7 · Hormones and alternative reproductive tactics in vertebrates

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CHAPTER SUMMARY

The wide diversity of alternative tactics of reproduction found among vertebrates offers a unique opportunity to study the endocrine mechanisms underlying the phenotypic variation of reproductive traits. Here, we first assess the existing conceptual frameworks on the mechanisms underlying the expression of alternative reproductive tactics (ARTs) by reviewing the available data on hormone levels in alternative phenotypes and on the effects of hormone manipulations in different vertebrate taxa. We then highlight recent studies that have opened new avenues of research on the neuroendocrine basis of ARTs, such as the use of functional genomics to study differential gene expression between morphs. Finally, we stress the need to integrate the study of ARTs with the mechanisms underlying the expression of alternative phenotypes and with functional studies of ARTs. Only such an integrative approach will allow a comprehensive understanding of the evolution and development of ARTs.

7.1 INTRODUCTION

7.1.1 Setting the scene

According to the classic paradigm of the endocrine control of vertebrate reproduction, the hypothalamus–pituitary–gonadal (HPG) axis controls gonadal maturation, the expression of secondary sexual characters, and reproductive behavior (Figure 7.1A). However, in some species there are males in which gonadal maturation and sperm production are dissociated from the expression of behavioral and morphological male traits (i.e., secondary sexual characters). They are males with male alternative reproductive tactics (ARTs), and they offer unique opportunities to study the proximate mechanisms of reproduction (Figure 7.1B). ARTs are also valuable models for the study of the causal mechanisms underlying individual variation in reproduction since within-sex variation in reproductive traits can be studied without the confounding effects of gender (Moore 1991, Godwin and Crews 2002).

Historically, typological classifications of ARTs have been based on the evolutionary processes underlying their expression (e.g., genetic polymorphisms vs. conditional tactics, Gross 1996; or Mendelian strategies vs. developmental strategies vs. behavioral strategies, Shuster and Wade 2003). In this chapter we will use a classification based on observed patterns of ARTs that does not require knowledge of their underlying processes (e.g., genetic vs. conditional strategies). The classification scheme is modified from that proposed by other authors (Caro and Bateson 1986, Moore 1991, Taborsky 1994, Moore et al. 1998, Brockmann 2001). We will consider alternative reproductive phenotypes as fixed if the individuals adopt one of the tactics for their entire lifetime or as plastic if individuals change their reproductive tactic. Within plastic ART phenotypes, we will distinguish between irreversible sequential patterns, when individuals switch from one tactic to another at a particular moment in their lifetime, and reversible patterns, when individuals can change back and forth between patterns (Moore 1991, Moore et al. 1998, Brockmann 2001) (see Figure 1.1).

A number of reviews on the proximate mechanisms of ARTs have been published lately, but each has a different focus from the present chapter. Moore and co-authors (1998) develop a conceptual framework for the role of hormones on tactic differentiation, Rhen and Crews (2002) provide an overview of mechanisms involved in ARTs in different vertebrate taxa, Knapp (2003) proposes a new generation of studies more focused on target tissues than on circulating levels of hormones, and Oliveira (2005) and Oliveira and co-authors (2005) focus on mechanisms operating in fish ARTs. So what can be added by another chapter on the causal mechanisms of ARTs?
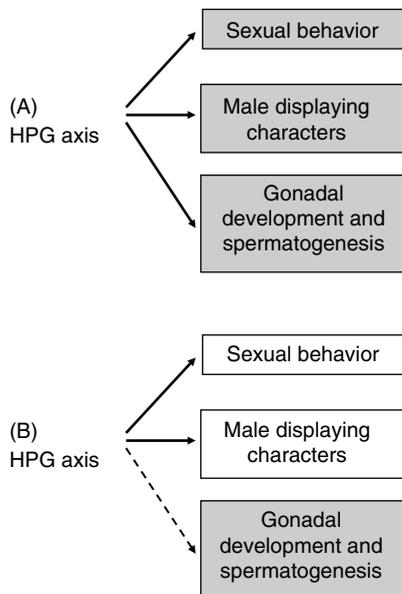
Figure 7.1 (A) Different reproductive traits share a common underlying causal agent (e.g., testosterone); (B) in species with ARTs a dissociation between the different traits may occur resulting in a phenotypic mosaic that can express both male and female traits (e.g., sneaker males that mimic female behavior and morphology in order to achieve fertilizations).

This chapter has two main objectives. The first is to present an exhaustive revision of the available data on hormone levels in alternative phenotypes and on the effects of hormone manipulations in different vertebrate taxa. This will provide the basis for the assessment of existing conceptual frameworks on the mechanisms underlying the expression of ARTs. The second objective is to highlight recent studies that have opened new avenues of research on the physiological basis of ARTs and its implications for understanding the evolution of ARTs (e.g., the study of differential hormonal-mediated costs of alternative phenotypes and the field of functional genomics to study differential gene expression between morphs).

7.1.2 Who's in the ARTs ark?

We will address only male ARTs since they are the most common and best-studied cases at a proximate level. In contrast to other recent reviews of ARTs, we also include species with cooperative breeding in which parentage is shared between breeders and helpers (e.g., acorn woodpecker, *Melanerpes formicivorus*: Haydock *et al.* 2001), in which there are behavioral observations of breeding attempts with the female of the pair by helpers (e.g., bell miner, *Manorina melanophrys*: Poiani and Fletcher 1994; but see Conrad *et al.* 1998), and in which helpers are non-breeders in their home group but attempt extra-pair copulations (EPC) with other group females (e.g., superb fairy-wrens, *Malurus cyaneus*: Mulder *et al.* 1994). In these cases we consider helping to be an alternative tactic to achieve breeding. According to these criteria we have included in our analyses the cooperative breeding species listed in Table 7.1. It should be noted that the use of these criteria assumes that observed mating episodes result in reproductive output, which may not always be the case. In contrast, we have discarded other cooperative breeding species for which detailed hormonal data are available when paternity analyses have revealed that the species are genetically monogamous (e.g., Florida scrub-jay, *Aphelocoma coerulescens*: Schoech *et al.* 1991, 1996, Quinn *et al.* 1999; red-cockaded woodpecker, *Picoides borealis*: Haig *et al.* 1994, Khan *et al.* 2001). In the white-browed sparrow weaver, *Plocepasser mahali* (Wingfield *et al.* 1991), for which there are hormone data for both breeders and helpers, the information on the helpers' behavior suggests that they do not try to sneak copulations (J. C. Wingfield, personal communication), and therefore this species was not included. Finally, there are species for which the available information regarding the reproduction of helpers is dubious or indirect. In the pied kingfisher (*Ceryle rudis*), two types of helpers occur: primary helpers that are offspring of the breeding pair and secondary helpers that are unrelated to breeders (Reyer 1980, 1984). Primary helpers have small, immature gonads and have lower testosterone levels than both male breeders and secondary helpers, and thus are not able to fertilize eggs (Reyer *et al.* 1986). In contrast, secondary helpers, which have mature gonads, sometimes fight with the breeder male to get access to the female of the pair (Reyer *et al.* 1986). Therefore, even without parentage data, we decided to consider secondary helping of the pied kingfisher as an ART and have included it in the analysis.

Two cooperatively breeding rodents in which helpers do not achieve reproductive success were also included, as they might be seen as special cases of ARTs: the naked mole-rat (*Heterocephalus glaber*) and the Mongolian gerbil (*Meriones unguiculatus*). In both cases subordinate individuals acting as helpers are incapable of direct reproduction and are

Table 7.1. *Cooperative breeding species in which helpers also breed*

Species	Evidence for breeding in helpers (reproductive success of helpers)	References
Fish		
Princess of Burundi, *Neolamprologus brichardi*	Genetic (10.8% of offspring)	Dierkes *et al.* 1999
Birds		
Seychelles warbler, *Acrocephalus sechellensis*	Genetic (15% of offspring)	Richardson *et al.* 2001
Mexican scrub-jay, *Aphelocoma coerulescens*	Genetic (low)	Bowen *et al.* 1995
Acorn woodpecker, *Melanerpes formicivorus*	Genetic (approx. 25% of offspring)	Haydock *et al.* 2001
Australian magpie, *Gymnorhina tibicen*	Genetic (high; up to 82% of extra-group paternity)	Hughes *et al.* 2003
Azure-winged magpie, *Cyanopica cyanus*	Behavioral (high)	De la Cruz *et al.* 2003; Valencia *et al.* 2003
Bell miner, *Manorina melanophrys*	Behavioral/genetic (genetic data indicates very low success)	Poiani and Fletcher 1994; Conrad *et al.* 1998
Superb fairy-wren, *Malurus cyaneus*	Genetic (within-group = 2.2%; extra-group = 76%)	Mulder *et al.* 1994
Pied kingfisher, *Ceryle rudis*	Behavioral (low)	Reyer *et al.* 1986
Harris's hawk, *Parabuteo unicinctus*	Behavioral (low)	Dawson and Mannan 1991
Mammals		
Ring-tailed lemur, *Lemur catta*	Behavioral (high)	Sauther 1991; Sussman 1991
Common marmoset, *Callithrix jacchus*	Behavioral/genetic (genetic data indicates very low success within the group)	Digby 1999; Nievergelt *et al.* 2000
Alpine marmot, *Marmota marmota*	Genetic (only subordinate helpers)	U. Bruns and W. Arnold, unpublished data in Dierkes *et al.* 1999
Dwarf mongoose, *Helogale parvula*	Genetic (24% of offspring)	Keane *et al.* 1994
Meerkat, *Suricata suricatta*	Genetic (low)	Griffin *et al.* 2003
Gray wolf, *Canis lupus*	Behavioral (low)	Creel 2005
African wild-dog, *Lycaon pictus*	Behavioral/genetic (low)	Girman *et al.* 1997; Creel and Creel 2002

obligate helpers, and thus their fitness is entirely indirect (Clark and Galef 2000, Faulkes and Bennett 2001) (see Box 7.1). In these two cases, it can be argued that helping is a conditional strategy, without which these individuals would have zero fitness.

In summary, this chapter will cover not only the usual ARTs but also the cooperative breeders that fit the conditions described above.

7.2 PROFILES OF ALTERNATIVE REPRODUCTIVE PHENOTYPES

In general, two alternative modes or tactics of reproduction can be found in species with male ARTs: a conventional or bourgeois tactic or an alternative or parasitic tactic. Whereas bourgeois males invest resources to attract mates (e.g., differentiation of morphological ornaments; expression

Box 7.1   Obligatory helping as an alternative reproductive tactic

In cooperatively breeding animals, it is usual that reproduction is monopolized by some group members resulting in a high within-group reproductive skew. Kin selection theory may explain indirect benefits for nonbreeding individuals that act as helpers in these groups, while direct benefits such as queuing to take over the breeding position when it is vacant have been advocated (see Solomon and French 1997). There are two extreme cases of obligatory helping that have been described among cooperatively breeding mammals: the naked mole-rat (*Heterocephalus glaber*) and the Mongolian gerbil (*Meriones unguiculatus*). In these two cases it can be argued that since their inclusive fitness equals their indirect fitness (i.e., the only chance that nonbreeding individuals have during their whole lifespan to get copies of their genes into the next generation is by helping kin to reproduce), individuals that specialize in alloparenting and/or helping behavior patterns can be seen as adopting an alternative tactic.

The naked mole-rat fits the eusociality definition derived from insects, since division of labor is present in the colony among the nonbreeding helpers, which is based on body size (Lacey and Sherman 1991). A single female, the "queen," is sexually active breeding with up to three breeding males (Bennett and Faulkes 2000). The queen controls the reproductive physiology of both sexes, maintaining the reproductive suppression of their subordinate colony mates (Faulkes and Abbott 1997). There is also evidence for the existence of castes, with a disperser morph among males and a morphologically distinct "queen" (O'Riain et al. 1996, 2000b). In addition, this mating system with high rates of inbreeding leads to a genetic structure similar to insect haplodiploidy, with intra-colony relatedness coefficients as high as 0.8, which is greater than the 0.75 achieved by the haplodiploid system (Reeve et al. 1990). This system seems to have evolved due to high costs of dispersal, and most subordinate individuals spend their whole lives as nonbreeding colony defenders.

