fitness benefits, the top-ranker is expected to increase its offspring numbers and/or survivorship by increasing the numbers of low-ranking helpers. As indirect fitness benefits, low-rankers are expected to increase their indirect fitness advantage through kin nest-mates, which is an assumed condition of AFR. Even if no kin relationship exists among nest-mates, an indirect fitness advantage is also expected for a low-ranker because reared non-kin immature individuals will become helper adults when the former low-ranker becomes a top-ranker.

In a species of hover wasp that inhabits Southeast Asia, *Liostenogaster flavolineata*, the colony cycle lacks seasonality. The colonies are basically formed by multiple females composed of a mother foundress and daughter helpers. In general, the second-ranker inherits the top position if the top-ranker disappears from the nest. The top-ranker is always a mated individual, but many lower-rankers are not. One research group applied several experimental manipulations (i.e., removing the top-ranker or attaching unoccupied nests to stimulate independent nesting by low-rankers) to examine the adaptive meaning of SQ. Their results demonstrated that low-rankers had reproductive capacity comparable to that of the top-ranker, but they did not leave and found nests on their own, probably because of strong ecological constraints associated with nesting alone (Field and Foster 1999). In addition, removal of a higher-ranker (i.e., increasing the chance of inheriting the nest) decreased the working frequency of the focal individual. In contrast, removal of a lower-ranker (i.e., decreasing the number of future helpers) increased the working frequency of the focal individual. These results appear to be consistent with the prediction that each ranker in SQ behaves according to their expected future fitness advantage (Field et al. 2006). Theoretically, AFR assumes the presence of genetic relations among nest-mates, but SQ does not require such relations (e.g., Kokko and Johnstone 1999). The absence of genetic relations among nest-mates, as mentioned above, is not always maladaptive because immature individuals of a top-ranker will become helper adults when the former low-rankers become top-rankers. It has been argued that the foundress associations of *P. dominulus* involve such a trade-off between present helping effort and future fitness (Cant and Field 2001; Shreeves et al. 2003; Cant et al. 2006; Zanette and Field 2009). In my opinion, the SQ framework currently describes these associations more successfully than does the RS framework.

In *Polistes* wasps, the CHC profile plays an important role in nest-mate recognition. However, facultative nepotism according to relatedness differences has not been observed in this genus (Queller et al. 1990; Strassmann 1996). In fact, only one study reports facultative nepotism among all social insects (Hannonen and Sundström 2003). Even among honeybees, the most extensively studied social insect, nepotistic behavior based on relatedness remains uncertain (Oldroyd and Rinderer 1990).

Sumana et al. (2005) reported that the CHC profiles of nest materials in *P. dominulus* did not change between the autumn and spring, suggesting that this stability is important for the philopatric tendency of foundresses. However, Dapporto et al. (2004b) reported that the CHC profiles were mixed among overwintering foundresses of *P. dominulus*. Recently, two peptides originating from the cuticle and poison gland of *P. dominulus* have been recognized as having an antibiotic role; concomitantly, the places where the peptides attached were likely to attract foundresses looking for favorite hibernation sites (Turillazzi et al. 2008). In general, such favorite sites appear to be limited in number, and the foundresses attracted to such sites likely originate from neighboring colonies, which could, in turn, lead to a philopatric tendency. Such a tendency could help to maintain sufficient relatedness among foundress groups, but each foundress is not likely to search actively for her kin.

4.3 Conflict Over Sex Ratio

4.3.1 Adaptive Sex Ratio Variation Under a Relatedness Framework

In hymenopteran insects, sex is determined by haplodiploidy, and relatedness between sisters becomes 0.75 in colonies with monandry and monogyny. Hamilton (1964a, b) first proposed the well-known kin selection theory under an inclusive fitness framework to explain the evolution of self-sacrificing behavior, which had been difficult to explain using the classic direct fitness framework. "Hamilton's rule" predicts that helping behavior is selected for if $rB - C > 0$, where B is a recipient's fitness benefit due to the donor's helping behavior, C is the donor's fitness cost due to the helping act, and r is the relatedness between the recipient and donor. Measuring relatedness is now quite easy using neutral genetic markers, whereas measuring B and C is complex and laborious even with contemporary methods.

The pioneering work of Trivers and Hare (1976) has provided a valuable tool with which to test the kin selection theory by measuring the population sex ratio instead of measuring B and C. Their theory predicts that the adaptive population sex ratio in monogynous and monandrous colonies is 3:1 (female/male) under worker control and 1:1 under queen control. Although this theory predicts an adaptive reaction of the sex ratio of the whole population, polymorphism in colony structure is often observed among colonies (e.g., queen-less colonies vs. queen-right colonies and monogyny vs. polygyny). A split sex ratio, which is a more general prediction of individual colony sex ratio variation associated with the variation of genetic colony structure, was proposed by Boomsma and Grafen (1991). The principal parameter for this theory is relatedness asymmetry (RA), which is the ratio between the life-for-life relatedness of workers to other sisters and that of workers to the males they rear. The theory predicts that colonies with an RA above the average RA of the whole population should invest only in females, whereas those colonies with an RA below the average should invest only in males. Empirical support for this theory has been reported for several ant species, in which RA differs among colonies owing to the facultative mating frequencies of the queen (Sundström 1994; Evans 1995; Sundström et al. 1996).

Relatedness asymmetry can vary with polymorphisms in the mating frequency of a queen and with other factors causing polymorphisms in the genetic colony structure among colonies. Polygyny is the prevailing system in swarm-founding wasps; and historically, kin selection theory could not readily explain its maintenance, as relatedness among workers is very low. However, under cyclical oligogyny (or seasonal monogyny) (West-Eberhard 1978), in which the number of queens decreases as the season progresses, daughter queens are produced only after a bottleneck in the number of old queens; consequently, these daughter queens are highly related, often as full sisters, elevating the relatedness among the worker progeny of the new queens and promoting cooperation (Fig. 4.4). Moreover, cyclical oligogyny is not contradicted by the split sex ratio theory; workers can enjoy their

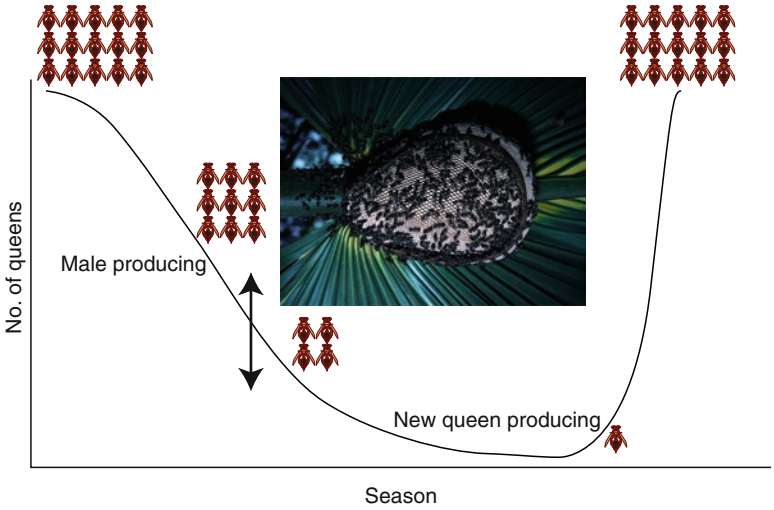

Fig. 4.4 Cyclical oligogyny in swarm-founding wasps. The number of queens seasonally fluctuates, and males are produced when the number is relatively high, whereas new queens are produced when the number is relatively low. The cycle does not synchronize among colonies. The number of queen illustrations reflects only relative numbers. The *arrows* indicate the difference between the male-producing stage and queen-producing stage. The photo shows a founding swarm of *Polybia paulista* in Brazil

optimum during the monogyny or oligogyny stage, when higher RA produces only gynes, and the polygyny stage, when lower RA produces only males (Strassmann et al. 1991, 1992, 1998; Gastreich et al. 1993; Queller et al. 1993; Hastings et al. 1998; Henshaw et al. 2000b; Tsuchida et al. 2000; Kudô et al. 2005). Although several unexplored questions concerning cyclical monogyny remain (e.g., how to continue to supply a large worker force by having a single-queen stage regardless of the substantial loss of productivity) (see Henshaw et al. 2000b; Kudô et al. 2005), studies so far have consistently shown high queen–queen relatedness relative to low worker–worker relatedness, thus supporting cyclical oligogyny. Therefore, a collective worker force appears to be an effective navigator for predicting the timing of the production of reproductives even in complicated polygynous colonies such as swarm-founding wasps.

4.3.2 Sex Ratio Variation Due to Factors Other Than a Relatedness Framework

Other than the sex ratio theory under a relatedness framework, two explanatory frameworks of individual sex ratio variation have been proposed: the ongoing queen–worker conflict hypothesis (Herbers 1984) and the resource availability hypothesis (Nonacs 1986). Herbers (1984) explained that the colony-level sex ratio

becomes parity as queen numbers increase because queens as a whole gain increasingly more power to control colony investment (see also Banschbach and Herbers 1996). On the other hand, Nonacs (1986) explained that a female-biased sex ratio in large colonies could be achieved by high resource availability due to higher worker numbers in such colonies. This theory predicts a male-biased sex ratio in small colonies because a female progeny becomes a worker (instead of a gyne) with relatively low resource acquisition (see also Rosenheim et al. 1996). Variation in resource availability also causes a split sex ratio in the presence of sex-biased local interactions (local resource competition, local mate competition, and local resource enhancement) (Frank 1987; Crozier and Pamilo 1996). Supporting evidence for this theory has been documented in a few ant species (Tsuji and Yamauchi 1994; Hasegawa and Yamauchi 1995). In *Formica exsecta*, a split sex ratio within colonies was observed in polygynous populations; however, the colonies producing only males did not have greater RA from the perspective of the adult workers that rear the brood (Brown and Keller 2000). Brown et al. (2002) proposed the queen-replenishment hypothesis, in which colonies produce gynes only when the queen number is so low that colony production of the brood is reduced or colony survival is threatened. These theories are also likely candidates for explaining a split sex ratio within populations. Recently, Kümmerli and Keller (2009) summarized four types of split sex ratios according to different combinations of factors determining colony kin structure, queen and worker control over the sex ratio, and the type of conflict between colony members.

4.3.3 Sex Ratio Variation in Eusocial Wasps

4.3.3.1 Relatedness Framework

Sex ratio studies of polistine wasps are relatively limited compared to those of ants. The main reason for this discrepancy is the lack of distinct morphological castes among wasps, which makes it difficult to divide females into workers and gynes. Three life-history strategies can create variation in RA among colonies of *Polistes*. First, an overwintered foundress generally constructs a nest either cooperatively or solely in the spring, generating variation among colonies, such as in monogyny and polygyny. In monogynous colonies workers should invest in a female-biased sex ratio due to high RA, whereas in polygynous colonies workers should invest in a male-biased sex ratio due to low RA (note that this prediction holds true only when foundresses are related, which is the case for most pleometrotic *Polistes*). Second, workers can mate with early males; and if these mated workers become replacement queens after the founding queen's death, the RA from the point of view of the workers changes. In other words, a colony headed by a replacement mated worker (parasocial colony) should invest in a male-biased sex ratio, whereas a colony headed by an original queen (eusocial colony) should invest in a female-biased sex ratio (Mueller 1991). Third, unmated worker reproduction decreases RA, and colonies

with more worker reproduction should invest in a male-biased sex ratio, whereas colonies with less worker reproduction should invest in a female-biased sex ratio.

Noonan (1978) reported that the population sex ratio of *P. fuscatus* was 1:1, which supports the queen control hypothesis. However, in single-foundress colonies, the sex ratio was slightly female-biased compared to that of multiple-foundress colonies. This trend does not contradict the RA hypothesis. Metcalf (1980) reported that sex ratios of both *P. metricus* and *Polistes variatus* were 1:1, supporting the hypothesis that the queen controls colony investment. All of these classical population sex ratio studies have indicated queen control of investment in *Polistes* colonies.

4.3.3.2 Production Schedule

In contrast to these classic studies, Strassmann (1984) observed a female-biased sex ratio of *Polistes exclamans* in years with low overall nest success due to bird predation, as the male production was likely to cease late in the season. She proposed the caste-plasticity hypothesis, in which females who will become workers or gynes are produced before males (protogynous production) whenever uncertainty exists in the timing of the production of reproductives. If unfavorable years shorten the production season of reproductives, the sex ratio becomes female biased. Suzuki (1986) pointed out that the sex ratio and production schedules of reproductives can be related (e.g., simultaneous production with an unbiased sex ratio, either protandrous production or protogynous production with a female-biased sex ratio or protogynous production with a female-biased sex ratio). In species with simultaneous production, observed sex ratios were 1:1, and worker reproduction was absent, suggesting that queen control over worker reproduction is effective. In species with protandrous production, in which males are produced before females, female-biased sex ratios were observed. This association can be explained within a sexual selection framework. That is, protandrous production favors a female-biased sex ratio when early reproductive females are of better quality than late ones (Bulmer 1983) or when the queen allows some worker control of investment while forcing workers to help rear her haploid eggs despite possible ongoing queen–worker conflict over male production (Bulmer 1981). In species with protogynous production, in which females are produced before males, female-biased sex ratios were observed in years during which colony cycles ended early (Strassmann 1984). Suzuki (1986) categorized 13 wasp species into the three production schedules mentioned above and pointed out that alternative explanatory frameworks other than relatedness context are also useful for considering sex ratio variation both from species to species and from colony to colony.

In *Polistes snelleni*, reproductives are produced protandrously, and cells of each nest are not reused. Therefore, the production sequence for each caste is easy to follow, and the precise sex ratio can be estimated based on the nests remaining at the end of the colony cycle. The sex ratios were dependent on colony size; a female-biased sex ratio was observed in large colonies (Inagawa et al. 2001). This trend could be explained by a power balance between the queen and workers; in large

colonies the collective worker force is strong and workers prefer a female-biased sex ratio, whereas in small colonies the queen has more power resulting in an even or a male-biased sex ratio. Alternatively, the colony-size dependence of the sex ratio could be explained by the resource availability hypothesis. Interestingly, mated workers were observed in queen-right colonies of *P. snelleni*, and they contributed to some female production in queen-less colonies (Suzuki 1985). If in some colonies mated workers succeed colonies after the death of the mother queen, variation in RA among colonies emerges, such that eusocial colonies with a mother–daughter relationship coexists with parasocial colonies that have a daughter (mated worker)–daughter (other worker) relationship (Mueller 1991; but see also Strassmann 1984). Further studies are needed to evaluate the genetic colony structures of *P. snelleni* and to determine how frequently mated workers become replacement queens and how many offspring such a worker can produce.

4.3.3.3 Worker Reproduction

In *Polistes chinensis antennalis*, frequent worker reproduction was observed even in queen-right colonies (Miyano 1980). Interestingly, early males are sometimes produced, but they lack reproductive potential, as they are diploids (Tsuchida et al. 2002, 2004). In monogynous and monandrous colonies, the relatedness among workers predicts that workers prefer to reproduce by themselves because the relatedness of a worker to her own sons is higher than that to her nephews and brothers. Tsuchida et al. (2003) studied sex ratio variation in this species for 3 years and found that large queen-right colonies invested more in males, whereas small queen-right colonies invested more in females. Population sex ratios were 1:1, suggesting queen control, even after allowing for male production by workers. Production schedules varied among colonies, such that larger colonies were protandrous and smaller colonies were protogynous. The queen in large colonies can produce her male haploid eggs early and can require her workers to rear them as well as giving partial allowance of reproduction to the workers, as predicted by Bulmer (1981). In small colonies, the queen may continue to lay diploid female eggs until relatively late in the season because females become both workers and gynes, resulting in delayed haploid egg production by the queen due to a lack of sufficient resources. In addition, worker reproduction was rarely observed in small colonies, suggesting that workers might refrain from ovipositing to avoid direct harsh conflict with the queen and/or as an expression of colony-level selection. Worker reproduction in small colonies should impose a larger cost than that in large colonies. Taken together, these results suggest that in *P. chinensis antennalis* factors other than relatedness (e.g., colony size, worker reproduction) are important determinants of the colony sex ratio.

As mentioned above, worker control of investment has never been confirmed in *Polistes* wasps, suggesting that the annual life cycle may limit the window for selection of the sex ratio based on variation in adaptive relatedness. In Vespinae wasps – which with the exception of *Provespa* are all believed to have an annual life cycle (Matsuura 1991) – current sex ratio data are severely lacking with a few exceptions

(e.g., Martin 1991, 1995). However, a split sex ratio likely occurs in vespid wasps in Japan (Takahashi, personal communication), a monogynous and monandrous species without relatedness variation among colonies. Recent theoretical work predicts that a split sex ratio is likely to evolve even in monogynous and monandrous species, which contradicts the original predictions under a relatedness framework (Ohtsuki and Tsuji 2009; Wiernasz and Cole 2009). These theories may help explain split sex ratios without polymorphisms in genetic colony structure.

In summary, conclusive evidence for worker control of the sex ratio in the primitively eusocial wasp *Polistes* is not likely to be found. The worker behaviors controlling sex ratios appear to be constrained by an annual life cycle and semelparity. Conversely, cyclical oligogyny, which is consistent with the collective sex ratio preferences of workers, has been observed in the advanced swarm-founding wasps (Strassmann et al. 1991, 1992; Gastreich et al. 1993; Queller et al. 1993; Hastings et al. 1998; Henshaw et al. 2000b; Tsuchida et al. 2000; Kudô et al. 2005). These results suggest that collective worker control is not active at the primitively eusocial stage with small colony size, and worker control might be facilitated with advancing eusocial stages involving increasing colony size and a perennial life cycle.

4.4 Conflict Over Male Production

4.4.1 Policing Theory

Other important aspects of social evolution include questions of why workers forego reproduction as well as how helping behavior (altruism) has evolved. Two explanations for why workers do not reproduce have been proposed. The first explanatory framework is the worker policing theory (Starr 1984; Woyciechowski and Lomnicki 1987; Ratnieks 1988). In monogynous and monandrous colonies, life-for-life relatedness of workers to their brothers, nephews, and their own sons is 0.25, 0.375, and 0.5, respectively (Fig. 4.5). If a queen mates doubly, the relatedness to her own sons and to her brothers is unchanged, whereas that to nephews becomes 0.25 [= $(n+2)/8n$, where n is the queen's mating frequency], the value of which decreases even more as the mating frequency of the queen increases. Therefore, the worker policing theory predicts that workers prefer to reproduce when the queen is singly mated unless worker reproduction imposes some extra cost on the colony and that workers kill nephew eggs when the queen mates more than twice. The second explanation involves the sexual deception hypothesis (Nonacs and Carlin 1990). This hypothesis assumes that a worker tries to destroy queen-laid male eggs and replace them with her own. Upon egg destruction, workers should discriminate queen-laid male eggs from queen-laid female eggs because the former ($r=0.25$) are less valuable to workers than the latter ($r=0.75$). If the queen conceals the sex of her offspring using a chemical substance, workers cannot discriminate the sex, and they refrain from destroying them to avoid the costly destruction of female eggs.

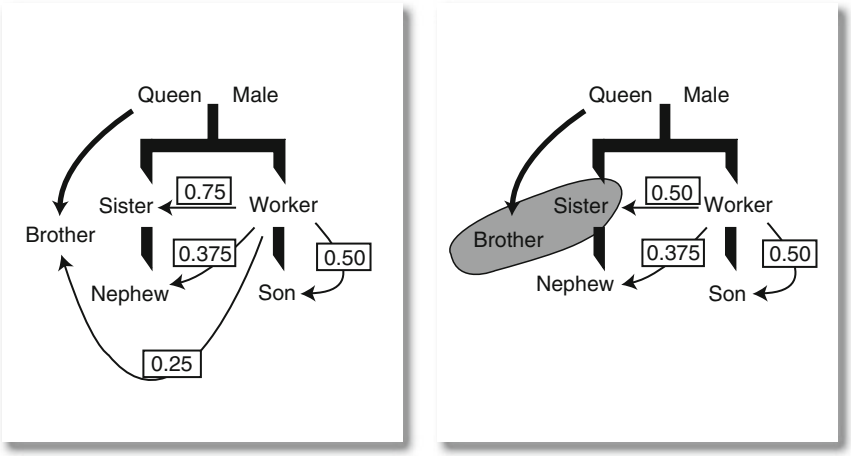

Fig. 4.5 Genetic relatedness (life-for-life relatedness) in a monogynous and monandrous colony. Life-for-life relatedness of workers to their brothers, nephews, and their own sons is 0.25, 0.375, and 0.5, respectively (*left panel*). If a queen mates doubly, the relatedness to her own sons and to her brothers is unchanged, whereas that to nephews becomes 0.25 [= $(n+2)/8n$, where n = queen mating frequency], the value of which decreases even more as the mating frequency of the queen increases. Therefore, the worker policing theory predicts that workers prefer to reproduce when the queen is singly mated unless worker reproduction imposes some extra cost on the colony and that workers kill nephew eggs when the queen mates more than twice. If a queen can chemically mask her sons and daughters (*right panel*), and workers cannot discriminate the sexual difference (*shaded area*) (sisters vs. brothers), workers refrain from destroying the queen's eggs and, instead, preferentially remove fellow workers' eggs

4.4.2 Positive Evidence for Policing Theory

Strong evidence supporting the worker policing theory has been reported for honeybees and a wasp species. Ratnieks and Visscher (1989) reported that worker-laid eggs are more swiftly removed than are queen-laid eggs in highly polyandrous honeybee colonies, which is consistent with the policing theory. Furthermore, in the facultatively polyandrous wasp *Dolichovespula saxonica*, the frequency of workers' sons was higher in singly-mated queen colonies than in multiply-mated queen colonies due to the frequent removal of worker-laid eggs in the latter colonies (Foster and Ratnieks 2000). However, the sample size of that study was small; a study of vespid wasps with a larger sample size revealed no effect of facultative differences in queen mating frequencies (Takahashi, personal communication).

Foster and Ratnieks (2001b) examined the relation between the queen's mating frequency and male production by workers in vespid wasps and found that, as predicted by the worker policing theory, male production by workers is uncommon in species with high queen mating frequency. The theory predicts that the frequencies of workers' sons are minimal in polygynous colonies with related queens. Consistent with this prediction, queens produced all of the males in swarm-founding

wasps (Hastings et al. 1998; Henshaw et al. 2000a). However, among the Vespinae, the predictions of Foster and Ratnieks (2001b) were not supported when new data from *Vespa* wasps were included (Martin et al. 2009).

Wenseleers et al. (2004a, b) analyzed the benefit of worker egg-laying using evolutionary stable strategy (ESS) models under queen-right and queen-less conditions and concluded that altruism based on relatedness alone (voluntary altruism) was insufficient to explain the suppression of worker reproduction. Instead, coercion by policing was more important, particularly among advanced eusocial insects (Wenseleers and Ratnieks 2006; Ratnieks and Wenseleers 2007; Ratnieks and Helanterä 2009).

4.4.3 Negative Evidence for Policing Theory

In contrast to the research discussed above, several studies have not supported the relatedness aspect of the worker policing theory. First, worker policing behavior has been observed in monogynous and monandrous colonies (Kikuta and Tsuji 1999; Iwanishi et al. 2003). Second, worker reproduction is absent in several monogynous and monandrous species (Arévalo et al. 1998; Walin et al. 1998; Foster et al. 2002; Takahashi et al. 2002, 2004a, b; Strassmann et al. 2003). Third, in contrast to the support for the policing theory observed in *D. saxonica*, facultative worker policing was not confirmed in a bumble bee, *Bombus hypnorum*, in which, similar to *D. saxonica*, mating frequencies of queens varied among colonies (Paxton et al. 2001). Fourth, using wider taxonomic groups than those of Foster and Ratnieks (2001a), Hammond and Keller (2004) showed that male parentage did not vary with relatedness, as predicted by the policing theory (see also Martin et al. 2009). They concluded that greater harmony and more complex regulation of reproduction exist in social insect colonies than would be expected from simple theoretical expectations based on relatedness alone.

Worker policing even in monogynous and monandrous wasps has become increasingly evident. Wenseleers et al. (2005a) reported that worker-laid eggs were policed by the queen in mostly monandrous colonies of *Vespula rufa*. Similarly, Wenseleers et al. (2005b) documented that worker-laid eggs were policed by both the queen and workers in monogynous and monandrous colonies of *Dolichovespula sylvestris*. In addition, queen-laid eggs survived better than worker-laid eggs, suggesting that the queen-laid eggs were likely marked with chemicals and could be identified. In *P. chinensis antennalis* most queen-laid eggs hatched successfully, whereas only a small proportion of worker-laid eggs did so. Worker-laid eggs were policed by both the queen and workers (Saigo and Tsuchida 2004). The queen oviposited both female and male eggs; therefore, the workers seemed not to discriminate the sex of queen-laid eggs. Analyses of CHC profiles on the egg surface revealed that the profiles differed between the eggs laid by queens and those laid by workers (Saigo and Tsuchida, unpublished data). The chemicals could also signal the fertility of the queen (honest signal). As with social insects (e.g., Peeters et al. 1999;

Endler et al. 2004; Martin et al. 2004), CHC and/or substances in Dufour's gland could serve as a fertility signal in *Polistes* wasps (Sledge et al. 2001, 2004; Dapporto et al. 2004a, 2005). In *P. fuscatus*, eggs experimentally rubbed with Dufour's extract from subordinate foundresses were destroyed by the dominant foundress, whereas eggs rubbed with a secretion from the dominant were not destroyed by the subordinate (Downing 1991).

4.4.4 Factors Other Than Relatedness That Regulate Worker Reproduction

Other than relatedness, two factors may help to explain worker policing in monogynous and monandrous insect societies: (1) colony efficiency (Ratnieks 1988) and (2) worker's male-egg removing concomitant with efficient sex ratio manipulation in favor of a worker optimum (Foster and Ratnieks 2001c). Tsuchida et al. (2003) found that the frequency of worker oviposition was positively correlated with colony size. This correlation could be interpreted to mean that worker reproduction in small colonies is relatively costly to the whole colony compared to that in large colonies, so the queen allows some reproduction by workers in large colonies. As mentioned above, workers among *Polistes* wasps seem not to manipulate the sex ratio effectively toward their optimum value. Therefore, the mechanism of Foster and Ratnieks (2001c) does not appear to play an important role in primitively eusocial wasps. It is noteworthy that in the three species (*D. sylvestris*, *P. chinensis antennalis*, *P. dominulus*) for which worker reproduction and worker and queen policing have been observed, workers appear to compete with each other upon ovipositing. In *D. sylvestris*, physical aggression to prevent egg-laying was directed toward workers that tried to oviposit. In *P. chinensis antennalis*, direct physical aggression was not observed, but worker-laid eggs, as a rule, were policed by the queen and other reproducing workers. In other words, policing workers are also reproducing workers. Among social insects, worker policing is generally defined as inhibition of the direct reproduction of other individuals by workers (Monnin and Ratnieks 2001). The above phenomena, as observed in annual Vespinae, fit this definition but do not quite match what Ratnieks (1988) originally called worker policing (i.e., mutual inhibition of worker reproduction in which workers that do not attempt to reproduce directly can hinder other workers in direct reproduction). In other words, the worker policing described by Ratnieks (1988) was a weakly spiteful behavior (as the actor cannot increase direct fitness), whereas those observed in *D. sylvestris* and *P. chinensis antennalis* were selfish behaviors. These lines of evidence strongly suggest that worker–worker competition for an oviposition site is another aspect of worker policing. Liebig et al. (2005) reported that in *P. dominulus*, the frequency of worker reproduction increased when some larvae were artificially removed. The authors suggested that workers laid eggs when they perceived a decline in queen power by means of an increment of empty cells that had been artificially introduced. In summary, in addition to colony size, mutual conflict among workers for

reproduction and individual direct assessment of queen fertility may play important roles in determining the magnitude of worker reproduction among annual wasps. Additional experimental studies are needed to evaluate the proximate factors governing worker reproduction in wasps.

4.5 Caste Differences Between Queens and Workers

4.5.1 Physiological Regulation of Caste Differences

4.5.1.1 Honeybees

Worker policing theory explains conflict and acquiescence between the queen and workers with regard to male production. Likewise, the theory motivates the fundamental question of how the queen and workers are physiologically differentiated. It is valuable to consider both proximate and ultimate mechanisms involved in regulating worker reproduction within a colony.

The most studied species with regard to physiological regulation of worker reproduction is the honeybee, *Apis mellifera*. The mandibular gland of the honeybee queen secretes queen mandibular gland pheromone (QMP), which signals queen presence and attracts workers. Recent studies have revealed that QMP suppresses the secretion of dopamine (DA), a biogenic amine, and JH, thereby impeding foraging behaviors (Pankiw et al. 1998; Beggs et al. 2007). In contrast, decreases in exposure to QMP are linked to the development of foraging behaviors via up-regulation of gene expression of foraging behavior (*Amfor*) and down-regulation of nurse behavior (Grozinger et al. 2003). Moreover, DA plays an important role in aversive learning (Beggs et al. 2007; Vergoz et al. 2007). Put simply, the age-dependent task shift from nurse to foraging is stimulated by a high JH titer, which exhibits a trade-off relation with the QMP effect. JH loses gonadotropic function in the honeybee queen but acquires a novel function of controlling the division of labor (Hartfelder 2000). Ovary development is stimulated by the egg yolk protein vitellogenine, which has an antioxidant function and extends the longevity of the queen (Corona et al. 2007). These results do not contradict the split function hypothesis under the ovarian ground plan (West-Eberhard 1996; see below).

4.5.1.2 Wasps

In contrast to honeybees, the JH titer functions to stimulate ovary development and behavioral dominance in primitively eusocial wasps belonging to *Polistes* and *Icariola* (Bohm 1972; Röseler et al. 1984; Agrahari and Gadagkar 2003; Sledge et al. 2004; Giray et al. 2005). Although a high JH titer elicits the advance of age polyethism (from in-nest tasks to outside tasks) in the highly eusocial swarm-founding wasp *Polybia occidentalis* (O'Donnell and Jeanne 1993) as in the honeybee, the

relation between JH titer and age polyethism in primitively eusocial wasps was not evident in *R. marginata* (Agrahari and Gadagkar 2003) but was confirmed in *Polistes canadensis* and *P. dominulus* (Giray et al. 2005; Shorter and Tibbetts 2009). The ovarian ground plan proposed by West-Eberhard (1996) explains that caste differences between the queen and workers in *Polistes* wasps can attain two extreme states, both of which exist in solitary wasps: One is in workers (ovary undeveloped), and the other is in the queen (ovary developed) and is triggered by differences in the JH titer or JH sensitivity. In *P. chinensis antennalis*, worker reproduction under queen-right conditions was positively associated with a high DA titer (Sasaki et al. 2007, 2009). The JH titer has not yet been measured, but high levels are likely associated with a high DA titer and could stimulate both ovarian development and the development of age polyethism. Further studies are warranted to clarify how JH and DA activities function in several age stages of primitively eusocial wasps.

4.5.1.3 Bumblebees

Similarly, in bumblebees, which are also primitively eusocial insects, JH still functions to stimulate gonadotropic activity (Bloch et al. 1996, 2000; Bloch and Hefetz 1999). In bumblebees, workers frequently reproduce under queen-right conditions at the late colony stage; and in some instances the queen is killed owing to conflict over reproduction with workers. Such reproducing workers have high JH titers and behave dominantly, which is a phenomenon similar to that seen in independent-founding *Polistes* wasps (Larrere and Couillaud 1993; Bloch et al. 1996, 2000; Bloch and Hefetz 1999). However, age-dependent age polyethism is independent of the JH titer (Cameron and Robinson 1990). On the other hand, body size-dependent polyethism has been observed in bumblebees, in which larger workers tend to forage more frequently than smaller ones (Goulson et al. 2002). Large workers have extended facet diameters in conjunction with reduced interommatidial angles. Thus, both overall sensitivity and image resolution are superior in such individuals, resulting in more efficient foraging in *Bombus terrestris* (Spaethe and Chittka 2003; Spaethe et al. 2007).

4.5.2 Gene Expression

Recent advances in molecular techniques, such as analyses of gene expression, can also provide new insights into JH and the development of the division of labor. An orthologous gene encoding for the cyclic guanosine monophosphate (cGMP)-dependent protein kinase G (PKG) family in the fruit fly (*foraging* gene in *Drosophila melanogaster*) has been found in honeybees (*Amfor*), and gene expression is higher in foraging workers than in nurse bees (Ben-Shahar et al. 2002). Interestingly, the expression of orthologous genes *foraging* and *Amfor* is low in foragers of the ant *Pogonomyrmex barbatus* and yellowjacket wasp *Vespula vulgaris* (Ingram et al. 2005; Tobback et al. 2008). Kodaira et al. (2009) reported that gene expression of *Bifor*, which is also an orthologous gene encoding for PKG, was

low in foragers of the bumblebee *Bombus ignitus*. Put simply, the relation between gene expression of the PKG-encoding gene and the development of foraging behavior in honeybees is the reverse of the relation for the three other eusocial insects. Future analyses of gene expression in several eusocial stages as well as the solitary stage could reveal the causal genes for social evolution.

4.5.3 Worker Totipotency

As mentioned previously, JH and CHC play important roles in the determination of dominance in foundress groups of wasps. In *P. dominulus*, queen removal after worker emergence results in a CHC profile of the replacement queen similar to that of the original queen (Dapporto et al. 2005). Thus, the daughter worker is implicitly believed to possess a totipotency to function as a queen.

We analyzed differences in CHC profiles between the original queen and reproducing workers under queen-right conditions in *P. chinensis antennalis*. The CHC profiles of queens apparently differed from those of reproducing workers, and differences between the two castes were observed on the egg surface as well as in Dufour's gland (Saigo and Tsuchida, unpublished data), suggesting that such differences serve as a policing mechanism, whereby the eggs oviposited by workers are selectively killed by both the queen and reproducing workers (Saigo and Tsuchida 2004). The CHC profiles of new gynes before hibernation were similar to those of nonreproducing workers, and a few constituents of the CHC were lacking in queens. The CHC profiles of reproducing workers differed from those of nonreproducing workers, indicating that the CHC profiles in workers reflect their reproductive ability but do not become similar to those of queens.

On the other hand, a portion of daughter workers became mated under queen-right conditions in *P. snelleni* (Suzuki 1997), and these mated workers can produce diploid offspring. We compared the CHC profiles of the queen, nonreproducing workers, and reproducing workers (Yamasaki and Tsuchida, unpublished data), and the results indicated that the CHC profiles differed significantly among the three groups.

In *P. dominulus*, a peptide in the cuticular surface differed between the queen and workers, and this difference was maintained when the original queen was removed and the workers were induced to reproduce (Dapporto et al. 2008). These results indicate that the queen and workers in *Polistes* wasps qualitatively differ and that mating is an obligatory prerequisite for the ontogeny of a fertile signal.

4.6 Conclusions

Published works concerning conflict resolution in *Polistes* wasps are summarized in this chapter in light of kin selection frameworks, with particular regard to foundress groups, sex ratio, and male parentage. Nepotistic group forming was not

evident among foundress associations. Although theoretical work has adequately explained sex ratio variation both at the population and colony levels in some eusocial species, most of the observed sex ratio variation in eusocial wasps has not been consistent with theoretical predictions, with certain exceptions in swarm-founding wasps. The observed frequencies of worker reproduction also have not always matched those predicted from the policing theory in a relatedness framework. Overall, the results indicate that kin selection has limited power to explain adaptive conflict resolution among nest-mates in *Polistes* wasps in all three areas (foundress groups, sex ratio, male parentage). Studies of proximate mechanisms determining caste differences in *Polistes* wasps have revealed that mating could be an obligatory prerequisite for generating such caste differences.

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Part II
Evolutionary Bases and Practical Implications of Animal Personality and Temperament

Chapter 5
How to Measure Animal Personality and Why Does It Matter? Integrating the Psychological and Biological Approaches to Animal Personality

Sonja E. Koski

5.1 Introduction: What Is Animal Personality?

During the last few years individual differences in nonhuman animal (hereafter "animal") behavior have been a subject of rapidly growing research interest (reviews in Réale et al. 2007; Sih and Bell 2008). This has met the much older research tradition of personality psychology, which includes human and, more recently, animal personality (Gosling 2001). Individual differences in behavior and their underlying psychology are now increasingly relevant research fields in several species of animals.

Individuals in many species, from invertebrates to lizards, fish, birds, and mammals, differ in their behavior from each other. This variation is often temporally consistent, meaning that an individual's general behavioral tendency stays similar over time (Sih et al. 2004b). Behavioral tendencies generalize to some extent across situations, so an individual shows limited plasticity in its responses (Sih et al. 2004a, b). Behavioral tendencies are heritable (van Oers et al. 2005), have significant fitness consequences (Smith and Blumstein 2008), and may be organized hierarchically so multiple traits correlate to form higher organizational levels (Réale et al. 2007; Sih and Bell 2008). Consistent behavioral variation can be named "personality," following the human personality psychology research tradition. Also, other terms (e.g., temperament, coping style, behavioral syndrome) have been applied to consistent interindividual behavioral variation, each having its own particular connotation (Réale et al. 2007; Sih and Bell 2008). In this chapter, however, I treat them as synonyms and define personality as consistent interindividual variation in behavior. With this definition, I take no stand regarding the proximate-level mechanisms, including psychological ones, underpinning behavior.

Consistent variation in behavior is evolutionarily puzzling because natural selection would be expected to winnow out any variation that has fitness consequences, so

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that over time the optimally adaptive level of a behavioral trait would be the prevailing phenotype in a population. For example, if bold individuals locate food faster than shy individuals, bolder individuals could be expected to have higher fitness and thus, in time, boldness should be favored over shyness. Consistency in behavior is also challenging to understand because it limits an individual's flexibility to adjust its behavior to deal with a situation in an optimal way. Moreover, behavioral variation often occurs in suites of correlated behaviors; for example, individuals that are relatively bolder are also relatively more aggressive (Sih and Bell 2008), evoking questions about why such covariation should exist.

The booming research on animal personality applies various approaches and methodologies. There has been much debate over how to best assess animal personalities (e.g., Gosling and Vazire 2002; Itoh 2002; Réale et al. 2007; Vazire et al. 2007; Uher and Asendorpf 2008). In this chapter, two fields of animal personality research are discussed – psychological and biological – that drastically differ from each other in their approaches. However, despite the seemingly fundamental differences, common ground can be found. Although their particular paradigms may differ, the aims of the two approaches are in the end similar: to map the depth and width of individual differences in behavior; to understand the structure of this variation; to understand its evolutionary consequences and underlying mechanisms; and to facilitate predictions, which in turn are helpful in a broad range of applications. Indeed, several researchers have stressed the benefits of such integration (Gosling 2001; Nettle 2006; Sih and Bell 2008; Uher 2008a; Brosnan et al. 2009). To achieve integration and synergy, we need to understand each other's approaches and their conceptual and practical consequences. Therefore, I discuss some of the conceptual, methodological, and practical issues that have been put forward in the biological and psychological animal personality literature in recent years. I propose that with increased methodological care and clarity in reporting, the two fields can benefit one another. Thereafter, I highlight some areas of research in which common ground can be found and suggest prospects for future animal personality research.

5.2 Two Approaches to Animal Personality

Animal personality research is roughly dichotomized between the human-oriented personality psychological tradition and the animal-oriented biological tradition. The "psychological" and "biological" approaches to personality differ at conceptual, methodological, and practical levels.

The *psychological approach* is adopted by comparative personality psychologists who apply the well-established human personality theory and methodology to animal personality research. The aim is to understand similarities and differences in human and animal personality regarding the structure, underlying neuropsychological mechanisms, and evolutionary history. In humans, personality is understood as a psychological construct that influences behavior and is organized in a hierarchical structure (Maltby et al. 2007; see also, e.g., Allport 1961; Fast and Funder 2008 for

various definitions). According to the widely accepted Five-Factor Model (FFM), human personality consists of five stable superordinate domains or constructs labeled Extraversion, Openness, Conscientiousness, Neuroticism, and Agreeableness, each of which includes a number of subordinate facets (Costa and McCrae 1992; McCrae and Costa 2008; but see Eysenck 1991, 1992; Ashton and Lee 2007 for alternative models). Personality dispositions are heritable (Bouchard and Loehlin 2001) and associated with important life outcomes, such as subjective well-being, health, mortality, mating success, quality of social relationships, occupational performance, psychopathology, and the likelihood of serious injuries (Franken et al. 1990; Neeleman et al. 2002; Nettle 2005; Ozer and Benet-Martinez 2006; Roberts et al. 2007). Although traditionally the evolutionary significance and underlying mechanisms of human personality have received limited attention (Nettle 2006, 2008), advances have been made in recent years (e.g., personality genetics: Bouchard and Loehlin 2001; Ebstein 2006; Penke et al. 2007; brain substrates: Gardini et al. 2009; evolutionary significance: Nettle 2005, 2006; Smith and Blumstein 2008).

Some personality psychologists have become interested in comparative personality research and have described animal personality within the framework of human personality psychology (e.g., Gosling and Vazire 2002; Weiss et al. 2007; King et al. 2008). This has wide-ranging benefits for personality psychology research, such as clarifying the phylogenetic history of personality, as well as the effects of genetic dispositions, development, and environment that are challenging to tackle in research on humans alone (Gosling 2001; Gosling and Greybeal 2007). The concept of personality as a hierarchical psychological construct is extended to animal personality, with the expectation that animal personality exhibits a similar structural organization (e.g., King and Figueredo 1997). The framework of the FFM has been taken as the starting point, and the methods are adopted from those used in human personality research.

In human personality research, the most common method of obtaining data is self-rating, in which people evaluate themselves on lists of descriptive terms ("items"). However, assessment by knowledgeable informants (e.g., peers, parents, teachers) is also accepted and widely used (Boyle et al. 2008); and self- and other-rating can also be used in conjunction (e.g., Fast and Funder 2008). Assessing animals by knowledgeable informants (e.g., animal care-takers) is considered as a logical continuation of these methods (King and Figueredo 1997; Gosling and Vazire 2002). Thus, people rate animals on questionnaires that list descriptor items, which can be adjectives or behavioral descriptions, such as "curious" or "subject often touches new objects at great length" (Uher and Asendorpf 2008). So long as certain criteria – most importantly interrater reliability and construct validity (see definitions below) – are fulfilled, the results are considered to reflect subjects' personality traits (Gosling and Vazire 2002; Vazire et al. 2007).

This work has revealed that personality of animals can successfully be characterized within the FFM framework (Gosling and John 1999; Gosling 2001; Capitanio and Widaman 2005). Personality constructs of animals vary in their degree of similarity to the human FFM, from highly similar (e.g., neuroticism in orangutans) (Weiss et al. 2006) to very different (e.g., dominance in chimpanzees) (King and

Figueredo 1997). This work has also led to further studies on heritability and cross-population consistency of ape personality and on apes' subjective well-being (Weiss et al. 2000, 2002, 2006, 2007, 2009).

The *biological approach*, as practiced by behavioral biologists, aims at finding out the mechanisms underlying, and the evolutionary forces maintaining, variation in personality traits. This approach builds on the traditions of ethology, behavioral biology, and evolutionary and theoretical ecology. Therefore, it relies on quantifying outward behavior. Whereas individual differences in animal behavior have been recognized as long as people have systematically observed animals (e.g., Yerkes 1939; Pavlov 1951; Stevenson-Hinde et al. 1980), in biological research variation was long considered the raw material for natural selection to act upon, rather than being adaptive in and of itself (e.g., Dall et al. 2004; Sih et al. 2004a, b; van Oers et al. 2005).

Variation around the assumed optimal mean was thought of as "noise" (Wilson 1998), and behavioral research aimed to find these optimal means for species, age, and sex categories. However, following increased interest in individual-based approaches and improved analytical methods, research has shown "noise" to be actively maintained by evolutionary processes (e.g., Dall et al. 2004; Wolf et al. 2007; Garamszegi et al. 2008; McNamara et al. 2009). This realization has led to efforts to quantify individual variation in behavioral traits. Usually behavioral personality research makes use of experimental testing, in which variation in a trait can be quantified by subjecting individuals to varying conditions or stimuli, such as a novel environment or a predator model (Réale et al. 2007). In addition, some biological personality research uses behavioral observations in natural or captive circumstances without experimental manipulation (Anestis 2005). Experimental and nonexperimental observations of behavior are coded to yield quantitative data on behavioral frequencies. This research has shown that in many species particular traits (e.g., aggressiveness, exploratory tendency, boldness, general activity) vary consistently among individuals (Sih et al. 2004b; Réale et al. 2007). Consistent variation is suggested to be maintained by frequency-dependent selection, mutation-selection balance, spatiotemporal variation in environmental conditions, and trade-offs between alternative strategies (Dall et al. 2004; Dingemanse et al. 2004; Wolf et al. 2007; Sih and Bell 2008; McNamara et al. 2009).

Largely, research on the evolutionary mechanisms of personality is still in its infancy, and much more additional theoretical and empirical work is needed to clarify the observed patterns in variation and consistency. What is clear, however, is that these findings have brought consistent individual differences into the focus of behavioral research. Recognition of the relevance of an individual as an explanatory level has large repercussions for studies on, for example, theoretical ecology, learning, cognition, mating behavior, and cooperation (Dall et al. 2004; Sih and Bell 2008; McNamara et al. 2009). Moreover, the concept of behavioral syndromes (i.e., consistently correlated behavioral traits) draws attention to limited plasticity in behavior, carry-over effects, and connections between behavioral traits that have traditionally not been studied together (Sih et al. 2004a, b; Sih and Bell 2008; Smith and Blumstein 2008).

The psychological and biological approaches have, at the outset, little in common. The gulf is further widened by the tradition of publishing in separate journals and attending different conferences. Consequently, the results are not easily comparable, and the advances in research in the respective fields often remain unrecognized by researchers taking different approaches. However, these approaches need not be so far apart.

5.3 Concepts in Personality Research: A Matter of Definitions and Analytical Levels

Conceptualization of personality in research is fundamentally a question of definitions and of the level of analysis. Nevertheless, it is by no means a trivial issue. Recently, Uher (2008a, b) highlighted it as one of the three critical issues of comparative personality research: how to conceptualize, identify the domains of, and measure individual variation.

The psychological and biological approaches to personality differ in their conceptualizations of personality. In the psychological tradition, the definition of personality allows multiple levels (psychological, situational, and behavioral) (Funder 2006). Conceptually, the trait hierarchy is emphasized, which demands knowledge of multiple traits and their relative externalizations. Personality is seen as a complex, hierarchical structure of narrow trait dimensions nested within broader trait dimensions. On the other hand, personality defined biologically, as consistent interindividual variation at the level of behavior, tends to ignore psychological influences and the hierarchical structure of traits (although the concept of behavioral syndromes is close to the concept of hierarchical structure in personality psychology) (Sih and Bell 2008; see also below). Although mechanisms are examined, they are not part of the definition of personality. Fundamentally, however, the differences between psychological and biological conceptualizations are minor, as both agree that personality describes stable interindividual variability in traits within a population (Sih and Bell 2008; Uher 2008a). The necessary criteria, consistency within, and variation between individuals can be assessed at any trait organizational level, including psychological dispositions (see discussions on trait organization in, e.g., Réale et al. 2007; Uher 2008a).

More problematic conceptual issues arise in comparative personality research, as species' biology strongly determines their behavior; consequently, comparing particular behavioral traits may lead to a "comparing apples with oranges" problem (cf. Gosling 2001). For example, an antelope's vigilance and easily triggered escape response may give an impression of a "shy" species if compared to a lion and a hornet's tendency to attack may give the impression of an "aggressive" species if compared to a sheep. However, the antelope's "shyness" and the hornet's "aggressiveness" result from particular ecological selection pressures leading to species-typical behavior. Within these species, aggressiveness and shyness can be characterized as personality traits if they exhibit greater between- than

within-individual variation that is sufficiently stable in a population. How, then, are we to compare the aggressiveness of hornets and sheep? Uher (2008a, b) proposed that we borrow conceptualizations for comparative personality research from cross-cultural personality psychology. That is, we should aim at identifying population-specific personality traits, weak universal personality traits, and strong universal personality traits. Population-specific personality traits are specific to a given species and thus cannot be compared across species. Universal traits are those that show consistent variation across species and are therefore comparable; strong universals are those that show significant differences between species' mean and variance of the trait distribution, and weak universals are those in which the mean and variance of the trait distribution are the same across species. When trait distributions differ among species, a mathematical standardization of the scores is necessary to allow comparisons.

The shyness–boldness continuum has been proposed as a universal trait due to substantial evidence of its existence in several species, including humans (Beaton et al. 2008; Sih and Bell 2008), but little is known of its trait value means and distributions across species. Uher's (2008a) framework is applicable to comparative research (but see Realo and Allik 2008). However, the framework puts little emphasis on how differences arise as a consequence of proximate determinants and evolutionary selection pressures. Even if a trait, such as boldness or aggressiveness, exhibits interindividual variation in a broad range of species, the proximate mechanisms may be different in different species. Moreover, the mean and variance of the trait distribution are likely to be strongly affected by species' ecology; consequently, particular traits are subjected to different selection pressures in different species. Therefore, in comparative research, identification of variation in a trait should ideally include determining proximate-level mechanisms and accounting for the species ecology and the selection pressures acting on the trait in the target species (Réale et al. 2007).

5.4 Methodologies in Personality Research

5.4.1 How Are Candidate Personality Traits Selected, Extracted, and Analyzed?

The methodological core issues concern how to (1) identify and (2) measure the domains of behavioral variation (cf. Uher 2008b). Identification, or selection, of candidate personality traits refers to the a priori process of deciding which traits are sampled as potentially personality-relevant ones. First, the definition and hierarchical level of a candidate trait are to be decided. One problem lies in the fact that the biological and psychological definitions of the term "trait" differ: Biologists understand a trait to be any (quantifiable) characteristic (see discussion of the term "characteristic" by Wagner 2001), whereas psychologists apply the term to internal

dispositions that influence behavior (Larsen and Buss 2005). Throughout this chapter, the term trait is used with its biological meaning and is limited to behavioral characteristics, following my focus on behavioral variation. Clarifying the definition of trait would reduce misunderstandings between the behavioral and psychological personality literature (see recent discussion of confusion about terminology by Carere and Maestripieri 2008; Uher 2008a, b; van Oers 2008).

The hierarchical level of the candidate trait is also relevant in the selection process. For example, maternal behavior is a composite trait consisting of nursing, carrying, protecting, grooming, and so on. These behaviors could be defined as individual traits or as measures of one composite trait. Each trait level is structurally connected to others below and above it (Réale et al. 2007). It depends on the question and the scale of the research on which organizational level the candidate traits are chosen. For example, if one is interested in behavioral syndromes, selection of candidates should include several lower-level traits that are examined for their interdependence, whereas if the goal is to identify fitness consequences of a particular trait, starting from a higher hierarchical level may prove more useful.

Uher (2008a) has summarized various approaches to selecting candidate traits. A "nomination approach" relies on the human ability to choose appropriate traits based on our perception of variation in animals, which allows a researcher to name the candidate traits. The better the species' behavior is known, the more likely meaningfully varying behaviors are selected. An "adaptive approach" assesses the trait's biological relevance in ecology or evolution of the species, so traits with the most significant fitness consequences in the past or present are assessed. A "top-down approach" takes personality traits found in other species and seeks similarities and differences in the target species. It can select a singular trait or base the selection on a broader hierarchical model of personality. Finally, a "bottom-up" approach starts from the study species and identifies candidates from its behavior or underlying mechanisms (see Uher 2008a, b for a thorough discussion).

Although this is an insightful categorization of the candidate trait selection approaches, some of these approaches are in practice close to each other and used in combination. For example, exploratory tendency and aggressiveness have been found to influence individual's fitness in great tits (*Parus major*: Dingemanse et al. 2004), and consequently other studies have examined variation in these traits in other species (e.g., mouse lemur *Microcebus murinus*: Dammhahn 2009; dog *Canis familiaris*: Svartberg et al. 2005; guppy *Poecilia reticulata*: Burns 2008; starling *Sturnus vulgaris*: Minderman et al. 2009; collared flycatcher *Ficedula albicollis*: Garamszegi et al. 2008). Selecting exploration tendency as a candidate trait in starlings combines the adaptive approach and the top-down approach and is possibly also influenced by knowledge that such behavior is part of the species' behavioral repertoire thus adding the nomination approach to the list. Also, Weiss and Adams (2008) have pointed out that whereas rating animals on an adjective descriptor item list (for which the items were derived from the human personality model) utilizes personality traits of another species by the top-down approach, the item descriptions are often adjusted to each species' particular behavioral repertoire, thus combining the top-down, bottom-up, and nomination approaches.

I combine and simplify Uher's (2008a) classification as mutually nonexclusive "know your species," "traits relevant in other species," and "compare to humans" approaches to candidate trait selection. The know your species approach relies on a broad knowledge base of the species' behavioral repertoire, socioecology, life history, and evolutionary history. When species' behavior has been sampled over years, in some cases decades, covering multiple individuals in multiple contexts and multiple populations, I consider it justified to select a range of naturally occurring behaviors as personality trait candidates. The benefit of this approach is that the selected candidate traits are likely to be ecologically relevant and part of the natural behavioral repertoire of the species. The drawback is a potential danger of selecting more easily accessible traits at the expense of rare or less conspicuous traits. The traits relevant in other species approach is an exploratory approach to test specific traits that have shown significance as personality traits in other species, either closely or more distantly related. The benefits of this approach are that it allows an assessment of the trait's phylogenetic history and mapping of the trait's generality across species (Gosling and Greybeal 2007). Also, it provides a time-saving shortcut to personality of a previously unstudied species. The most obvious drawback is that it may fail to account for traits relevant for the particular study species. Finally, the compare to humans approach seeks to identify those personality traits in animals that are known in humans, putting the focus on human–nonhuman similarities and differences. The drawback is that it may exclude traits that are absent in humans but biologically relevant for the target species (Uher 2008b; Uher and Asendorpf 2008; cf. Gosling and John 1999; Gosling 2001).

Based on ecological relevance and generality across species (i.e., know your species and traits relevant in other species approaches), Réale et al. (2007) nominated five categories for candidate personality traits in animals: shyness–boldness, exploration–avoidance, activity, aggressiveness, and sociability (see also Bell 2007). This proposition does not specify the genetic or neurophysiological mechanisms nor on which structural level at which the trait should be measured. These trait categories are likely to be ecologically and evolutionarily relevant regardless of the species' particular ecological conditions (Sih and Bell 2008). A possible drawback of keeping to these five categories is that it may limit research efforts to only these trait categories at the expense of other candidates. Moreover, in many species, some of these trait categories are correlated, suggesting structural dispositions among them (Sih and Bell 2008). Therefore, the five trait categories should not be presupposed to be independent.

All the aforementioned approaches have their own pros, cons, and justifications depending on the particular goal of the research. A common drawback in the outlined selection processes concerns the structural analysis. Understanding the structural hierarchy of personality traits is relevant because it clarifies the connections between traits and directs us to the mechanisms underlying these connections (Réale et al. 2007; Sih and Bell 2008). However, all selection processes necessarily limit structural analysis to the assessed traits only and may neglect other traits that are potentially more biologically significant for a given species. The traits relevant in other species approach identifies variation often only in one or two candidate traits

(e.g., boldness or aggressiveness) and disregards the structural organization altogether. The know your species approach may nominate easily observable traits in the target species' repertoire at the expense of less easily identified traits, again leading to a potentially incomplete structure. The compare to humans approach examines animals for those traits that are relevant for the human personality model, potentially biasing the structure toward that of humans. Furthermore, we must acknowledge that none of the selection methods produces an all-inclusive list of personality traits in any given species. For example, if boldness is identified as a personality trait in great tits, we cannot argue that we have found all there is to be found in great tit personality. Similarly, if human personality traits are used as a template to identify personality in chimpanzees, and subsequently some or all of these traits are shown to exhibit consistent variation in chimpanzees, it does not mean that chimpanzee personality only includes variation in those traits. So long as researchers are aware of the drawbacks and state clearly the reasons for and methods of their selection of candidate traits, comparisons with other studies are possible.

The second methodological concern is how the chosen traits are measured. The biological approach relies on coding expressed behavior. Biologists observe animals and extract quantitative data from these observations. The situation may be experimentally induced, or a trait may be coded in nonmanipulated living conditions in the wild or captivity. Once the candidate traits are chosen, appropriate paradigms for measuring the target trait are designed, and standard observational techniques are applied. For example, to test exploration tendency, an animal is put into a novel environment and its response is measured by, for example, latency to visit a particular part of the novel environment (Verbeek et al. 1994). To test boldness–shyness, a novel object is introduced into the environment, and the subject's latency to approach the object is recorded (Wilson et al. 1993). These tests are repeated over time for each subject. Data are analyzed for within- and between-subject variation and temporal consistency. In observations of nonexperimental situations, behavioral data of candidate traits are collected over a significantly longer period of time and across several contexts. The most common methods involve focal and scan sampling (e.g., Capitanio 1999; Uher et al. 2008). Precautions must be taken to avoid bias to particular individuals or to variation in behavioral patterns due to circadian rhythm and care-taking events by randomizing the order of focal individuals and observing every animal repeatedly at different times of the day and in several contexts, all of which are standard practices in behavioral research (Martin and Bateson 1993). To ensure that the sample is representative of real behavior, a sufficient amount of data from each individual and a rigorous observational technique are key criteria. Nevertheless, in nonexperimental settings, it may be difficult to obtain data for a particular trait as it occurs in nonsystematized contexts and is likely to be influenced by other factors than those we are interested in studying (Réale et al. 2007). On the other hand, observing behavior as it occurs without experimental stimulation avoids the problem of artificial or ecologically irrelevant situations.

The psychological approach relies on knowledgeable people rating the study subjects. The item lists consist of trait-relevant descriptors sometimes accompanied

by explanatory descriptions of the terms (e.g., Weiss et al. 2007). For example, the item "sympathetic" is given with an explanation "(s)ubject seems to be considerate and kind toward others as if sharing their feelings or trying to provide reassurance" (Weiss et al. 2006). Subjects' item scores are analyzed for interrater reliability, which refers to agreement among raters in their assessment of a particular individual. The data are then subjected to data reduction methods (usually factor analysis or principal components analysis), which yield information on multiple traits and their taxonomic structure. The rating method relies on people's intuitive ability to mentally collate and hold information of an animal's characteristics in meaningful categories (Gosling and Vazire 2002; Uher 2008a). The benefit of this method is its practicality, as large numbers of animals can be sampled in a short time. In addition, cross-situational consistency and reliability are argued to be superior in the rating to that of the behavioral coding method because ratings incorporate information over time and contexts, whereas behavioral codings are necessarily more limited for time and are vulnerable to the influence of context (Vazire et al. 2007; see also below). However, the potential drawbacks of rating include unclear correspondence with behavior, unclear ecological relevance of rated items, and the ever-looming possibility of anthropomorphic projections (see below).

5.4.2 Diagnostic Criteria: Validity, Reliability, Repeatability

In Gosling's (2001) comprehensive review on animal personality encompassing a broad range of species, more than 70% of animal personality research had relied on coding observed behavior. The rating method, however, was proportionately more often (42%) favored in studies on primates and domesticated animals. This reflects one of the key differences between the coding and rating methods: Rating animals is possible only when we can mentally represent an animal's behavioral characteristics as impressions that translate to the descriptor items. This is likely to be easier when we find the behavior intuitively "understandable," which is more probable in closely related species and species with which we have associated for a considerable part of human evolution (Gosling et al. 2003; Weiss and Adams 2008; Uher 2008b). It is thought that we hold less-clear representations of the behavior of animals that are taxonomically more distant from us or otherwise less familiar, and thus rating these animals with descriptive items enhances the risk of interpretational errors. Coding behavior, in contrast, is possible with any organism so long as there is an a priori agreement of what exactly is being coded (i.e., the definition of the behavior is clear).

The danger of anthropomorphizing animal behavior is present when we rate animals based on intuitive impressions and presumably more so when items are derived from human personality theory. The issue of anthropomorphism has been hotly debated in the animal personality literature, and it is beyond the scope of this chapter to summarize all of the arguments (e.g., Gosling and John 1999; Gosling and Vazire 2002; Itoh 2002; Réale et al. 2007; King et al. 2008; Uher 2008a, b). In

behavioral research, evaluating animals based on impressions of qualities labeled by human terms without quantified information of the corresponding behavior and its generality and frequency of occurrence is considered dubious. In contrast, the psychological research tradition puts little emphasis on behavioral frequency. Instead, the emphasis is in the psychometric properties of the emerging personality scores, which, when acceptable, are considered to reduce the likelihood of anthropomorphism. However, personality psychology is also well aware of the relevance of construct validity (i.e., the extent to which ratings reflect the corresponding real-life behavior and other corresponding and meaningful outcomes). Note that in this chapter the term "validity" is used as construct validity, and not face or concurrent validity, and convergent and discriminate validity is not separated – cf. Maltby et al. 2007. Construct validation is necessary to ensure that rated personality traits are reflected in quantifiable differences within the species' behavioral repertoire (e.g., Gosling 2001; Uher and Asendorpf 2008). Yet, thus far, behavioral validation of ratings is not the standard procedure (Gosling 2001).

Animal personality studies that have assessed construct validity have generally reported high correspondence between ratings and observed behavior (Feaver et al. 1986; Pederson et al. 2005; Konečná et al. 2008). In a study on rhesus macaques (*Macaca mulatta*), behavioral scores correlated moderately with the corresponding rated scores (Capitanio 1999). Moderate to high correspondence was also found in two studies on captive chimpanzees (*Pan troglodytes*) (Pederson et al. 2005; Vazire et al. 2007); but in another study (Uher et al. 2008) where behavioral data were obtained both experimentally and nonexperimentally, the correspondence between rated adjective-item data and coded behavioral data was low. Sometimes the obtained validity results are difficult to evaluate owing to biological or methodological challenges. In a thorough study on wild-ranging male Hanuman langurs (*Semnopithecus entellus*), ratings corresponded well to measured behavior, and the principal components analysis (PCA)-derived personality dimensions obtained by behavior coding and ratings agreed with each other (Konečná et al. 2008). However, coded and rated scores were influenced by male rank, which in langurs is labile (i.e., the rank position changes during the lifetime) (Borries 1997). This evokes a question regarding to what extent currently observed behavior is consequent of (unstable) rank position rather than personality. Furthermore, how do personality and rank position interact? As short-term studies are snapshots in time, the causal relations between rank position, behavior, and personality are difficult to assess. A methodological issue is the independence of rating and behavior coding; to truly test the construct validity of ratings, the behavioral data should be obtained independently (i.e., by different people), but this has not always been the case (e.g., Feaver et al. 1986; Capitanio and Widaman 2005; Konečná et al. 2008). Validations have also been done by rating observed behavior, rather than quantifying frequency, duration, and so on of the target behaviors (Gosling et al. 2003), and have been based on limited sampling efforts (Vazire et al. 2007). Finally, researchers may rely on the assumption that if someone has validated (some of) the personality traits in the same species (even if in a different population), it is unnecessary to perform behavioral coding (e.g., Weiss et al. 2002; King et al. 2008). Although cross-population

rating studies give converging results supporting the generality of personality (King et al. 2005; Weiss et al. 2007, 2009), the actual behavioral frequencies may well differ among populations. In sum, the importance of rigorous behavioral validation cannot be overemphasized. As a standard procedure, it would provide data with a common metric (i.e., occurrences and frequencies of strictly defined behavioral traits) that not only would facilitate comparisons across populations (and potentially across species), thus complementing data obtained by ratings, but also comparisons with behavioral personality studies. By including quantified behavioral measures of rated personality, it is possible to assess directly the similarities with studies conducted by behavioral coding.

In contrast to ratings, the problem of construct validity is less of an issue in behavioral coding as the actual behavior is quantified. Behavioral research is not free from validation challenges, as there is always an a priori expectation of the correspondence between the target personality trait (e.g., shyness) and the behavior (e.g., latency to approach an object), which may or may not be a correct assumption. To ensure construct validity in behavioral research, knowledge of the species' behavioral repertoire and the functions of measured behaviors are imperative. Furthermore, some researchers (Vazire et al. 2007) have raised a concern that observers may interpret behavior wrongly (e.g., code submissive behavior as play), which would undermine the value of behavioral coding. Of course, as in any behavioral research, coders must be trained well to be familiar with a species' behavior so they recognize the traits they code and record the observations reliably (see below for discussion on reliability). If knowledge of species' behavior and functions of target traits, as well as coders' sufficient training in recognizing and obtaining behavioral data are ensured, I consider construct validity of behavior coding to be, by default, high.

Biologists stress the importance of another kind of validity, namely ecological validity (Réale et al. 2007; Burns 2008). That is, the test design and the behavioral measures should be ecologically relevant for the species. For example, a novel object that an animal reacts to is likely to be different for a bird than for a fish. Furthermore, response in a test should translate into responses in the corresponding real-life situation. In a recent study, mouse lemurs exhibited personality variation in standard novel object and open environment tests designed to assess variation in boldness and exploration tendencies (Dammhahn 2009). However, when the same animals were tested in a realistic situation posing varying degrees of risk – feeding on the ground (risky situation) versus feeding on a higher platform (safe situation) – the responses were not attributable to the measured personality differences. The author hypothesized that exploration tendency and boldness do not influence the survival component of fitness in this species. Alternatively, the test situation may not tap into personality differences exhibited in the real-life situation, thus illustrating the potentially low ecological relevance of the standard tests for this species. To ensure salience of the test situation, experimental setups should account for species' ecology and natural behavior. In addition, traits ideally are assessed by several tests on the same trait (Burns 2008). In nonexperimental studies, ecological validity is vulnerable to the effects of context (Weiss and Adams 2008), which can

be overcome by sufficient sampling efforts. In rating studies, the ecological validity may be left implicit (Uher 2008a), especially for terms that have a less clear behavioral meaning. However, ecological validity can be ensured by showing that the rated items have an equivalent in naturally occurring behavior.

Another key criterion is sufficient reliability (i.e., agreement in assessment between raters/coders) to minimize the chance of rater and coder biases. In rating studies, this is ensured by multiple raters whose assessments are tested for correlation. The items that do not meet the criterion are excluded from further analyses. Rating studies have shown remarkably high interrater reliabilities in various species, reflecting a high agreement between people on the animals' characteristics (or, rather, between peoples' impressions thereof) (Gosling 2001). However, interrater reliabilities are shown to be lower for items that have a behaviorally less clear connotation – such as eccentric, jealous, sensitive – compared to items that are behaviorally clearer, such as dominant, aggressive, and playful (Gosling 2001; Vazire et al. 2007; Dutton 2008). Reliability in rating thus seems to at least partly depend on how behaviorally clear the semantic meaning of the item descriptor is. Behavior coding studies have been noted for having low reliability or for not reporting reliability (Vazire et al. 2007). Indeed, reliability is often not tested or reported in behavioral personality studies, leaving unclear how many people coded the behavior and whether interobserver reliability was tested. This is in contrast to behavioral research outside of the personality realm, where testing for interobserver reliability is a standard procedure (observational research: e.g., Parr et al. 2005, Koski et al. 2007; experimental research: e.g., Call et al. 2005, Silk et al. 2005). The absence of reliability reporting in behavioral personality research is unfortunate. It remains crucial that interobserver reliability is habitually assessed in personality studies that rely on behavioral coding.

To solve the debate between coders and raters as to which approach is better in animal personality research, Vazire et al. (2007) conducted a study using both methods. A total of 52 captive chimpanzees were rated on a 34-item list (a subset of item lists used in research on rhesus macaque and spotted hyena personality by Capitanio 1999 and Gosling 1998, respectively), as well as coded on a range of behaviors by one observer, obtaining 2–3 h of behavioral data per chimpanzee. The resulting scores of rated items and a selected subset of observed behaviors were correlated. The level of agreement between rated items and coded behavior per conceptually equivalent category varied greatly, from negligible to highly significant, implying that impressions on behavior and quantification of the corresponding behavior did not consistently match. Reliability of the rated data concerned, as is customary, the interrater correlation of the rated item scores. This was high, indicating that people shared their impressions about the chimpanzees' personality. In contrast, the reliability of the coded data was tested by treating each 15-min focal observation of a chimpanzee as an independent observation, which was correlated with other singular focal samples of the same chimpanzee (allowing calculation of the intraclass correlation coefficient). However, this procedure hardly represents good behavioral research practice for two reasons. First, a total of 2–3 h of observation of a mammal with a complex behavioral repertoire is a very limited sample of

its general behavior patterns. Second, one focal observation cannot be considered as an independent and representative sample of an animal's overall behavior. With small sample sizes, the risk of over- or underestimating behavioral parameters is considerably inflated (Martin and Bateson 1993). Moreover, testing intraclass correlations of singular focal samples is not an equivalent reliability test to inter-rater agreement in rating. To obtain a reliability measure comparable to the one of rated items, behavioral observations of several people obtained simultaneously should have been compared to each other.

In sum, rating studies often suffer from a lack of independent and quantitative behavioral validation, which also leaves the ecological relevance of (some) rated items unclear. Coding studies have potential for a high construct and ecological validity, depending on the trait selection, experimental procedures, and sampling effort. Reliability among raters/observers is usually high in rating studies, whereas in coding studies it thus far is often left untested.

Yet another diagnostic measure of a personality trait is repeatability or consistency over time. Behavioral consistencies have been analyzed in many ways (Hayes and Jenkins 1997). The current standard in behavioral research is to calculate a trait's repeatability (i.e., an estimate of the variation within and between individuals). Repeatability is calculated with an analysis of variance (ANOVA), with individuals as a fixed factor and with a minimum of two measures of a trait for each individual (Lessells and Boag 1987; Bell et al. 2009). Behaviors that show low within-individual variation but high between-individual variation are more repeatable. In a recent meta-analysis, Bell et al. (2009) showed that across a large range of taxa and behaviors the average repeatability was significantly greater than 0, although there were species and sex differences. Individual differences accounted for roughly 37% of the variation. Also, psychological studies on animal personality have used various methods to test temporal consistency – for example, Cronbach's alpha (Uher et al. 2008) and intraclass correlation coefficients (e.g., King and Landau 2003), which is statistically identical to repeatability. Thus, the importance of behavioral consistency is agreed upon in both approaches.

5.5 Finding Common Ground

This chapter focuses on the differences between the psychological and biological approaches to animal personality. I have also stressed that it is possible to overcome the differences by appropriate methodological practices and improved clarity in reporting. Below are some aspects that I believe are of interest to both psychological and behavioral personality research and that would likely benefit from communication across disciplines.

Noted earlier is the importance of hierarchical structure in the psychological personality research tradition, which is largely ignored in the behavioral research tradition. However, as behavioral syndromes are now in the forefront of behavioral research (cf. Sih and Bell 2008), they directly link with the question of structure of

personality traits. Which personality traits form syndromes and whether those syndromes are conceptually similar to constructs from the human personality theory are interesting avenues for future research. We also need to understand whether and when behavioral correlations are stable and which mechanisms underpin them. Understanding how multiple behavioral syndromes influence overall behavior and how they are dependent on each other is important in its own right (cf. Sih and Bell 2008), but unraveling structural hierarchy of animal personality also allows direct comparisons with human personality structure.

Animal and human personality research would make significant advances by addressing the four questions posed by Tinbergen (1963): causation, function, ontogeny, and evolutionary history (Bell 2007; Nettle 2008). Causation of behavior is about the proximate mechanisms underlying behavior. Both biological and psychological work has revealed a number of interesting mechanisms of personality. The genetic base of personality has been confirmed by establishing a significant heritability of numerous traits (Bouchard and Loehlin 2001). Direct connections between certain personality traits and their genetic and neurochemical correlates have also been proposed – for example, between a polymorphic dopamine receptor gene (*DRD4*) and novelty-seeking behavior (Roussos et al. 2009; but see Klueger et al. 2002) and between a serotonin transporter gene (*5-HTT*) and anxiety-related traits (Ebstein 2006) in humans. Several other neuropeptides and hormones have been connected to personality, including testosterone (androgen receptor polymorphism: Westberg et al. 2009; circulating testosterone: Rowe et al. 2004), vasopressin (Bartz and Hollander 2006), and cortisol (Hauner et al. 2008). Polymorphisms in the *DRD4* and *5-HTT* genes have also been identified in some animals, including nonhuman primates (Livak et al. 1995; Seaman et al. 2000; Bailey et al. 2007; Inoue-Murayama et al. 2008), and they may influence their personality traits much as in humans (Inoue-Murayama et al. 2006, 2008; Bailey et al. 2007; Spinelli et al. 2007; Inoue-Murayama 2009). Establishing the genetic and physiological bases of human personality is one of the key challenges in human psychology (cf. Penke et al. 2007), and animal models are an important part of this work. However, it is equally important to establish mechanisms of animal personality in their own right. Illuminating links between genetics, brain functions, behavioral endocrinology, and personality traits is important to advance our understanding of both human and animal personalities for fundamental and applied reasons.