In Mongolian gerbils male fetuses vary in their intra-uterine positions, and this variation is reflected in adult testosterone levels. Males gestated between two males (2M males) have higher testosterone levels when adults than their brothers that were gestated between two females (2F males) (Clark et al. 1992b). This intrauterine position has a major impact in the development of male sex characters and sexual behavior: 2F males have reduced bulbocavernosus muscle mass (involved in penile erection) and alterations in their copulatory and scent-marking behavior, achieving a lower reproductive success than their 2M siblings (Clark et al. 1990, 1992a). Conversely, 2F males express more paternal behavior than the 2M males (Clark et al. 1998). Among 2F males some individuals that have extremely low levels of circulating testosterone (similar to those of females) show no interest in receptive females, failing to impregnate them when they are paired. Therefore, nonbreeding 2F males are incapable of direct reproduction and are obligate helpers (Clark and Galef 2000).

of visual, chemical, or acoustic courtship signals; defense of breeding territories) (see Chapter 1 and Taborsky 1997), parasitic males, in contrast, exploit the investment made by the bourgeois males to get access to mates (e.g., female mimicry, sneaking, satellite) (see Chapter 1 and Taborsky 1997). Therefore, the traits selected in the two male types are usually divergent. In bourgeois males, traits related with mate attraction and monopolization will be favored by selection, while in parasitic males, traits that increase the probability of stealing fertilizations from bourgeois males will prevail. This disruptive selection acting on a constellation of phenotypic traits may result in the creation of phenotypic mosaics in which both male and female traits are expressed in the same individual, as is the case with parasitic males that mimic female morphology and behavior to get access to fertilization events (e.g., female mimicry in sneaker males of the peacock blenny, *Salaria pavo*: Gonçalves et al. 1996, Gonçalves et al. 2005). In this example, the expression of male reproductive behavior and male secondary sex characters become dissociated from the differentiation of a functional male gonad. Classically, male sexual differentiation involves the action of androgens (e.g., testosterone), which, in a cascade of events, promote the masculinization of different body parts (see Box 7.2 on sexual differentiation in vertebrates). However, ARTs offer the possibility to gain insight into the proximate mechanisms underlying sexual differentiation, since in the parasitic tactic, gonadal maturation and spermatogenesis can be dissociated from the expression of behavioral and morphological male traits (Figure 7.1). The decoupling of different male traits in parasitic males may be achieved by different means (e.g., by variation in the local micro-environments in target tissues, as a result of differential

Box 7.2 Sex determination in vertebrates

What determines sex in an individual starts with a blueprint laid out in the genetic material organized in chromosomes, referred to as *genetic sex*. In most vertebrates, sex chromosomes contain the most important genes required for the developing gonad to differentiate according to the genetic plan into an ovary or a testis, referred to as *gonadal sex*. As the gonads develop they start to secrete hormones that will act on the urogenital system, central nervous system, and external features to promote the secondary sexual characteristics originating what we recognize from behavior and appearance as the *phenotypic sex*.

During early development two urogenital ridges along the entire length of the dorsal body wall originate from the vertebrate mesoderm; the mid portion of these ridges differentiates into a single genital ridge from which a bipotential gonad originates. The urinary and reproductive systems are therefore closely associated, and in more primitive vertebrates, they share common ducts.

In eutherian (placental) mammals, maleness is determined by the Y-chromosome being present in normal individuals. This chromosome contains one-third of the number of genes present in the X-chromosome, some inactive, and includes *SRY* (<u>S</u>ex determining <u>R</u>egion on <u>Y</u>). SRY protein acts on the bipotential gonad to initiate a cascade of gene expression leading to the development of the testis (Morrish and Sinclair 2002). One of the essential factors expressed specifically in the testis differentiation pathway is *SOX9*, an autosomal gene also involved in cartilage and bone formation. Both *SOX9* and *SRY* are thought to have derived from *SOX3*, located in the X-chromosome. As soon as a testis is formed, Sertoli cells start secreting antimüllerian hormone (AMH), which inhibits the differentiation of Müllerian ducts into female reproductive tract structures (fallopian tubes, uterus, and part of the vagina), and Leydig cells secrete testosterone, which promotes the differentiation of the Wolffian ducts into seminiferous tubules, vas deferens, and seminal vesicle. However, for the differentiation of the external genitalia (prostate, scrotum, and penis), testosterone needs to be converted to 5α-dihydrotestosterone through the action of 5α-reductase.

In the female differentiation pathway, *SRY* is absent and DAX1, the product of a gene located in the X-chromosome, is thought to inhibit *SOX9* expression and therefore inhibit the male differentiation pathway (Swain *et al.* 1998). The expression of DAX1 itself is upregulated by WNT4, a factor that is also essential for Müllerian duct formation and steroidogenesis (Mizusaki *et al.* 2003). In the mammalian female, differentiation of the ovary and external genitalia proceeds without the intervention of sex steroid hormones, which led to the notion that female differentiation is "passive." However, it is, like the male pathway, an active process in which failure in one step can lead to partial or total phenotypic sex reversal.

Phenotypic sex reversal can happen as a result of gene duplication, deletion, inversion, or mutations, which originate a higher or lower formation of gene product. This is the concept of sex related to gene dosage (number of copies of a gene), which is thought to be the ancestral form of sex determination. For example, any of these conditions originate a female phenotype in XY individuals: absence of *SRY*, two copies of *DAX1*, one copy of *SOX9*, or one copy of *SF1* (steroidogenic factor 1, a factor required for steroidogenesis). Three copies of *SOX9* in XX individuals will also originate a male phenotype. In marsupial mammals, gonadal sex is also determined by the presence of a Y-chromosome, but the development of female pouch versus male scrotum depends on X-chromosome dosage (Vaiman and Pailhoux 2000).

Sex determination mechanisms evolve rapidly, and this has resulted in the independent development of sex chromosomes throughout the vertebrates. The monotremes (egg-laying mammals) appear to have a hybrid between the mammalian XY chromosome system and the avian WZ/ZZ system (Grutzner *et al.* 2004). In birds WZ/ZZ sex chromosomes are universal (female heterogamety). Male and female heterogamety is present in reptiles, amphibians, and fish. Environmental sex determination (ESD) is common in reptiles, but it is also present in amphibians and fish. Parthenogenesis has been reported in reptiles and fish, and polygenic systems are present in several fish species (Kraak and Pen 2002).

Only mammals, except monotremes, have the master sex determining gene *SRY*. In other species only in medaka fish (*Oryzias latipes*) has a master sex-determining gene been found – *DMY*, related to *DMRT1* (also important in the male sex-differentiation pathway) (Matsuda *et al.* 2002, Nanda *et al.* 2002). However, it is absent in some populations of the same species and other fishes (Volff *et al.* 2003).

Other than *SRY*, it appears that most of the above factors indicated as important in mammalian sex differentiation are also present and are expressed at the appropriate time

during development in nonmammalian vertebrates, which may indicate common mechanisms (Smith and Sinclair 2004). However, unlike in mammals, in birds and in other vertebrates, steroids are required for the development of the female pathway – androgens promote testicular development and estrogens ovarian development. Thus, the non-mammalian female gonad expresses aromatase, which converts testosterone to estradiol-17β inducing its feminization (Sarre *et al.* 2004).

The most common form of ESD is through the action of incubation temperature (TSD). The temperature at which embryos are incubated influences the activity of steroidogenic enzymes, in particular aromatase. The inhibition of aromatase leads to the accumulation of testosterone and masculinization, while optimum temperatures for aromatase activity favor the ratio of estrogen to androgen and feminization (Pieau and Dorizzi 2004). Socially induced ESD will ultimately influence steroidogenic enzymes to promote sex change in fishes (Devlin and Nagahama 2002).

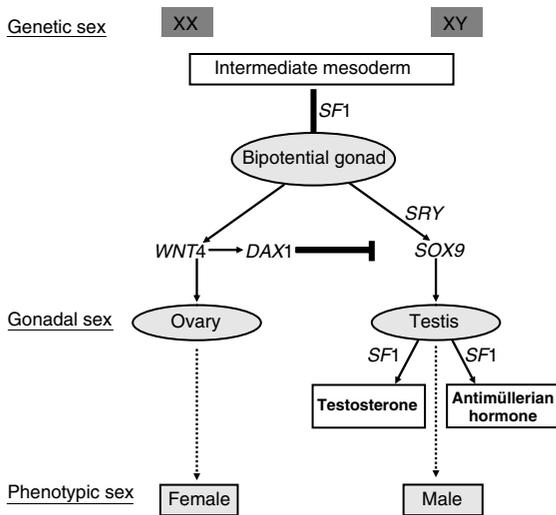Figure 7.2 shows a schematic representation of the sex determination pathway in mammals.

Figure 7.2 Schematic representation of the sex determination pathway in mammals.

expression of receptors or of differential levels of activity of steroidogenic enzymes that modulate the availability of the active hormone) (see Section 7.6).

7.3   PROXIMATE CAUSES OF PHENOTYPIC PLASTICITY: NEURAL AND ENDOCRINE MECHANISMS

7.3.1   Neural structural reorganization versus biochemical switching

Structural reorganization and biochemical switching have been recognized as the major mechanisms underlying behavioral plasticity (Zupanc and Lamprecht 2000). Structural reorganization of neural networks underlying behavior may include processes such as neurogenesis, synaptogenesis, apoptosis, and changes in the dendritic structure of neurons that lead to the differentiation of new neural circuits. These processes are not necessarily restricted to early developmental phases, since adult neurogenesis, for example, has been demonstrated to occur in a variety of vertebrates including humans (Alvarez-Buylla and Lois 1995, Zupanc 2001, Ming and Song 2005). Neural structural reorganization leads to changes in the properties of the networks and therefore in their behavioral output. Functional changes in neural networks activity may also be achieved by alterations of glia cells. For example, changes in astrocyte volume may alter the area of neuronal membrane that is juxtaposed in adjacent neurons. Therefore, glial withdrawal (which can be induced by water deprivation) could increase the area of contact between neurons, potentially leading to an increased excitability of these cells (Zupanc and Lamprecht 2000). In summary, structural reorganization can occur at different life-history stages and involves the modification of the structure of neurons and/or glial cells. As a result, behavioral changes that depend on this mechanism are expected to be slow, long-lasting, and drastic.

In contrast, biochemical switching involves the modulation of synaptic transmission within circuits that are not being rearranged. The main neuromodulators that have been identified include catecholamines, serotonin, and neuropeptides (Zupanc and Lamprecht 2000). Since neuropeptides and catecholamines can be released in a non-synaptic fashion, they may act on larger areas of the central nervous system by diffusion, which would allow them to influence more than one behavioral system at a time. Biochemical switching is thus a mechanism that allows for reversible behavioral output and underlies faster, gradual, or transient changes (Zupanc and Lamprecht 2000).

These potential neural mechanisms underlying phenotypic plasticity have a parallel in hormonal mechanisms: structural (re)organization of neural circuits can be influenced by organizational effects of hormones during well-defined, sensitive periods in the life of an individual, while biochemical switches can be driven by activational effects of hormones on central pathways underlying behavior (for a review on organizational vs. activational effects of hormones in vertebrates see Arnold and Breedlove 1985).

Therefore, it is predicted that reversible tactics that require rapid and transient changes in neural activity are mediated by biochemical switches influenced by hormones in an activational fashion, whereas fixed and sequential tactics, which involve, in the first case, an organization of the phenotype early in the development or, in the second case, a post-maturational reorganization of the phenotype, are mediated by structural reorganization of neural networks. Concomitantly, the role of hormones in the expression of the different types of tactics should differ: organizational (or reorganizational) effects should be associated with fixed and sequential tactics, activational effects with reversible tactics.

7.3.2   Organizational versus activational effects of hormones

The action of hormones, in particular sex steroids, on behavior has been classically divided into activational and organizational effects. Activational effects are transient and occur throughout the lifespan of the individual, while organizational effects are long-lasting and occur early in ontogeny, typically during a critical period of development (Arnold and Breedlove 1985). This dichotomy of sex hormone action was initially proposed by Phoenix and co-authors (1959) and assumes that activational effects act through the activation of neural circuits that are already present, whereas organizational effects require the organization of new neural circuits at critical periods during development.

The use of the dichotomy between activational and organizational effects of hormones has also been proposed by Moore (1991) as a conceptual framework for the hormonal basis of ART, and it is known as the *relative plasticity hypothesis*. The rationale behind this hypothesis is that the effects of hormones in the differentiation of alternative reproductive tactics are equivalent to their effects in primary sex differentiation (Moore 1991). Thus, by making a distinction between fixed alternative phenotypes (in which individuals adopt one of the tactics for their entire life) and flexible alternative phenotypes (in which individuals may switch tactics during their lifetime), Moore (1991) proposed an organizational-like role for hormones in the former case and an activational-like role in the latter case. Two predictions can then be extracted from this hypothesis (Moore 1991). (1) In species with plastic ARTs, hormone levels should differ between adult alternative morphs; in species with fixed ARTs, adult hormone profiles should be similar among alternative morphs, except when morphs experience different social environments (Moore 1991). (2) In species with plastic ARTs, hormone manipulations should be effective in adults but not during early development (activational effect); in fixed ARTs hormone manipulations should be effective during early development but not in adults (organizational effect). More recently, a second generation of the relative plasticity hypothesis has been proposed (Moore *et al.* 1998). This revised version emphasizes the distinction between reversible and irreversible phenotypes among plastic tactics and between conditional and unconditional fixed tactics. Accordingly, the plastic, reversible tactics would be the true equivalents of activational effects of hormones, and thus, the original predictions of the relative plasticity hypothesis would only apply to this type of alternative tactic. The plastic, irreversible (i.e., sequential) ARTs would represent a post-maturational reorganization effect, in which the phenotypic outcome would be produced immediately (Moore *et al.* 1998). Thus, hormone differences needed to differentiate the two alternative phenotypes need not be permanent and may only be present during the transitional phase. Among the fixed ARTs, the distinction between conditional and unconditional fixed tactics has no consequences for the predictions concerning the endocrine mechanisms of ARTs, with organizational actions being predicted in both cases (Moore *et al.* 1998). Thus, the predictions of Zupanc and Lamprecht (2000) for the neural mechanisms underlying phenotypic plasticity and those of the relative plasticity hypothesis are in good agreement (Table 7.2).

7.3.3   Endocrine candidates: sex hormones, glucocorticoids, and neuropeptides

Sex steroids, glucocorticoids, and neuropeptides emerge as candidates to play a major role in the differentiation and maintenance of alternative reproductive morphs. As mentioned above, sex steroids have an essential role in sexual differentiation and in the control of male reproduction in vertebrates (e.g., Dixon 1998, Wilson *et al.* 2002, Nelson

Table 7.2. *Neural and hormonal mechanisms of alternative reproductive tactics in vertebrates*

ART type	Neural mechanism (Zupanc and Lamprecht 2000)	Hormonal mechanism following the relative plasticity hypothesis v.1 (Moore 1991)	Hormonal mechanism following the relative plasticity hypothesis v.2 (Moore *et al.* 1998)
Fixed	Structural organization	Organizational effect	Organizational effect (post-maturational)
Sequential	Structural reorganization	Activational effect	Organizational effect
Reversible	Biochemical switching	Activational effect	Activational effect

2005). In particular androgens participate in the differentiation of primary and secondary sex characters, in the expression of reproductive behavior, in the feedback regulation of the hypothalamus and pituitary, and in spermatogenesis (Nelson 2005, Oliveira 2005). These pivotal roles in reproduction make them the preferential target for studies of endocrine correlates of male ARTs. However, as discussed below, the development of male ARTs is likely to be influenced by the neuroendocrine system in addition to gonadal steroids.

Glucocorticoids play an important role as mediators of interindividual variation in social behavior. One classic example of such an effect is provided by a series of studies on the relationship between social status and cortisol levels among free-living male olive baboons (*Papio anubis*) in an African national park (Sapolsky 1983, Sapolsky and Ray 1989, Virgin and Sapolsky 1997). In stable social hierarchies, dominant males have lower basal cortisol concentrations than do subordinates, but these differences disappear at times of social instability when all males show elevated basal cortisol levels and suppressed cortisol responsiveness to stress (Sapolsky 1983). Moreover, within high- and low-ranking males, individuals adopting different behavioral profiles also share different endocrine profiles. Among dominant males, only those with a high degree of social skill (e.g., those that are able to distinguish between threatening and neutral interactions with rivals and therefore more likely to initiate fights in the first but not in the latter case) had lower basal cortisol titers. Dominant males lacking these skills had cortisol levels as high as subordinates (Sapolsky and Ray 1989). Also among low-ranking males, a subset of individuals with high rates of consortships had higher cortisol levels than subordinates who had high rates of surreptitious copulations. This might reflect the stress experienced by the former subset of subordinates, which adopt a precocious strategy of open reproductive competition with the dominant males (Virgin and Sapolsky 1987). Overall, these studies suggest that glucocorticoid profiles are associated with distinctive behavioral styles. Moreover, glucocorticoids can interact with the HPG axis and thus modulate the expression of reproductive traits (Sapolsky *et al.* 2000).

Finally, studies of two forebrain neuropeptide systems may help us to understand the differentiation of ARTs: gonadotropin-releasing hormone (GnRH) and arginine vasopressin (AVP; or arginine vasotocin [AVT] in non-mammalian vertebrates). GnRH plays a central role in the control of vertebrate reproduction by orchestrating the functioning of the HPG axis (Parhar 2002) and AVP/AVT influences the expression of social behavior patterns, including courtship behavior, in a wide range of vertebrates (Goodson and Bass 2001). Since both neuropeptide systems have been reviewed in the light of ARTs (Foran and Bass 1999, Bass and Grober 2001) and will be addressed in a separate chapter in this volume (see Chapter 6), we will limit this review to the evidence for the involvement of sex steroids and glucocorticoids in ARTs in the next two sections.

7.4 SEX HORMONES AND ARTs: THE RELATIVE PLASTICITY HYPOTHESIS AND BEYOND

7.4.1 Testing the relative plasticity hypothesis: the first prediction

In order to look for associations between patterns of circulating sex hormone levels (i.e., gonadotropins, androgens, estrogens, and progestogens) and the expression of alternative reproductive morphs in the different classes of vertebrates, we have surveyed the published literature (see Table 7.3).

Table 7.3. *Comparison of hormone levels between alternative reproductive tactics in male vertebrates*

CLASS/Family/Species	Alternative phenotypes	Intrasexual dimorphism	Pituitary tropic hormones	Androgens	Estrogens	Progestogens	Glucocorticoids	References
TELEOSTEI								
Batrachoididae								
Lusitanian toadfish, *Halobatrachus didactylus*	Fixed?	+	?	KT: B > P T: B = P	E2: B = P	B = P	?	Modesto and Canário 2003a
Plainfin midshipman, *Porichthys notatus*	Fixed: type I calling vs. type II noncalling males	+	?	KT: B > P T: B < P	E2: B = P	?	?	Brantley *et al.* 1993b
Blenniidae								
Peacock blenny, *Salaria pavo*	Sequential: nest-holders vs. female-mimic sneakers	+	?	KT: B > P[a] T: B > P[a]	?	?	?	Oliveira *et al.* 2001b
Rock-pool blenny, *Parablennius parvicornis*	Sequential: nest-holders vs. satellites	+	?	KT: B > P T: B = P	?	?	?	Oliveira *et al.* 2001c
Centrarchidae								
Bluegill sunfish, *Lepomis macrochirus*	Fixed: parentals vs. sneakers and satellites	+	?	KT: B > P T: B = P	?	?	?	Kindler *et al.* 1989
Longear sunfish, *Lepomis megalotis*	Fixed?	+	?	KT: B > P T: B = P	?	?	F: B < P	Knapp 2003
Cichlidae								
Princess of Burundi, *Neolamprologus pulcher*	Sequential: breeders vs. helpers	-	?	KT: B = P[b] T: B = P[b]	?	?	?	Oliveira *et al.* 2003
Mozambique tilapia,	Reversible: territorial courting vs.	+	?	uKT: B > Pu T: B > P	?	uP: B > P	?	Oliveira *et al.* 1996, Oliveira

Species	Type of tactic							References
Oreochromis mossambicus	nonterritorial female mimics							and Almada 1998a
St. Peter's fish, *Sarotherodon galilaeus*	Reversible: monogamous vs. polygynous males	−	?	KT: B=P / T: B=P	?	B=P	?	Ros et al. 2003
Labridae								
Corkwing wrasse, *Symphodus melops*	Fixed: territorial vs. female mimics	+	?	KT: B>P / T: B<P	E2: B<P	?	?	Uglem et al. 2000, 2002
Rainbow wrasse, *Coris julis*	Sequential: initial-phase vs. terminal-phase males	+	?	KT: B>P[c]	?	?		Reinboth and Becker 1984
Saddleback wrasse, *Thalassoma duperrey*	Sequential: initial-phase vs. terminal-phase males	+	?	KT: B>P / T: B=P	E2: B=P	B=P	?	Hourigan et al. 1991
Poeciliidae								
Sailfin molly, *Poecilia velifera*	Reversible: large, courting vs. small, noncourting males	−	?	KT: B=P[b] / T: B=P[b]	?	?		R. F. Oliveira, D. M. Gonçalves, and I. Schlupp, unpubl. data
Salmonidae								
Atlantic salmon, *Salmo salar*	Fixed: mature parr vs. anadromous males	+	?	KT: B>P[d] / T: B<P[d]	?	B=P[d]	?	Mayer et al. 1990
Scaridae								
Stoplight parrotfish, *Sparisoma viride*	Sequential: initial-phase vs. terminal-phase males	+	?	KT: B>P / T: B>P	E2: B<P	?	?	Cardwell and Liley 1991
Serranidae								
Belted sunfish, *Serranus subligarius*	Reversible: streakers vs. pair spawners in a simultaneous hermaphrodite	−	?	KT: B>P	?	B>P	?	Cheek et al. 2000

Table 7.3. (*cont.*)

CLASS/Family/Species	Alternative phenotypes	Intrasexual dimorphism	Pituitary tropic hormones	Androgens	Estrogens	Progestogens	Glucocorticoids	References
AMPHIBIA								
Bufonidae								
Great plains toad, *Bufo cognatus*	Reversible: callers vs. satellites	–	?	DHT: B=P T: B=P	?	?	B: B>P	Leary *et al.* 2004
Woodhouse's toad, *Bufo woodhousii*	Reversible: callers vs. satellites	–	?	DHT: B=P T: B=P	?	?	B: B>P	Leary *et al.* 2004
Ranidae								
Bullfrog, *Rana catesbeiana*	Reversible: callers vs. satellites	–	?	DHT: B<P T: B<P	?	?	B: B>P	Mendonça *et al.* 1985
REPTILIA								
Iguanidae								
Marine iguana, *Amblyrhynchus cristatus*	Sequential: territorial vs. satellite vs. sneaker	+	?	T: B>P1=P2	?	?	?	Wikelski *et al.* 2005
Gekkonidae								
Leopard gecko, *Eublepharis macularis*	Fixed: males from male-biased incubation temperature vs. males from female-biased incubation temperature (more sexually active and less aggressive)	+(males from male-biased incubation temperature with larger snout–vent length)	?	T: B=P DH T: B=P	E2: B<P	?	B: B=P	Flores *et al.* 1994, Tousignant and Crews 1995, Crews *et al.* 1998
Phrynosomatidae								
Tree lizard, *Urosaurus ornatus*	Fixed: territorial vs. nomadic rovers/sedentary satellites	+(dewlap color: orange with blue spot, orange)	?	T: B=P T: $P_1 < P_2$	?	?	B: B=P, $P_1 > P_2$	Thompson and Moore 1992, Moore *et al.* 1998, Knapp *et al.* 2003