The fitness consequences of and the evolutionary mechanisms maintaining human personality are gaining attention (MacDonald 1995; Nettle 2005, 2006, 2007; Penke et al. 2007). As empirical research on the costs and benefits of personality in humans is still limited, it can benefit from the active research on these questions in animal personality research. Identifying fitness consequences of personality traits is one of the main goals of biological personality research. It has been shown that many heritable personality traits have significant fitness consequences in terms of reproductive output and survival (Dingemanse and Réale 2005; Smith and Blumstein 2008). This has evoked a new set of questions about the evolutionary significance of personality. For example, most animal studies have addressed the fitness effects of single traits but not of correlated traits (Smith and Blumstein 2008; but

see Sih and Watters 2005). How the different organizational levels of traits influence the overall behavior and how they influence individual's fitness are interesting future questions. Also, although some aspects of the environment's influence on fitness consequences of animal personality have been shown (e.g., spatiotemporal variation in resource availability) (Dingemanse et al. 2004), there is much to be done to unravel the role of environmental conditions – e.g., predation pressure, social conditions, fluctuations therein – in determining fitness effects of personality traits (Sih and Bell 2008).

Studying personality in social species is an especially interesting direction of research as some have suggested that social environments promote consistency in behavior (Fishman et al. 2001; Dall et al. 2004; McNamara et al. 2004) and maintain interindividual variation in continuous behavioral traits through frequency-dependent selection (McNamara et al. 2009). Social environments also influence how personality traits manifest. For example, in rainbow trout (*Oncorhynchus mykiss*) observations of another's shyness made bold individuals shyer, whereas shy individuals became bolder (Frost et al. 2007); and among zebra finches (*Taeniopygia guttata*) individuals' exploration tendency increased in the company of an exploratory individual (Schuett and Dall 2009). In addition to the effects of the social environment on the manifestation of personality traits in general, sociability as a personality trait has been surprisingly little studied in animals. In the common lizard (*Lacerta vivipara*), individual variation in sociability has been shown to influence survival and reproductive success (Cote et al. 2008). Similar, but indirect, evidence comes from primates: Baboon (*Papio cynocephalus*) females' social network size correlates positively with their fitness (Silk et al. 2003, 2009). Whether and how network size is dependent on personality in baboons is unknown. However, in young rhesus macaques, personality (i.e., activity and calmness) predicts the number of social relationships (Weinstein and Capitanio 2008). In chimpanzees (Anestis 2005) and vervet monkeys (*Chlorocebus aethiops*) (Fairbanks et al. 2004), some personality traits (i.e., aggressiveness and reactivity in chimpanzees, impulsivity in vervets) predict male rank, thus influencing males' fitness. Furthermore, differences in chimpanzee alpha males' dominance "styles" regarding their social grooming patterns have been identified, likely reflecting differences in personality traits (Foster et al. 2009), but it is yet unknown whether they have consequences for their fitness.

Humans, like most other primates, are a highly social species. In human personality, sociability is, like excitement-seeking, a facet of extraversion (Costa and McCrae 1992), which is shown to predict sexual promiscuity (Schmitt 2004; Nettle 2005) and social network size (Swickert et al. 2002). Furthermore, sociability has been shown to increase the likelihood of having children (Jokela et al. 2009). These findings indicate that sociability has evolutionary relevance in us as well. Therefore, the social environment, the particular personality traits it favors and constrains, and its influence on fitness are intriguing questions for human and animal personality research.

Ontogeny of human personality has traditionally been the realm of developmental psychology. Personality during early years is often referred to as temperament (McAdams and Olson 2010). The continuity from temperament to the five

personality constructs has been scarcely studied, although some studies have proposed a developmental scheme from the early temperament dispositions to personality factors (reviewed by Caspi et al. 2005; Rothbart 2007; McAdams and Olson 2010). Later in life, personality develops in predictable ways: mean trait levels of neuroticism decrease, agreeableness and conscientiousness increase, and openness first increases and then decreases during adult life (Roberts et al. 2006). Early ontogeny and later development of personality traits has barely been studied in animals (cf. Stamps 2003). King et al. (2008) described the development of personality constructs in chimpanzees as nearly identical to that of humans. In three-spined stickle-backs (*Gasterosteus aculeatus*), boldness and aggression were stable through individual development in one study population but not in another (Bell and Stamps 2004). For great tit nestlings, handling stress (i.e., fear response to being handled by a human) at the age of 14 days correlated with the response 6 months later (Fucikova et al. 2009). Developmental aspects in animal personality deserve more research as the age-related changes in animal personality are poorly understood. Moreover, they can illuminate the effects of gene–environment interactions on personality (cf. Caspi et al. 2005; Roberts et al. 2007).

Finally, research into the evolutionary history of personality traits will benefit the most from comparative personality research and thus from an integrative phylogenetic framework (Gosling and Greybeal 2007). The process of identifying similarities and differences in personality across the animal kingdom, including humans, has only just started. Some personality traits, such as exploration tendency and boldness, appear to be important for a whole host of species and analogous (or homologous) with human personality traits within the constructs of openness and extraversion, respectively (Gosling and John 1999; Beaton et al. 2008). However, we are far from understanding how personality traits have evolved in various taxa, which of the similarities are due to homology and which to convergence, how the differences can be explained, and how this relates to a range of other aspects, such as species' life history, population dynamics, cognition, learning, and social structure, to name but a few. Animal personality research is coming of age, and as it grows it will significantly affect our understanding of human and animal behavior. The first tentative steps toward a unified approach to animal personality have been set. Aligning the concepts in animal personality research by different approaches will greatly enhance the advances in this rapidly growing field of research.

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Chapter 6
Evolutionary Genetics of Personality in Nonhuman Primates

Mark James Adams

6.1 Introduction

Our whole conception and acknowledgement of personality – both scientific and quotidian – is based on the notion of difference. A personality is precisely that which distinguishes one individual from another. These differences have consequences for behavior, health, and well-being, but we are mostly ignorant of their evolutionary roots. For humans and other primates, evidence is coalescing around a common structure that describes personality differences usefully categorizable in terms of shared versus derived traits and consistent with known species differences (Gosling and John 1999; Weiss et al. 2006). Although functional and genomic studies begin to hint at the proximate genetic and environmental factors that mix to produce differences in personality, we are still left with this wondrous puzzle: Why do these basic differences persist over evolutionary time scales as primates have speciated and evolved?

This problem runs up against one of the unendingly contentious issues in quantitative genetics: How is trait variation maintained? This question comes out of a basic mathematical result in genetics with its origin in animal breeding. The result says that natural and artificial selection reduces the heritability of a trait in a population. Much ink has been spilled on theoretical treatments of variation in primate (mostly human) personality and other psychological traits (e.g., Tooby and Cosmides 1990a; Nettle 2006; Penke et al. 2007). What we need are good data.

But which way forward? Which data? In evolutionary psychology, the usual tact is to identify past conditions within a lineage that explain present-day adaptation and variation (Tooby and Cosmides 1990b). This is mistaken in that it ignores a key insight of evolutionary biology: it is only through a phylogenetically informed approach that we can determine when traits arose and changed within a lineage (Gosling and Graybeal 2007). For determining when different features of personality originated in each primate lineage, this comparative approach is sound. To explain why differences

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within populations persist, however, something else is needed. Evolutionary quantitative genetics fills this gap. This branch of biology deals with the effects of evolutionary processes on continuous traits and the genetic and environmental factors underlying them. Most promising and relevant for the explorations of primate personality is the development of techniques for studying evolution in wild populations using pedigree data (Kruuk and Hill 2008). Given the length of time many primate populations have been investigated (Goodall 1986; Rawlins and Kessler 1986; Fedigan and Asquith 1991; Nishida et al. 2002), the identification of individuals (de Waal 2003), and the resolution of pedigree structure (particularly through matrilineal kin e.g., Fairbanks et al. 2004; Blomquist 2009b), it is a wonder that these techniques have not been more widely applied to nonhuman primate behavior.

Revealing the evolution of nonhuman primate personality requires first understanding how personality variation is defined and how differences among species are informed by phylogenetic relations. The evolvability of personality within a species is proportional to the heritability of each personality trait, which has already been estimated in several primate species. Making usable inferences about the evolution of personality first requires choosing a method for estimating heritability appropriate to the data (Kruuk and Hadfield 2007). Knowing what heritability really is will lead to a consideration of the exact role that the resemblance between parents and offspring, as captured by heritability, plays in random drift and selection in wild populations. Pinning down the fitness implications of personality differences requires more than just the genetic structure of personality but offers the opportunity to integrate many threads from psychological and behavioral–ecological approaches to personality.

6.2 Nonhuman Primate Personalities

Nonhuman primate personality has been examined from a number of stances, but integrating these different attitudes is still a major challenge (Clarke and Boinski 1995; Itoh 2002; Uher 2008) (see Chap. 5 for a full discussion). These methods include, broadly, impressionistic ratings using adjectives describing personality; observational measures and codings of differences in the presence, frequency, and duration of behaviors; and impressionistic ratings of behavior–situation units (Uher and Asendorpf 2008). Although methodological differences can shroud comparisons among species, Gosling and John (1999) found broad support for the basic personality dimensions related to sociality, anxiety, and cooperativeness in a number of other animals, from octopuses to chimpanzees. Although species-specific dimensions outside of those that differentiate humans exist (Uher 2008; Uher and Asendorpf 2008) and personality should encompass not only people but also behaviors and situations (Funder 2009), descriptions of stable, between-human personality differences as rendered in factor models usefully orient explorations of nonhuman primate personality structure. Differentiating individuals along basic personality dimensions provides a platform of traits for initial quantitative genetic

analyses of personality in primates. They are also good candidates for fitness correlates in evolutionary studies because these broad personality dimensions consistently relate to differences in health, longevity, and other social outcomes in humans (Roberts et al. 2007).

Studying multiple species with the same instrument also aids phylogenetic comparisons of personality structure by revealing the historical patterning of the emergence and modification of personality dimensions (Weiss and Adams 2008). This chapter next reviews factor model perspectives before considering how personality traits can evolve. However, behavioral and functional approaches make their appearance later when we need to causally connect broad personality variation to fitness (see Sect. 6.5.3). Assessments of behavioral profiles (Shoda and Mischel 2000; Uher et al. 2008), in particular, show promise for reaction-norm studies of personality evolution (see Sect. 6.6).

6.2.1 Models

One of many models for human personality describes personality differences in five independent dimensions (Digman 1990): generally speaking, differences in sociability and assertiveness are called Extraversion; variation in trust and cooperation are grouped as Agreeableness; Conscientious describes differences in discipline, planning, and self-control; variation in curiosity and creativity is captured by Openness; and a dimension called Neuroticism differentiates individuals in terms of anxiety, emotional stability, and stress response. Reasonably, it is referred to as the Five-Factor Model (FFM). A single individual is characterized by a stable density distribution along each of these dimensions (Fleeson 2001). The FFM is robust across cultures (McCrae et al. 2005) and emerges whether people are measured on items that are adjectival descriptors (Digman 1990) or cognitive-affective reactions to situations (Denissen and Penke 2008). This factor-model description of human personality has served as the starting point of several investigations of nonhuman primate personality.

Chimpanzees share with humans the broad dimensions of the FFM, with the addition of Dominance, which describes differences in competitive facility (King and Figueredo 1997). As a personality trait in primates, Dominance should be distinguished from social dominance or rank as the latter is an outcome rather than an aspect of personality (Hinde 1978; Buss 1988; Gosling and John 1999). The chimpanzee dimensions Agreeableness and Openness were given names identical to their human homologues. Although the labels differ, the remaining three traits map human equivalents: Surgency onto Extraversion, Emotionality onto Neuroticism, and Dependability onto Conscientiousness. Although chimpanzee Conscientiousness is more narrowly defined than its counterpart in humans (it does not include facets related to trustworthiness and duty) the Conscientiousness personality dimension seems to be a derived character in humans and chimpanzees, as it has not appeared

as a "pure" construct in any other species investigated (Gosling and John 1999; Weiss et al. 2006; Weiss et al. in press). Chimpanzees can also be differentiated from each other by their behavioral signatures, including propensities to set upon or affiliate with conspecifics, anxiety and arousal in stressful situations, curiosity toward novel foods and objects, impulsivity, goal pursuit, and physical and sexual activity (Pederson et al. 2005; Uher et al. 2008; Uher and Asendorpf 2008).

Gorillas likewise can be differentiated by their behavioral repertoires, similar to chimpanzees (Uher et al. 2008). Gorillas have also been described under the rubric of the human FFM using the dimensions Extroversion, Understanding (i.e., Agreeableness), Fearfulness (i.e., Neuroticism), and Dominance (Gold and Maple 1994). Salient in their absence from gorilla personality are homologues of human and chimpanzee Openness and Conscientiousness. Do gorillas really not differ in levels of curiosity and self-control, or were researchers just not looking for variation in these traits (Gosling and John 1999; Weiss et al. 2006)?

The importance of being more thorough can be seen in Weiss et al.'s (2006) portrayal of orangutan personality using a similarly broad instrument previously applied to chimpanzees (King and Figueredo 1997). Orangutans can be described with the dimensions Dominance, Extraversion, Agreeableness, Neuroticism, and Intellect. Intellect appears to be a blend of Openness and Conscientiousness.

A variety of models using impressionistic ratings have emerged to describe rhesus macaque personality. Some early studies revealed, alternatively, three dimensions: Fear, Hostility, Affiliation (Chamove et al. 1972) or Excitability, Sociability, Confidence (Stevenson-Hinde and Zunz 1978; Stevenson-Hinde et al. 1980). Later studies derived four dimensions: Tense–Fearful, Aggressive, Solitary, Curious–Playful (Bolig et al. 1992) or Sociability, Confidence, Excitability, Equability (Capitanio 1999). Rhesus macaques can even be described in as many as six dimensions: Confidence, Friendliness, Dominance, Anxiety, Openness, Activity (Weiss et al. in press). These results demonstrate the vagaries of measuring personality with instruments that have been incompletely adapted from studies of other species (Uher and Asendorpf 2008). That said, despite the various labels and differing numbers of components, many of these dimensions describe the same constructs. The primate dimensions of Extraversion are captured by Affiliation/Sociability/Solitary, Agreeableness by Hostility/Aggressive/Friendliness; Neuroticism by Fear/Excitability/Tense–Fearful/Confidence–Anxiety; Openness by Curious–Playful/Openness; and Dominance by Confidence/Dominance (Gosling and John 1999; Weiss et al. in press). This lumbering development matches the slow growth and refinement in characterizing broad dimensions of human personality chronicled by Digman (1990). We will not get there all in one go.

Using behavioral codings, Rouff et al. (2005) identified three dimensions of overall behavioral variation and four of between-individual differences in the personalities of lion-tailed macaques. The components that differentiated individuals (in contrast to behavioral occasions irrespective of the individual exhibiting them) map roughly onto the rhesus macaque dimensions Friendliness, Dominance, Activity/Confidence, and Anxiety. Although methodological and sample-size differences between these studies make for a knotty comparison, they suggest that several broad

features are conserved in the genus *Macaca*. It also shows that basic dimensions can shine through even if the instrument or ethogram is not specifically designed to find them. For instance, Rouff et al. (2005) chose behaviors that defined Neuroticism-like bipolar facets, namely, Anxious–Relaxed and Reactive–Unreactive. Each pole of these facets, however, did not group together. Reactive clustered with the Confidence-like component, and Relaxed and Unreactive loaded on the Anxiety-like component. This tallies with the claim that primate Neuroticism can become uncoupled into two independent dimensions describing free-floating versus situationally determined anxiety (Weiss et al. in press). Further work on lion-tailed, rhesus, and other macaque species is needed to clarify personality structure within this genus.

Whole personality structures have been educed in other Old World monkeys. Vervet monkey personality consists of three dimensions – Social Competence, Playful, Curious, Opportunistic Self-Serving (McGuire et al. 1994) – which map to the great ape domains of Dominance, Openness, and Agreeableness, respectively (Gosling and John 1999).

Konečná et al. (2008) extended the search for nonhuman primate personality structure to colobines. They investigated male Hanuman langur personality using both impressionistic descriptors and behavioral codings. Male langur behavior exhibits a three-dimensional structure consisting of Dominance, Involvement, and Activity. Impressionistic ratings also revealed three dimensions, called Agreeableness, Confidence, and Extraversion. High Agreeableness was expressed behaviorally by low Dominance; high Confidence by high Dominance and Involvement and by low Activity; and high Extraversion by elevated Activity. Again, these dimensions broadly match those found in other primate species, and the absence of other distinct dimensions (such as Openness) have reasonable ecological explanations (e.g., langurs are opportunistic foragers).

Over the years other, more specific aspects of personality and temperament have been examined in nonhuman primates (Clarke and Boinski 1995). Factor models and behavioral profiles by no means cover all the facets of primate personality that have been discovered. Attempts to describe all the features of between-individual personality differences, however, are starting to pay dividends by distinguishing the separate threads that we need to weave the historical patterns of primate personality evolution.

6.2.2 Building Blocks

Gosling and John (1999) showed that dimensions analogous (and perhaps homologous) to the five human factors appear in other species, with the addition of two dimensions, Dominance and Activity. Although Dominance is a salient dimension across many species, they found little evidence for Activity as a separate dimension. Nonetheless, activity is a common trait explored in behavioral–ecological investigations of personality (Réale et al. 2007) and was found to define a separate dimension in

wild langurs (Konečná et al. 2008) and rhesus macaques (Weiss et al. in press). Furthermore, in humans, although this dimension is subsumed under Extraversion in adults, it can emerge as a separate feature in adolescent males (John et al. 1994).

As it is possible for traits that normally vary together to become uncoupled during development (Groothuis and Carere 2005), we can consider the developmental evolution and phenotypic integration of personality dimensions. Correlated variation in the rudimentary personality structures of humans, chimpanzees, orangutans, and rhesus macaques can be described with a set of eight "basic" and five "blended" personality traits (Weiss et al. in press). The basic traits are called Sociability, Activity, Altruism, Anxiety, Confidence, Dominance, Openness, and Conscientiousness. The other traits are combinations of these components. In humans, chimpanzees, and orangutans, Sociability and Activity positively covary to form Extraversion; and Anxiety and Confidence negatively covary as Neuroticism. In rhesus macaques, in contrast, Sociability fluctuates with Altruism and is denoted as Friendliness. Meanwhile, in humans, Altruism and Dominance negatively covary in the dimension that describes cooperative behavior (i.e., Agreeableness), whereas orangutans have an interesting blend of Openness and Conscientiousness called Intellect. Positing these different basic traits follows the suggestion of Réale et al. (2007) to start defining possible categories of correlated suites of behavior beyond those already considered in work on behavioral syndromes (i.e., shyness–boldness, exploration–avoidance, activity, aggressiveness, sociability). These basic traits may be the result of opportunities for adaptive behavioral variation for meeting the social, ecological, and developmental challenges faced by big-brained, gregarious, long-lived mammals. Factor models for each species are the first step in hypothesizing the building blocks constituting primate personality structures.

Why we should find this historical patterning in primates or even whether we have the right "basic" dimensions are big questions. When thinking about the evolution of personality dimensions, it might seem strange at first to consider the evolution of something that is only a construct describing differences between individuals. Extraversion, for instance, describes differences between individuals in their sociality and action. Unlike a new behavior or organ, a personality dimension is not an obvious thing that a single individual has. However, this thinking takes a rather narrow view of what evolution is or how it effects populations. Selection does not act only on the mean level of a trait. Evolutionary change can occur on higher moments (e.g., variance, skew, kurtosis) of the population distribution of a trait as well as its covariance with other traits (Rice 2004). The genetic and environmental factors contributing to personality can start and stop covarying as the population evolves.

Before worrying too much over these complications, let us start more simply. When a population experiences selection, how does it respond? Let us go into the wild and find a troop of apes that differ in Extraversion. We measure their personalities and find, as it happens, that only individuals who are a value of S below the population mean in Extraversion are having children. For the moment, do not worry about why this might be the case. How sociable should we expect these offspring to be? Here, S is the selection differential (the amount that the parents producing

offspring deviate from the average trait value), and we want to know by how much the offspring will also differ from the parental average (or the response to selection, R). We are asking $R = ? \times S$ and the answer should have something to do about the resemblance between parents and their offspring.

6.3 Heritability

Heritability captures the resemblance between relatives. Heritability (h^2) is the proportion of the difference in phenotypes attributable to differences in inherited genes and thus ranges from 0 to 1.0. When considering two traits, we can also ask to what extent they are influenced by the same set of genes and what the direction of this relationship is. This is the genetic correlation (r_A) and can extend from −1.0 to +1.0. Behavioral traits are generally less heritable than morphological traits (Stirling et al. 2002). The heritability of personality and related traits has been established in several species of nonhuman primates. However, it is important to keep in mind that genes are not the only factor of interest in explaining variation: for certain problems, other types of environmental variance may be equally compelling to the researcher. As we shall see, although all sources of variation should be examined, differences caused by the additive effects of genes (called heritability) hold special status in the origin of both adaptive and neutral variation among individuals. The first step is to consider the extent of heritability in nonhuman primate personality.

Weiss et al. (2000) estimated the heritability of the six factors of chimpanzee personality. Of these factors, only Dominance was found to be significantly heritable ($h^2 = 0.63$). The estimate for Dependability was 0.21; although not detectably greater than 0, this suggests low to moderate heritability. The remaining traits showed little or no heritability. Importantly, this study of zoo-housed chimpanzees also established that very little of the differences in personality could be accounted for by differences among zoos. A later study using a different estimation technique (see Sect. 6.3.1) confirmed the high heritability of Dominance ($h^2 = 0.66$) and established the high genetic correlation with subjective well-being ($r_A = 1.00$) (Weiss et al. 2002).

The heritability of facets of personality and other related traits has also been investigated in nonhuman primates. Williamson et al. (2003) estimated the heritability of fearfulness and anxiety in rhesus macaques. Several aspects of their responses, such as a tendency to explore novel environments (latency to leave the protection of their mother during a Free Play Test) or to approach novel objects (in this case, a kiwi fruit) had estimated heritabilities of 1.0. These high estimates of heritability in these types of trait were confirmed in a later study with a similar measure of vigilance ($h^2 = 0.98$) (Rogers et al. 2008). Latency to approach strangers (measured as a Social Impulsivity Index) is also heritable in vervets, but only moderately so ($h^2 = 0.34 \pm 0.11$) (Fairbanks et al. 2004). There was no effect from the maternal environment, which given how it was estimated includes nonadditive genetic variance

from dominance and epistatic effects as well as the influence of maternal care and the mother's genotype. The Social Impulsivity Index consisted of two subscales measuring approach–avoidance and aggressiveness that were themselves highly genetically correlated ($r_A = 0.78 \pm 0.12$), suggesting that the two facets are influenced by a similar set of genes.

These results are not surprising given that the heritability of personality dimensions in humans has been estimated to be in the range of 0.4–0.8 (Riemann et al. 1997; Bouchard and Loehlin 2001), depending on the population and whether personality is assessed with self-reports, peer-reports, or both and are of similar magnitude in other animal species (van Oers et al. 2005a). The lack of a maternal effect in vervet impulsivity is also consistent with the small influence of shared environment (e.g., maternal care experienced by siblings) on personality in humans (Bouchard 1994; Rowe 1994).

6.3.1 Estimating Heritability

It is worth taking a step back and considering what heritability is and how it can be estimated. The basic question is how do parents and offspring resemble each other; that is, what is the covariance between mean offspring and mean parental phenotypes? Second, what proportion of variation among the offspring is caused by variation inherited from their parents? This value is found in the coefficient from a linear regression of offspring on parental phenotypes (Falconer and Mackay 1996), or

$$\beta_{z_O, z_P} = \frac{\mathrm{cov}(z_o, z_P)}{\mathrm{var}(z_P)},$$

where $\mathrm{cov}(z_O, z_p)$ is the covariance between the phenotypes of offspring and their parents, and $\mathrm{var}(z_p)$ is the phenotypic variation of the parents. This quantity describes how traits are selected (see Sect. 6.3.2) (Rice 2004).

Like the derivation of many basic statistical terms (e.g., "split-plot") from agricultural experimentation, the meaning of many of the concepts surrounding the estimation of heritability are clearer once their origins in animal and plant breeding are understood. If you are raising livestock and are picking individuals to mate with one another to produce a new generation, what information do you want about these parents? What interests you is not the phenotype of each parent but, rather, the average phenotype of a parent's offspring. An individual's "breeding value" is a score representing their offspring's expected phenotype when mating is random (Falconer and Mackay 1996). Breeding values act additively – which is to say that an offspring's expected breeding value is the average of its parents' – and are thus thought of as caused by genes (not genotypes) that are passed from parents to their offspring. The effects of these genes act additively because they influence the phenotype independent of the constitution of the rest of the genotype, which is not the case for dominance or epistatic interactions.

The part of differences in phenotypes that can be attributed to breeding values is called the additive genetic variance of a trait. The ratio between additive genetic (V_A) and phenotypic variance (V_P) is an estimate of heritability

$$h^2 = \frac{V_A}{V_P}$$

because, assuming certain conditions apply, these genes are what determine the parent–offspring resemblance (Rice 2002). These assumptions are the following: (1) an individual's phenotype is a combination of the additive genetic effects from both its parents plus an effect from the environment (there is no influence from dominance or epistasis); (2) mating is random; (3) genotypes are independent of the environment in which they are expressed; and (4) parents do not transmit their environment to their offspring. To the extent that these conditions hold, V_A/V_P can be used to estimate β_{z_O, z_P}, and in practice this is what is done.

Heritability can be estimated in a number of other ways, depending on the relationship between the individuals measured, such as twins (Martin and Eaves 1977) or half-siblings (Falconer and Mackay 1996). Entire pedigrees – describing not just the relatedness between parents and offspring or among siblings but between all relatives – can even be combined into a single analysis using the squared differences of phenotypes between all individuals (Grimes and Harvey 1980), which was shown to be an improvement over analysis of variance-based estimation techniques (Bruckner and Slanger 1986a, b). Like other, more advanced methods, this requires determining the relatedness of all individuals in the study population from a pedigree.

More recently, animal breeders and evolutionary quantitative geneticists have begun to favor variance component analysis, also known as random effects or mixed effects models, for estimating genetic and environmental sources of individual differences (Henderson 1950, 1975; Shaw 1987; Lynch and Walsh 1998; Kruuk 2004). These models still use all relationships in the pedigree; but, rather than pairing or nesting individuals together as in the techniques described above, breeding values are determined for each individual. Because the analysis occurs at the level of individual animals, this model was dubbed the "animal model" (Lynch and Walsh 1998). This set of equations can also be described as a mixed-effects model because it differentiates fixed effects (which account for mean differences among groups of individuals) from random effects (which partition the remaining variance between individuals). Breeding values are the typical random effect of interest. Although the meaning of "fixed" versus "random" effects are quite varied (and confused) in the literature (Gelman 2005), it is by these terms that evolutionary geneticists are trying to distinguish known causes of differences between classes of individuals (e.g., sex and age) from those that govern a trait's variance and for which each individual has its own value. An advantage of the animal model is that it can incorporate, and therefore estimate, other sources of variance. (See Chap. 7 for effects of interest in animal personality research.) Animal models have been used successfully on data from wild populations to estimate components of

variance in addition to heritability (Kruuk 2004; Kruuk and Hadfield 2007) and are particularly suitable when trying to distinguish genetic from environment effects (Kruuk and Hadfield 2007). Variance components and breeding values for the animal model can be estimated with restricted maximum likelihood (REML) methods (Shaw 1987; Lynch and Walsh 1998) or using Bayesian analysis (Sorensen and Gianola 2007; O'Hara et al. 2008; Hadfield et al. 2010).

In quantitative genetics of natural populations, these effects can only be identified if they differ between individuals so variance component decomposition does not provide a complete causal account of how a trait comes to be (component terms such as V_A or V_E are also referred to as causal components of variance) (Falconer and Mackay 1996). Take a look at maternal effects such as those from early rearing experience: work by Harlow (1969) showed the importance of a mother's love for the behavior and adjustment of an individual later in life. The mother clearly has an "effect." Although such differences can be induced in experimental conditions, there still might not be any maternal effects in the wild. Just because close maternal contact is developmentally necessary for proper fear and anxiety reactivity does necessarily mean that differences in rearing style influence offspring phenotypes. This lack of difference is what is meant if no maternal effect is found on a trait.

Estimates of heritability in nonhuman primates have drawn on all of these techniques, but it pays to use the method most suited to the available pedigree data. For estimating heritability in primate populations, the animal model is to be preferred. This is primarily because it can handle the arbitrary but interconnected pedigree structure of primates in different zoos (Weiss et al. 2000) as well as tolerate unknown relatedness such as missing paternity information common in studies of wild primates (de Ruiter and Geffen 1998). Furthermore, using all relationships from a pedigree improves estimates of genetic correlations (Åkesson et al. 2008). The ability of Bayesian methods to handle small sample sizes (O'Hara et al. 2008) and confounding variables (Ovaskainen et al. 2008) makes it suitable for analyses involving the hundreds of subjects available for primate research rather than the thousands typical in agricultural settings, for which REML procedures have been developed and refined. Bayesian methods are also good for evolutionary questions because the uncertainty in the prediction of breeding values can more easily be carried on to estimates of evolutionary change (Hadfield et al. 2010).

Whichever technique is used, it is important to realize that these are simply models of the transmission of traits from parents to offspring (Rice 2004). Estimating heritability is a process of fitting statistical parameters to data, and these estimates are influenced by more than just the variation in additive genes (Stirling et al. 2002). Many of the modeling assumptions (random mating, no gene–environment correlations) required to estimate heritability from a parent–offspring regression are unlikely to hold in primates. Furthermore, variance from the environment in these models is actually just the residual variance, or the error. This error includes all the causes of differences between individuals for which we do not know how to account. Even when we are assigning a name to a key component of variance, such as V_A, the most general descriptions of the parent–offspring resemblance do not

make any assumptions about what is being inherited. It is usually assumed that the transmission of DNA sequence variants accounts for this resemblance, but epigenetic sequences can be transmitted across generations and contribute to additive genetic variance in the same way (Johannes et al. 2008, 2009). Primate parents and offspring can resemble each other for nongenetic reasons as well, such as abusive rearing styles in rhesus macaques (Maestripieri 2005). That environments can be transmitted is a distinct possibility that is not without utility for evolutionary model building (Odling-Smee et al. 2003). As in the remake of a 1970s horror film, these snags in understanding heritability (Feldman and Lewontin 1975; Visscher et al. 2008) are the "undead" of quantitative genetics, particularly in the psychological sciences (Taylor 2010).

It is thus important in any discussion of heritability to have a handle on how it is being estimated and whether the model or design being used is appropriate to the data (Kruuk and Hadfield 2007; Hadfield et al. 2010). Similarly, animal models can be sensitive to the inclusion of fixed effects (Wilson 2008). Additive genetic variance estimates can change when adding a fixed effect that is genetically correlated with the trait.

When interpreting heritability as a statistic, there is little practical use in *P* values associated with testing the hypothesis that $h^2 > 0$. First, almost all psychological traits are heritable (Turkheimer and Gottesman 1991), so finding significant additive genetic variance should not come as a shock. Second, the sample sizes available for most primate populations often do not give enough power to distinguish heritability from zero, even if heritability is actually moderate. Finally, evolutionary geneticists are not interested in the predictive utility of heritability as it is practiced in animal and plant breeding, where a particular point estimate for h^2 is sought. What we are, instead, interested in is the range of likely values for h^2 that are supported by the data and by the model (typically the 95% coverage or confidence interval) to indicate whether heritability is low, moderate, or high.

6.3.2 Why Care About h^2?

In the age of molecular genetics, heritability may seem like an old fashioned or even outdated concept (Visscher et al. 2008). It may also appear quirky to put so much focus on genes (without naming specific ones) rather than on genotypes. Would we not like to know the specific genes that interact with each other and with the environment to determine an individual's personality? On a practical level, even if an investigation revolves around nongenetic variables, carrying out an analysis within an animal model framework allows estimates of the effects of these variables to be conditioned on familial resemblance. For answering evolutionary questions, heritability gets at those differences in genes that are required for the change of phenotypes through both random drift and natural selection and are therefore fundamental to the debate over how phenotypic differences are maintained in populations.

Going back to our hypothetical troop of more-or-less extraverted primates, heritability captures how much offspring are expected to resemble their parents. A linear regression, such as that of offspring on mid-parent phenotype, is also a model for predicting an offspring's phenotype from those of its parents. It can be used to predict the average personality level of the next generation from the mean level of the selected parents. Heritability thus answers our question of how the offspring of the less Extraverted parents will differ from their parents' generation. The potential for the mean phenotypic value of a trait to respond to selection is proportional to the magnitude of selection on the trait times its heritability. The equation expressing this relationship is called the breeder's equation,

$$R = h^2 S,$$

stating that a population's response (R) to selection (S) is limited by the heritability of the trait being selected. This equation can be expanded to more than one trait, in which case the response to selection of one trait is a function of its genetic variance and its covariance with other traits being selected (Lande 1979; Turelli 1988; Falconer and Mackay 1996), given by

$$\Delta z = \mathbf{G}\beta,$$

the multivariate breeder's equation, where $\mathbf{G}$ is a matrix of additive genetic variances and covariances of the traits, β is a vector of selection gradients on each trait, and Δz is a vector of responses to selection for each trait (see Blows 2007 for a review). When studying the evolution of personality, then, it is important to estimate not just the heritability of each dimension but also the genetic correlations among the dimensions and between personality and other traits (see Chap. 7). Thus, genetic correlations between behaviors is one way in which personality traits can be defined (Dingemanse and Réale 2005).

The centrality of heritability to the problem of quantitative variation comes from a basic mathematical result: both random drift and selection reduce additive genetic variance (Falconer and Mackay 1996). Much work on the evolution of personality has gone into developing theories about how individual differences in personality are maintained.

6.4 Persistence of Variation in Psychological Traits

The maintenance of heritable variation in traits is a long-standing problem in biology (Barton and Turelli 1989; Barton and Keightley 2002). Processes that maintain additive genetic variation in a trait may come through direct action on the trait or through indirect action on a genetically correlated trait (Robertson 1967).

In discussions of the "amount" of additive genetic variation, it is often pointed out that, as a ratio, the magnitude of heritability is as much a function of all other sources of variance (nonadditive genetic and environmental) as it is of V_A. To make

heritability comparable between traits and species, Houle (1992) defined the coefficient of additive genetic variation as

$$CV_A = 100\frac{\sqrt{V_A}}{\bar{X}},$$

which standardizes V_A by the phenotypic mean, $\bar{X}$. However, calculating CV_A requires that the phenotype is measured on a ratio scale, meaning that it has a true zero value. Personality constructs in primates are typically formed on ordinal or interval scales, however. This coefficient, therefore, has little utility for comparisons among the heritability of many psychological traits. Furthermore, it loses the key interpretation of heritability as the covariation of parent and offspring phenotype, which is so key to the evolvability of a trait.

6.4.1 Processes Maintaining Variation

All genetic differences ultimately arise through mutation, so it is possible for genetic variance to be maintained by a balance between its introduction by mutation and its removal by selection (Lande 1979) or random drift (Barton and Turelli 1989). In biology, most of the debate involves theoretical considerations about the distribution of mutation effect sizes, the number of loci influencing the trait, and the extent of pleiotropy (Johnson and Barton 2005; see Penke et al. 2007 for a review of alternative models from the perspective of human personality evolutionary genetics). The problem with applying these models to the maintenance of genetic variation in nonhuman primate personality traits is that the data required to evaluate them are not available so the arguments are restricted to theoretical considerations. Until such a time as data are available, evolutionary studies of personality will focus on phenotypic and quantitative genetic data.

Even without comprehensive molecular genetic data, fitness trade-offs are essential to consider in the evolution of any trait (Lande 1982; Charnov 1989; Roff and Fairbairn 2007). Such incompatibilities arise when a change in one trait that increases fitness is accompanied by a change in a second trait that decreases fitness. Trade-offs are a particular focus of life-history theory where, for example, there might be alternative choices between fecundity and survival (Williams 1966b; Partridge and Sibly 1991). Because selection will have eroded variation that influences both traits positively, components of fitness tend to have negative genetic correlations even if the phenotypes are positively correlated (Lande 1982). The evolutionary effect that such trade-offs have is typically explored through genetic covariations (Roff and Fairbairn 2007). This brings us back to the multivariate breeder's equation (see Sect. 6.3.2): the potential response of a trait to selection is constrained by selection on other, correlated characters, expressed in the **G** matrix.

The interpretation of **G** as an expression of trade-offs between traits is not without controversy (Pigliucci 2006) because functional trade-offs between two traits (e.g., in resource allocation) can sometimes have a positive genetic correlation

(Houle 1991). In personality research, some of these broader problems can be avoided because we are not interested in predicting long-term responses to selection, which is the crux of much of Pigliucci's (2006) critique of evolutionary quantitative genetics. Trade-offs that can be posited by considering genetic covariances can also be seen when selection of correlated characters produces scenarios where different combinations of traits have equal fitness, potentially maintaining genetic variation in each trait (Roff and Fairbairn 2007). Beyond this, traits can be entangled developmentally through higher orders of epistasis in addition to genetic correlation (Rice 2004).

6.4.2 Evolving and Resolving Explanations

Evolutionary psychologists have given many explanations for the persistence of variation in human personality. These explanations have been grouped into three categories: adaptive, nonadaptive, and maladaptive differences (Buss and Greiling 1999). In evolutionary genetic terms, the categories can be rephrased. When speaking of adaptive or maladaptive differences, one is interested in traits that are causally related to fitness, without regard for "where" the variation is coming from (genes or the environment). Nonadaptive sources of difference include neutral variation that, although it may correlate with fitness, does not cause fitness differences; and "by-products of adaptive variation" (Buss and Greiling 1999) that come about through the correlated selection of some other trait. Given the recent shared ancestry and common personality structures between humans and nonhuman primates, explanations offered by evolutionary psychologists are a reasonable starting point for addressing variation in nonhuman primate personality.

Tooby and Cosmides (1990a) were the first to place personality squarely within a modern evolutionary framework, arguing that individual variation was the result of neutral evolution. Most of the variation in the traits that psychologists consider as personality would evolve by drift if behavioral tendencies that are stable across situations are not adaptive; this is because such general tendencies would not be solving any particular problem and thus be causally unconnected with fitness, that is, evolving neutrally. Although the effective population size in humans is large enough that drift is inadequate at reducing genetic variance in neutral traits, all the evidence connecting personality to differences in health, longevity, and reproductive success contradicts the required complete selective neutrality (Penke et al. 2007). MacDonald (1995, 1998) and Nettle (2006) argued instead that variation is maintained by balancing selection for personality differences as alternative behavioral strategies. Human personality dimensions can be cast as trade-offs (Nettle 2006) between mating success and exploration versus risk (Extraversion); vigilance versus the health consequences of stress (Neuroticism), mate attraction versus psychosis (Openness); short-term versus long-term fitness benefits (Conscientiousness); and altruism versus selfishness (Agreeableness). If this is the case, similar trade-offs are likely to manifest in nonhuman primates. As several of these mechanisms are being

investigated in primates (e.g., stress and cooperative behavior), an appreciation of individual differences would reveal whether these fitness trade-offs exist. For example, most primate interaction networks support the emergence of cooperation (Voelkl and Kasper 2009), so primate societies might contain a mix of cooperators and defectors who differ in Agreeableness. Other trade-offs that have not been put forward for humans have been observed in primates, such as decreased vigilance over infants displayed by rhesus macaque mothers while engaged in allogrooming (Maestripieri 1993).

A basic life-history trade-off has also been theorized to underlie human personality and intelligence differences (Rushton 1985; Figueredo et al. 2005; Rushton et al. 2008). This within-species difference in a developmental strategy of investing in fecundity or survival was theorized to extend in humans to family size, interbirth interval, and parental care (Rushton 1985). An individual would either pursue a risky life of multiple mates, large families, and little parental investment or a slow-paced existence with one mate, few children, and long life. Humans disposed toward the latter strategy were found to be less neurotic, more extraverted, more agreeable, and more conscientious (Figueredo et al. 2005). Rushton et al. (2008) combined this with the postulation of a general factor of personality (GFP) underlying the five human dimensions (Musek 2007) to suggest this single factor (capturing differences in cooperativeness and prosociality) is under directional selection along with the associated life-history traits (contra Figueredo et al. 2005, who proposed balancing selection). Although selection has not been estimated for the GFP, genetic analysis of twins showed that all of the genetic variance was attributable to dominance effects (Rushton et al. 2008), which matches a theoretical prediction of long-term directional selection (Falconer and Mackay 1996) and the finding that life-history traits have higher dominance variance (Crnokrak and Roff 1995).

The existence of a general personality factor in humans is a bit tender in its psychometric joints (Ashton et al. 2009), but this does not invalidate the study of life-history traits and personality in nonhuman primates. Personality traits may be separately linked to different life-history variables. In comparison with most other mammals of the same size, primates take longer to gestate and mature, have fewer offspring, and live longer lives (Strier 2003). There is also a significant amount of variation in life-history variables among primate species concerning the speed of gestation, development, and maturation adjusting to fit differences in body size, which is an adaptation to local ecology (Harvey and Clutton-Brock 1985). Trade-offs, then, exist at the within-species level. This can be seen in rhesus macaques, which exhibit a positive genetic correlation between age at primiparity and longevity, so females who start reproducing earlier have a shorter lifespan (Blomquist 2009b), suggesting that a fitness trade-off in life-history strategies potentially exists in nonhuman primates.

The life-history perspective on personality is also favored in theoretical work by behavioral ecologists (Dall et al. 2004; Stamps 2007; Wolf et al. 2007; Biro and Stamps 2008). Personality differences are again conceived of as distinct behavioral strategies (Dall et al. 2004). This body of theory allows us to imagine under what conditions we would not have personalities at all. Two basic "personalities" can

coexist as stable types of competing strategies under frequency-dependent selection (Maynard Smith 1982). However, the same stable situation can emerge if each individual plays a mixture of both strategies. In this case, no personalities exist because each individual is expressing exactly the same behavioral tendency. Individual differences in behavior that can be dubbed personality can emerge, however, if these differences are tied to life-history trade-offs (Wolf et al. 2007; Biro and Stamps 2008). More generally, the fitness of a particular trait may depend on the frequency of other traits being expressed in the population rather than on the frequency of alleles affecting the target trait (Reeve and Dugatkin 1998). For example, the fitness implications of exploratory behavior might depend on conspecifics' aggression rather than on one's own level of exploration–avoidance. The output of such theory has so far been applied exclusively to studies of nonprimate animal personality from the framework of behavioral syndromes (Sih et al. 2004).

For rhetorical reasons, explanations of the persistence of variation in personality are often set up as mutually exclusive possibilities. This need not be the case and is probably an artifact of how selection is usually presented and contrasted (Rice 2004). Directional and stabilizing selection can co-occur on the same trait, changing different moments of the phenotypic distribution. Such possibilities should be exploited when considering how personality evolves (see Sect. 6.6).

Which approach is applicable for nonhuman primates? Both their close affinity with humans and the rich literature on their behavioral ecology (Strier 2003) suggest that combining methodologies from evolutionary psychology and behavioral ecology perspectives are feasible. From an evolutionary genetic perspective, the apparent commonality of several aspects of primate personality structure, such as dimensions related to sociality and anxiety, suggest that certain evolutionary equilibria are maintained over long periods of time in primates. If true, evolutionary genetic processes can be fruitfully investigated using phenotypic data (see Chap. 7). Resolving alternative explanations for the persistence of variation in nonhuman primate personality is particularly exciting because we can compare species that are closely allied because of phylogenetic affinity (e.g., macaques) or socioecological similarity (e.g., chimpanzees and spider monkeys). Nonhuman primates also offer a window through which to chase the evolutionary genetics of personality into the wild.

6.5 Evolution in the Wild

Studying the evolution of personality in primates means eventually studying personality in wild primates. Investigating the selection of personality can proceed along two courses: by relating personality to fitness differences or by indirect inference in comparing how correlations among personality traits differ between populations in varying environments (Dingemanse and Réale 2005). Evolutionary genetic studies in the wild have progressed tremendously through the use of extensive pedigree information, long-term data collection, and the identification of individuals (Kruuk and Hill 2008). Recognizing individual animals in the wild has been central

to traditions in primatology for at least 60 years (Matsuzawa and McGrew 2008). This acknowledgment of individuality and family life eventually led some primatologists to start tracking familial lineages (Kawai 1958; Kawamura 1958; DeVore 1962; Yamada 1963; Carpenter 1964; Goodall 1986). Given the many decades these pedigrees have been curated at some wild primate sites (e.g., Arashiyama, Cayo Santiago, Gombe, and Koshima), it is a wonder that this information has been used only sparingly for evolutionary and quantitative genetic research (the exceptions are, notably, captive populations, those at the Vervet Research Colony and the Southwest National Primate Research Center). More typically, pedigree information is used for the purpose of determining reproductive success, mate choice, and social relationships among kin (see Chap. 3). Quantitative genetic studies of wild primates offer rich, low-hanging fruit of which primatologists are now beginning to partake (Blomquist 2009a, b).

6.5.1 Pedigree Construction

In primates, maternity can be reliably inferred from behavioral data, as infants initially associate exclusively with their mothers (Strier 2003). Paternity is more tricky and typically requires exclusion or likelihood assignment using genetic markers. Currently, the most prevalent molecular markers for pedigree construction are microsatellites (Jones and Ardren 2003). The advantages of microsatellites are that they are relatively easy to discover in new species, are codominant (both alleles can always be recognized, if they differ), are highly variable (making it easier to distinguish individuals), and can be obtained from wild samples (Pemberton 2008). With these markers, a number of algorithms and statistical techniques can be used to assign paternity (Jones and Ardren 2003; Pemberton 2008). For evolutionary genetics, pedigree accuracy is a constant concern because errors lead to imprecision in heritability estimates (Kruuk 2004).

Building pedigrees also allows detection of inbreeding. In primates, inbreeding is primarily a concern in isolated, endangered, or captive populations (e.g., Alvarez et al. 2009). Although the role of inbreeding depression in personality has not been investigated directly (Penke et al. 2007), there is evidence suggesting that it is a possibility (Rebello and Boomsma 2007).

6.5.2 Fitness Is Not What You Think It Is; Rather, It Is Exactly What You Think It Is

"Fitness" is an inconsistently used term in evolutionary studies, with evolutionary psychology being no exception. Many workers have taken definitions of fitness that attempt to distinguish the effect of random drift from that of natural selection. Writing on the subject, authors often adopt, knowingly or unknowingly, Williams's (1966a)

definition of fitness as the average reproductive success of a given "design." For example, Grafen (1988) acknowledged Williams in distinguishing individual lifetime reproductive success from fitness, and Penke et al. (2007, p. 553) described fitness as a property of a genotype, with "its statistical propensity for successful reproduction." Yet these distinctions are not necessary. The cleanest definition marks fitness as an individual's contribution to the next generation, and it is thus a property of individuals and not of genotypes or of alleles (Rice 2004). This interpretation includes both selection and drift in an individual's reproductive success, the difference being whether the covariance between genotype and fitness is random (drift) or nonrandom (selection). It is thus a question of causality.

Lifetime reproductive success is therefore the canonical measure of fitness, but individual differences leading to reproductive success can enter at any stage in an organism's life – the where and when having considerable practical import. Four general components of fitness include survival to breeding age, reproductive lifespan, fecundity, and offspring survival (Brown 1988). Assuming parentage can be assigned, these data can be (and are being) tracked in wild primates. Whichever component of fitness is used, selection is measured with it in the same way. The first step is to test whether the trait of focus is significantly related to fitness (Mitchell-Olds and Shaw 1987) by regressing the trait on fitness. Because annual and lifetime breeding success are not normally distributed but, rather, follow a zero-inflated Poisson or negative binomial distribution, where each year in an animal's life is a chance to "fail" at having an offspring, a generalized linear model should be used instead of an ordinary least-squares regression (Kruuk et al. 2002). The next step is to estimate the strength and mode of selection by regressing the standardized trait on relative fitness as the linear coefficient (for directional selection) or twice the quadratic coefficient (for stabilizing or disruptive selection) using an ordinary least-squares method (Arnold and Wade 1984; Stinchcombe et al. 2008). Because there are competing hypotheses about the role of selection in maintaining variation in personality, it is essential to avoid the publication bias that plagues estimates of the strength of selection (Kingsolver et al. 2001). Given the present state of knowledge on personality in the wild, the absence of selection is as interesting as its presence (Dingemanse and Réale 2005) because we would like to know under what ecological conditions personality differences are adaptive in primates and when they are only neutral.

Selection coefficients of personality traits are already being estimated in wild populations of nonprimate animals (Dingemanse and Réale 2005), so primatologists should follow the lead of behavioral ecologists in applying the tools of evolutionary biology to personality (in contrast to doing psychology with their evolutionary-paradigm beanie on). A difficulty in following this path is that in current studies of nonhuman primate personality, lifetime reproductive success is usually not available simply because the study subjects are still alive. Research on living individuals must then use other components of fitness, such as age at primiparity, interbirth interval, annual reproductive success, or infant survival. Investigations of personality in populations of wild primates are barely embryonic, but a future goal of this research should be longitudinal studies that ultimately measure the implications of personality differences for lifetime reproductive success.

6.5.3 G Matrices Gone Wild

The use of the additive genetic variance–covariance matrix runs into a spot of trouble when taken out of the farm and into the jungle. In agricultural and laboratory conditions, the predictive value of the breeder's equation works because we decide which traits to select. In the wild, however, we can never be certain that we are including all the characteristics that are being selected (Lande and Arnold 1983; Endler 1986). This is one explanation for why, in wild populations, the phenotypic response to selection can be either zero or even opposite of what is predicted from the **G** matrix and the vector of selection gradients (Merilä et al. 2001).

Another general difficulty that must be resolved in the particular is the leap from the estimation of selection gradients to inferences about adaptation (Grafen 1988). Here, we are seeking functional and causative accounts for how personality and life-history variables lead to differences in reproductive success (Pigliucci 2006). In the troop of primates where we find only the introverts having children, is the negative correlation between Extraversion and breeding success chance sampling variation (i.e., genetic drift), or is this connection causal, meaning that there is selection for low Extraversion? In building causal models to distinguish direct selection from indirect selection or random drift, it is essential to have a more complete functional and behavioral understanding of personality differences. In nonhuman primates, personality dimensions based on adjectival descriptors do not enjoy a one-to-one mapping with independent aspects of behavior (Konečná et al. 2008). In langurs, both Confidence and Extraversion correlate with the behavioral dimension Activity, but Confidence is also related to the Dominance and Involvement behavioral components. It is likely that impressionistic dimensions capture personality traits that can be expressed through different aspects of the same behavior, such as the frequency and bout length of grooming sessions. This is precisely the point where behavioral repertoire and syndrome approaches will be of most use in the evolutionary genetics of nonhuman primate behavior, where a syndrome or profile can identify the situational and behavioral units that correlate. Such procedures promise to untangle the ecological variables defining the situations in which personality is differentially expressed and provide testable paths through which trait personality differences might be affecting life-history outcomes and reproductive success.

6.5.4 Into the Wilds of Personality

Although personality researchers can borrow techniques for estimating heritability and selection from evolutionary quantitative geneticists, they are still faced with the problem of collecting personality data on wild animals. Studies that specifically take ecological or evolutionary paths to discovering and defining personality traits are sorely lacking on nonhuman primates (Uher 2008). In addition to highlighting the need to investigate species-specific differences in personality constructs (Uher 2008; Uher et al. 2008), we also need to be open to the possibility of

the same species exhibiting alternative personality structures in different ecological environments (Bell 2005; Uher 2008). Another barrier to educing a whole personality structure of a species in the wild is the number of different populations that can be studied. The incorporation of Openness-like facets into the Extraversion dimension of Hanuman langurs (Konečná et al. 2008) could be peculiar to the population rather than to the species. The small sample of langurs ($n = 27$) is not necessarily fatal, as a fully informative factor structure can be recovered from small samples if the number of factors is small and the number of items large (de Winter et al. 2009). Furthermore, as the chimpanzee factor model replicated in different populations (King et al. 2005; Weiss et al. 2007, 2009), there are unlikely to be broad structural differences between primate populations of the same species, as defined by factor models of personality. Understanding the ecologically relevant differences in primate personality expression, then, requires the finer lens of behavioral repertoire and allied approaches.

A standard adjective rating instrument, such as the Hominoid Personality Questionnaire,[1] can be used to obtain an initial impression of species whose personality has not been previously measured. First, this allows initial integration into other findings about personality structure and will help resolve unknown questions about the historical patterns of personality evolution. Second, as a practical matter, impressionistic ratings can be gathered from raters who, although familiar with the individual animals in the study population, may not have been studying their behavior. Finally, behavioral repertoires might differ between populations (because of slight differences in ecological situations) more than the personality structure is likely to differ.

6.6 Personality As a Norm of Reaction

An interaction between a genotype and a set of environments is called a reaction norm (Dobzhansky 1955; Platt and Sanislow 1998 and references therein). Plots showing hypothetical reaction norms of different genotypes in different environments litter psychology textbooks (Platt and Sanislow 1998) and come up repeatedly in contentious debates about nature and nurture (e.g., Sternberg and Grigorenko 1997). Accounting for these effects will take real work, not just chatter about *Arabadopsis*, *Drosophila*, and *Mus*. Models that estimate variance from $G \times E$ interactions have started making their way into psychological research (Johnson 2007). The concept of a behavioral syndrome explicitly incorporates the idea of personalities as norms of reaction (i.e., a correlated suite of responses across environments) (Sih et al. 2004) and captures the idea that personality depends on context (van Oers et al. 2005b). Envisioning personality in this way may allow behavioral ecologists

[1]Available from Alexander Weiss.

to bypass much of debate between person–situation and ordinary trait perspectives on personality (Penke et al. 2007). However, a norm-of-reaction approach offers a much greater potential for integrating between- and within-individual variation in personality to the intrepid primate psychologist willing to grapple with a few more complexities in their models.

Looking at personality as a reaction norm may fuse various perspectives on personality when we incorporate tools from quantitative genetics (van Oers et al. 2005a). Mischel and Shoda's (1995) person×situation perspective on personality, which looks for stable behavioral profiles, can be recast in terms of reaction norms (Penke et al. 2007). Penke et al. (2007) also noted that Mischel and Shoda (1995) focused their theory on describing individual reaction norms but that as aspects of these behavioral profiles are heritable (Borkenau et al. 2006) it is more appropriate to examine the $G \times E$ level of reaction norm differences. However, the individual reaction norm should not be discarded, even if what we are interested in is genetics.

The $G \times E$ reaction norms are typically investigated using experimental designs that subject a set of genotypes to different environments to assess the phenotypic plasticity of each genotype (Via et al. 1993). What do we do with primates, however, who are generally not keen on being cloned or grown in experimental plots? To the extent that a personality trait varies within an individual, it is a labile trait, changing throughout the course of life (Gomulkiewicz and Kirkpatrick 1992; Lynch and Walsh 1998).

Because personality can be measured multiple times, either in different situations or as the individual ages, it can also be examined using individual reaction norms (Nussey et al. 2007). Individual reaction norms encompass all of the situations and environments in which an individual expresses a trait throughout life. Reaction norms can be scrutinized at both the individual phenotypic and genotypic levels. An individual reaction norm covers person×situation (or environment) variance at the phenotypic level, whereas the genetic reaction norm describes $G \times E$ interactions. Differences in reaction norms may exist at either level (or none or both). These reaction norms have a physiological basis, as seen in rhesus macaques, who have stable serotonin concentrations in early life as they experience a number of stressful events during emigration (Mehlman et al. 1995). The nongenetic variance in individual reaction norms is attributed to permanent environment effects, which for personality could include the influence of early development or of learning. Individual reaction norms can be studied with quantitative genetics using a random regression animal model (Gomulkiewicz and Kirkpatrick 1992; Nussey et al. 2007), which, using repeated measures of personality in different situations and pedigree data, can distinguish the permanent from the genetic sources of interaction variance.

The chief practical difficulties of this approach for the evolutionary genetics of primate personality are twofold. The first is the partition of environments. What situations are considered the same environment in which a personality trait is being expressed? Plus what is a situation? Second, the sample sizes needed to obtain good estimates of these parameters will be arduous to muster for nonhuman primates. Hopefully, primatologists are up to this challenge (van Oers et al. 2005a).

6.7 Conclusion

Reaction-norm representations of the expression, development, and evolution of behavior may be able to separate out the genes, situations, and vagaries of existence that go into determining an individual primate's personality. However, these approaches by themselves have little hope of putting an end to the nature–nurture debate because we cannot fathom that nature + nurture is a model.

The focus here has been on variance component models that break down the causes of personality and of measuring selection on the phenotypic level because this is what the data from most current studies of wild nonhuman primates can support. Researchers familiar with their primate subjects, even if they do not study behavior, are a resource for getting initial impressionistic ratings from which a personality structure can be defined. Once this structure is known and compared within its phylogenetic context, researchers can ask the salient behavioral and ecological questions of why we find a particular personality structure in each species. Many primates live together in groups where kin can be identified and tracked as individuals throughout their lives, supplying information about genetic relatedness and life history needed for evolutionary genetic studies of personality. Ethological investigations of more specific aspects of personality can be used to connect personality differences to fitness-relevant outcomes. To the extent that personality hinders or helps individual or group adaptation to habitat disruption, a thorough understanding of nonhuman primate personality may aid conservation efforts.

Taking evolutionary quantitative genetics more broadly, we should aim to investigate primate personality through population genetics, genomics, and molecular ecology. These techniques are already being used to study the evolution of primate phenotypes, such as coloration, for which specific gene variants have been identified (see Chap. 14). Comparing homologous and convergent personality traits among primate taxa would highlight ecological conditions that pattern structural divergence between species as well as guide the evolutionary study of psychological traits out of the morass of "environments of evolutionary adaptedness" (Symons 1979; Tooby and Cosmides 2005). This would further involve finding gene variants and quantitative loci underlying personality differences, detecting differences in gene frequencies among populations of the same species, and looking for molecular signatures of past selection and demographic changes that explain extant variation in primate personality.

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Chapter 7
Toward a Basis for the Phenotypic Gambit: Advances in the Evolutionary Genetics of Animal Personality

Kees van Oers and David L. Sinn

7.1 Introduction

Individuals of many species, including humans, differ consistently in the way they behave. These consistent behavioral differences among individuals are collectively known as animal personality (Gosling 2001), behavioral syndromes (Sih et al. 2004a), behavioral strategies (Benus et al. 1990), or behavioral profiles (Rodgers et al. 1997). Each of these terms, to some extent, describe an emergent phenomenon of the total biases in behavioral reactions an individual expresses compared to other individuals within the same population or species. In other words, animal personality, in addition to referring to consistent differences between individuals, also refers to correlated behaviors. These correlations (usually defined at the level of populations of individuals) can occur through time (an individual that is bold at one time is also bold at another), across different functional contexts (an individual that is bold toward a predator is also aggressive toward conspecifics), or some combination of time and context (juvenile exploratory behavior is related to adult sociability). Although there is some debate on terminology (e.g., Réale et al. 2007; Gosling 2008), we use the term "animal personality" throughout this chapter.

Interest in animal personality has vastly increased (Fig. 7.1), and recent evidence suggests that consistent between-individual behavioral variation often co-varies with several important indicators of fitness, such as growth, reproduction, and survival (Smith and Blumstein 2008). For example, personality has been shown to influence settlement patterns in Western bluebirds (*Sialia mexicana*), where males aggressively compete for territories. Males with more aggressive personalities are

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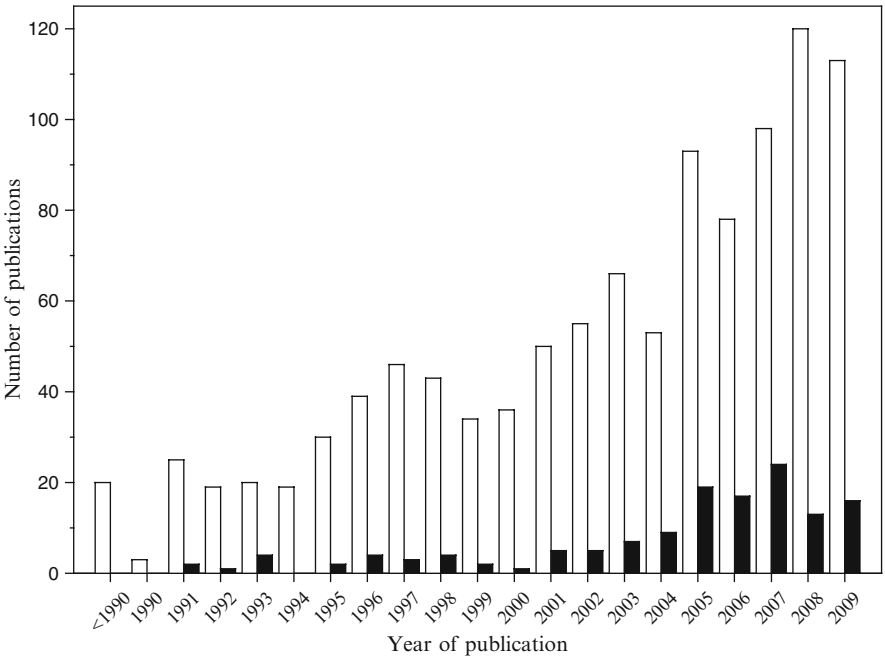

Fig. 7.1 Number of publications for the period 1959–2009 using the Web of Knowledge search engine with the following search terms (01-05-2010): for the white bars (personality): personality, behavi* syndrome, animal temperament, coping style*; for the black bars (personality + heritability): personality AND heritabilit*, behavi* syndrome AND heritabilit*, animal temperament AND heritabilit*, coping style* AND heritabilit*. The number of publications for the years before 1990 are lumped together. We excluded all duplicates, all human studies and all studies that were related to behavioural disorders

more successful in preferred habitats with multiple nesting possibilities per territory (Duckworth 2006b). The physical habitat characteristics of these areas, however, also induce correlated selection on morphology: in preferred areas (where agility is favored) there is positive selection on longer tarsi and tails, whereas in less preferred habitats (where agility was not as important) there was no morphological selection on physical characteristics (Duckworth 2006a).

As this example illustrates, animal personality constructs represent an exciting framework for studying how variation in life-history characteristics relates to variation in fitness across a wide range of taxa (Groothuis and Carere 2005; Réale et al. 2007; Biro and Stamps 2008). Many behavioral ecological studies on personality focus on a so-called phenotypic approach. However, how much of an evolutionary response (i.e., gene frequency changes at the population level) might we expect from, for example, the above-mentioned studies of Western bluebirds? To answer this question, one needs to understand what genetic influences there are on traits, and what genetic covariances might exist between different traits, including behavioral, morphological, and life-history characteristics.

Genetic approaches have proven to be important to answer questions about the adaptive significance and the evolution of life-history traits. However, the genetic basis of behavioral traits in studies in an ecological or evolutionary context has largely been neglected (Sokolowski 2001; Boake et al. 2002; Higgins et al. 2005). Reasons for this may be the lack of genetically tractable natural systems (Wolf 2001) or the behavioral ecologist's focus on field studies, in which genetic analyses can be difficult (Boake et al. 2002). Additionally, abiotic sources of noise (e.g., temperature) or physiological processes (e.g., age and stress) can also generate experimental noise in behavioral assays (Boake 1994; Higgins et al. 2005). Nevertheless, most behavioral traits are expected to be at least partially heritable (Lynch and Walsh 1998; Turkheimer 1998). What is currently unknown, however, is how the genetic architecture of animal personality traits may influence, and is influenced by, the life-history characteristics and diversity of behavior that is currently witnessed in populations of wild animals (Stirling et al. 2002). More importantly, what is needed is an understanding of the degree and genetic nature of the observed phenotypic variation that is currently observed in studies of natural selection on animal personality. The genetic nature of animal personality traits is an essential component of our understanding of both how and why personalities may have evolved in humans and other animals.

7.2 General Definitions of Quantitative Genetic Terms for Delineating Genetic Effects on Animal Personality Traits

Many methods exist for partitioning phenotypic variance into various genetic and nongenetic components. All current methods are based on the principle that phenotypic resemblance among relatives can provide information on the degree of genetic influence on a particular phenotypic trait of interest. Quantitative geneticists attempt to reduce the observed phenotypic variation between individuals into several categories, with the standard approach that the phenotypic value of a trait for an individual, z, is the sum of the genotypic effects of all loci on a trait (G), and an environmental effect (E). Both G and E can be further partitioned into more specific components (some of which are described below), which allows clearer understanding of the ultimate processes that result in observed phenotypic patterns in populations.

- *Additive genetic variation*: This refers to the phenomenon whereby genotypic influences are defined by the additive effects of the alleles inherited from parents or, in other words, the tendency for offspring to resemble their parents regarding the trait of interest. In a simple case where a phenotypic trait is influenced by two alleles at a single locus with complete additivity, the genotypic

values of a homozygous offspring is 2a1 and 2a2, and the genotypic value of the heterozygote would be a1 + a2. Additive genetic variation can also be considered an estimate of the efficiency of a population's response to selection. It is possible for the total genetic variance to be completely additive in nature, but this is probably not a realistic assumption for many personality traits (van Oers et al. 2004c).

- *Dominance genetic variation*: This describes the phenomenon where genes exhibit dominant gene action such that they mask the genetic contribution of the recessive allele at that locus. In the two-locus case given above, if one allele (A) is dominant to the other (a), the phenotypic value of the Aa heterozygote would not be phenotypically distinguishable from an AA homozygote. The dominance effect of a gene locus can be defined as the deviation of the observed genotypic value from the expected genotypic value based on additive effects only. Dominance is a component of a broader class of phenomena whereby genes interact with one another in a nonadditive way. This broader class of nonadditive gene interactions is termed "epistasis." It is worth noting that gene × gene interactions can introduce complex dynamics into researchers' ability to predict evolutionary changes.
- *Maternal effects*: Maternal effects are indirect effects of the maternal phenotype that are expressed in offspring. Although they may have a genetic component (e.g., variation in milk production properties among female mammals), maternal effects are environmental sources of variation from the standpoint of offspring. Maternal effects mean that the phenotypic traits of a population can be heavily influenced by the environmental conditions of the previous generation, such as food availability. Maternal effects have an added level of complexity because maternal environmental sensitivity can also be a function of a female's age.
- *Environmental effects*: All traits are influenced by environmental factors, at least to some extent. As with genetic variation, environmental effects can also be partitioned into several sources. Lynch and Walsh (1998) partition environmental effects into two broad categories. General environmental effects are environmental effects shared by groups of individuals. Experimental treatments or shared patches of habitats are examples of these effects, but these effects can also be experienced in smaller groups (e.g., maternal environmental effects, whereby mothers have general effects on their offspring above and beyond the direct transmission of genes through maternal care). Special environmental effects are deviations from the expected phenotype that are expected based on genetic effects and general environmental effects. These effects are unique to individuals, based on microenvironmental variation and resulting in individual developmental pathways. Ideally, the phenotypic variation among individuals can be conceptualized as the sum of its genotype and its developmental environment, $z = G + E$. However, in reality, different personality phenotypes respond to environmental change in different ways, a phenomenon known as genotype (or individual) × environment interaction (e.g., van Oers et al. 2005; Dingemanse

et al. 2010; Stamps and Groothuis 2010). For further details and definitions of quantitative genetic terms, refer to Chap. 6 of this volume or Lynch and Walsh (1998).

7.3 Phenotypic Gambit

To date, behavioral ecologists have largely assumed that there is heritable variation available for the evolution of behavior by natural selection (Owens 2006). Along with this assumption is the implicit idea that the observed phenotypic patterns of behavior accurately reflect the underlying genetic patterns and that the details of the genetic architecture does not seriously influence the evolution of those behaviors. This phenomenon was first identified by Krebs and Davies (1978) and later named the "phenotypic gambit" by Grafen (1984). On the one hand, the phenotypic gambit should be safe when studying a behavior that is at long-term evolutionary equilibrium because evolution is predicted to lead to behavioral adaptation irrespective of the underlying genetic architecture over evolutionary time (Owens 2006). In most other cases, however, the difference between phenotypic and genetic patterns can be crucial. For example, any relation between phenotypic and genotypic correlations depends heavily on the heritabilities of the two traits and on their environmental covariance (Kruuk et al. 2008). As is detailed below, heritabilities and environmental covariances of personality traits are likely to vary along with changing environments, the latter being a ubiquitous feature of nature.

Two points are relevant here to establish whether the phenotypic gambit is a "safe" bet for studies of animal personality. First, for any particular study system, one needs to understand what part of the phenotypic variation can be attributed to additive genetic variation, genetic dominance, genetic maternal effects, and environmental effects. Second, we need to know something about the way behavioral variation in one trait co-varies with variation in other traits. In other words, personality profiles are likely shaped by multivariate selection resulting from complex fitness landscapes rather than directional, disruptive, or stabilizing selection on any one personality trait in isolation.

Currently, in most animal personality research from wild populations, phenotypic correlations (r_p) form the basis of evolutionary interpretations. As mentioned above, however, phenotypic correlations are often different from genetic correlations (r_g) because r_g also depends on the extent of age- and environment-specific additive genetic expression for each trait. Selection theory predicts that genetic correlations among traits are likely to be common and that these correlations can affect the evolution of individual traits (Lande 1982; Lande and Arnold 1983). This idea of correlated selection also lies at the core of animal personality research (Sih et al. 2004a, b; Dingemanse and Réale 2005; van Oers et al. 2005; Réale et al. 2007). We therefore in this chapter specifically ask the question of whether the phenotypic gambit holds. We do it by reviewing the current standing of the field of quantitative genetic studies on animal personality. We try to show that when we are interested in the evolutionary dynamics of behavior, understanding the genetic structure of behavioral traits becomes potentially important.

7.4 Two Caveats

Before we begin to evaluate whether the phenotypic gambit is a reasonable assumption in studies of animal personality, we first make two caveats. First, although Mendelian genetic models of inheritance have been instrumental in describing observations of biological inheritance, it is being increasingly recognized that the models of modern evolutionary theory that underpin population and quantitative genetics (i.e., whereby inheritance is solely due to the transmission of DNA from parents to offspring) is incomplete (Uller 2008; Bonduriansky and Day 2009). Several studies now suggest that nongenetic mechanisms of inheritance occur in many taxonomic groups across a wide range of phenotypic traits. For example, parental and ancestral genetic influences can be altered through learning, cultural transmission, maternal environmental effects, or other mechanisms of developmental plasticity (Mousseau and Fox 1998; West-Eberhard 2003). Furthermore, transfer of epigenetic or cytoplasmic components between parents and offspring can have important evolutionary influences on phenotypic traits (Jablonka and Lamb 1995, 2006). Although nongenetic mechanisms of inheritance no doubt are an exciting field of study in behavioral and evolutionary ecology, we have chosen not to cover them here, largely because many of these aspects of nongenetic mechanisms of inheritance have not been studied with regard to animal personality traits (but see Daisley et al. 2005; Tobler and Sandell 2007; Groothuis et al. 2008).

Our second caveat is that here we consider genetic studies of animal personality from wild populations only or from studies that examine genetic inheritance in laboratory offspring from wild-caught parents. Currently, most information available on the structure of inheritance of personality traits comes from humans and other nonhuman primates (e.g., Bouchard 2004; Fairbanks et al. 2004; Savitz and Ramesar 2004), rodents (e.g., Sluyter et al. 1996), and domesticated species (e.g., Burrow 1997). Although genetic studies of human personality have been immensely valuable in demonstrating genetic influences on personality traits, interpreting patterns of genetic variation of personality in humans in evolutionary terms remains difficult (Bouchard 1994; Nettle 2005, 2006).

Animal models, on the other hand, have proven more useful for understanding the underlying genetic mechanisms of behavioral traits (e.g., Wehner et al. 2001). Unfortunately, most genetic studies on personality traits in animals to date have been on populations that were bred in captivity over long periods (e.g., laboratory animals and domesticated species). Although studying laboratory model organisms offers tremendous advantages in terms of control, replication, and convenience, laboratory studies also provide novel, stable, uniform, benign environments where selection is unlikely to operate as it would in wild populations (Merilä and Sheldon 2001). In cases where laboratory populations have been maintained through time (sometimes only for several generations), and with sufficient competition, genetic adaptation to laboratory environments has presumably occurred (e.g., Blanchet et al. 2008). Laboratory estimates of heritability may therefore not be good predictors of heritability in natural populations owing to the reduction in environmental

variability (but see Riska et al. 1989; Drent et al. 2003). Note that this does not mean that artificial selection is fundamentally differently from natural selection or that measures of selection in captive populations are not useful. Selection processes in natural populations might, however, be important for generating and maintaining specific patterns of genetic variation and covariation in and between particular personality traits. Studies of artificial selection therefore may give important insight into the selection process and its outcomes but not necessarily on the strength of natural selection and corresponding genetic response given a particular set of population genetic and environmental conditions observed in wild populations.