Side-blotched lizard, *Uta stansburiana*	Fixed: ultraterritorial vs. territorial vs. nonterritorial female mimics	+(throat color: orange, blue and yellow morphs)	?	T: $B > P_1 = P_2$	?	?	Sinervo et al. 2000
Colubridae Red-sided garter snake, *Thamnophis sirtalis parietalis*	Sequential: he-males vs. she-males	+(skin lipids)	?	T: $B < P$	?	?	Mason and Crews 1985
AVES Brown-headed cowbird, *Molothrus ater*	Reversible: paired vs. unpaired (EPC)	—	?	T: $B > P$	?	?	Dufty and Wingfield 1986
House finch, *Carpodacus mexicanus*	Fixed: dominant and no parental care vs. subordinate and parental care	+(plumage: dull vs. redder)	?	T: $B < P$	?	?	Duckworth et al. 2004
White-throated sparrow, *Zonotrichia albicollis*	Fixed: dominant singing vs. subordinate and less singing	+(plumage: white stripe vs. tan stripe)	?	T: $B > P$	?	?	Maney et al. 2005
Pied flycatcher, *Ficedula hypoleuca*	Reversible: polyterritorial vs. home-territorial	—	LH: $B > P$	T: $B > P$	?	?	Silverin and Wingfield 1982
Seychelles warbler, *Acrocephalus sechellensis*	Sequential: primary males vs. subordinate males (cooperative breeding, EGC)	—	?	T: $B > P$	?	?	Crommenacker et al. 2004
Florida scrub-jay, *Aphelocoma coerulescens*	Sequential: nest-owners vs. non-nesting (EPC)	—	?	T: $B = P$	?	?	Vleck and Brown 1999
Acorn woodpecker, *Melanerpes formicivorus*	Sequential: breeder vs. helper (cobreeder)	—	?	T: $B = P$	?	?	Koenig and Dickinson, this volume
Australian magpie, *Gymnorhina tibicen*	Sequential/ reversible: breeding adults vs.	+(plumage between adult	?	T: $B1 = P1$ $B2 > P2$	?	?	Schmidt et al. 1991

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Table 7.3. (*cont.*)

CLASS/Family/ Species	Alternative phenotypes	Intrasexual dimorphism	Pituitary tropic hormones	Androgens	Estrogens	Progestogens	Glucocorti- coids	References
	nonbreeding adults vs. breeding subadults vs. nonbreeding subadults	and subadult morphs)	LH: B1 = P1 B2 > P2	DHT: B1 = P1 B2 > P2				
Azure-winged magpie, *Cyanopica cyanus*	Reversible: breeder vs. helper (EPC)	—	?	T: B = P	?	?	?	De la Cruz *et al.* 2003
Bell miner, *Manorina melanophrys*	Sequential: breeder vs. helper (EPC)	—	?	T: B > P	?	?	?	Poiani and Fletcher 1994
Superb fairy–wren, *Malurus cyaneus*	Sequential: dominant group breeder vs. paired vs. helper (EGC)	—	?	T: B > P1 = P2	?	?	?	Peters *et al.* 2001
Pied kingfisher, *Ceryle rudis*	Sequential: breeders vs. primary helpers vs. secondary helpers	—	LH: B = P1 = P2	T: B = P2 > P1	?	?	?	Reyer *et al.* 1986
Harris's hawk, *Parabuteo unicinctus*	Sequential: adult breeders vs. adult-plumaged helpers vs. juvenal-plumaged helpers	+(plumage)	LH: B = P1 > P2	T: B = P1 > P2	E2: B = P1 = P2	?	B: B = P1 = P2	Mays *et al.* 1991
MAMMALIA Hominidae Human, *Homo sapiens*	Reversible: polygynously married Swahili men *vs.* monogamously married Swahili men	—	?	$_s$T: AT1 > AT2[e]	?	?	?	Gray 2003

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Taxon	Alternative mating tactics	Secondary sexual traits	Other hormones		T		F	References
Chimpanzee, *Pan troglodytes schweinfurthii*	Reversible: dominant vs. subordinate (surreptitious matings)	—	?	?	$_u$T: B > P $_f$ T: B > P	?	$_u$F: B > P	Muehlenbein *et al.* 2004, Muller and Wrangham 2004a, 2004b
Orang-utan, *Pongo* spp.	Sequential: territorial flanged males (courting with long call vocalization) vs. nomadic unflanged males (forced copulations)	+(facial flanges, long, thick hair and a vocal throat sac in territorial males)	$_u$LH: B > P $_u$FSH: B = P $_u$GH: B > P $_u$TSH: B = P	?	$_u$T: B > P $_u$DHT: B > P	?	$_u$F: B = P	Maggioncalda *et al.* 1999, 2000, 2000, 2002
Cercopithecidae Mandrill, *Mandrillus sphinx*	Reversible: social fatted males vs. solitary nonfatted males (sneak matings in group incursions)	+(brilliant red and blue skin in the face and fat rumps in alfa social males)	?	?	T: B > P	?	?	Setchell and Dixson 2001
Olive baboon, *Papio anubis*	Sequential: dominant (maintain consort-ships) vs. subordinate (stolen copulations)	—	?	?	T: B > P^f	?	F: B > P^f	Sapolsky 1983, Virgin and Sapolsky 1997, Ray and Sapolsky 1992
Rhesus macaque, *Macaca mulatta*	Plastic: dominant vs. subordinate (surreptitious matings)	—	?	?	T: B > P	?	?	Rose *et al.* 1971
Japanese macaque, *Macaca fuscata*	Plastic: dominant (multiday consortships with estrous female) vs. subordinate (forced copulations)	—	?	?	$_f$T: B = P	?	$_f$F: B > P	Barrett *et al.* 2002
Cebidae Mantled howling monkey, *Alouatta palliata*	Sequential: dominant group mating vs. subordinate (opportunistic mating)	—	?	?	$_f$T: B > P	?	?	Jones 1995, Zucker *et al.* 1996

Table 7.3. (*cont.*)

CLASS/Family/ Species	Alternative phenotypes	Intrasexual dimorphism	Pituitary tropic hormones	Androgens	Estrogens	Progestogens	Glucocorti- coids	References
Tufted capuchin monkey, *Cebus paella nigritus*	Reversible: dominant (courtship followed by multiple mounts) vs. subordinate (rapid copulation)	–	?	$_f$T: B = P	?	?	$_f$F: B = P	Lynch *et al.* 2002, Lynch Alfaro 2005
Callitrichidae								
Common marmoset, *Callithrix jacchus*	Sequential: dominant breeder vs. subordinate helper (copulation with extragroup females during intergroup encounters)	–	?	T: B = P	?	?	?	Abbott and Hearn 1978, Baker *et al.* 1999, Digby 1999
Indridae								
Sifaka, *Propithecus verreauxi*	Plastic: dominant (scent marking and mate guarding) vs. subordinate	–	?	$_f$T: B > P	?	?	?	Kraus *et al.* 1999
Lemuridae								
Ring-tailed lemur, *Lemur catta*	Plastic: dominant *vs.* subordinate (group transfer?)	–	?	$_f$T: B > P	?	?	?	Cavigelli and Pereira 2000, Gould 2005
Cricetidae								
Mongolian gerbil, *Meriones unguiculatus*	Fixed (intrauterine position): 2M males vs. 2F males (reduced copulatory behavior and scent marking; alloparenting)	+(reduced bul- bocavernosus muscle mass)	?	T: B > P	?	?	?	Clark and Galef 2000

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Sciuridae							
Alpine marmot, *Marmota marmota*	Sequential: dominant territorial vs. subordinate kin vs. subordinate nonkin	−	?	T: B=P1 >P2	?	GC: B=P2 >P1	Arnold and Dittami 1997
Bathyersidae							
Naked mole-rat, *Heterocephalus glaber*	Fixed?: colony defenders vs. dispersers	+(higher girth-to-body length and more fat in the neck region)	LH: B < P	?	?	?	O'Riain *et al.* 1996
	Plastic: within–colony breeders vs. nonbreeders	−	?	$_u$T: B > P	?	$_u$F: B = P	Clarke and Faulkes 1998
Elephantidae							
African elephant, *Loxodonta africana*	Reversible: musth vs. sexually active without musth	−	?	$_f$EA: B > P	?	$_f$GC: B = P	Ganswindt *et al.* 2005
Rhinocerotidae							
White rhino, *Ceratotherium simum*	Sequential: territorial vs. nonterritorial (satellite)	−	?	$_f$T: B > P	?	?	Rachlow *et al.* 1998
Equidae							
Plains zebra, *Equus burchelli*	Sequential: harem breeding vs. bachelor	−	?	$_u$T: B > P	?	?	Chaudhuri and Ginsberg 1990
Grevy's zebra, *Equus grevyi*	Sequential: territorial vs. bachelor	−	?	$_u$T: B > P	?	?	Chaudhuri and Ginsberg 1990
Shetland pony, *Equus caballus*	Sequential: harem stallion vs. bachelor	−	?	T: B > P	?	?	McDonnell and Murray 1995
Misaki feral horse, *Equus caballus*	Sequential: harem stallion vs. bachelor	−	?	T: B > P	?	?	Khalil *et al.* 1998
Przewalski horse, *Equus ferus przewalski*	Sequential: harem stallion vs. bachelor	−	?	$_f$T: B > P $_f$EA: B > P	$_f$E2: B > P	?	Schwarzenberger *et al.* 2004

Table 7.3. (*cont.*)

CLASS/Family/ Species	Alternative phenotypes	Intrasexual dimorphism	Pituitary tropic hormones	Androgens	Estrogens	Progestogens	Glucocorti-coids	References
Bovidae								
Plains bison, *Bison bison*	Sequential: tending vs. nonguarding	−	?	$_f$T: B > P	?	?	?	Mooring *et al.* 2004
Bighorn sheep, *Ovis canadensis*	Sequential: tending vs. coursing/blocking	+	?	$_f$T: B > P	?	?	?	Pelletier *et al.* 2003
Cervidae								
Impala, *Aepyceros melampus*	Sequential: territorial vs. bachelor	−	?	T: B > P	?	?	?	Illius *et al.* 1983
Viverridae								
Dwarf mongoose, *Helogale parvula*	Sequential: dominant breeder vs. subordinate helper	−	?	$_u$T: B = P	?	?	?	Creel and Waser 1994, Creel *et al.* 1992
Meerkat, *Suricata suricatta*	Sequential: dominant breeder vs. subordinate helper	−	LH: B = P	$_f$T: B = P$_u$ T: B = P	?	?	?	O'Riain *et al.* 2000a, Moss *et al.* 2001
Felidae								
African lion, *Panthera leo*	Sequential: dominant vs. subordinate	+(darker mane vs. lighter mane)	?	T: B > P	?	?	?	West and Packer 2002
Canidae								
Gray wolves, *Canis lupus*	Sequential: dominant vs. subordinate helper	−	?	?	?	?	$_f$F: B = P	Sands and Creel 2004
African wild dog, *Lycaon pictus*	Sequential: dominant vs. subordinate	−	?	$_f$T: B > P	?	?	$_f$F: B > P	Creel *et al.* 1997

Phocidae

Harbor seal, *Phoca vitulina*	Sequential: larger hauled-out alone vs. medium-sized with low fidelity to haul-out site	—	?	?	T: B=P	?	?	Coltman et al. 1999
Weddell seal, *Leptinychotes weddelli*	Sequential: territorial vs. nonterritorial	—	?	?	T: B>P	?	F: B>P	Bartsch et al. 1992

References: For each species we give the reference that has reported the relative hormone levels of the alternative tactics. In cases in which the endocrine data is given in a paper that does not mention the ARTs, we also cite a paper that documents the occurrence of alternative tactics in that species (e.g., one paper reports that dominant males of a given species have higher testosterone levels than subordinates and an independent paper suggests that subordinate males of that same species use an alternative mating tactic).

Hormone abbreviations: LH, luteinizing hormone; FSH, follicle stimulating hormone; GH, growth hormone; TSH, thyroid stimulating hormone; T, testosterone; KT, ketotestosterone; DHT, dihydrotestosterone; EA, epiandrosterone; E2, estradiol; P, progestogen (can vary across taxa, e.g., progesterone in mammals and various kinds among teleost fish); F, cortisol; B, corticosterone; GC, antibody used had higher cross reactivity with more than one glucocorticoid.

Hormone prefixes: u, urinary levels; f, fecal levels; s, salivary levels.

Alternative tactic abbreviations: B, bourgeois tactic; P, parasitic tactic (P1 and P2 are used when there is more than one alternative tactic); EPC, extra-pair copulation; EGC, extra-group copulation.

[a] Testicular androgen levels.

[b] Steroid levels in fish holding water.

[c] In vitro gonadal production from [^{14}C] T incubation.

[d] Values for late summer, when GSI values peak.

[e] In some species alternative tactics occur that do not match the functional classification of bourgeois vs. parasitic males, e.g., polygynously vs. monogamously married Swahili men; in these cases we have used AT1 and AT2 as abbreviations for alternative tactic 1 and alternative tactic 2.

[f] Only present in stable hierarchies.

A clear association exists between androgen levels and the expression of one of the alternative reproductive tactics (Table 7.3). For the majority of the species, the conventional morph has higher levels of androgens than the alternative morphs, but in many other cases, there are no significant differences in androgens between the two alternative morphs, and in some cases the parasitic males may even have higher androgen levels than the bourgeois males (Table 7.3). How can such variability be explained?

Could this variability be explained by the first prediction of Moore's reproductive plasticity hypothesis – that hormone profiles should differ in plastic adult morphs but not in fixed ones?

Unfortunately, the relative plasticity hypothesis is flawed. Androgen levels not only influence behavior (and thus can be expected to play an activational role in species with plastic ARTs), but they can also be influenced by the social environment in which the animal lives (Wingfield et al. 1990, Oliveira et al. 2002, Oliveira 2004). This means that any conclusions derived from finding different levels of androgens in alternative reproductive morphs (either fixed or plastic) are suspect. Moore (1991) argued that in fixed ARTs, adult hormone profiles should be similar among alternative male phenotypes, except when alternative morphs experience different social environments (see also Thompson and Moore 1992). Therefore, positive associations, negative associations, and even the lack of an association between androgen levels and the ART type are to be expected. As a result, the study of androgen levels in species with plastic ARTs is far more informative. In fact, among plastic species androgen levels should differ between the alternative morphs, and any negative result (lack of difference) cannot be explained by differential influences of the social environment on the androgen levels of the alternative phenotypes. Thus, the most robust estimate of this prediction is to compute the percentage of plastic species in which there are no differences in circulating levels between the bourgeois and the parasitic morph. In order to make this exercise easier and to avoid potential phylogenetic bias (i.e., bias introduced by some patterns being more characteristic of some vertebrate classes than others), the raw data from Table 7.3 were reorganized into contingency tables for each vertebrate class (the data for amphibians and reptiles were pooled into a single table owing to the low number of species for which endocrine data on ARTs are available) (Tables 7.4 through Table 7.7). In these tables, the shaded background cells represent cases that support the first prediction of the relative plasticity hypothesis and the white background cells

Table 7.4. *Test of the first prediction of the relative plasticity hypothesis in fish*

Androgen levels	ART type	
	Fixed	Plastic
Bourgeois > Parasitic	Plainfin midshipman Lusitanian toadfish Bluegill sunfish Corkwing wrasse Atlantic salmon	Peacock blenny Rock-pool blenny Stoplight parrotfish Rainbow wrasse Saddleback wrasse Mozambique tilapia Belted sunfish
Bourgeois = Parasitic		Princess of Burundi St. Peter's fish Sailfin molly
Bourgeois < Parasitic		

Table 7.5. *Test of the first prediction of the relative plasticity hypothesis in reptiles and amphibians*

Androgen levels	ART type	
	Fixed	Plastic
Bourgeois > Parasitic	Side-blotched lizard	Marine iguana
Bourgeois = Parasitic	Tree lizard	Great plains toad Woodhouse's toad
Bourgeois < Parasitic		Bullfrog Red-sided garter snake

represent those that reject it. The tables illustrate that by using this conservative estimate from the relative plasticity hypothesis, we cannot explain 30% of the occurrences of plastic ARTs in fish, 40% of those in amphibians and reptiles, 54.5% of the plastic ART cases in birds, and 19.4%

Table 7.6. *Test of the first prediction of the relative plasticity hypothesis in birds*

Androgen levels	ART type	
	Fixed	Plastic
Bourgeois > Parasitic		Brown-headed cowbird
		Pied flycatcher
		Seychelles warbler
		Bell miner
		Superb fairy-wren
Bourgeois = Parasitic		Mexican scrub-jay
		Acorn woodpecker
		Australian magpie
		Azured magpie
		Pied kingfisher
		Harris's hawk
Bourgeois < Parasitic	House finch	

Table 7.7. *Test of the first prediction of the relative plasticity hypothesis in mammals*

Androgen levels	ART type	
	Fixed	Plastic
Bourgeois > Parasitic	Mongolian gerbils	Human
		Chimpanzee
		Orang-utan
		Mandrill
		Olive baboon
		Rhesus monkey
		Mantled howling monkey
		Sifaka
		Ring-tailed lemur
		Alpine marmot
		Naked mole-rat
		African elephant
		White rhino
		Plain zebra
		Grevy's zebra
		Shetland pony
		Misaky feral horse
		Przewalski horse
		Plains bison
		Bighorn sheep
		Impala
		African lion
		African wild dog
		Harbor seal
		Weddell seal
Bourgeois = Parasitic		Japanese monkey
		Common marmoset
		Tufted capuchin monkey
		Dwarf mongoose
		Meerkat
Bourgeois < Parasitic		

of mammalian plastic ARTs. This means that the model can potentially explain over 80% of the ART cases in mammals, where sex is genetically determined, males are the heterogametic sex, and the expression of their secondary sexual characteristics is androgen dependent. Among other vertebrate classes, where the mechanisms of primary sex determination vary from those present in eutherian mammals, the model loses its predictive power. In birds, females are the heterogametic sex and the expression of male ornaments, a typical bourgeois trait, is, in most cases, not androgen dependent (e.g., male breeding plumage: Owens and Short 1995; but see Kimball and Ligon 1999). In amphibians, reptiles, and fish, primary sex determination mechanisms are more labile and open to influences from the environment, such as temperature or the social context (environmental sex determination, ESD), even though sex chromosomes may be present (Crews 1998). For example, genetic sex determination (GSD) mechanisms in fish, which are present in approximately half the species that have been studied using cytogenetical data, are very diverse. They range from polygenic systems to systems with dominant sex-determining factors, to sex chromosomes with either heterogametic males (XY) or females (ZW) (Devlin and Nagahama 2002). Interestingly, the number of species that display male heterogamety is twice the number of those

with female heterogamety (Devlin and Nagahama 2002), a fact that could, to a degree, explain why fish appear as the second best fit of the model. In summary, an association between the mechanisms of sex determination operating in each animal class and the role of sex hormones on the expression of ARTs seems to be present, which in turn

suggests that differences between alternative reproductive morphs within a sex are based on the same mechanisms that generate sex differences within a species (Godwin and Crews 2002). Crews (1998) already pointed out the relationship between the sex-determination mechanism and the type of ART displayed, suggesting that species with fixed tactics should have GSD, whereas species with plastic tactics should have either GSD or ESD (but see Oliveira 2005 for a review of this issue among teleost fish yielding different results). The parallels between the processes of sex differentiation (i.e., males vs. females) and the differentiation of discrete alternative reproductive phenotypes within the same sex further support a role for sex steroids in the differentiation of intrasexual alternative phenotypes.

How can we explain species with fixed ARTs in which androgen levels differ between the alternative phenotypes? As mentioned above differences in sex hormone levels between alternative reproductive male types might not reflect different hormone profiles due to an activational effect on the expression of the bourgeois tactic, but rather might reflect the responsiveness of these hormones to the expression of the tactic itself (Thompson and Moore 1992). That is, they are a consequence and not a cause of the expression of alternative mating tactics. This can be the case if the alternative phenotypes experience different social environments, which is very likely since by definition bourgeois males defend resources to get access to mates and thus are expected to face higher levels of social challenges than parasitic males. For example, in the peacock blenny, nest-holder males show an increase in androgen levels during the breeding season that is positively correlated with an increase in sneaking attempts to which they are exposed (Oliveira et al. 2001a). In only three cases does the parasitic tactic have a higher testosterone level than the bourgeois tactic: the house finch (*Carpodacus mexicanus*), the bullfrog (*Rana catesbeiana*), and the red-sided garter snake (*Thamnophis sirtalis parietalis*). In the house finch, the dull and less ornamented males are dominant over redder males, but the redder males pair earlier and provide more parental care than the dull males (Duckworth et al. 2004). In addition, the higher testosterone levels found in free-living, dull males are probably the result of dull males having a higher motivation to access food resources and are not a direct cause for the differentiation of alternative phenotypes (Duckworth et al. 2004). In the bullfrog, the lower levels of androgens present in calling (bourgeois) males have been interpreted as a stress-related cost due to frequent combat to defend territories (Mendonça et al. 1985). In the red-sided

garter snake, higher androgen levels in recently emerged she-males (which is a phase through which apparently all males go after emerging from winter dormancy: Shine et al. 2000) can be a consequence of the twofold higher mating activity that they experience compared to conventional males (Mason 1992).

Data on progestogens are available for six species with ARTs, all of them teleosts (Table 7.3). Interestingly, progestogens are never higher in the parasitic morph than in the bourgeois morph (they are higher in the bourgeois males than the parasitic males in two species, and no differences are present in the other four species). However, the progestogen(s) measured varied from species to species. For example 17,20β,21-trihydroxy-4-pregen-3-one (17,20β21P), 17,20α-dihydroxy-4-pregen-3-one (17,20αP), and 17,20βP were measured in the Lusitanian toadfish (Modesto and Canário 2003a); 17,20αP and 17,20βP were assayed in the Mozambique tilapia (Oliveira et al. 1996); 17,20β21P and 17,20βP were determined in the belted sunfish (Cheek et al. 2000); whereas only 17,20βP has been monitored in the saddleback wrasse (Hourigan et al. 1991), in the St. Peter's fish (Ros et al. 2003), and in the Atlantic salmon (Mayer et al. 1990). The available data suggest that 17,20β21P in the toadfish, 17,20βP in the saddleback wrasse, and 17,20βP in the Atlantic salmon may play a role in male reproduction (e.g., spermiation). In the Mozambique tilapia, territorial males have higher levels of both 17,20αP and 17,20βP than nonterritorial, female-mimicking males, but only a 17,20αP increase in the plasma concentration in the presence of females when courtship behavior is expressed by the males (Oliveira et al. 1996), suggesting that 17,20αP may play a major role in spawning behavior and/or spermiation in this species. In the belted sandfish, 17,20β21P rather than 17,20βP seems to be associated with male reproductive behavior (Cheek et al. 2000). In summary, progestogens appear to be associated with the expression of bourgeois reproductive traits, but for most species it is difficult to disentangle potential effects of progestogens on male courtship behavior from effects on spermiation. It is also interesting to note that in the tree lizard, a species with fixed ARTs determined early in ontogeny (see Section 7.4.2), progesterone peaks twice during the critical period, and on both occasions the levels are bimodal at the population level, suggesting a potential involvement of progesterone on morph differentiation (Moore et al. 1998). This is further supported by the fact that approximately 90% of the individuals that received a single injection of progesterone on the day of hatching

differentiated into the bourgeois morph (Moore *et al.* 1998). Future studies should examine the role of progestogens on the expression of ARTs.