7.5 First Evaluative Criterion for the Phenotypic Gambit: Consistency of Heritabilities

Currently, most genetic studies on animal personality traits focus on establishing at least some sort of genetic basis for their phenotypic traits of interest (see Table 7.1 for a list of studies), and there is now evidence that there are significant genetic influences on many animal personality traits (van Oers et al. 2005; van Oers and Sinn 2010). However, most nonhuman animal studies estimate heritability at one point in time or use a pedigree (e.g., Quinn et al. 2009) to calculate an overall heritability for a single population; they therefore often neglect the fact that heritability is not necessarily a static property of a population (Dingemanse et al. 2009). Importantly, the expression of genetic variation is often dependent on the quality or predictability of the environment (Hoffmann and Merilä 1999; Wilson et al. 2006; Dingemanse et al. 2009). In this case, differences in heritability may be caused by covariation between gene expression and environmental conditions.

In a recent meta-analysis, Charmantier and Garant (2005) estimated genetic parameters across heterogeneous environments in wild populations. They reported an emergent trend of higher heritability under more favorable conditions, which was statistically significant for morphological but not life-history traits. This indicates that environmental conditions can have important consequences for predicted responses to selection (see also Quinn et al. 2009). Comparisons across environments indicate that there is often significant covariance between the expression of genetic variance and measures of environmental quality; however, there appears to be no strong generalization as to how heritability is likely to change in direction with regard to environmental conditions (Hoffmann and Parsons 1991; Weigensberg and Roff 1996). Studies on the response of heritability of personality traits to changing environments are sorely needed (but see Dingemanse et al. 2009). Almost nothing is known about the mechanisms by which environmental factors alter phenotypes by modifying gene expression (Bateson et al. 2007).

Another way in which heritability can vary is when genes are differentially expressed depending on an animal's age or sex. In many cases, population-level phenotypic variation for traits and their underlying gene expression in individuals

Table 7.1 Studies that have estimated heritability estimates for animal personality traits using wild subjects or offspring from wild-caught parents

Author-Year	Taxon	Age	N_{est}	N_{traits}	Trait name	Sex	h^2	r_g [a]	N_{sub}
Bakker (1994)	Teleostei	Ju	5	2	Aggression	M	0.37	0.50	128
		Ju			Aggression	F	0.25	1.05	171
		Ad			Aggression	M	0.23		250
		Ad			Aggression	F	0.31		296
		Ad			Dominance	M	0.34	ND	88
Bell (2005)	Teleostei	NG	6	3	Activity	NG	0.05	0.70	29
					Activity		0.17	0.85	42
					Aggression		0.01	0.84	29
					Aggression		0.14	0.26	42
					Boldness		0.04	0.75	29
					Boldness		0.00	0.65	42
Colvin and Gatehouse (1993)	Lepidoptera		1	1	Total flight time		0.39	NA	120
Dingemanse et al. (2002)	Passeriformes	Co	5	1	Exploratory behavior	M,F	0.22	NA	42
		Co			Exploratory behavior		0.31		59
		Co			Exploratory behavior		0.34		63
		Ju			Exploratory behavior		0.61		50
		Ju			Exploratory behavior		0.37		33
Dingemanse et al. (2009)	Teleostei	Ju	24	6	Exploration novel environment	M,F	0.22	0.65	577
					Exploration novel environment		0.33		577
					Exploration novel environment		0.17	0.84	464
					Exploration novel environment		0.14		464
					Activity 2 h post-release		0.12		577
					Activity 2 h post-release		0.02		577
					Activity 2 h post-release		0.31	0.78	464
					Activity 2 h post-release		0.24		464
					Activity 4 h post-release		0.03		577
					Activity 4 h post-release		0.13		577

					Activity 4 h post-release		0.30	1.17	464
					Activity 4 h post-release		0.25		464
					Sociability		0.05		577
					Sociability		0.24		577
					Sociability		0.20	0.74	464
					Sociability		0.42		464
					Exploration novel conspecific		0.19		577
					Exploration novel conspecific		0.03		577
					Exploration novel conspecific		0.33	0.83	464
					Exploration novel conspecific		0.22		464
					Boldness		0.27	0.87	577
					Boldness		0.18		577
					Boldness		0.35	0.81	464
					Boldness		0.28		464
Drent et al. (2003)	Passeriformes	Ju	3	1	Exploratory behavior	M,F	0.54	NG	414
		Co			Exploratory behavior	M,F	0.25		414
		Co			Exploratory behavior	M,F	0.33		414
Duckworth and Badyaev (2007)	Passeriformes	Co	1	1	Aggression	M	0.45	NA	231
Gervai and Csanyi (1985)	Teleostei	Ad	7	6	Exploration of novelty	M,F	0.97	NG	358
					Defense in novel environment		0.96		358
					Timidity		0.92		358
					Territoriality		0.12		358
					Activity 1		0.55		358
					Activity 2		0.89		358
					Emotionality		0.88		358
Han and Gatehouse (1993)	Endopterygota	Ad	1	1	Duration of flight	M,F	0.27	NA	31
Philipp et al. (2009)	Teleostei	Ad	1	1	Feeding boldness	M,F	0.15	NA	4,141
Pruitt and Riechert (2009)	Agelenidae	SA	1	1	Social tendency	M	0.32	NA	30

(continued)

Table 7.1 (continued)

Author-Year	Taxon	Age	N_{est}	N_{traits}	Trait name	Sex	h^2	r_g [a]	N_{sub}
Réale et al. (2000)	Ruminatia	Co	1	1	Boldness	F	0.21		35
Réale et al. (2009)	Ruminatia	NG	2	2	Boldness	F	0.39	–	135
					Docility		0.65	0.38	
Sinn et al. (2006)	Cephalopoda	Ju	6	3	Boldness in threat context	M,F	0.21	ND	147
					Activity in threat context		0.67		147
					Reactivity in threat context		0.89		147
					Boldness in feeding context		0.08		147
					Activity in feeding context		0.05		147
					Reactivity in feeding context		0.00		147
van Oers et al. (2004b)	Passeriformes	Ju	2	1	Risk taking	M,F	0.19	0.84	73
					Risk taking		0.32		92
Wright et al. (2003)	Teleostei	NG	1	1	Shoaling tendency	M,F	0.23	NA	80

Subject age classifications were classified into either juvenile (Ju), subadult (SA), or adult (Ad) life stages where possible from original reports. Co = stages combined; NG = not given; NA = not applicable; ND = not detectable

Sample sizes for studies that used selection lines to estimate heritability include all subjects that contributed to estimates from all selection lines. N_{est} = number of reported estimates; N_{traits} = number of traits; N_{sub} = number of subjects; h^2 = heritability; r_g = genetic correlation

[a]Note: For Bakker (1994), genetic correlations reported represent estimates between the same trait across two ages (i.e., juvenile and adult aggressiveness). The genetic correlation estimate between aggression and dominance was not different from zero. For Bell (2005), correlation estimates correspond to the next trait listed. For example, the first two estimates are the genetic correlation between activity and aggression for two populations, etc. For Dingemanse et al. (2009), genetic correlations given represent estimates between the same trait from the same population of individuals across different predator versus non-predator developmental environments (i.e., no across-trait correlations are given). For Sinn et al. (2006), correlations between the same trait in different contexts was not detectable, and correlations of different traits within the same context were not estimated. For van Oers et al. (2004a), the correlation estimate is taken from van Oers et al. (2004c) and represents the correlation between risk-taking and early exploratory behavior

changes with age, and constancy of estimates of genetic contributions to traits across the lifespan is probably not a realistic assumption. For example, heritability for many morphological traits is known to vary over ontogeny (Réale et al. 1999; Badyaev and Martin 2000; Uller et al. 2002). For many morphological traits, estimates of additive genetic variation also differ between the sexes, suggesting significant sex differences in genetic architecture (Coltman et al. 2005; Kruuk et al. 2008). Sex differences in the genetic architecture of fitness-related traits appear to be linked to mating systems and may be most pronounced in highly polygynous species with high degrees of sexual dimorphism. Note that basic patterns of sex-specific additive genetic variation are central to understanding antagonistic pleiotropic effects and, therefore, the maintenance of variation in personality. For example, it is conceivable that some alleles would be associated with enhanced male fighting success but reduced female fecundity due to their antagonistic effects on levels of hormones in each sex (e.g., testosterone) (Kruuk et al. 2008).

Currently, although many animal personality traits have a significant genetic basis (van Oers and Sinn 2010), there is a basic lack of understanding of environmental, age-specific, and sex-specific effects on contributions of genetic variation to animal personality traits. Further to this is how these parameters may have differential effects on some personality traits but not others within an animal system (Weiss et al. 2000; Sinn et al. 2006). Information on environmental qualities and age- and sex-specific effects on the relative difference in the amount of additive genetic variation between different animal personality traits could give information on the selection pressures and fitness consequences that act or have been acting on these traits (Kimura 1958). Currently, most animal personality studies report the heritability for a single trait only and are hampered by small sample sizes and thus lack of statistical power. It is probably unlikely that most animals express behavioral variation along only a single trait axis. Further work is needed on differences in genetic influence on various personality traits through time and across environments using known-pedigreed individuals in longer-term studies (see Réale et al. 2007 for a proposed framework). Recently developed statistical methods, (i.e., "animal models") should allow for increased power in at least some study systems (Kruuk 2004) (see Chap. 6).

7.6 Second Evaluative Criterion for the Phenotypic Gambit: Genetic Correlations

Whereas narrow-sense heritability quantifies the amount of variation that can be attributed to additive genetic variation, genetic correlations measure the degree to which traits have genes in common or when genes are co-inherited due to linkage disequilibrium (Roff 1996). Genetic correlations, therefore, can arise through pleiotropy or can be caused by linkage disequilibrium. In the case of pleiotropy, individual genes have effects on several traits. The effects of a gene on two traits might themselves be independent or structurally linked (de Jong 1990).

Independence of the effect of a gene on different traits is usually assumed in quantitative genetics rather than structural pleiotropy. Linkage disequilibrium exists when traits are affected by different sets of genes, but a selective force generates and preserves particular combinations of alleles at a particular locus (Price and Langen 1992; Falconer and Mackay 1996; Lynch and Walsh 1998). Genetic correlations between traits can constrain evolutionary change of singular traits, as during selection on one trait genetic correlations influence the selection response of the other (Gromko 1995). However, a genetic correlation does not act as a constraint if the effects of a gene on two traits are themselves independent. If consistent individual differences are adaptive (Wilson 1998; Buss and Greiling 1999), the coherence between different personality traits could well be a product of adaptive evolution as well (Wolf et al. 2007).

The existence of trade-offs between different components of fitness is fundamental to the concept of correlated suites of traits and therefore personality. Prerequisites for trade-offs to act as evolutionary constraints from a quantitative genetic standpoint is that: (1) the two traits have a genetic basis, and (2) there is either antagonistic pleiotropy where the same gene or genes are involved in both traits or there is a genetic correlation between the traits (Lande 1982; Kruuk et al. 2008). The word "constraint" as used here should not be confused with an absolute evolutionary constraint (Roff and Fairbairn 2007). Additive genetic effects and genetic correlations are states in an evolutionary trajectory, not necessarily absolute constraints that might hamper the evolution of a trait (e.g., Bell 2005; Dingemanse et al. 2007; Roff and Fairbairn 2007). Genetic covariance only limits evolution of possible trait combinations when the genetic correlation between two traits is −1 and constant because independent selection does not have any effect on the separate traits (Roff and Fairbairn 2007). As heritability for most quantitative traits is variable, this scenario is highly unlikely for personality traits. Hence, a particular genetic structure for a personality profile is most likely not an absolute constraint for an adaptive evolutionary response but, instead, may represent a consequence of past evolutionary processes.

Estimates of genetic correlations are fundamental to understanding the evolution of behavioral constructs such as personality. The functional architecture of personality traits has been debated in various approaches to human personality research (see McCrae et al. 2001 for references), but most human personality approaches share commonality in that they report an underlying genetic structure that induces the genetic inheritance of a suite of personality traits (Bouchard and Loehlin 2001; McCrae et al. 2001).

Several animal personality studies have now reported moderate to high genetic correlation values ranging from 0.4 to 0.9 (van Oers et al. 2005, Table 7.1; Moretz et al. 2007; van Oers and Sinn 2010). What effect these correlations have on behavioral evolution is currently a point of discussion. Moretz et al. (2007) pointed out that the evolutionary influence of strong genetic correlations may be small, as over time strong genetic correlations might decrease differences among individuals in a population. Therefore, unless correlational selection is acting on the two traits simultaneously or there are temporal fluctuations in environmental conditions

(Sinervo and Svensson 2002), the originally strong phenotypic or genetic correlations are likely to disappear. In other words, selection against particular combinations of traits causes other combinations to be more frequent, and this eventually decouples the correlation. Hence, unless correlational selection is strong and chronic (Bulmer 1989; Falconer and Mackay 1996), linkage disequilibria built up by correlational selection is expected to weaken rapidly and therefore would not constrain evolution of the separate traits (Dingemanse and Réale 2005; Penke et al. 2007). In contrast, mutations most likely counteract these effects as they are more likely to be of the rare type. Moreover, selection in both human and animal personality traits has been found to fluctuate over time and space (Moretz et al. 2007), possibly causing genetic correlations to fluctuate (see above), thereby preventing erosion of genetic variation in the two traits simultaneously. On the other hand, weak, but continual, genetic correlations may have a profound evolutionary impact when considered over long periods of time, especially for quantitative behavioral traits (Sgro and Hoffmann 2004). Here, strong genetic linkage between some of the genes involved may produce only weak phenotypic correlations, which over long periods of time have profound effects on the direction of evolutionary change. The next step, therefore, in animal personality research is to begin measuring genetic correlations in various environments and then track these correlations over several generations to understand how they may change owing to variation in selection pressures and direction.

In a study on exploration in a natural population, Dingemanse et al. (2004) found a second indication for selection regarding sets of behavioral traits. They showed differences in selection pressure on exploratory behavior for males and females and different selection pressures over three different years. Considering the differences in selection pressure together with the prerequisites of correlational selection, the genetic correlations found are built up and maintained by correlated selection only if variation in natural selection on one trait covaries with variation in selection on another trait. Because this seems unlikely, structural pleiotropy seems to be a potential explanation for genetic correlations but see Sinn et al. (2010). This does not exclude differences in correlational selection to be a major factor for explaining differences among populations, however. Phenotypic and genetic correlation among traits in a population can also be the result of selection on sets of traits when certain combinations of traits are more fit than others. Nevertheless, the absence of phenotypic correlations in a population does not automatically imply that the existence of a correlation in other populations is caused by correlational selection. For example, in a study by Bell and Sih (2007), no correlation between boldness and aggression was found in a population of stickleback fish before a predation event. In a controlled predation experiment, predation was found to act on only one trait, (i.e., boldness); and although there was no selection on the combination of the two traits, the surviving population showed a positive phenotypic correlation between the two traits (boldness and aggression). Hence, the existence of genetic correlations is not proof for an absolute constraint or lack of a potential response to selection, and the presence of correlated selection does not imply independent evolution of personality traits.

As personality traits are likely to have complex genetic correlations with other traits (e.g., Merilä and Sheldon 1999, 2000), the potential for evolutionary change

to be limited by a lack of genetic covariance in the multidimensional direction favored by selection is in need of investigation (Blows and Hoffmann 2005; Moretz et al. 2007). To date, however, there is a surprising dearth of studies on genetic correlations of animal personality traits in variable environments in populations of wild animals, whereas genetic correlations are known to vary and even switch signs over environments for morphological and life-history traits (Sgro and Hoffmann 2004). The calculation of genetic correlations for behavioral traits is often complicated by the notoriously high standard errors (e.g., van Oers et al. 2004b). In short-term studies of wild populations this is problematic; but with information on multiple generations and pedigree files, the calculation of meaningful genetic correlations is not impossible. As well, simpler methods, such as full- or half-sibling mean correlations have on some occasions proven to be valuable (Astles et al. 2006).

7.7 Is the Phenotypic Gambit a Safe Bet?

Given the collection of studies amassed in Table 7.1 as well as taking into consideration what is known from the larger field of evolutionary biology, what can we conclude about the phenotypic gambit? On the one hand, it is probably a safe bet in the sense that it is likely that of many animal personality traits probably have detectable levels of genetic variation (Dobzhansky et al. 1977; van Oers and Sinn 2010; Table 7.1). In this sense, natural selection may have predictable gene frequency effects on populations through time. On the other hand, the phenotypic gambit may be dangerous as so little is known concerning how (or if) animal personality trait heritabilities are age-, sex-, and environment-specific. Because the variability in genetic covariation between traits partly depends on the variability of the heritability of each of these traits, genetic correlations are also likely to fluctuate over time and possibly among (sub)populations. The existence of genetic correlations is therefore not proof of an absolute constraint or lack of a potential response to selection. In conclusion, there is no identifiable pattern in the genetic structure of personality traits in natural populations to date. Previous to predicting responses of animal personality traits to natural selection, more studies of the factors that may influence the additive and nonadditive genetic variation and genetic correlations of personality traits are urgently needed.

Because the expected response to natural selection on any trait depends on its underlying genetic structure, quantitative and molecular genetics are integral components of our understanding of studies on the fitness, natural selection, and evolution of animal personality traits. These complex aspects of personality genetics are in common with many quantitative traits, and therefore we believe the field of animal personality genetics is well poised to make significant contributions to a greater understanding of evolutionary biology. Although it is probably fair to say that the genetic analysis of animal personality traits, especially in wild populations, is in its infancy, we believe that it is also true for relatively descriptive questions, yet to be adequately addressed – How much genetic variation is there for a personality trait in

wild populations? What is the genetic architecture of a personality profile? – will prove fruitful not only to our understanding of the evolution of personality but also to a larger understanding of the processes involved in evolution by natural selection.

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Chapter 8
Applications of Personality to the Management and Conservation of Nonhuman Animals

David M. Powell and Marieke Cassia Gartner

8.1 Introduction

A growing body of literature over the last two and a half decades has shown us that, like humans, nonhuman animals demonstrate consistent behavioral differences from one another and sometimes from one population to another. These differences have been termed personality (e.g., Gosling and John 1999), temperament (e.g., Hansen and Møller 2001), and behavioral syndromes and types (e.g., Sih et al. 2004). These concepts have come from a variety of disciplines, including comparative psychology, behavioral ecology, evolutionary biology, ethology, and population genetics. Although people who work with animals regularly have known for some time that animals demonstrate these consistent behavioral traits, it has not been until recently that scientists have formally recognized the phenomenon in animals and actively engaged in research in this area. What were historically considered curious differences between individuals and populations are now thought to be of major significance in understanding how animals make decisions, how they interact with individuals of their own and other species, and how their populations evolve. It has also been suggested that these differences have or will have an influence on the persistence of populations in the face of anthropogenic environmental change (e.g., McDougall et al. 2006) and the likelihood that populations of some species can be reestablished in the wild (e.g., Bremner-Harrison et al. 2004).

We are still only beginning to understand (1) the extent of variation in individual differences in behavior within and between species; (2) the methods by which this variation can be measured; (3) the impact that this variation has on individual survival, reproductive success, and well-being; and (4) the impact that this variation

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has on the evolution of populations. In this chapter, we survey the theories, methods, and findings from personality research in nonhuman animals and discuss some of its current applications in management and conservation settings. Finally, we suggest some areas for future research and speculate on how personality could be more broadly utilized in the management of captive and free-ranging wildlife.

8.2 Personality in Nonhuman Animals

Although the scientific study of personality in animals is relatively new (Gosling and John 1999), it has engendered a large body of literature. Studied species include water striders (*Aquarius remigis*) (Sih and Watters 2005), three-spined stickleback (*Gasterosteus aculeatus*) (Bell 2005), felids [Wielebnowski 1999 for cheetahs (*Acinonyx jubatus*) and Wielebnowski et al. 2002 for clouded leopards (*Neofelis nebulosa*)], giant pandas (*Ailuropoda melanoleuca*) (Powell and Svoke 2008; Powell et al. 2008), orangutans (*Pongo pygmaeus/Pongo abelii*) (Weiss et al. 2006), chimpanzees (*Pan troglodytes*) (Weiss et al. 2007), gorillas (*Gorilla gorilla*) (Gold and Maple 1994), black rhinoceros (*Diceros bicornis*) (Carlstead et al. 1999a, b), rhesus monkeys (*Macaca mulatta*) (Stevenson-Hinde et al. 1980a, b), swift foxes (*Vulpes velox*) (Bremner-Harrison et al. 2004), and hyenas (*Crocuta crocuta*) (Gosling 1998), among others. Overwhelmingly, these studies have found that individual differences in behavioral tendencies or personalities do exist in nonhuman species (Gosling and John 1999).

The definitions of the terms used to describe these tendencies vary. The term personality is almost always used in reference to humans, and some argue that it should also be used for nonhuman animals (Gosling 2008). Temperament, although often used synonymously with personality, has also been defined as mainly having a genetic basis (Box 1999). A behavioral syndrome – a suite of correlated behavioral traits (Sih et al. 2004) – is defined on the species or population level. For example, one population may be more aggressive than another: populations of funnel web spiders with low food availability evolved higher aggression levels across contexts than populations with abundant resources (Riechert 1993). Sih et al. (2004) also discuss behavioral types, which are reflected in the behavior of individuals (a more aggressive animal versus a less aggressive one). Although the wording of definitions for temperament or personality varies from scientist to scientist, these terms are generally described as consistent behavioral differences in individuals over time and across contexts. It seems to us much more useful to think of them as consistent behavioral tendencies because personality characteristics likely exist along a continuum rather than in absolute dichotomous states (see discussion by Gosling and John 1999). Despite the varying terminology and definitions used, it is clear that these behavioral tendencies are real and are quantifiable in a variety of experimental and observational settings. In addition, hypotheses and predictions can be tested regarding the impact of personality on behavior, reproduction, survival, and well-being. In this chapter, we use the term personality for the sake of consistency.

8.2.1 Assessing and Measuring Personality

Assessment of personality in animals has historically been carried out in three ways: recorded behavior, observer ratings, and behavioral tests (Manteca and Deag 1993) (see Chap. 5) (Fig. 8.1).

The three classes of methods have their own advantages and disadvantages. Recording behavior of an individual in its "home" environment and/or social group (e.g., Bard and Gardner 1996) arguably provides the most reliable and comprehensive picture of what its consistent behavioral tendencies are in a variety of settings. The difficulty emerges when trying then to understand which behaviors are the most important for distinguishing individuals or how to compile behaviors into some kind of composite score (Altman 1974). Also, these methods require significant amounts of time so the animal can be observed in a variety of situations and the behaviors observed can be considered reliable responses.

In response to some of these issues, many studies have made use of observer ratings, behavioral tests, and in several studies a combination of the two. An observer familiar with the individual(s) should theoretically be able to provide feedback on the personality of the animal(s) because they have spent considerable time with the animal already and have seen its responses to a variety of situations (Vazire et al.

Fig. 8.1 Common behavioral tests of personality in animals often involve exposing them to novel objects, mirrors, or other challenges designed to assess reactivity. (Photos: Jessie Cohen, Mehgan Murphy, Smithsonian's National Zoo)

2007). Their observations can be used much more quickly to produce a sketch of the animal's personality. The challenges in this class of methods have been to (1) validate that what the observer says really reflects behavioral differences among individuals and (2) design surveys that incorporate and define anthropocentric terms that can be clearly understood by respondents and applied to animals (e.g., What does "confidence" look like in a lion?). Several studies have been able to validate observer ratings in terms of their reflection of behavioral differences (Carlstead et al. 1999a, b; Wielebnowski 1999; Powell and Svoke 2008), but in some cases researchers have found a lack of concordance between some behavioral traits and a surveyed characteristic or that a surveyed characteristic does not apply to the studied species (Gartner and Powell, submitted; Phillips and Peck 2007).

Behavioral tests have a long history in the field of psychology (Archer 1973). These tests are relatively easy to conduct, and the testing methodology can usually be standardized across subjects, a factor that is not always possible or practical to achieve in recorded behavior studies. As these methods have been used for some time, there is also a large body of literature from which to draw guidance and an understanding of comparative aspects of animal personality. However, these tests arguably measure only a narrow selection of personality traits (e.g., "reactivity" or "fear"). By design, these tests measure how animals respond to environmental challenges that may be considered threatening, and in most cases they test single individuals, so they cannot tell us about personality traits that relate to relationships and interactions with conspecifics (e.g., "sociable" or "playful"). There is also still the question of what variables behavioral tests actually measure (e.g., latency to approach a novel object) and how to interpret the behaviors observed during the test (e.g., playing with a novel object versus sitting on or next to it) (Fig. 8.2).

We see several viable lines of future research regarding the methodology of studying animal personality. First, in studies of recorded behavior, how much observation is needed to provide a reliable snapshot of behavior, and can indices or

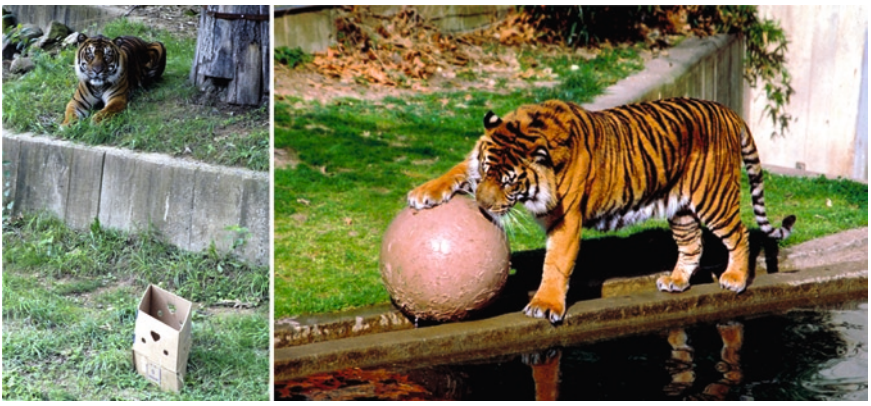

Fig. 8.2 Although novel object tests are a common tool for assessing animal temperament, it is not always clear what behavioral variables should be measured during the test and how they should be interpreted. (Photos: Jessie Cohen, Smithsonian's National Zoo)

composite scores be developed based on those observations that provide a holistic measure of personality? For observer rating studies, can a standardized set of personality traits or adjectives be identified and empirically defined that can be used across taxa or at least a subset of related species (e.g., felids)? This would significantly facilitate the comparison of findings across studies. Similarly for behavioral tests, is there a set of standardized variables to measure that are the most informative, and can we agree on their interpretation? Our review of the literature demonstrates that much of the animal personality work that has been done has focused on mammals, and it remains to be seen how well these methods of assessment work for other taxa.

8.2.2 Theoretical Treatment of Animal Personality

Until recently, the theoretical framework of personality was based largely on and applied to humans, without a corresponding body of personality theory for animals. Some psychologists are therefore looking into how transferable human theories of personality are to animals, and they are developing new theories that include nonhuman animals. Behavioral ecologists have taken theoretical and empirical approaches to understanding animal personality as well.

Gosling and John (1999) reviewed 19 studies of personality across 12 nonhuman species using the Five-Factor Model, a hierarchical model of personality that was developed from studies of humans and is one of the generally accepted theories of personality. Each of the five factors represents a broad, abstract level of personality, which is comprised of more specific traits, each of which can be described by certain behaviors. For instance, animals that are outgoing would be labeled social or active, and these traits would fall under the broad factor "extraversion versus introversion"; the four remaining factors are "neuroticism versus emotional stability," "agreeableness versus antagonism," "open versus closed to experience," and "conscientiousness versus carelessness." The authors found that three of these factors – extraversion versus introversion, neuroticism versus emotional stability, agreeableness versus antagonism – generalized the most across species. Open versus closed to experience followed, with seven of the species studied showing such traits. Finally, the factor labeled conscientiousness was found only in chimpanzees.

Recently, behavioral ecologists have developed a theory of personality around behavioral syndromes, or suites of correlated behaviors that are consistent across different contexts (Sih et al. 2004). It is posited that these syndromes can have both ecological and evolutionary implications (Sih et al. 2004). One aspect of the existence of behavioral syndromes is behavioral plasticity. If an individual with active tendencies always has active tendencies, a context that calls for cautiousness (e.g., a nearby predator) may not be met with the optimal behavior. Behavioral syndromes therefore explain "inappropriate" behaviors, but Sih et al. (2004) also argue that these syndromes are adaptive. In addition, behavioral syndromes can affect species distribution, tendencies of species to respond to environmental change, and speciation rates (Sih et al. 2004). For instance, activity syndromes can limit distribution in that very active animals typically stay in predator-free habitats,

whereas less active animals utilize predator-heavy habitats. Behavioral syndromes can affect a species' response to environmental change negatively: As mentioned earlier, the limited plasticity implied by the presence of a behavioral syndrome can lead to more predation but also to the decline of the species if too many "inappropriate" behaviors are exhibited, especially in a rapidly changing environment. However, if a mix of behavioral types is present among individuals, a species as a whole may be able to respond more appropriately because different survival strategies may then be exhibited. Finally, speciation rates are affected by behavioral syndromes in birds; for example, those that were more exploratory (specifically, showed more feeding innovations) had higher speciation rates (Webster and Lefebvre 2000 in Sih et al. 2004). According to Sih et al. (2004), the innovative behavior was socially transmitted and enabled the population to access new habitats, resulting in speciation.

Although personality is an individual attribute that likely has an effect on fitness (Biro and Stamps 2008) (see Chap. 6), the performance of individual behavioral types within a population depends partly on the mixture of behavioral types in that population because some behavioral types are more likely to cooperate with one another whereas others are more antagonistic. Researchers are just beginning to consider what impact personality has on overall group dynamics and the long-term stability and survival of groups. Sih and Watters (2005) found that the mixture of behavioral types in a group affects both individual and group fitness, which can depend on the social environment. By experimentally manipulating the behavioral types that comprised groups of water striders, the authors showed that the mix of behavioral types in the group affected the group outcome (e.g., a group of low activity/aggression males led to the creation of a hyperaggressive male, which inhibited mating in the group) and individual outcomes (the hyperaggressive males were less likely than the other males to mate).

8.3 Management and Conservation of Wildlife

Zoos and aquariums strive to maintain genetically and demographically healthy populations of animals for the long term. To this end, husbandry and management protocols are developed to keep individuals and populations physically and mentally healthy and capable of successful reproduction and rearing of offspring. The species in these settings have not evolved in the environments in which they now live, and they have not been subject to a long history of intense artificial selection for behavioral and/or physiological traits as is the case for domesticated animals. Working with comparatively small collections (as compared to laboratories and farms) of rare and endangered wildlife requires a keen ability to be able to predict how animals will cope with and respond to challenges from the physical and social environment. Because an animal's personality can predict how it will respond to different situations in which it is put, it can be used to understand and promote well-being (Vazire et al. 2007).

A central component of captive animal husbandry in zoos is environmental enrichment, which is the practice of providing stimulating environments for animals that

promote the expression of species-typical behavior and provide opportunities for animals to have choices and control over their environment (Association of Zoos and Aquariums Behavior Advisory Group 2009). Enrichment encompasses the design of appropriate exhibits, the management of species-typical social groups, and the introduction of stimuli (sights, sounds, smells, objects) to the animal's environment. Personality likely has a major influence on how animals respond to new environments (e.g., new exhibits or holding areas), to familiar and unfamiliar conspecifics and individuals of other species, and to changes in their surroundings. If the animal's personality, or the species' behavioral syndrome, is taken into account when designing environments and husbandry practices, well-being should be optimized.

Gartner and Powell (submitted) assessed personality in snow leopards (*Uncia uncia*) by examining their reaction to a novel object and comparing it to keeper assessments of personality via a survey. Their results suggested that personality could be used to design management programs, including assessing the value of enrichment and decreasing stereotypies; for instance, a shy animal should be given more places to hide, and a bold animal might need more novel items to explore.

Several authors have suggested that temperament be considered during the process of introducing animals to new exhibits and to each other (Gold and Maple 1994; Barlow et al. 2006; Powell 2010). For example, Gold and Maple (1994) identified four personality dimensions in captive gorillas and suggested that individuals with high scores on extroverted and low scores on dominant be used in the formation of bachelor groups (Fig. 8.3).

Scientists have also shown that personality plays a role in how captive animals react to zoo visitors. High densities of zoo visitors can cause stress for captive primates (Hosey 2000); however, other factors may come into play in regard to how animals react to visitors, and visitors may even act as enrichment in some cases (Hosey 2000). When captive Diana monkeys (*Cercopithecus diana*) were exposed to high visitor density, the personalities of various monkeys affected how they responded. Some individuals became more aggressive and exhibited abnormal behaviors, whereas others exhibited more affiliative behaviors (Barlow et al. 2006). Animals that were rated by observers as solitary, irritable, and aggressive demonstrated increased abnormal behavior when visitor density was high, whereas animals rated as active, playful, and excitable exhibited an increase in species-typical behaviors such as play. Thus, personality can be used to decide which animals go on exhibit during heavy visitor hours or during other potentially stressful events.

Personality has also been used to promote breeding success in endangered species that historically have had trouble breeding in captivity. Powell et al. (2008) studied personality in giant pandas using a novel-object test and correlated personality with sociosexual behavior. The authors found that high scores on shyness correlated with poor sociosexual performance. Based on that finding, the authors suggested that altering enclosures (providing environmental enrichment and more dens), increasing comfort levels with keepers, and reducing stress could improve reproductive success as these manipulations might reduce shyness (Fig. 8.4).

Fig. 8.3 Understanding animal personality and its behavioral manifestations are important in captive husbandry of wild animals – in this case understanding how lions respond to one another during an introduction. (Photos: Julie Larsen-Maher, Wildlife Conservation Society)

Fig. 8.4 Shyness correlates with poor sociosexual performance in giant pandas. Researchers suggest that improving relationships between giant pandas and their caretakers could reduce shyness. (Photos: Jessie Cohen, Mehgan Murphy, Smithsonian's National Zoo)

Wielebnowski (1999) suggested that assessing personality could allow predictions of reproductive success on an individual level. Using a mirror-image stimulation test and a keeper survey, she found three personality components in cheetahs: tense-fearful, vocal-excitable, and aggressive. Animals that did not breed successfully scored higher in tense-fearful than those that did breed, suggesting they had less ability to cope with the captive environment. The author suggested that the tense-fearful animals therefore may need more seclusion and more places to hide to breed successfully.

Carlstead et al. (1999a, b) found that the personality of the black rhinoceros included six components: olfactory behaviors; chasing/stereotypy/mouthing (a composite of aggressive and abnormal behaviors); fear; friendly to keeper; dominant (to conspecifics); and patrolling. Females that scored higher on dominant than the male they were paired with were more successful in breeding. Unsuccessful females also scored higher on chasing/stereotypy/mouthing, suggesting that either these females are incompatible with their mate or are behaviorally compromised by some other factor.

Personality may also play a role in parental care. Maestripieri (1993) found that individual differences influence maternal behavior in captive rhesus macaques. Using behavioral measures of anxiety (visual monitoring and scratching), he showed that visual monitoring of the infant and of other monkeys by the mother was correlated with maternal protectiveness and that the former was a better predictor of individual differences than age, experience, dominance rank, number of young in the group, or sex of the infant (Fig. 8.5).

These studies suggest that personality be formally added to the array of factors considered in the design of zoological facilities and husbandry protocols, particularly regarding species for which captive breeding is essential to their conservation. Breeding programs that make recommendations based on genetic compatibility should also consider compatibility in personality. Although more studies are needed, there is evidence that parental care is also influenced by personality; because captive maternal behavior is often problematic (Wielebnowski 1998), personality may be used to assess whether a problem is likely and then address it.

In addition to captive propagation, conservation plans frequently include reintroduction and/or translocation programs, which often are unsuccessful (Beck et al. 1994). Recent research suggests that personality could be a tool in planning such programs in addition to training animals that are to be released into the wild to cope with specific challenges that their new environment might present (McDougall et al. 2006). Personality has been shown to be a good predictor of survival in the wild, and it also can aid in handling animals before their release (e.g., Watters and Meehan 2007). Some studies have found that successful reintroduction programs can be informed by personality traits and suggest that although the use of this tool is not widespread it should be.

Bremner-Harrison et al. (2004) quantified the responses of 49 swift foxes to four novel stimuli and found two personality types: bold and cautious. They found that the swift foxes they had assessed as bold were not good candidates for reintroduction to the wild, as they had predicted they would be, as those animals died within 6 months

Fig. 8.5 Studies of nonhuman primates demonstrate that personality affects maternal behavior, specifically maternal protectiveness of infants. (Photo: Julie Larsen-Maher, Wildlife Conservation Society)

of reintroduction (two were killed by motor vehicles and the cause of death of the remaining animals classified as bold is unknown). Cautiousness was found to be more advantageous to fox survival in the wild. The authors concluded that this type of assessment is an important tool in predicting survival in released animals and should be used for animal selection and preparation.

However, Watters and Meehan (2007) argued that a one-size-fits-all theory of reintroduction may not always work and may be to blame for the high rates of failure associated with reintroduction of captive animals to the wild. Instead, the authors suggest that a range of personalities be introduced and monitored so it can become more apparent which personality types best equip a reintroduced animal to survive in a new environment. Those data can then be used for ongoing reintroduction programs and subsequent release.

For example, Sih and Watters (2005) assessed the personality of male water striders and then formed 12 groups based on the results: The most aggressive males were in one group, the next most aggressive in the next group, and so on until the last group, which was comprised of the least aggressive males. As discussed earlier, the authors found that the behavioral type of the group affected group outcomes.

The authors recommended that further studies be done on mixing behavioral types in groups to better understand the effects of such a mix on group outcomes.

The idea of a mix of behavioral types improving reintroduction success can also be applied before their release. Watters and Meehan (2007) argued that variation in behavioral types can be promoted by environmental factors, so attention to them before release may aid in successful reintroductions. The authors recommend that zoo managers provide different environmental contexts when rearing captive animals using environmental enrichment techniques, thereby promoting variation and developing a group more ready for reintroduction. In addition, they suggest that captive animals' personality be assessed and responses from each behavioral type to different environmental contexts be studied.

Other suggestions for establishing a community of animals in the wild include considering the family or neighborhood group as the reintroduction or translocation unit because such groups probably represent a compatible mix of personality types, which might influence their success, as Sih and Watters (2005) suggested. Shier (2006) found that prairie dogs that were translocated with their family groups intact were five times more likely to survive (predator success was decreased) and had better reproductive success than those that did not. It is possible that family groups that exhibit certain behavioral syndromes would fare even better, but that has yet to be studied.

Interestingly, group reintroduction is effective with typically solitary animals as well. Shier and Swaisgood (2009) found that Stephens' kangaroo rats (*Dipodomys stephensi*) that were translocated with neighbors fared better than those translocated with unfamiliar animals. The former did not travel as far from their release site and had higher rates of survival. Again, it is possible that certain personality types that are translocated with neighbor groups would fare even better, or that neighbor groups are successful because they contain a compatible mix of personality types. This should be studied to further the success of reintroduction programs.

8.4 Future Directions for Research and Application

Starting with the belief that only humans have personalities, to the acknowledgment that nonhuman animals do as well, to the use of personality as a conservation, management and well-being assessment tool – what more do we need to know and where can personality take us next? One important area of research is establishing an understanding of how much the physical and social environment affects personality and to what extent personality is plastic. Given that some individuals fare better in managed environments (e.g., zoos and reintroduction/translocation programs), can we somehow change those environments and associated protocols to improve how all of the individuals fare? Is it possible to produce more dominant female black rhinoceros and less shy giant pandas? We suggest that longitudinal studies of personality be carried out to assess how it may or may not change over time and what factors or events coincide with the changes. We also suggest that

researchers assess the impact of rearing environments (physical and social) on personality so even if personality is a life-long consistent trait we might have some ability to send individuals down differing developmental paths that culminate in different personalities (Fig. 8.6).

Similarly, groups of related and unrelated animals should be reared in standardized environments, and the heritability of personality traits should be measured to determine the extent of a genetic component to personality. More work should focus on the fitness or viability of populations that vary in regard to either dominant personality traits (e.g., a generally "bold" population) or in the composition of personality types (see reviews by Dingemanse and Réale 2005; Réale et al. 2007; Smith and Blumstein 2008). These basic studies would have obvious management and conservation applications.

In terms of captive animal well-being, might it be possible to further incorporate personality into veterinary care? Do different behavioral types respond differently to treatment? When immobilizing animals for treatment, veterinarians make every effort to keep the animals as calm as possible during the process so the anesthetic drugs have an optimal effect. In these situations, individual personality probably plays some role in keeping the animal calm before the anesthetic is administered. Might this also be true for therapeutic medications? In humans, studies have shown that some people who have a better outlook on life follow treatment plans better and demonstrate faster recovery times from some diseases (for depression, MacLeod and Moore 2000; for cancer, Greer et al. 1979; but see Wilkinson and Kitzinger 2000) – is there also a relation between personality and morbidity or mortality in zoo populations?

A common reason for mortality in reintroduced or translocated populations is dispersal from the reintroduction site (Fischer and Lindenmayer 2000). What is mediating this dispersal drive? Individuals might be dispersing in an attempt to find a familiar landscape (Stamps and Swaisgood 2007), because they cannot integrate into the resident population (Kleiman 1989), or it may simply be due to stress. It is possible that personality is a factor. Different types may be more or less able to find

Fig. 8.6 The extent to which the environment affects the development of personality is an avenue of future research that would be beneficial for the management and conservation of wild animals. (Photos: Jessie Cohen, Smithsonian's National Zoo)

resources (e.g., "curious" types), establish their own territories (e.g., "bold" individuals), form social relationships (e.g., "calm," "sociable" types), or cope with stress. It is likely that the "right" type of individual for reintroduction or translocation will vary by species and the ecological characteristics of the site, including the demographics and personality composition of the resident population, if one exists.

We are in the midst of an extinction crisis that is unprecedented in scale. The survival of many species depends on the extent to which they can endure anthropogenic environmental change and in some cases become commensal with humans (e.g., gray squirrels, *Sciurus carolinensis*; "temple monkeys," *Macaca* spp. and *Presbytis* spp.). It is possible that different personality types fare better than others in the face of these selective forces. Alternatively, the degree to which personality is plastic may be the deciding factor regarding whether a species or population adapts. It has also been suggested that personality plays a role in the likelihood that a species becomes invasive (Réale et al. 2007). For example, is it a bold type or behavioral syndrome that is more likely to invade owing to its aggressive nature, or would it be a cautious or timid type that would survive the hazards of the unknown environment? Is it possible that personality affects whether an individual becomes a nuisance or problem animal (e.g., man-eating large carnivores, crop-raiding animals, campsite-raiding bears)? If so, could we then attempt to shape personalities away from those tendencies, or could we identify these "problem types" in advance and proactively relocate or control them some other way?

8.5 Conclusion

The concept of personality in animals is maturing as we continue to document the diversity of personality types and characteristics in different species and learn how to measure them. Theories from psychology and behavioral ecology are enriching our understanding of animal personality and are allowing us to make predictions about the impact it has on behavior and evolution. The knowledge we have gained on animal personality has already begun to be put to good use in the management of captive animals, but there is significant room for more application. Animal personality has rarely been considered in conservation and wildlife management. We hope that our discussion here stimulates more theoretical and empirical work and expands the application of our current knowledge of animal personality to finding ways to conserve and live harmoniously with nonhuman species.

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Chapter 9
Developing and Validating Measures of Temperament in Livestock

Simon P. Turner, Jenny M. Gibbons, and Marie J. Haskell

9.1 Introduction

The extension of the concept of temperament to livestock dates back as far as the beginning of the twentieth century. Temperament in livestock has been shown to influence productivity, survival, ease of handling, and handler safety. A variety of terms are used to refer to different aspects of temperament (or personality) traits in livestock. Despite the variety of definitions and adjectives used, the underlying principle that animals behave in consistent ways over time and situations is the main defining characteristic of a trait. In this chapter, we explore the motivations behind the desire to assess livestock temperament. We review and summarize fearfulness, a major trait that has been widely studied in livestock to describe the complexities inherent in measuring aspects of temperament. We highlight the importance of temperament from economic and welfare viewpoints and illustrate with a number of studies that have demonstrated links between temperament and production. Finally, we evaluate the constraints of breeding for temperament traits and discuss what the future may hold in this area.

9.2 Defining Temperament for Livestock Research

Individuals of many species of animals have been shown to react in a consistent manner. This consistency in behavior can be assessed at many levels. The basic level is where there is consistency within an individual in regard to its reaction to stimuli in a single situation (e.g., a beef cow runs quickly away from a handling

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area). The next level is where the individual reacts consistently in a number of related situations (e.g., the cow struggles when she is being restrained; she shows fearfulness by running away quickly from the handling area and is fearful when humans approach). The highest level is where different types of behavior are correlated across situations (for instance, we might find that not only is the cow fearful in many situations but she also shows low levels of exploration in novel situations and low levels of aggression).

In the behavioral ecology literature, the animal's behavior is said to show "repeatability" when it is consistent when tested on two or more occasions within a short or medium-term time period and within a single situation. When behavior is consistent across situations, the animal is said to be a certain behavioral type, whereas the term "behavioral syndrome" refers to the situation where different behavioral types are correlated across situations (Sih et al. 2004a, b). The idea that animals show consistent behavior patterns over a number of situations is similar to the concept of personality in humans (Wilson 1998) and nonhuman animals (see other chapters noted later). Indeed, this term has been used by some scientists in the context of farm or domestic animals (e.g., Erhard and Mendl 1999; Gosling 2001).

Research in farm animals has looked for consistency and correlations at each of these levels, but the terminology used to describe behavioral consistency has not always differentiated between the different levels at which the terms can be applied. Much work has focused at the behavior of individuals in a single situation (repeatability) level, as many traits of interest are primarily important only in a single situation. For instance, the reaction of dairy cows to milking (Uetake et al. 2004) or the response of mink or beef cattle to human handling (Burrow et al. 1988; Hansen 1996), are all-important to the productivity of the farm or welfare of the animals. These single behavior, single situation responses are sometimes referred to as "temperament" (e.g., milking temperament) but are also referred to as behavioral or temperament traits (e.g., Hansen 1996; Müller and Schrader 2005) or as individual differences (e.g., Kilgour et al. 2006). However, the term temperament is also used at the behavioral type level in livestock research (e.g., Grignard et al. 2001; Lansade et al. 2008). This is also the definition used in some studies of wild animals (Réale et al. 2000; McDougall et al. 2006). At the behavioral syndrome level, researchers have also found sets of behavioral traits that are correlated. Similar to that found in rodents (Benus et al. 1980), a proactive/reactive behavioral strategy construct was found in pigs (Hessing et al. 1993, 1994; Ruis et al. 2000). However, there is some disagreement about the existence of behavioral syndromes in farm animals, as others have not been able to verify their existence (e.g., Forkman et al. 1995) or indeed to find evidence of consistency at the cross-situational level (e.g., Van Reenen et al. 2004; Gibbons et al. 2009).

What is apparent is that there are many and varied ways of assessing behavioral consistency in livestock species, with the major consensus being that an underlying consistency of behavior does exist in individuals and that it can affect the productivity, health, and welfare of the animal as well as the welfare of its conspecifics and human handlers. Hereafter, this chapter uses the terms temperament trait and

behavioral trait interchangeably. We also discuss why assessing temperament traits in livestock is important, how it can be done, and what use can be made of the information to improve livestock management and welfare.

9.3 Why Measure Temperament Traits in Livestock?

9.3.1 The Drivers

The expression of undesirable temperament traits in farmed livestock has the potential to significantly compromise farm viability, animal welfare, human safety, and labor efficiency. Research in this field is therefore motivated by economic drivers, concerns over animal welfare, and ethical concerns, in addition to intrinsic interest. At the heart of these issues is the observation that individuals vary in their response to certain stressors in predictable ways. Because of the economic and welfare issues, it is the undesirable responses that have received the most attention. There are a number of issues that have been investigated. One of the major issues is the relative fearfulness shown by animals in response to human handling. A large number of studies in various livestock species have demonstrated a link between behavioral responses indicative of fearfulness of humans and a range of economically important production traits. Fearfulness of humans in beef cattle has been associated with lower weight gain (Burrow and Dillon 1997; Voisinet et al. 1997; Petherick et al. 2002), poorer feed conversion efficiency (Petherick et al. 2002), poorer meat eating quality (Reverter et al. 2003), and delayed onset of puberty (Stahringer et al. 1990). In other livestock species, fearfulness of humans has been linked to poorer milk yield from dairy cows (Drugociu et al. 1977), reduced rate of eggs laid by laying hens (Barnett et al. 1992), and poorer mothering ability in sheep (Lambe et al. 2001) and pigs (Janczak et al. 2003; Marchant-Forde 2003). The diversity of species and quantity of studies testifies to the importance of this trait in livestock production and management.

The behavioral expression of temperament may also compromise the welfare of the actor and/or receiver, either directly through its own performance of the behavior or through the management methods used to reduce the severity of the outcome. High aggressiveness, for example, has consequences for both the aggressor and the recipient, and mismothering associated with fearfulness can have fatal consequences for neonatal lambs (Lambe et al. 2001). There are other significant but more specific problems, such as the damaging tail biting in pigs and feather pecking in laying hens that have also been investigated. These behavioral traits appear to be consistent within individuals (Savory and Griffiths 1997; Keeling et al. 2004). Tail docking of piglets and beak trimming of laying hens, both increasingly brought to public attention, are routinely performed on whole populations to minimize the consequences of tail biting and feather pecking/cannibalism performed by a small number of individuals. Although the genetic and environmental interactions that

cause one individual and not another to show these damaging behaviors are not fully understood, it is only by altering the genetics of the animals or the farm environment that these serious welfare problems can be solved.

9.3.2 Use Made of Temperament Data

There are two major ways in which information on animal temperament is used in livestock production. First, problems on a farm or in a farming system can be highlighted when an undesirable behavioral trait is found to be expressed by many individuals. Action can then be taken to improve the environment. For example, much effort has been directed at understanding the etiology of tail biting and feather pecking, and management approaches to minimize their occurrence and consequences have derived from this information. One of the outcomes of this process has been the advent of legislation requiring the provision of manipulable substrates to pigs [e.g., The Welfare of Farmed Animals (England) (Amendment) Regulations 2003]. As well as changing the environment, we might breed animals to have a more suitable temperament for the prevailing farming system, although ethical considerations must be taken into account. It is generally thought that selective breeding to reduce fearfulness and aggressiveness in animals is ethically sensible. In recent years, the heritability of many temperament traits has been estimated, and in some cases genomic regions with an influence on trait variability have been identified (discussed further in Sect. 9.6). There are cases where breeding values are now routinely calculated for behavioral traits, such as the responses of dairy cows to milking (Brotherstone 1995) and of beef cattle to restraint in a handling crush (Donoghue et al. 2006).

9.4 Measuring Temperament Traits on Farms: Challenges and Expectations

9.4.1 Constraints to Measuring Behavior On-Farm

For livestock, researchers are typically measuring temperament traits in groups of animals on experimental or commercial farms. Compared to a more controlled experimental laboratory environment, these environments offer both opportunities and constraints to measuring and understanding temperament traits. Farms are a source of large numbers of animals whose pedigree links with others on the farm and elsewhere are frequently known. Within any given farm, animals may also have experienced a fairly standardized rearing process. However, one farm differs from another in many respects, such as the quality of human interaction, the level of nutrition provided, and the social groups the animals are kept in, among others,

all of which could affect temperament. For instance, to assess aggressiveness in individual pigs when groups of pigs are mixed with others, a test must be used in which all animals are mixed with a standardized number of unfamiliar animals, within a controlled weight range, and into pens of the same size. Such standardization can be achieved only on some farms. All of these factors mean that farms cannot be treated as statistical replicates, and each farm must be regarded as unique.

Another constraint to conducting research on farms is that researchers are usually required to ensure that their observations are compatible with normal farm routines and cause minimal disturbance and additional handling of the animals. Observation of animals in outdoor conditions, particularly extensive environments, has additional challenges. Extensively managed animals are rarely handled and may show extreme fearfulness of humans. Observations of undisturbed behavior may need to occur from some considerable distance or after a prolonged period of familiarization to a human presence. Standardization of the outdoor physical environment is usually impossible, and behavior is likely to be affected by ambient temperature, precipitation, and wind speed (e.g., Morgan et al. 2009). Identifying individual animals when managed in large groups can also be problematic, as eartags or identifying brands may be difficult to see at a distance. In practice, the choice of behavioral recording method is therefore strongly influenced by what can feasibly and reliably be achieved on farm. The decision is typically a pragmatic one and is a compromise between accuracy and speed or ease of measurement.

It is important that explanatory variables (e.g., breed, age, physical and social environmental conditions), including the identity of the group are taken into account in temperament studies. To illustrate this point, intraspecific pig aggressiveness is affected by breed, age, floor space allowance, method of feed delivery, and time since social regrouping. Even in groups composed of animals with the same rearing history and given largely identical physical environments, considerable group-level effects on aggressiveness are apparent, suggesting that the behavior of individuals is affected by that of others in the group (e.g., Turner et al. 2009). At another level, the very presence of other conspecifics, irrespective of who they are, can alter how an individual responds. For example, withdrawal from an approaching human as a measure of fearfulness can be affected by the location of other group members (Rennie et al. unpublished data).

9.4.2 Trait Definition and Situation Specificity

As mentioned previously, livestock temperament research is primarily problem-driven. However, a clear definition of exactly how to quantify or objectively describe the problematic behavioral trait or traits has sometimes been lacking. As previously described, the problem is often initially described as something like “there are a lot of skin injuries occurring when two groups of pigs are put together for the first time” or “some beef cattle struggle violently in the weighing crate or

restraint crush, and others don't." The experimental challenge is then to define the exact behavior or behaviors that are important and how they can be quantified.

As an example we consider the research that has been carried out to find suitable methods for assessing fearfulness in beef cattle. Beef cattle are generally farmed extensively; they spend most of their time at pasture and therefore may have limited contact with humans. However, at various times they are moved into handling facilities for veterinary treatments, artificial insemination, weighing, or transfer to market. Animals that struggle, attempt to escape, or strongly resist these handling procedures are at risk of injuring themselves or their handlers and may experience high levels of stress. Improved handling facilities have been designed to reduce injury and stress (e.g., Grandin 1980). The other modification methods include the use of "training programs" to habituate the animal to the handling practices and implementation of a selective breeding program or other genetic methods. Training programs to reduce fear have been deemed ineffective owing to the individual attention each animal needs and the time it would require. Therefore, reduction of fear through selective breeding has been seen as a viable option (Burrow 1997). To do this, however, requires an assessment of fearfulness that can be applied to individuals, is of short duration so hundreds of animals can be assessed, and can be easily incorporated into routine farm procedures. This necessitates the use of a behavioral test or standardized observation method to quantify individual fearfulness (some of which are considered below). However, fearfulness may be shown toward a wide range of stimuli (Fig. 9.1).

Although correlations exist between some of these contexts (see Burrow 1997 for a review; see also Sect. 9.2), the behavioral response shown by different individuals in the same situation may be driven by different motivational stimuli. For

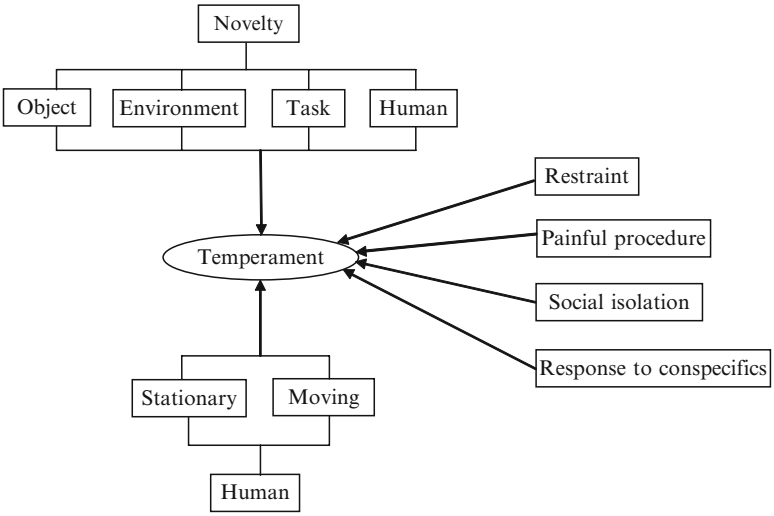

Fig. 9.1 A wide range of anxiogenic stimuli can contribute to the manifestation of a fearful temperament

instance, we might use the degree to which an animal struggles in the handling crush as a measure of its fearfulness. However, this struggling may be due to the separation from its herdmates, the proximity of humans, the physical containment, or a combination of these factors. Moreover, the exact eliciting factors may vary among animals showing the same level of response. Fearfulness shown toward different sets of external stimuli might have independent genetic influences despite being manifest through similar behavior (e.g., Popova et al. 1993). This may require the use of different assessment methods in each case and demand different interpretations of the same behaviors. It is perhaps because of these motivational and genetic disparities in different situations that attempts to find a single universal measure of cattle fearfulness have proved unsuccessful. This has required a redefinition of the problem to focus specifically on fearfulness in situations of greatest importance on farms. In practice, the development of temperament tests has necessarily focused on the measurement of behavior in one or only a small number of situations (e.g., fearfulness of growing animals in a handling crush). Our expectation is that selection using behavior measured in one of these paradigms will lead to a reduction in the expression of fear in some but not all situations of relevance on-farm. This experience with beef cattle fearfulness therefore highlights the premise that management decisions or breeding goals that aim to alter animal behavior must begin with a clear description of the target behavior and context.

In the rest of this section, we provide a summary of the main tests of fearfulness in cattle as an example of how tests are developed. The main types of test are the open-field test (Kilgour 1975), the approach/avoidance or docility tests (Boivin et al. 1992b), the crush test (Tulloh 1961; Grignard et al. 2001), and the flight time test (Burrow et al. 1988). For each, we allude to some of their limitations and consider the motivational systems invoked. This ought to provide a taste for how complex the selection of temperament assessment methods is in reality. However, it is important to realize that there are multiple variants of each of these approaches.

9.4.2.1 Open-Field Test

The open-field test has been used extensively in rodents, where it is used to assess locomotor and exploratory behavior in response to an anxiogenic novel environment (Denenberg 1969). The behavioral response to this situation has been assessed in cattle by scoring ambulation, vocalization, and elimination (Kilgour 1975; Kilgour et al. 2006) and the ease with which cattle could be sorted from their herd mates to enter the open field (Boivin et al. 1992a). The interpretation of behavior in the test has been challenged (Walsh and Cummins 1976; Manteca and Deag 1993) because any response is likely to reflect a number of different underlying motivational states, including a desire to escape the handler who moved them into the arena and a desire to reinstate contact with companions. The behavior observed is likely to be an expression of a combination of these motivational states, in addition to ones relating more directly to neophobia and exploration, which is what we really wish to assess.

9.4.2.2 Approach/Avoidance and Docility Tests

The approach/avoidance tests assess how closely an animal approaches a stationary human or avoids a moving human (reviewed by Waiblinger et al. 2006) (Plate 9.1). The tests are often performed when the cattle are grouped with others in a familiar environment, which means that the behavior is not likely to be confounded by novelty or social isolation. An element of exploration may be involved, particularly if the particular human is associated with the delivery of food. Cattle approach/avoidance test responses have been measured in a range of experimental conditions including at pasture (Murphey et al. 1980), in the home pen (Waiblinger et al. 2006), and in an open field (Jago et al. 1999; Kilgour et al. 2006). These tests have more recently been adapted for use on commercial farms as part of on-farm welfare assessment audits (Waiblinger et al. 2003; Windschnurer et al. 2008). Again, caution is needed when interpreting data from approach/avoidance tests, as they can be influenced by habituation to the human presence, such as that resulting from people walking past the pen in which animals are housed. Additionally, the quality of the handling experience the animal has previously received from humans, the location and behavior of other animals, and the calf-rearing system used can affect the animal's willingness to withdraw (Jago et al. 1999; Waiblinger et al. 2003; Breuer et al. 2005; Gibbons 2009).

The docility test (Plate 9.2) involves an experimenter attempting to restrain an animal for 30 s in a corner of a testing pen with only his or her outstretched arms. A docility score is calculated by combining different behaviors measured during the

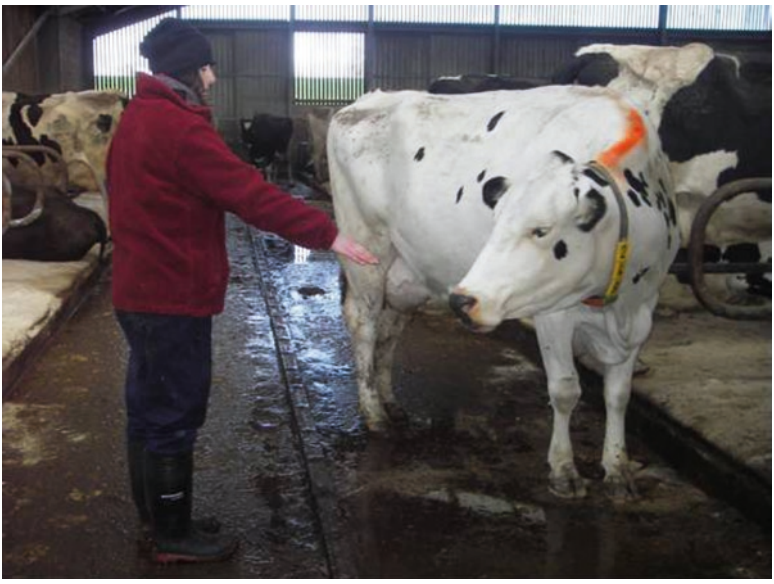

Plate 9.1 An approach test involves approaching an animal to determine at what distance or level of interaction the animal moves away from the approaching human

Plate 9.2 The docility test, in which a handler attempts to restrain an isolated animal in the corner of an arena using outstretched arms

test, such as the number of attempts to escape from the corner and aggressiveness toward the handler (Boivin et al. 1992a, b). Motivations involved are likely to be an amalgamation of those invoked in the open-field and approach/avoidance tests. The approach is time-consuming and can be dangerous to the handler. However, this is one of the few tests quantifying a temperament trait that has been taken up by the farming and cattle breeding industry. In France, the docility test has been used to select for improved temperament in Limousin bulls since 1992.

9.4.2.3 Crush Tests and Flight Speed

The crush test (Plate 9.3) involves measuring the resistance of cattle to restraint in a handling crush in the presence of humans, typically using a rating scale of five to seven categories (e.g., Grandin 1993; Kilgour et al. 2006). In an early example, Ewbank (1961) attached the descriptors docile, alarmed, greatly alarmed, or submissive, respectively, to a four-point rating scale. These scales were criticized for their reliance on subjective descriptors of each category, the interpretation of which can vary from observer to observer. Despite this limitation, similar descriptive categorical scales have been used in tests in recent years, and a number of beef cattle breeding networks use this approach to provide estimates of genetic merit for this trait for individuals [estimated breeding values (EBVs); see Sect. 9.6] for cattle fearfulness during handling (Donoghue et al. 2006). This trait is usually referred to by the Australian beef industry solely as "temperament." The validation of subjective scales is discussed further in Sect. 9.5.2.

In contrast, "flight speed" or "flight time" (Plate 9.4) provides an automatically recorded objective measurement of the time taken to move between two sensors positioned several meters apart after release from restraint (Curley et al. 2006; Kilgour et al. 2006; Müller and von Keyserlingk 2006). This technique has been

Plate 9.3 The crush test, in which the degree of restlessness of the animal is scored on a categorical rating scale

Plate 9.4 Flight speed is recorded as the time (in hundredths of a second) to travel between the vertical poles

used in research on beef cattle temperament and is also now used to produce EBVs for this trait (Brahman cattle: breedplan.une.edu.au). Both this and the crush test, however, are sensitive to the slipperiness of the ground, the position of other cattle and humans, and the age and agility of the animal. The motivational systems

involved include escape from a human presence, escape from restraint, and social reinstatement. However, researchers have shown reasonable repeatability and heritability of the trait (Kadel et al. 2006), which has supported its consideration by the beef cattle breeding industry.

9.5 Measuring Temperament Traits On-Farm: Validation of Methods

9.5.1 State or Trait? Importance of Repeatability (Test–Retest Reliability)

In the short-term, the behavior of an animal can be affected by immediate proximate factors such as hunger or illness. Consider, for instance, a usually sociable individual that has become ill. During the period of illness it becomes reclusive and introverted (behavioral state) even though this does not reflect its more usual, underlying sociable disposition (behavioral or temperament trait). Although these short-term behavioral states can provide important information about how animals perceive certain aspects of their environment, they may not be informative of underlying longer-term traits. To determine whether a particular observational method is useful for measuring a temperament trait, it must be demonstrated that it is capable of detecting across-time repeatability in behavior. This validation is frequently lacking from reports on livestock temperament traits. The threshold that distinguishes a behavioral response as a trait rather than a state is not always clear.

The variance of behavior can be partitioned into a component within individuals, measuring the differences in performance within the same individual over time, and a component between individuals, measuring more permanent differences between individuals (Falconer and Mackay 1996). Analysis of repeatability attempts to extract the variance due to temporary environmental effects from longer-term effects resulting from individual differences. It is calculated from the between-animal and within-animal variance components as follows (Lessells and Boag 1987).

$$\text{Repeatability} = \sigma_b^2/(\sigma_b^2 + \sigma_w^2) \tag{5.1}$$

where σ_b^2 is the variance component between animals, and σ_w^2 is the variance component within the individual animal.

High repeatability requires both variation within the population and consistency at the level of the individual over time. The individual animal need not have exactly the same score at each testing, but the rank order differences between animals should remain largely the same.

When a test is repeated, some animals habituate to the test situation more rapidly than others, and the magnitude of their response then declines. Other animals may become sensitized, and the level of their response increases. In these cases, the "ability to habituate" trait adds statistical "noise" to measures of the focal trait and

so reduces the level of repeatability. The repeatability estimate can also be reduced by changes in the environment itself over time (e.g., with seasons) or the way in which the test is conducted or responses scored (perhaps due to observer drift; but see Sect. 9.5.3), despite our best efforts to control for these factors. When an animal is repeatedly tested, the second response may be more predictive of the third and subsequent response than is the first response (e.g., Kilgour et al. 2006). This is perhaps unsurprising given that the situation is novel to the animal on its first exposure. It is also common to find that repeatability declines as the time between test days increases (e.g., Erhard and Mendl 1997). Repeatability values vary substantially among studies using the same test scenario. For instance, in the flight speed test, repeatabilities of 0.36 and 0.68 were shown by Halloway and Johnston (2003) and Petherick et al. (2002), respectively, suggesting that the tests are highly sensitive to the specific animals and management environments used.

Behavior may also change in the medium term. During development, some degree of change is adaptive and is to be expected. The behavior of animals also changes with experience. Temperament traits in both humans and livestock species are believed to be in a state of flux when young (Goldsmith and Bihun 1997; Van Reenen et al. 2004), and observations delayed until after weaning may be more reliable predictors of adult behavioral traits than those taken prior to weaning. Again, it is important to note that when a trait has a high repeatability estimate it does not necessarily mean that the exact temperament scores for individuals do not change with age or environmental conditions. It does mean, however, that rank order differences between individuals are largely maintained (Roberts and DelVecchio 2000; Réale et al. 2007).

These considerations illustrate that despite the limitations discussed above the selection of a test protocol should not be based only on what can most easily be implemented on farm and on what test best invokes the behavior at the center of a research question. It is equally important to observe behavior that is most reflective of the animal's underlying temperament rather than simply a reflection of short-term behavioral states. Furthermore, binary traits or categorical scales in which most of the animals receive the same score provide little opportunity to discriminate between individuals and, as such, have little information content or statistical power.

9.5.2 Understanding What Is Recorded

Interpretation of behavioral data is necessary at three levels. At its most fundamental, there is a need to interpret the motivational factors behind the behavioral response observed correctly, as some features of the environment affect responses. For instance, as discussed in Sect. 9.4.2.3, the response during a flight time test depends on the proximity of other animals. Temperament assessment in livestock has made considerable use of paradigms first designed for rodents, such as the open-field test (discussed above) and elevated plus maze. These tests have been used extensively to assess exploration and aspects of fearfulness, such as risk aversion and neophobia. They have been criticized, as the animals' behavior needs to be interpreted with care;

a well-cited case in point is the meaning of immobility in an open field situation, which could be indicative of extreme fearfulness or a low motivation to escape. However, used with caution, such tests have a role to play. As examples, Rutherford et al. (2009) examined the effect of aversive early life experiences on pig behavior in the elevated plus maze, and Erhard et al. (1997) studied individual differences in aggressiveness of pigs using a version of a resident–intruder situation first developed in rodents (Denenberg 1969). Physiological parameters such as heart rate (Le Neindre 1989; Grignard et al. 2001; Kilgour et al. 2006) and cortisol levels (Munksgaard and Simonsen 1996) can be called on to interpret the significance of behavioral responses in tests such as these. Both of these measures have been used to validate that flight time and crush tests in beef cattle are likely to relate to fear or the perception of stress brought about by the test scenario (Watts and Stookey 2001; Curley et al. 2006).

There may also be a need to understand how observers interpret complex behavioral sequences to assign categorical temperament scores to animals. This is particularly important when using rating scales whose categories are open to subjective interpretation, such as those used to score the response to restraint in the crush test described above. To understand exactly what aspects of the animal's behavior observers use to classify its response as docile, alarmed, or very alarmed, it is first necessary to explore associations between these categorizations and objective observations of behavior itself.

Additionally, some approaches to assess a goal trait do so by measuring an objective indicator; thus, it is clearly necessary to demonstrate that the indicator predicts the goal trait. As an example, pigs show intense aggressive behavior when mixed with unfamiliar individuals. This is problematic in commercial production as it compromises growth rate, efficiency of feed use, immunocompetence, and meat eating quality (Tan et al. 1991; Morrow-Tesch et al. 1994; Warriss et al. 1998). Fighting involves the very rapid exchange of complex behavioral sequences that are difficult to observe in real time. To observe the aggressiveness of large numbers of animals, Turner et al. (2009) used a count of the skin lesions present 24 h after mixing into new social groups. Lesions are an outcome of involvement in both fighting and the receipt of one-sided bullying. To understand what lesions tell us about aggressiveness, it has been necessary to correlate counts of lesions with involvement in these behaviors using time-lapse video-recorded data. This has allowed refinement of the approach and quantification of the error associated with using this particular indicator of aggressiveness, which has allowed it to be used in larger-scale trials to assess the heritability of the trait (Turner et al. 2008), which is discussed further in Sect. 9.6.

9.5.3 Quantifying Observer Error

Lastly, it is important to show that the error inherent in observing and recording behavior is acceptably low. In practice, this means that a single observer should be consistent over time regarding the observation methods; and, as a rule, multiple observers should agree on at least 80% of occasions. In the case where a single

observer takes the measurements, it is good practice (although not always done) for that person to score behavior from a set of video clips at the start of the recording period, and then to score them again in random order some time later to ensure that their own scoring system has not "drifted." Similarly, it is good practice, and a standard procedure in other areas of temperament or personality research, for multiple observers to simultaneously but independently score behavior to check their agreement at the beginning of a study. It is common practice for observer identity to be included as a factor in statistical analyses to take into account any differences among observers.

9.6 Genetics and Temperament Traits in Livestock

9.6.1 Principles, Progress, Constraints, and Possibilities

9.6.1.1 Principles

As already briefly discussed, temperament research on livestock is often driven by what are seen as problematic behavior patterns, such as high levels of fear of humans or high levels of aggression toward conspecifics. These problems require a solution, so attempts have been made to reduce the occurrence or impact of behaviors by altering the physical environment (e.g., introduce better handling facilities, train handlers in appropriate techniques) or by using selective breeding programs to reduce the number of animals showing the focal behavior (e.g., increase docility in beef cattle). Selection programs using quantitative genetic methods have been used extensively in the past to achieve production goals, such as increasing milk production by cows and the growth rate of pigs.

There are a number of requirements for a selection program to be used for a particular trait (Simm 1998). First, it must be possible to measure reliably the trait of interest in individual animals. As many animals must be assessed, the trait must be measurable quickly and/or automatically. To overcome this issue in behavioral traits, we can design a time-efficient test, or proxy measures can be developed and validated (e.g., lesion scoring as a proxy for mixing aggression in pigs). Once this is done, sufficient animals with a known pedigree must be measured to calculate heritability (proportion of observed variation due to additive genetic effects). This is used to derive individual estimated breeding values (EBVs), which are an estimate of the animal's genetic merit for the trait of interest. EBVs are valuable tools that breeders can use when selecting elite breeding stock or culling animals with poor values. At the next level, breeders can use selection indices to make genetic progress in a number of traits at the same time by combining several EBVs (e.g., production, health, and fitness) to maximize response in the overall breeding objective (e.g., profitability). As discussed previously, EBVs are available for docility in beef cattle and milking temperament in dairy cows. These traits do not appear to have been incorporated into selection indices and so are probably used as stand-alone

values when choosing breeding animals. The use of a selection index also requires estimates of genetic correlations among the traits. A profitability index requires economic values for each trait. For temperament traits, some of this information is not yet available (Haskell et al. 2008). These issues are discussed further below.