Estrogens have also been measured in alternative morphs of five teleosts and in one mammal. Among fish estradiol titers are never higher in the bourgeois morph (they are lower in two cases and equal in the other three; see Table 7.3). In contrast, fecal estrogen levels are significantly higher in stallions than in bachelor males of Przewalski horses (Table 7.3). However, it should be stressed that, in all cases, estrogen levels are almost always very low, suggesting that high circulating estrogen levels are incompatible with the expression of the bourgeois tactic, at least among teleost fish.

Finally, data are available on luteinizing hormone (LH) for seven species (four birds and three mammals). One of the cases for which an LH level is available is an interesting type of ART in which a dispersive morph has been described in naked mole-rats (see Box 7.1). Since it is not clear that the colony defenders are playing a bourgeois tactic and the dispersers a parasitic tactic, no clear prediction can be made for this case; however, it has been found that dispersers exhibit higher LH circulating concentrations than colony defenders (O'Riain *et al.* 1996). In the remaining six cases in which the adopted functional dichotomy bourgeois-vs.-parasitic tactic seems to be valid, LH levels are never lower in the bourgeois morph (it is higher in two cases and similar in the other four) than in the parasitic morph. In all of these cases, LH perfectly mirrors the differences in androgen levels between morphs (Table 7.3). Therefore, a direct involvement of LH in the differentiation of alternative tactics is not plausible, and the most parsimonious hypothesis for its action upon morph differentiation is through sex steroids.

7.4.2   Testing the relative plasticity hypothesis: the second prediction

As mentioned above, according to the second prediction of the relative plasticity hypothesis, in species with fixed ARTs, hormone manipulations should only be effective early in development (i.e., should have organizational effects), whereas in species with plastic ARTs, the exogenous administration of hormones should be effective in adults (Moore 1991, Moore *et al.* 1998). Unlike the first prediction, the second prediction does not suffer from epistemological flaws and provides, therefore, a stronger test for the assessment of the relative plasticity hypothesis. Unfortunately, hormone

levels of alternative phenotypes have been manipulated in only 12 species (see Table 7.8 for a survey of the available literature on hormone manipulations in species with ARTs).

In only one case, the tree lizard, have the effects of early administration of androgens to males of a species with fixed ARTs been evaluated. Males treated with testosterone implants the day they hatched developed into the orange-blue morph in a significantly higher proportion than sham-operated males. Conversely, males castrated at the same age preferentially developed into the orange phenotype (Hews *et al.* 1994). These data support an organizational effect of androgens in the expression of tree lizard ARTs and suggest a well-defined critical period for this effect in the ontogeny of the species. Tree lizard males begin to express their color morphs between days 60 and 90 post-hatching (Moore *et al.* 1998). Testosterone implants on day 1 and on day 30 were effective in directing morph differentiation, while those performed on day 60 had no effect, indicating the presence of a critical period that ends between day 30 and day 60 post-hatching (Hews and Moore 1996). Another case demonstrating that early exposure to hormones manipulates the expression of ARTs is the Mongolian gerbil. In this species an intrauterine position effect has been described in which males gestated between two females (2F males) have lower testosterone levels when adults than their brothers gestated between two male fetuses (2M males) (see Box 7.1). Some of the 2F males that display exceptionally low levels of circulating testosterone (i.e., similar to those of females) do not express male sexual behavior when exposed to females in oestrus but, in contrast, overexpress allopaternal behavior. Therefore, the early exposure to androgens determines the tactic adopted by male Mongolian gerbils, with some 2F males becoming asexual and obligate helpers (Clark and Galef 2000). These two examples strongly support a straightforward organizational effect of androgens on the development of fixed alternative phenotypes.

The evidence compiled for hormone manipulations in adulthood yields much less clear results (Table 7.9). Of the 11 species that have been studied, only five support Prediction 2. Of the five supportive cases, in two of them (one reptile and one cooperatively breeding bird), the administration of testosterone to the parasitic morph of species with plastic ARTs induced a tactic switch (see Tables 7.8 and 7.9). In a third case, the inhibition of testosterone production reduced the sexual activity of juvenile males that tried to steal copulations in Soay sheep, *Ovis aries* (Stevenson and Bancroft 1995). In the other two cases, there was no effect of the administration of testosterone on the

Table 7.8. *Effects of hormone manipulations on adult alternative (i.e., parasitic) reproductive morphs in vertebrates*

CLASS/Species	Alternative phenotypes	Hormone manipulation (manipulated sex type)	Effects on behavior	Effects on morphology	References
TELEOSTEI					
Plainfin midshipman	Fixed	KT (type II males)	–	+ (sonic muscle)	Lee and Bass 2004
Peacock blenny	Sequential	KT implant (sneakers)	–/+ (no effect on bourgeois behavior but inhibits sneaking behavior)	+ (anal gland and genital papillae)	Oliveira et al. 2001d
Rock-pool blenny	Sequential	KT and MT implants (satellite males)	–/+ (MT treatment increases the time satellite males spend in independent nests)	+ (anal gland and genital papillae)	Oliveira et al. 2001e
Sailfin molly	Reversible	MT injections (small males)	–	Not determined	R. F. Oliveira, I. Schlupp, D. M. Gonçalves, and A. V. M. Canário, unpubl. data
REPTILES					
Marine iguana	Sequential	Testosterone IP injection (satellite males)	+ (establishment of temporary territories)	–	Wikelski et al. 2005
		Testosterone IP injection (sneaker males)	+ (leave female groups and start behaving like satellite males)	–	Wikelski et al. 2005
		Androgen receptor blocker + aromatase inhibitor IP injection (territorial males)	+ (decrease head-bob patrolling, territory size and number of females in the territory)	–	Wikelski et al. 2005
Tree lizard	Fixed	Castration (neonatal males)	Not determined	+ (increased the frequency of orange males as adults)	Hews et al. 1994
		Testosterone implant (neonatal males)	Not determined	+ (increased the frequency of orange-blue males as adults)	Hews et al. 1994
		Testosterone implant (males at days 1, 30, and 60 after hatching)	Not determined	+ (d1 and d30 treatments increased and d60 had no effect on the frequency of orange-blue males as adults critical period)	Hews and Moore 1996

Species	Fixed/Plastic	Manipulation	Effect	Effect	Reference
Side-blotched lizard	Fixed	Progesterone injection (neonatal males)	Not determined	+ (increased the frequency of orange-blue males as adults)	Moore et al. 1998
	Fixed	Testosterone implants (blue and yellow morphs)	+ (increase endurance [measured in a treadmill], activity, home-range size, and control over female territories)	–	DeNardo and Sinervo 1994b, Sinervo et al. 2000
AVES					
Ruff, *Philomachus pugnax*	Fixed	Testosterone implants (satellite males)	– (satellite males did not express territorial behavior but increased satellite behavior)	Not determined	D. B. Lank, unpubl. data in Rhen and Crews 2002
		Castration (independent males)	?	+ (lack growth of male displaying feathers)	Van Cordt and Junge 1936 in Lank et al. 1999
		Testosterone implants (females)	+ (male courtship)	+ (increase in body mass and development of male display feathers)	Lank et al. 1999
House finch	Fixed?	Testosterone implants (males)	+ (increased dominance)	–	Duckworth et al. 2004
Azure-winged magpie	Plastic	Testosterone implants (males at the beginning of the breeding season)	– (likelihood of becoming a helper or a breeder)	–	De la Cruz et al. 2003
Superb fairy-wren	Plastic	Testosterone implants (helpers)	+ (increases helper courtship behavior towards own female)	–	Peters et al. 2002
MAMMALS					
Soay sheep, *Ovis aries*	Plastic	Inhibition of testosterone production by medroxy-progesterone acetate injections (juveniles that attempt copulations by harassing consorting pairs)	–P (reduces harassing behavior)	–	Stevenson and Bancroft 1995

For scientific names of each species and information on their taxonomy consult Table 7.3. +, transformations towards the bourgeois phenotype; –, no transformations towards the bourgeois phenotype; –/+, partial transformation towards the bourgeois phenotype; –P, inhibition of parasitic tactic.

Table 7.9. *Testing the second prediction of the relative plasticity hypothesis (shaded cells represent cases that support the prediction)*

Manipulation of androgen levels in parasitic males		ART type	
		Fixed	Plastic
Early in development	Effective	Tree lizards	
	No effects		
In adults	Effective	Side-blotched lizards	Marine iguanas
		House finch	Superb fairy-wren
			Soay sheep
	No effects	Plainfin midshipman	Peacock blenny
		Ruff	Rock-pool blenny
			Sailfin molly
			Azure-winged magpie

parasitic morph of the "fixed" type species (one fish and one lek-breeding bird; see Tables 7.8 and 7.9). Of the six cases that do not support the second prediction, two correspond to positive effects of testosterone administration in "fixed" species (one lizard and one bird), and the other four to the absence of effects of testosterone administration in "plastic" species (three fish and one cooperatively breeding bird) (see Tables 7.8 and 7.9). Therefore, overall, the validity of the second prediction of the relative plasticity hypothesis is only present in 50% of the species studied so far. Unfortunately, in the vertebrate taxa for which the hypothesis is probably most adequately applied, the mammals, there is only one species for which data are available (and it supports the hypothesis).

Interestingly, of all the hormone manipulations performed on vertebrates with the objective of unraveling the physiological mechanisms of ARTs, only in one case (the marine iguana) has the reversibility of the transformation from parasitic to bourgeois male in "plastic" species been tested. In the experiment, territorial males were implanted with an androgen receptor blocker (flutamide) together with an aromatase inhibitor (1,4,6-androstatrien-3,17-dione; ATD) in order to block the direct (i.e., testosterone acting on an androgen receptor) and indirect (i.e., testosterone being aromatized into estradiol, which would activate the behavior) effects of testosterone on the expression of bourgeois behavior (Wikelski *et al.* 2005). Treated males decreased the expression of their territorial behavior, had their territories reduced in size, and suffered a decrease in the number of females present on their territories, but they did not develop the full expression of parasitic behavior. These results

suggest that the blockage of androgens in bourgeois males can reduce the expression of bourgeois behavior but cannot induce a tactic change to a parasitic morph in a "plastic" species with sequential tactics. This conforms to the expectation that plasticity in alternative morphs should only be permissible in directions that correspond to normal sexual differentiation (i.e., parasitic males can transform into bourgeois males but not the reverse).

In summary, although the relative plasticity hypothesis provides a tentative conceptual framework for the study of the hormonal basis of ARTs and has been elegantly developed (Moore *et al.* 1998), it does not seem to apply across vertebrate taxa. One of the major reasons for this mismatch may reside in the fact that this hypothesis, derived from the organizational paradigm of mammalian sex differentiation, is not common to other vertebrate classes and, in particular, is not found in those with labile sex-determining mechanisms.

7.4.3   Beyond the relative plasticity hypothesis: the "making of" alternative phenotypes

It is also important to be able to distinguish whether alternative phenotypes diverge only in terms of behavioral traits, or if they also differ in the expression of morphological traits. Since behavior is often more labile than morphology and anatomy, the mechanisms underlying the expression of behavioral variation are expected to be more flexible than those underlying morphological and anatomical variations. It follows that alternative reproductive tactics that only involve differences in behavior should differ in the activation of

different neural substrates but not necessarily display different hormonal profiles. In contrast, alternative reproductive phenotypes that also show a divergence in morphological traits (i.e., intrasexual polymorphisms), in which the differentiation of sexual characters between the alternative morphs needs a whole-organism control system, are expected to have different hormone profiles to account for these differences. It could be argued that differences in hormone levels should only be present at the period of the differentiation of the tactic, if their effects were to be organizational. However, there are several pieces of evidence suggesting that androgen-dependent traits, typical of bourgeois males, need continuous exposure to androgens to be maintained. For example, in adults androgens inhibit the shrinkage of motorneurons in the spinal nucleus of the bulbocavernosus that controls penile erection in rodents (Breedlove and Arnold 1981, Forger *et al.* 1992, Watson *et al.* 2001). Also, castration induces the regression and exogenous administration of androgens restores the development of sonic muscles in vocalizing male fish (Brantley *et al.* 1993a; but see Modesto and Canário 2003b). The hypothesis that androgens may play differential roles in the differences between male morphs across different phenotypic traits (i.e., behavioral, morphological, and gonadal) will be discussed below.

HORMONES AND DIFFERENCES BETWEEN ALTERNATIVE PHENOTYPES IN SECONDARY SEX CHARACTERS

Since androgens play a major role in the induction of secondary sex characters in male vertebrates (Nelson 2005), differences in androgen levels among morphs may be of little importance in species with alternative tactics lacking major tactic-specific morphological specializations (such as the expression of male secondary sex characters in bourgeois males). Among the species displaying ARTs and intrasexual dimorphism, 100% of the fish, 66.6% of the reptiles, 75% of the birds, and 100% of the mammals (i.e., 90.9% of all studied species) displayed significant differences in circulating androgen levels, with the bourgeois morphs having consistently higher levels than those of the parasitic males (Table 7.3).

Recently, the association between the degree of phenotypic specialization of the alternative tactics and the magnitude of the difference in androgen levels between alternative male types was investigated among teleost fish (Oliveira 2005). In all species for which androgen levels are known and for which the ART involves a morphological intrasexual dimorphism (apart from differences in body size), the levels of 11-ketotestosterone (KT, the most potent androgen in fish) are higher in the bourgeois than in the parasitic male, irrespective of the type of ART displayed (Oliveira 2005). This suggests a parallel to the androgen correlates of sex-changing fish, in which androgens may play a major role in morphological differentiation during sex change but are not essential for behavioral sex change (Godwin *et al.* 1996, Grober 1998, Reavis and Grober 1999). These results, together with the data presented here, suggest a major role for androgens in the differentiation of morphological traits typical of the bourgeois tactic.

HORMONES AND DIFFERENCES BETWEEN ALTERNATIVE PHENOTYPES IN REPRODUCTIVE BEHAVIOR

In species with reversible ARTs without morphological modifications, changes in the activity of neural pathways underlying the behavioral changes are to be expected rather than differences in androgen levels (Zupanc and Lamprecht 2000; see Section 7.3.1). This could explain, for example, the lack of differences in KT levels between polygynous and monogamous males in the St. Peter's fish (Ros *et al.* 2003) and between callers and satellites in toads (Leary *et al.* 2004). Hence, reversible ARTs lacking intrasexual dimorphisms may have been emancipated from a sex-differentiation mechanism ruled by sex hormones. In this respect, it is interesting to note that in the peacock blenny, where sneaker males mimic female courtship behavior, castrated sneakers (that mimic females) continue to exhibit female courtship (D. M. Gonçalves, J. Alpedrinha, and R. F. Oliveira, unpublished data), indicating that gonadal steroids are not crucial for the behavioral expression of the parasitic tactic in this species.

HORMONES AND DIFFERENCES BETWEEN ALTERNATIVE PHENOTYPES IN GONADAL ALLOCATION

For a large number of species with ARTs, in particular among fish, the parasitic morph has relatively larger gonads, a phenomenon which has been explained by the sperm competition hypothesis (Taborsky 1998). This is intriguing from a physiological perspective since androgens are also involved in spermatogenesis. There are several possible explanations for this paradox.

(1) In the particular case of teleost fish, KT and testosterone (T) have different roles in the control of spermatogenesis: KT stimulates germ cell proliferation

and maturation, and T is involved in the negative feedback mechanisms needed to control KT-dependent spermatogenesis. Thus, a balance between T and KT is critical for the control of spermatogenesis (Schulz and Miura 2002). A plot of the KT to T ratio as a function of the relative size of the gonad (GSI) shows that in species in which the magnitude of the ratio between bourgeois and parasitic is larger, there is a smaller difference in GSI (Oliveira 2005). This means that a higher GSI among parasitic males is associated with a lower KT : T ratio, which allows them to have larger testis without a linked expression of bourgeois male secondary sex characters and behavior (Oliveira 2005).

(2) In the case of other vertebrates, a potential alternative explanation is differential density of gonadal receptors among morphs, so that the gonads of parasitic males may become particularly reactive to the same levels of gonadotrophic hormones when compared with those of bourgeois males.

(3) In vertebrates direct innervation of the gonads has been demonstrated, and this might allow for an alternative route for controlling gonadal function in alternative phenotypes. In all vertebrates, both afferent and efferent neural connections between the gonad and the hypothalamus have been described, with the efferent fibers terminating on steroidogenic cells of the gonad (for references see Crews 1993). Moreover, de-innervation of the gonad causes gonadal atrophy whereas the electrical stimulation of these fibers induces variations in gonadal steroid secretion and sperm release (Demski 1987, Damber 1990). Thus, a private channel between the brain and the gonads is present that might allow for a control of gonadal activity in parasitic males independent of the systemic action of the HPG axis.

In summary, the relative importance of different physiological mechanisms for the differentiation of tactic-specific traits might vary among behavioral, morphological, and gonadal traits. If this occurs in species with ARTs, it would challenge the classic paradigm of androgens controlling, in a whole-organism fashion, the expression of the entire set of reproductive characters that distinguish each tactic.

7.5 STRESS, GLUCOCORTICOID LEVELS, AND ARTs

One of the axioms of the current ART theory is that alternative morphs have a lower competitive ability and therefore a subordinate status if in direct competition with bourgeois morphs. Dominance relationships are also known to have a differential effect on glucocorticoid (GC) levels, and for a long time it was assumed that circulating concentrations of a subordinate's GCs should be higher than those of dominant individuals and that these differences should mediate the effects of social rank on reproductive physiology (Creel 2005). This belief has led to the concept of social status as almost synonymous with stress for subordinates in a social group. This concept was built on a logical inference using three independent pieces of evidence: (a) in staged fights both winners and losers experience an increase in circulating levels of GCs, but there is a higher magnitude in the loser's response; (b) GCs suppress the HPG axis; and (c) social stress leads to the suppression of reproduction in subordinates (for references see Creel 2005). However, it has become increasingly clear that in most free-living species, either there is no difference in GC levels according to social status, or there is a trend for dominant males to have higher circulating levels of GCs than subordinates (Creel 2001, 2005, Abbott *et al.* 2003; however, these reviews included only bird and mammalian studies). In fact, the winner–loser effects on GC levels do not predict differences between dominant and subordinate individuals in free-living groups that conform to different social systems, and there is no parsimonious argument that allows one to predict whether dominants or subordinates are more stressed in the wild. While dominants are expected to face the stressful situation of having to fight harder and at higher rates to keep their status, subordinates, in turn, are exposed to the stress of repeated defeats (although in the wild they can often spatially avoid being exposed to dominant individuals or even take the option of dispersal) (Creel 2005). Based on a meta-analysis of rank differences in cortisol levels among primates, Abbott and co-authors (2003) proposed that two conditions should explain the relationship between social status and GC levels. According to this analysis, subordinates should have higher GC titers than dominants (1) when subjected to higher rates of stressors, either physical (e.g., food availability, exposure to predators and to pathogens, likelihood of facing aggressive challenges) or psychological (e.g., control access to resources, exposure to aggression, establish stable and predictable social relations) or (2) when they experience decreased opportunities of social support.

According to the rationale proposed by Creel (2005), in species with ARTs, the bourgeois morph, characterized by its investment in the monopolization of access to mates, should face more social challenges and therefore would be

expected to have higher circulating levels of GC than the parasitic morph. However, an analysis of Table 7.3 does not support this prediction. In fact, the three possible relationships between GC levels and ART type are present: of the 16 species studied so far, levels are higher in bourgeois males in 37.5% of the cases, are higher in the parasitic morph in 12.5% of the cases, and there are no differences in the remaining 50%. Moreover, the differences in GC levels are independent of the type of ART expressed (fixed vs. plastic; ACTUS – simulation statistics for contingency tables with low expected values – $P < 0.05$), indicating that the first prediction of the relative plasticity hypothesis also does not conform to the available data on GCs. However, this result should be taken with caution since in most studies only basal levels were reported. Glucocorticoids act through a dual receptor system where two receptor types are present in target tissues: type I receptors (or mineralocorticoid) and type II receptors (or glucocorticoid) (de Kloet et al. 1993). Since type I receptors have a higher affinity for glucocorticoids than type II, at baseline levels most GCs are bound to type I receptors. This receptor subtype mediates permissive actions of GCs (i.e., actions that are already present before the stressor and that prime the stress defenses of the organism). When GCs increase in response to a stressor and type I receptors become saturated, then there is a binding shift towards type II receptors, which mediate suppressive actions of GCs mainly outside the HPA axis, such as reproductive suppression (Sapolsky et al. 2000). The disruption of the HPG axis by glucocorticoids can be achieved by several different mechanisms, namely by decreasing both the hypothalamic release of GnRH, and the LH secretion from the pituitary, as well as by reducing the gonadal responsiveness to LH and the local density of LH receptors (Sapolsky et al. 2000). For a clearer picture of a potential role of GCs on ARTs, we need to look for differences in GC responses to challenges between alternative tactics and to confirm that the dual GC receptor system described in mammals is also present in the other vertebrate classes. Below we illustrate some known examples of the involvement of GCs on the expression of ARTs in different vertebrate taxa.

In the tree lizard, *Urosaurus ornatus*, two fixed reproductive phenotypes exist: territorial males display an orange dewlap with a blue spot (orange-blue males), and non-territorial males have an orange dewlap (Moore 1991, Moore et al. 1998). Within the orange morph, the males may switch between a sedentary satellite tactic and a nomadic tactic, depending on the environmental conditions they
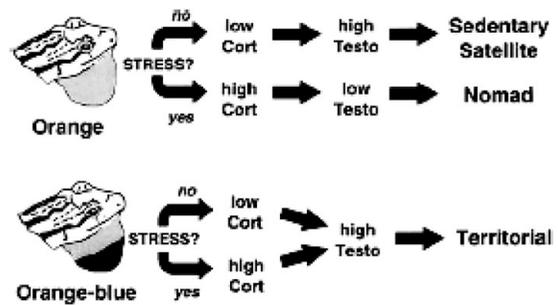

Figure 7.3 Proposed endocrine mechanism for tactic switching in the tree lizard. Males have a two-step reaction to stress. Both morphs increase their corticosterone levels in response to a stressor. However, orange-blue male testosterone levels are corticosterone resistant, while testosterone levels of orange males are sensitive to suppression by corticosterone. Therefore, orange-blue males express territorial behavior independently of exposure to stress, whereas orange males switch their tactic from satellite (with low corticosterone) to nomad (with high corticosterone) depending on the environmental conditions. (Reprinted with permission from Knapp et al. 2003.)

face, thus representing plastic ARTs (Moore et al. 1998). The corticosterone response to stress seems to be the key factor triggering this switch within the orange morph (see Figure 7.3). In harsh conditions, corticosterone levels increase causing a decrease in testosterone concentrations, which leads to a lack of site attachment (cf. DeNardo and Sinervo 1994a, b) and a concomitant switch from the satellite to the nomadic tactic (Figure 7.3). Apparently the orange-blue males are resistant to testosterone suppression by corticosterone, and thus, independently of the environmental conditions, continue to express the territorial tactic (Knapp et al. 2003).