Where there are single genes that exert a large effect, animals may be genotyped when young, with animals carrying the unfavorable genotype not used for breeding. This information can also be used in marker-assisted selection programs, which combines EBVs and marker information in a selection index. There are a few studies that have identified the genomic regions underlying a temperament trait (e.g., cattle fearfulness: Ball et al. 2002; Gutiérrez-Gil et al. 2008; pig aggressiveness: Murani et al. 2009; feather pecking: Buitenhuis et al. 2003), but few of these regions account for sufficient variation in the trait to be used in a definitive screening program.

9.6.1.2 Progress

The possibilities and practicalities of using selective breeding for temperament traits in livestock, together with the ethical and economic consequences of doing so, have been much debated in recent years by researchers and breeders. Most behavioral traits examined to date have been found to have a significant heritability (Turner et al. 2008) (see also Chap. 6, Sect. 3.1). Heritability varies on a scale of 0–1, in which a low value denotes a small genetic contribution to trait variation. As a crude guide, most temperament traits in livestock fall between the heritabilities of reproductive traits (commonly around 0.1) and growth traits (usually around 0.4), both of which are currently used in selection indexes. This level of heritability indicates that the heritable variation in behavioral traits is large enough to allow a moderate rate of response to selection. Many problematic behavioral traits have now been assessed for their likely response to selection, including feather pecking and cannibalism in poultry; poor maternal care in sheep; fearfulness during handling in beef cattle; poor responses to milking in dairy cattle; and in pigs, tail biting, aggression at regrouping, and savaging and crushing of piglets by sows (Knap and Merks 1987; Kjaer and Sørensen 1997; Buitenhuis et al. 2003; Breuer et al. 2005).

Apart from beef cattle, fearfulness by certain breed societies in certain countries, and more commonly dairy cattle behavior during milking (Heringstad et al. 2001), it is sobering that selection has not yet been implemented in any of the other cases. This is not simply because these issues have only recently reached the attention of breeders or because the heritabilities have only recently been estimated. Indeed, behavioral approaches to measure fearfulness in beef cattle and its heritability estimates were first reported several decades ago, and yet calculation of EBVs is relatively recent. Additionally, where EBVs do exist, the trait has frequently not been integrated into existing multitrait indexes but remains stand-alone. This means that no reference is made to interactions between temperament and existing economic traits during selection. This situation hints less at a lack of will on behalf of breeders and more at the need to overcome practical constraints and a lack of demand by farmers. The methods to overcome the practical and technical difficulties are discussed below.

9.6.1.3 Constraints in Scoring Phenotypes

To calculate EBVs, a large number of animals must be scored using a well-designed protocol for a defined temperament trait (for more detail see Chap. 5). This usually requires that scoring take place on a number of farms. We return to the example of pig aggression to illustrate some of the barriers to implementation and how they may, in time, be overcome. The first constraint is the ability to identify a sensitive measure of the temperament trait of interest that can be recorded rapidly on large numbers of animals and during normal farming routines. The use of skin lesions as an indicator of involvement in fighting described by Turner et al. (2006) represents an acceptable compromise between accuracy of prediction of a goal trait (fighting behavior) and speed of measurement. In this case, measurement requires less than 2 min per pig, as was demanded by the breeding industry. Such time constraints immediately rule out the use of many test scenarios used in traditional research settings to assess the temperament trait. For example, resident–intruder tests of aggression that require extensive additional handling of animals would need modification before being taken up by the industry. However, the flight time and docility test examples in beef cattle illustrate that, given sufficient research effort and will on the part of the agricultural sector involved, time-efficient methods can often be found. Developing automated methods of collecting behavioral data, such as using GPS tracking devices and other movement and behavior-monitoring technologies may be used to reduce the time taken to collect behavioral trait data.

As discussed in Sect. 9.4.1, another constraint to scoring temperament traits on large numbers of individuals is the need to standardize the testing situation when working on a number of farms. In many tests, where the response being measured is sensitive to environmental factors, this can be a problem. It can be overcome by careful selection of the farms included in the assessment.

9.6.1.4 Interactions with Existing Economic Traits and Estimation of Economic Weights

The addition of any new trait into an existing multitrait selection index dilutes or reduces the selection pressure that can be applied to each individual trait which means the genetic progress on each trait is slower. Therefore, we must be convinced of the value of the new trait (e.g., a temperament trait) before including it in an index. Currently, a major barrier to the inclusion of temperament traits in selection indexes is the fact that the genetic relation between these traits and others in the index is not yet known (e.g., how cattle fearfulness is related to growth rate). Therefore, the effect it has on genetic progress of other economically important traits is, as yet, unknown. There is a risk that the behavioral trait may be unfavorably genetically correlated with the existing traits that confer profitability, meaning that selection for an improvement in one may directly result in an opposite and undesirable response in the other. Although this situation is not insurmountable, as animals

usually exist in a population that counter this trend (i.e., the two traits are beneficially genetically correlated), it is nonetheless an undesirable situation and greatly reduces the speed of genetic progress, or the number of individuals that can be used for breeding. In the case of our aggression trait, there appears to be no genetic association with the economic traits assessed to date.

Traits in a profit index are weighted based on their economic value. To be incorporated into an index requires that the economic value of a temperament trait must also be estimated. Some economic consequences of a trait such as aggressiveness ought to be quantifiable, such as its impact on the growth rate. Other aspects that affect the viability of farming systems are less easy to estimate, such as the effect on immunocompetence and the subsequent risk of illness. However, as well as an economic value, these traits also have a noneconomic value. In the context of aggressiveness, this represents the ethical good, in monetary terms, of improving animal welfare, which is likely to be seen as a benefit by society at large. Estimating the economic and noneconomic benefits of implementing selection on behavioral traits is therefore complex. To complicate matters further, these benefits may not remain static. For instance, the spread of postweaning multisystemic wasting syndrome in many pig-producing countries was accompanied by a realization that aggression associated with regrouping significantly exacerbated the severity of clinical signs and triggered renewed efforts to avoid regrouping altogether. Following the emergence of this disease, the benefits, both economic and noneconomic, of selecting to reduce aggression have increased. Similar arguments can be made following European Union legislation banning confinement housing of individual breeding female pigs and enforcing group housing and therefore regrouping. These societal benefits are difficult to quantify and may necessitate the use of a "desired gains" approach. With this method, rather than include a monetary value in the weighting of each trait in the index, the required change in a trait is specified without calculating its relative economic weight (e.g., we specify that aggressiveness must fall by 5% per generation). This method could offer a better way of making progress in selection for temperament traits and may be suitable for use with pig aggressiveness and other temperament traits. In reality, the uptake of selection for traits such as this is still likely to depend on farmers seeing tangible evidence of direct and significant effects on economic profitability. It is thus likely to be market led by farmers themselves, irrespective of whether methods such as desired gains are used.

Selection for reduced fearfulness of beef cattle, as measured in variants of the crush test and docility tests described earlier, is now being realized, usually as a stand-alone trait. Evidence that fearfulness is associated at the genetic level with growth and particularly meat tenderness, coupled with an appreciation of its effects on human safety, animal safety, labor efficiency, and a negative perception of the fearfulness of certain breeds, has been a sufficient catalyst to create the market for less "temperamental" cattle from these breeds. This has occurred without the economic and noneconomic effects on human and animal safety and labor use being formally estimated and there currently being little financial reward for meat quality traits in many countries. Significant benefits could also be achieved by implementing this selection in other breeds where there is perceived to be less of a problem but where

considerable variation in fearfulness exists. That this is not yet occurring highlights the importance of the perception of farmers in driving selection on behavioral traits. Perhaps in time this drive will overcome some of the current constraints to implementing selection on other behavioral traits. Research has a major part to play in informing producers about the impact that animal behavior, and their role in molding it, has to play on profitability.

9.6.2 Wider Effects of Selection on Behavior and Welfare

9.6.2.1 Favorable Outcomes

A major step in assessing the benefits of selection on a temperament trait is to understand to what extent a behavioral trait is predictive of temperament in a wider sense. It is common for behavior to correlate between two contextually similar scenarios, such as the crush test and flight speed test. More rarely, behavior correlates across situations that involve a different array of anxiogenic stimuli, such as the crush test and response to a novel object. However, where these positive associations are found, the value of improving behavior in one context is heightened if it leads to desirable changes in behavior in other contexts. Selection on skin lesions now appears to be more worthwhile because we have shown that it leads to a reduction in aggression in stable social groups in addition to immediately after unfamiliar groups are mixed (Turner et al. 2009).

9.6.2.2 Unfavorable Outcomes

There are many examples of unfavorable effects on nontarget traits resulting from selection on a narrow range of economic traits in both livestock and companion animals (Sandøe et al. 2003). For instance, selection for increased milk yield is unfavorably genetically correlated with fertility in dairy cattle (Pryce et al. 1997). The risk of this occurring through selection on behavioral traits is just as great, operating either through pleiotropy (in which one gene has effects on multiple traits) or linkage (in which genes are spaced close together and tend to be inherited together). Quantifying the effect, in both magnitude and direction, on nontarget traits ought to be done as a precaution before implementing selection on behavioral traits.

To illustrate this point, we return for a final time to our examples of pig aggression and beef cattle fearfulness. We know that involvement in aggressive behavior has a heritability of around 0.4 and that it should respond to selection (Turner et al. 2009). However, to understand fully the implications of selection on the overall welfare of the animal and the population, it is important to understand the motivational goals and behavioral mechanisms through which some pigs achieve lower aggressiveness. Do such pigs avoid fighting new individuals by being more lethargic,

being more fearful of novelty, or by some other route? To date, we have demonstrated that unaggressive pigs are no less active than their aggressive counterparts but do respond differently to a novel handling situation in subtle, but genetically determined, ways (D'Eath et al. 2009). This handling test involved components of novelty, human presence, physical restraint, and social isolation. The heritability of the response to this challenge was low, which suggests that the rate of change would accumulate slowly as a result of selection to reduce aggression. However, if the correlated response in this test reflects a more general difference in the way that unaggressive pigs respond to challenges, it may have either beneficial or undesirable implications for welfare and perhaps needs to be investigated further.

As described, progress has been made in selecting against fearfulness during handling of young cattle. In several species, a phenotypic relationship has been found between reduced fearfulness before giving birth and subsequently heightened willingness to defend their offspring aggressively. This is of relevance in beef cattle as aggressive defense of the calf by beef cows can result in serious and fatal injuries to producers, veterinarians, and members of the public who unwittingly walk through calving fields. If this phenotypic relation is confirmed at the genetic level in beef cattle, it may caution that reducing fearfulness in young stock could have undesirable consequences if the animals enter the breeding herd. Selecting young cattle intermediate in their expression of fear or including selection against handler-directed aggressiveness itself in breeding programs may transpire to be more beneficial for handler safety than selecting for extremely fearless individuals. The two examples of pig aggressiveness and cattle fearfulness highlight the importance of understanding the correlated response in other behavioral traits, even where strong economic and ethical arguments can be made favoring selection.

9.7 Future Developments

There is a danger of assuming that selection on behavioral traits is impossibly difficult. It is undeniable that they pose additional layers of complexity and practical hurdles in comparison to more conventional production traits. It is also undeniable, however, that the behavioral disposition of the animal can reduce or preclude the animal from obtaining its full genetic potential in economic traits in both the animal itself and its group members (e.g., an aggressive pig may not grow as fast as it could, and the growth rate of its penmates may also be affected). Furthermore, as the selection response in economically important traits (e.g., production or reproductive traits) begins to plateau, the value of selecting behavioral traits as a means of indirectly gaining further economic improvement will increasingly be appreciated. Much work has already demonstrated the overall economic benefits to be gained by broadening breeding goals for dairy cattle to include health traits (Stott et al. 2005).

Routine phenotyping of complex behavioral traits is one of the most obvious costs of quantitative selection. However, as discussed in Sect. 9.6.1.1, few genomic regions with a large influence have been found; and similar to many other traits of

interest, it appears that tens or hundreds of loci are involved in the expression of each trait. Genome-wide selection in which the small contributions of many loci across the genome are combined to give a genomic breeding value for a trait could make use of these small individual contributions. This approach still requires some phenotyping of an initial resource population to identify genomic regions with an influence on trait expression but subsequently does not require routine phenotyping of all animals. This approach is currently being developed for production traits for many species, and it is hoped that it will be extended to temperament traits.

Multilevel selection is an alternative approach that arguably requires no phenotyping at all once it is set up but may have dramatic effects on behavioral phenotypes nonetheless. This approach has been trialed in commercial pig breeding. The method, described by Bijma et al. (2007), places selection pressure not only on an individual's own growth, as in conventional breeding, but also on the heritable effect that an individual has on the growth of others in its group. As this effect on others is likely to operate through behavior, multilevel selection can be expected to affect social and potentially nonsocial temperament traits (e.g., Canario et al. 2008). Among other mechanisms, this approach could favor animals that are less aggressive, less inclined to direct vices (e.g., tail biting, feather pecking) at others, or less active and therefore less likely to disturb others, causing unnecessary expenditure of energy. Clearly, to appreciate the impact of this approach on behavior and welfare, the behavioral mechanism or mechanisms through which individuals affect each others' growth and that would be altered by selection must be understood. Once again, this requires at least an initial period of behavioral phenotyping.

9.8 Conclusions

The achievement of widespread acceptance of selection on temperament traits depends on demonstrating the economic benefits in the widest sense, showing that deleterious consequences can be avoided and finding solutions to practical constraints such as the costs of phenotyping. The potential benefits of selection are significant, cumulative, and permanent; and the cost of implementation to individual farmers is small in comparison to changing management methods. Where improvements in management methods have ground to a halt and fallen short of what is ideal, animals could be selected that thrive better and have improved welfare in such suboptimal housing. However, selection should not become an excuse to reduce efforts to find economically tenable ways of improving management conditions or, worse, to allow existing conditions to deteriorate. An open dialog between breeders, farmers, and the wider society on the ethics and economics of each issue is required to find an acceptable solution.

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Part III
Molecular Genetic Bases of Personality and Temperament

Chapter 10
Genetic Variants of the Dopaminergic System in Humans and Model Organisms

Kouta Kanno and Shoichi Ishiura

10.1 Introduction

Human personality is shaped by both genetic and environmental factors. Molecular genetics has begun to identify specific genes for quantitative traits. The first candidate genes investigated were components of the monoamine neurotransmitter pathways, such as serotonin and dopamine. The serotonergic system is involved in mood, anxiety, and aggression. Temperamental predisposition and behavior are likely to be influenced by genetic variations of serotonergic genes – i.e., serotonin-metabolizing enzymes, tryptophan hydroxylase and monoamine oxidase (MAO), catechol-*O*-methyltransferase (COMT), 14 kinds of serotonin receptor (5-hydroxytryptamine, or 5HT) and serotonin transporter (SERT).

The dopaminergic system is involved in the brain's reward system and addictive behavior. Human or animal behavior is also influenced by dopaminergic genes such as tyrosine hydroxylase (TH), dopamine receptors (DRD), and dopamine transporter (DAT). Noradrenergic and γ-aminobutyric acid (GABA)ergic genes are also involved in behavior.

It has been reported that single nucleotide polymorphisms (SNPs) and simple microsatellites in and around the coding regions of the dopamine- and serotonin-related genes – e.g., DRD3, DRD4, DAT1 (SLC6A3), TH, COMT, brain-derived neurotrophic factor (BDNF), 5HT2A, MAOA, and SERT (5-HTT, SLC6A4) (D'Souza and Craig 2008) – are important factors in human neuropsychiatric disorders and behavior.

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Here, we primarily focus on the dopaminergic systems and review reports, including our recent studies, on functional DAT1 polymorphisms.

The dopaminergic nervous system plays important roles in regulating locomotion, cognition, reward, addiction, and hormone release (Jackson and Westlind-Danielson 1994; Missale et al. 1998; Bannon et al. 2001; Uhl 2003). Dopamine and its related genes are thought to be involved in neuropsychiatric disorders and behavioral traits. The human dopamine transporter (*DAT1*) gene is involved in many dopamine-related disorders. Levels of DAT are reduced in Parkinson's disease (PD) and elevated in attention deficit hyperactivity disorder (ADHD), Tourette's syndrome, and major depression (Madras et al. 1998; Muller-Vahl et al. 2000; Brunswick et al. 2003; Krause et al. 2003). Additionally, several psychoactive drugs, including cocaine, amphetamine, and methylphenidate, are known to inhibit dopamine reuptake by the DAT protein (Giros et al. 1991, 1992; Giros and Caron 1993; Kilty et al. 1991; Shimada et al. 1991).

10.2 Functional Genetic Polymorphism of *DAT1*: The Variable-Number Tandem Repeat

The dopamine transporter, which is a major tuner of synaptic dopamine levels, is a 620-amino-acid protein belonging to the family of Na^+/Cl^--dependent neurotransmitter transporters with 12 putative transmembrane domains and is located on axon terminals (Uhl 2003). A functional genetic polymorphism exists in part of the 3′-noncoding region included in exon 15 of the *DAT1* gene (Michelhaugh et al. 2001). As shown in Fig. 10.1, this 3′-UTR contains a 40-bp variable-number tandem repeat (VNTR) polymorphism ranging from 3 to 11 repeats, with 9 and 10 repeats being the most common alleles (Vandenbergh et al. 1992; Michelhaugh et al. 2001). We identified 6-, 7-, 9-, 10-, and 11-repeat alleles and their sequences in a Japanese population (Fuke et al. 2005) (Fig. 10.1). The repeats' unit sequences with 9 and 10 repeats were the same as those reported by Mill et al. (2005).

This VNTR polymorphism is known to be associated with many neuropsychiatric disorders such as ADHD, PD, and drug abuse (Cook et al. 1995; Vandenbergh et al. 2000; Ueno 2003; D'Souza and Craig 2008). Many genetic studies have reported significant associations between disorders and addictions with these genotypes. However, discrepancies exist among the studies, although a recent meta-analysis showed a small but significant association between the 10-repeat allele and ADHD (Yang et al. 2007).

If the VNTR is associated with these diseases, what is the mechanism? One possible answer is the different levels of DAT expression among the genotypes. In fact, modified gene expression, depending on the genotype, was observed in vivo (Heinz et al. 2000; Jacobsen et al. 2000; Martinez et al. 2001; Mill et al. 2002; D'Souza and Craig, 2008). We first demonstrated modified gene expression in vitro in Cos-7 cells using the luciferase reporter assay (Fuke et al. 2001), and since then several groups have confirmed the results in mammalian cell lines (Inoue-Murayama et al. 2002;

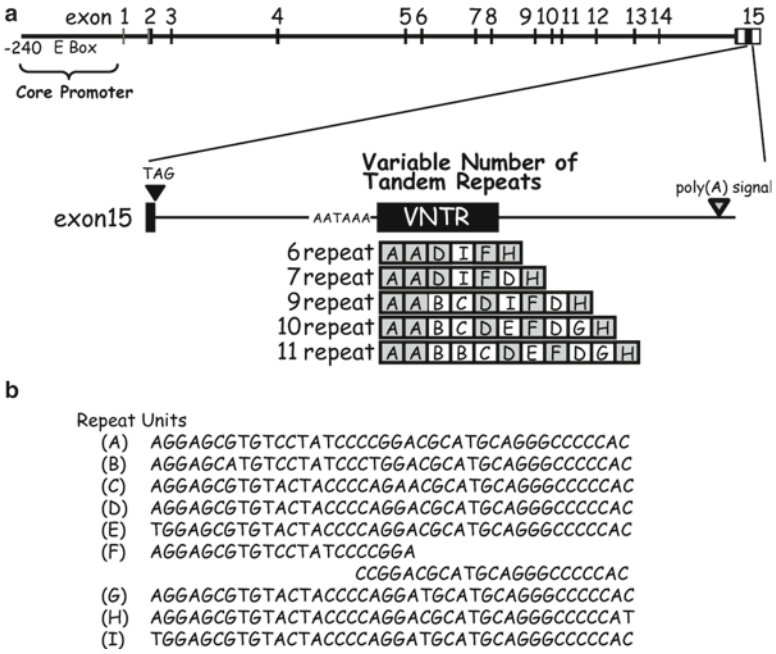

Fig. 10.1 Genomic structure of the *DAT1* gene and allelic variants of variable-number tandem repeat (VNTR) polymorphism in exon 15. (**a**) Coding region (*black box*), noncoding region (*open boxes*), VNTR, and constant repeat units (*gray boxes*) are shown. Exon 15 of the *DAT1* gene contains a stop codon (*black arrowhead*) and polyadenylation signal (*open arrowhead*). Upstream of the VNTR are six nucleotides, AATAAA, that resemble a polyadenylation signal. The allelic variants of VNTR polymorphism indicate repeat units type (*A–I*) in each allele. (**b**) Nucleotide sequence of each unit of VNTR polymorphism in the 3′-UTR of the *DAT1* gene

Miller and Madras 2002; Greenwood and Kelsoe 2003; Mill et al. 2005; VanNess et al. 2005; D'Souza and Craig 2008). However, these studies also generated conflicting results, both in vivo and in vitro.

For example, in in vivo single-photon emission computed tomography (SPECT) studies in the striatum, Jacobsen et al. (2000) reported that DAT availability was higher in the brain of the 9-repeat (r) group than in the 10r group, whereas Heinz et al. (2000) reported that the value in the 10/10r group was higher than that in 10/9r. Martinez et al. (2001), on the other hand, detected no significant difference among genotypes. In a study of postmortem brain tissue, reverse transcription polymerase chain reaction (RT-PCR) evaluation showed that DAT expression in the samples of 10r was higher than that in 9r (Mill et al. 2002; Brookes et al. 2007).

In in vitro studies, possible reasons for these discrepancies include differences in methodology, such as in the cell lines and promoters used in the reporter assay and the location of the 3′ untranslated region (UTR) in the reporter vectors. We observed differing results depending on the cell lines used. The 3′-UTR, including the VNTR, decreased luciferase activity with the DAT1 core promoter in SH-SY5Y,

Neuro2A, and Cos-7 cells but did not do so in HEK293 cells (Fuke et al. 2005). Such differences could be the result of differential expression of regulating factors in each cell, but the molecular and neural bases remain unknown because no factor interacting with the VNTR has yet been characterized, although it is expected that proteins bind to the region (Michelhaugh et al. 2001).

10.3 HESR1: A Protein Binding to the 3′-UTR of DAT

To clarify the molecular mechanism of DAT gene regulation via the VNTR, we screened proteins that bound to the 3′-UTR using a yeast one-hybrid system and identified HESR1 (the hairy/enhancer of split related transcriptional factor 1 with YRPW motif) protein as a *trans*-acting factor through the 3′-UTR of the *DAT1* gene (Fuke et al. 2005). We then showed that HESR1 bound directly to the region by electrophoretic mobility shift assay (EMSA) and repressed expression of the endogenous *DAT1* gene in a mammalian cell line (by RT-PCR assay) (Fuke et al. 2006).

However, it is possible that other factors affect *DAT* gene expression via the VNTR, as it is expected that more than one factor would bind to such a region (Michelhaugh et al. 2001). The HESR family genes – *HESR1*, *HESR2*, *HESR3* – were characterized as a direct transcriptional target of the Notch signaling pathway involved in neural development (Kokubo et al. 1999; Leimeister et al. 1999; Nakagawa et al. 1999, 2000; Henderson et al. 2001; Iso et al. 2001, 2003; Wang et al. 2002; Sakamoto et al. 2003).

The HESR family genes encode a basic helix–loop–helix (bHLH) domain that is essential for DNA binding, an Orange domain, and a YRPW motif. HESR proteins bind to E boxes or N boxes, which are known bHLH-binding consensus sites, and repress expression of target genes (Nakagawa et al. 2000; Iso et al. 2001, 2003). The bHLH domain sequences among the HESR family are highly conserved (Steidl et al. 2000). In fact, human HESR1 and HESR2 (Belandia et al. 2005) and mouse Hesr1 and Hesr2 (Kokubo et al. 2007) repress gene expression at the same genome site in reporter assay systems. Thus, not only HESR1, but also HESR2 and HESR3, may be candidate regulating factors for DAT expression via the VNTR.

10.4 HESR Family Genes: Candidate Regulating Factors for DAT Expression

Recently, we performed luciferase reporter assays to examine whether HESR2 and HESR 3 could affect DAT gene expression via the 3′-UTR including the VNTR region in human neuroblastoma SH-SY5Y cells (Kanno and Ishiura 2009). We found that HESR1 and HESR2 inhibited reporter gene expression via both the core promoter and 3′-UTR, whereas HESR3 enhanced it only via the core promoter. We did not expect the HESR family to affect the core promoter region because HESR1 was

identified as a protein binding to the 3′-UTR, but the core promoter does also contain an E box, known to be a bHLH consensus binding site. Additionally, a functional −67 A/T SNP in this promoter region has been reported to be associated with personality traits such as ADHD and bipolar disorder (Greenwood and Kelsoe 2003; Ohadi et al. 2006, 2007; Shibuya et al. 2009). HESR family proteins may also interact with this SNP. Only HESR3 increased reporter luciferase activity via the DAT core promoter. We also found that HESR1, including the Leu94Met SNP in the second helix of the bHLH domain, lacked inhibitory activity (Fuke et al. 2005). The latest study demonstrated that an SNP transformed HESR1 from an androgen receptor co-repressor to an activator (Villaronga et al. 2009).

Furthermore, HESR1 and HESR2 may differentially alter DAT expression patterns depending on VNTR alleles. Relatively strong inhibition of luciferase activity with 10r was observed with HESR1. In general, our results in these reporter assays showed a tendency for luciferase activity with 9r to be higher than that with 10r, although the difference was not statistically significant, and the highest activity was with 7r. Human HESR2, but not mouse Hesr2, diminished the difference in luciferase activity between 9r and 10r. These findings basically support our idea that different DAT expression levels can be altered by factors in each cell, depending on VNTR alleles. This may explain the discrepancies between the many previous studies described above.

10.5 Behavioral and Neurochemical Aspects of the Hesr Family

We also reported increased expression of the *DAT* gene in the brains of *Hesr1* knockout (KO) mice (Fuke et al. 2006). The KO mice showed decreased spontaneous locomotor activity, reduced exploration of novelty, and enhanced anxiety-like behavior in the open-field test and the elevated plus-maze test (Fuke et al. 2006). This is consistent with our in vitro data because HESR1 is thought to be an inhibitory factor for *DAT*. Additionally, the expression of several dopamine receptor genes, *D1*, *D2*, *D4*, and *D5*, the main targets of synaptic dopamine responsiveness, were enhanced in the *Hesr1* KO mice. Although we did not directly measure synaptic extracellular dopamine levels, decreased activity and increased dopamine transporter and receptors seem to indicate a low synaptic dopamine level in the KO mice. These phenomena are the opposite of those in *DAT* KO mice (Fig. 10.2). Mice lacking the *DAT* gene show decreased intraneural storage of dopamine, spontaneous hyperlocomotion, and down-regulation of several dopamine-related genes, such as dopamine receptor D1 and D2 (Giros et al. 1996; Caine 1998; Jaber et al. 1999; Fauchey et al. 2000; Gainetdinov et al. 2002). This indicates the importance of Hesr1 in the dopaminergic system in vivo.

We also conducted an immunohistochemical analysis to investigate the localization of Hesr family proteins in the mouse midbrain dopaminergic region (Fig. 10.3). Immunostaining for tyrosine hydroxylase (TH), a DA neuron marker, and each Hesr were conducted from the anterior (−3.04 to −3.49 relative to bregma) to the posterior part (−3.94 from bregma) of the midbrain dopaminergic regions: ventral

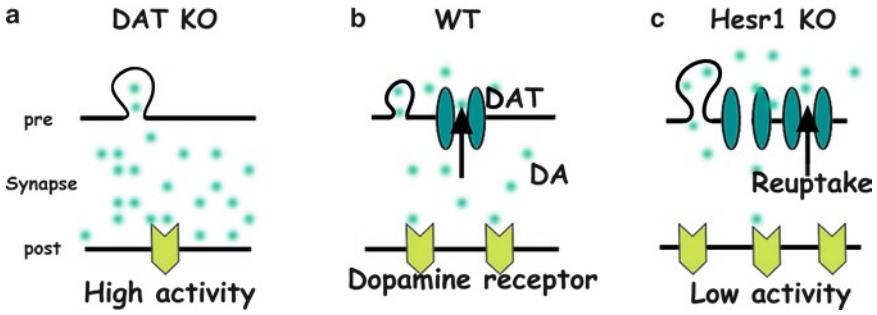

Fig. 10.2 Synapses in *DAT* or *Hesr1* knockout (*KO*) mice. (**a**) *DAT* KO mouse. This indicates increased synaptic extracellular dopamine and decreased dopamine receptors. (**b**) Wild-type mouse. This indicates the normal synaptic state. (**c**) *Hesr1* KO mouse. This indicates possibly decreased synaptic extracellular dopamine and increased dopamine receptors

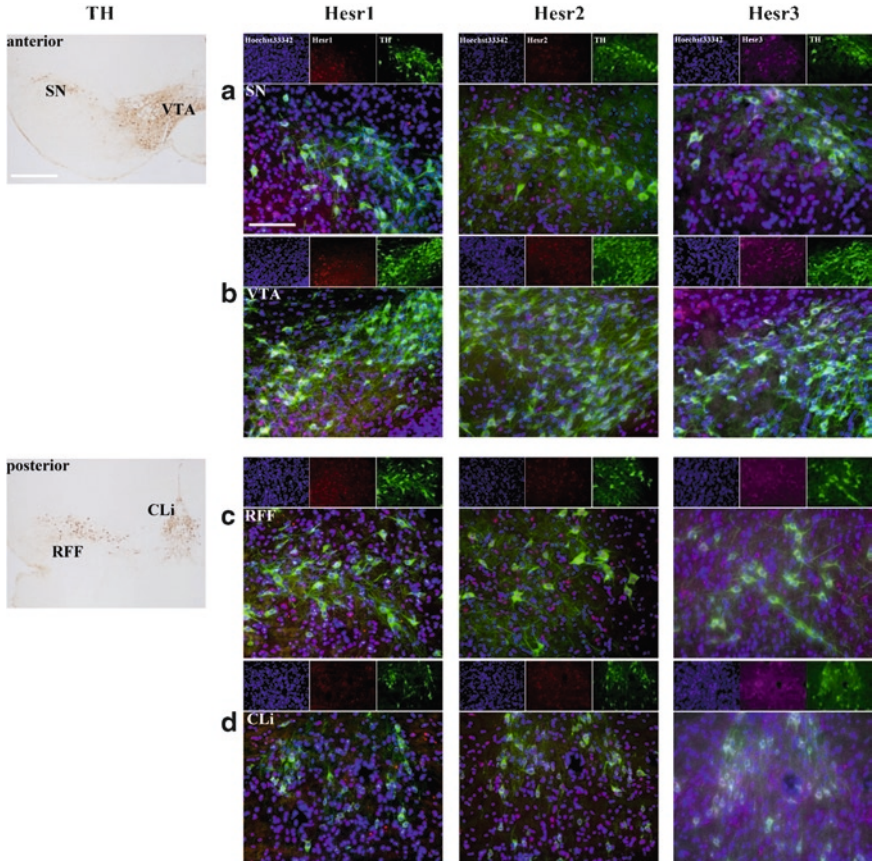

Fig. 10.3 Immunohistochemistry for tyrosine hydroxylase (*TH*) and Hesr family. TH (*green*, Cy2); Hesr1/2 (*red*, Cy3); Hesr3 (*magenta*, Cy3); nucleus (*blue*, Hoechst 33342). *VTA*, ventral tegmental area, *SN*, substantia nigra; *RFF* (RFF/A8), retrorubral field and A8 DA cells; *CLi*, caudal liner nucleus of raphe. *Bars* 500 μm for immunoenzymatic staining for TH; 100 μm for immunofluorescence staining

tegmental area (VTA), substantia nigra (SN), retrorubral field and A8 DA cells (RFF/A8), caudal liner nucleus of raphe (CLi). Each Hesr was expressed in almost all dopaminergic neurons (TH-ir cells) in the mouse midbrain. Thus, Hesr family proteins may affect *DAT* gene expression, as was observed in transfected cells. Further investigation of the in vivo functions of Hesr family members, especially Hesr2 and Hesr3, in the dopaminergic system is needed.

Unique dopamine neurons have recently been found in which DAT expression is relatively low. Lammel et al. (2008) identified a type of dopaminergic neuron within the mesocorticolimbic dopamine system with unconventional fast-firing properties and low DAT/TH mRNA expression ratios that selectively projects to the prefrontal cortex and nucleus accumbens core and medial shell as well as to the basolateral amygdala. Could Hesr family proteins be involved in such a neuron, generating diversity in dopaminergic neurons? Our immunohistochemical study found differential cellular localization between the Hesr family proteins. Hesr1 and Hesr2 were primarily expressed in the nucleus, whereas Hesr3 was cytoplasmic (Fig. 10.3). Additionally, it is possible that cellular localization of Hesr1 is altered depending on the hormonal state (Belandia et al. 2005). A combination of chemical, neuroanatomical, and molecular studies is needed to understand Hesr function in the brain. Such studies may help explain conflicts in the previous in vivo neuroimaging studies (Heinz et al. 2000; Jacobsen et al. 2000; Martinez et al. 2001) and ex vivo RT-PCR analyses (Mill et al. 2002; Brookes et al. 2007).

Although it seems clear from transfection culture studies that the VNTR has a role in regulating DAT1 expression, at the same time, discrepancies have been noted in the differential effects of the various alleles. In the future, an in vivo approach using transgenic mice (e.g., *DAT-9r* or *DAT-10r* knock-in mice) may provide a clearer and more direct approach to characterizing the mechanisms of DAT transcriptional regulation. If such animals are generated, our data from luciferase assays with the mouse Hesr family can add a molecular basis to the research.

Our recent findings of HESR family function regarding *DAT* may suggest new strategies for the treatment of *DAT*-related disorders. Functional VNTR polymorphism also exists in the SERT gene located in intron 2, and two transcription factors, Y box-binding protein 1 (YB-1) and CCTC-binding factor (CTCF), were found to be responsible for the modulation of VNTR function (Klenova et al. 2004). YB-1 and CTCF are targets of lithium (LiCl), a mood stabilizer (Roberts et al. 2007). LiCl modified the levels of CTCF and YB-1 mRNA and protein. HESR proteins may also be a target of drugs.

10.6 Conclusions

Our studies and others indicate that the VNTR in the 3′-UTR of the *DAT* gene affects gene expression. Ex vivo RT-PCR studies and in vivo human neuroimaging studies have demonstrated differential DAT expression depending on the alleles, primarily focusing on 9r and 10r, although the results are conflicting.

More genetic and personality studies combined with neuroimaging should be done to clarify the relation between psychological and neurological states, especially DAT expression levels or function. Further molecular biological studies are also necessary to clarify the mechanism of modification of DAT expression and its signaling pathway, which may also help find new neuropsychological drug targets.

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Chapter 11
Molecular Behavioral Research in Great Apes

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11.1 Introduction

Since the initial reports linking human "novelty seeking" to the dopamine receptor D4 (*DRD4*) locus (Benjamin et al. 1996), there have been multiple studies examining associations between human personality traits and specific genotypes of neurotransmitter-related proteins (Ebstein 2006) (see Chap. 10). Recently, research on the genetic bases of personality traits has been extended to studies of within- and between-species studies of nonhuman primates. Personality is defined as those characteristics of individuals that describe and account for temporally stable patterns of affect, cognition, and behavior. As is the case in humans, these individual differences have a biological and experiential basis (Gosling 2008). Studies examining the relation between genotypes and personality in nonhuman animals started with experimental models such as mice, which enabled researchers to evaluate the functions of specific genes (Hohoff 2009) (see Chap. 10). Later studies examined a wider range of species, such as dogs (see Chap. 12) and even birds (see Chaps. 7 and 13). Nonhuman primates are subject species for comparative research as they are evolutionary neighbors of humans and exhibit a wide range of individual differences in behavioral traits. During the past decade, studies of nonhuman primates have primarily focused on rhesus macaques (Barr et al. 2003, 2004; Miller et al. 2004; Newman et al. 2005; Wendland et al. 2006; Spinelli et al. 2007; Jarrell et al. 2008; Rogers et al. 2008). Recently, however attention has been focused on our closest nonhuman relatives, the great apes.

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Great apes exhibit complex social behaviors and a diverse array of social structures. Chimpanzee (*Pan troglodytes*) and bonobo (*Pan paniscus*) social structures are characterized by multi-male, multi-female groups; gorilla (*Gorilla*) social structure is mostly characterized by uni-male harems; and the social structures of the more distantly related orangutans (*Pongo*) or gibbons (*Hylobates*) are semi-solitary and pair-based, respectively. Together with other factors such as ecology and phylogeny, social structure affects or reflects species difference in personality, such as levels of aggression, affiliation, and pair-bonding. Similarly, social structures could also be reflected in genetic differences.

Recently, the personalities of captive chimpanzees, gorillas, and orangutans have been reliably assessed (King and Figueredo 1997; Kuhar et al. 2006; Weiss et al. 2006, 2009) (see Chaps. 5–9). In addition, the genotypes of these species have been characterized (Table 11.1) (Inoue-Murayama 2009). However, no study to date has examined the relation between the personalities of these species and their genotypes. Human personality domains are related to well-being, mental and physical health, and mortality across a wide range of samples (Ozer and Benet-Martínez 2006). Thus, assessing the personalities of captive animals may very well have implications for animal welfare in that caretakers would be able to identify at-risk individuals whose health and well-being should be more closely monitored. Furthermore, if the genetic bases of personality in apes are identified, we may be able to genotype individuals for markers related to personality and therefore health.

In this chapter, we provide an overview of our current research on the personality and genotypes of great apes and other primate species. At first we provide an overview of the great apes kept in Japan. We then introduce findings from molecular genetic studies of human personality and the results of studies of corresponding loci in nonhuman primates. Here, we examine differences among species, groups, and individuals. We then focus on species differences in the personalities of chimpanzees and gorillas as examined using ratings and molecular genetic approaches. Finally, we discuss future directions for this work.

11.2 Great Apes in Japan

In Japan, there are a total of 342 chimpanzees (*P. troglodytes*) in 53 facilities, 24 gorillas (*Gorilla gorilla*) in 10 facilities, and 45 orangutans (*Pongo pygmaeus* and *Pongo abelii*) in 23 facilities (December 2009, Great Ape Information Network http://www.shigen.nig.ac.jp/gain/index.jsp). In the wild, chimpanzees and gorillas live in large groups. However, although there are notable exceptions such as the Tama Zoological Park in Tokyo, captive great apes kept in Japan live in small groups. In 2004, for example, 34% of facilities kept one or two chimpanzees, and 13% of facilities kept ten and more chimpanzees, although not all of the latter facilities kept their chimpanzees in a single group (Ochiai-Ohira et al. 2006). Also, among the 212 founder chimpanzees, approximately 60% could reproduce, and six male founders had 137 descendants (58.8% of all chimpanzees born in Japan). These situations have led to lower

Table 11.1 Candidate genes for behavior in primates

Gene	Abbreviation	Polymorphic region	Repeat unit/ SNP	Personality/ disorder	Reference (humans)	Reference (nonhuman primates)	Species in which polymorphism was reported
Domamine receptor D4	*DRD4*	Exon3	48 bp (16aa)	Novelty seeking	Benjamin et al. (1996)	Inoue-Murayama et al. (1998, 2000b)	Gorilla, Hylobates, Marmoset
		Exon1	12 bp (4aa)	Delutional disorder	Catalano et al. (1993)	Seaman et al. (2000)	Gorilla, Orangutan, Hylobates, Macaque
		Intron	3 bp	?	Shimada et al. (2004)	Shimada et al. (2004)	Chimpanzee, Orangutan, Hylobates, Macaque
Dopamine transporter	*DAT*	3′UTR	40 bp	Novelty seeking	Sabol et al. (1999)	Inoue-Murayama et al. (2002)	Chimpanzee, Rhesus macaque
Serotonin transporter	5HTT	Promoter	20 bp	Anxiety	Lesch et al. (1996)	Inoue-Murayama et al. (2000a)	Gorilla, Hylobates, Macaque
		Intron	18–20 bp	Anxiety	Vormfelde et al. (2006)	Inoue-Murayama et al. (2008)	Chimpanzee, Gorilla, Orangutan, Hylobates, Macaque
Tryptophan hydroxylase 2	*TPH2*	Exon	A (Gln) → G (Arg)	Depression	Zhang et al. (2005)	Hong et al. (2007b)	Chimpanzee
Monoamine oxidase A	*MAOA*	Promoter	30 bp, 18 bp	Aggression	Alia-Klein et al. (2008)	Wendland et al. (2006), Inoue-Murayama et al. (2006)	Chimpanzee, Gorilla, Orangutan, Macaque
		Intron	CA	Bipolar disorder	Furlong et al. (1999)	Hong et al. (2008)	Chimpanzee, Gorilla, Orangutan
Monoamine oxidase B	*MAOB*	Intron	GT	Parkinson's disease	Mellick et al. (2000)	Hong et al. (2008)	Chimpanzee, Gorilla, Orangutan, Hylobates, Macaque
Androgen receptor	*AR*	Exon	3 bp (Gln)	Aggression	Comings et al. (199a)	Hong et al. (2006)	Chimpanzee, Gorilla, Hylobates
			3 bp (Gly)	Neuroticism	Westberg et al. (2009)	Hong et al. (2006)	Chimpanzee, Gorilla, Hylobates
Estrogen receptor alpha	*ESRa*	Promoter	TA	Anxiety	Comings et al. (1999b)	Hong et al. (2007a)	Chimpanzee, Gorilla, Hylobates
Estrogen receptor beta	*ESRb*	Intron	CA	Alzheimer's disease	Forsell et al. (2001)	Hong et al. (2007a)	Chimpanzee, Gorilla, Orangutan, Hylobates
Vasopressin receptor	*AVPRla*	Promoter	GATA	Pair bonding	Walum et al. (2008)	Rosso et al. (2008), Hong et al. (2009)	Chimpanzee, Gorilla, Orangutan, Hylobates, Macaque

rates of reproduction among chimpanzees in Japan; and as a consequence of these factors, the number of captive chimpanzees in Japan is decreasing (Morimura et al. 2008). Recently, in an attempt to improve the welfare of captive apes, most captive facilities in Japan have begun to enact enrichment programs that attempt to reproduce the natural environments of these species (Morimura et al. 2008). These facilities have also enthusiastically contributed data to studies that seek to understand the mental states of great apes by asking caretakers to rate their personalities and well-being on well-validated scales (Weiss et al. 2009).

11.3 Molecular Genetic Studies of Human Personality

Molecular genetic studies of human personality are well on their way. These studies focus on monoamine neurotransmitters, such as dopamine or serotonin, which are released into the synaptic cleft and bind to receptors. These neurotransmitters are then taken up by transporters and degraded by monoamine oxidase or reused. Individual differences in genes that code for these proteins, polymorphisms of tandem repeats, insertions, deletions, or single nucleotide polymorphisms (SNPs) in their exon, promoter, or untranslated regions, can affect personality. For example, the human *DRD4* is polymorphic in repeat numbers of the 48-bp unit in the third exon corresponding to the third cytoplasmic loop of the receptor. Individuals with longer repeats in the gene scored higher in "novelty seeking" than did those with shorter repeats (Benjamin et al. 1996). In addition, the promoter region of the serotonin transporter gene (*5-HTT*) includes tandem repeats based on 20- to 23-bp units, and two major alleles with 14 (short) and 16 (long) repeats have been found in humans. This polymorphism has been related to anxiety, as individuals with short alleles displayed higher scores for anxiety-related traits such as neuroticism than did those with long alleles (Lesch et al. 1996). A reporter gene assay showed that promoter activity of the short allele was lower than that of the long allele, suggesting that the smaller number of serotonin transporters in the synapses of individuals with short alleles is responsible for the personality differences. Finally, variation in genes associated with hormone-related proteins such as androgen receptors are associated with aggression in humans; individuals with short repeat of glutamate in androgen receptor tend to be more aggressive (Rajender et al. 2008).

Polymorphisms in neurotransmitter-related genes are related not only to personality but also to psychiatric disorders, including schizophrenia and depression (D'Souza and Craig 2008). Therefore, surveying these genes in our closest nonhuman relatives might be useful for understanding the evolutionary bases of and treating these disorders in humans and nonhuman primates. Unfortunately, attempts to replicate these personality–genotype associations in other countries and ethnic groups have failed. These failures have been attributed to small sample sizes, polygenic inheritance patterns, or the measures of personality (Ebstein 2006). Studies of animal models might yield insights that lead to a breakthrough in understanding the relations between genotypes and personality.

11.4 Comparison of Allele Distribution and Its Effect Among Species

Table 11.1 summarizes the polymorphism of regions corresponding to those of humans in several nonhuman primate species. We focused on the variable number of tandem repeats (VNTR) because this polymorphism is easily detectable. The number of observed alleles in apes and Japanese macaques (*Macaca fuscata*) is summarized in Table 11.2. We found that not all of the corresponding regions were polymorphic in all nonhuman primate species. Furthermore, the range of variation in the number of repeats among nonhuman primates was sometimes outside the range found in humans, indicating possible functional differences for these alleles in nonhuman primates. For example, the repeat sequence of the 3′ untranslated region of dopamine transporter (*DAT*) is reported to be related to novelty seeking (Sabol et al. 1999). In the corresponding region of chimpanzees and gorillas, alleles with one or two repeats were detected, whereas in humans nine or ten repeats are common (Inoue-Murayama et al. 2002). In the reporter gene assay of the polymorphic regions, luciferase activity of chimpanzees and gorillas was twice that of humans (Inoue-Murayama et al. 2002). In the VNTR in the promoter region of monoamine oxidase A gene (*MAOA*), compared to humans, the luciferase activity of transfected cells was found to be lower in chimpanzees and higher in gorillas (Inoue-Murayama et al. 2006).

The results of these assays enable us to estimate the signal transduction of humans, chimpanzees, and gorillas. The number of dopamine transporters in the brain is estimated to be higher in chimpanzees and gorillas than in humans. Therefore, the signal duration should be shorter in apes than in humans. However, compared to humans, the expression of oxidase is lower in chimpanzees and higher in gorillas. Therefore, the signal strength is likely strongest in chimpanzees and weakest in gorillas, with

Table 11.2 Number of alleles observed in primates

		Chimpanzee (*P. t. v*)	Gorilla (*G. g. g.*)	Orangutan (*P. p. p.*)	Siamang (*S. s.*)	Macaque (*M. f. f.*)
Gene	Region	$n=56$	$n=16$	$n=20$	$n=17$	$n=30$
DRD4	Exon1	1	2	3	3	2
	Intron	3	1	2	4	2
	Exon3	1	2	3	2	1
DAT	3′UTR	2	1	1	1	2
5-HTT	Promoter	1	4	3	1	1
	Intron	2	5	2	2	2
MAOA	Promoter	2	4	2	1	–
	Intron	4	6	4	–	1
MAOB	Intron	4	6	4	–	6
AR	Exon (poly-Q)	12	3	1	1	2
	Exon (poly-G)	6	2	1	6	1
ESRa	Intron	4	3	1	1	–
ESRb	Intron	8	7	2	6	–
AVPRla	Promoter (RS1)	6	7	5	7	4

humans showing intermediate signal strength. Thus, even in our closest nonhuman relatives, the neural transmission systems greatly differ. These differences in expression may be one cause of species differences in behavior.

Other genes displayed a change in frequencies across species suggesting that they were linearly related to the hominization (Inoue-Murayama et al. 2001). The frequencies of the longer repeat allele of *DRD4* tended to increase as a function of the phylogenetic proximity to humans. On the other hand, the repeat number in the promoter region of *5HTT* tended to decrease as a function of phylogenetic proximity to humans. These changes suggested that during hominization alleles related to greater novelty seeking and anxiety were favored in the human ancestors who left the rainforest for new environments teeming with opportunities and hazards.

11.5 Genetic Basis of Behavior in Nonhuman Primates

The association of genes with behavioral traits has primarily been studied in rhesus macaques (*Macaca mulatta*) (Barr et al. 2004). This species has a repeat polymorphism in a region corresponding to human *5-HTT*, and functional differences of each allele are similar to those in humans (Heinz et al. 1998). Rhesus macaques with short alleles tended to be more anxious than those with long alleles (Barr et al. 2003; Rogers et al. 2008).

In addition, a gene for monoamine metabolism (*MAOA*) has been studied as a candidate gene for aggressive behavior. The repeat region in the *MAOA* promoter region included functionally different alleles in rhesus macaques: Mother-reared monkeys with low-activity long alleles were more aggressive than mother-reared monkeys with high-activity short alleles; peer-reared monkeys with low-activity alleles were less aggressive than peer-reared monkeys with high-activity alleles (Newman et al. 2005). Associations of SNPs of mu-opioid receptors and aggression have also been reported in rhesus monkeys (Miller et al. 2004). In vervet monkeys (*Cercopithecus aethiops*) novelty seeking was associated with the *DRD4* genotype (Bailey et al. 2007).

Group differences in Japanese macaques were surveyed (Inoue-Murayama et al. 2010). We genotyped two candidate loci: the promoter region of *MAOA* and an androgen receptor exon (*AR*) in samples from eight regions of Japan to examine population differences of allele frequencies. The Awajishima group, which are known to be highly tolerant of each other in the feeding area (Koyama et al. 1981), differed greatly from other groups in the allele frequencies of *MAOA* and *AR*. However, these genetic differences have not yet been related to individual differences in behavior in populations. Because detailed pedigrees and behavioral records are available, Japanese macaques are an excellent subject species for quantitative behavior–genetic studies. However, because Japanese macaques have only a few polymorphic genes (Table 11.2), prior to conducting molecular genetic studies more candidate genes with polymorphisms should be identified.

11.6 How to Evaluate Personality in Apes

Great apes clearly exhibit individual personalities (Fig. 11.1). To find the relation between genotype and personality requires reliable and valid measures of personality. Uher et al. (2008) broadly classified these methods as being either "top-down" or "bottom-up." The former method involves basing nonhuman primate personality or well-being measures on existing personality measures, whereas the latter method involves assessing personality or well-being via behavioral observations (see Chap. 5 for a detailed discussion). An example of a top-down study includes a recent study of 146 captive chimpanzees in Japan rated on 54 adjectival personality descriptors and four well-being items (Weiss et al. 2009). The personality scale in this study was partly based on measures of the human Five-Factor

Fig. 11.1 Possible behavioral manifestations of personality traits in chimpanzees: (**a**) angry displays, (**b**) reconciliation, (**c**) greediness, and (**d**) exploratory behavior. (Courtesy of Toshifumi Udono of the Chimpanzee Sanctuary Uto)

Model (e.g., Goldberg 1990) (Table 11.3). The well-being items in this study were based on measures of human happiness (Diener and Emmons 1984; Pavot et al. 1991; Cantor and Sanderson 1999). An example of the bottom-up approach involved behavioral observations in which durations of observed behaviors were recorded in 20 zoo-housed great apes: bonobos, chimpanzees, gorillas and orangutans (Uher et al. 2008).

11.7 Species Differences in the Personalities of Apes

Using a top-down method, we conducted a set of preliminary analyses to test for mean-level differences in the personality dimensions of chimpanzees and gorillas. Table 11.3 shows the factor scores for chimpanzees and gorillas assessed using the same 54 trait descriptor adjectives and four subjective well-being items. We obtained ratings on 14 individuals from three zoos. We then identified a sex- and age-matched sample of 14 chimpanzees and compared their scores with those of the gorillas. To rule out the possibility that mean differences reflected different facilities or raters, we also compared a subsample of four gorillas and five chimpanzees that lived in the same zoo and were therefore rated by the same caretakers. Compared to chimpanzees, gorillas were higher in Conscientiousness ($P<0.05$). The same tendency was observed among individuals who lived in the same zoo and were rated by the same raters. In addition, Agreeableness was higher in gorillas than in chimpanzees. Finally, Neuroticism and Openness were higher in chimpanzees than gorillas, and this held for the total sample as well as the subsample of individuals in the same facility. Conscientiousness is defined by a positive loading on the item predictable and negative loadings on the items impulsive, defiant, reckless, erratic, irritable, aggressive, jealous, disorganized, thoughtless, distractible, quitting, and clumsy (Weiss et al. 2009). Although these findings are preliminary, they clearly merit future studies involving larger samples rated by a common set of raters. Moreover, similar studies of phenotypic differences can guide the search for candidate genes in molecular genetic studies that are related to species differences in behavior.

Chimpanzee and gorilla personality traits and subjective well-being can also be compared with respect to the amount of variance in these traits. Compared to chimpanzees, gorillas showed more variation (as indicated by a higher standard deviation) in Dominance, Extraversion, Neuroticism, Openness, and subjective well-being. For Conscientiousness and Agreeableness, more variance was observed in chimpanzees. These differences suggest differences between those traits that best capture individual differences in chimpanzees and gorillas.

11.8 General Discussion and the Way Forward

In this chapter, we highlighted the findings of studies that explored the genetic bases of personality differences in nonhuman primates. Finding similar personality–genotype relations at the species, group, and individual levels in nonhuman

Table 11.3 Factor scores of chimpanzees and gorillas

			Dominance	Extraversion	Conscientiousness	Agreeableness	Neuroticism	Openness	SWB
Total	Chimpanzee ($n=14$)	Cavg	4.3±0.50	4.19±0.64	4.65±0.67	3.67±0.80	3.64±0.70	3.89±0.79	4.43±0.75
	Gorilla ($n=14$)	Gavg	4.13±0.75	3.79±1.06	5.15±0.61	3.81±0.77	3.45±0.92	3.43±1.16	4.16±1.12
		P value	0.496	0.243	0.047*	0.654	0.545	0.235	0.454
One zoo	Chimpanzee ($n=5$)	Cavg	4.07±0.23	3.96±0.68	4.49±0.55	3.54±0.72	4.11±0.71	3.95±0.76	3.80±0.49
	Gorilla ($n=4$)	Gavg	4.16±0.54	4.67±0.32	4.88±0.28	3.99±0.62	3.70±0.44	3.81±0.90	4.46±0.59
		P value	0.758	0.098	0.235	0.364	0.353	0.810	0.108

*: $P<0.05$

primates would provide strong evidence supporting the present findings. On the other hand, differences between humans and nonhuman primates in how genotypes are related to personality could suggest the presence of species-specific factors that are related to personality.

In the preliminary study reported here, we described mean personality differences between chimpanzees and gorillas. The fact that chimpanzees display lower Conscientiousness scores than gorillas is intriguing. This dimension contains a facet previously labeled "tameness" (King et al. 2008), which is comprised of traits such as (not) aggressive. Aggression-related traits have been associated with *MAOA* and *AR* genotypes in humans and macaques (Table 11.1). Although not conclusive, the fact that chimpanzees have lower levels of Conscientiousness and lower *MAOA* expression than gorillas (Inoue-Murayama 2009) suggests that the species difference in Conscientiousness has its origins in the different *MAOA* genotypes. On the other hand, the fact that the short allele of *AR*, which is related to aggression in humans (Comings et al. 1999a), is less frequent in chimpanzees than gorillas is not consistent with the Conscientiousness differences between species. Clearly, more studies looking for parallels between species differences in genotypes and personality phenotypes are needed to reach a firm conclusion. Variation in personality scores within species was large enough to measure individual differences in chimpanzees and gorillas. Molecular genetic studies of personality within species of great apes are currently underway. For example, we found that an SNP (Q468R) in the tryptophan hydroxylase 2 gene (*TPH2*) coding region was related to enzyme activity. Chimpanzees whose *TPH2* was characterized by the 468R allele had higher serotonin levels than chimpanzees whose *TPH2* was characterized by the 468Q allele (Hong et al. 2007b). This functional difference might be related to personality traits related to serotonin activity such as neuroticism.

The present findings were based on ratings of captive animals. It is possible that in the wild various environmental influences affect group composition and social interaction and that these factors might result in the genetic selection of specific levels of personality traits. All gorillas in Japan are western lowland gorillas (*G. gorilla gorilla*), and most chimpanzees are western chimpanzees (*P. troglodytes verus*). Comparisons of personality in wild chimpanzees from different regions or different subspecies might provide information to understand how specific social interactions and group compositions are selective pressures for personality.

In addition, we hope that future studies explore other possibilities. For example, the relation between the *5HTT* genotype and personality was greater among humans exposed to life stressors (e.g., losing family members) (Caspi et al. 2003). A similar effect was found in rhesus macaques: *5HTT* genotype was related to aggressive behavior in peer-reared but not mother-reared monkeys (Barr et al. 2004; Spinelli et al. 2007). In chimpanzees, the rearing environment affects the quality of social interactions, cognitive abilities, tool use, and other aspects of chimpanzee behavior. These behaviors are important and may contribute to their quality of life. Given that the environmental histories of many captive-born apes are documented, gene x environment studies of ape personality could provide useful information for improving the welfare of great apes.

Studies relating personality or genetic polymorphisms to other outcomes in nonhuman primates could lead to a better understanding of the physiological and genetic pathways underpinning personality. Prior research in this area has found that the *5HTT* genotype in rhesus macaques is related to measures such as plasma cortisol level as an indicator of stress (Jarrell et al. 2008) and that chimpanzee cortisol levels were related to a personality dimension labeled "mellow" (Anestis et al. 2006). Other studies have shown associations between personality traits in rhesus macaques and immunocompetence (Capitanio et al. 1999, 2008). Moreover, Danese et al. (2004) have shown that the constitution of intestinal flora is related to human personality dimensions; this relation can be explored potentially in chimpanzees (Irbis et al. 2008). Finally, brain imaging of labeled ligand distribution in conscious marmosets has found associations between brain activation and personality-like traits (see Chaps. 18 and 19).

Research on other individual differences, such as cognitive abilities (Deary et al. 2009), could also benefit from comparative genetic studies such as those reported in this chapter. Moreover, tests used to study cognitive functioning may be adopted for the study of personality. For example, to study the role of serotonin transporter genes in social interactions, Watson et al. (2009) assessed social reward and punishment via responses to photographs of high-status males. Finally, it would be worthwhile to assess great ape personality using a variety of other behavioral tests (e.g., Uher et al. 2008).

Given the relatively small number of chimpanzees in captivity, it is unlikely that genome-wide association studies will sweep over the landscape of great ape personality research as they have in human research. However, genome-wide association studies in humans (e.g., Terracciano et al. 2008) can undoubtedly inform candidate gene studies in great apes. These candidate gene studies may provide information useful for identifying at-risk individuals and maximizing their well-being, and they certainly will be an effective tool for understanding personality's evolutionary origins.

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Chapter 12
"Genetics and the Social Behavior of the Dog" Revisited: Searching for Genes Relating to Personality in Dogs

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12.1 New Concepts in Dog Research

Without a doubt, the scientific interest in dogs has grown tremendously. Previously, dogs were considered "artificial" animals and of little interest to biologists studying the causes of behavior. However, things have changed, partly due to parallel developments of thoughts and research efforts in ethology, genetics, and evolutionary biology. Many researchers have realized that if scientific questions are asked in the right way, the biological study of dogs could provide valuable (and even generalizable) answers. These new insights have put dogs in the forefront of biology, and this is particularly the case for medical and behavioral genetics.

Behavioral genetics researchers traditionally used rodent models, mainly because of the rodents' short lifespans and tolerance of laboratory environments. However, the social structure of mice and rats differ greatly from that of the top predator, humans. Canids' social systems show more similarities and therefore as a species are better models for humans in several aspects. Dogs' genetic heritage from the ancient wolves includes the ability to maintain life-long relationships with their mates, food-sharing, context-dependent hierarchies, and complex communicative behavior – all of which are similar to the abilities of humans (Miklósi 2007). Domestication may have facilitated the emergence of different social cognitive skills in dogs, enhancing their chances of survival in human families. Exposure to the human social environment results in individual experiences that in many respects correspond to that of human children. Therefore, the comparison of human infants and dogs enables one to determine how two organisms with very different evolutionary paths behave after having been exposed to a similar social environment

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(Gomez 2005). Moreover, the genetic sequence information of dogs is considered a standard for comparison to the human genome system (Wayne and Ostrander 2004). Dog's phenotypic diversity and the large number of genetic diseases common to humans (Wayne and Ostrander 2007) provide a unique possibility to model biologically relevant questions of basically human interest.

12.1.1 Ethology: Human and Dog Behavioral Parallels

Wherever they live in the world, most dogs develop some ties with the local human populations. In some cases this could be very close (e.g., if dogs and humans share their resources in a family setting). These dogs are usually described as pets and are considered family members by humans (Kubinyi et al. 2009). For many years, researchers have argued that this co-habitation would not be possible without some genetically based changes that allow dogs to form complex social relationships with humans (see Miklósi 2007 for a review). Recent findings (some of which have been supported in independent laboratory investigations) reveal that dogs have the ability to rely on complex human visual and acoustic communicative cues, can obtain information by observing human companions, may understand some aspects of human intention, and engage in complex cooperative interactions (see Miklósi 2007 for a review). The corpus of data suggest that in a broader framework the behavioral changes in dogs could be viewed as convergent in relation to functionally similar human traits (Topál et al. 2009a). During their evolution, dogs adopted patterns of behavior that to some extent functionally matched those of their human partners. Based on this theory, we expect dogs to show behavioral differences from those of their ancestor, the wolf, which enhance their chances of survival in a human setting. At the same time, the behavior of dogs shares some similarity with that of their human counterparts (e.g., Topál et al. 2009b).

In 2000, Overall as well as Ostrander and her colleagues introduced the concept of the dog as a natural model of human behavior in respect to mental disorders (Ostrander et al. 2000; Overall 2000). It soon became obvious that this idea could be extended to personality traits of healthy individuals as well.

12.1.2 Domestication: Evolutionary Process

Although Darwin used many examples of domesticated animals for making plausible suggestions about the hypothesized evolutionary processes to the inexperienced readers of his time, it took some time to realize the truth in these arguments. For many years, domestication was considered artificial selection for animals living in captivity. Price (1984) was one of the first to define domestication as an adaptation to captive environments that is achieved through genetic changes that occur over generations, environmental stimulation, and experiences during an animal's lifetime.

In line with this concept, Coppinger and Coppinger (2001) suggested that the habitat overlap between wolves and humans facilitated the emergence of subpopulation(s) of wolves that, through their closer contact with humans, became slowly isolated from the "wild" wolf population. This early separation into two populations was followed by a diverse selection for different phenotypes, which became the ancestors of today's dogs. This so-called two-stage process has recently gained support in the field of evolutionary biology. The statistical analysis and modeling of linkage disequilibrium in dogs suggested two population bottlenecks during the course of domestication (Lindblad-Toh et al. 2005). However, from an evolutionary point of view, there could be some differences. The first bottleneck might have been associated with directional selection for certain "general" dog-specific morphological and behavioral traits that are still characteristic for present-day dogs. The second bottleneck might have emerged during a diversifying selection process when some clades of dogs as well as the ancient genetic stock of modern breeds became established. A similar two-stage process has been suggested on the basis of behavioral differences between wolves and dogs (Hare and Tomasello 2005). Accordingly, the first-phase selection favored individuals who showed specific actions in a wide range of social behaviors (changes in emotional reactivity), and the second phase favored individuals expressing specific behavioral skills (e.g., hunting performance) (Brenoe et al. 2002).

Recent estimates of dog–wolf differences at the DNA level suggest a value of about 0.2% (Wayne 1993). Naturally, at present, it is difficult to judge the functional genetic nature of such a difference. The 25,000–50,000 years since their divergence might not have been enough for the emergence and spreading of novel mutations in dogs. However, there are some findings that counter such views. Recently, Parker et al. (2009) found that a second copy of an existing gene, *FGF4* (fibroblast growth factor 4), was inserted at the other end of the same chromosome (no. 18) and retarded the growth of short-limbed dogs. The trait is dominant, so it could have manifested relatively rapidly in the dog population. A second source for the difference could come from changes in allele frequencies. During domestication, dogs with some types of allele could have been selected for in the anthropogenic environment. In certain cases, some existing but rare alleles could become increasingly frequent in dog populations (see also below).

12.1.3 Genetics: Dog Genome

Two years after the publication of the first generation of dog DNA sequence (Kirkness et al. 2003) the high-quality draft genome sequence of the domestic dog was made public (Lindblad-Toh et al. 2005). This information on the genetic structure not only paved the way for studying the functional significance of genes in dogs but also allowed a comparison with the genomic structures of humans and mice.

These analyses revealed that despite the fact that the ancestors of dogs were separated much earlier from the joint primate–rodent clade in some respects,

compared to rodents, the genomic structure of dogs may be more similar to that of humans (see Haitina et al. 2009). Genes that are shared between dog and human, on average, show approximately twice the sequence similarity as is observed between human and mouse genes (Wayne and Ostrander 2004). This likely reflects the more rapid rate of sequence evolution in the mouse compared to human and dog (Kirkness et al. 2003). The dog sequence recovers slightly more human genes (18,473) than does that of the mouse (18,311).

In summary, it seems that in addition to the more recent convergent evolutionary process that affected the behavior and probably the genetics of the dog, the particular evolution of the Canidae clade (including the wolf and the dog) retained an increased similarity to the genome of primates (including humans). As a result, we should expect a mixture of both homologies and convergences at various levels of biological organizations such as genes and behavior traits.

12.2 In the Footsteps of Giants

Scott and Fuller's *Genetics and the Social Behavior of the Dog* (1965) became the bible of dog researchers. The work covered by the book was aimed at providing information about the genetic background of morphological and behavioral features of the dog. Scott, Fuller, and their colleagues applied a traditional Mendelian method for the genetic analysis, but they also carefully controlled for environmental influences. They worked with various breeds of dog that were similar in size and were maintained and socialized in similar ways. They were particularly careful when choosing the breeds for comparison. Basenijs represented the "ancient dogs," and Beagles, Shetland sheepdogs, Cocker Spaniels, and Fox Terriers represented groups of dog breeds that have been selected for various behaviors and forms. There are many lessons to be learned from these efforts. Taken together, this work did not differ much from laboratory studies of rodents. This approach constrained the chances of researchers to demonstrate the uniqueness of dogs. Without providing a full list, here are a few points for consideration.

Although all dogs were socialized to the human laboratory staff, no individual social relationships developed. Understandably, the large number of animals prohibited a more intensive social contact between humans and dogs; but, then, these subjects could not be considered to have experienced "normal" environmental input. Nevertheless, they missed simple, ethologically based behavioral traits assessing dog–human relationships (e.g., attachment and social cognition). Instead, they preferred to use laboratory learning tests established for testing rats or monkeys (e.g., T-mazes and string pulling). Although the biological parallels between dogs and humans was a fundamental issue for Scott and Fuller, their approach for testing behavioral phenotypes in dogs prevented direct comparative accounts due to the lack of complementary data on people.

Scott and Fuller reported considerable differences among breeds, but they also found remarkable variation in individual behavior in the tests. Notably, the authors

concluded that "it is impossible to generalize about any one breed from experience with one dog or even one strain of dogs" (Scott and Fuller 1965, p. 378). It seems that the morphological similarity of dog breeds may be deceptive, leading many researchers to assume a similarly homogeneous behavioral phenotype and physiology, which is clearly not the case.

Scott and Fuller also noted that that there are relatively few general behavioral traits in dogs. Dog breeds could be considered as behavioral mosaics of special characteristics, many of which reflect their selection history.

The work and research strategy started by Scott and Fuller has been continued on rodents. Dog research should not and cannot compete with these efforts for theoretical, methodological, and practical reasons. However, for the very same reasons, research on privately owned family dogs could provide a valuable model for human behavior. First, as pointed out previously, one of the most unique additions of dogs to research is their specific evolutionary history with humans. This can be investigated only if dogs are observed (and reared) in their natural environment (see above). Second, if comparative behavioral work is envisaged (whether it concerns "normal" or "pathological" traits), observations and tests should be comparable to those of humans (e.g., Vas et al. 2005; Lakatos et al. 2009). Third, from both the welfare and financial points of view, it is problematic to keep a large number of dogs in a laboratory setting.

12.3 Dogs in Their Natural Environment

12.3.1 Practicalities of Dog Research

One of the most important reasons dogs have become a favorite animal for behavioral scientists is the practical aspect. The natural environment of dogs is the human social setting, which means that studies can be conducted anywhere – from the home with a family to an empty room of the laboratory. In the home, however, there is no need for animal housing, trained animal care staff, and so on. The natural environment of dogs varies greatly and can be manipulated more easily than that of humans. For example, it would be possible to conduct cross-fostering studies of dogs. The health care service for dogs is almost comparable to that of humans. The existence of breeds and the careful mapping of individual relatedness provide natural opportunities for studying gene–environment interactions. Moreover, the lifespan of dogs is notably shorter than that of humans: 1- to 2-year-old dogs are adults.

In recent years, scientists from a variety of backgrounds have begun to study dogs. As a result, there has been some confusion with respect to the methods used and the interpretation of findings. There is therefore an urgent need for standardized testing and identification of the genetic and environmental variables that affect dog behavior (Diederich and Giffroy 2006; Miklósi 2007). The study of dogs is also complicated because of the many uncontrolled environmental variables. Researchers therefore need to assess the generalizability of their results via replication.

Instead, despite the fact that experiments are often conducted in a single country, region, or city, findings are often assumed to apply to dogs in general. However, one should not forget that dog-keeping practices, owners' perceptions of their dogs, and in fact the dogs themselves may vary around the world; and researchers might consider replicating behavioral research with dogs from different populations as well before firmly concluding that the findings are applicable to all dogs (Wan et al. 2009).

12.3.2 Niche of Dogs in Western Cultures

Presently, most dogs in Western societies live as family pets in a complicated, often uncertain environment while being highly dependent on their owner. In Western societies, only a small proportion of dogs are free-ranging; for example, in the United States, shelters admit approximately 4% of the total population as "strays" (Patronek and Glickman 1994). Research on dogs in cultures where dogs function mainly as food or pelt are underrepresented.

The percentage of dog-owning households varies across countries. Whereas approximately 40% of households in the Czech Republic and Australia include a dog (Marston and Bennett 2003; Houpt et al. 2007), only 14% of Austrian households do so (Kotrschal et al. 2004). The number of dogs kept per household depends on many factors (urbanization, economic situation). In a German-speaking sample with 14,004 individuals (Kubinyi et al. 2009) 33.1% of the owners reported to have more than one dog.

There are marked differences even among Western cultures in dog-keeping practices. For example, we found that German shepherd owners in the United States were more likely than those from Hungary to (1) keep their dogs indoors during the day and night, (2) report that their dogs were kept as pets, and (3) engage their dogs in a greater number of training exercises, such as conformation or agility training (Wan et al. 2009). It is also worth noting that more women have dogs than men; for example, in our German-speaking sample (Kubinyi et al. 2009) 79.6% of the respondents were women, and in an Australian population it was 85.0% (Bennett and Rohlf 2007).

12.3.3 Breeds As Genetically Isolated Populations

Having approximately 400 recognized breeds presents a major advantage in studying dogs. Selective breeding resulted in a great variety among dogs in terms of their appearance and behavior. Most current breeds are approximately 100–200 years old, but despite this fact, dogs can be correctly assigned to their respective breeds on the basis of their genotype (Parker et al. 2004; Sundqvist et al. 2006). According to Parker et al. (2004) variation among breeds accounts for more than 27% of the

total genetic variation. Therefore, breeds are inbred, genetically isolated units, with reduced genetic heterogeneity (Lindblad-Toh et al. 2005; Saetre et al. 2006). According to Ostrander and Comstock (2004), "The development of dog breeds by selection for rarefied traits represents one of the greatest experiments in biological variation ever done by man" (p. R99). There are over 350 inherited diseases in dogs, many associated with just a few breeds. By studying the affected breeds, genes underlying complex diseases can be mapped.

However, not only genetic diseases, but certain typical behavioral phenotypes are associated with breeds: personality trait differences among breeds were detected in several studies (e.g., aggressiveness: Bradshaw and Goodwin 1998; Svartberg 2006; Notari and Goodwin 2007; Duffy et al. 2008; playfulness: Svartberg 2006; sociability: Seksel et al. 1999; Svartberg 2006; trainability: Bradshaw and Goodwin 1998; Serpell and Hsu 2005; Ley et al. 2009; boldness: Svartberg and Forkman 2002; Svartberg 2006).

12.4 Personality Studies in Dogs

12.4.1 Concept

Personality is often defined as an individual's distinctive pattern of behavior (other than feeling and thinking) that is consistent across time and situations (e.g., Pervin and John 1997). An individual's personality is based on a set of traits. A personality trait has contributions from more than one quantifiable behavioral item or variable. For example, activity–impulsivity items can consist of lack of self-control, fidgeting, difficulty controlling the behavior, and strong motivation for playing and running (Vas et al. 2007).

Personality or temperament studies in dogs have become very popular during the last decade (Jones and Gosling 2005; Kubinyi et al. 2009). In addition to its theoretical interest, dog personality is a matter of public concern and has a wide range of practical applications, including significant influence on the dog–human bond. Despite the increased interest, at present there is neither standard methodology nor standard terminology in dog personality studies (Diederich and Giffroy 2006).

In a meta-analysis, Jones and Gosling (2005) identified seven main personality dimensions that characterizes dogs: reactivity, fearfulness, sociability, responsiveness to training, aggression, dominance, and activity. As a result of different methods used, researchers suggested different numbers of potential personality traits for dogs (e.g., 11 personality traits by Hsu and Serpell (2003); 2 personality traits – shyness–boldness and aggressiveness – by Svartberg and Forkman (2002)). Recently, based on the human Big Five questionnaire, we found four traits, all but one of which can be related to the human counterparts (Kubinyi et al. 2009). There was no evidence for conscientiousness in dogs, in line with Gosling and John (1999), who noted that conscientiousness appears only in chimpanzees and humans.

So far, dog personality research has focused on (1) developing tools for characterizing behavior (e.g., Sheppard and Mills 2002; Hsu and Serpell 2003; Ley et al. 2008); (2) investigating breed-related genetic differences (e.g., Wilsson and Sundgren 1997a, b; Svartberg and Forkman 2002; Strandberg et al. 2005; van Oers et al. 2005; Svartberg 2006); and (3) studying the effect of development or stability of the behavioral characteristics over an extended time. In the latter case, individuals are repeatedly tested during early puppyhood, at a juvenile age (time of sexual maturation), and later in adulthood with the aim of evaluating the predictability of certain early behavioral characteristics (e.g., Wilsson and Sundgren 1998; Slabbert and Odendaal 1999).

12.4.2 Methods in Personality Studies

Two methods are typically used for recording information about the behavior of individual animals: behavioral coding and subjective ratings (Gosling 2001). These methods reflect different resolutions to the supposed trade-off between quantifying behavior in terms of objective acts and using humans to record and collate information more subjectively (Kubinyi et al. 2010).

Behavioral coding is rooted in the tradition of ethology and aims to capture as faithfully as possible what an animal does on a particular occasion. For example, researchers might count the number of times an animal performs an act (e.g., charges at another), the latency to do something (e.g., time taken to approaching a novel object), or the duration of a behavior (e.g., time spent looking at another animal). Coding approaches are widely believed to not be influenced by observer biases. In dog personality studies, breed clubs' or working dogs' character tests or working field trials provide large sample sizes and support the investigation of dogs over a long period of time (Goddard and Beilharz 1986; Wilsson and Sundgren 1997b; Ruefenacht et al. 2002; Strandberg et al. 2005; Saetre et al. 2006). In these studies, because of the standard circumstances and the large number of dogs, evaluation of behavior is based on the subjective judgment of several observers or judges. Although the judges are mostly well trained, there could be significant differences in their assessments (e.g., Murphy 1995; Ruefenacht et al. 2002; Lindberg et al. 2004).

Rating approaches are rooted in the tradition of psychology and aim to capture what an animal does at a higher level of abstraction. For example, rather than record the number of times an individual engages in specific acts of aggression, raters use their judgment to rate the general frequency of aggressive acts (e.g., a rating from "rarely" to "often") or to rate an animal's standing on a trait (e.g., a rating from "unaggressive" to "aggressive"). Rating approaches, which intrinsically rely on the experience and judgment of observers, are widely considered less objective than coding approaches; indeed, they are often referred to as "subjective ratings" (e.g., Stevenson-Hinde and Zunz 1978). This is based on the assumption that each owner assesses or interprets the dogs' behavior differently depending on their age, sex, experience level, and so on. As a result, ratings are sometimes thought to be an inappropriate method for scientific measurement (Vazire et al. 2007; Uher et al. 2008). However, several researchers argue that aggregated observations of multiple

observers are reliable and independent of the peculiarities of individual observers. Many studies have argued that owners' rating are a reliable information source about dogs' behavior and could be useful in ethological surveys (Gosling et al. 2003; Kwan et al. 2008; Meagher 2009). For intrinsically broad constructs such as personality, collating information about animals from experienced observers via broad ratings is more efficient than the relatively time-consuming behavioral coding.

Questionnaire-based personality surveys are frequently used in psychology, so there are elaborated criteria and judgment procedures (Gosling and Vazire 2002). In the case of dogs, owners are generally considered experienced observers. Owners have multiple experiences with their dog; thus, by involving owners, researchers have the possibility to collect information about the dog's behavior outside the testing situations. By means of questionnaires, researchers can survey aspects of the character that are difficult to assess via behavioral tests (e.g., dogs often fail to show certain types of aggressive behavior in staged tests) (Duffy et al. 2008). Additionally, by using questionnaires, we can investigate sample sizes that far exceed those obtained with traditional testing methods. Finally, the owners observe the dog's behavior continuously, so they can assess the dog on the basis of many similar situations and conduct a "mental factor analysis" (Miklósi 2007). These features make questionnaires a useful tool for measuring traits (e.g., personality) that are stable and consistent across time and situations (Pervin and John 1997).

There is evidence on the relations between owners' rating and behavioral observations (Gosling and Bonnenburg 1998; Gosling et al. 2003; Hsu and Serpell 2003; Svartberg 2005; Vas et al. 2007; Kubinyi et al. 2010). These correlations are usually relatively weak (0.2–0.3) but not weaker than in human studies (Gosling et al. 2003). In any case, the questionnaires used should be checked for criteria of reliability and validity (Gosling 2001; Taylor and Mills 2006; Kubinyi et al. 2010; Meagher 2009). Personality traits or factors are usually identified from factor analysis or principal component analysis (or other data reduction method) by examining the correlation pattern between narrow behavioral variables (test variables or questionnaire items).

12.5 Genetic Association Studies in Dogs

We have provided an overview on the benefits of using dogs as the functional model of some aspects of human behavior and personality. In the following, we show how this approach can be turned into action when one is interested in the underlying genetic factors that influence personality traits in dogs.

12.5.1 What Genes and Why

Quantitative studies (e.g., Wilsson and Sundgren 1998; Ruefenacht et al. 2002) are able to assess the heritability of certain behavioral traits and may provide estimates

of the number of underlying genes. However, these studies cannot reveal the genetic background of the traits. Candidate gene analysis assumes that the phenotypic trait is determined to some extent by genes that have detectable effects. Candidate genes are usually related to the neurotransmitter and hormonal systems, and the aim is to find a significant association between variation in the phenotype and the allele polymorphism. In recent years this approach has become widespread in research on humans (Reif and Lesch 2003).

In research on dogs, the strategy has been to find allelic variation in dog candidate genes for which some effect can be hypothesized based on human studies. Although several polymorphisms have been found in dogs, it is important to note that the genetic nature of the polymorphism in dogs might differ from that of humans. For example, variability could affect different exons or introns, the length, and/or the number of repeats; moreover, single nucleotide polymorphisms (SNPs) likely have a different position in the DNA sequence (see below for the case of the *DRD4* gene). Even if the variability is present in similar regions (e.g., exon 3 in the *DRD4* gene), it does not ensure a similar effect on the phenotype (Héjjas et al. 2007b).

Importantly, genetic association studies have some pitfalls. Principally, there is a high chance of getting false-positive results. Also, individuals from genetically isolated populations (e.g., breeds) are more likely to show behavioral differences due to their population-specific background, not due to the presence of a single gene (Hamer and Sirota 2000). Thus, candidate gene analysis should be carried out within single breeds and include unrelated individuals.

Another pitfall is identification of the phenotype. A widely used method includes relying on breed stereotypes provided by experts such as dog trainers – not direct phenotypic measures of the individuals (Jones et al. 2008; Chase et al. 2009). To reveal a valid association between genetic factors in the association studies, precise behavioral phenotyping at the individual level is definitely as important as accurate genotyping.

12.5.2 Behavioral Associations with the DRD4 *Gene*

Some personality traits are supposed to be homologous in vertebrates, including humans; and therefore the underlying neurobiological and neuroendocrine factors should be similar. A significant association in the animal (dog) model supports the external validity of human findings and offers the multiple advantages associated with animal models.

The *DRD4* gene was the first candidate gene for which it was implied that allelic differences are associated with different patterns of human behavior (Ebstein et al. 1996). The dopamine D4 receptor is highly abundant in the limbic system, which is responsible for emotions and cognitive functions. Since 1996, the *DRD4* exon 3 has been one of the most studied candidate gene polymorphisms. The most thoroughly investigated polymorphism of the gene is located on exon 3, where the repeat number of a 48-base pair (bp) long segment varies from 2 to 10. A large number of studies deal with this variable number of tandem repeats as a possible

risk factor for several psychiatric disorders (Ebstein 2006). Importantly, several independent laboratories found associations between attention deficit and hyperactivity disorder (ADHD) and the *DRD4* gene (Faraone and Mick 2010).

12.5.2.1 Looking for a "Good" Phenotype: Activity/Impulsivity Trait

Our research group successfully adapted a human parental ADHD questionnaire (DuPaul 1998) for measuring activity–impulsivity and attention deficit traits in dogs (Vas et al. 2007). This activity–impulsivity and inattention questionnaire (Dog-AIA-Q) is short and simple: owners are asked to rate the frequency of 13 behavioral traits of their dog, such as, "My dog fidgets all the time" and "My dog's attention can be easily distracted." The Dog-AIA-Q showed satisfactory test–retest and interobserver reliability, internal consistency, and external validity on a sample of 220 individuals drawn from 69 breeds. Thus the phenotype obtained by the questionnaire seemed to be suitable for a genetic study. Based on human data, we chose to investigate the possibility of an association between polymorphism of the dopamine receptor *D4* gene and activity–impulsivity traits in dogs.

12.5.2.2 Looking for a Polymorphism: *DRD4* Exon 3 in Dogs

In 1999, a Japanese research group found seven length-variant polymorphisms in *DRD4* exon 3 in dogs (Niimi et al. 1999, 2001). Similar polymorphisms do not exist in rodents (e.g., O'Malley et al. 1992) but are present in nonhuman primates (Bailey et al. 2007). Furthermore, an SNP polymorphism was found in horses (Momozawa et al. 2005).

Niimi et al. (1999) and Ito et al. (2004) suggested that *DRD4* gene variations are related to behavioral traits such as excitability, aggression, and reactivity. In Ito et al.'s study (2004), allele frequencies of 23 dog breeds were determined and correlated with behavioral differences. The breeds were divided into two main groups based on the allele frequencies of the *DRD4* exon 3 polymorphism. Dogs belonging to group A had a higher frequency of alleles named 2 and 3a, whereas the alleles 3b, 5, and 6 were more frequent among the animals of group B. Phenotypes of the dogs were analyzed by means of a questionnaire for dog professionals. They found that dogs in group B obtained a higher average score of aggressiveness and a lower value of reactivity compared with individuals in group A.

However, as mentioned above, to avoid false positives in single gene–behavior trait associations it is advisable to use within-breed comparisons instead of between-breed comparisons to avoid the effect of population stratification (Hamer and Sirota 2000).

Although the associations of Niimi et al. (1999) and Ito et al. (2004) might be spurious, the frequency data are valuable and facilitate cross-country comparisons. After genotyping 655 individuals from three dog breeds and 44 European wolves, we found that the allele frequencies of the *DRD4* exon 3 in German shepherds and

Table 12.1 Frequency of *DRD4* exon 3 genotypes in domestic dogs and wolves

Genotype	Belgian Tervueren ($n=100$)	Belgian Groenendael ($n=105$)	Belgian Malinois ($n=50$)	German Shepherd Dog ($n=308$)	Siberian Husky ($n=91$)	European wolf ($n=44$)
DRD4 exon 3						
2/2	14.00	29.52	18.00	41.56	3.30	15.91
2/3a	47.00	43.81	36.00	42.53	5.50	2.27
3a/3a	39.00	26.67	46.00	15.91	5.50	4.55
2/5	0	0	0	0	1.10	13.64
3a/5	0	0	0	0	18.70	0
5/5	0	0	0	0	40.60	15.91
8/8	0	0	0	0	11.00	22.73
3a/8	0	0	0	0	14.30	4.55
2/8	0	0	0	0	0	18.18
5/8	0	0	0	0	0	2.27

The results are expressed in percents

Data from Héjjas et al. (2007a, b, 2009) and unpublished results

Siberian huskies were similar in Japanese and Hungarian samples (Ito et al. 2004). These frequencies are reported in Table 12.1, and for comparison we also report frequencies for Belgian shepherds (Tervueren, Groenendael, Malinois). In a recent study, we found a new allele in Siberian huskies and European wolves that was not identified by the Japanese researchers. According to gel electrophoresis, this allele is the longest variant of all; therefore we labeled it as the "eighth" allele (Héjjas et al. 2008).

By using a single breed to avoid population stratification, we found that police German shepherds having at least one 3a allele showed significantly higher scores on the activity–impulsivity dimension of the Dog-AIA-Q (Vas et al. 2007) than dogs lacking this allele (Héjjas et al. 2007b) (Fig. 12.1). The same genotype–phenotype association could not be demonstrated in pet German shepherds living with their owners. It was hypothesized that various environmental effects (e.g., the attitude of the owners, the quality of training) overshadow the subtle genetic effects of the *DRD4* polymorphism. In contrast, the police dogs lived and were trained in a homogeneous environment in that they all went through the same special training, are kept in similar environmental conditions, and experience similar stressors. Similar findings in humans were described by Lahti et al. (2005). They found that childhood sociodemographic characteristics (e.g., the mother's education) moderated the association between certain *DRD4* variants and novelty-seeking during adulthood. Importantly, another animal model, rhesus macaque (*Macaca mulatta*) demonstrated gene–environment interactions repeatedly that translated to the human condition (Schwandt et al. 2010). According to a recent finding, early adversity in males (but not females) carrying the short allele of the serotonin transporter gene (*5-HTTLPR*) was associated with higher frequencies of contact aggression toward a conspecific intruder (Schwandt et al. 2010). These findings suggest that gene–environment interactions are an important factor in behavioral genetic studies (Caspi and Moffitt 2006).

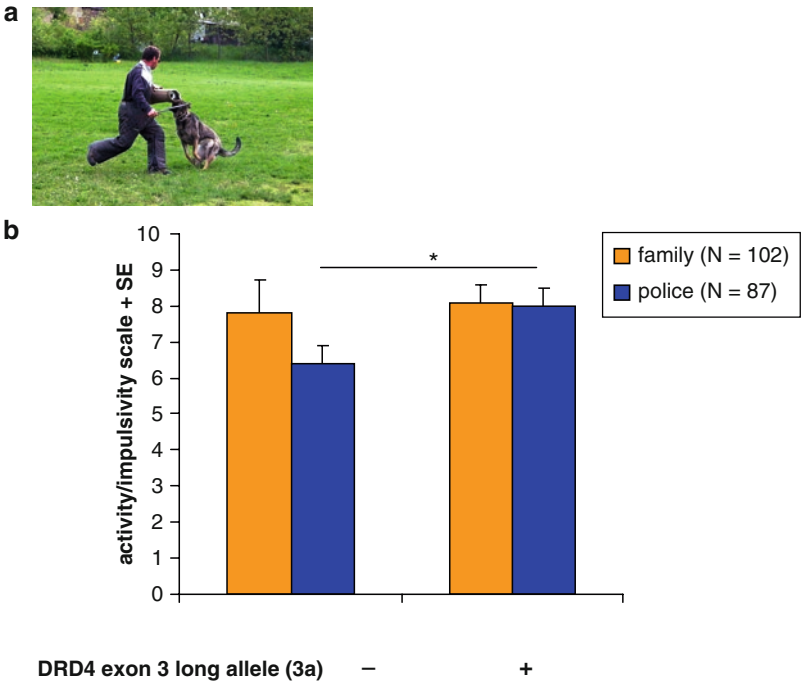

Fig. 12.1 (**a**) Activity–impulsivity in police and family German Shepherds according to their *DRD4* exon 3 genotypes. (**b**) The phenotype measure was derived from the Dog-AIA-Q filled in by the owner of the dog. The mean scores are plotted in the genotype categories. Groups were defined based on the presence (+) or absence (–) of the long allele. (Based on the results of Héjjas et al. 2007a)

12.5.2.3 Other Behavioral Associations with Genes

The serotonin transporter (*5HTT*) genes play a role in anxiety through the signal transduction of serotonin (Reif and Lesch 2003). Maejima et al. (2007) reported a weak association between distractibility trait and a *5HTT* haplotype in Labrador Retrievers trained to detect drugs. Distractibility was derived from ratings by trainers on the traits "obedience training" (negative), "affection demand," and "aggression toward dogs." Similar studies might clarify factors related to the aptitude of working dogs to assess whether candidate dogs are suitable for the specific training programs.

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the synthesis of dopamine, which is a precursor of norepinephrine and epinephrine (Reif and Lesch 2003). Héjjas et al. (2007a) reported that there is a 36 bp long sequence in the intron 4 region of the *TH* gene that is present either as a single copy (short allele 1) or in a duplicated form (long allele 2). Allele 1 was rare in German Shepherds, Malinois, and gray wolves; but it was fairly frequent in Tervuerens and Groenandaels.

Recently, we have found a significant association between a *TH* intron 4 polymorphism and the activity–impulsivity trait in German Shepherds living in human

families (Vas et al. 2009). Activity–impulsivity and a related trait, Liveliness, were assessed based on two validated owner-questionnaire scales (Vas et al. 2007; Wan et al. 2009) combined with a novel test battery consisting of seven subtests. The results of the three instruments correlated with each other, suggesting that they measure the same trait. Importantly, the *TH* genotype was significantly associated with all scales. Heterozygote dogs had higher owner-rated scores on the questionnaire and higher codings in the behavioral test battery by independent observers. We concluded that the *TH* and activity–impulsivity association is consistent with the human data, and that the test battery is a reliable and valid instrument for measuring activity–impulsivity in German Shepherds kept by families. At present, we can only speculate on the molecular pathway involved. Any difference in the activity of the TH enzyme may influence the availability of dopamine at particular places of the brain.

Behavioral analysis of 96 unrelated German shepherds showed that polymorphisms at the exon 3 and intron 2 variable number of tandem repeats (VNTR) of the *DRD4* gene contributed to the social interest of German shepherds (Fig. 12.2), which manifests in approaching and following behavior while encountering a friendly but unfamiliar experimenter. This behavior could be an important aspect of a dog's personality, especially if it plays a role in adjusting social behavior to the demands of human society where such contacts with strangers frequently occur (Hejjas et al. 2009).

The *DRD4* intron 2 VNTR in the dog *DRD4* gene was described by Nara et al. (2005). It is an insertion/deletion of a 17 bp long region; however, the detailed structure of the polymorphism was not reported. Based on a thorough sequence analysis, we found that the 17-bp region was present in triplicate in the longer (Q) allele, and the middle module was deleted in the shorter (P) variant. Both long and short alleles were detected in Belgian and German Shepherds and Siberian Huskies. Interestingly, in the 22 European gray wolves tested, no Q allele was found (Héjjas et al. 2009).

12.5.3 Molecular Functional Analysis of DRD4 Intron 2

In association studies, it is essential to demonstrate by independent methods that there is a difference in the functional aspects of the polymorphic alleles. Therefore, we decided to conduct a functional analysis on the *DRD4* intron 2.

It is obvious that the nonexonic regions of the genome are exposed to a lower rate of selection and therefore show higher variability among species. Nevertheless, intronic sequences might have an important regulatory role in gene expression, as was shown for a VNTR in intron 2 of the human serotonin transporter gene (Fiskerstrand et al. 1999).

As mentioned above, a short and long forms of the intronic variation were identified in 678 unrelated dogs from five breeds and in 22 wolves. For molecular analysis, the intron 2 region was cloned into a promoterless luciferase reporter vector that led to an elevation in transcriptional activity. Moreover, an allelic difference in promoter activity was detected, as was a repressive effect of the long allele

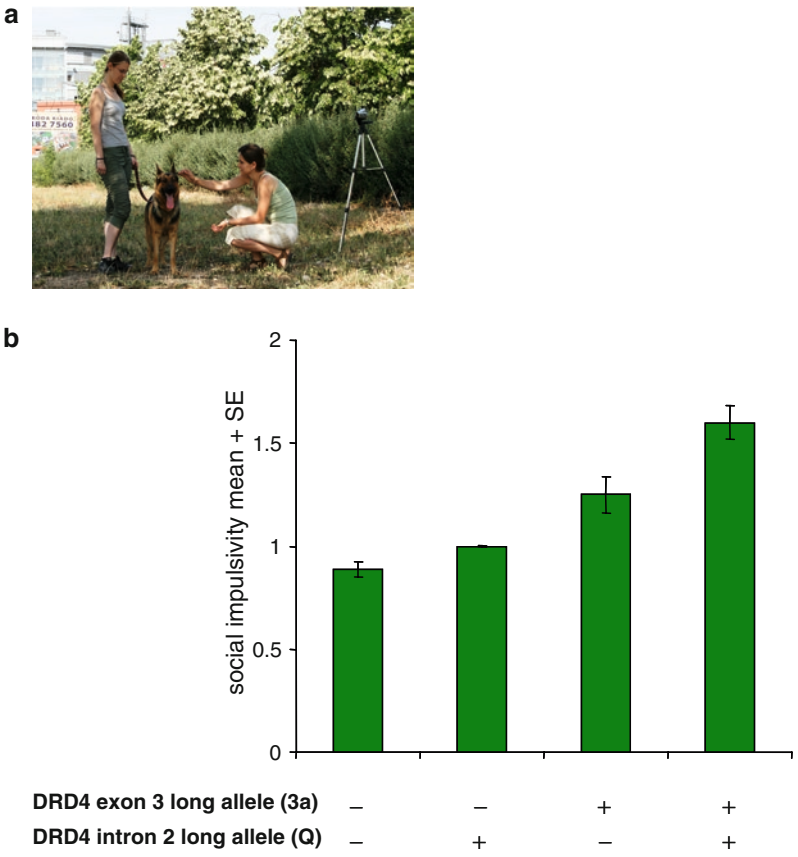

Fig. 12.2 Social interest in German Shepherds in a family setting according to their *DRD4* exon 3 and intron 2 genotypes. Dogs participating in the Greeting Test (**a**) had a score of 1 if they approached the experimenter plus another score of 1 if they followed the experimenter when she stepped away. The mean scores are plotted by the genotype categories. (**b**) Groups were defined based on the presence (+) or absence (–) of the long allele at the intron 2 and exon 3 VNTRs, respectively. (Based on the results of Héjjas et al. 2009)

(Héjjas et al. 2009). Although these findings suggest that the two intron variants of this gene may also have a different effect in vivo, more studies are needed to establish the functional significance of the nonexonic polymorphisms of the dopaminergic genes of the dog genome.

12.6 Conclusions

The chapter presented the most recent candidate gene studies in dogs. The results indicate that dogs can be a useful model species for the study of genetic effects on behavior and personality. Thus, we share the opinion of Scott and Fuller (1965, p. 4):

"The dog is a veritable genetic gold mine…. Anyone who wishes to understand a human behavior trait or hereditary disease can usually find a corresponding condition in dogs." Although these association studies are still in their early stages, it is already obvious that these methods can offer outstanding possibilities for those who are looking for the genes' underlying behavior. Researchers should never forget, however, that molecular genetic methods and detailed behavioral assessment should go hand in hand.

We have to underline here that as a result of the significant health care and social impact of dogs on human life, genetic studies of dogs have an applied aspect as well. Revealing the genetic background of complex traits could have important consequences for dog breeding or selecting which dogs are suitable for certain training programs. For example, attention skills are relevant to trainability and the communicative behavior of dogs, both of which contribute to the everyday challenges of dog–human interaction. Our results suggest that in the future the process of selecting a dog for a definite purpose (e.g., therapy, sports, police work) may be based partly on the animal's genetic composition and that a large investment could be safe-guarded if individuals with appropriate genotypes are chosen.

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Chapter 13
Personality-Associated Genetic Variation in Birds and Its Possible Significance for Avian Evolution, Conservation, and Welfare

Andrew Fidler

13.1 Introduction

13.1.1 Birds As Study Subjects in Behavioral Research

Studies of bird behavior, in captivity and in the wild, have featured prominently in the development of scientific theories concerning the evolution and ecological significance of animal behaviors (Lack 1968; Konishi et al. 1989; Houck and Drickamer 1996). Doubtless, the abundance of bird studies reflects the inherent attractiveness of birds. However, more objective and scientific criteria also justify the relative prominence of bird behavioral studies. Such criteria include the relative ease with which marked birds can be observed in the wild, often over multiple years and generations, and that field-based observational studies can be complemented by more controlled experiments using captive birds (Konishi et al. 1989). The phylogenetic position of birds with respect to mammals make bird behavioral studies instructive from a comparative perspective. In addition, as birds have diversified into a vast number of diverse ecological niches they offer abundant examples of complex behaviors that have evolved in response to differing selective pressures (Konishi et al. 1989). In more recent years, birds have gained prominence as sentinel species for detecting the biological and ecological affects of climate change (Charmantier et al. 2008; Visser 2008). Finally, as the modern poultry industry involves housing vast numbers of birds under far-from-natural conditions, increased understanding of avian behavior may assist in addressing the significant animal welfare issues that have arisen from the industrialization of bird-based agriculture (Flint and Woolliams 2008; Flisikowski et al. 2009; Jensen et al. 2008).

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13.1.2 Scientific Study of Animal Behavioral Syndromes or Personalities

An aspect of animal behavior that is attracting increasing experimental and theoretical attention is the existence of "behavioral syndromes" or "animal personalities" (Gosling 2001; Sih et al. 2004a, b; Bell 2007; Réale et al. 2007; Wolf et al. 2007, 2008; Sih and Bell 2008). Indeed animal personalities' have been reported across a wide taxonomic range – from primates to insects and molluscs (Gosling 2001; Sih et al. 2004a, b). Although terminology in this area has proven somewhat contentious, the working definition of animal "personality" used here is "consistent individual differences in suites of correlated behaviors" that are similar to what is known as personality in humans (Gosling 2001; Sih et al. 2004a, b; Bell 2007; Réale et al. 2007). For example, some individual animals tend to be more aggressive than the population average throughout their life and across a wide variety of contexts (Gosling 2001; Sih et al. 2004a, b; Bell 2007; Réale et al. 2007; Wolf et al. 2007, 2008; Sih and Bell 2008).

The idea of animal personality is familiar to anyone who lives or works closely with animals (e.g., farmers and pet owners), so it is somewhat surprising, at least to me, that only during the past decade has animal personality become the subject of widespread and respectable scientific study. The apparent reluctance of scientists to approach this subject probably reflects it requiring a conceptual shift away from conventional behavioral ecological thinking, which tends to view such interindividual variation as simply statistical variance about some theoretical adaptive optimum (Wilson 1998). However, there is now growing acceptance of the idea that intraspecies variation in behavioral phenotypes is intrinsically adaptive, with different personalities having differing selective advantages under different circumstances (Dall et al. 2004; Wolf et al. 2007, 2008; Biro and Stamps 2008; McNamara et al. 2009; Quinn et al. 2009). I should add that there are perhaps two other, often unspoken but highly influential social reasons for the scientific neglect of animal personality. One is a fear among professional scientists of being viewed as "anthropomorphizing" animal behaviour and thereby lacking scientific rigor. The second is a widespread social taboo against ascribing interindividual behavioral differences to inheritance, which probably arose in reaction to nineteenth and twentieth century quasi-scientific ideologies concerning class and racial superiority (Paul and Spencer 1995).

13.1.3 Existence and Significance of Bird Personality

Passerine taxa are among the most intensively studied in avian ecology and evolution (Lack 1968; Bennett and Owens 2002). Among the passerines, the great tit (*Parus major*), a common Northern Hemisphere species, is emerging as a model species for studying both the proximate and ultimate factors influencing animal

personality (Drent et al. 2003; Both et al. 2005; Dingemanse et al. 2004; van Oers et al. 2004, 2005; Dingemanse and Réale 2005; Groothuis and Carere 2005). Selective breeding has demonstrated that novelty-seeking behavior in great tits has a significant inherited component (Drent et al. 2003; van Oers et al. 2005). Furthermore, the novelty-seeking component of great tit personality has been shown to influence individual survival (Dingemanse et al. 2004), mate choice (van Oers et al. 2008), and breeding success (Both et al. 2005). Hence, there exists considerable evidence for the selective/evolutionary significance of great tit novelty-seeking behavior. However, the current view is that adaptive evolution results from, at the most fundamental level, changes in allele frequencies at functionally significant loci due to the differential reproduction of different genotypes (Hurst 2009; Nei and Kumar 2000; Orr 2005, 2009). Molecular characterization of loci influencing great tit novelty-seeking behavior would open up the possibility of studying personality evolution at its most fundamental level: changing allele frequencies at personality-associated loci.

In the next section is a commentary on aspects of a study with which I am familiar (Fidler et al. 2007) which looked for an association between sequence variation in a neurotransmission-associated gene, more specifically a dopamine receptor, and interindividual variation in great tit novelty-seeking. It is hoped that this study proves instructive regarding general issues associated with studies of avian personality molecular genetics and provides an encouraging example of the challenges, limitations, rewards, and promise of research into bird personality molecular genetics.

13.2 Avian *DRD4* Polymorphisms and Novelty-Seeking Variation

13.2.1 Personality Trait Quantification

Whatever the methodologies used, any genotype–phenotype association study requires the phenotype to be clearly defined and consistently quantified. Great tit novelty-seeking behavior has been quantified through the measurement of early exploratory behavior (EEB) as described in Drent et al. (2003). Briefly, birds were scored for EEB levels using two tests: (1) a novel environment exploration test in which the time a bird took to visit a fourth tree in a standard room was converted to a scale of 0–10 (Fig. 13.1a) and (2) two tests of a bird's reaction to two different novel objects placed in its home cage, with the results of both novel object tests quantified on a scale of 0–5 (Fig. 13.1b). The final EEB score was the sum of all three tests: range 0–20 (Drent et al. 2003). Two great tit lines had been bidirectionally selected over four generations for either high or low EEB scores, which proved invaluable for the subsequent genotype–phenotype association study (Drent et al. 2003).

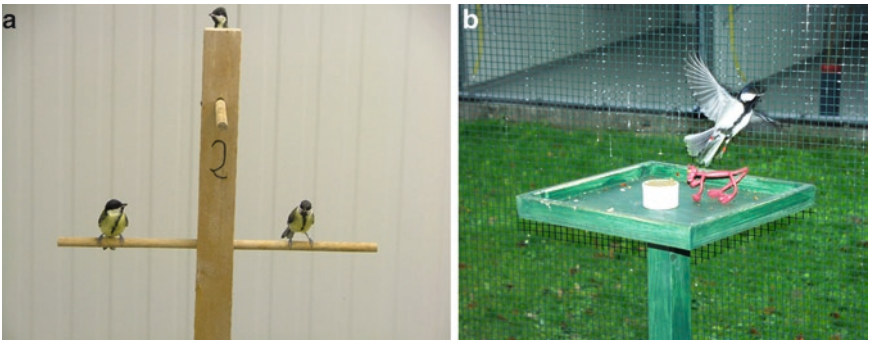

Fig. 13.1 Measurement of great tit (*Parus major*) early exploratory behavior (EEB). Birds were scored for EEB levels based on two behavioral tests. (**a**) Novel environment exploration test in which the time a bird took to visit a fourth tree in a standard room was converted into a scale of 0–10. (**b**) The bird's reaction to two novel objects placed in its home cage, with the results of both novel object tests converted into a 0–5 scale (Drent et al. 2003)

13.2.2 Candidate Gene Selection

Broadly speaking there are two approaches to the molecular characterization of genetic loci functionally influencing complex trait variation: either by mapping (at various levels of resolution) or by the selection of candidate genes. In a limited but rapidly increasing range of organisms for which the appropriate molecular genetic resources exist, it is possible to use either quantitative trait locus (QTL) or association mapping to localize loci to a genetic map (Anholt and MacKay 2004; Gibbs and Singleton 2006; Ellegren and Sheldon 2008; Kruglyak 2008; Mackay et al. 2009). Having localized the critical loci, there are a range of molecular genetic approaches to identifying DNA sequence variation functionally linked to observed phenotypic variation (Gibbs and Singleton 2006; Kruglyak 2008). Although having much to commend them, such mapping approaches are applicable only to species for which suitable molecular genetic resources exist. However, recent technological advances mean that QTL and association mapping studies of "nonmodel" organisms, including birds, are becoming more feasible (Backström et al. 2008a, b; Ellegren and Sheldon 2008; Mackay et al. 2009; van Bers et al. 2010). Fidler et al. (2007) took the candidate gene approach, which is based on the assumption that over restricted evolutionary distances (e.g., within the vertebrata) both the sequences and functions of genes are conserved (Fitzpatrick et al. 2005).

When the Fidler et al. (2007) study was initiated (i.e., 2002), there was a body of intriguing, but equivocal, evidence linking polymorphisms in the human dopamine receptor D4 (*DRD4*) gene with variation in novelty-seeking levels (Kluger et al. 2002; Schinka et al. 2002; Reif and Lesch 2003; van Gestel and van Broeckhoven 2003; Savitz and Ramesar 2004; Ebstein 2006). Over subsequent years, this association was also reported in horses and a nonhuman primate (Momozawa et al. 2005; Bailey et al. 2007; Inoue-Murayama 2009). Taking a candidate gene approach, which in retrospective seems to me to have been naïvely optimistic,

we sought to determine if any association could be detected between allelic variation in the great tit *DRD4* gene and EEB values.

13.2.3 Sequencing the Great Tit DRD4 Gene

In 2002, the red jungle fowl (*Gallus gallus*) genome sequence was not publicly available nor were there any published avian *DRD4* gene sequences. Undeterred, we carried out BLAST searches of the existing public expressed sequence tag (EST) databases and identified two chicken ESTs that appeared to encode *DRD4* orthologues. From these sequences, polymerase chain reaction (PCR) primers were designed to amplify, from great tit genomic DNA, a sequence that appeared to be a

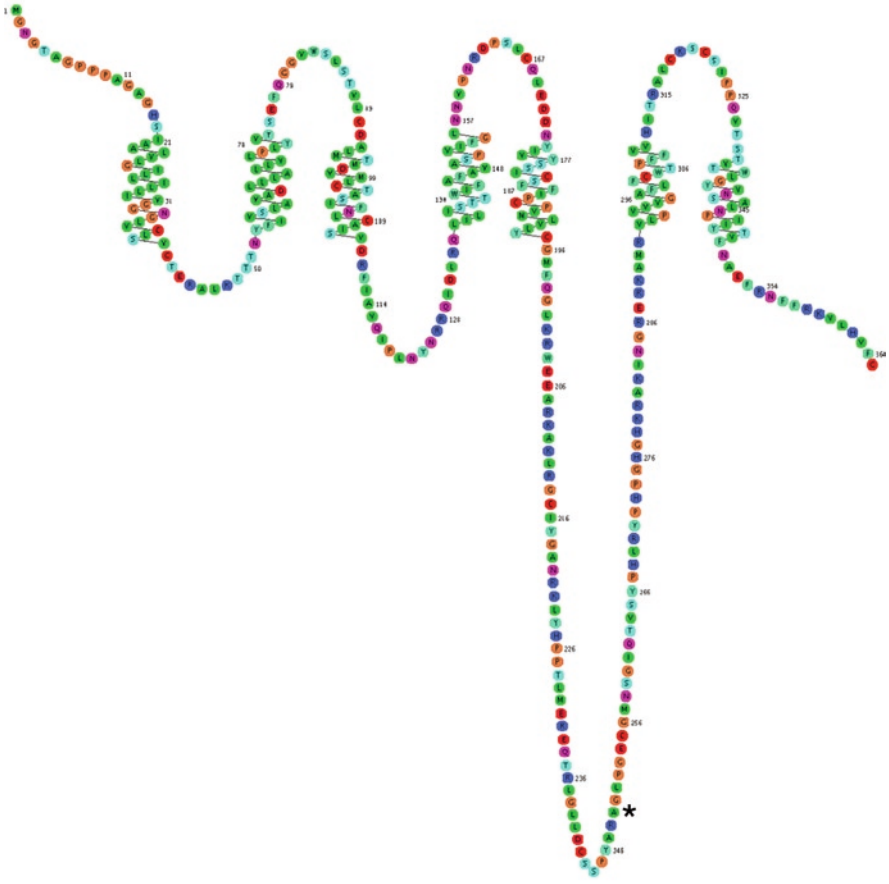

Fig. 13.2 Snake-plot of the great tit DRD4 protein. The predicted topology of the great tit DRD4 protein conforms to the typical G-protein coupled receptor pattern of seven hydrophobic regions corresponding to transmembrane regions. *Asterisk* indicates the alanine residue (Ala_{249}), in the third intracellular loop, encoded by the codon containing the SNP830 polymorphism

partial *DRD4* orthologue sequence. 5′/3′RACE was then used to amplify a corresponding full coding sequence from great tit brain cDNA. This cDNA sequence contained an open reading frame encoding a 365-residue protein predicted to adopt the typical G-protein-coupled receptor topology, as expected for a dopamine receptor (Fig. 13.2). The sequence's status as a probable *DRD4* orthologue was convincingly established by alignment with known vertebrate DRD4 proteins and database searches. I should note that we were greatly assisted in our analyses by the opportune publication of an annotated draft of the red jungle fowl (*G. gallus*) genome (Hillier et al. 2004).

13.2.4 Great Tit DRD4 Gene Polymorphisms

Having obtained the great tit *DRD4* orthologue coding sequence, we looked for polymorphisms in it. We decided to direct our attention toward the exon encoding the third intracellular loop (Fig. 13.2), as polymorphism in this region had been associated with novelty-seeking variation in three mammalian species: humans, horses, and monkeys (Kluger et al. 2002; Schinka et al. 2002; Reif and Lesch 2003; Van Gestel and Van Broeckhoven 2003; Savitz and Ramesar 2004; Momozawa et al. 2005; Ebstein 2006; Bailey et al. 2007). Using the genomic DNA of birds from two EEB-selected lines (Drent et al. 2003), a single nucleotide polymorphism (SNP) was identified: C/T at position 830 of the *DRD4* cDNA sequence (GenBank accession no. DQ006802) and denoted SNP830. Conceptual translation indicated that the SNP830 polymorphism was synonymous with the two alternative codons both encoding Ala_{249} (Fig. 13.2).

13.2.5 Detection and Interpretation of DNA Sequence Polymorphisms

It is now necessary to address the conceptual and technical issues that had arisen by this stage of our study. The conceptual issue concerned attributing functional significance to DNA-level sequence polymorphisms. In an ideal world, the outcome of a study of this type would be the prompt detection of one (or more) sequence polymorphism(s) in the candidate gene to which one could confidently attribute functional significance. However, it is rarely possible to assign functional significance to allelic variation on the basis of sequence data alone, with frame-shift variants perhaps providing an exception to this generalization. With respect to the great tit *DRD4* SNP830 synonymous polymorphism I implicitly assumed, and kept reassuring my co-workers, that as it was synonymous it could have no direct functional/phenotypic consequence. However, I subsequently learned that some synonymous polymorphisms are not functionally equivalent and may be under selection (Chamary et al. 2006; Goymer 2007; Komar 2007).

Specific details aside, this example exemplifies the general problem that ascertaining the functional consequences of gene polymorphisms is not usually possible from the DNA sequence data alone; rather, functional significance must be determined by associations with organism-level phenotypic differences and/or through biochemical experiments (Dean and Thornton 2007; Dalziel et al. 2009; Slate et al. 2009).

The technical issue that arose at this stage of the work concerned reliable, inexpensive SNP genotyping. In the Fidler et al. (2007) study, we were remarkably fortunate, given our resource limitations, that the SNP830 polymorphism resulted in the presence (5′-GCCGGC-3′) or absence (5′-GCTGGC-3′) of a *Nae*I restriction site and, consequently, a cleaved amplified polymorphic sequence (CAPS) assay could be developed for SNP830 genotyping. However, ever-improving technologies are making more generic genotyping methods accessible to small to medium-sized research groups; while DNA sequencing has become so inexpensive that repeated sequencing of amplification products may, at least in some contexts, prove to be a cost-effective genotyping method (Backström et al. 2008a, b; Bonneaud et al. 2008; Ellegren 2008a, b; Kahvejian et al. 2008; Slate et al. 2009; van Bers et al. 2010).

13.2.6 DRD4 SNP830 Allele Frequencies in the EEB-Selected Lines

It appears that linkage disequilibrium (LD) in wild bird populations extends over some thousands to millions of base pairs (Backström et al. 2006; Li and Merilä 2010). We speculated that the synonymous SNP830 polymorphism might be in LD with a functionally significant polymorphism perhaps within *DRD4* itself. We looked for associations between the SNP830 genotype and EEB levels in two contexts: (1) in the lines selectively bred for divergent levels of EEB (Drent et al. 2003) and (2) in hand-raised birds taken from a wild great tit population. Frequencies of the three *DRD4* SNP830 genotypes (C/C, C/T, T/T) were found to differ significantly between birds of the Slow and Fast-EEB lines, with the Slow-EEB line having fewer birds of the C/T and T/T genotypes and more of the C/C genotype than the Fast-EEB line. Furthermore the SNP830 genotype frequencies in the Slow-EEB line, but not in the Fast-EEB line, differed significantly from genotype frequencies among birds from an unselected natural great tit population, with the T/T and C/T genotypes being scarcer in the Slow-EEB line than among the unselected birds. These results were highly encouraging and were consistent with the EEB-based selection regimen having selected against the C/T and T/T genotypes in the Slow-EEB line. However, such data should be interpreted with great caution as, like all small populations, selectively bred lines are subject to random influences, such as founder effects and genetic drift (Nei and Kumar 2000). We were very conscious that the relatively low frequency of the SNP830T allele in the Slow-EEB line could have arisen from such random effects.

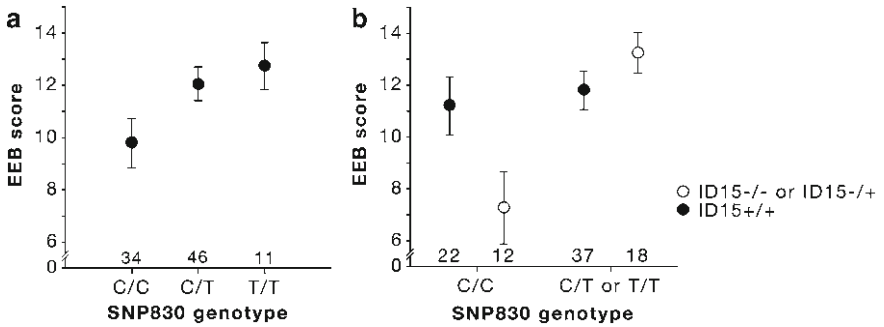

Fig. 13.3 *DRD4* gene polymorphisms are associated with personality variation in *Parus major*. (**a**) EEB scores of unselected birds genotyped for the *DRD4* SNP830 polymorphism. The most prominent difference in EEB scores is between the genotype C/C birds and the other two genotypes (C/T and T/T), indicating a partially dominant effect of the SNP830T allele. (**b**) EEB scores of unselected birds genotyped for both the *DRD4* SNP830 and ID15 polymorphisms. Groups represent individuals with or without the SNP830T allele and with or without the short (-) ID15 allele. The interaction between the SNP830 and the ID15 polymorphisms was significant in a mixed-effects model ($p = 0.016$). Data shown are mean EEB values ± SEM. Sample sizes (number of individuals) are indicated above the x-axis. (**a**, **b**) ©Royal Society (London), reproduced with permission

13.2.7 DRD4 SNP830 Genotypes and EEB Phenotypes Among Unselected Birds

Among unselected birds (i.e., taken from natural populations and hand-raised) the mean EEB score of SNP830C/C homozygotes was significantly lower than the mean EEB scores of both SNP830C/T heterozygotes and SNP830T/T homozygotes (Fig. 13.3a). This finding was consistent with the SNP830 allele frequencies from the EEB-selected lines. Thus, selecting for the Slow-EEB phenotype may have resulted in selection against both SNP830C/T and SNP830T/T genotypes, leading to the lower frequency of the SNP830T allele in the Slow-EEB line.

13.2.8 Search for Additional DRD4 Polymorphisms

Taken together, the results from both the EEB selected and unselected birds provided support for the original hypothesis that *DRD4* sequence variation may be associated with variable novelty-seeking tendencies in great tits. However, we still lacked a plausible molecular mechanism linking the *DRD4* SNP830 genotype and the EEB phenotype. [On a personal note, I suspect that because I was trained as a molecular biologist the lack of such a molecular mechanism probably troubled me more than my behavioral ecology-trained colleagues.] *DRD4* sequences were amplified from great tit genomes homozygous for the SNP830 polymorphism (i.e., either SNP830C/C or SNP830T/T), and these sequences were examined for additional

polymorphisms. Obtaining the complete *DRD4* genomic sequence entailed using the technique of "genomic walking," which proved to be a significant technical challenge, one bravely met by Sylvia Kuhn.

The great tit *DRD4* gene appears to be highly polymorphic, and we identified 73 polymorphisms (66 SNPs and 7 indels) from our limited sampling. However, no further polymorphisms (i.e., in addition to SNP830) were found within the *DRD4* coding region. We found no evidence that any of the intronic polymorphisms showed any linkage with the SNP830 polymorphism, nor were any deemed likely to be of functional significance. However, a 15-bp indel, denoted ID15, located 1,036 bp from the tentatively assigned transcription initiation site was judged to be of possible functional significance particularly in light of reports associating polymorphisms in the *DRD4* promoter region with variation in human behavior (Kluger et al. 2002; Schinka et al. 2002; Reif and Lesch 2003; Van Gestel and Van Broeckhoven 2003; Savitz and Ramesar 2004; Ebstein 2006).

13.2.9 DRD4 ID15 Polymorphism and EEB

We looked for evidence of an association between the ID15 polymorphism and EEB score. As with the SNP830 polymorphism, we were fortunate that ID15 genotyping was technically straightforward, with the two alleles generating PCR products differing in length by 15 bp. The Slow- and Fast-EEB lines did not differ significantly in the frequencies of the three possible ID15 genotypes. However, the Slow-EEB line did differ significantly in ID15 genotype frequencies when compared with the unselected bird population. However, it is perhaps not surprising that the Slow-EEB line differed significantly from the unselected birds for both the SNP830 and ID15 genotype frequencies, as the two sites are separated by only 9,359 bp and selection was only over four generations. Therefore, any allelic associations (i.e., particular haplotype combinations) between SNP830 and ID15 polymorphisms in the EEB-selected founder birds are likely to remain among their descendents. Among the unselected birds, no association was found between the EEB score and the *DRD4* ID15 genotype. However, a statistically significant interaction was found between the ID15 and SNP830 genotypes: more specifically, the association between the absence of the SNP830T allele and lower EEB scores described above (Fig. 13.3a) was predominantly found in those unselected birds that carried at least one copy of the short ID15 (-) allele (Fig. 13.3b). In reporting this result, we were very conscious that interpreting statistical associations between multiple sequence polymorphisms and phenotypic traits is problematic. Nonetheless we cautiously speculated that genotypes combining *DRD4* haplotypes with particular ID15 and SNP830-linked polymorphisms might account for much of the observed association between *DRD4* SNP830 genotype and EEB score. However, as far as we could ascertain, within the unselected bird population examined there was no significant LD between the SNP830 and ID15 polymorphisms.

13.2.10 Concluding Comments and Supporting Research

We provided data consistent with the hypothesis that *DRD4* gene polymorphisms are associated with variation in the personality trait of novelty-seeking in a wild bird species. Such a finding also suggested the intriguing possibility that an association between *DRD4* gene variation and novelty-seeking variation may predate the divergence of the mammalian and avian lineages, which in turn suggests that such variation is retained through long periods of evolution and presumably confers some sort of selective advantage.

Notwithstanding these very positive outcomes, I should caution that this study also made me very aware of the complexities of behavior–gene association studies, particularly in a genetically poorly characterized, free-living species. In short, a skeptical reader can justifiably question how confident we could be that the *DRD4* polymorphism–EEB association reported was "real" and not simply a statistical artifact or a consequence of population structuring. Furthermore, the Fidler et al. (2007) study could not eliminate the possibility that the *DRD4* SNP830–EEB association observed arose from the SNP830 polymorphism being in LD with a nearby gene. Such caveats are a general feature of personality–genetic polymorphism association studies and probably account for at least some of the inconsistencies in the extensive human personality genetics literature (Kluger et al. 2002; Schinka et al. 2002; Reif and Lesch 2003; van Gestel and van Broeckhoven 2003; Savitz and Ramesar 2004; Ebstein 2006; Munafò et al. 2008).

In this context, it is also perhaps appropriate to mention ongoing concerns over publication bias. In short, are those studies that find an association between a genetic polymorphism and phenotypic variation more likely to be published than those that do not (Munafò et al. 2004, 2008)? Perhaps the most robust test of any behavior–gene association is replication (i.e., Is the same association found by other workers in other populations of the same, or related, species?) In the case of the *DRD4*–novelty-seeking association, the human personality literature is large and often contradictory, but meta-analyses have concluded that an association of some type is indeed real (Munafò et al. 2004, 2008).

While preparing this chapter, I was fortunate that two recently published avian personality genetics studies broadly supported the hypothesis that *DRD4* polymorphisms are associated with novelty-seeking variation in birds (Flisikowski et al. 2009; Korsten et al. 2010). Flisikowski et al. (2009) examined if *DRD4* polymorphisms are associated with a tendency toward feather-pecking in domesticated chickens. The rationale for this investigation was that one of the etiological hypotheses for feather pecking is that it is an abnormal manifestation of natural exploratory pecking behavior (Rodenburg et al. 2008; Flisikowski et al. 2009). Flisikowski et al. (2009) found strong evidence for an association between *DRD4* haplotypes and feather-peaking tendencies in both commercial chicken lines with differing feather-peaking tendencies and two chicken lines selectively bred for divergent tendencies to feather-pecking. However, LD analyses indicated that the associations extended beyond *DRD4* to an adjacent gene, deformed epidermal autoregulatory

factor 1 (*DEAF1*), which has a role in serotonergic signaling pathways. Furthermore, as with the Fidler et al. (2007) study, no polymorphisms found in either the *DRD4* or *DEAF1* genes were clearly of functional significance, leading to a fascinating suggestion that both the *DRD4* and *DEAF1* genes may be involved through physical interaction of their transcripts. In conclusion, the Flisikowski et al. (2009) study did, broadly speaking, support the conclusions of Fidler et al. (2007), although many questions remain unresolved. At a more general level, the Flisikowski et al. (2009) study highlights the enormous benefits of having a reference genomic sequence, albeit incomplete, of the study organism and the feasibility of using resequencing for comprehensive genotyping.

In a second recent study, Korsten et al. (2010) tested if the great tit *DRD4*–EEB association, first found using hand-raised birds taken from the wild, is also present in wild-raised birds and if it is replicable across four geographically separated European great tit populations. Briefly, Korsten et al. (2010) found the *DRD4* SNP830 genotype - EEB association when they examined wild-raised birds from the same Dutch great tit population as was studied by Fidler et al. (2007). In contrast, in the other three European great tit populations, the SNP830 genotype–EEB association appeared to be either absent or very weak (i.e., statistically nonsignificant). However, it is perhaps to be expected that the relative strength of genotype–phenotype associations will differ between populations due to different environmental influences and/or different polymorphisms/loci contributing to the observed behavioral variation. Korsten et al. (2010) found no evidence of an association between the ID15 genotype and the EEB phenotype.

In summary, the results of Flisikowski et al. (2009) and Korsten et al. (2010) provide support, with a number of important caveats, for the conclusions of Fidler et al. (2007), although many questions remain concerning underlying biochemical mechanisms and interpopulation differences.

13.3 Future Directions in Avian Personality Genetics

13.3.1 Avian Personality Genetics: Beyond Genotype–Phenotype Association Studies

Research into the molecular genetics of free-living, non-"model" animals is experiencing rapid growth, in no small part because of the technological advances that have made such studies both technically and economically feasible (Ellegren 2008a, b; Ellegren and Sheldon 2008; Slate et al. 2009). Not unexpectedly, many of the pioneering vertebrate studies have focused on relatively simple phenotypes (e.g., pigmentation) where credible mechanisms linking observed variable phenotypes with DNA sequence variation can be proposed (Mundy 2005; Hoekstra 2006; Gratten et al. 2008; Pointer and Mundy 2008; Protas and Patel 2008). However, there have been some impressive studies of molecular genetic variation associated

with behavioral variation in free-living invertebrate species (Fitzpatrick et al. 2005, 2008; Krieger 2005; Kiontke 2008; Dalziel et al. 2009) and with vertebrate physiological variation (Dalziel et al. 2009). Following the lead of these studies, it is clear that for some time a major goal of avian personality genetics research is the identification of major loci associated with significant variation in personality parameters.

As avian personality molecular genetics matures beyond simply tracking down functionally significant DNA sequence variation, it is hoped that questions regarding the genetic mechanisms and selective pressures underpinning personality variation and their ecological and evolutionary implications can be addressed. The molecular characterization of genetic variation functionally associated with well-characterized, quantifiable personality parameters would provide a firm foundation for describing, and possibly explaining, any associated microevolutionary changes at the most basic level – changes in allele frequencies (Nei and Kumar 2000; Orr 2005, 2009; Anisimova and Liberles 2007). Perhaps the most pressing question to address is why animal personalities exist at all and how (if at all) personality variation contributes to adaptation and speciation (Sih et al. 2004a, b; Réale et al. 2007; Wolf et al. 2007, 2008; Sih and Bell 2008). Conventional explanations for genetic diversity in natural populations range from variants of the neutral theory (i.e., the variation is wholly or largely selectively neutral) to frequency-dependent selection to proposals that in the spatially and temporally variable conditions of the real world natural selection does not favor any theoretical "ideal" phenotype but, rather, maintains a range of adequately adapted phenotypes (Nei and Kumar 2000; Sih et al. 2004a, b; Réale et al. 2007; Wolf et al. 2007, 2008; Sih and Bell 2008). The latter explanation, evoking varying selection pressures, has received support from studies of the differential survival and reproductive success of differing "personality types" in wild great tit populations (Dingemanse et al. 2004; Quinn et al. 2009). Another intriguing question is whether personality-associated loci of social birds harbor greater diversity than their orthologues in more solitary species. One could imagine that within social groupings there are different "social niches" for which differing selective pressures favor different personalities (Cote et al. 2008). Provisioned with DNA-level genotyping data, researchers would also be in a much stronger position to investigate the roles personality-associated genetic variation may play in mate choice and reproductive success (Both et al. 2005; van Oers et al. 2008).

13.3.2 Alternative Avian Species for Personality Genetics Research

Extensive behavioral trait variation among chicken breeds, in combination with a public access red jungle fowl (*G. gallus*) genome sequence, are likely to reinforce the chicken's status as a "model" species for avian behavioral genetics studies (Buitenhuis et al. 2005; Siegel et al. 2006; Cogburn et al. 2007; Wang et al. 2005). However, the small size, ease-of-care, and great fecundity of the zebra finch (*Taeniopygia guttata*) suggest that it could emerge as an "avian mouse" in which

the selective breeding and maintenance of strains by academic researchers is feasible (Forstmeier et al. 2007; Schuett and Dall 2009). The zebra finch's rising status is likely to receive added impetus from the release of a draft zebra finch genome sequence (http://www.ncbi.nlm.nih.gov/genome/guide/finch/) (Replogle et al. 2008; Stapley et al. 2008). Although the chicken and zebra finch will perhaps remain the avian species of first choice for phenotype–genotype association studies, I venture to suggest that neither the chicken's progenitor species (*Gallus* spp.) living in the forests of Asia (Eriksson et al. 2008; Kanginakudru et al. 2008), nor the free-living zebra finch, an opportunistically breeding species of the expansive Australian grasslands, is likely to prove particularly suitable for field-based personality studies. Rather, I suggest that abundant, widespread, visible passeriform species such as the great tit (*P. major*), but also the blue tit (*Cyanistes caeruleus*), house sparrow (*Passer domesticus*), and highly social and intelligent corvids, are the most suitable species for Northern Hemisphere field studies (Emery and Clayton 2004; Schloegl et al. 2009; Korsten et al. 2010; van Bers et al. 2010). Given my Southern Hemisphere-biased world-view, I cannot resist suggesting that highly visible, long-living, social parrots such as the common Australian cockatoo species and the exceptionally intelligent and neophilic New Zealand alpine parrot, the kea (*Nestor notabilis*), could also be fruitful research subjects (Diamond and Bond 1999; Huber and Gajdon 2006; Auersperg et al. 2009; Range et al. 2009; Schloegl et al. 2009). Indeed, I would go so far as to suggest that if a third avian genome is considered for complete sequencing it should be from a psittaciform species as not only would this be useful from the perspective of avian phylogenetics it would provide an invaluable resource for parrot genetics. Finally, when selecting any study species, it should be borne in mind that such studies are greatly enhanced by comparisons between subspecies, or closely-related species, that display contrasting behavioral phenotypes, as this opens up the possibility of comparative genomic studies (Nair and Young 2006; Donaldson and Young 2008; Ellegren 2008b; Li et al. 2008).

13.3.3 Applications of Bird Personality Genetics Research

There is growing awareness that human "harvest" of free-living animals could be having significant evolutionary consequences (Allendorf and Hard 2009). It is certainly apparent that some methods of catching/trapping wild birds are nonrandom with respect to personality (McDougall et al. 2006; Biro and Dingemanse 2009; Garamszegi et al. 2009). In more extreme cases, the birds removed from a wild population may actually be selected, albeit unconsciously, on the basis of personality type. A possible example is the highly neophilic, and endangered, kea (*N. notabilis*) of the New Zealand mountains. While the antics of highly inquisitive kea (Fig. 13.4a) provide free entertainment for tourists (at least for those who are not having their possessions wrecked and stolen!) such bold, exploratory behavior has gained the kea a negative reputation. Following the introduction of sheep farming into New Zealand, the kea gained a (partly deserved) reputation as "sheep-killers"

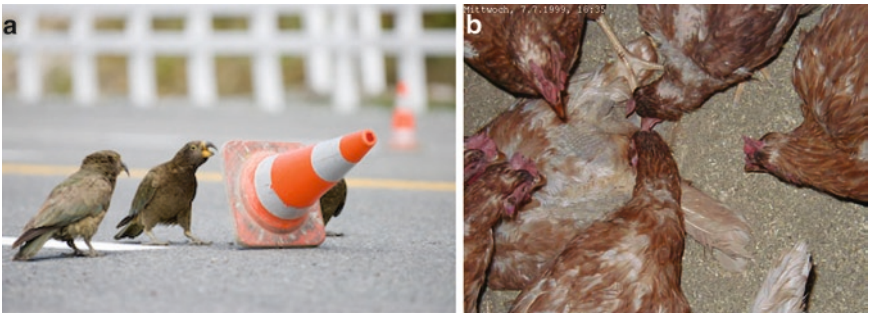

Fig. 13.4 Conservation and agricultural husbandry-related issues associated with avian novelty-seeking/exploratory behavior. (**a**) The extreme neophilia of kea (*Nestor notabilis*) has allowed adaptation to life in the challenging alpine environment of New Zealand's South Island. However the same novelty-seeking/exploratory tendencies probably led to some kea attacking live sheep, primarily to obtain fat, and to general nuisance behaviors around human settlements. Sadly, the human response was organized persecution of kea for more than a century. (**b**) Feather-pecking is a persistent and serious welfare problem among domesticated chickens housed at high density. One theory proposes that feather-pecking is a manifestation of misdirected normal exploratory-pecking behavior. (**a**) © Andrew Walmsley (www.wildfocus.org), reproduced with permission. (**b**) © Technical University of Munich, reproduced with permission

and, encouraged by a New Zealand government bounty, an estimated 150,000 kea were killed over a century (1868 to ca. 1970) (Diamond and Bond 1999). Anecdotal evidence suggests that a few dominant males initiate sheep attacks in a given area and the behavior then spreads culturally; thus, it is possible that those kea displaying more exploratory, risk-taking, personalities were targeted for culling. In 1986, kea belatedly received full legal protection; however, as part of the political compromise associated with the kea's improved legal status, the New Zealand Department of Conservation is obliged to move, or take into captivity, any birds considered to have taken up the sheep-attacking habit or who are proving a significant nuisance around human settlements and ski fields. Consequently, there are reasonable grounds to suspect that the current captive kea population was selected nonrandomly with respect to personality genetics.

Much has been written about the need to preserve genetic diversity in the relatively small gene pools that are characteristic of endangered species (Kohn et al. 2006; Primmer 2009). When quantifying genetic diversity, there is a general consensus that in lieu of anything better the diversity of putatively selectively neutral loci (e.g., microsatellites) is a satisfactory proxy for diversity at functionally important loci (Kohn et al. 2006; Primmer 2009). However, such a "broad brush" approach has its limitations; and endangered species conservation programs may seek to preserve genetic diversity at defined genetic loci, including those of the major histocompatibility complex (MHC), the diversity of which is thought to provide populations with resistance to a wider range of pathogens (Sommer 2005; Acevedo-Whitehouse and Cunningham 2006). In an analogous manner personality/behavior-associated

genetic variation may provide a population with evolutionary flexibility; and it would be prudent for conservation workers to at least be cognizant of a need to preserve this variation. In this context, it should be noted that in addition to the evolutionary effects of small and genetically skewed founder populations behavioral evolution may be an intrinsic feature of small captive populations – an issue of particular importance when the long-term objective is to release captive bred animals to supplement or reestablish wild populations (Hakansson and Jensen 2005, 2008; Hakansson et al. 2007; Frankham 2008; Pelletier et al. 2009).

Although ecotourism does not involve the actual "harvest" of animals, we should be aware that animals of differing personality types may differ in their responses to close human contact; and this, in turn, may result in differential reproduction and associated evolutionary pressures (Martin and Réale 2008; Ellenberg et al. 2009).

The twentieth century industrialization of agriculture has provided great benefits as measured by the abundance and relatively low prices of many foods, including animal proteins. However, these changes in farming practices were accompanied by a general tendency to view farmed animals as psychologically inert "production units." Happily, there now appears to be a reappraisal of this attitude and a trend toward more consideration of the psychological welfare of farmed animals (Flint and Woolliams 2008). In the case of birds, such welfare issues largely pertain to poultry farming. Although there is considerable room for improvement in poultry husbandry, there is also a reasonable case to be made for the breeding of birds whose temperament/personality is better suited to intensive agriculture. Molecular characterization of genes associated with deleterious behaviors and/or maladapted personality types should assist in breeding poultry better adapted to husbandry conditions constrained by both economics and public attitudes (Jensen and Andersson 2005; Keeling et al. 2004; Flisikowski et al. 2009; Mormede 2005). Such agricultural breeding efforts may become reliant on conservation efforts, as domestication may have depleted the gene pools of modern farm animals of "useful" alleles retained in traditional breeds and their related wild species.

13.4 Conclusion

In providing justifications for further avian personality molecular genetics research, I suggest that, in addition to the conservation and welfare implications outlined above, such studies may also provide useful insights into human mental health-related questions. In particular, the personality genetics of free-living animals may help us to understand better why genetic variation associated with human behaviors now characterized as pathological may have persisted over time, possibly by their conferring some selective advantage(s) in some context(s) (Keller 2008; Pawlak et al. 2008).

I also reiterate my support for increased dialog between poultry scientists and academic avian behavioral ecologists, to the benefit of both groups. It is my hope

that future avian personality molecular genetics studies will provide many examples of how productive such industry–academia relationships can be.

Whatever utilitarian justifications may be offered for bird personality genetics research, I also believe that such work has intrinsic cultural value by increasing our understanding and appreciation of birds. It is to be hoped that such knowledge will improve human stewardship of birds during an uncertain time when unrelenting "progress" is transforming humanity's diverse economic, ethical, spiritual, and intellectual values into, arguably, the main drivers of evolutionary change.

Acknowledgments My thanks go to the following people for providing photographs: Kees van Oers (Fig. 13.1a, b), Andrew Walmsley (Fig. 13.4a), and Ruedi Fries (Fig. 13.4b) and to the Royal Society (London) for permission to reproduce the data shown in Fig. 13.3. Many thanks to Silke Steiger for generating the snake plot shown in Fig. 13.2, to Kees van Oers and Peter Korsten for very helpful comments on a draft version of this chapter, and to Miho Murayama for her patient editorial assistance. I am grateful to my wife, Petra, for her patience and support during the writing of this chapter and to my children, Finn and Ella, for constantly reminding me how great the mystery surrounding human personality really is.

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Part IV
Evolution of Coloration and Visual Opsin Genes in Vertebrates

Chapter 14
Evolutionary Genetics of Coloration in Primates and Other Vertebrates

Nicholas I. Mundy

14.1 Introduction

Coloration is a fundamental trait of vertebrates and important in the lives of most species.[1] Coloration can have many functions, but in the context of behaviour it is particularly important for predator–prey interactions between species and social and sexual interactions within species. There are a number of properties of vertebrate coloration that make it an attractive system to study the evolutionary genetics of adaptation, including its evolutionary lability, ease of quantification, and, at least for some forms of coloration, knowledge of the underlying genetic networks. Coloration provides an excellent opportunity to obtain a detailed understanding of the mechanisms of phenotypic evolution from genetics to development and physiology through to behaviour and adaptation (Hoekstra 2006).

This chapter briefly introduces the proximate basis of coloration and types of colour variation before discussing progress in identifying the molecular basis of colour variation in wild vertebrates. It then considers the progress that has been made to date in primates. Functional explanations of coloration largely lie beyond the scope of this chapter. For recent reviews of the function of coloration in mammals, birds, and primates, respectively, see (Caro 2005; Hill and McGraw 2006b; Bradley and Mundy 2008).

[1]Note for the nonspecialist reader: The vertebrates show an astonishing diversity of coloration, from the showy display of the peacock's tail to the bright red and blue face of a male mandrill monkey. Current research is now beginning to uncover the genes responsible for this diversity. This chapter explains the current progress in this research field. A notable finding is that the same genes can be responsible for mutations causing similar coloration from species as diverse as mammals, birds, and fish.

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14.1.1 Physicochemical Basis of Vertebrate Colours

The colour of a vertebrate (or indeed any object) depends on the spectrum of the incident light, the physicochemical makeup of the object, and the colour vision system of the observer (see Chaps. 15 and 16). The colour properties of an object are conveniently quantified and standardized in the reflectance spectrum, but other methods are required to quantify patterning. The two basic ways in which coloration is achieved are by the presence of one or more pigments and in the physical structure of the integument (Hill and McGraw 2006a).

There is a large range of pigments utilized for coloration by vertebrates, but the most important categories are melanins (red/yellow phaeomelanin and black/brown eumelanin), carotenoids (yellow to red), pteridines (red to brown), and haemoglobin (red to brownish red). Pigments are frequently, but not always, deposited in specialized pigment cells. Mammals and birds have a single class of pigment cell in the integument, melanocytes, which synthesize melanins and transfer them to epidermal keratinocytes in skin, hairs, and feathers. Teleost fish, amphibians, and reptiles have several classes of dermal pigment cell specialized for different pigments, which are retained in the cell of origin: melanophores (eumelanin), xanthophores (pteridine and carotenoid), erythrophores (pteridine and carotenoid), and iridophores (reflective crystals of guanine) (Kelsh 2004).

The physical structure of the integument can also affect coloration. Notably, microstructures such as air bubbles or arrays of keratin or collagen fibres can lead to changes in spectral composition of reflected light by selective reflectivity for particular wavelengths (often in the blue or ultraviolet range). There is no simple dichotomy between pigment-based and structural colours. They are often used in combination (e.g., carotenoids and structural colours) (Prum and Torres 2003), and many structural colours require the presence of pigmented melanin granules.

14.1.2 Temporal Variation in Coloration

Coloration of individual vertebrates can of course change dramatically over time. Such changes may be intrinsic, relating to the physiological state of the individual, or be under environmental control. Considering the first category, colour changes can be age-related, such as ontogenetic colour change from juveniles to adults. They may be seasonal, as in the well-known winter coats, providing concealment in several Arctic species – e.g., arctic fox (*Alopex lagopus*), ptarmigan (*Lagopus minutus*) – which are due to moulting to hairs/feathers with low melanin content. Changes in colour of sexual skin occur in relation to the ovarian cycle, often signalling female receptivity, and this is an important feature in many primates (Dixson 1998). On even shorter time scales of hours down to minutes, coloration can change in certain fish, amphibians, and reptiles in relation to, for example, background matching (Bentley 1998). Temporal variation in coloration during the life of a single individual is under physiological control, especially via hormone levels. Coloration may also be affected

by environmental factors, which is particularly the case when coloration is dependent on pigments taken up from the diet, as is the case for carotenoids.

14.1.3 Sexually Dimorphic Coloration (Sexual Dichromatism)

The presence of coloration differences among conspecific individuals of different sexes, or sexual dichromatism, is highly prevalent but not universal. Sexual dichromatism is of course strongly related to sexual selection (Andersson 1994). It is often associated with morphological changes that complement the colour differences to form an integrated signal – e.g., the male peacock's train (*Pavo pavo*) or the ridges on the face of a male mandrill (*Mandrillus sphinx*). Sexual dichromatism may be present year round or occur with the annual breeding cycle. Sexual dichromatism is frequently under control of the sex hormones.

14.2 Genetic Basis of Vertebrate Colours

The starting point for the genetic analysis of coloration was the presence of numerous stably inherited colour forms in domesticated mammals and birds. Although humans have presumably been selecting for colour of many domesticated species for millennia, it is mice that were pivotal for the development of coloration genetics. Mutant lines of mice with different coloration were already being systematically analyzed during the early twentieth century. Today, more than 120 loci that affect mouse coat coloration have been identified, and more than half of these have been identified at the molecular level (Bennett and Lamoreux 2003; Hoekstra 2006). Many of these loci have also been associated with coloration in other mammals (Jackson 1997). Among other vertebrate groups, good progress is being made in the molecular genetics underlying coloration in teleosts, such as zebrafish (Parichy 2003); and an increasing number of loci affecting colour in chicken and quail are being identified at the molecular level (Kerje et al. 2004; Nadeau et al. 2008). However, it is important to note that there is currently a strong bias towards loci involved in controlling melanogenesis, and there is a paucity of information on the genetics of coloration based on carotenoids (but see Eriksson et al. 2008), pteridines, and structural colours, which is directly attributable to the lack of these types of coloration in mice.

14.2.1 Evolutionary Genetics of Colour Variation Within Species

Species in which individuals of the same sex and developmental stage show large variation in coloration can be tractable systems for investigating the molecular basis of coloration. If there is natural interbreeding between colour forms, such as occurs

in a colour polymorphism or along a cline, associations between genotype and colour phenotype can be investigated or colour inheritance studied in families. In some cases, it is possible to set up experimental crosses and perform quantitative trait locus (QTL) analyses, which involve genome-wide linkage analyses for the colour trait of interest using large numbers of genetic markers. Association studies are particularly suited to studying candidate loci in which potentially important loci are identified using a priori information, whereas QTL analyses have the benefit of not requiring such information. If significant associations between colour variation and variation in candidate loci are found, it is important to exclude the possibility that the association has arisen simply because of population history. Whatever method is used, it is useful to perform follow-up studies on the function of the variants found.

Table 14.1 summarizes studies in which loci involved in colour variation in free-living vertebrate populations have been identified to date. All of these cases involve light or dark melanin-based variation, and most relate to polymorphic species in which colour variation is inherited in a quasi-Mendelian fashion. It is apparent that one locus dominates in Table 14.1 – *MC1R* (melanocortin-1 receptor) – and it is striking that this locus is implicated in natural colour variation across all vertebrate groups investigated. The MC1R protein is expressed in the cell membrane of melanophores in skin and melanocytes in skin, hairs, and feathers; it regulates the amount and type of melanin synthesized. When MC1R is stimulated by the extracellular hormone α-melanocyte-stimulating hormone (α-MSH), it leads to enhanced eumelanin synthesis in the melanocyte. In the absence of α-MSH or the presence of the inhibitor agouti signalling protein (ASP or ASIP), MC1R activity is low, leading to phaeomelanin synthesis or reduced synthesis of eumelanin. *MC1R* was originally described as the gene at the classic *extension* locus in mice (Robbins et al. 1993). The repeated involvement of *MC1R* in colour evolution may well be partly related to the relatively low negative pleiotropy at this locus (Mundy 2005); however, there is undoubtedly some bias as this locus is easy to assay because it has a single coding exon. In an interesting extension to extinct species, *MC1R* variation in the mammoth (*Mammuthus primigenius*) is consistent with a role in colour variation in this species (Römpler et al. 2006).

In recent years, several other pigmentation loci have been implicated in coat colour variation in wild species (Table 14.1), with the greatest diversity present in mammals. These loci include *ASIP*, the inhibitor of *MC1R*; *BCD103*, a novel activator of *MC1R*; *TYRP1*, which encodes an enzyme involved in eumelanin synthesis; and *KITLG* and *C-KIT*, which encode a ligand–receptor pair important in melanocyte/melanophore development.

Some of the studies in Table 14.1 go well beyond simply documenting genotype–phenotype associations to show the wide spectrum of evolutionary questions that can be answered with a detailed knowledge of the genetic basis of phenotypes. For example, in a study of the genetics of adaptive pale coloration in beach mice, which live on white sand, epistasis was found between alleles at *ASIP* and *MC1R*: The *MC1R* allele causing pale coloration only did so in an individual that was homozygous for the *ASIP* pale allele (Steiner et al. 2007). Thus, the study revealed how

Table 14.1 Loci implicated in colour variation in free-living vertebrate species

Taxon	Colour variation	Locus	Reference
Teleosts			
Mexican cavefish (*Astyanax mexicanus*)	Brown → pale	*OCA2, MC1R*	Protas et al. (2006), Gross et al. (2009)
Three-spined stickleback (*Gasterosteus aculeatus*)	Dark → pale	*KITLG*	Miller et al. (2007)
Reptiles			
Little striped whiptail (*Aspidoscelis inornata*)	Dark → pale	*MC1R*	Rosenblum et al. (2004)
Birds			
Bananaquit (*Coereba flaveola*)	Black → grey-yellow	*MC1R*	Theron et al. (2001)
Lesser snow goose (*Anser chen caerulescens*)	Dark → pale	*MC1R*	Mundy et al. (2004)
Arctic skua (*Stercorarius parasiticus*)	Dark → pale	*MC1R*	Mundy et al. (2004)
Red-footed booby (*Sula sula*)	Dark → pale	*MC1R*	Baião et al. (2007)
Chestnut-bellied monarch (*Monarcha castaneiventris*)	Black → reddish brown	*MC1R*	Uy et al. (2009)
Mammals			
Rock pocket mouse (*Chaetodipus intermedius*)	Dark → pale	*MC1R*	Nachman et al. (2003)
Beach mouse (*Peromyscus polionotus*)	Dark → pale	*ASIP*, *MC1R*, *c-Kit*	Steiner et al. (2007)
Deer mouse (*Peromyscus maniculatus*)	Dark → pale	*ASIP*	Kingsley et al. (2009), Linnen et al. (2009)
Grey squirrel (*Sciurus carolinensis*)	Dark → grey	*MC1R*	McRobie et al. (2009)
Soay sheep (*Ovis aries*)	Dark → pale	*TYRP1, ASIP*	Gratten et al. (2007, 2010)
Jaguar (*Panthera onca*)	Dark → spotted	*MC1R*	Eizirik et al. (2003)
Jaguarundi (*Felis yaguarundi*)	Dark → pale	*MC1R*	Eizirik et al. (2003)
Black bear (*Ursus americanus*)	Dark → pale	*MC1R*	Ritland et al. (2001)
Gray wolf (*Canis lupus*)	Dark → pale	*BCD103*	Anderson et al. (2009)

selective pressure on a locus (*MC1R*) could change according to the genetic background and indicated the likely order of fixation of functional mutations at different loci, with *ASIP* preceding *MC1R*. In the case of melanism in wolves, a variant allele at the *BCD103* locus that was first identified in domestic dogs was shown to be associated with dark coat colour in wolves. Detailed analysis of population genetics at *BCD103* in both wolves and dogs showed that the melanic allele at the *BCD103* locus has likely introgressed from domesticated dogs (Anderson et al. 2009). In soay sheep, variation at the *TYRP1* locus was associated with dark coloration, with the dark allele dominant. The ability to score dark individuals as homozygotes or

heterozygotes at *TYRP1* was then used to dissect the cause of patterns of selection on body size and coloration (Gratten et al. 2008). It was found that coat colour is negatively genetically correlated to a QTL affecting fitness, which could explain why the frequency of dark sheep is falling in the population, even though dark coat colour is positively correlated with body size, which is beneficial.

14.2.2 Evolutionary Genetics of Colour Variation Among Species

One of the key questions in evolutionary genetics is whether the same genetic mechanisms are involved in microevolution (variation within species) as in macroevolution (variation among species). Therefore, it is of considerable interest to investigate whether the loci involved in colour polymorphisms in Table 14.1 are also implicated in the evolution of coloration among species. This requires different methodology, however, because unless two species are closely related enough to be able to hybridize direct demonstration of the role of a locus in interspecific coloration is not possible. There are various ways to overcome this difficulty. Comparative methods can be used to find correlations over a phylogeny between colour phenotype and genotype. The pattern of natural selection on colour loci can be investigated in relation to the evolution of coloration. Further evidence can come from knowledge of the functional effects of specific mutations at a locus.

There are relatively few studies in this area (see also later discussion of primates). In a comparative study of the evolution of sexual dichromatism in galliform birds (e.g., chicken, pheasants, partridges), we investigated sequence evolution in four pigmentation loci (*MC1R*, *TYR*, *TYRP1*, *DCT*), a candidate pigmentation locus (*AGRP*), and a control locus (*CYTB*) in 35 taxa (Nadeau et al. 2007). We found a robust and significant relation between the ratio of nonsynonymous to synonymous substitution rates (dN/dS) and the degree of plumage dichromatism across the phylogeny for *MC1R*; none of the other five loci were associated with dichromatism. Furthermore, some of the mutations present in *MC1R* are at known functional sites and are expected to contribute to the relatively dark phenotype in some species. The results strongly suggest that *MC1R* is important for interspecific evolution of dichromatism in galliforms and implies that sexual selection acting on plumage coloration during galliform evolution has left an imprint at the *MC1R* locus (Nadeau et al. 2007).

Another comparative study of *MC1R* variation across swans (*Cygnus*) identified *MC1R* mutations associated with sexually monomorphic differences in melanin content of plumage (Pointer and Mundy 2008). Interestingly, the two species with substantial black patches in the plumage (the black swan *Cygnus atratus* and the black-necked swan *Cygnus melancoryphus*) had *MC1R* mutations that are predicted to lead to increasing amounts of melanin. However, the interpretation in this case is complicated by the finding that one of these same mutations is found in the distantly related all-white coscoroba swan (*Coscoroba coscoroba*), which can perhaps be explained by epistatic effects at another locus.

In cichlid fish, an “eggspot” evolved on the anal fin of males that increases fertilization efficiency. In a comparative study of evolution of the *csf1ra* locus, this

known pigmentation locus was shown to be expressed in the eggspot and to show some evidence for positive selection in the lineage on which eggspots evolved (Salzburger et al. 2007).

In summary, in response to the question posed at the beginning of this section, it does seem that one locus at least (*MC1R*) is involved in coloration differences both within and between vertebrate species. However, far more evidence is required to assess the generality of this result.

14.3 Primate Coloration

Primates are among the most colourful of all mammals, and the variation and function of coloration in primates was recently reviewed (Bradley and Mundy 2008). Primate coat colours vary from almost pure white to completely black, with greys, yellow, and reds in between. They are often strikingly patterned – e.g., the pied coloration seen in black-and-white ruffed lemurs (*Varecia varecia*), indris (*Indri indri*), and black and white colobus (*Colobus guereza*), among others. Apart from hair coloration, however, a prominent feature of primates is the coloration of bare body parts. For example, primate faces may be pale or dark (melanin variation), bright red due to haemoglobin in the underlying blood supply, or blue due to structural colours. Eye colour also varies among primate species, with the iris colour varying from blue to pale brown to dark brown; but this area has received little attention, and we know little about the function of this variation. From a functional standpoint, bare body part coloration is far better understood than hair coloration; and, for example, recent experiments have shown the importance of the redness of bare body parts in sexual signalling in rhesus macaques (*Macaca mulatta*) and mandrills (*M. sphinx*) (Gerald 2001; Setchell et al. 2006).

Coloration varies in primates in ways similar to those seen in other mammals. Ontogenetic colour change during development is a common feature and is sometimes dramatic, as in the bright orange infants in several colobine species. Sexual dichromatism in bare body parts is common, whereas sexual dichromatism in coat coloration is rare and patchily distributed, occurring, for example, in several species of *Eulemur*, the black-and-gold howler (*Alouatta caraya*), and several gibbons (*Hylobates*, *Nomascus*). Quantitative variation in coloration within populations appears quite common, but true polymorphisms (i.e., cases showing simple Mendelian inheritance of colour variation) in primates are rare, the best example being the Javan langur (*Trachypithecus cristatus*), where the adults of either sex can be dark grey or orange.

14.3.1 Evolutionary Genetics of Primate Coloration

The first studies on the genetic basis of coloration in primates were in humans. *MC1R* variants were shown to cause red hair and pale skin in European populations (Valverde et al. 1995); more recently, polymorphisms in *ASIP* and *SLC24A5*

(which encodes a solute carrier protein) were associated with skin colour (Kanetsky et al. 2002; Lamason et al. 2005). With the availability of genome-wide single nucleotide polymorphism (SNP) data in several human populations there has recently been an explosion of studies implicating pigmentation-related genes in positive selection, with more than ten loci identified, including *SLC24A5* (Sabeti et al. 2002; Voight et al. 2006; Norton et al. 2007; Williamson et al. 2007; Pickrell et al. 2009). Although intriguing, it is still too early to say if most of these loci have been involved in adaptive evolution of skin colour in humans because an association with phenotype has not generally been demonstrated.

In nonhuman primates, most work on the evolutionary genetics of coloration has taken a comparative, interspecific approach and has been largely confined to coat colour variation. In our own work, we have assayed variation at two key candidate loci – *MC1R* and *ASIP* – across the primate order to examine whether sequence variation is associated with coat colour differences (Mundy and Kelly 2003, 2006). To maximize the chances of detecting phenotypically relevant genetic changes, we sampled from several primate groups in which closely related taxa show dramatic differences in eumelanin/phaeomelanin distribution: macaques (*Macaca*), langurs (*Trachypithecus*), lion tamarins (*Leontopithecus*), howler monkeys (*Alouatta*), and ruffed lemurs (*Varecia*). In addition, we sampled a few other notable cases of all-black or all-red species – chimpanzee (*Pan troglodytes*); gorilla (*Gorilla gorilla*); orangutan (*Pongo abelii*); Goeldi's monkey (*Callimico goeldii*) – and, to increase the phylogenetic perspective, species from the major clades of catarrhines (Old World monkeys and apes) and platyrrhines (New World monkeys).

The full coding regions of *MC1R* (single exon, ~1 kb) and *ASIP* (three coding exons, 400 bp total) were sequenced. In general, no simple relation was found between variation in these two candidate loci and coloration differences among primate taxa. There are several examples where species with radically different distributions of eumelanin and phaeomelanin have identical coding sequences at *MC1R* and/or *ASIP*. For example, pig-tailed (generally buff) and lion-tailed (generally black) macaques (*Macaca nemestrina* and *M. leonina*, respectively) have identical *MC1R* sequences, as do red-ruffed lemurs (*Varecia rubra*) and black-and-white ruffed lemurs (*Varecia variegata*); notably, Goeldi's monkey (*C. goeldii*) and the common marmoset (*Callithrix jacchus*) have identical *ASIP* sequences. Thus, a role of *MC1R* and *ASIP* coding variation in phenotypic change can be excluded in these cases. A similar result was reported for *MC1R* in a larger sample of macaque species (Nakayama et al. 2008).

Not surprisingly, more distantly related taxa tended to show more divergence at both *MC1R* and *ASIP*. For example, the orangutan has unique *MC1R* and *ASIP* sequences compared to other primates. Some indication of the functional significance of the substitutions involved can be obtained from the good knowledge of structure–function relations, particularly for *MC1R*. In general, most of the *MC1R* and *ASIP* mutations in primates do not occur at known functionally important sites, and their fixation is mostly attributable to genetic drift. A dramatic loss of the entire *ASIP* locus in a 100-kb genomic deletion was recently reported in gibbons (Nakayama and Ishida 2006), but the consequences of this deletion regarding coloration are unclear.

The overall absence of a genotype–phenotype association at *MC1R* in primates is somewhat surprising, given its association with polymorphic colour variation in many mammals and other vertebrates (Table 14.1). There is no indication of any basic differences in MC1R function between primates and other mammals. One possible explanation for the discrepancy is that evolutionary genetic mechanisms for intraspecific and interspecific melanism are different. To investigate this further, we examined the biochemical function of MC1R of a broad range of primate species in vitro (Haitina et al. 2007). The results revealed considerable diversity of MC1R function. Of particular interest are a number of species, such as the black-and-white ruffed lemur, in which the MC1R has lost the ability to bind α-MSH but, instead, shows a high level of constitutive activity. The results show that evolution of MC1R function has occurred repeatedly in primates, although it has a stronger relation to the primate phylogeny than to the current coat colour phenotype.

The absence of an association between *ASIP* variation and coat colour in primates is difficult to interpret. Coding changes at the *ASIP* locus are associated with colour differences in several domestic species (e.g., fox, horse, and cat (Våge et al. 1997; Rieder et al. 2001; Eizirik et al. 2003) and one wild species, *Peromyscus maniculatus* (Kingsley et al. 2009). However, most of the functional alleles at *ASIP* in mice are due to mutations in the large (>100 kb) regulatory region, which was not assayed in our study and which is implicated in two cases in the wild: in soay sheep and *Peromyscus* (Gratten et al. 2010; Kingsley et al. 2009). Because of the pivotal role of *ASIP* in regulating phaeomelanin bands on hairs and dorsoventral difference in coloration, it seems likely that regulatory mutations in *ASIP* have indeed played a role in primate coat colour evolution. An analysis of molecular evolution of *ASIP* in primate lineages in which two important *ASIP*-controlled phenotypes were lost (agouti hairs and dorsoventral differences) provided some evidence for a change in constraint on the locus in these lineages: lineages reconstructed as lacking *ASIP*-controlled phenotypes had a higher average dN/dS than did lineages that retained the *ASIP*-controlled phenotypes (Mundy and Kelly 2006).

A recent study investigated whether the mutations implicated in the blue-eyed phenotype in humans also occurs in association with blue eyes in a subspecies of the black lemur (*Eulemur macaco flavifrons*) (Bradley et al. 2009). The mutations upstream of the *OCA2* locus were absent in the lemur, showing an independent origin for blue eyes in the two lineages.

14.3.2 MC1R, ASIP, and Colour Variation in Lion Tamarins

Lion tamarins (*Leontopithecus*) have some of the most dramatic colour variation of any primate genus. The close relations among *Leontopithecus* species combined with a lack of sexual dichromatism or ontogenetic change in coloration make them an excellent group for studying the genetic basis of interspecific colour differences. Each species has a characteristic distribution of patches of solid orange (phaeomelanin) and solid black (eumelanin) hairs, ranging from the completely orange golden lion

tamarin (*Leontopithecus rosalia*) to the black lion tamarin (*Leontopithecus chrysopygus*), which has small orange patches on its thighs. The habitats occupied by the species are quite similar, and a role in social function seems the most likely explanation for the colour differences, although there are few relevant data. The evolution of *MC1R* and *ASIP* in lion tamarins show several peculiarities compared with other primates that together imply a role for these loci in coat colour evolution (Fig. 14.1).

The *MC1R* of lion tamarins shares a derived deletion in the 3′-end that leads to the presence of a novel stop codon. The closely related black lion tamarin (*L. chrysopygus*) and golden lion tamarin (*L. rosalia*) have identical *MC1R* coding sequences, but there are as many as seven coding mutations separating this pair from the golden-headed lion tamarin (*Leontopithecus chrysomelas*). Six of these are point mutations, but the most interesting is a 24-nucleotide deletion, which leads to the loss of eight amino acids in the MC1R protein. Remarkably, a conserved eight-amino-acid deletion at the same position has also been reported in the MC1R of jaguarundis (*Felis yaguarundi*) (Eizirik et al. 2003). Coat colour is polymorphic in these cats, and the *MC1R* deletion is associated with dark (eumelanic) coat colour. The deletion occurs in the relatively melanic golden-headed lion tamarin, and it seems likely that it plays a functional role in the dark coloration of this species.

Of further interest are the results of analyses to estimate the strength of selection on the *MC1R* gene in lion tamarins. The (dN/dS) estimated for *MC1R* among the lion tamarins was close to 1.0, which is significantly greater than that of any other primate genus investigated (range 0.089–0.229) (Mundy and Kelly 2003). This suggests that whereas purifying selection is the dominant mode of evolution of *MC1R*

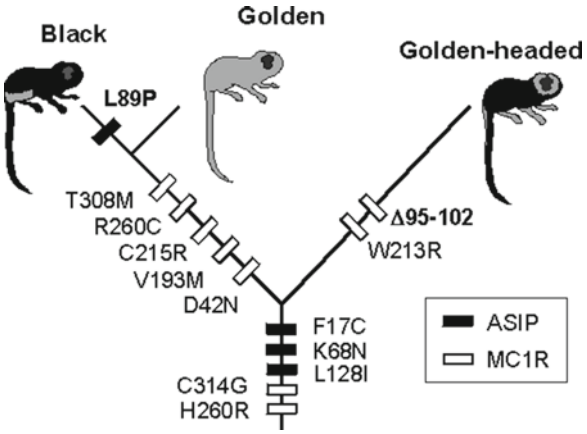

Fig. 14.1 Reconstructed evolution of the amino acid sequence of melanocortin-1 receptor (MC1R) and agouti signalling protein (ASIP) in lion tamarins. Mutations are shown in single-letter amino acid codes, numbered according to the human protein sequence. Mutations in the root are since the common ancestor with other Callitrichid primates and include a deletion at the 3′-end that led to both a C314G and premature stop codon. The eight-amino-acid deletion in golden-headed lion tamarins that is shared with jaguarundi cats is shown as Δ95-102

in primates there was a change in selection pressure in lion tamarins. The most plausible scenario is that of a mixture of positive selection and purifying selection on *MC1R* during lion tamarin evolution, which further strengthens the case that *MC1R* is important for interspecific differences in coloration.

The pattern of evolution of *ASIP* among lion tamarins is in strong contrast to that of *MC1R*. There is little variation in *ASIP*, and the sequences in the golden and golden-headed lion tamarins are identical. The only mutation present occurs in the black lion tamarin and results in a leucine-to-proline amino acid change at position 89 in the ASIP protein. This is therefore the only coding difference in *MC1R* and *ASIP* among the black and golden lion tamarins, and it could potentially be of functional importance. It should also be pointed out that the large regulatory region of *ASIP* lion tamarins was not examined and may contain functionally relevant mutations.

To summarize these results, we identified derived mutations that are plausibly related to melanization of coat colour in lion tamarins – the eight-amino-acid deletion in *MC1R* of the golden-headed lion tamarin and the leucine-to-proline mutation at amino acid position 89 of *ASIP* of the black lion tamarin. It will be interesting to test this hypothesis by investigating in vitro activity of the variant *MC1R* and *ASIP* sequences. If this scenario is correct, it has interesting implications for attempts to reconstruct the coat colour of the ancestral lion tamarin. In particular, the presence of a melanizing mutation in the golden-headed lion tamarin suggests that its ancestor had a paler coat.

14.4 Conclusions and Prospects

Good progress has been made in identifying the genes and mutations underlying colour evolution in vertebrates. The major conclusion from the studies to date is that there is considerable convergence in the genetic mechanisms across broad phylogenetic scales. In contrast, the genes underlying colour variation in many nonhuman primates are poorly known. A combination of several factors, including new genomic resources, should enable more rapid progress in this field in the near future. Equally important, however, is the continuing work into the adaptive function of coloration in primates, including its role in behaviour.

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Chapter 15
Speciation of Cichlid Fishes by Sensory Drive

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15.1 Why African Cichlid Fishes?

Speciation is a process where the gene flow (gene exchange) between closely related populations ceases (Coyne and Orr 2004). Speciation is mainly classified into three types: allopatric, parapatric, sympatric. Allopatric speciation occurs by complete geographic isolation, such as by continental drift or island formation. Parapatric speciation occurs under a limited extent of gene flow. The distribution of species along an environmental cline[1] (e.g., temperature, salt concentration, light environment) results in populations that have adapted to their local environment. This gradient of local adaptation is the beginning of this type of speciation. Most species are composed of populations with limited gene flow and therefore have a potential for parapatric speciation. Sympatric speciation does not require geographic isolation. With this type of speciation, a new species emerges within a freely breeding population. Sympatric speciation is theoretically possible but is generally considered uncommon in nature. Reproductive isolation is classified into prezygotic and postzygotic ones. Prezygotic isolation is caused by any behavioral, anatomical, or physiological trait that leads to preventing fertilization, such as mating avoidance, mate choice, and morphological mismatch of reproductive organs. Postzygotic isolation is caused by physiological processes that prevent normal development of zygotes after fertilization and normal fertility of offspring.

To date, many theoretical models of speciation have been proposed in which conditions leading to different types of speciation are studied (Coyne and Orr 2004). The ecological speciation hypothesis is one in which the reproductive isolation evolves as a consequence of ecological adaptation of traits to different environments (Schluter 2001). This type of speciation might occur in allopatry, parapatry, and sympatry. Ecological speciation occurs when a trait diverges between populations

[1] Clines consist of forms of species that exhibit gradual phenotypic differences over a geographic area as a result of environmental heterogeneity.

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inhabiting different environments and leads to reproductive isolation between them. In this model, reproductive isolation is a by-product of adaptation of the trait to the environment. When the trait is a sense, the model is called "speciation by sensory drive." In sensory-driven speciation, adaptation of a sense to different habitats leads to a mating preference to more conspicuous individuals than others for the sensor, resulting in premating (prezygotic) isolation (Boughman 2002).

Speciation is believed to be an engine that drives biodiversity. Despite its biological importance, speciation was once thought to be difficult to study because of belief that speciation takes too long to complete. Recently, however, African cichlid fish have become a model system for understanding the genetic basis of vertebrate speciation (Kocher 2004).

Cichlids (Family Cichlidae; Order Perciformes) are tropical freshwater fish that are found in Africa, South America, India, and Madagascar (Fryer and Iles 1972; Stiassny 1991; Kocher 2004). In Africa, Lakes Victoria, Malawi, and Tanganyika in the East African Rift Valley harbor roughly 500, 500, and 250 endemic species of cichlid fishes, respectively (Fryer and Iles 1972; Snoeks et al. 1994; Konings 1995; Seehausen 1996; Turner et al. 2001). These fishes have fascinated evolutionary biologists studying taxonomy, ecology, and molecular biology as a spectacular example of explosive adaptive radiation[2] of living vertebrates (Fryer and Iles 1972). Lake Tanganyika is the oldest of the African Great Lakes, with an age of 9–12 million years (Cohen et al. 1993, 1997) followed by Lake Malawi with an age of 2–5 million years (Delvaux 1995). Lake Victoria is the youngest, with an estimated age of between 250,000 and 750,000 years (Johnson et al. 1996). Moreover, geological evidence suggests that Lake Victoria dried up at the end of the Pleistocene and refilled only 15,000 years ago (Johnson et al. 2000).

Molecular analyses have revealed phylogenetic relations among the major lineages of African cichlids (Meyer et al. 1990; Nishida 1991; Sturmbauer and Meyer 1992; Kocher et al. 1993; Mayer et al. 1998; Takahashi et al. 2001; Salzburger et al. 2002, 2005; Terai et al. 2003a). The fishes in each lake underwent several independent radiations after they colonized in each lake (Sturmbauer and Meyer 1992; Salzburger et al. 2005; Genner et al. 2007a). The comparison of adaptive radiations with different ages among different lakes is one approach to studying speciation in African cichlids (Fig. 15.1).

The radiation in Lake Victoria is the most recent (Nagl et al. 2000). The genetic distances between species in mitochondrial DNA (mtDNA) are very short, tenfold shorter than those in Lake Malawi (Joyce et al. 2005). Most of the genetic variations – including point mutations, insertions, and deletions – in the genome is expected to be neutral with respect to the effect of natural selection (Kimura 1983). An "allele" is defined as a variant DNA at a genomic locus. In a short evolutionary time frame such as in Lake Victoria, neutral alleles in ancestral founder populations in the lake are expected to have persisted in its current descended species, although with varying frequencies. In other words, the current species are on the path of differentiation of

[2] Adaptive radiation is rapid evolutionary radiation characterized by an increase in the morphological and ecological diversity of a single lineage.

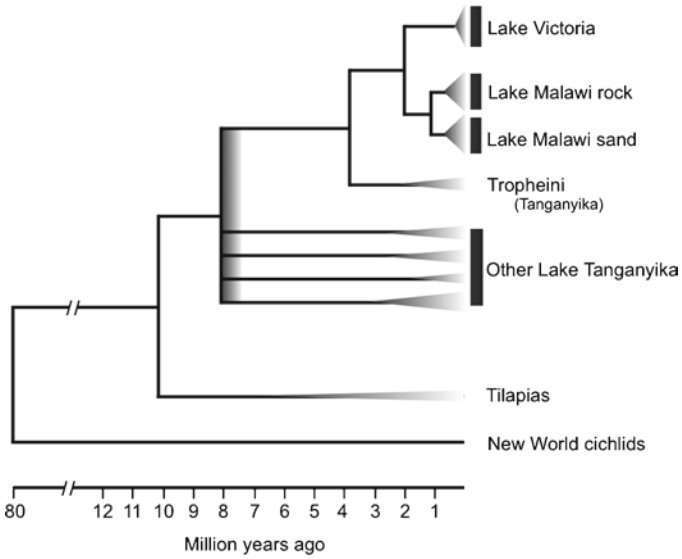

Fig. 15.1 Phylogenetic tree of cichlid lineages and their age. Adapted from Kocher (2004)

allelic composition but have not yet reached completion where different alleles are fixed in different species (Nagl et al. 1998, 2000; Terai et al. 2004; Seehausen et al. 2008; Maeda et al. 2009) ("neutral mutations" in Fig. 15.2). In contrast, if an allele is more advantageous than others (i.e., "is positively selected") in a species, the allele is expected to be fixed in the species more quickly than alleles with no selective advantage (Terai et al. 2002a) ("adaptive mutation" in Fig. 15.2). Thus, if an allele of a locus is fixed in a species, whereas most of the other loci are polymorphic with alleles shared among species, the locus is a candidate gene enabling the adaptive radiation in Lake Victoria.

15.2 Mate Choice by Color Vision

One interesting feature of cichlids is the way in which females care for their eggs and larvae (Fryer and Iles 1972). In most species in Lakes Malawi and Victoria, the female protects its eggs and larvae by holding them in her mouth (Salzburger et al. 2007). This behavior is called "mouth breeding." On the other hand, species in tribe Lamprologini in Lake Tanganyika lay their eggs on substrate (e.g., rocks and shells); both the male and the female protect them and are thus known as "substrate spawners" (Fryer and Iles 1972). Females of polygynous[3] species invest in parental

[3]Polygyny is a form of mating in which a male mates with two or more females.

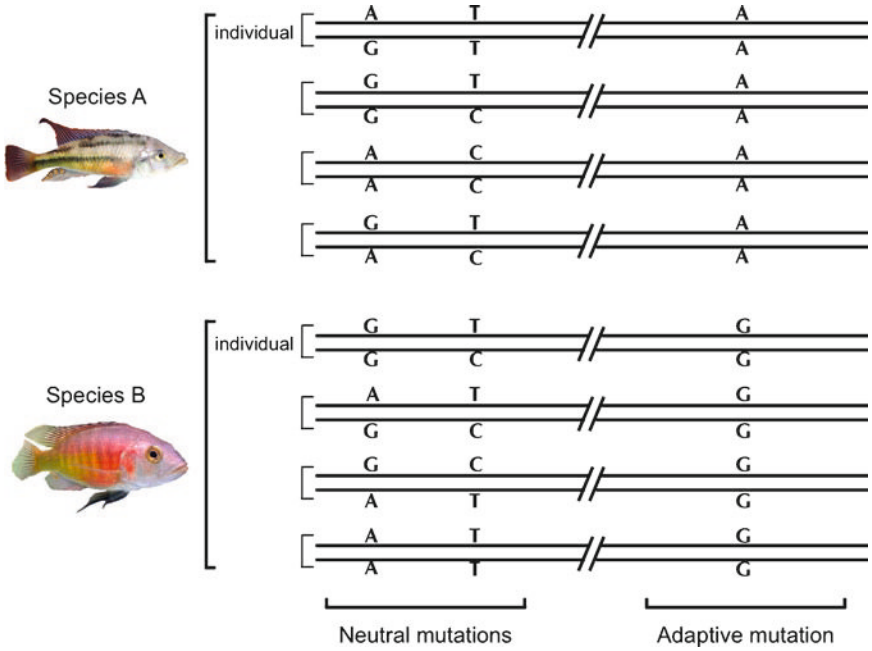

Fig. 15.2 Genetic background in Lake Victoria cichlids. Let us suppose that genetic variation is present in a common ancestral population between the two species. If the speciation occurred recently enough, a neutral portion of the ancestral polymorphism is still expected to be shared between them with different frequencies. In contrast, if an allelic nucleotide at a nucleotide site is adaptive in one species and the other allelic nucleotide is adaptive in the other species, these changes are expected to spread quickly and be fixed in each species by the aid of natural selection

care much more than males because only the female protects her eggs. This strong asymmetrical investment is conductive to sexual selection on male breeding characteristics and leads to sexual dimorphism[4] (Seehausen et al. 1999). Thus, like many polygynous mouth-breeding species, males exhibit conspicuous and colorful body color to attract females, whereas females' coloration is cryptic (Seehausen 2000). Male breeding coloration is one of the most amazingly diverse phenotypic traits among African cichlids. In the absence of postzygotic isolation, sexual selection on male breeding coloration by female preference has been thought to be a driving force of species diversity in cichlids. Evidence for this hypothesis is the presence of correlation between species richness and sexual selection, as we can see in African cichlids whose species-rich lineages are polygynous and that have sexually dimorphic conspicuous breeding coloration (Seehausen 2000).

[4]Sexual dimorphism is the systematic difference in form between individuals of different sex in the same species.

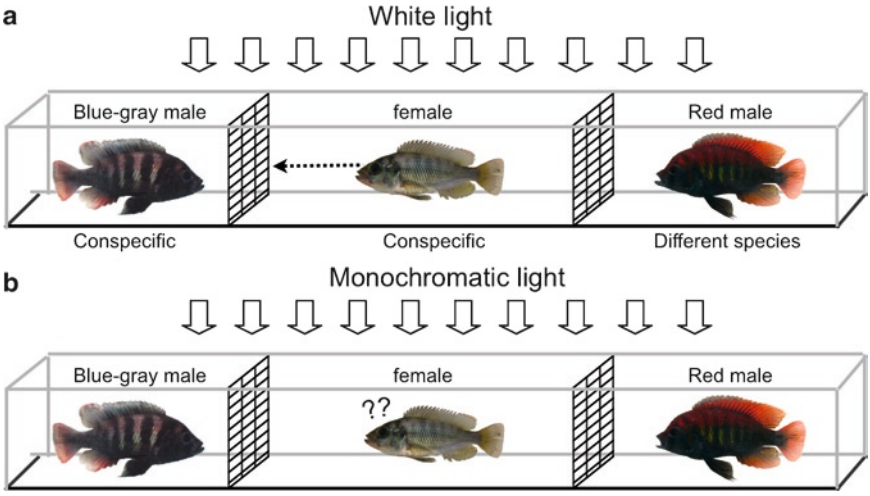

Fig. 15.3 Mate choice experiment. (**a**) Under white light, females chose conspecific males. (**b**) Under monochromatic light, where color vision is disabled, females did not show preference for the conspecific male. Adapted from Seehausen et al. (1998)

How do females choose males? Male breeding color display is important in female mate choice of Lake Victoria cichlids (Seehausen et al. 1997; Seehausen and van Alphen 1998; Maan et al. 2004). In laboratory experiments, females chose conspecific males under a white light condition where their color vision was available, but females could not choose them under the monochromatic light where their color vision was not workable (Seehausen and van Alphen 1998) (Fig. 15.3). In other laboratory experiments, females preferred mating with the more conspicuously colored males (Maan et al. 2004). Hence, female mating preference is affected by color vision (Seehausen and van Alphen 1998; Maan et al. 2004).

To determine what color females prefer, Maan et al. (2006) conducted a series of laboratory experiments. They used a closely related species pair of red and blue-gray colorations and measured the sensitivity to different light color by behavioral light detection thresholds. Red species had lower detection thresholds for red light (high sensitivity to red light), and blue-gray species had had lower detection thresholds for blue light (Maan et al. 2006). These results indicate that females' preferred breeding color is that to which their sensory system is most sensitive. This sensitivity to light is determined by opsin, a light-absorbing protein (see below; also see Chap. 16).

15.3 Opsins in African Cichlid Fish

As we described in Sect. 15.1, the neutral genetic diversity among Lake Victoria species is very low. In contrast, there is marked diversity in visual sensitivity among these species. The cone cells (see Chap. 16) of cichlids are composed of two cell types.

The first is cells with short-wavelength-sensitive single cones, and the second is cells with long- and middle-wavelength-sensitive double cones. The maximum absorption spectra (λmax) for double cone cells with long- and middle-wavelength sensitivity varied from 565 to 594 nm and from 522 to 538 nm, respectively (Van der Meer and Bowmaker 1995; Smit and Anker 1997). At the time of those studies, the genetic differences responsible for those differences in λmax had not been solved; later, analysis of opsin genes resolved this question.

Visual pigments in the photoreceptor cells of the retina consist of a light-absorbing component, the chromophore, and a protein moiety, the opsin (Shichida 1999; Yokoyama 2000). The light sensitivity of a visual pigment is determined by the chromophore [11-*cis*-retinal (vitamin A_1 aldehyde) or 11-*cis*-3,4-dehydroretinal (vitamin A_2 aldehyde)] and by its interaction with amino acid residues, forming the retinal binding pocket of the opsin in which the chromophore lies (Yokoyama 2000). The difference between 11-*cis*-retinal and 11-*cis*-3,4-dehydroretinal is an additional carbon–carbon bond in the latter that shifts the λmax to a longer wavelength (Harosi 1994) (see Chap. 16 for opsins and chromophores).

Eight opsins have been found in African cichlids (Fig. 15.4). Seven are cone opsins: ultraviolet (UV)-sensitive SWS1 (λmax 368 nm) (Carleton et al. 2000); SWS2B (423 nm) (Carleton and Kocher 2001; Parry et al. 2005); SWS2A (452–455 nm) (Carleton and Kocher 2001; Parry et al. 2005); RH2B (484 nm) (Parry et al. 2005); RH2Aβ (519 nm) (Parry et al. 2005); RH2Aα (528 nm) (Parry et al. 2005); *LWS* [(Carleton and Kocher 2001), (A1 pigments: 544–559 nm) (Terai et al. 2006;

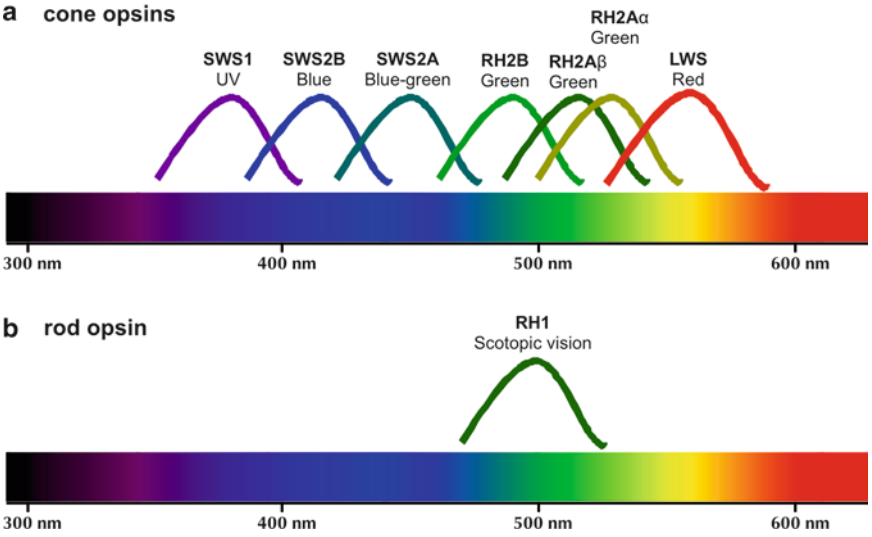

Fig. 15.4 Cichlid cone opsins (**a**) and rod opsin (**b**). Each *line* indicates the absorption spectra of each opsin pigment. The names of opsin genes (*bold*) are indicated above the peaks of the spectra, with their conventional naming by *color*. Note that the color names do not necessarily mean the color of the corresponding wavelengths of light

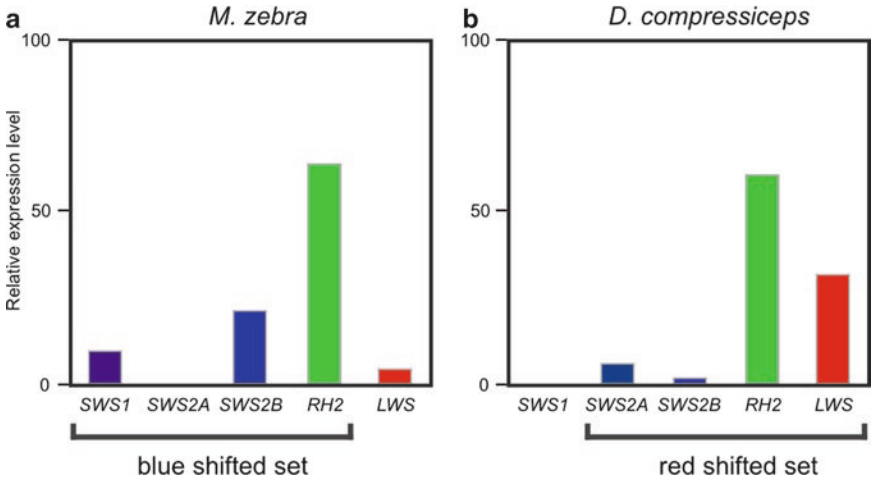

Fig. 15.5 Relative expression level of opsin genes. (**a**) *M. zebra* expresses a blue-shifted opsin gene set. (**b**) *D. compressiceps* expresses a red-shifted set. Adapted from Carleton and Kocher (2001)

Seehausen et al. 2008), (A2 pigments: 604–611 nm) (Terai et al. 2006)]. The other is a rod opsin, *RH1* (484–505 nm) (Sugawara et al. 2005).

African cichlids primarily express three or four of the seven cone opsins, and the expressed opsin sets differ among species (Carleton and Kocher 2001) and among developmental stages (Carleton et al. 2008). For example, in Lake Malawi, *Metriaclima zebra* expresses *SWS1*, *SWS2B*, and *RH2A*, a blue-shifted set (Fig. 15.5a), whereas *Dimidiochromis compressiceps* expresses *SWS2A*, *RH2A*, and *LWS*, a red-shifted set (Fig. 15.5b) (Carleton and Kocher 2001). The riverine cichlid *Oreochromis niloticus* changes a set of opsin genes expressed from the larval stage through the juvenile stage and to the adult stage. In contrast, some Lake Malawi species show only slow changes of the expression set, and other species show no change from the larval stage to the adult stage (Carleton et al. 2008).

Opsin genes have been highly diversified during adaptive radiation of cichlids. As described above, the nucleotide difference in mtDNA among Lake Victoria species is tenfold lower than that among Lake Malawi species, reflecting that the cichlid radiation in Lake Victoria is much younger than that in Lake Malawi (Joyce et al. 2005). In contrast, the difference in *LWS* gene between Lake Victoria species is five times higher than that between Lake Malawi species (Terai et al. 2002a). This opposite pattern of *LWS* gene to mtDNA suggests that the large divergence of the *LWS* gene among species in Lake Victoria was driven by natural selection (Figs. 15.1 and 15.6). Similarly, the *RH1* opsin gene has been diversified over the neutral divergence in Lakes Tanganyika and Malawi cichlids, and several convergent replacements of amino acids are observed between the species in the two lakes (Sugawara et al. 2002, 2005).

Site / Allele	1 3 2	1 5 7	1 8 4	2 4 3 9	3 3 3	3 9 1	4 1 0	4 6 5	5 0 3	5 2 3	5 2 9	5 3 5	5 7 2	6 4 5	6 4 7	6 6 4	6 7 6	6 7 9	6 8 8	8 1 4	8 2 3	8 2 4	8 4 4	8 9 1	9 4 9
01	C	G	G	C	A	T	C	T	C	G	G	G	A	C	A	C	A	T	G	G	T	G	A	C	A
02	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
03	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04	-	-	-	-	-	-	-	-	G	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-
05	-	-	-	-	-	-	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-
06	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	A	-	A	T	G	-	-
07	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	A	-	A	T	-	-	-
08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	A	T	-	-	-
09	-	-	-	-	-	-	-	-	-	-	-	-	-	G	T	-	-	-	A	-	A	T	-	-	-
10	-	-	-	-	-	-	G	-	-	-	-	-	-	G	T	-	-	-	A	A	A	T	-	-	-
11	T	-	A	T	G	-	G	-	G	-	T	T	T	-	-	A	-	A	-	-	-	-	-	T	G
12	T	-	A	T	G	-	G	-	G	-	T	T	T	-	-	A	T	A	-	-	-	-	-	T	G
13	T	-	A	T	G	-	G	-	G	-	T	T	T	-	-	A	-	A	T	-	-	-	-	T	G
14	T	T	A	T	G	G	G	-	G	-	T	T	-	-	-	A	T	A	T	-	-	-	-	T	G

Fig. 15.6 Nucleotide variation of *LWS* alleles among Lake Victoria cichlids. The numbers indicate nucleotide positions in the *LWS* gene (*top*). The numbers *01–14* are the ID numbers of allele types. A *dash* indicates identity with the top sequence (allele 01). Nonsynonymous substitution sites in which amino acid replacement changes or might change the absorption spectra of opsin pigments are highlighted in *black*; and the remaining nonsynonymous sites are highlighted in *gray*. Adapted from Terai et al. (2002a)

Natural selection on opsin genes is supported by the following evidence. Spady et al. (2005) analyzed cone opsin genes from three Great Lakes and compared amino acid altering ("nonsynonymous") and nonaltering ("synonymous") nucleotide substitution rates. This study found statistical evidence for positive natural selection in all opsin genes except *SWS1* and *SWS2B*. These studies showed that natural selection pressures acted on opsin genes during the long-term radiation of cichlids.

15.4 Speciation by Sensory Drive

In the sensory drive hypothesis, the mating signals (signals for communication between males and females) are designed to attract mates. Signals that are more easily detected are likely to be preferred (Endler 1992). Consistent with this hypothesis, females often prefer conspicuous males. The sensory drive hypothesis for speciation predicts two processes. First, the sensory systems adapt to particular environments (Boughman 2002). This adaptation is predicted to occur in sympatry, parapatry, and allopatry (Boughman 2002; Kawata et al. 2007). Second, the mating signals diverge to match with adapted sensory systems. The mating signals are evolutionarily modified through sexual selection to be most easily sensed by the sensory system, which leads to differentiation of the mating signal and eventually to reproductive isolation between populations (Boughman 2002). The conditions of

the environmental heterogeneity that enable this process have been well studied (Kawata et al. 2007). Recently, two studies of Lake Victoria cichlids demonstrated a case of sensory drive speciation by clarifying these two processes (Terai et al. 2006; Seehausen et al. 2008).

In Lake Victoria, water is a dense medium that generates a highly heterogeneous light environment to which cichlid species may need to adapt their visual systems (Van der Meer and Bowmaker 1995; Seehausen et al. 1997, 2008). Terai et al. (2006) examined four species in a rocky habitat with a cline of different water clarity (35–258 cm) (Fig. 15.7a) that generates a heterogeneous light environment. Four species occupy different water depths (Seehausen et al. 1998) (Fig. 15.7b): *Neochromis rufocaudalis* and *Pundamilia pundamilia* are in shallow water, *Mbipia mbipi* in deeper water, and *Neochromis greenwoodi* (including its offshore incipient species *Neochromis omnicaeruleus*) in even slightly deeper water.

In *N. greenwoodi* populations, two main allele groups, H and L (see Fig. 15.8, below), of the *LWS* gene were found. An allele group was defined as a group of alleles sharing two amino acid replacements at positions 177 and 275 (nucleotide sites 529 and 823–824) (Figs. 15.7c and 15.8). Within an allele group, there are other synonymous and nonsynonymous nucleotide variations. The H and L allele groups were almost fixed in populations that lived in high- and low-transparency environments, respectively (Fig. 15.7c). The authors then analyzed population differentiation using upstream and downstream sequences of the *LWS* gene from the populations with high and low transparencies. They used a measure of population differentiation (Fst) that evaluates the level of genetic differentiation based on allele frequency data. High and low Fst values indicate high and low population differentiation, respectively. The analysis of Fst clearly showed that the two populations diverged in the *LWS* gene region ($Fst > 0.8$) but not in upstream and downstream regions ($Fst < 0.2$) (Fig. 15.9). Populations in high- and low-transparency water are strongly differentiated from one another only in the *LWS* gene region, suggesting two populations diverged through natural selection on their *LWS* gene allele groups, H and L, respectively (Fig. 15.9).

Then, what is the functional difference between the H and L allele groups on which the natural selection acted? By in vitro reconstitution of the H and L allelic LWS photopigments using A1 and A2 retinal chromophores, Terai et al. (2006) showed that the λmax of the L allele group was 7 nm longer than that of H allele group when the A2 retinal was used as a chromophore (Fig. 15.7d). Highly transparent water transmits broad spectra, whereas turbid water selectively scatters and absorbs light of short wavelengths, leading to a shift in spectral composition toward longer wavelengths (Seehausen et al. 1997). Therefore, fixation of the red-shifted L allele group of *LWS* in red-shifted turbid waters is likely an adaptation to an environment in which longer wavelengths are dominated.

The breeding colorations also diverged into blue-black and yellow-red morphs in the populations of high and low transparencies, respectively (Fig. 15.7c). However, the yellow-red breeding coloration was not completely fixed in low-transparency populations. In the relatively turbid waters of Lake Victoria, yellow and red light travel farther than blue light; therefore, the main light component in

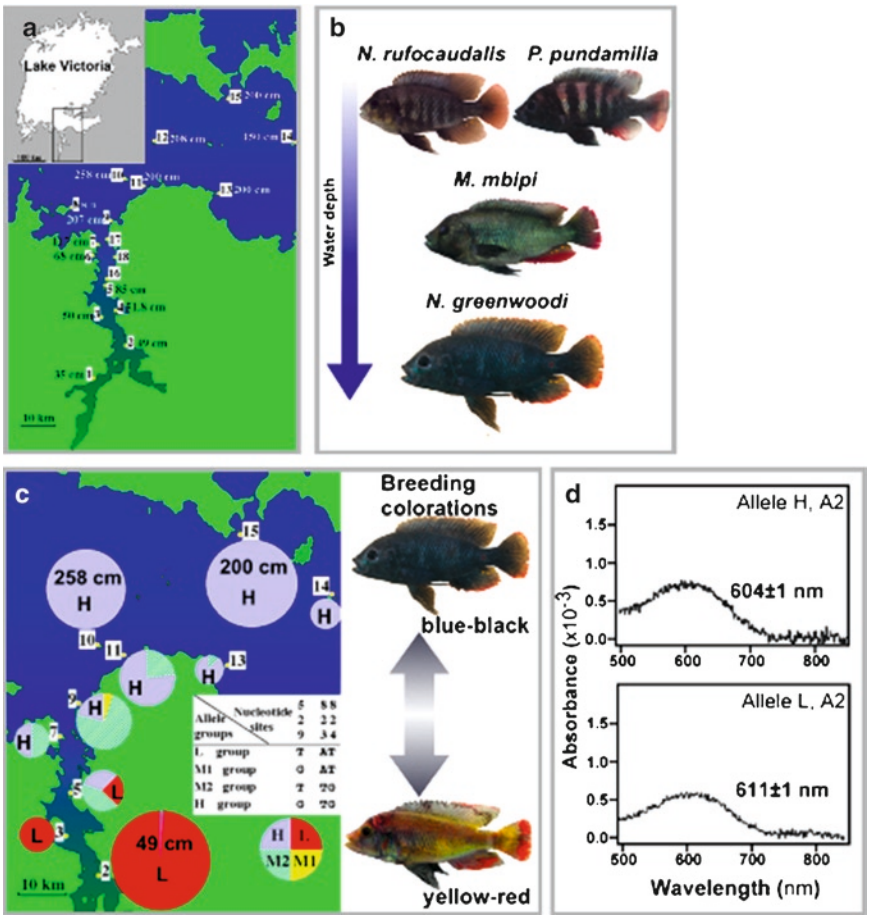

Fig. 15.7 Geographic maps, inhabiting cichlid species, their *LWS* allele frequencies, and their breeding coloration. (**a**) The study area is southern Lake Victoria. *Arabic numerals* indicate stations at which cichlids were collected. The water transparency (centimeters) at each station is shown in *parentheses*. (**b**) Distribution of the studied species by water depth. (**c**) *LWS* allele group frequencies in the populations of *N. greenwoodi*. The size of the pie chart reflects the relative number of haplotypes sequenced among study stations. The allelic nucleotides at three important nucleotide sites are shown for the major allele groups. The breeding colorations of *N. greenwoodi* (blue-black and yellow-red morphs) are shown to the *right side*. The yellow-red morph was not fixed in the population with low transparency. (**d**) Absorption spectra of the LWS pigments evaluated by the dark–light difference spectra. The LWS pigments were reconstituted from the H allele with A2 retinal (*upper panel*) and L allele with A2 retinal (*lower panel*). The λmax values are indicated with their standard errors. Adapted from Terai et al. (2006)

relatively deep water becomes yellow and red (Maan et al. 2006). In the yellow and red light environments, blue color cannot reflect light because of no blue light in the light component, whereas yellow and red colors are bright because these colors can reflect yellow and red light. Hence, yellow and red colors may be perceived as

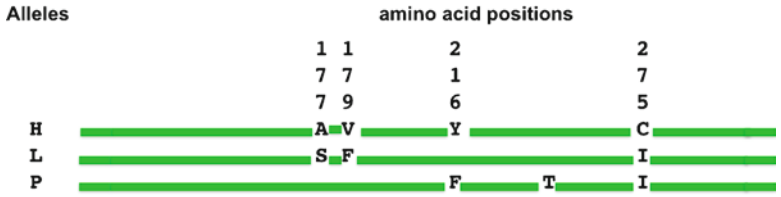

Fig. 15.8 *LWS* alleles with amino acid repl acements

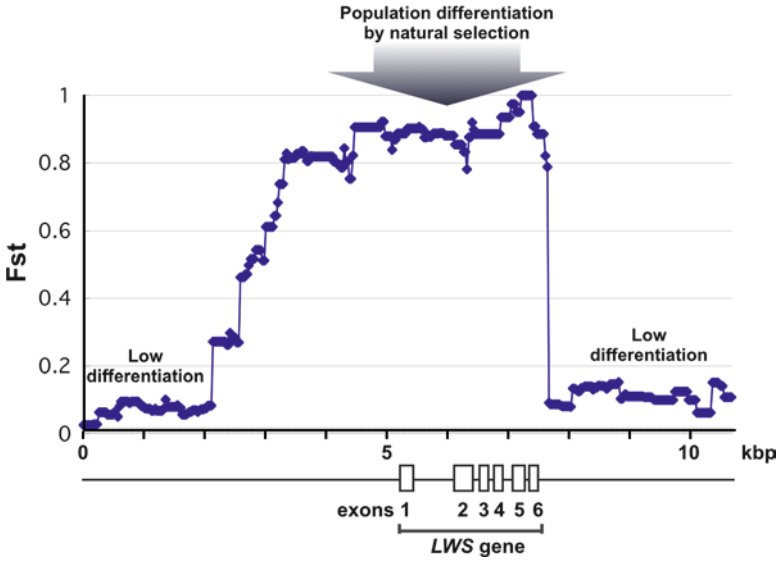

Fig. 15.9 Detection of natural selection by sliding-window analysis of Fst between populations with high and low transparency. The exons of the *LWS* gene are indicated by *rectangles*. The low (<0.2) Fst values for upstream and downstream of the *LWS* gene (0–2 and 8.0–10.5 kbp, respectively) indicate low differentiation between populations. In contrast, high (>0.8) Fst values for the *LWS* gene indicate much higher population differentiation than those for the supposedly neutral regions and thus are likely caused by natural selection. The operation of the natural selection was supported further by several other tests (Terai et al. 2006). Adapted from Terai et al. (2006)

brighter than blue colors in relatively deeper water. This effect is stronger when water becomes more turbid (Seehausen et al. 1997). A similar pattern of divergences of *LWS* alleles and breeding colorations into high and low-transparency populations were also observed in *M. mbipi*. The transparency of shallow water is less heterogeneous. Consistently, such divergence was not observed in two shallow-water species, *N. rufocaudalis* and *P. pundamilia*. These findings indicate that light environment, shaped by water depth and turbidity, affects divergences of *LWS* alleles and breeding colorations between populations. Hence, the adaptive divergence of LWS and the divergence of male breeding coloration in *N. greenwoodi* were consistent with the model of sensory drive.

Seehausen et al. (2008) analyzed species in two closely related rocky habitats whose water clarity differed. Two species occupy different water depths: *P. pundamilia* are in shallow water and *Pundamilia nyererei* in deeper water (Fig. 15.10a). Specimens were collected from five localities with different transparencies; locality 2 was 58 cm, 4 was 50 cm, 5 was 96 cm, 6 was 78 cm, and 10 was 225 cm (locality numbers are described in Fig. 15.7a). In water with middle to high transparency from the surface (78–225 cm), the major light component gradually shifts with water depth from blue on the surface to green, yellow, orange, and red in deeper water (Fig. 15.10a). Two *LWS* allele groups (P and H) (Fig. 15.8) were found from these two species, and P and H allele groups were almost fixed in *P. pundamilia* and *P. nyererei*, respectively (Fig. 15.10a). The authors then analyzed population differentiation using upstream and downstream sequences of the *LWS* gene from *P. pundamilia* and *P. nyererei* populations (locality 5 in Fig. 15.7a). The analysis of Fst showed greater divergence in the *LWS* gene and in 2 kilobases (kb) of the upstream sequence (Fst > 0.8) than in the downstream sequences (Fst < 0.15). The differentiation in the *LWS* gene region but not in the downstream region suggests that two populations diverged through natural selection on their *LWS* gene allele groups, H and P, respectively.

The absorption spectra of P and H LWS pigments were measured, showing that the λmax of the P pigment is shorter than that of the H pigment (Fig. 15.10a). Therefore, the fixation and divergence of P and H alleles is likely an adaptation to

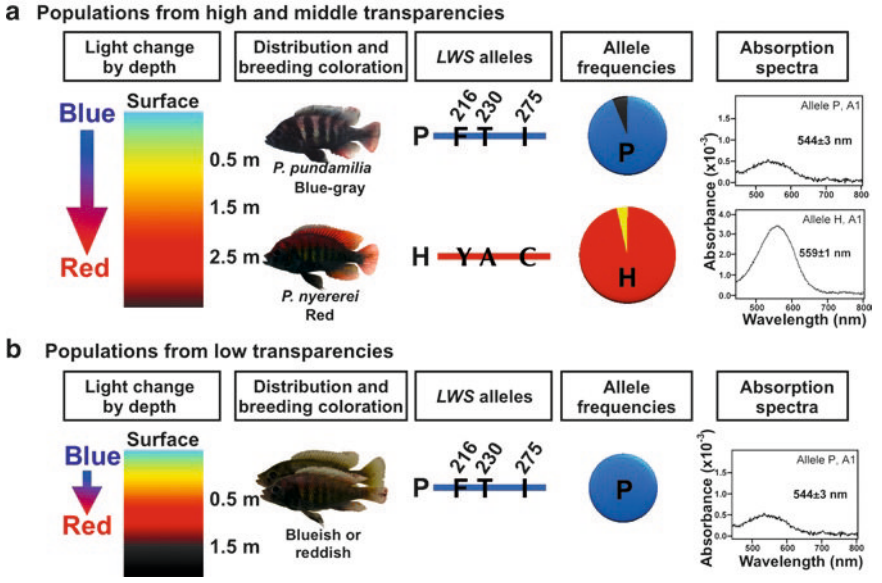

Fig. 15.10 Divergences of color vision and breeding coloration between *P. pundamilia* and *P. nyererei*. The major light component by water depth, *LWS* allele frequencies, and absorption spectra of the allelic opsin pigments are summarized for populations with (**a**) high and middle transparency and (**b**) low transparency. Adapted from Seehausen et al. (2008)

blue and red light environments, respectively. The breeding colorations diverged such that the breeding colors were most visible to their visual system: blue-gray in *P. pundamilia* and red in *P. nyererei*. However, the major light components change more steeply in water of low transparency from the surface (50–53 cm), as a function of depth, than in higher-transparency water (Fig. 15.10b). In the low-transparency water, the P allele of *LWS* was almost fixed, although it is difficult to distinguish species, as they appear mixed with bluish, intermediate, and reddish phenotypes (Fig. 15.10b). Thus, species did not diverge in the low-transparency environments.

In general, many animals use mating signals, such as songs, pheromones, and color. When a species is distributed along a particular environmental gradient (1) the sensors adapt to different environments in the gradient by natural selection (Fig. 15.11a) and (2) signals diverge to adapt to the sensors (Fig. 15.11b). After the adaptation of sensors and divergence of signals, the recognition of mating signals between two populations becomes weaker. Finally, reproductive isolation occurs when the individuals of one population no longer recognize those of another as potential mates (Fig. 15.11c). As described above, this speciation mechanism was hypothesized as speciation by sensory drive (Endler 1992; Boughman 2002). Speciation by sensory drive has recently been formulated theoretically as one of the plausible mechanisms of speciation in the condition that the environmental gradient is not too steep (Kawata et al. 2007). The divergence of sensory systems and colorations of cichlids in a highly heterogeneous light environment is well consistent with this hypothesis.

The speciation by sensory drive has also been suggested for threespine sticklebacks *Gasterosteus* spp. Males from the limnetic population are red and those from the benthic population are black (Boughman 2001). Females prefer males with red breeding coloration in the environment where red is conspicuous (Boughman 2001).

Although Terai et al. (2006) and Seehausen et al. (2008) showed that interspecies divergence of the visual sensory gene (i.e., *LWS*) was driven by natural selection, it remains speculative whether the species difference in body coloration was indeed

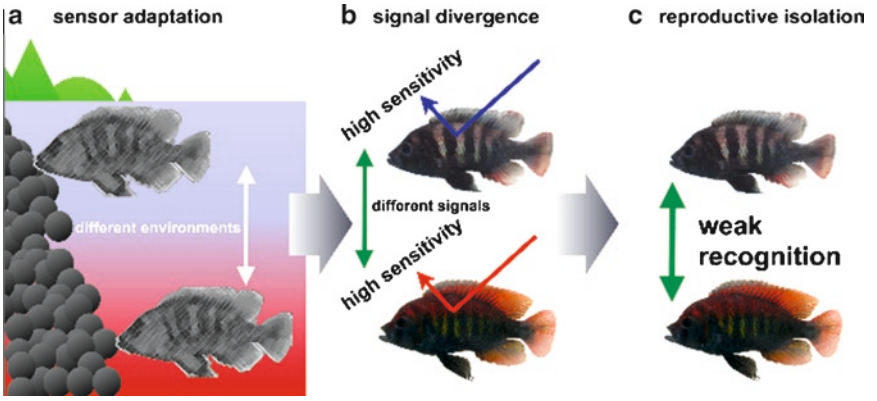

Fig. 15.11 Summary of sensory-driven speciation process. (**a**) Adaptive divergence of sensors to the environment. (**b**) Divergence of mating signals to be more easily detected by the sensor. (**c**) Reproductive isolation by weak recognition between populations

driven by some form of selection as well. For this understanding, it is necessary to identify genes responsible for body coloration. Coloration in cichlids, including breeding coloration, is shaped by pigment cells such as melanophores, iridophores, and xanthophores (Fujii 2000). Several studies have reported candidate genes responsible for evolution of coloration in cichlids (Terai et al. 2002b, 2003b; Sugie et al. 2004; Salzburger et al. 2007). Further analysis of these genes will be of great importance to evaluate whether selection has operated on diversifying theses gene (see Chap. 14).

15.5 Reproductive Isolation by Other Sensors

In the previous section, we described the first explicit example of speciation by sensory drive. In this case, color vision works as a driving force for differentiating body color and reproductive isolation. However, light may not be the only signal for mating communication in cichlids. Is there any possibility in which other sensors affect reproductive isolation?

In one laboratory experiment, females of *Pseudotropheus emmiltos*, a rocky habitat species in Lake Malawi, were given the choice of a spawning site next to a conspecific male or a male of the closely related sympatric *Pseudotropheus fainzilberi* species. Females exhibited a significant preference for conspecific males when both olfactory and visual cues were available but not when only a visual cue was available (Plenderleith et al. 2005). Another experimental study found that when masking the distinctive male dorsal fin hues (blue or orange) or making the body color cryptic by illuminating it with monochromatic light, these manipulations did not significantly affect the females' mating preference for conspecific males (Blais et al. 2009). In another experiment, Verzijden and ten Cate (2007) showed that mating preference in two closely related species in Lake Victoria was affected by brooding environment, suggesting the effect of learning olfactory cues. In their study, the researchers exchanged the eggs in females' mouths between species, and later scored the mating preference of these offspring. The females of the offspring developed a sexual preference for males of their foster mothers' species. The authors suggested that learning these olfactory cues might help maintain reproductive isolation (Verzijden and ten Cate 2007). These studies suggest that olfactory sensors may affect mate choice and reproductive isolation. The olfactory genes responsible for mating signals and the ligands for those genes are still unknown, but it is hoped that further analysis of olfactory systems in cichlids will reveal the mechanism of reproductive isolation by olfaction.

The species inhabiting Lake Malawi's rocky habitats use sound during courtship. The courtship sounds are emitted during male courtship display. They consist of rapidly repeated pulse units, which differ among species (Amorim et al. 2004). During agonistic displays[5] between males, the males produce long sounds with more pulses

[5] Agonistic display is the combative or territorial behavior of an animal that feels threatened by another animal of the same species.

(Simões et al. 2008). The role of the sounds is still unclear, but the courtship sounds and the sound sensor may be involved in the sound-based reproductive isolation.

Another possible sensor is dim-light (scotopic) vision (see Chap. 16). One opsin, *RH1*, is responsible for the scotopic vision and achromatic image formation (Yokoyama and Yokoyama 1990, 1996). Deep-water cichlid species from Lakes Tanganyika and Malawi have adapted to the narrow-light spectrum (470–490 nm) environment in the deep water. Because the light spectrum is strictly limited in deep water, it was proposed that deep-water species are reproductively isolated only by achromatic images to recognize conspecific males (Genner et al. 2007b).

15.6 Conclusions

In this chapter, we reviewed speciation by sensory drive, in which the adaptation of a sensor for environmental heterogeneity drives the divergence of mating signals, leading to reproductive isolation. Cichlids use color vision and breeding coloration as a sensor and a mating signal, respectively. When a species is distributed along an environmental gradient, color vision adapts to different light environments by natural selection, and the breeding colorations diverge so as to reflect light more sensitive to the color vision through sexual selection. This process leads to poor recognition between different populations and eventually to reproductive isolation. Thus, adaptation of the sensory system triggers speciation by sensory drive. Further studies of visual sensory systems and colorations in other animals as well as other cichlid species will reveal a more general role of mating signal communication in the speciation processes.

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Chapter 16
Evolutionary Diversification of Visual Opsin Genes in Fish and Primates

Shoji Kawamura

16.1 Introduction

Color vision is the ability to perceive different light wavelengths. Better color vision means that more colors can be perceived by certain animals (i.e., that animal would have a larger "color space"). Color vision is involved in a variety of behaviors, such as foraging for food, escaping predators, mating, and navigating their environment. It thus could facilitate evolution and diversification of animal coloration (see Chaps. 14 and 15). The types and nature of color vision is primarily determined by how many spectral types of color sensor cells (cone visual cells in vertebrates) an animal has in its retina and how much overlap there is among the ranges of wavelengths to which cells are sensitive. Humans have three spectral types of cone visual cell in the retina and thus have three-dimensional color space (i.e., trichromatic color vision). The three types of cone cell are most sensitive to wavelengths (λmax) of about 560, 530, and 420 nm and are often called long (L, or red), middle (M, or green), and short (S, or blue) wavelength-sensitive cones, respectively. Because the ranges of wavelengths to which L and M cones are sensitive largely overlap, the actual number of colors humans can perceive in the three-dimensional color space is quite limited.

In contrast, birds typically have four types of cone cell, one type more than humans, making their color space four-dimensional (tetrachromatic). Moreover, unlike humans, the spectral separations between spectrally adjacent cone types are quite even among the four and the spectral overlaps between them are small, with one type sensitive in the ultraviolet (UV) range. This arrangement of the spectral characteristics of cone types enables birds to use nearly fully their four-dimensional color space. Mixtures of light wavelengths invoke varieties of color sensations via the activation of different spectral types of cone cell. Combinations of output magnitudes from four cone types can be much greater than those from three cone

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types. This difference is further enhanced by the small spectral overlap between spectrally adjacent cones in birds and the large overlap between L and M cones in humans. Thus, birds can likely see many more colors than humans.

Television (TV) monitors display all colors visible to humans by combining outputs from red, green, and blue monochromatic light emitters. However, for birds, TV images of natural scenes must appear impoverished as they cannot represent gradients perceivable by one of the birds' four color sensors. It is a pity that humans cannot even imagine how rich the color world must be for birds.

Surprisingly, fish, which are one of the most primitive vertebrates, have an even richer and more complex color vision system than birds. This is especially the case among fish living in shallow waters, such as at the coasts and rivers. These species have rich repertoires of cone opsins (color sensor molecules produced in cone cells). Some fish, in fact, have eight cone opsin genes, usually including a UV-sensitive opsin. Some fish switch the opsins used throughout their development from larvae to adults. Some fish use different opsins in different areas of the retina, possibly enabling them to have different color vision at different visual angles.

Just glancing at these examples provides the realization that the general view of evolution where humans are placed at its apex does not apply to color vision. Aside from the self-centered human worldview, it leads to the realization that better color vision might not necessarily be more advantageous for some animals including ourselves. For example, it might benefit nocturnal, cave-dwelling or deep-sea animals to have fewer cone types as this would enable them to better distinguish between wavelengths under dim lighting (Vorobyev 2004). Even among some diurnal animals, certain colors may be less important than others, and there may be no benefit in being able to perceive those colors. As with any biological trait, color vision at present is a historical product that may have been modified or preserved during evolution as a consequence of natural selection and phylogenetic constraints. As noted above, human color vision is not at all excellent compared to that of birds and fish. To understand why human color vision is in the present form, we need to remember that humans are mammals and primates.

Among placental mammals, only primates have three spectral types of cone. Thus, trichromatic color vision is not a human but a primate feature. Unlike primates and humans, among the vertebrates mammals generally have poorer color vision, typically having only two spectral types of cone cell (dichromatic color vision). As described in the following section, the common ancestor of vertebrates had four types of cone opsin gene and thus is presumed to have tetrachromatic color vision. Mammals have only two of the four types of cone opsin gene and thus are considered to have lost the other two. This occurred possibly during the Mesozoic Era when mammals were mostly nocturnal animals (Ahnelt and Kolb 2000). During the Cenozoic Era after the extinction of the dinosaurs, only the ancestors of primates created an additional spectral type of cone opsin gene from one of the remaining types of cone opsin gene in mammals (Jacobs and Nathans 2009). Why could only primates regain high-dimension color vision? Possible answers include the fact that early primates became arboreal, diurnal, fed on a variety of resources (e.g., fruits, leaves, insects), and were highly social in the

canopy world of broad-leaved trees that developed during early Cenozoic global warming (Dominy et al. 2003b).

Shadows generally yield strong variation in the intensity of illumination. However, a comparison of chromatic signals from different spectral types of cone provides a value that remains constant across different levels of illumination intensity (Foster and Nascimento 1994). Thus, color vision is especially useful for object detection under conditions of patchy and changing illumination or against dappled backgrounds. In addition, color facilitates the identification of an object by its surface reflectance irrespective of spectral distribution of the illuminant, a phenomenon known as "color constancy" (Pokorny et al. 1991). Patchy, spectrally varying illumination is common in shallow water and in forests. Therefore, these are the places where color vision would be strongly selected for and be the most diverse (Vorobyev 2004). This article focuses especially on fish and primates and reviews recent work on the evolution and diversity of vertebrate color vision.

16.2 Vertebrate Visual Opsins

16.2.1 Visual Cells and Visual Pigments

Vertebrate retinas contain two types of visual photoreceptor cell (visual cells): rods and cones. Rods allow dim-light vision, and cones allow daylight and color vision. Photosensitive molecules called visual pigments are located on the outer segments of these cells. The absorption spectrum of a visual pigment is bell-shaped when plotted against wavelengths. The wavelength of maximal absorbance is called "λmax" and is used as a measure to represent the whole absorption spectrum. A visual pigment consists of a protein moiety, opsin, and a chromophore, either 11-*cis*-retinal or 11-*cis*-3,4-dehydroretinal (vitamin A_1 or A_2 aldehyde, respectively) (Nathans 1987). In general, opsins are retinal-mediated light-sensing proteins found in a variety of organisms from bacteria to vertebrates. These proteins have various visual and nonvisual functions and belong to a multigene superfamily called G-protein coupled receptors (GPCRs), which commonly form a characteristic seven-transmembrane structure. In this review, opsins for visual function are referred to as "visual opsins."

Amino acid sequences of the opsins modulate absorption spectra of the chromophore. With an identical opsin, the A2 chromophore absorbs longer wavelengths than the A1 chromophore (Nathans 1987). The difference of absorption spectra between A1 and A2 chromophores is greater when combined with longer-wavelength opsins. In diurnal birds, reptiles, and lungfish, colored oil droplets located in cones act as color filters to further modulate absorption spectra of the cones (Walls 1942; Robinson 1994). These color filters reduce the overlap in sensitivity between spectrally adjacent cones and hence increase the number of distinguishable colors

(Govardovskii 1983). Thus, opsins, chromophores, and oil droplets together define the spectral properties of cones. However, the A2 chromophore is not used in mammals, birds, or most reptiles; and oil droplets are absent in many groups of animals (Lythgoe 1979; Goldsmith 1990). Thus, it can be said that opsins play a universal and pivotal role in the evolution of color vision. In addition, a major advantage to molecular evolution studies of choosing to focus on opsins lies in the feasibility of functional assays of opsins, coupled with site-directed mutagenesis, by transfection of opsin cDNAs to cultured cells, reconstitution of functional photopigments in vitro with a chromophore, and spectral measurement of the purified pigments (Yokoyama 2000b).

16.2.2 Color Vision and Visual Opsins

Color vision is based on the ability to discriminate light by differences in the wavelengths (or hue), and at least two spectral types of cone cell are necessary in the retina to compare signals from these wavelengths. Thus, to achieve color vision, it is essential that animals have a set of spectrally differentiated cone opsins and that different cone opsin genes are expressed in different cone cells. Animals with only one spectral type of cone cannot discriminate among wavelengths and are completely colorblind (however, a low level of color discrimination is possible under a dim-light condition where both rods and cones are workable). Animals with two spectral types of cone (or opsins if expressed in different cones) process color information based on signals from the two types of cell and are dichromats. Likewise, those with three and four spectral types of cones are trichromats and tetrachromats, respectively.

16.2.3 Visual Opsin Repertoires of Vertebrates

The visual opsins in vertebrates are classified into five phylogenetic types: RH1 (rod opsin or rhodopsin) and four types of cone opsin – RH2 (RH1-like, or green), SWS1 (short-wavelength-sensitive type 1, or ultraviolet-blue), SWS2 (short-wavelength-sensitive type 2, or blue), and M/LWS (middle- to long-wavelength-sensitive, or red-green) (Yokoyama 2000a). It is well established that these five types were present in the common ancestor of all vertebrates including jawless fish (Yokoyama 2000a; Collin et al. 2003; Davies et al. 2009). Thus, early vertebrates could already have had tetrachromatic color vision in their shallow aquatic habitat during the early Cambrian, approximately 540 million years ago (Maximov 2000). The current repertoires of visual opsins of vertebrates are based on these five types: Some lost part of the five and some increased their repertoire by gene duplications or allelic differentiation of these five types (Fig. 16.1). In the latter case, the sister opsins that emerge by gene duplication or allelic differentiation are called subtypes.

	Red-Green type (M/LWS)	Green type (RH2)	Blue type (SWS2)	Blue-UV type (SWS1)	Rod type (RH1)
Fish	◎	◎	◎	◎	◎
Amphibians	○	?	○	○	○
Reptiles	○	○	○	○	○
Birds	○	○	○	○	○
Monotremes	○	×	○	×	○
Marsupials	○	×	×	○	○
Placental mammals	○	×	×	○	○
Primates	◎ (L-M)	×	×	○ (S)	○

Fig. 16.1 Distribution of the five visual opsin types among vertebrates. *Circles*, *double circles*, and *crosses* indicate the presence of only one gene, presence of two or more genes (subtypes), and absence of the gene in the genome, respectively, for the five types of opsin. Subtypes by gene duplications have been reported for all five opsin types in fish. It has not been clear if amphibians have the RH2 opsin gene. Among mammals, only primates have subtypes in the M/LWS type by gene duplication or allelic differentiation

Many species of birds and reptiles retain one of the four cone opsin types (and a rod opsin) and are tetrachromats (Ebrey and Koutalos 2001) (Fig. 16.1). Mammals are believed to have lost RH2 and either the SWS2 (placental mammals and marsupials) or SWS1 (monotremes) opsin genes in a nocturnal ancestor that lived during the Mesozoic period (Ahnelt and Kolb 2000; Davies et al. 2007). As a result, extant placental mammals are basically dichromatic with only the rod opsin and the SWS1 and M/LWS cone opsins (Jacobs 1993). Primates are the sole exception among placental mammals in that they regained trichromatic vision by diversifying the M/LWS opsin gene through either gene duplication or allelic diversification (Jacobs 1999).

16.3 Wide Variety of Visual Opsins in Fish

16.3.1 Gene Duplications and Spectral Differentiation

In contrast to other vertebrates, many fish have a rich and varied repertoire of visual opsins, including two or more opsin subtypes within the five types (Fig. 16.1), presumably reflecting their evolutionary adaptation to diverse aquatic light environments

(Levine and MacNichol 1982). All visual opsin genes have been isolated and characterized for a variety of fish species, including zebrafish (*Danio rerio*) (Chinen et al. 2003), medaka (*Oryzias latipes*) (Matsumoto et al. 2006), and cichlids (Family Cichlidae) (Terai et al. 2002, 2006; Parry et al. 2005; Seehausen et al. 2008). Zebrafish and medaka are small surface-swimming freshwater species and have been studied as model animals for developmental genetics (Westerfield 1995; Wittbrodt et al. 2002). Both of these species are also now the focus of separate genome projects (Kasahara et al. 2007). Cichlids are well known animal models for the study of speciation as they achieved rapid speciation in lakes and have attracted evolutionary biologists (see Chap. 15). For these three species, photopigments have been reconstructed for all of the visual opsin genes and measured for their absorption spectra.

Zebrafish have nine visual opsin genes consisting of two spectrally distinct M/LWS and four spectrally distinct RH2 opsin subtypes and single-copy SWS1, SWS2, and rod (RH1) opsin genes (Chinen et al. 2003). Medaka also have nine visual opsin genes with three spectrally distinct RH2 subtypes and two spectrally distinct SWS2 subtypes in addition to two spectrally undifferentiated M/LWS subtypes and single-copy SWS1 and RH1 opsin genes (Matsumoto et al. 2006). Cichlids have eight visual opsin genes with three spectrally distinct RH2 subtypes and two spectrally distinct SWS2 subtypes in addition to single-copy M/LWS, SWS1, and RH1 opsin genes (Carleton and Kocher 2001; Parry et al. 2005). Molecular phylogenetic studies using the nucleotide or amino acid sequences have revealed that the creation of subtype opsins via gene duplications has occurred many times in various phylogenetic groups during fish evolution (Matsumoto et al. 2006).

Molecular phylogenetic study can also reveal which amino acid replacements occurred where in the reconstructed phylogenetic trees. By in vitro reconstitution of the inferred ancestral opsins from the phylogenetic analysis and by introducing mutations to the opsin cDNAs, one can identify amino acid replacements by which spectral differentiation of the opsins occurred (Yokoyama 1997, 2000b; Yokoyama et al. 2000, 2008; Shi and Yokoyama 2003). By this approach, amino acid replacements have been identified that differentiated absorption spectra of fish visual opsins between subtypes and between species (Chinen et al. 2005a, b).

16.3.2 Differential Expression of Visual Opsin Subtypes

Having eight and seven cone opsin genes, respectively, are zebrafish and medaka octachromatic and cichlids heptachromatic? In some species of cichlids, only three cone opsin genes are primarily expressed in the retina at a given developmental stage, and the expressed repertoires differ among species in different habitats (Parry

et al. 2005). In addition, some species also switch primary opsin genes expressed in the retina during development (Spady et al. 2006; Carleton et al. 2008). However, opsins with low gene expression could play an important role in vision. For example, in human retina, S cone cells comprise a minority among all the cone cells, but trichromatic color vision is not realized without this minor S cone population. Little is known about the contribution of opsins with low amounts of gene expression in the cichlid retina, and further studies are awaited to elucidate their biological significance.

There are many other examples of fish that change the expression pattern of opsin genes throughout their developmental stages. In particular, such changes are found in fish that migrate between rivers and the ocean. The ocean looks blue because the short-wave light is better transmitted by ocean water whereas other visible light is scattered or absorbed; in contrast, rivers look greenish because longer-wave light is better transmitted there. In general, marine fish use only A1 retinal as the visual pigment chromophore, whereas freshwater fish use both A1 and A2 chromophores. This is understood as an evolutionary adaptation of fish to their light environments because A1 chromophore absorbs shorter wavelengths than A2 chromophore when combined with the same opsin. Many migratory fish are reported to use the A1 chromophore in the sea and switch to the A2 chromophore in the river. In addition to the change of chromophore, eels change their opsin subtypes of rod visual pigments: they use a shorter-wave subtype in the sea and longer-wave subtype in rivers (Archer et al. 1995; Zhang et al. 2000). Salmon undergo a similar chromophore exchange, but they are also reported to switch gene expression from UV-sensitive SWS1 opsin to blue-sensitive SWS2 opsin in the same cells when adult fish migrate from the sea into a river for spawning (Cheng and Novales Flamarique 2004).

In zebrafish, in situ hybridization studies have revealed that the four RH2 and the two M/LWS opsin genes are expressed in different areas in the retina (Takechi and Kawamura 2005) (Fig. 16.2). For RH2 and M/LWS, shorter-wave subtypes are expressed in the central to dorsal retina, and longer-wave subtypes are expressed in the ventral and peripheral retina, circumscribing the shorter-wave area. This suggests that spectral sensitivity and possibly color vision differ as a function of visual angles. In water, spectral composition can vary greatly depending on the directions from which light comes. In shallow water, the spectral composition of the light coming directly through the surface is similar to that of sunlight in the air, whereas the spectral composition of light from deeper water or that coming horizontally is distorted by scatter and absorption by water. The different coloration of body areas in fish could also be an adaptation to being viewed by fish in the light gradient in mating or alarm. Future studies will undoubtedly focus on whether the ringed expression pattern of opsin subtypes in the zebrafish retinas is found in other species and how this pattern is related to the behavior and ecology of species. The zebrafish study is also a good example of how an opsin gene that is not highly expressed and is expressed in only a limited area of the retina could have a specific visual function.

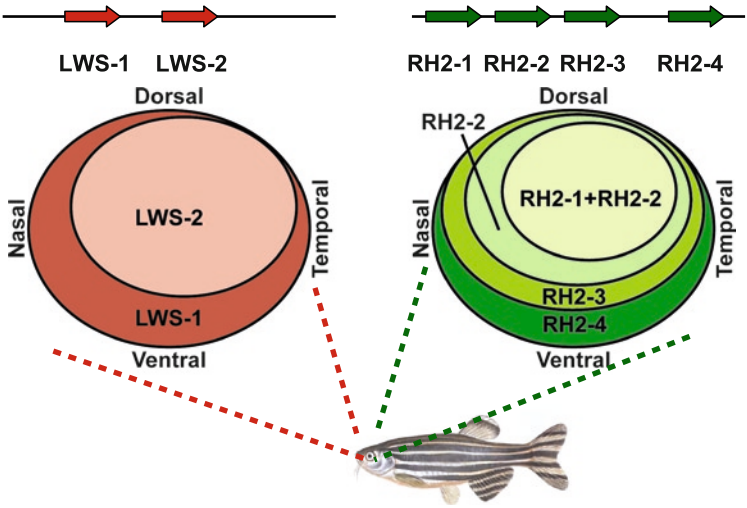

Fig. 16.2 Concentric expression pattern of opsin subtypes in zebrafish retina. Zebrafish have two M/LWS genes [*LWS-1* (λmax 558 nm), *LWS-2* (λmax 548 nm)] and four RH2 genes [*RH2-1* (λmax 467 nm), *RH2-2* (λmax 476 nm), *RH2-3* (λmax 488 nm), *RH2-4* (λmax 505 nm)] in the genome (Chinen et al. 2003). In both M/LWS and RH2 types, short-wave-sensitive subtypes are expressed in the central to dorsal area in the retina, whereas long-wave subtypes are expressed surrounding it, especially in the ventral area (Takechi and Kawamura 2005)

16.3.3 Regulatory Mechanism of Differential Expression of Subtype Opsin Genes

All subtype opsin genes thus far known for fish are the products of local gene duplication but not of genome duplications that are known to have occurred during fish evolution. It appears that subtype opsin genes created by genome duplications have been lost from the genome. This could be because subtype genes created by local gene duplications might be more controllable for differential and coordinated expression among subtypes than those arisen by genome duplications. Genes that have arisen by local duplications may have more chance to access and share *cis* regulatory elements[1] between duplicates. The regulatory region of the opsin genes has been intensively studied on zebrafish with its feasibility to employ transgenic technology using a living color reporter such as the green fluorescent protein (GFP) (Kennedy et al. 2001; Asaoka et al. 2002; Hamaoka et al. 2002; Takechi et al. 2003, 2008; Luo et al. 2004; Kawamura et al. 2005).

An example of the regulatory region has been found for zebrafish RH2 opsin genes that is relevant for differential expression of subtype opsin genes that arose

[1] *Cis* regulatory element (or region): A region of DNA that regulates the transcription of genes which are physically linked to the region. It is often a binding site of transcription factors and is often located in the upstream region to the gene it controls.

by local gene duplications (Tsujimura et al. 2007). The four RH2 opsin genes of zebrafish are arrayed in tandem and are controlled by a 0.5-kb regulatory region called RH2-LCR (locus control region), which is located approximately 15-kb upstream of the gene array (Tsujimura et al. 2007). The RH2-LCR is necessary and sufficient to drive gene expression in a specific type of cone cell (short member of double cones) in the zebrafish retina. When the position of RH2-LCR is changed in the RH2 gene array, the expression area of one RH2 gene in the retina is affected by its relative distance from RH2-LCR.

Interestingly, expression of the primate M/LWS opsin gene array, consisting of L and M opsin subtype genes, is also controlled by a single regulatory region, called LCR, located upstream of the gene array. It should be noted that the zebrafish RH2-LCR serves for the concentric expression pattern in the retina among the subtypes and enables differential color vision among visual angles. On the other hand, the primate LCR of the M/LWS gene array leads to nearly random distribution of L and M cone cells in the retina, enabling trichromatic color vision. It is impressive that a similar mechanism yielded different visual systems between fish and primates that are adaptive in the aquatic environments of fish and in the arboreal environments of primates.

It is of great importance for future studies to describe the spatiotemporal expression pattern of subtype opsin genes in the retinas of fish species belonging to different phylogenies or living in different ecologies. It is also important to evaluate the regulatory mechanisms of the concentric expression pattern or other patterns found and how these expression patterns are related to ecological demands for the fish.

16.4 Trichromatic Color Vision in Primates

16.4.1 Prerequisites for Trichromatic Color Vision

Among dichromatic mammalian ancestors, why could only primates develop trichromatic vision? We could include key factors such as a diurnal and arboreal lifestyle in forests of the patchy and changing illumination. However, these are clearly not the only factors: Squirrels, for example, although diurnal and arboreal forest dwellers, are dichromats. Addressing this question requires two answers: one regarding the mechanics of color vision and the other regarding the adaptive significance of color vision.

From the viewpoint of mechanics, duplication of the M/LWS opsin gene and spectral differentiation between duplicates, resulting in the L and M opsins, are not sufficient to realize primate trichromacy. A mechanism is required to express the L and M opsin genes in a mutually exclusive manner to generate distinct L and M cone cells. In addition, a mechanism that enables comparison of outputs from the L and M cones with high spatial resolution is needed. In primates, these mechanisms were made possible by three entities that were not particularly developed for color vision.

- *X chromosome locality*: In mammals, the M/LWS opsin gene is located on the X chromosome. Males have only one X chromosome in the genome, and females have one of the two X chromosomes randomly inactivated in a cell. Thus, only one M/LWS allele is always expressed in a given cone cell; thus, there was no need for the evolution of a special mechanism to achieve allelic exclusion. Indeed, trichromatic color vision in most New World monkeys[2] and some of prosimians is attained by this X-chromosomal locality in females having different alleles of the M/LWS opsin gene in spectral sensitivity.
- *LCR*: Catarrhines (Old World monkeys, apes, and humans) have L and M opsin genes in tandem on the same X chromosome as a result of a gene duplication. In catarrhines, an additional mechanism is required to express selectively only one gene from the M/LWS gene array on the X chromosome. A regulatory region (an enhancer) for the original single-copy M/LWS opsin genes is located upstream of the gene. Because the gene duplication did not involve the enhancer, the LCR controls expression of both L and M opsin gene loci (Wang et al. 1992). The LCR of the primate M/LWS opsin genes interacts with only one of the genes via its promoter in a given cell. The choice of the gene is random, and the interaction does not switch to another gene once the interaction begins. It is not known whether or how this randomness and stability of the interaction between the LCR and a promoter is controlled. However, when a DNA fragment is artificially introduced into mice mimicking the gene duplication of L and M opsin genes with the LCR placed upstream of the gene array, almost mutually exclusive expression between the two genes is realized (Smallwood et al. 2002). Thus, when this gene duplication occurred on an X chromosome in a catarrhine ancestor, the mutually exclusive expression between the L and M opsin genes from one X chromosome was likely to be simultaneously realized. The X chromosome locality and this LCR system ensure that only one M/LWS subtype is expressed in a cone cell.
- *High-acuity spatial vision*: One characteristic of primate vision is three-dimensional vision due to forward-facing eyes and a high degree of visual acuity in the central retina. One mechanism ensuring the high spatial acuity is the one-to-one midget ganglion pathway. The pathway receives input from only one M/LWS cone cell in the center of the receptive field of a midget ganglion cell. The pathway thus compares the center input with inputs from surrounding M/LWS cones (Martin 1998). A more primitive midget ganglion pathway is seen even in nocturnal prosimians (bushbabies) (Yamada et al. 1998). In bushbabies the cone–ganglion convergence ratio in the central retina is higher than one (five cones per one ganglion) but is still much lower than that, for example, in cats (~30 cones per ganglion). Thus, the stereoscopic and high-acuity vision of nonhuman primates is considered to be an adaptation to their predominantly arboreal life, which requires agile movements and salutatory locomotion from branch to

[2]New World monkeys: a group of primates, platyrrhines, that inhabit Central and South America. The group contains three families – Atelidae, Pitheciidae, Cebidae – that separated from the ancestor of catarrhines (Old World monkeys, apes, and humans) about 40 million years ago.

branch. In higher primates (simians: New World monkeys and catarrhines), the eye is surrounded by a bony cup called the postorbital plate. The most important function of the postorbital plate is to prevent the chewing muscles from disrupting eye position, thereby improving visual acuity (Fleagle 1999; Heesy et al. 2007). Simians also have a specialized area in the center of the retina, called the fovea, where cone cells are densely packed, allowing high visual acuity.

The midget ganglion pathway of simians also provides a color opponent mechanism, which is the neural basis of primate trichromacy, if the center-surrounding inputs are from different spectral types of M/LWS cones (Martin 1998). In other mammals, both the receptive-field center and its antagonistic surroundings receive input from multiple cone cells. This neural wiring provides only poor spatial acuity but enables high sensitivity to light and is thus considered to be an adaptation to nocturnal life.

When mice are manipulated genetically so females have the human L opsin gene on one X chromosome and the native M opsin gene on the other X chromosome, enhanced long-wavelength sensitivity and a new capacity for chromatic discrimination was observed even though mice lack a midget ganglion pathway, fovea, and postorbital plate (Jacobs et al. 2007). This chromatic discrimination could be because these genetically engineered mice extracted chromatic information based on the difference in total M and L input between the center and surrounding regions. The degree of chromatic difference between the two regions should vary stochastically and could be subtle. It is not clear how the variable and subtle improvement of color discrimination in the coarse spatial image is useful and adaptive for mice.

As described above, prosimians lack a fovea and postorbital plate. Their midget system is relatively unspecialized. Trichromacy by the L–M gene polymorphism is less common in prosimians than in New World monkeys (Tan and Li 1999). Thus, the selection pressure to maintain trichromacy may not have been strong enough for the mammals with poor spatial resolution of the visual image. Without the midget ganglion pathway, evolution of trichromacy might not have been possible in other mammals even if a similar spectral differentiation of opsin subtypes would have occurred (Surridge et al. 2003; Vorobyev 2004). Thus, as in the genetic mechanism for the exclusive gene expression, the necessary neural circuitry for trichromatic color vision was provided serendipitously from a preexisting system for spatial vision.

16.4.2 What Are the Advantages of Having Trichromatic Vision?

It has long been hypothesized that primate trichromacy was selected for finding ripe fruits against a background of mature leaves (“fruit theory”) (Allen 1879; Osorio and Vorobyev 1996). Recently, however, there have been arguments against this explanation because many fruits eaten by primates are, in fact, also distinguishable from background leaves by dichromats via the blue-yellow (S vs. L/M) signal and luminance signal (Dominy and Lucas 2001). Furthermore, some fruits do not

develop conspicuous colors and yet constitute a significant portion of primate diets. In addition, a study of the 12 plant species most commonly consumed by the primates of the Kibale Forest in Uganda revealed that fruit color was not a nutritional cue (Dominy 2004). Many fruits are also highly seasonal and become scarce during the dry season.

Figs and palm nuts are not seasonal and can function as keystone resources during the periods of fruit scarcity (Terborgh 1986). Cryptic coloration is frequent in figs and palms, and it is suggested that early primates in the warm Paleocene–Eocene forests, which were characterized by figs and palms (Morley 2000), relied on these fruits as keystone resources (Dominy et al. 2003b). However, the global cooling and drying during the Eocene–Oligocene interval (about 30–40 million years ago) coupled with increasing seasonal fluctuations dramatically reduced the density and availability of figs and palms, especially in Africa (Morley 2000), where early simians evolved (Fleagle 1999). Africa is still highly seasonal, with a phenology characterized by alternating periods of fruiting and leafing.

Another theory regarding the evolution of trichromacy is the "young leaf theory." This theory states that, given the seasonality of Africa, young leaves provide a critical fallback resource during periods of fruit shortage (Lucas et al. 1998). Regardless of tree species, young leaves are tender and rich in proteins and free amino acids (Dominy and Lucas 2001). Young leaves are often reddish and thus distinct from mature leaves only via the red-green color channel of trichromats. Hence, the ability to discern between young and mature leaves may have been a major selective force for primate trichromacy (Lucas et al. 1998; Dominy and Lucas 2001; Lucas et al. 2003). The young leaf theory is strengthened in the context of the historical biogeography of figs and palms; in Africa, where early catarrhines evolved, figs and palms are scarce; and routine trichromatic vision was selected for exploiting proteinaceous young leaves as a replacement resource. However, in the Neotropics and Madagascar, where polymorphic color vision is seen in most New World monkeys and some prosimians, figs and palms remained abundant; and some New World monkeys (e.g., marmosets) do not depend on young leaves at all (Dominy et al. 2003b). Thus, the young leaf theory does not seem to explain the evolution and maintenance of trichromacy outside Africa.

Another long-standing hypothesis to explain the evolution of trichromacy has been the detection of social signals or the detection of predators (Allen 1879; Surridge et al. 2003; Vorobyev 2004; Changizi et al. 2006). A recent study showed, however, that the primate trichromacy appeared before the evolution of red pelage and red skin as well as gregarious mating systems; and therefore the social signals could not be a factor in the evolution of trichromacy from dichromacy (Fernandez and Morris 2007).

Yet another hypothesis recently put forward could be named the "foliage hypothesis" (Sumner and Mollon 2000). For trichromatic primates, perceived color (i.e., chromaticity) can be described as a ratio of the quantum catch among their L, M, and S cones and expressed as a point in color space[3] analogous to the MacLeod–Boynton

[3]Color space: a conceptual space – such as red-green-blue (RGB), CIE, and MacLeod-Boynton – in which a color is defined numerically as a point.

diagram (MacLeod and Boynton 1979) consisting of L/(L+M) and S/(L+M) axes. The former axis represents a ratio of quantum catch of L cones to that of L and M cones, whereas the latter represents that ratio for S cones to L and M cones (Regan et al. 1998). L/(L+M) indicates the redness that is provided by the "red-green" chromatic channel – for which only trichromats are equipped – and subserved by the midget ganglion cells. The S/(L+M) indicates the blueness that is provided by the "blue-yellow" chromatic channel for which all mammals are equipped. This more ancient system is subserved by the small bistratified ganglion cells. Colorimetric measurements of natural scenes in forests reveal that the chromaticity of mature leaves falls in a very narrow range of L/(L+M) values but spreads widely along the S/(L+M) axis and also in luminance values. Thus, chromaticity of fruits, young leaves, pelage, and skin often deviate from mature leaves in their L/(L+M) value but largely overlap with them in S/(L+M) and luminance values (Regan et al. 1998; Sumner and Mollon 2000, 2003; Regan et al. 2001). This leads to the hypothesis that primate trichromacy could be adaptive and have evolved for detecting *anything* differing from the background foliage in L/(L+M) value. This hypothesis appears to be superior to the others in being based on the general characteristics of the forest environment, not being specific to any particular visual targets, and being able to account for diverse feeding and social ecologies among primate species that still have similar sets of visual opsins.

On the other hand, recent studies have found that dichromatic vision may be advantageous to primates under some conditions (e.g., finding cryptic fruits or insects or for detecting cryptic predators, such as snakes) (Caine et al. 2003; Saito et al. 2005b). The conceptual basis for this hypothesis is that trichromatic vision compromises the acuity of other visual systems. The neural system of trichromatic individuals must combine signals from the L and M photoreceptors to obtain the luminance signal used for achromatic "color-blind" tasks such as spatial vision and the perception of shape, texture, and motion (Morgan et al. 1992; Kelber et al. 2003). The different spectral inputs from the two receptors can cause corruption, resulting in a weaker overall signal. Additionally, color may compete with texture information, or trichromats may learn to rely on color at the expense of information to be gained by texture. Therefore, dichromats may have an advantage over trichromats in achromatic (color-blind) tasks, such as defeating camouflage and depth perception.

Behavioral experiments that compared feeding efficiency between vision types in laboratory settings with artificial targets have suggested that there is a selective advantage of trichromacy when foraging on colored foods (Caine and Mundy 2000; Smith et al. 2003b). On the other hand, studies have found that dichromats are better than trichromatic primates at detecting camouflaged stimuli (Caine et al. 2003; Saito et al. 2005b). It should be noted that such comparisons evaluate whether the difference in visual ability is consistent with the difference in color vision phenotypes but do not evaluate whether one phenotype is more advantageous than another (Saito et al. 2005a). In other words, these experiments should only be regarded as tests that determine visual phenotypes, although they do provide useful predictions about the potential foraging advantages of these vision phenotypes.

16.4.3 *Behavioral Observation for Wild Populations of New World Monkeys*

Whatever the theories and laboratory experiments predict, the adaptive value of primate trichromacy (or dichromacy) can only be evaluated in light of behaviors seen in the wild. Hence, it is important to compare behaviors between free-ranging dichromats and trichromats and to evaluate whether and how the contrast between a visual target and its background are correlated with behavioral differences in these two types of primate. New World monkeys are an excellent model in which to test this because of the allelic polymorphism of the L–M opsin gene that results in dichromatic and trichromatic individuals within the same population (Mollon et al. 1984) (Fig. 16.3).

Despite the predicted advantage of trichromacy, behavioral observation of wild primate populations has provided only limited support. In a study of a wild mixed-species troop of saddleback (*Saguinus fuscicollis*) and mustached (*Saguinus mystax*) tamarins, during vigilance trichromats are further from their neighbors than their dichromatic conspecifics. This is explained as a result of the potentially better perception of predation risk in trichromats (Smith et al. 2005). However, results of other behavioral observations of wild primates have produced equivocal results or results contradictory to the predictions from the trichromat advantage hypothesis.

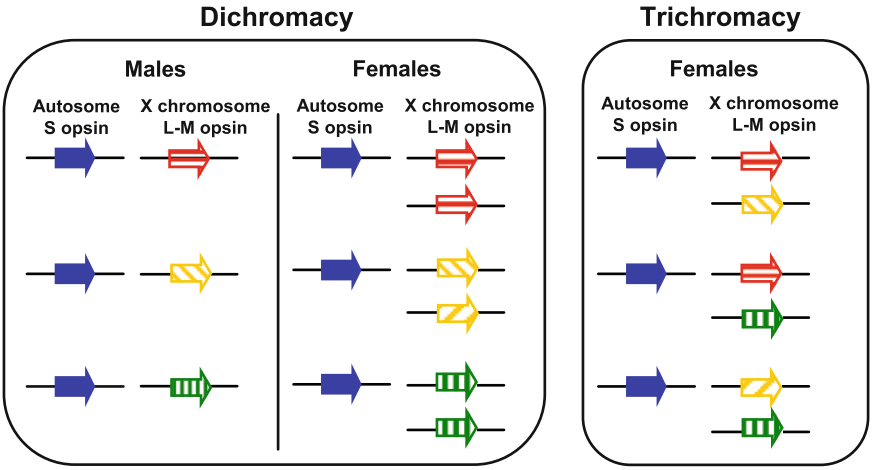

Fig. 16.3 Color vision polymorphism of New World monkeys. Typically, three alleles are found in the X-linked M/LWS opsin gene. Males have only one X chromosome and are therefore obligate dichromats, having a single M/LWS opsin allele on the X chromosome and the single autosomal S opsin gene. However, there are three types of dichromatic male in the same species each having different M/LWS allele types. If a female has the same M/LWS opsin allele on both X chromosomes, she is also dichromatic, like the males. If a female has two different M/LWS alleles, she is a trichromat. There are three types of trichromatic female in the species because three heterozygote combinations are possible in a triallelic system. In total, six color vision phenotypes can exist in one species if there are three M/LWS alleles

The study of the wild mixed-species troops of tamarins showed that the color-vision types (dichromatic or trichromatic) did not have a consistent effect on the leadership of the troops to feeding trees (Smith et al. 2003a). Another study of tamarins (*Saguinus imperator imperator* and *S. fuscicollis weddelli*) found no significant difference between females (thought to consist of trichromats and dichromats) and males (all dichromats) in their ability to locate or discriminate between feeding sites (Dominy et al. 2003a). In a population of capuchin monkeys (*Cebus capucinus*), there was no significant difference between trichromats and dichromats in feeding or energy intake rates (Vogel et al. 2007). In another population of the same capuchin monkey species there was no difference between dichromats and trichromats in the time spent foraging on different food types (Melin et al. 2008). Some modeling studies based on field observations have found that many fruits eaten by spider monkeys (*Ateles geoffroyi*) or squirrel monkeys (*Saimiri sciureus*) are similarly discernible or similarly indiscernible from background foliage for both trichromats and dichromats (Riba-Hernandez et al. 2004; Stoner et al. 2005; De Araujo et al. 2006). A field study of free-ranging spider monkeys (*A. geoffroyi*) measuring their foraging efficiency on fruits and colorimetric properties of fruits and background leaves revealed that dichromats are not inferior to trichromats in frequency, accuracy, or unit-time intake efficiency of detecting fruits (Hiramatsu et al. 2008). The study showed that this is because the luminance contrast of fruits to background leaves is the main determinant of fruit detection by both dichromats and trichromats. A study of the same social group of spider monkeys also showed that irrespective of color vision phenotypes the monkeys sniff visually cryptic fruits more often than visually conspicuous fruits (Hiramatsu et al. 2009). This indicates that color vision is not the sole determinant for ingestion or rejection of fruits. A field study of capuchin monkeys (*C. capucinus*) has even demonstrated a dichromat advantage in foraging for surface-dwelling insects (Melin et al. 2007, 2010). These behavioral observations suggest that the superior ability of trichromats to see the red-green color contrast may not translate into a selective advantage because the use of a variety of sensory modalities may compensate for the inferiority of any one sense (Hiramatsu et al. 2009).

16.4.4 Future Directions of the Study of Primate Color Vision

As we have seen, field observations of the foraging behaviors of New World monkeys have thus far either demonstrated dichromat advantage for insect foraging or failed to detect clear advantage of trichromats for fruit foraging. This leaves a fundamental question unanswered regarding what maintains trichromatic vision in New World monkeys because trichromacy (i.e., heterozygosity on the L–M opsin alleles) would have disappeared without a selective force acting to maintain allelic variations of the L–M opsin. Population genetics should be a powerful tool to test whether such natural selection is indeed operating by comparing genetic variation between the L–M opsin gene region and other genomic regions (Hiwatashi et al. 2010).

On the other hand, catarrhines other than humans are almost uniformly trichromatic, suggesting extremely strong selective advantages for trichromacy in nonhuman catarrhines. The advantages of trichromacy might be manifested in tasks for which behavioral data have yet to be gathered, such as long-distance detection of reddish objects under dappled foliage (Sumner and Mollon 2000), foraging on reddish ripe fruits during severe dry seasons when these fruits might be scarce (Dominy and Lucas 2001), or the recognition of social signals (Changizi et al. 2006; Fernandez and Morris 2007).

For polymorphic color vision, we should also investigate by behavioral observations and experiments a possible selective advantage for the coexistence of individuals with different color vision phenotypes in the same population. There is a clear advantage of monkey and ape dichromats, as well as human dichromats, in detecting color-camouflaged objects (Morgan et al. 1992; Caine et al. 2003; Saito et al. 2005b), including surface-dwelling insects (Melin et al. 2007, 2010), an important food source for many primates. Given such potential selective advantages in dichromats, we then need to ask why dichromats are so rare in nonhuman catarrhines. We should also ask whether the selective advantages in dichromats is applicable to humans. Interdisciplinary studies combining genetics, behavioral ecology, and visual physiology will continue to provide a wealth of data for furthering our understanding of the evolution of primate color vision.

16.5 Evolutionary Implications to Human Color Vision Variations

Among trichromatic catarrhines, humans constitute a notable exception. Approximately 3–8% of males have "color vision defects" mainly due to unequal meiotic recombination between L and M opsin genes (Deeb 2006). The distribution of this defect in the population can be due to relaxation from some selection pressure that selects for trichromacy, although the nature of this pressure remains unclear. Another possibility is that the persistence of dichromats in the human population may reflect, as noted above, some advantage to having different color-vision morphs in a population. Perhaps from the apes' point of view, humans are weird primates, having left the leafing forest some million years ago. Then, approximately two million years ago, members of this primate started to devise stone tools and included hunted meat as a considerable portion of their diet. Finally, the increased brains eventually led to the development of agriculture some thousand years ago and the building of a modern industrial society only a few hundred years ago. The persistence of color vision morphs in humans could be related to any of these major events: Life outside the forest reduces the need for color vision, hunting might have benefited by the presence of dichromatic group members, or large agricultural or industrial societies could isolate humans from selection against dichromacy. It is also important to know when the color vision polymorphism is spread into the population as today in human evolution.

16.6 Conclusion

To understand animal color vision is to understand human color vision. Nonhuman primates are a good reference point for comparison; studies of New World monkeys are particularly important to understand a condition where color vision can be polymorphic in the population. Fish species demonstrate how versatile the visual system is in coping with differing light environments and are an important reference point for understanding color vision in primates. Since cone opsin genes were isolated during the mid-1980s (Nathans et al. 1986), our understanding on the evolution of color vision has rapidly progressed. This is largely due to the fact that these studies encompass research on genes, physiology, and behaviors. Further interdisciplinary studies will continue to produce a wealth of findings.

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Part V
Chemical and Neural Probes for Studying Social Behaviors

Chapter 17
Effect of Endocrine-Disrupting Chemicals on the Development of Macaque Socialization

Takamasa Koyama and Akiko Nakagami

17.1 Environment, Pollution, and Disease: Problems of Modern Society

Since Rachel L. Carson published *Silent Spring* in 1962, several scientists have suggested that synthetic chemical products might adversely affect living organisms (Carson 1962). Colborn, Dumanoski, and Myerds published *Our Stolen Future* in 1996 with a preface by then Vice President of the United States Al Gore (Colborn et al. 1996). This book was translated into Japanese in 1997, and around this time discussion about "environmental hormones" began to be noticeably prevalent in Japan.

The Environmental Agency of Japan started the Project on Exogenous Endocrine-Disrupting Chemicals (EDCs) in 1997, and in 1998 they officially published a Strategic Plan against EDCs (SPEED'98) in which they listed 65 chemical products considered potentially disruptive to endocrine functioning. Of these chemicals, the agency tested 28 and found toxic effects only for nonylphenol, octylphenol, and bisphenol A (BPA) when tested in Japanese medaka (*Oryzias latipes*). Because of these limited findings, the Japanese government reversed its policy, and the list itself disappeared over the course of time. Media coverage gradually decreased, leaving the impression that the environmental hormone problem no longer existed. However, in April 2008, The Ministry of Health in Canada designated BPA a hazardous substance and banned the import, selling, and advertisement of polycarbonate nursing bottles. The U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) reexamined the safety standards of their risk assessment lists. In Japan, the Ministry of Health, Labor, and Welfare requested that the Food Safety Committee in the Cabinet Office perform a risk assessment of BPA because the National Institute of Health Sciences had reported abnormal sexual cycles of female rats subjected to low doses of BPA (see Vom Saal et al. 2007 for a general discussion).

The Ministry of the Environment in Japan has been more sensitive to issues regarding dioxins and polychlorinated biphenyls (PCBs) as they had been grappling with them

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for a longer time than other pollutants. In 1968, Japanese experienced the rice oil disaster, which was followed in 1976 by the Seveso disaster in Italy (Eskenazi et al. 2002; Kanagawa et al. 2008). It became obvious that PCBs and dioxins had strong toxicity and teratogenicity. In 1997 the Ministry of the Environment amended the law to prevent air pollution, and in 1999 they began to enforce the law concerning special measures against dioxins. In 2003, the Ministry issued special measures for the appropriate treatment of PCB waste. Recently, researchers have observed that PCBs affect the development of nerve cells via thyroid hormone and that PCBs are related to developmental disorders such as learning disability (LD), attention deficit hyperactivity disorder (ADHD), and autism (Rice 1997, 2000; Rice and Hayward 1997).

We began a behavioral assessment of dioxin toxicity by observing the emotional reactions of rhesus macaques and their peer interactions (Negishi et al. 2006). In addition to this assessment, we observed the behaviors of cynomolgus infants born to mothers exposed prenatally to BPA, which was said to function like estrogen. Kuroda showed that mothers' PCBs passed through their placenta, and he found that they decreased concentrations of thyroxine in their fetuses (Kawahara and Kuroda 2002). Moreover, it is well known that thyroid hormone deficiency increases the likelihood of LDs and/or ADHD; thus, we initiated our observations of cynomolgus infants and their PCB-exposed mothers to elucidate the effects of PCBs on macaque socialization.

We used primate subjects for research on EDCs for two main reasons. First, development of the nervous system varies among species; therefore, it would be difficult to extrapolate results from nonhumans to humans (the thalidomide disaster demonstrated this fact most emphatically). Because primates are closest to humans in terms of brain function, structure, and metabolism (Yoshikawa 2005), they could be expected to serve as a good model for conducting in vivo research on the effects of EDCs. Second, we have been interested in the development of social behavior and individual intelligence because social behaviors require higher cerebral functions for adaptation to real life (Byrne 1995) and because they more readily reflect sex differences (Mitchell 1968, 1979; Mitchell and Brandt 1970). Therefore, we chose primates as a suitable model to assess human brain disorders associated with EDC contamination, even though the study of primates is more expensive and time-consuming.

In the following sections, we review the results of research on mother–infant and peer relationships using three kinds of EDCs: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), BPA, and PCBs. All three EDCs are very familiar in our daily life: 2,3,7,8-TCDD is used as the standard of toxicity in dioxins; BPA is contained in plastic products such as compact disks (CDs) and cellular phones; and PCBs are the most widely prevailing chemicals, from the tropics to the Arctic.

17.2 Gestational and Lactational Exposure to 2,3,7,8-TCDD Affects Social Behaviors of Rhesus Infants

Forty colony-bred adult rhesus females were divided into two groups: One group was injected with 2,3,7,8-TCDD (30 ng/kg) on day 20 of gestation, and the other group was treated with 300 ng/kg on the same day. The two groups received additional

injections of 1.5 ng/kg and 15.0 ng/kg, respectively, every 30 days until postnatal day 90. A control group ($n = 20$) received only the vehicle, a mixture of toluene and dimethylsulfoxide (DMSO), on the same schedule (see Negishi et al. 2006 for more details). Each pregnant monkey was housed in a stainless steel cage ($69 \times 61 \times 75$ cm) until weaning of offspring. Two male and two female infants from each group (chosen by order of body weight at birth) were selected to encounter each other in pairs in a $69 \times 122 \times 75$ cm observation cage (Fig. 17.1a) approximately 30 days after weaning. We conducted the tests twice for each pair: once at the age of 12–15 months and again at the age of 24–27 months.

The cage was partitioned into two equal rooms by a stainless steel slide and transparent acrylic panels. At first, two subjects were placed separately in each room. We allowed them to spend 10 min alone so we could observe their individual behavior in a novel environment. After 10 min, we removed the steel panel so they could see each other through the acrylic panel. After 5 min, the transparent panel was removed, and the two subjects stayed together in the cage for 15 min. Subjects from three experimental groups were observed for a total of six times per group. Behaviors of subjects were recorded with a digital video camera, and 40 behavioral categories were counted by the one-zero sampling method at 5-s intervals (Martin and Bateson 1990; Lehner 1996).

Visual exploration, stereotypy, mutual proximity, self-directed behavior, and outward interest showed significant effects of TCDD exposure (Fig. 17.1b). Both groups exposed to TCDD showed more visual exploration and mutual proximity at the first-year assessment, less stereotypy at both the first- and second-year assessments, and less self-directed behavior at only the second-year assessment compared with the unexposed control group. The 300 ng/kg group showed more outward interest than the control group at the first-year assessment. Stereotypy usually represents inhibition of social interest or sociality by animals that are tense and autistic. The exposed subjects therefore were less tense and more sociable than the control subjects. The results of visual exploration, mutual proximity, self-directed behavior, and outward interest do not contradict this characteristic of the experimental group subjects; more occurrence of visual exploration and mutual proximity was evidence of more social interest by the other subjects, and the occurrence of self-directed behavior showed that the control subjects were more tense than the exposed subjects. Furthermore, the outward interest values indicated that the most exposed group was more relaxed and interested in their surroundings. However, we did not find a clear dose effect in these results.

The observed behaviors were analyzed by canonical discrimination (Fig. 17.2). In the first eigenvector Z1, visual exploration was a positive contributing factor, whereas stereotypy, fear grimace, and vocalization were negative contributing factors. In the second eigenvector Z2, mutual proximity and mutual passive contact were positive contributing factors; and environmental exploration, self-directed behavior, and stereotypy were negative contributing factors (Fig. 17.2a). Thus, we considered that the values of Z1 and Z2 represented negative tenseness and negative self-centeredness, respectively (Negishi et al. 2006). For the first-year encounter test, the location of the three groups was clearly separated in the Z1 (negatively tense) and Z2 (positively self-directed) dimensions. A characteristic of both the 30 ng/kg

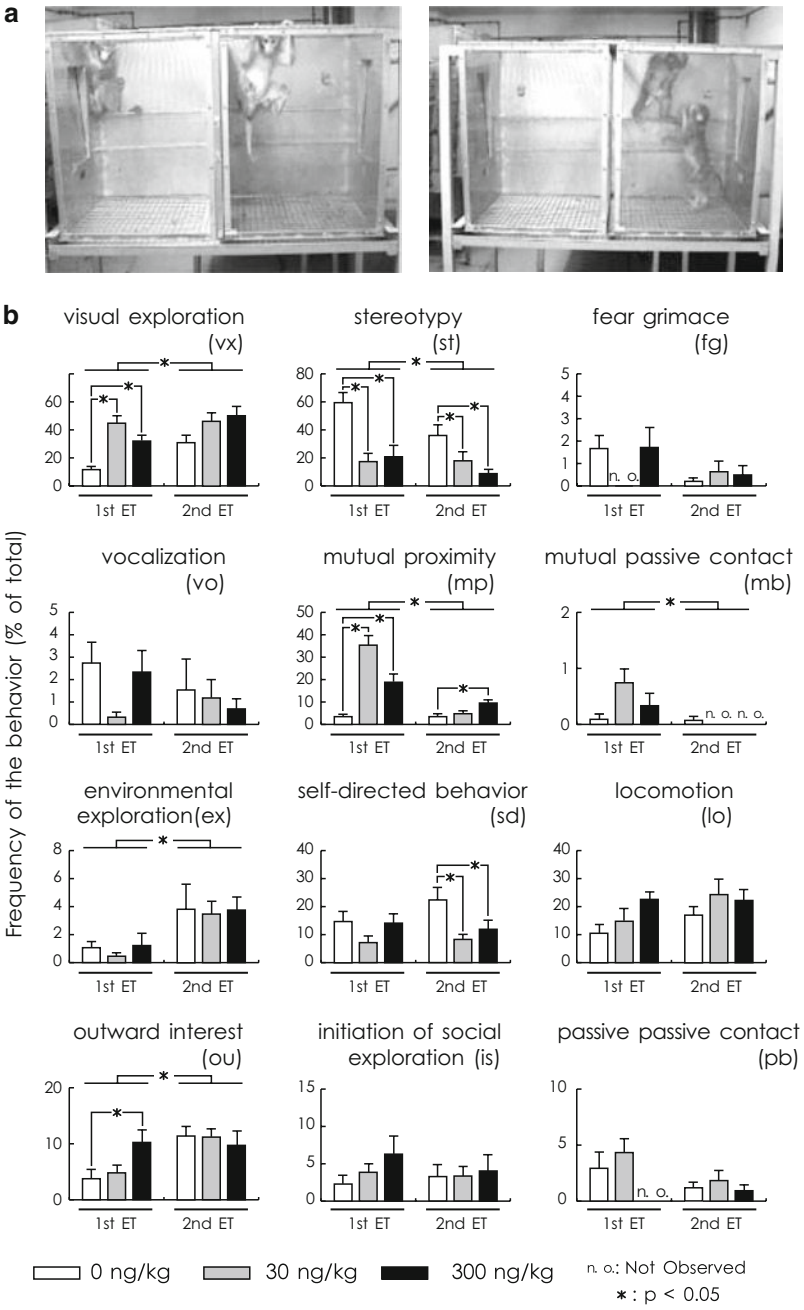

Fig. 17.1 (**a**) Encounter test apparatus. Two subjects were first placed in rooms separated from each other (*left panel*). They interacted after removal of the acrylic wall (*right panel*). (**b**) Among 40 behavioral categories, the frequencies of 12 were studied: visual exploration (*vx*), stereotypy (*st*), fear grimace (*fg*), vocalization (*vo*), mutual proximity (*mp*), mutual passive contact (*mb*), environmental exploration (*ex*), self-directed behavior (*sd*), locomotion (*lo*), outward interest (*ou*), initiation of social exploration (*is*), and passive contact (*pb*) (Negishi et al. 2006)

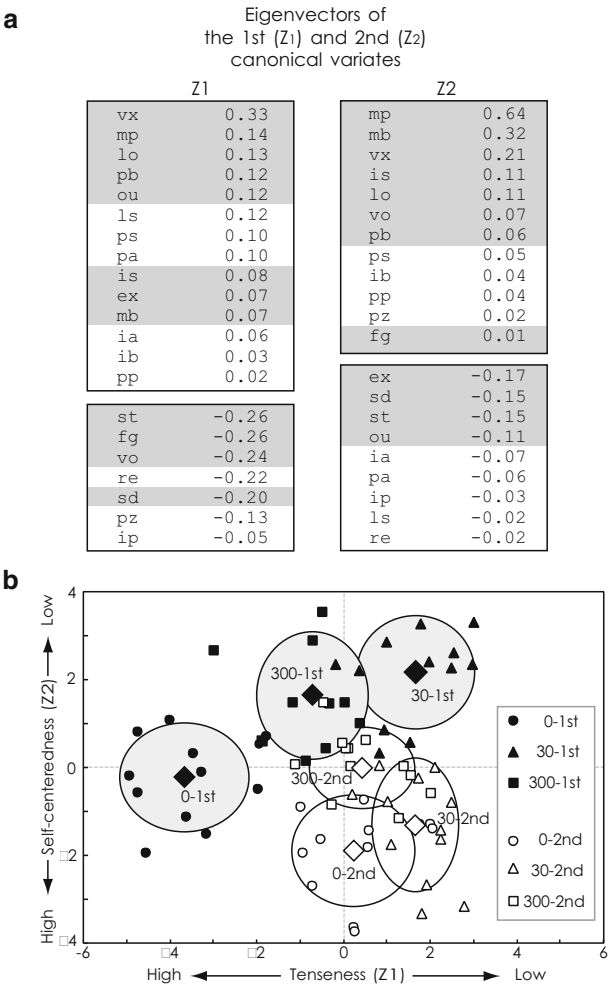

Fig. 17.2 (**a**) Eigenvectors of the first (Z1) and second (Z2) canonical variates in the canonical discrimination. In Z1, visual exploration (*vx*) was a positive contributing factor, whereas stereotypy (*st*), fear grimace (*fg*), and vocalization (*vo*) were negative contributing factors. In Z2, mutual proximity (*mp*) and mutual passive contact (*mb*) were positive contributing factors, whereas environmental exploration (*ex*), self-directed behavior (*sd*), and stereotypy (*st*) were negative contributing factors. Gray-masked items are those graphed in Fig. 17.1b. (**b**) Scatter diagram of the canonical discrimination in the encounter tests (see the legend of Fig. 17.1a and the text in p. 355). The *large diamond* and *circle* represent the center of balance and the area of 80% confidence, respectively for each group (Negishi et al. 2006)

and 300 ng/kg groups was that they were less tense and more sociable than the control group. In the second-year tests, the data points became closer to each other, and the animals looked more self-directed than they did during the first-year tests. Thus, we can conclude that canonical discrimination successfully distinguished TCDD-exposed infants from control ones at both stages of development (Fig. 17.2b).

17.3 Development of Mother–Infant Interactions in Cynomolgus Monkeys Exposed to BPA

BPA has been used to produce polycarbonate plastics and epoxy resins that are commonly used in our daily life as materials for CDs, cellular phones, paint, and frames. Governments of developed nations have declared that BPA is safe, but it is well known that its structure is similar to that of estrogen. Kubo and colleagues (2001, 2003) and Fujimoto et al. (2006) showed that BPA abolished and inverted sex differences in open-field behavior and in the locus ceruleus (LC) in rats. Normally, female rats are more active and have a larger LC than males. However, BPA-exposed rats show a normal phenotype of sexual organs. Hence, we hypothesized that the behavioral regulatory system in the brain is likely to be more sensitive to EDCs than the systems that regulate reproduction (Nakagami et al. 2009). We therefore began studying the effects of low-dose BPA on the social development of infants in mother–infant and peer interactions. Harlow and colleagues (Harlow and Mears 1979; Mitchell 1979), Itoigawa (1973), and Minami (1974, 1997) described sex differences in the social development in macaques: Males were more active, maintained a greater distance from their mother, and spent more time in rough-and-tumble play. We thought that a behavioral analysis of mother–infant and peer interactions would be a suitable paradigm to test for subtle effects of BPA on sexual behavior regulation by the brain.

A total of 18 adult female, colony-bred cynomolgus macaques were housed in a stainless steel cage (69×61×75 cm) until weaning of offspring. All animals were pregnant and primiparous. The animals received BPA (10 μg/kg/day) in a mixture of *N*,*N*-dimethylacetamide and polyethylene glycol (400) (1:1) through Alzet osmotic pumps. The pumps were surgically implanted in the dorsal subcutaneous tissue of each macaque, and each pump released a fixed amount of the solution (6 μl/day) from the 20th day of pregnancy until delivery (see Nakagami et al. 2009 regarding BPA volume and serum concentration). In all, 19 control pregnant females received only the vehicle solution using the same osmotic pumps.

We observed mother–infant interactions twice a week during the first 90 days after birth and once a week during the period from 90 to 180 days after birth. The front mesh of the cage was exchanged for Plexiglas, and the behavior of the mother–infant pair was recorded for 20 min with a digital video camera. Nine social behaviors and six nonsocial behaviors of infants were checked every 5 s using the one-zero sampling method.

We implemented canonical discriminant analysis on age-pooled data sets to discriminate four groups behaviorally: BPA-treated male and female groups and control groups of both sexes. Discrimination scores for all subjects were plotted in two dimensions (Fig. 17.3). Function 1 (Z1) represents a measure of the static versus dynamic mother–infant relationship categories, consisting of outward interest, locomotion, orientation, ventral contact, and social exploration. It accounted for 89.3% of the cumulative contribution, whereas that of function 2 (Z2) accounted for only 7.8% of the cumulative contribution. The control male group was clearly

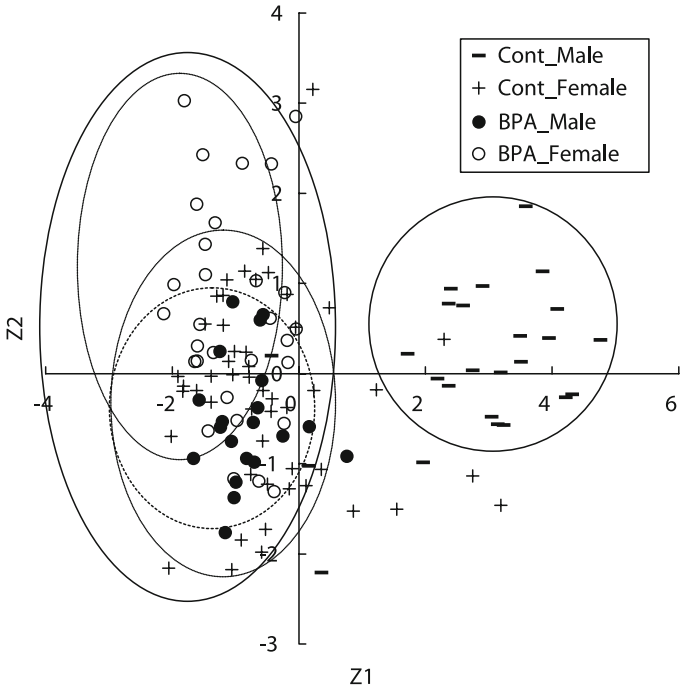

Fig. 17.3 Canonical discriminant analysis (four groups). Subjects were classified into four groups [males and females of the bisphenol A (BPA) group and males and females of the control group (i.e., *cont male* and so on)] in consideration of the sexual differences in behaviors. Z1 represents the measure of the static versus dynamic mother–infant relationship (Nakagami et al. 2009)

located separately from the other three groups on the function 1 axis (Z1), whereas scores for control females, BPA-treated females, and BPA-treated males were close to each other. Figure 17.3 shows that BPA-treated males were shifted to the location of control and BPA-treated females and that the control males were quite different from the other three groups (the BPA-treated male and female groups and the control female group). The canonical discriminant analysis thus suggested behavioral feminization of BPA-treated males.

Developmental data on locomotion, outward interest, ventral contact, and social exploration showed different tendencies of the four groups (Fig. 17.4). These four behavioral categories mainly contributed to function 1 of the canonical discriminant analysis discussed above, and all categories showed a significant interaction (treatment × age) in a three-way analysis of variance (ANOVA) (treatment × sex × age). Outward interest refers to behaviors reflecting an infant's interest in the outer environment, such as "looking out of the cage from the gap between the front Plexiglas and the wall, observing the wire on the floor." Control females were much more interested in the outer environment than control males; however, control males were catching up with control females in this behavior pattern by the end of the observation period. On the other hand, the patterns of outward interest in all groups except the control

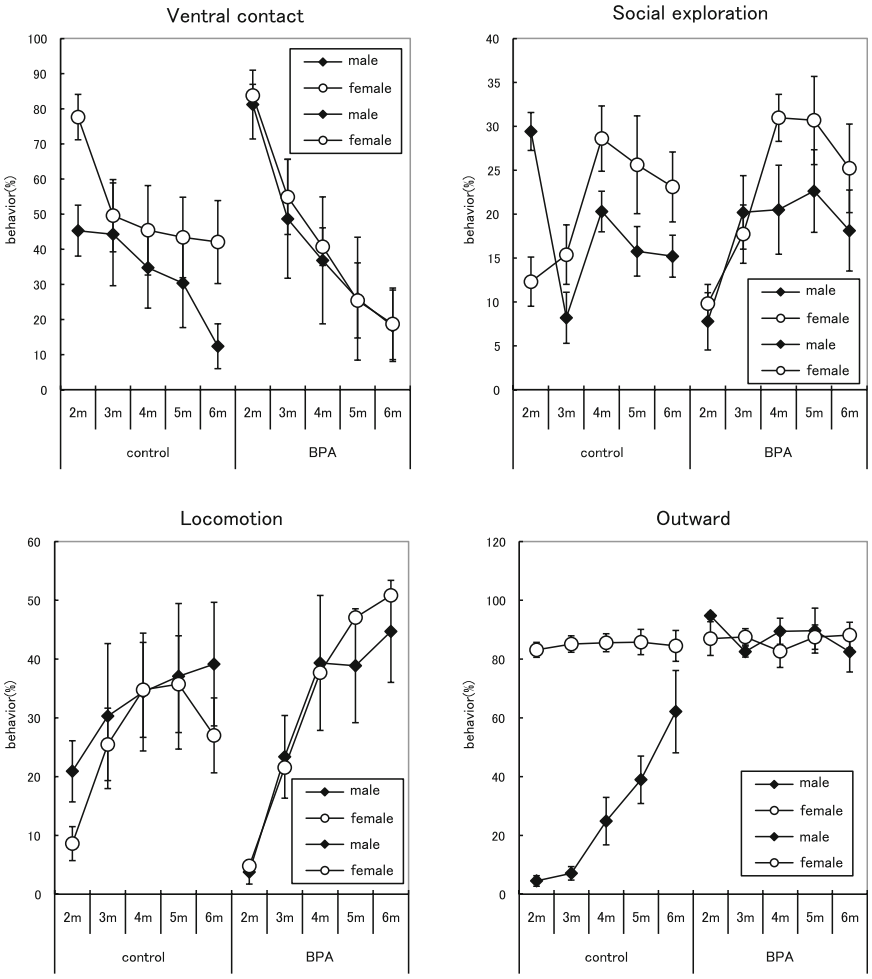

Fig. 17.4 Developmental changes of infant behaviors (Nakagami et al. 2009)

male group were similar to each other. This result showed that BPA-treated males had shifted to the developmental pattern of both control and BPA-treated females.

A decreasing pattern of ventral contact (i.e., abdominal clinging to the mother) was consistent with that in previous mother–infant studies (Hinde and Spencer-Booth 1967; Mason 1970; Hinde 1974; Harlow and Mears 1979; Minami 1997), but we found a difference in the pattern between the BPA-treated and control groups. The patterns of BPA-treated females and males were similar, but control females showed more contact than control males, and the decreasing pattern of the females was more gradual than that of the males, which was steeper. Thus, exposure to BPA might attenuate sex differences in infants. Social exploration (i.e., interaction with the mother, such as licking and visual exploratory action toward her) showed a different pattern: Both BPA-treated and control females showed a similar changing pattern,

whereas the BPA-treated male group revealed less social exploration during the second month because of its ventrally contacting the mother as much as both female groups did and significant difference in "treatment × age interaction," as shown by two-way ANOVA. Locomotion, representing activity of the infant such as moving on foot or brachiating by clinging to tall limbs and branches, showed another example of the effect of BPA on sex differences. There was no sex difference between BPA-treated females and males during the first 3-month period. In contrast, the control males were more active for the first 2 months and immediately before weaning (at the age of 6 months). BPA-treated females became more active than control females as they matured, so, in a sense, BPA-treated females began behaving similarly to the males.

17.4 Correlation Between Plasma Concentration of PCBs in Cynomolgus Mothers and Behavioral Tendencies of Their Offspring

It is well known that wild polar bears have already been contaminated by PCBs, which may be orally ingested as a result of the food chain. Although the toxicity of PCBs has been recognized for a long time, there has been a failure to prevent their introduction into the global environment. We asked the Shin Nippon Biomedical Laboratories (SNBLA) to assay the plasma concentration of PCBs in 30 cynomolgus pregnant female subjects imported from China, and we found that they had already been contaminated with various PCBs. We selected 10 mothers from among the 30 females by order of highest total PCB concentration so that half of the mothers had male offspring and the remaining half had female offspring. We also selected ten mothers from the lowest PCB concentration group in the same way. The ten mothers with the highest PCB concentrations and the ten mothers with the lowest PCB concentrations were divided at the concentration point of 15 pg/g. The average concentration in the low-PCB-concentration mothers of male offspring was 10.126 ± 1.50 pg/g and that of the high-PCB-concentration mothers was 21.216 ± 3.83 pg/g; the average of the low-PCB-concentration mothers of female offspring was 10.214 ± 1.46 pg/g and that of the high-PCB-concentration mothers was 23.530 ± 6.35 pg/g. The PCB concentration was significantly different between the high- and low-concentration groups ($F_{1,16} = 40.128$, $P < 0.001$). Thus, we were able to categorize five mothers with male offspring and five mothers with female offspring in both high-concentration and low-concentration groups.

We observed 21 behavior categories of the mother–infant interaction in the same manner as in the BPA study discussed earlier (Nakagami et al. 2010). We first implemented a discriminant analysis to prove that the infants of the high-concentration-group mothers were in fact behaviorally distinct from the infants of the low-concentration mothers. The categories contributing to the discriminant score were locomotion (positive) and visual exploration (negative), with the low-concentration-group infants having a tendency to be more active than those in the high-concentration group.

We conducted principal components analysis to identify the behaviors that made the two groups distinct from each other. We obtained seven factors that accounted for 74.714% of the variance. All subjects' principal component scores were analyzed by a three-way ANOVA (PCB concentration × age × sex); it showed a significant difference in PCB concentrations and a significant interaction (PCB × age) in PC1 and PC3. This means that the infants of the high-concentration mothers developed differently from infants of the low-concentration mothers during the early stage of life.

Behavior categories contributing to PC1 were approach, proximity, look, and locomotion. All behaviors except locomotion represent mother–infant interaction: Approach is reaching a hand to the mother or moving toward her; proximity is being close to the mother, within reach of one hand; and look is facing the mother to look at her face. Locomotion represents other activities of the infant. The three-way ANOVA (PCB concentration × sex × age) showed no sex difference in the four behavioral categories. We then implemented a two-way ANOVA and found a significant interaction between PCB concentration and age (i.e., the first 3 months and the last 3 months during the 6-month observation period). The occurrence of these four categories (proximity, look, locomotion, approach) was significantly different only during the later period, between 4 and 6 months, but not in the earlier months. There was no increase in behavior from the earlier stage of development until the later stage in either sex for offspring of high-concentration mothers (Fig. 17.5). We can see that the interaction between the low-concentration mother and infant

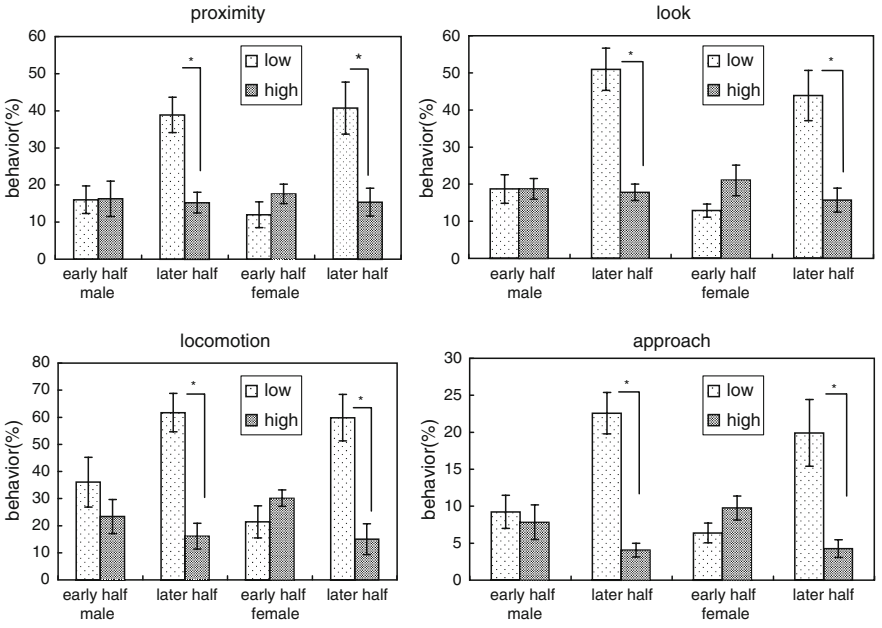

Fig. 17.5 Developmental change of four behavioral items (proximity, locomotion, look, approach) shown by offspring of high- and low-polychlorinated biphenyl (PCB)-contaminated mothers during the nursing period. *Asterisk* shows a significant difference ($P < 0.05$) (Nakagami et al. 2010)

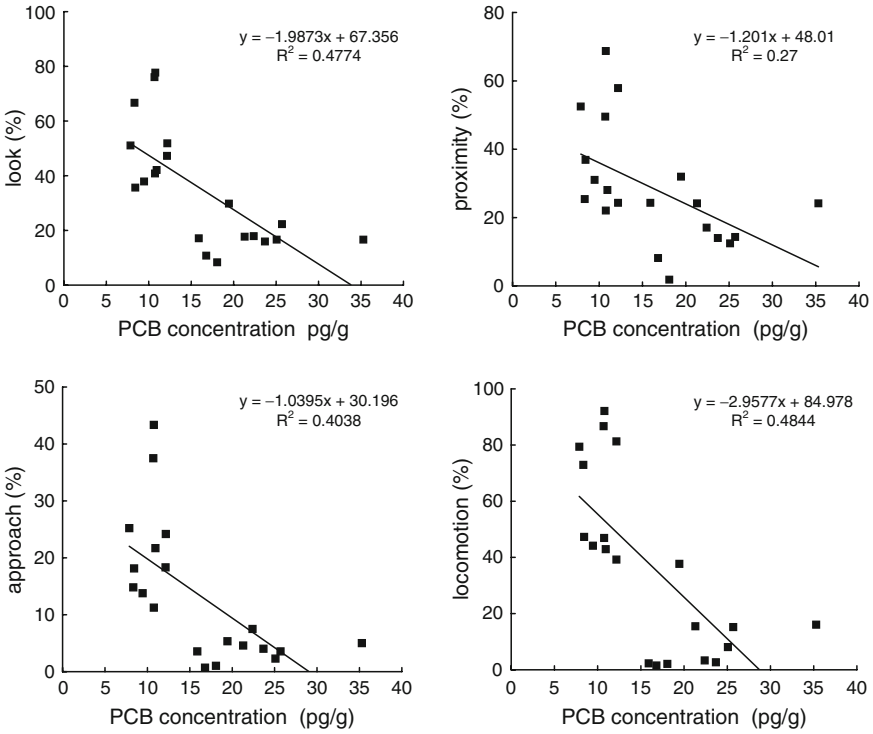

Fig. 17.6 Correlation between mother's level of PCB contamination and the occurrence of her offspring's four behaviors (Nakagami et al. 2010)

becomes more active during the later half of the nursing period, which is not the case between the high-concentration mother and her infant.

This conclusion was bolstered by the observation of a negative relation between PCB concentration and occurrence of these four behaviors (Fig. 17.6). The plots of all subjects and the regression lines show weak but significant negative correlation between a mother's level of PCB contamination and her offspring's behaviors (e.g., approach, proximity, look, locomotion). The offspring of high-concentration mothers did not follow the normal developmental route. They seemed to be less active, did not approach their own mothers, and did not look at the mother at the beginning of their development.

17.5 How We Assessed Behaviors Affected by EDCs and Recommendations for Further Studies

We can draw some conclusions from the above-reviewed studies. Dioxin is less poisonous to the brain than its toxicity might indicate, and this result was consistent with the findings reported by Schantz et al. (1992). BPA in low doses, however, is more

toxic than proclaimed by the government; exposure to low-dose BPA affected the offspring of mothers contaminated by BPA during the perinatal period. Environmental PCBs have disseminated much faster and more widely than expected, and the effect of this contamination has brought, is bringing, or might keep bringing serious results to human life. Despite our small samples, the 30 cynomolgus female monkeys collected as subjects were observed to be already contaminated by PCBs, some seriously. The higher the accumulation of PCBs in the mother's body, the less evidence there was of sociability in her offspring.

It is becoming clear that BPA not only functions like estrogen but that it also affects monoamine transmitters, which act on estrogen receptors. Furthermore, PCBs disrupt thyroid hormones and monoamine transmitters in the brain other than affecting the sex hormone secretion. BPA disturbs the thyroid hormone and prevents normal development of the brain (Zoeller et al. 2005). Other EDCs affect the brain in a similar complex fashion; therefore, the study of EDCs is undoubtedly important to society.

The long history of psychology includes many studies by behavioral pharmacologists on the effects of chemical products on animals. Typically, this research is carried out using procedures that follow a learning experiment or simple observation paradigm, as in sexual behavior or by activity or emotional responses in an open field test. As a result, most investigations have been performed on rodents. Carrying out experiments on the effects of PCBs on animals in social situations allow us to generalize these behavioral findings to human society. Animals selected for such experimental studies should be higher primates such as macaques because their cerebral structure is similar to that of humans and their social structure is complicated. Higher primates are therefore a good model of human society, especially in studies of mother–infant relations or peer relations, as many historic primate studies have shown (Harlow and Mears 1979). Schantz and colleagues performed important research on the effects of EDCs in Wisconsin (Schantz et al. 1986, 1992; Schantz and Bowman 1989). Schantz also paid attention to toxic chemical pollutants such as lead and dioxin during the early days. She conducted some experiments by observing macaque behaviors and concluded that dioxin (2,3,7,8-TCDD) did not have such serious effects on mother–infant interactions and peer relations, but PCBs caused some learning ability deficit. Recently, some researchers have postulated a relation between exposure to EDCs and developmental disorders such as ADHD, LD, or other behavioral disorders (Rice 1997; 2000; Rice and Hayward 1997). Further behavioral studies of primates are needed to investigate the effects of EDCs on human society and the natural environment.

We have observed mother–infant and peer interactions in a laboratory. Compared with previous behavioral studies, our research was unique in that we deliberately used multivariate analyses such as discriminant analysis, canonical discriminant analysis, and principal component analysis. By using these analytical methods, we successfully detected differences between contaminated groups and intact groups as well as the presence or absence of sex differences in 15–40 discrete variables of behavior. We proved that the behavioral observation paradigm is useful and that it

successfully enabled us to find key variables to study the subtle influences of low-dose EDCs on the brain; thus, we stress the validity of this paradigm in the psychobiological field.

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Chapter 18
Functional Association Between the Brain and Physiological Responses Accompanying Negative and Positive Emotions and Its Regulation by Genetic Factors

Hideki Ohira

18.1 Introduction

A seventeenth century philosopher, Benedict de Spinoza, defined emotions as follows.

> By emotion I mean the modifications of the body, whereby the active power of the said body is increased or diminished, aided or constrained, and also the ideas of such modifications.
>
> (*Ethics*, part 3, definition 3)

Here, emotions are considered as changes in bodily states caused by external or internal stimuli and, at the same time, awareness of such bodily changes. In Spinoza's thoughts, both mind and body are critical for emotions. Additionally, in modern neuroscience, the mind is a phenomenon that functions of the brain should produce. Motivated by such thoughts, this chapter discusses aspects of emotions in a perspective of associations between the brain and body.

Emotions have been developed though evolutional processes and thus must have been beneficial for the survival of animals, including humans. For survival, we have to detect potentially threatening stimuli rapidly in environments and elicit appropriate behaviors to it (e.g., approach versus avoidance, fight versus flight). In these situations, brain and bodily associations should be critical for executing efficient coping behaviors. Furthermore, for appropriate behaviors according to demands of the emotional situation, the regulation of strength and the temporal length of the emotional response are also important. Thus, neural and physiological bases of processing and regulation of emotions are discussed herein on the basis of recent findings.

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18.2 Brain and Physiological Responses in Negative Emotions

Roles of emotions are more dominant in domains of negative emotions. Emotions such as fear, anger, and stress are directly linked to our survival. In this section, recent findings about neural and physiological bases of processing and regulation of negative emotions are introduced.

18.2.1 Emotional Responses and Emotion Regulation

18.2.1.1 Automatic Response of the Amygdala and Its Regulation

Probably the most important region in the brain that is related to emotions is the amygdala. The amygdala is an almond-shaped group of nuclei located deep within the medial temporal lobes of the brain. The amygdala is believed to play critical roles in detecting emotional stimuli, initiating emotional responses, forming emotional memories, and eliciting emotional behaviors (Whalen and Phelps 2009).

One of prominent properties of the amygdala is that it can work rapidly and without conscious awareness. Especially, recent neuroimaging studies using functional magnetic resonance imaging (fMRI)[1] have indicated that the amygdala is involved in "subliminal perception"[2] of fearful and angry faces (Whalen et al. 1998; Morris et al. 1998), probably via a subcortical pathway to the amygdala, including the superior colliculus and pulvinar (Morris et al. 1999). These findings suggest that we cannot necessarily identify the origins of emotions and sometimes are not even aware of emotional responses evoked in ourselves. In addition, those unconscious emotions can drive automatic behaviors. Indeed, some social psychologists have argued that most of our social behaviors are regulated through such automatic emotions and motivations without elaborative thoughts (Bargh 1997).

We explored roles of the amygdala in such unconscious emotions and those implicit influences on perceptions and behaviors by using event-related fMRI (Nomura et al. 2004). This neuroimaging technique enabled us to examine brain activities time-locked to the onset of stimulus presentation and processing of the

[1] Functional magnetic resonance imaging (fMRI) is a neuroimaging technique that measures the hemodynamic responses (changes in blood oxygen levels) related to neural activity in the brain. Contrary to the conventional "block-design fMRI," which detects integrated brain activity within a several-minute period, "event-related fMRI" can detect temporal brain activity related to a specific event of stimulus onset or initiation of processing with temporal resolution of several seconds.

[2] Subliminal perception is a psychological phenomenon that one can detect or be affected by any stimuli below a sensory threshold for conscious perception. For visual subliminal perception, stimuli are presented with a short duration (several milliseconds) and are masked by other stimuli. Although this concept has been controversial in scientific fields, studies in cognitive psychology and cognitive neuroscience have shown that humans can perceive stimuli without conscious awareness in some cases.

stimulus with a temporal resolution of 2 s. We used a task based on the paradigm of subliminal affective priming[3] (Murphy and Zajonc 1993). Specifically, the paradigm of the present study consisted of two experimental conditions. Either a facial expression of anger or an affectively neutral facial expression was randomly presented as a prime for 35 ms and was masked by an ambiguous and weak-anger facial expression as a target for 500 ms (Fig. 18.1a). The required task was to categorize the facial expression of anger, neutrality, or happiness each target face

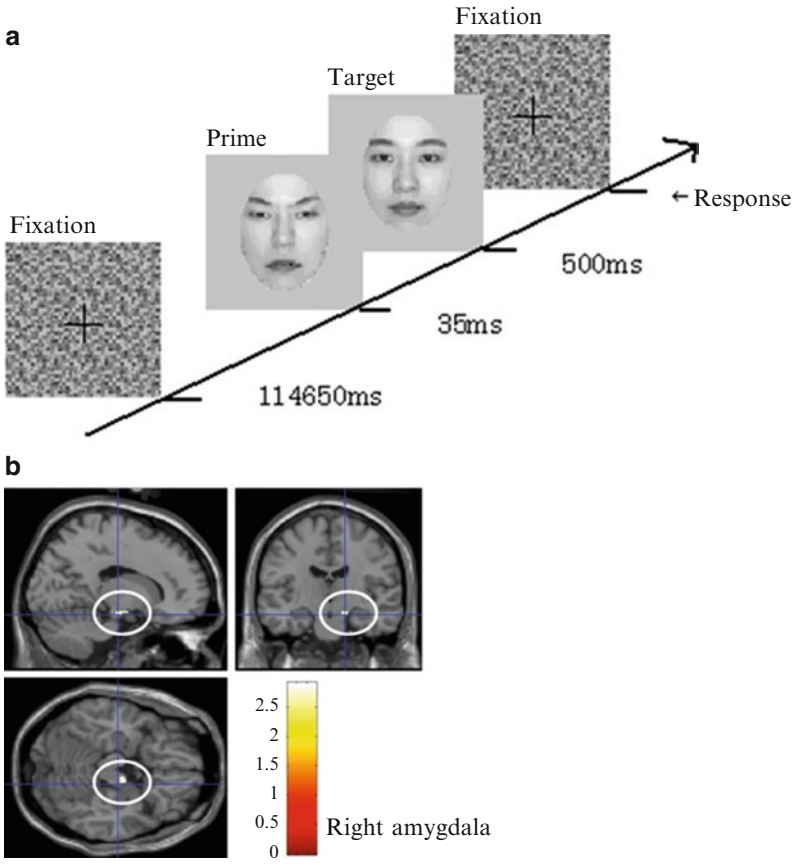

Fig. 18.1 (**a**) Procedure for subliminal affective priming. Angry or neutral faces were presented for a duration of 35 ms and masked by neutral faces. (**b**) Angry faces subliminally presented activated the right amygdala without conscious awareness

[3] Affective priming is a psychological phenomenon that a preceding stimulus with affective valence can influence on processing of a following stimulus. When the preceding stimulus and the following stimulus have the same affective valence (e.g., positive or negative), speed and efficiency of processing for the following stimulus is facilitated. Additionally, evaluation of the following stimulus (e.g., good or bad) can be shifted to the direction according to the affective valence of the preceding stimulus.

showed. This technique can make conscious perception of the prime faces impossible. Additionally, we predicted that perception of the target faces that expressed ambiguous and weak anger can be unconsciously shifted to a perception of anger by the subliminally presented primes of angry faces.

Results showed that the right amygdala was significantly more activated in the anger prime condition than in the neutral prime condition (Fig. 18.1b). This result is consistent with those of previous studies reporting involvement of the amygdala, especially on the right side, in processing negative facial expressions such as fear and anger presented subliminally (Morris et al. 1998; Whalen et al. 1998). More importantly, we showed that the rate of judgment of anger to the target faces was positively related to activity in the right amygdala; that is, individuals who exhibited prominent activation in the right amygdala when they received a subliminal emotional signal with negative valence (anger) probably evoked implicit negative emotional responses and unconsciously utilized the inner representation of negative emotions as a cue to interpret current stimuli, resulting in an increased possibility of interpreting the current stimuli in ways congruent with their emotional states (judgment of the target face as anger).

These sensitive and powerful functions of the amygdala might be a double-edged sword. The merit, of course, is rapid detection of a potential threat and coping to it. However there is the possibility of a mistake that a nonthreatening stimulus can be regarded as dangerous and wrongly addressed. Once the mistake happens, it is difficult to correct it because the processes progress automatically and unconsciously. Therefore, such a sensitive system must be regulated appropriately. We also found that the ventrolateral prefrontal cortex (VLPFC) is the site of such regulation over the amygdalar activity: Activity of the VLPFC showed a negative correlation with activity of the amygdala (Fig. 18.2). It has been widely argued that the right VLPFC is involved with inhibition of responses (Garavan et al. 1999; Konishi et al. 1999). Our findings suggested that the VLPFC can work to inhibit emotional drives to responses even when we are not aware of its existence.

18.2.1.2 Voluntary Regulation of Emotions

The inhibitory control of the VLPFC over amygdalar activity is, in a sense, automatic regulation that has been embedded in the emotional neural system. This regulatory mechanism has been developed through evolution and thus is not specific to humans. However, humans live in social environments where additional regulation of emotions is necessary. Imagine that you are being severely scolded by your superior. Even if the superior is stupid and his or her remark is irrational, you probably try to regulate your own emotions: you might try not only to inhibit behavioral responses such as aggression or escape but also to conceal any symptoms of negative emotions including trembling, blushing, and tension. How can we complete the emotion regulation voluntarily? What neural bases are there for the voluntary emotion regulation?

To examine those research questions, we conducted a combined neuroimaging study where we simultaneously recorded brain activity using ^{15}O-water positron

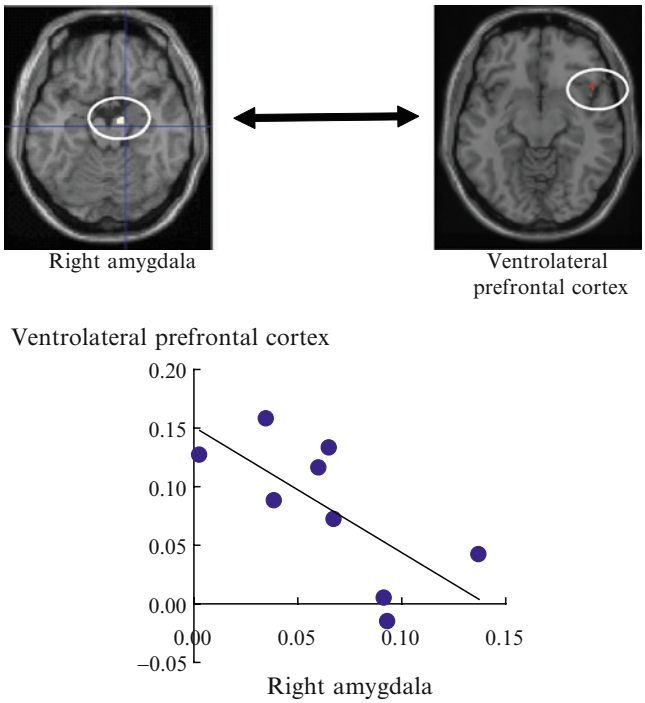

Fig. 18.2 Activation of the ventrolateral prefrontal cortex (VLPFC) negatively correlated with activation of the amygdala, suggesting that the VLPFC has a role in inhibitory control over amygdalar activity

emission tomography (PET)[4] and autonomic [heart rate (HR) and skin conductance response (SCR)] and neuroendocrine [adrenocorticotropic hormone (ACTH)] activities during emotional experiences and voluntary suppression of emotions (Ohira et al. 2006). One limitation of PET is a less temporal resolution: We can only examine integrated brain activity (regional blood flow) over several minutes using ^{15}O. However this technique has the advantage that simultaneous recording of peripheral physiological responses is easy, which is difficult if not impossible with fMRI.

In this study, participants viewed affectively positive, neutral, and negative color pictures in separated 2-min blocks. The pictures were selected from the International Affective Picture System (Lang et al. 1995), which is a validated and standardized set of emotion-inducing pictures and is often used in neuroimaging studies on emotions. Under natural (attending) conditions, participants viewed the pictures and presumably

[4] Positron emission tomography (PET) is a method of neuroimaging using some radioactive chemical tracers. Although PET is usually utilized to scan the distribution of some receptors of neurotransmitters in the brain and metabolism of glucose in the brain, PET using ^{15}O as a tracer has better temporal resolution and can detect changes of blood flow in the brain (details of PET techniques are provided in Sect. 5.3 of this volume).

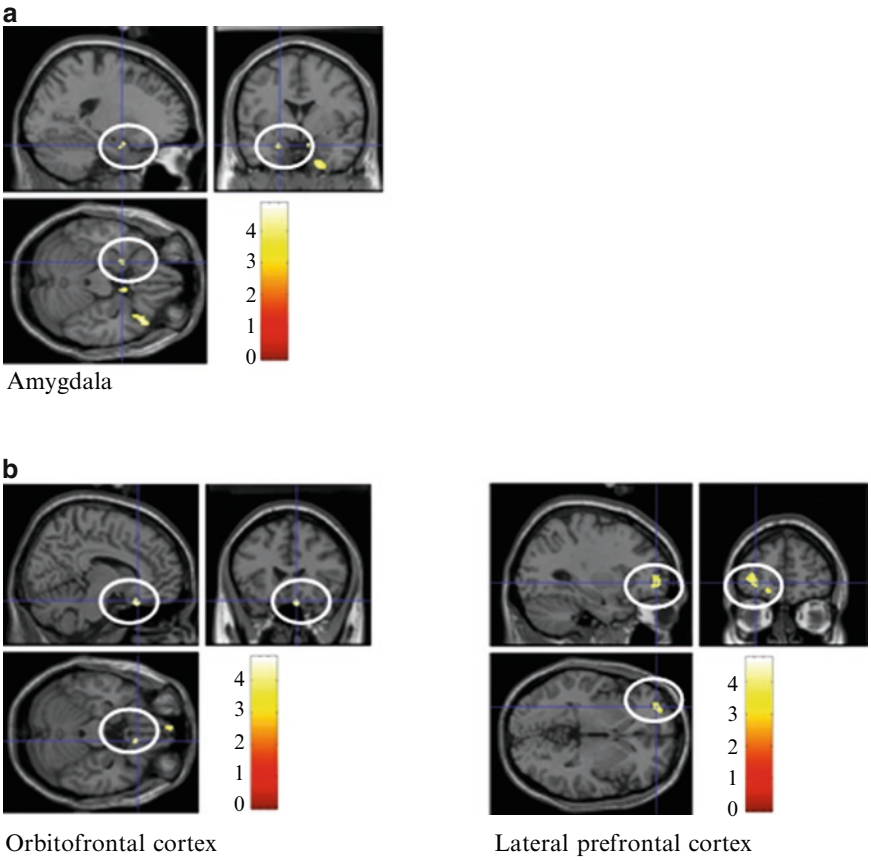

Fig. 18.3 (**a**) Attending to emotional pictures activated the amygdala. (**b**) On the other hand, voluntary suppression of emotion activated some prefrontal regions

experienced positive, neutral, or negative emotions. In a suppression condition, they were required to conceal any emotional responses including physiological responses. The left amygdala was robustly activated during the attending task (Fig. 18.3a). Furthermore, activation in the amygdala positively correlated with magnitudes of the SCR and ACTH responses in this condition. These results suggested that the amygdala elicited autonomic and endocrine responses, probably via direct neural projections to the hypothalamus and midbrain regions. On the other hand, these responses were abolished in the suppression condition. Instead, activation was observed in the left lateral prefrontal cortex (LPFC) and medial orbitofrontal cortex (OFC) during the suppression condition (Fig. 18.3b).

The LPFC, through its meta-cognitive/executive top-down processes (Goldman-Rakic 1987; Fuster 1999), might maintain the goal of regulating one's own inner states to desired outcomes. The OFC, especially its medial area, is thought to have an inhibitory control function over the amygdala (Price 1999, 2003) through its

direct neural projections to the amygdala (Cavada et al. 2000). Because the OFC also has direct neural connections to the LPFC (Price 1999; Kringelbach and Rolls 2004), it might play a pivotal role in harmonizing emotion regulation. Taken together, our results suggested that different regions of the prefrontal cortex play specific roles to accomplish the top-down regulation of emotions.

18.2.2 Acute Stress and Controllability

Stress is defined as a set of biological responses to defend organisms against challenges from environments; it is usually accompanied by affectively negative emotions. Acute stressors stimulate the sympathetic-adrenomedullary (SAM) system and the hypothalamic–pituitary–adrenal (HPA) axis, leading to physiological responses that can be interpreted as recruitment of energy to cope against a threat for survival. Thus, stress reactivity is essential for environmental adaptation; and, conversely, dysfunction of this reactivity can be a cause of physiological and psychiatric disorders (McEwen 1998; Charmandari et al. 2005). Another biological response accompanying acute stress is rapid changes in the redistribution of lymphocytes in blood. Specifically, circulating numbers of lymphocytes representing innate immunity, such as natural killer (NK) cells, increase during acute phases of psychological stress (Dhabhar et al. 1995; Isowa et al. 2004, 2006; Kimura et al. 2005). Increasing numbers of peripheral innate immune cells that can nonspecifically react to any antigens might be interpreted as a preparation step for potential invasion by bacteria from injuries accompanying fight–flight behaviors (Engler et al. 2004). Numerous studies have shown that redistribution of NK cells during acute stress situations was mediated by activation of both the SAM and the HPA axis (Mills et al. 1995; Bosch et al. 2005).

However, a stable pattern of physiological responses to acute stress would be less effective. Rather, continuous assessment of environmental demands and dynamic modulation of responses to deal with those demands are critical for adaptation. Psychological models of stress adaptation (Lazarus and Folkman 1984; Blascovich et al. 1999) have focused on cognitive appraisal of such processes. In particular, in response to a stressful event, whether the event is impactful is first assessed (primary appraisal). Then, controllability of the event and the individual's coping resource to the event – or whether the event is a threat or challenge to the individual – is evaluated (secondary appraisal). As a result of such a series of appraisal processes to the stressor, subjective feelings and behaviors can be affected. Previous studies have shown that autonomic, endocrine, and immune systems can react differently to a particular stressor on the basis of the appraisal of controllability (Peters et al. 1999, 2003; Maier and Watkins 2005; Isowa et al. 2006).

Therefore, we examined the neural basis of modulating physiological stress responses that accompany appraisal of the controllability of an acute stressor (Ohira et al. 2008). We used the simultaneous recording of ^{15}O-water PET and cardiovascular [HR and blood pressure (BP)] and immune (proportion of NK cells in peripheral blood) activities during a typical laboratory acute stress task: a continuous mental

arithmetic task with time pressure. The degree of controllability of the task was manipulated by feedback about subjects' performance during each trial: Feedback indicating a correct answer or feedback indicating an error exactly corresponding to the subject's performance represented a high controllability condition, whereas bogus feedback was irrelevantly given to the subject's performance with some probability in a low controllability condition. With the low controllability condition, subjects would experience a gap between subjective perception about their own performance and feedback about performance, resulting in experiences of lower controllability for the task.

We found that regions in the prefrontal cortex, especially the OFC, medial prefrontal cortex (MPFC), and anterior cingulate cortex (ACC), were activated specifically in the low controllability condition (Fig. 18.4). These results suggested that prefrontal regions are involved in cognitive appraisal of controllability of the acute stressor. Interestingly, these regions were also involved in modulation of peripheral physiological responses. Specifically, the degrees of both autonomic (HR and BP) and immune (increased NK cell proportion) responses were suppressed in the low controllability condition compared to the high controllability condition. This phenomenon has been repeatedly found in our studies (Kimura et al. 2007; Ohira et al. 2009a) and can be interpreted as a kind of energy-saving coping, because it is risky to allocate much

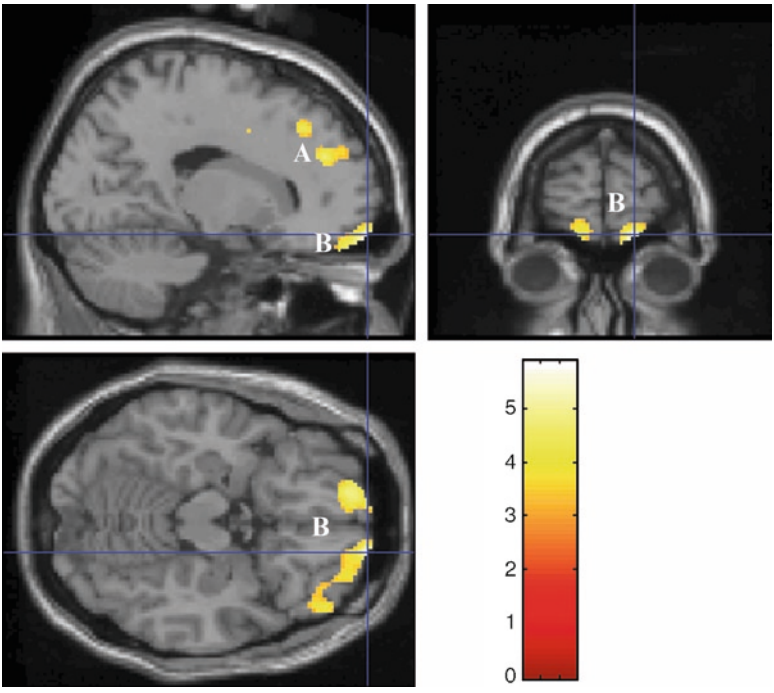

Fig. 18.4 Cognitive appraisal of low controllability in an acute stress situation activated the medial prefrontal cortex (**A**) and orbitofrontal cortex (**B**)

biological energy when the situation is uncontrollable and the coping strategy that would be best is uncertain. Activation of the prefrontal neural network (OFC, MPFC, ACC) correlated with HR, BP, and even the proportion of NK cells. The MPFC and ACC have direct neural projections to limbic and midbrain areas that regulate autonomic activities (Kringelbach and Rolls 2004). Additionally, animal studies revealed that secretion of dopamine and serotonin in the OFC and MPFC areas was a key factor for behavioral changes in uncontrollable stress situations (Bland et al. 2003; Amat et al. 2005). Therefore, the neural network including the prefrontal regions should be the center of any appraisal of stressor controllability and, at the same time, modulation of peripheral physiological activities on the basis of the results of the appraisal.

Taken together, our studies (Nomura et al. 2004; Ohira et al. 2006, 2008) illustrated dynamic functional associations between corticolimbic regions of the brain and the peripheral body's accompanying emotional processes. These brain and body associations should be useful for adaptation to environments.

18.3 Brain and Physiological Responses in Positive Emotions

Traditionally, psychological research on emotions has mainly focused on negative emotions, such as fear, anger, and sadness. This is natural because treatment of those negative emotions and emotional dysfunctions such as depression and clinical anxiety has been important. However, recent psychological studies have revealed the significance of positive emotions in regard to our well-being and health. For example, it has been reported that individuals with a great tendency to experience positive emotions, such as happiness and joy, are less vulnerable to viral infections (Cohen et al. 2003; Marsland et al. 2006). Furthermore, the proportion of circulating NK cells increases after positive emotions are experienced due to sexual arousal (Haake et al. 2004). However, the knowledge about associations of the brain and body accompanying positive emotions is limited.

Thus, we attempted to examine psychological, neural, and physiological responses – including central nervous, endocrine, and immune parameters – accompanying positive emotions (Matsunaga et al. 2008). It is relatively more difficult to elicit positive emotions than negative emotions in experimental settings. This is probably due to wide individual differences of stimuli inducing positive emotions. For example, aversive pictures such as those of snakes, spiders, or an injured body can robustly elicit negative emotions. However, whether pictures of beautiful natural scenes or pretty babies and animals can elicit positive emotions depends highly on the individual's subjective preferences. Therefore, we used "order-made" stimuli to elicit positive emotions. Seeing one's favorite person such as a love interest or favorite actor/actress may evoke positive emotions and occasionally lead to a feeling of elation (Esch and Stefano 2005; Stefano and Esch 2005; Planalp et al. 2006). Thus, in this study, male participants themselves selected female persons (actresses, singers, or television talents) whom they found attractive, and positive emotions were manipulated by their viewing a film featuring these attractive persons.

We simultaneously recorded various parameters such as mood states, brain activity, peripheral circulating NK cell activity, and the serum levels of catecholamines (epinephrine, norepinephrine, dopamine) while the subject was viewing a films that induced positive emotions.

Results confirmed that this technique successfully induced robust positive feelings in the participants. Interestingly, NK cell activity was elevated, and the peripheral dopamine level increased significantly after exposure to the positive affective stimuli. It is widely known that secretion of dopamine is related to reward; thus, the positive affective stimuli in our study should have worked as reward for the participants. We speculated that increased dopamine in peripheral blood might reflect transportation of dopamine that was secreted in the brain as a result of exposure to reward via dopamine transporters. Despite a lack of direct evidence, our speculation is supported by the finding that the concentration of homovanillic acid in the plasma (which has been used as a peripheral marker for central dopaminergic neurotransmission) positively correlated with the density of dopamine transporters in the brain measured by single photon emission tomography (SPECT) (Bowers et al. 1998). On the other hand, epinephrine and norepinephrine, which are typical neurotransmitters related to stress or negative emotions, did not increase after exposure to the positive stimuli. Furthermore, the peripheral dopamine level was positively correlated with NK cell activity, which suggests that NK cells were activated via dopamine receptors distributed in the surface of NK cells. This interpretation is supported by the fact that expression of various dopamine receptors has been found in lymphocytes, including NK cells (McKenna et al. 2002), and by the finding that NK cell activity was suppressed by dopamine receptor antagonists in vitro (Won et al. 1995). In addition, the change in the dopamine level was positively correlated with positive mood scores (Fig. 18.5), suggesting that positive emotions induced by viewing the films of favorite persons can work as a reward. Thus, positive emotions elicit a totally different profile of psychological and physiological responses from those elicited by negative emotions.

Elevation of innate immune function (NK cell activity) probably is beneficial for adaptation. Importantly, this can be completed not by epinephrine and norepinephrine, which have strong effects but are somewhat risky for organisms, but by dopamine, which is less powerful but safer for the organisms.

For brain activity, the rostral MPFC was especially activated while viewing the films inducing positive emotions. Furthermore, activation in the same region of the brain positively correlated with NK cell activity and the peripheral dopamine level while watching the positive film. Indeed, the MPFC, especially its rostral region, is a portion of the brain reward system. There are many projections of dopamine neurons into the rostral MPFC. Additionally, as described above, the MPFC is a portion of the neural network of cognitive reappraisal and top-down modulation over peripheral physiological responses.

The results in our study consistently indicated that the dopaminergic reward system in the brain plays a key role in experiences of positive emotions. Moreover,

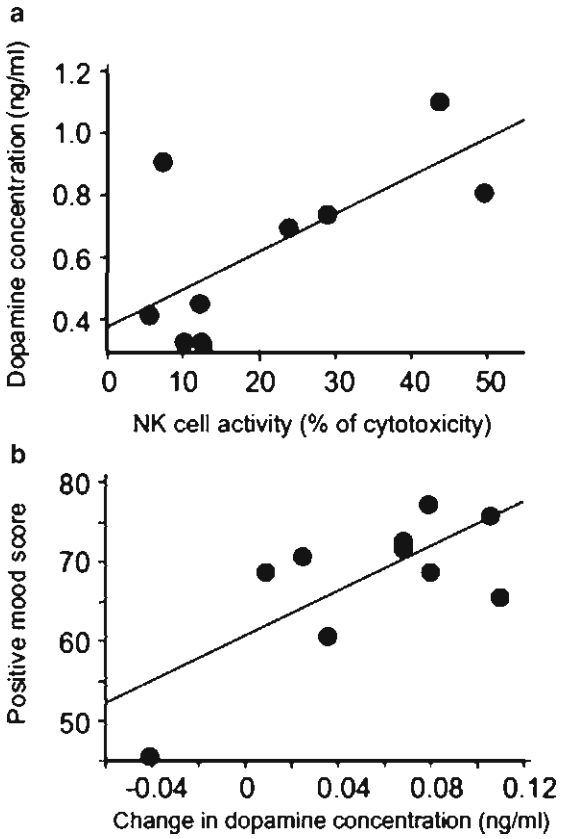

Fig. 18.5 (**a**) Concentration of peripheral dopamine after exposure to a positive emotional stimulus correlated positively with natural killer (NK) cell activity. (**b**) At the same time, the degree of positive emotions determined dopamine secretion. Reproduced from Matsunaga et al. (2008), with permission

it plays a role in the modulation of bodily states to facilitate psychological and physiological well-being, which should be beneficial for adaptation.

18.4 Genetic Modulations of Negative and Positive Emotional Responses

As we experience in everyday situations, there are wide interindividual differences and relatively intraindividual consistencies in emotional responses. Such characteristics lead us to infer that at least some portions of individual differences of emotions can be explained by genetic factors. In many chapters of this volume, significant findings have been introduced about genetic factors affecting personalities, including emotional aspects in animals (e.g., Chaps. 11–13) and humans (Chap. 10).

18.4.1 Polymorphism of the Serotonin Transporter Gene

18.4.1.1 Acute Stress and Serotonin Transporter Gene Polymorphism

At present, one major genetic factor determining individual differences in emotional reactivity is a polymorphism of a gene coding the serotonin (5-hydroxytryptamine, or 5HT) transporter (5HTT). 5HTT plays a critical role in regulating 5HT levels in the brain by transporting 5HT from the extracellular space into the neuron. The human 5HTT gene is encoded on chromosome 17q11.1-q12 (Ramamoorthy et al. 1993) and has a polymorphism in the 5′-flanking promoter region termed the serotonin transporter gene-linked polymorphic region (5HTTLPR) (Heils et al. 1995). In lymphoblast cell lines containing the promoter sequence [long (L) or short (S) form of 5HTTLPR], the promoter activity of the 5HTT gene is dependent on these allelic variants (Heils et al. 1996). Transcriptional activity of the L allele was more than twice as high as that of the S allele (Collier et al. 1996). Thus, the S promoter allelic variant is linked to lower expression of 5HTT mRNA, resulting in less serotonin reuptake when compared to the L allelic variant (Lesch et al. 1996).

In animal studies, rhesus macaques carrying an S allele of 5HTTLPR exhibited exaggerated behavioral and neuroendocrine responses to acute stress and abnormalities in 5HT metabolism (Barr et al. 2004). Accordingly, 5HTT knockout mice showed facilitated catecholamine responses to brief and mild stressors when compared to wild-type controls (Tjurmina et al. 2002). Additionally, Yokoyama and Onoe (see Chap. 19) showed that binding potential values of 5HTT in the common marmoset brain measured by PET were positively correlated with sociability and negatively associated with social anxiety, suggesting that individual differences in5HTT traits determine animal personalities related to emotions. In humans, individuals with one or two copies of the S allele exhibited higher rates of depression and suicidal tendency as a function of exposure to increasing levels of stressful events than did individuals with two copies of the L allele (Caspi et al. 2003). The S allele carriers also showed enhanced secretion of cortisol in response to acute psychological stressors (Gotlib et al. 2008). The S allele was also associated with greater heart rate reactivity to laboratory stress, especially in women (McCaffery et al. 2003). Importantly, the polymorphism of 5HTT can regulate activity of emotion-related brain regions. Hariri et al. (2002) was the first to indicate that the S allele carriers showed enhanced activation of the amygdala while viewing fearful faces. Furthermore, S allele carriers showed uncoupling in the cingulate and amygdala circuit, which is critical for emotion regulation (Heinz et al. 2005; Pezawas et al. 2005). Because the amydgala has direct neural connections to the hypothalamus and midbrain, hyperactivity of the amygdala in the S allele carriers might be a rational cause of their hyperactive physiological responses, such as cortisol and HR, observed in previous studies.

However, to date, there has been no direct test for effects of 5HTTLPR on the reactivity of the brain or peripheral physiology to emotional challenges. Thus, we examined this possibility regarding a negative emotion: acute stress (Ohira et al. 2009b). More specifically, we predicted that S allele carriers would show greater activation

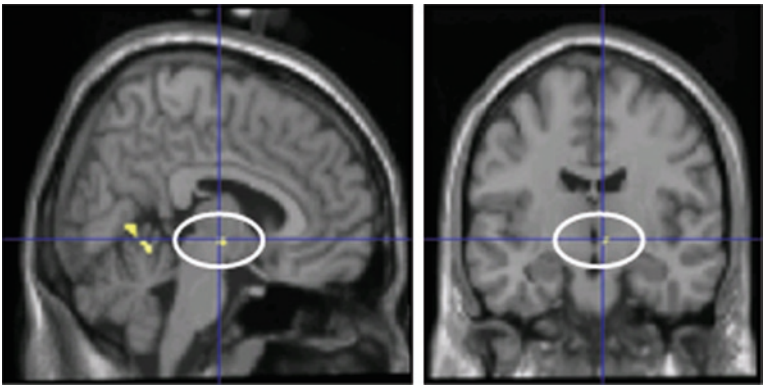

Fig. 18.6 Individuals who had the short variant of alleles of the serotonin transporter gene exhibited more activation of the hypothalamus in an acute stress situation than did individuals who had other genotypes of the serotonin transporter gene

in stress-related brain regions and more prominent enhancement of physiological responses to an acute stressor. We examined this hypothesis by simultaneous measurement of ^{15}O-water PET and cardiovascular (HR, BP) and neuroendocrine (epinephrine, norepinephrine, ACTH) indices during a typical experimental acute stress task: mental arithmetic with time pressure (Ohira et al. 2008). This task is identical to the one described in an earlier section. Participants conducted the mental arithmetic task with time pressure in three blocks that lasted 2 min each. Results supported our hypotheses: carriers of double S alleles (SS), compared to carriers of single or no S alleles (SL and LL), showed greater secretion of epinephrine and norepinephrine during the acute stress task, especially in its initial stage (block 1). Furthermore, the SS carriers indicated greater activation of the hypothalamus – which is the center of autonomic activity during all blocks of the task – than the SL and LL carriers (Fig. 18.6). No activation of the amygdala was observed. The cause of this lack of amygdalar activation might be due to a limitation of temporal resolution of imaging using PET. The amygdala is one of the most sensitive regions in the brain and shows rapid activation and fast habituation. The amygdala in the SS carriers might have been activated only at the initial stage of the stress task, with its activation being rapidly habituated; thus, it could not be detected by PET imaging. Further tests should be conducted to clarify this issue using fMRI and the same stress task.

18.4.1.2 Positive Emotions and Serotonin Transporter Gene Polymorphism

The S allele of the 5HTT gene has been linked to maladaptive emotional characteristics, such as depression and dysfunctional emotion regulation in previous studies conducted in Western countries. Influences of this gene polymorphism on positive aspects of emotions have not been considered at all. For example, the amygdala is

sensitive not only to negative emotional stimuli but also to positive emotional stimuli (Murray 2007). Thus, if the SS carriers of the 5HTT gene had hyperactivity in their amygdala they might show greater emotional responses not only in negative, but also in positive, emotional situations. To test this speculation, we conducted a combined study (Matsunaga et al. 2010) of ^{15}O-water PET and innate immune activity (proportion of NK cells in peripheral blood) during induction of positive emotions induced by seeing a film clip of a favorite opposite-sex person, which is the same paradigm with our previous study (Matsunaga et al. 2008) described above. Either an emotionally neutral film or a film featuring women who male participants considered attractive was screened for 4 min on a display. PET was performed during the first 2–3 min of the screening. Blood samples were obtained before and after the screening to measure the proportion of NK cells among peripheral lymphocytes. Interestingly, the male SS carriers, compared to their SL and LL counterparts, showed stronger activation of the amygdala when viewing the video of their favorite women. Furthermore, this activity of the amygdala was positively correlated with increases in the NK cell proportion among peripheral lymphocytes (Fig. 18.7). Thus, the polymorphism of the 5HTT gene influences the associated interactions between the central nervous and immune systems in affectively positive situations.

These results suggest that S allele carriers are not necessarily anxious or depression-prone people but emotionally sensitive people in both positive and negative directions. Therefore, whether they are adaptive or maladaptive might depend highly on their environment. In situations where they have any threats, they are more anxious and affectively more negative. However, in situations where they can have significant rewards, they are happier and affectively more positive. In this sense, the relation between the polymorphism of the 5HTT gene

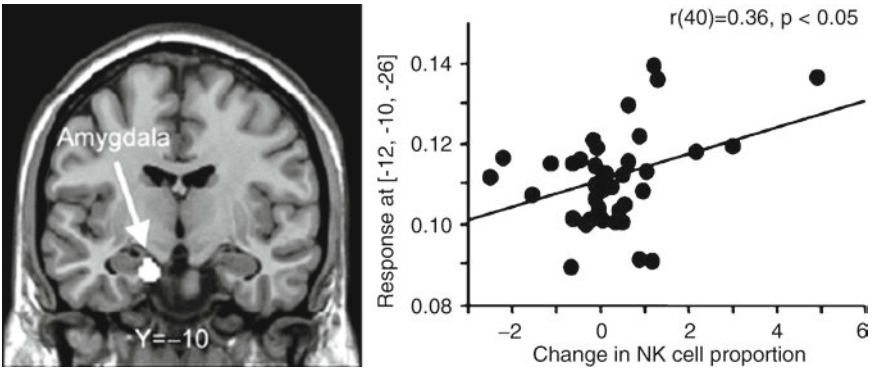

Fig. 18.7 Individuals who had the short variant of alleles of the serotonin transporter gene exhibited a correlation between activation of the amygdala and enhancement of the proportion of peripheral NK cells in a positive emotional situation. This correlation was not observed in individuals who had other genotypes of the serotonin transporter gene. From Matsunaga et al. (2010) with permission

and adaptation is influenced by cultures. A dominant dimension that colors cultures is individualism–collectivism.[5] In individualistic cultures (e.g., American and Western), one must be independent and have much chance and take much risk. In those environments, S allele carriers might be maladaptive. On the other hand, in collectivistic cultures (e.g., Japanese and Eastern), people are interdependent and harmonizing with other people is important. In those environments, S allele carriers might be at an advantage because they are more sensitive to other people's emotions and good at mind reading and cooperation. One item of supporting evidence for this speculation is the fact that the distribution of 5HTT genotypes is highly different among ethnic groups. The frequency of the L allele is 50–60% in Caucasians and less than 30% in Asians (Williams et al. 2001; Mizuno et al. 2006). This seems to indicate that it has been more advantageous for S allele carriers in the history of evolution in Eastern cultures. Furthermore, consistent with the above speculation, Chiao and Blizinsky (2010) showed a clear correlation between the frequency of the S allele of the 5HTT gene and tendencies of individualism–collectivism across more than 20 countries: more S allele and more collectivism.

18.4.2 Polymorphism of the μ-Opioid Receptor Gene

Even without specific emotional events, we sometimes feel some affective states, such as pleasantness and unpleasantness. Those relatively weak and ambiguous affective states are called "moods" and can influence our mental and physical health states. One of the important factors determining mood states in everyday life is the inflammatory cytokines.[6] Peripheral circulating inflammatory cytokines, which are the immune signaling molecules that promote systemic inflammation, such as tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), and most prominently interleukin-6 (IL-6), reach the brain via leaky regions in the blood–brain barrier, active transport molecules, and afferent nerve fibers (Dantzer et al. 2008). Through those mechanisms, cytokine signals can induce a syndrome called sickness behavior, whose features (including anhedonia, anorexia, impaired sleep, and reduced locomotor activity) overlap with those of major depression. Therefore, circulating inflammatory cytokine levels may be associated with physical and mental health-related quality of life (QOL).

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[5] Individualism and collectivism are concepts that are often used to describe personalities in social psychology. Individualism is a tendency that emphasizes the independence of each person within social groups and focuses importance of one's own goals and self-esteem. Collectivism is a tendency that emphasizes the interdependence of persons in social groups and sometimes focuses on the priority of group goals in preference to individual goals.

[6] Cytokines are proteins and peptides that are secreted by immune cells such as macrophages. They carry signals between immune cells and have effects on the whole-body orgasm. Inflammatory or proinflammatory cytokines are subfamilies of cytokines that enhance cellular immunity and induce inflammation.

The endogenous opioid peptide β-endorphin is known to inhibit IL-6 secretion from the spleen through a μ-opioid receptor-dependent mechanism (Straub et al. 1997). There is a single nucleotide polymorphism (SNP) at position 118 (A118G) in the coding region of the μ-opioid receptor gene in humans. This SNP codes for a change from asparagine to aspartic acid at position 40, resulting in threefold stronger binding of β-endorphin to the μ-opioid receptor (Bond et al. 1998). It is suggested that carriers of the G allele may show more dominant responses mediated by β-endorphin (e.g., analgesia, euphoria, sedation) because of the sensitive μ-opioid receptor (Chou et al. 2006; Lötsch et al. 2006). For example, carriers of at least a G allele display a stronger alcohol-induced euphoria than do individuals without the G allele (Ray and Hutchison 2004).

Therefore, it is suggested that the A118G polymorphism may be involved in secretion of inflammatory cytokines from peripheral immune cells and in the carrier's QOL. On the basis of the above logics, we predicted that carriers of the G allele may have lower peripheral inflammatory cytokine levels and higher QOL than individuals without the G allele. We compared the serum concentrations of several inflammatory cytokines [interleukin-2 (IL-2), IL-6, TNFα, interferon-γ (IFNγ)] and QOL between μ-opioid receptor genotypes (AA, AG, GG) in a healthy population ($n = 123$) (Matsunaga et al. 2009). Consistent with this prediction, concentrations of IL-6, TNFα, and IFNγ in the serum were significantly lower and the score of subjectively rated general health that might reflect QOL was significantly higher in carriers of the G allele than in individuals without the G allele. Furthermore, a correlation analysis indicated that the general health score negatively correlated with the concentration of IL-6 in the serum. These results suggest that the sensitive endogenous opioid system in carriers of the G allele may suppress inflammatory cytokine secretion from peripheral immune cells, consequently improving mood states, and finally be linked to higher perception of good health states.

This finding is interesting because it seems to reflect a relation between happy moods and health. In so-called folk psychology, it is sometimes believed that happy or optimistic people are healthier and can enjoy longer lives. Our study suggested that a biological interindividual variation such as polymorphism of μ-opioid receptor gene can underlie that phenomenon and can provide scientific evidence to support the folk psychology.

18.5 Conclusion and Future Directions

As described in this chapter, mechanisms of the brain and body determine emotional processes. The amygdala can detect emotional stimuli sometimes rapidly and without conscious awareness; and it can automatically bias subsequent perception and behaviors related to emotions (Nomura et al. 2004). The amygdala is involved in the detection of not only affectively negative stimuli but also affectively positive stimuli (Matsunaga et al. 2010). In this sense, the amygdala is a significance detector that can react to any important stimuli. Furthermore, the amygdala can elicit bodily

responses that are probably helpful for coping behaviors during emotional situations. However, patterns of elicited bodily responses are different between those of negative emotions and those associated with positive emotions. In negative emotional situations, the integrated activation of sympathetic, endocrine, and innate immune responses is caused probably for fight–flight behaviors to cope with stressful and threatening stimuli (Ohira et al. 2006). This pattern of responses is modulated by the brain on the basis of cognitive appraisal of the emotional situation (e.g., controllability) (Ohira et al. 2008). In positive situations, secretion of dopamine through the brain reward system marches into action, activating NK cells in the peripheral body; and sympathetic and endocrine responses typically observed during negative emotions are not seen (Matsunaga et al. 2008). In addition, responses of the brain and body are affected by genetic factors: Both negative and positive emotional responses are more enhanced in individuals who have the S allele of the 5HTT gene (Matsunaga et al. 2010; Ohira et al. 2009b), and everyday perception of well-being is boosted in individuals who have the G allele of the μ-opioid receptor gene (Matsunaga et al. 2009).

Finally, I suggest some research questions for future studies. First, a causal relation between activities of brain regions, physiological responses, and subjective emotional experiences, and behaviors should be examined. Combined studies of PET and physiological responses described in this chapter are powerful methods, but they are correlational. Simultaneous fMRI and monitoring physiological responses during emotion and emotion regulation tasks would be helpful. In addition, advanced analysis techniques, such as functional connectivity and dynamic causal modeling, appear promising to explore this issue.

Second, adaptive values of brain and bodily responses accompanying negative and positive emotions should be empirically tested. In this chapter, I tried to interpret the brain and body associations in emotions from evolutional perspectives. However, those arguments are still speculative and remain to be examined. For example, is the increase of NK cells in peripheral blood that accompanies acute stress beneficial to defend against potential infections of antigens?

Third, the significance of positive emotions and accompanying physiological responses should be examined in more detail. For example, why is enhanced innate immune function, reflected by NK cell activity, necessary in positive emotional situations? More fundamentally, how are positive emotions beneficial for our survival?

Fourth and finally, the psychological and biological factors that determine individual differences in emotional processes should be more precisely identified. Examination of the effects of genetic factors should be promoted. Endeavors to clarify those issues should be primary and can ultimately promote our understanding of emotions.

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Chapter 19
Molecular Brain Imaging of Personality Traits in Nonhuman Primates: A Study of the Common Marmoset

Chihiro Yokoyama and Hirotaka Onoe

19.1 PET Imaging as a Tool for Mapping Brain Molecules Underlying Behavior

In vivo brain imaging enables the chronological and comprehensive assessment of the living, intact, whole brain. Among brain imaging techniques, positron emission tomography (PET) using positron-labeled tracers, which are intravenously injected into the body as biologically active and/or specifically binding molecules, enables quantitative visualization of the neurochemical aspects of brain function. The location of a PET tracer can be identified by detecting pairs of gamma rays emitted at 180° angles during the annihilation reaction between a positron and an electron. Images are reconstructed, and three-dimensional images with tracer concentrations are then processed with a computer system (Fig. 19.1). The PET imaging technique enables highly accurate quantitative analysis of even minute amounts of imaged molecules with a minimum burden on the body because of low radiation exposure due to the short half-lives of the tracers used. It is thus a highly sensitive and accurate means of in vivo molecular imaging.

19.1.1 Human Studies

Human PET studies using PET tracers that bind to receptors and transporters in the brain, including those related to dopamine and other neurotransmitters, have revealed abnormalities of neurotransmission in patients with psychiatric disorders (Sedvall et al. 1988). PET imaging makes possible the study of not only alterations in binding densities of receptors and transporters in patients but also the mechanisms

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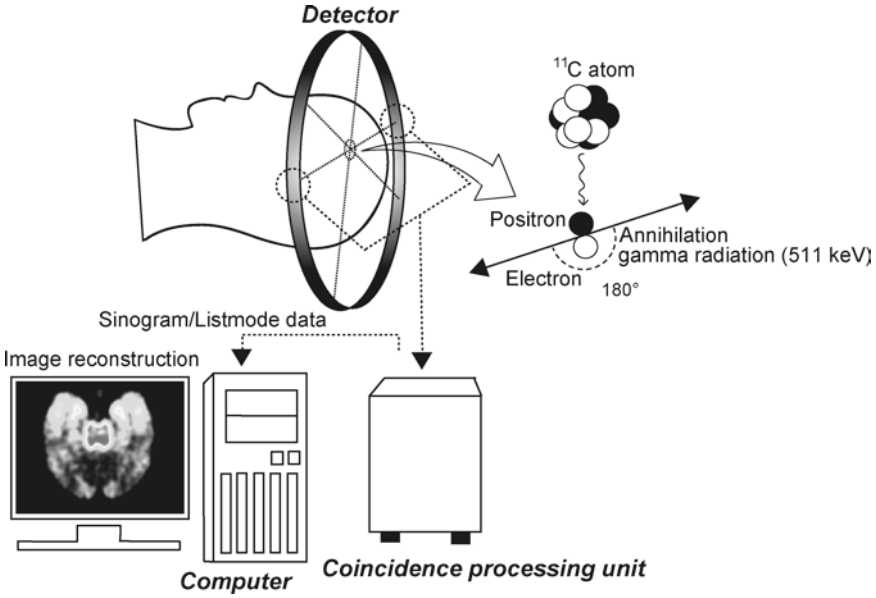

Fig. 19.1 Principles of positron emission tomography (PET)

and sites of action of therapeutic drugs. It thus aids both the development of practical methods of clinical diagnosis and elucidation of the neuropathology of psychiatric disorders.

In studies of individual differences in personality traits, PET imaging using specific radiotracers has enabled analysis of specific neurotransmitter systems over a wide range of human behavior. For example, dopamine has long been of interest in regard to its role in determining personality traits (Ebstein et al. 1996; Depue and Collins 1999). PET imaging has been used to relate the brain's dopamine system with human personality traits including Novelty Seeking and Social Detachment (Breier et al. 1998; Laakso et al. 2000, 2003; Suhara et al. 2001; Reeves et al. 2007). Relations between serotonin and suicidal and/or impulsive aggression have been demonstrated by biochemical measurements of the concentration of 5-hydroxyindoleacetic acid, a serotonin metabolite, in cerebrospinal fluid and hormonal responses to acute challenge with serotonergic agents (Coccaro 1992; Coccaro et al. 1997). Genotyping serotonin transporter (SERT) and receptor gene polymorphisms has been reported to predict anxiety-related and impulsive behaviors (Lesch et al. 1996; Sen et al. 2004; Nomura et al. 2006). In addition, PET imaging studies have found relations between the serotonergic system and traits such as Neuroticism, Harm Avoidance, and Openness as well as a spiritual zeal described as Self-transcendence (Tauscher et al. 2001; Moresco et al. 2002; Takano et al. 2007; Kalbitzer et al. 2009).

Human populations exhibit polymorphic genetic variations in receptors, transporters, and synthetic and catabolic enzymes related to dopamine and serotonin,

which may be related to the transcriptional regulation and functional activity of various molecules. Genetic polymorphisms of monoamine neurotransmission have been found to be associated with personality traits; for example, Novelty Seeking and Extraversion are associated with dopamine receptor D4 polymorphism (Benjamin et al. 1996; Ebstein et al. 1996; Ebstein 2006), and Neuroticism and Harm Avoidance are associated with SERT promoter region polymorphisms (Lesch et al. 1996; Ebstein 2006). However, it is difficult to confirm the findings of such studies because there are many steps between gene expression and complex behavioral phenotypes (Paterson et al. 1999; Kluger et al. 2002; Schinka et al. 2002, 2004; Willis-Owen et al. 2005; Ebstein 2006). In fact, the effects of allelic variations on human behavior are strongly influenced by gene–gene interactions (Ebstein et al. 1997; Benjamin et al. 2000) and environmental factors such as life stress (Caspi et al. 2002, 2003; Manuck et al. 2004; Kim-Cohen et al. 2006). Along these lines, molecular brain imaging has revealed important aspects of the determination of neurochemical endophenotypes[1] mediating events that occur between the genetic level and behavior (Kim-Cohen et al. 2006; Alia-Klein et al. 2008). However, findings of associations between genetic variations and binding densities of some neurotransmitters measured by PET imaging are still inconsistent (Pohjalainen et al. 1998, 1999; Jonsson et al. 1999; Shioe et al. 2003; Parsey et al. 2006; Fowler et al. 2007; Praschak-Rieder et al. 2007; Reimold et al. 2007; Hirvonen et al. 2009). Nevertheless, PET imaging could be exceedingly valuable for examining the function of neurotransmitters in vivo, the characteristics of which are influenced by genetic and environmental factors. Experimental evidence supporting the existence of gene–environment interactions in the determination of personality traits has been obtained in nonhuman primate studies.

19.1.2 Nonhuman Primate Studies

Similar to humans and other animals, nonhuman primates exhibit personality traits (Gold and Maple 1994; King and Figueredo 1997; Gosling and John 1999; Weiss et al. 2006) (see Chap. 5, Sect. 2 and Chap. 6, Sect. 2). Personality traits in the chimpanzee generalize across habitats (King et al. 2005; Weiss et al. 2007, 2009) (see Chap. 11, Sect. 2), exhibit high reliability, temporal stability, and heritability (King and Figueredo 1997; Weiss et al. 2000, 2007; Dutton 2008; King et al. 2008) (see Chap. 5. Sect. 4). Some of the personality dimensions in primates exist across species, including humans (King and Figueredo 1997; McCrae and Costa 1997; Gosling 2001) (see Chap. 5, Sect. 3 and Chap. 6, Sect. 2). Studies with macaque monkeys revealed that central monoamines, including serotonin and dopamine, play critical roles in personality traits related to aggressiveness and social dominance (Yodyingyuad et al. 1985; Mehlman et al. 1994; Kaplan et al. 2002; Morgan et al.

[1]Endophenotype: psychiatric concept for a special kind of biomarker that is a more stable and quantifiable component with a clear genetic connection than behavioral symptoms.

2002; Howell et al. 2007). Furthermore, SERT gene promoter variation affects brain serotonergic function and aggressive behavior in monkeys that have experienced early maternal deprivation but not in normally reared monkeys (Bennett et al. 2002; Barr et al. 2003) (see Chap. 11, Sect. 5). Monoamine oxidizing enzyme gene promoter variation is associated with aggressive behavior, and the relation is also sensitive to early rearing experience (Newman et al. 2005) (see Chap. 11, Sect. 5). These findings strongly indicate that heritable personality traits associated with serotonergic and dopaminergic neurotransmission could be masked and/or enhanced by environmental factors. Although recent methodological developments have permitted the use of experimental animals for in vivo brain imaging, few studies have examined the behavioral personality traits of nonhuman primates (Morgan et al. 2002; Heinz et al. 2003; Ichise et al. 2006). Using a specific tracer for SERT, PET studies with macaque monkeys have revealed that regional abnormalities in brain serotonergic systems may be related to behavioral abnormalities caused by early life stress (Ichise et al. 2006; Wrase et al. 2006), which is similar to that observed in a human PET study (Miller et al. 2009). PET imaging studies with human and nonhuman primates have thus acted as a bridge between basic and clinical research.

19.2 PET Imaging of Common Marmosets

The common marmoset (*Callithrix jacchus*) is a tropical monkey unique among primates for its small body size, rapid reproduction, and cooperative social behavior (Abbott et al. 2003). Although the common marmoset is a New World Monkey and thus less closely related to humans than apes or Old World Monkeys, it exhibits a high degree of tolerance in social dynamics, as represented by food sharing and altruistic responses, which are similar to those observed in humans (Burkart et al. 2007; Kasper et al. 2008). Because the common marmoset is noncompetitive and exhibits cooperative behavioral characteristics, its social learning ability has been studied extensively (Caldwell and Whiten 2004; Schiel and Huber 2006; Voelkl and Huber 2007; Pesendorfer et al. 2009). It is thus valuable as an animal model of human behavior with regard to social conformity, including personality traits related to social affiliation. Here we report in vivo brain imaging of the dopaminergic and serotonergic systems in the common marmoset using a newly developed PET imaging method without anesthesia that was originally established in our laboratory.

To assess accurately ligand–receptor binding pharmacologically and physiologically in animal PET imaging studies, several critical issues such as brain size (about 8.0 g in the common marmoset), the specific activity[2] of radiotracers, and the method used for immobilization during scanning need to be addressed. The PET scanner used in this study is specially designed for animal studies (microPET

[2]Specific activity: radioactivity per unit mass (Bq/g or Bq/mol) for its use in nuclear science.

Focus220; Siemens Medical Solutions, Knoxville, TN, USA). To obtain individual anatomical information, magnetic resonance imaging (MRI) was performed using a 3-T MRI scanner (Signa Horizon Lx VH3; General Electric Healthcare, Milwaukee, WI, USA) equipped with a customized eight-channel coil. To minimize the degree of receptor occupancy and achieve maximal specific binding, a PET tracer with high specific activity is required (Hume et al. 1998). In our laboratory, radiotracers are constantly prepared with high specific activity (50–100 GBq/μmol) and radiochemical purity (>95%), which result in approximately 5% maximum occupancy of binding sites. In addition to these physical and chemical issues, use of anesthetics in animal PET studies for immobilization of voluntary head and body movements during scanning sometimes obscures changes in physiological brain functioning. Because anesthetics have marked effects on cerebral circulation and neuronal activity in the animal brain (Onoe et al. 1994; Tsukada et al. 1999), we developed a PET imaging system for conscious common marmosets. Before the PET experiments, a tiny acrylic tip was surgically attached to the top of the animal's skull. The head tip was used for painless fixation of the animal's head during the PET scanning. The animal was placed in a sitting position with the head fixed to a custom-made chair and the scanner was tilted to 45°, allowing the animal to remain more comfortable than in a supine or prone posture without tilting while conscious (Fig. 19.2).

We performed PET brain imaging of common marmosets with two PET tracers, [^{11}C]-3-amino-4-(2-dimetylaminomethyl-phenylsulfanyl)-benzonitrile ([^{11}C]DASB) and *N*-(3-iodoprop-2E-enyl)-2β-carbomethoxy-3β-(4-methylphenyl)nortropane

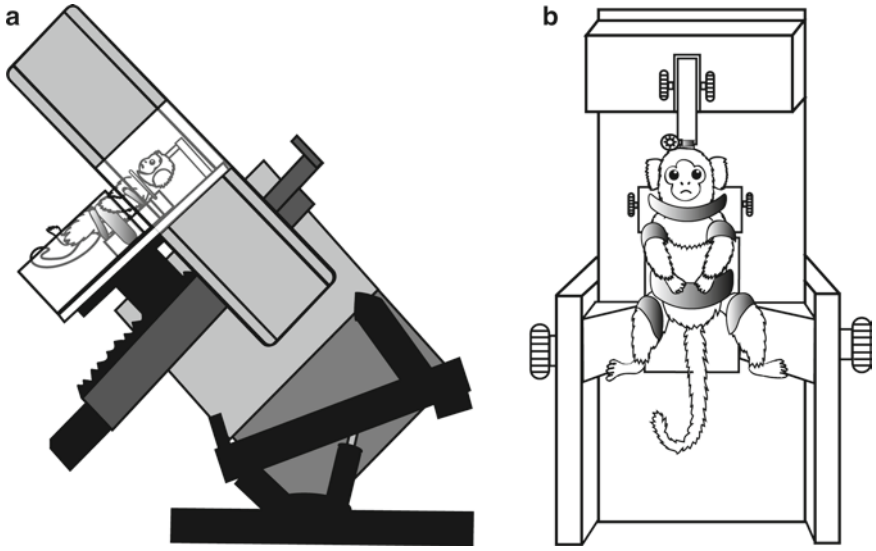

Fig. 19.2 PET system to assess conscious common marmosets. (**a**) PET scanner was tilted to 45°, with the animal sitting on a customized chair fixed to a moving table. (**b**) The animal sits on the chair with head fixation using specialized equipment

([^{11}C]PE2I), which specifically bind to serotonin and dopamine transporters, respectively (Wilson et al. 2002; Halldin et al. 2003). These transporters regulate net rates of serotonergic and dopaminergic transmission and have been related to personality traits in primates including humans (Laakso et al. 2000; Bennett et al. 2002; Heinz et al. 2003; Ichise et al. 2006; Takano et al. 2007; Kalbitzer et al. 2009). Presently, we assessed personality via behavioral responses to a social challenge in individual subjects using encounter trials between unfamiliar males. Finally, we analyzed PET imaging data in combination with personality traits in common marmosets.

19.2.1 Serotonin Transporters

SERTs are located in presynaptic nerve terminals and the cell bodies of serotonin neurons. SERTs play a pivotal role in regulating synaptic signal transduction. They are also target sites of widely used selective serotonin uptake inhibitors in a wide range of psychiatric patients, including those with depression, anxiety disorders, and personality disorders (Coccaro and Kavoussi 1997; Vaswani et al. 2003). The SERT promoter region polymorphism is associated with anxiety (Schinka et al. 2004; Willis-Owen et al. 2005; Ebstein 2006). Previous PET imaging of brain SERTs with [^{11}C]DASB revealed significant variation in SERT binding in normal subjects and in those with pathological depression (Cannon et al. 2006; Takano et al. 2007; Reimold et al. 2008; Kalbitzer et al. 2009). In a PET study with [^{11}C]DASB using macaque monkeys, maternal separation resulted in lower SERT binding in the brain during adolescence (Ichise et al. 2006). Similarly, the effects of childhood adversity on SERT binding were confirmed in a human PET study using patients with major depression (Miller et al. 2009).

In the present study, we performed PET brain imaging for 90 min in conscious common marmosets with [^{11}C]DASB to obtain dynamic histograms. Parametric images of binding potential (BP) were then generated using the two-parameter linearized reference tissue model (Ichise et al. 2003) in which the cerebellum is used as a reference. Several anatomical regions of interest (ROIs) in individual PET images aligned on individual MRI scans were manually drawn according to a stereotactic brain atlas of the common marmoset (Stephan et al. 1980). Regional BP values were also obtained from dynamic histograms of ROIs. The BP images and numerical BP values in ROIs revealed a wide distribution of [^{11}C]DASB binding in the common marmoset brain: high in the midbrain, pons, hypothalamus, thalamus, amygdala, and ventral striatum; moderate in the dorsal striatum, hippocampus, and cingulate, occipital and temporal regions of the cortex; and modest in other regions of the cortex (Fig. 19.3).

This pattern of SERT distribution was consistent with the pattern of serotonergic neurons and nerve terminals in common marmosets determined using traditional immunohistochemical techniques (Schofield and Dixson 1982; Hornung and Fritschy 1988; Hornung et al. 1990; Hornung and Celio 1992). PET imaging can now reveal various functional aspects of SERT in highly quantitative fashion in vivo, unlike in vitro histochemistry, which relies on using postmortem brains. The pattern of distribution of

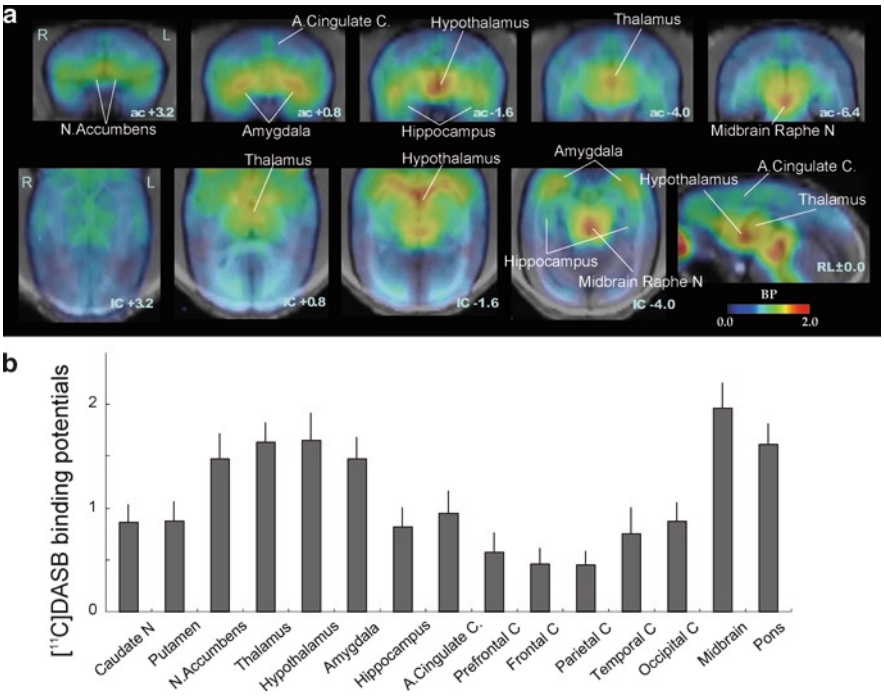

Fig. 19.3 (**a**) Representative parametric images of the binding potential (BP) of [^{11}C]DASB for serotonin transporters of an animal that were fused on magnetic resonance imaging (MRI) scans for anatomical identification. (**b**) Means and standard errors of [^{11}C]DASB BPs in regions of interest (ROIs) are shown on the graph ($n = 8$)

[^{11}C]DASB in common marmosets is almost the same as those in conscious macaque monkeys and humans (Kim et al. 2006; Yokoyama et al. 2010). However, the absolute BP values calculated from kinetic data between common marmosets and rhesus monkeys are inconsistent in several brain regions (Yokoyama et al. 2010). Compared to the brains of rhesus monkeys, common marmoset brains exhibited lower BP in subcortical regions and nearly equal or somewhat higher BP in cortical regions and the hippocampus. These species differences may be related to functional specialization of regional serotonergic activities in common marmosets.

19.2.2 Dopamine Transporters

The brain dopamine transporter (DAT) is also located in presynaptic nerve terminals and the cell bodies of dopamine neurons (see Chap. 10, Sect. 1). DAT is a major target site of cocaine and is linked to cocaine's acute behavioral effects (e.g., hyperactivity, restlessness, euphoria), which result from enhancement of dopaminergic activity by blocking the presynaptic uptake of dopamine. The relation between DAT

occupancy and the reinforcing effects of cocaine and other DAT blockers has also been revealed in PET studies of nonhuman primates (Villemagne et al. 1999; Howell and Wilcox 2002). In addition, DAT imaging by PET has been utilized as an objective in vivo means of quantifying the loss of nigrostriatal neurons in Parkinson's disease and a nonhuman primate model of it (Thobois et al. 2001; Bohnen and Frey 2003; Nagai et al. 2007; Muramatsu et al. 2009). Genetic polymorphism of DAT is known to be associated with neuropsychiatric diseases such as attention deficit hyperactivity disorder, drug abuse, and Parkinson's disease (Cook et al. 1995; Le Couteur et al. 1997; Ueno et al. 1999) (see Chap. 10, Sect. 1). Although there is evidence that dopamine neurotransmission plays a role in the determination of human personality traits (Ebstein et al. 1996; Breier et al. 1998; Depue and Collins 1999; Suhara et al. 2001; Laakso et al. 2003; Reeves et al. 2007), previous DAT imaging studies have revealed controversial results, which may be due to differences in imaging methods or characteristics of samples (Laakso et al. 2000; Schneier et al. 2009).

We performed PET scans with [^{11}C]PE2I for DAT in the same fashion as for [^{11}C] DASB and generated BP images using the Logan noninvasive model (Logan et al. 1996). BP images revealed the distribution of [^{11}C]PE2I binding in the common marmoset brain. Binding was extremely high in the striatum; moderate in the hypothalamus, amygdala, and ventral midbrain; low in the thalamus and hippocampus; and scarcely detectable in cortex (Fig. 19.4). Although common marmosets have also been used in research on Parkinson's disease (Eslamboli 2005), there has been no previous study of the relation between brain dopamine systems and personality traits. Our PET brain imaging with [^{11}C]PE2I demonstrated low but detectable levels of binding in extranigrostriatal limbic structures such as the hypothalamus, amygdala, thalamus, and hippocampus as well as extremely high levels in nigrostriatal regions. Previous PET studies of [^{11}C]PE2I in humans and macaque monkeys examined only the nigrostriatal DAT-rich regions (Nagai et al. 2007; Hirvonen et al. 2008).

19.2.3 Features of Molecular Brain Mapping Underlying Personality Traits

To characterize the social interactions of laboratory animals, the intruder challenge test, a test of social challenge for a dyad or group, has been widely used in rodents (Ferguson et al. 2002; Young 2002; Veenema and Neumann 2007) (see Chap. 5, Sect. 4). In macaque monkeys, behaviors of approach and aggression, which determine dominance, were measured in a test of social challenge involving dyads in a cage (Bachevalier et al. 2001; Hadland et al. 2003) and with long-term testing of a social group by the focal animal sampling method (Fairbanks et al. 2001; Kaplan et al. 2002; Newman et al. 2005). In common marmosets, prior studies examined the neurochemical underpinnings of affiliative and agonistic behavior (Kinnally et al. 2006), anxiety-related behaviors during confrontation with conspecific strangers

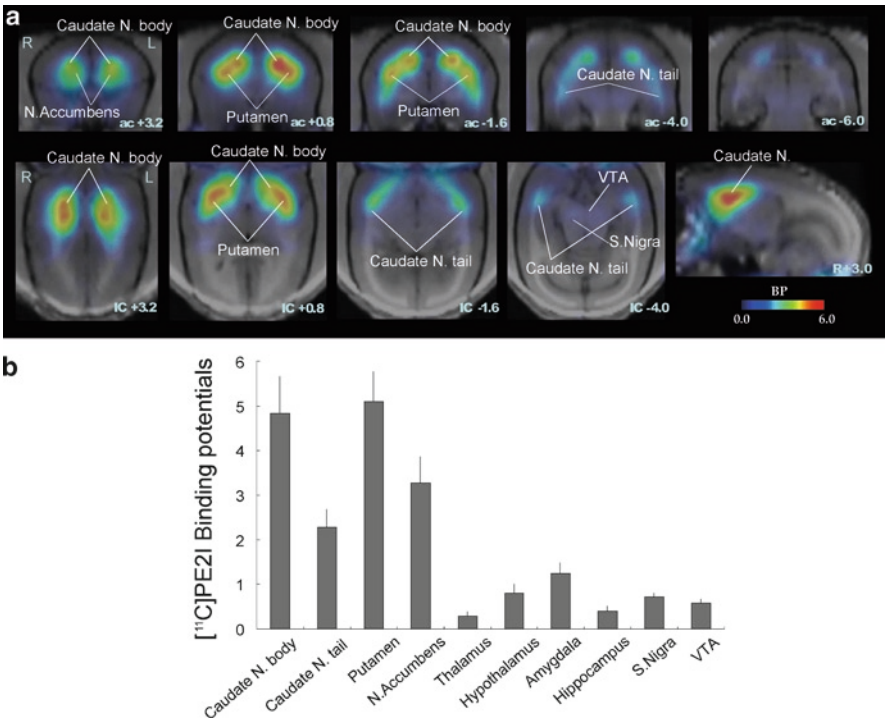

Fig. 19.4 (**a**) Representative parametric images of the BP of [^{11}C]PE2I for dopamine transporters of an animal were fused on MRI scans for anatomical identification. (**b**) Means and standard errors of [^{11}C]PE2I BPs in ROIs are shown on the graph ($n = 8$)

(Cilia and Piper 1997; Kinnally et al. 2006), and the propensity for monogamy (Gerber and Schnell 2004).

For integrated analysis of behavioral responses in a social challenge condition in common marmosets, encounter trials between two subjects unfamiliar with each other were conducted (Fig. 19.5) (Yokoyama et al. 2008). The subjects of the present study were 12 young adult male marmosets who were pair-housed in their home cages. The behavioral test involved placing two unfamiliar males together in a test cage for 5 min. Durations of specifically defined behaviors exhibited by focal animals were recorded, as shown in Fig. 19.5. Altogether 17 behaviors were identified, and the total times spent in the trial for each behavior were compiled and rated per 5 min as variables. Factor analysis of the behavioral measures identified three dimensions: Aggressiveness, Sociability, and Social Anxiety (Fig. 19.6). Factor scores for each trial were computed, and personality traits were denoted by numerical indices as representative factor scores for individual subjects.

To investigate neural correlates of personality traits in SERT function, the associations between regional SERT BP values and the three factor scores were analyzed with a multiple linear regression model. SERT BP values were positively correlated with Sociability in various ROIs, including the anterior cingulated cortex ($\beta = 0.12$,

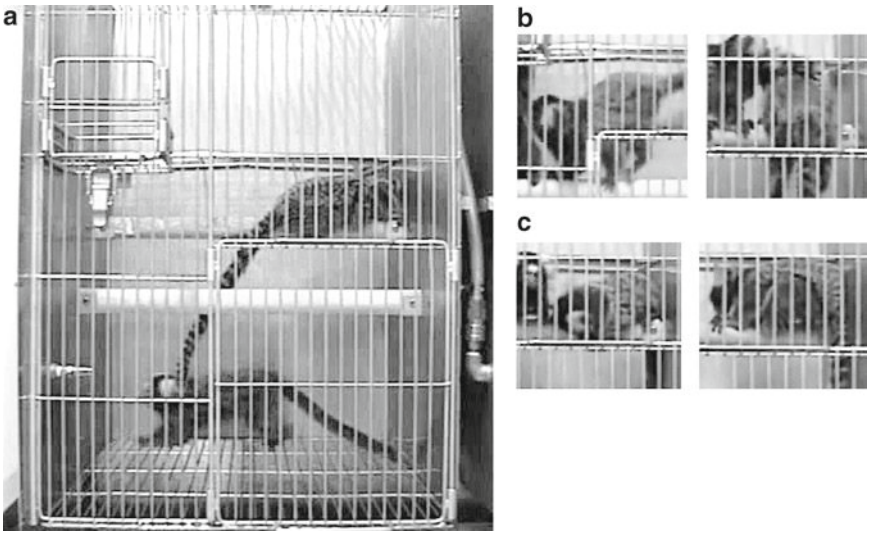

Fig. 19.5 (**a**) Encounter trial between two common marmosets unfamiliar with each other in the social challenge paradigm. (**b**, **c**) Snapshots of a subject approaching and sniffing its opponent and biting a perch and marking it

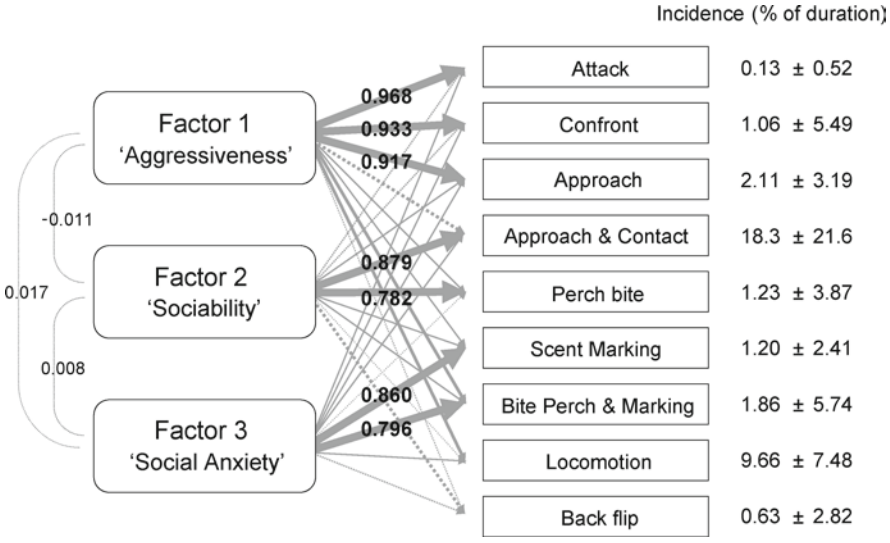

Fig. 19.6 Results of a factor analysis for behaviors in the social challenge paradigm. Behavioral items were selected based on the significance of the test-retest reliability. The incidence of each item is shown on the *right*. The respective factor loadings are presented on *arrows*. Correlation coefficients between three factors are presented on the *left*

SE = 0.03, $P < 0.05$), hippocampus (β = 0.20, SE = 0.06, $P < 0.01$), and caudate nucleus (β = 0.17, SE = 0.01, $P < 0.001$). In addition, SERT BP values in the caudate nucleus was positively correlated with Aggressiveness (β = 0.31, SE = 0.09, $P < 0.05$). A negative correlation was found between Social anxiety and BP levels in the putamen (β = –0.10, SE = 0.04, $P < 0.05$). This finding shows that serotonergic neurotransmission has distinct effects on different personality dimensions within a neuronal circuit consisting of limbic structures, which has been partially suggested in human PET studies (Takano et al. 2007; Kalbitzer et al. 2009). This circuit can be involved in information processing of memory, cognition and affection, which influences personality traits.

Neural correlates of personality traits in DAT function were also examined by statistical analysis of associations between regional DAT BP values and the factor scores. DAT BP value in the tail of caudate was negatively correlated with both Sociability (β = –0.19, SE = 0.05, $P < 0.05$) and Social Anxiety (β = –0.21, SE = 0.07, $P < 0.05$), but that in the putamen exhibited a significant positive correlation with Aggressiveness (β = 2.88, SE = 0.60, $P < 0.01$). The present findings are consistent with previous studies that have indicated an association between low dopaminergic activity and detachment (Breier et al. 1998; Laakso et al. 2000, 2003; Reeves et al. 2007).

Not only transporters but also various receptor subtypes regulate net rates of serotonergic and dopaminergic neurotransmission. In vivo receptor binding studies with subtype-specific PET ligands are therefore needed to clarify neurochemical phenotypes on the serotonergic and dopaminergic systems linked to personality traits in the common marmoset.

19.3 Conclusion and Future Directions

Among brain imaging techniques, PET imaging using radiotracers that specifically bind to target molecules enables in vivo quantitative visualization of neurochemical functions in the brain. The availability of this technique for human and nonhuman primates has enabled examination of the in vivo functions of specific neurotransmitter systems that underlie behavior. We have performed regional and quantitative analyses of SERTs and DATs using a newly developed technique for performing PET scans of conscious common marmosets in our laboratory. Furthermore, we have identified personality dimensions related to social behavior in common marmosets by examining behavioral responses in encounter trials. SERT BP values in the anterior cingulate cortex and hippocampus were positively correlated with Sociability, and that in the caudate nucleus was positively correlated with both Aggressiveness and Sociability. In addition, SERT BP value in the putamen was positively correlated with Sociability but negatively correlated with Social Anxiety. On the other hand, DAT BP value in the tail of caudate was negatively correlated with both Social Anxiety and Sociability, while that in the putamen was positively

correlated with Aggressiveness. These findings suggest that neural systems controlled by SERT and DAT play roles in the social behavior of common marmosets.

The combination of PET brain imaging with integrated behavioral descriptions of nonhuman primates is a powerful means for investigating the neural mechanisms of targeted behaviors and of interspecies and intraspecies differences among primates. The common marmoset could serve as a unique and useful model of human social behavior for biomedical and biological research in the neurosciences involving transgenic technology, which has recently been applied successfully in this primate (Sasaki et al. 2009). Using these newly developed techniques, studies with common marmosets can aid in elucidating the molecular mechanisms underlying personality and the social learning ability of individuals (Pesendorfer et al. 2009), which could be affected by both genetic background and environmental modifications.

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