In amphibians the *energetics–hormone vocalization* model has been proposed (Emerson 2001, Emerson and Hess 2001), which aims to explain transitions in vocal production (i.e., calling vs. noncalling) in anurans. It proposes that elevated levels of corticosterone due to the energetic demands of calling behavior inhibit androgen production which inhibits calling. Data are available for three anuran species with noncalling satellite males (Table 7.3). In two of these species, the Woodhouse and the Great Plains toads, although corticosterone levels are higher in the calling morph, there are no differences between morphs in androgen levels. These findings are contrary to a suppression of the HPG axis by increased levels of corticosterone in calling males and support the occurrence of direct effects of
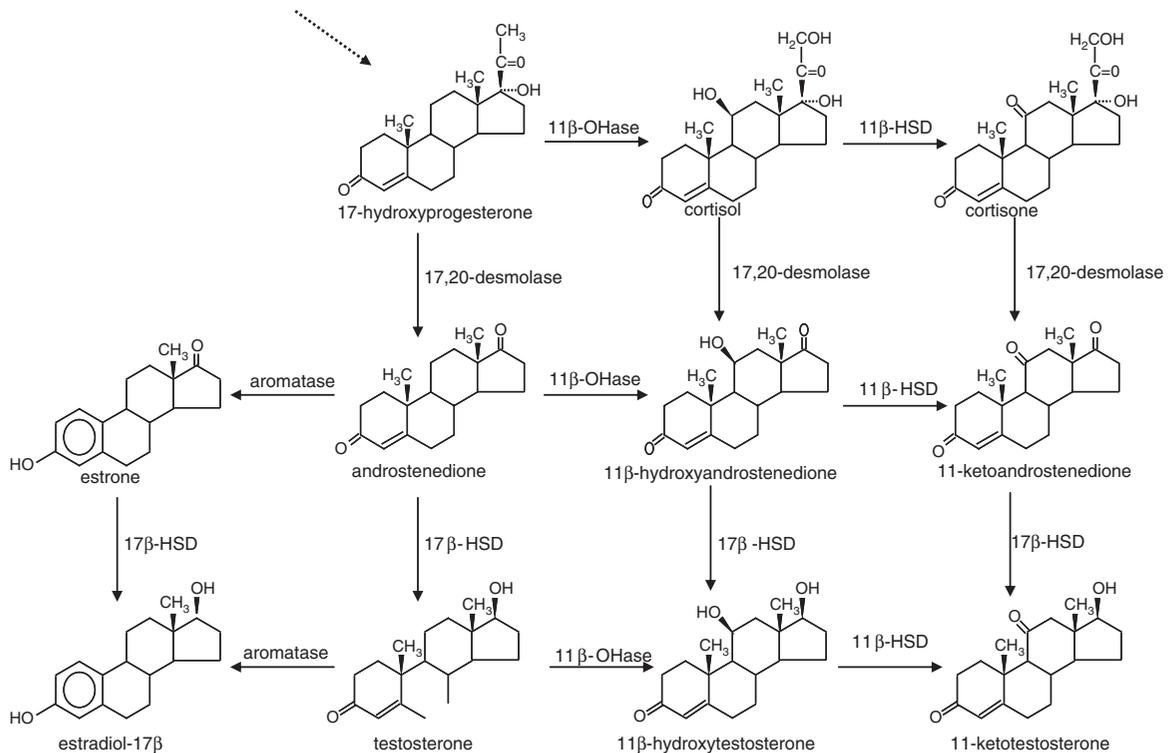
Figure 7.4 Steroidogenic pathways illustrating the similarities in the enzymes involved in androgen and glucocorticoid metabolism.

corticosterone on vocal brain nuclei that control calling behavior (Leary *et al.* 2004).

Finally, a model for the differentiation of alternative phenotypes in teleost fish based on glucocorticoid–androgen interactions has been proposed by Knapp and co-workers (Knapp *et al.* 2002, Knapp 2003). Since the same enzymes that participate in the synthesis of KT are also involved in the synthesis (11β-OHase = 11β-hydroxylase) and inactivation (11β-HSD = 11β-hydroxysteroid dehydrogenase) of GCs (see Figure 7.4), it is proposed that reciprocal competitive inhibition can regulate the activity of these enzymes (Knapp 2003). Consequently, in species with plastic ARTs, reciprocal inhibition creates the possibility that these enzymes may mediate the transduction of social into endocrine signals that will modulate the adoption of a certain ART (Knapp 2003). This model assumes that parasitic males have higher cortisol levels than bourgeois males, as a result of aggressive interactions among the two morphs. Competitive inhibition of 11β-OHase and/or 11β-HSD would yield lower levels of KT in parasitic males and result in an accumulation of T. The increased T could then be available to the enzyme aromatase for estrogen production. Therefore, higher levels of aromatase activity are predicted in parasitic males, as has been observed in midshipman fish type II males (Schlinger *et al.* 1999). A potential pitfall of this model is the assumption of competition for cortisol and KT production. However, this is only expected if occurring in the same tissue (i.e., gonad or adrenals). Data on cortisol levels in teleost species with ART are only available for the longear sunfish, where parasitic males have both higher levels of cortisol and lower levels of KT than bourgeois males, suggesting that parasitic males may have a lower activity of 11β-HSD both in the interrenal glands and in the testes relative to bourgeois males (Knapp 2003). A similar model has been independently proposed by Perry and Grober (2003) to explain the social modulation of sex change in sequential hermaphroditic teleosts. This model is supported by the fact that in the bidirectional, socially induced, sex-changing goby *Gobiodon histrio*, a glucocorticoid responsive element has been identified in the promoter region of the aromatase gene CYP19A1 (gonadal isoform) that could allow GC to act as an upregulatory transcription factor, ultimately promoting estrogen synthesis responsible for male-to-female sex change (Gardner *et al.* 2005). Thus cortisol could play a pivotal role when

subordinate males change back to females as a response to the stress of competition with dominant males (Munday and Jones 1998).

In vertebrates other than the teleosts, where KT is not present, a role for these steroidogenic enzymes is still possible. In mammals 11β-HSD plays a major role at the intracellular level in regulating the availability of GC to glucocorticoid receptors. This enzyme has two isoforms with different activities. Whereas 11β-HSD2 catalyzes the irreversible inactivation of GCs, leading to the formation of 11-keto-steroids (i.e., cortisone from cortisol and 11-dehydrocorticosterone from corticosterone), 11β-HSD1 can promote both the inactivation or the activation (by reduction of the 11-ketosteroids) of GCs (de Kloet et al. 1998, Sapolsky et al. 2000). In Leydig cells, 11β-HSD activity modulates the availability of intracellular GC to the type II receptors that in turn inhibit testosterone production (Gao et al. 1996a, b). Therefore, differential expression of the two isoforms in different tissues between alternative morphotypes can be a mechanism that explains intrasexual variation in the expression of reproductive traits. The lizard, anuran, and teleost examples illustrate the fact that GCs seem to act in the expression of alternative tactics, but their exact role may depend on the social systems and on particular GC mechanisms present in different taxa (e.g., the duality of GC receptors present in mammals).

7.6   BEYOND HORMONE PROFILES: FOCUSING ON TARGET TISSUES

The decoupling of different male traits in alternative reproductive phenotypes may be achieved by mechanisms other than differences in hormone levels, namely by varying the local microenvironment in the different target tissues. This could result from differential expression of receptors or differential levels of activity of catabolic enzymes that modulate the availability of the active hormone to specific targets (e.g., 11β-OHase and 11β-HSD, which metabolize testosterone into KT, are key steps in the expression of male secondary sex characters, in spermatogenesis, and in the modulation of the expression of reproductive behavior in male teleosts: Borg 1994). This focus on target tissues, when studying the mechanisms of intrasexual variation in reproduction, has rarely been used. One rare example of such an approach is a study on the relative levels of brain steroid receptors between alternative reproductive phenotypes in the protogynous wrasse *Halichoeres trimaculatus*. In this

species it was found that by using competitive reverse transcriptase–polymerase chain reaction, the levels of androgen receptor (AR) transcripts were significantly higher in the brain of terminal-phase males (bourgeois tactic) than in initial-phase males (parasitic tactic) (Kim et al. 2002). No other significant differences in gene expression were observed, either for AR in the gonads or for estrogen receptor (ER) in the brain and in the gonads. Thus, by regulation of the expression of AR in specific tissues (by varying AR density in different tissues such as brain vs. gonad) of bourgeois males (in this case terminal-phase males), the sensitivity to circulating androgen levels in specific targets (the brain) can be increased, and the effects of androgens compartmentalized (Ketterson and Nolan 1999). This mechanism hypothetically makes it possible to activate the expression of an androgen-dependent reproductive behavior in bourgeois males without having the associated costs of increasing spermatogenesis or expressing a sex character, since the androgen action can be independently modulated at each compartment (brain vs. gonad vs. morphological secondary sex character).

Another level at which the availability of steroid hormones to target tissues can be differentially modulated between alternative phenotypes is through steroid-binding globulins (SBGs). SBGs can regulate the availability of circulating steroids to target tissues, since only the free (unbound) fraction is biologically active. To our knowledge, there is only one published study in vertebrates that documents differences in binding capacity of an SBG among alternative morphs (Jennings et al. 2000). In the tree lizard two SBGs have been identified: one with a high affinity to androgens and estradiol (i.e., a typical sex-hormone-binding globulin), and another with a high affinity to androgens, progesterone, and corticosterone, thus named androgen–glucocorticoid–steroid-binding globulin (AGBG: Jennings et al. 2000). Whereas the capacity of the former SBG does not differ between the two morphs, the AGBG capacity is much larger in the orange-blue males, resulting in higher levels of free (i.e., unbound) corticosterone in the orange morph (Jennings et al. 2000). Consequently, testosterone levels in the orange morph are more sensitive to negative feedback by corticosterone, especially during periods of stress (e.g., staged male–male encounters: Knapp and Moore 1996, 1997). Thus, at least for tree lizards, SBGs can act as mediators of the environmental effects on the differentiation and expression of alternative morphs. Further studies focusing on target tissues are thus a major avenue for future research in this area.

7.7   ARTs IN THE GENOMICS ERA: A HOLISTIC APPROACH TO THE PROXIMATE MECHANISMS OF ARTs

Functional genomics tools now provide a new approach to understanding the proximate mechanisms of ARTs. Using microarray technology, the activity of large sets of genes (thousands) can be monitored simultaneously in key tissues (e.g., brain, gonads). It is therefore possible to identify genes and regulatory networks that are consistently upregulated or downregulated in each morph. These differentially expressed genes are then taken as likely candidates involved in the expression of the alternative morphotypes (Hofmann 2003). Only two studies have been published that used microarray techniques to study alternative phenotypes. In the honeybee (*Apis mellifera*), workers socially regulate the division of labor, with younger individuals acting as hive workers and older individuals as foragers. The transition between these two alternative (sequential) phenotypes is associated with differential gene expression in 39% of the approximately 5500 genes tested (Whitfield *et al.* 2003), indicating a link between different profiles of brain gene expression and the occurrence of behavioral plasticity. In a second study, the only one of a vertebrate species, gene expression profiles were compared between sneaker males and immature juveniles (of the same age) of the Atlantic salmon, *Salmo salar* (Aubin-Horth *et al.* 2005). Males that will reproduce as sneakers do not migrate to the sea and attain sexual maturity earlier (1–3 years old) than migratory males that return later to the breeding grounds as large, anadromous individuals (3–7 years old) (Fleming 1998). Thus, the immature males represent the anadromous phenotype before migration, and they are the same age as the sneakers (in order to avoid age-related differences in gene expression). A differential expression of 15% of the 2917 genes tested has been detected between the sneaker and the juvenile immature males (Aubin-Horth *et al.* 2005). Most of the upregulated genes in sneakers are associated with reproduction and associated processes (e.g., gonado-tropins, growth hormone, prolactin, and POMC genes), and the upregulated genes in immature males are mainly associated with somatic growth (e.g., genes involved in transcription regulation and protein synthesis, folding, and maturation). These differences reflect, at the cellular level, the life history trade-off between reproduction and growth that is found in these two alternative phenotypes (Aubin-Horth 2005). Interestingly, genes involved in neural plasticity (e.g., genes coding for synaptic function and for cell-adhesion glycoproteins that have been implicated in memory formation) and neural signaling (i.e., genes coding for nitric oxide synthesis, a neurotransmitter involved in the regulation of neuropeptide action) were upregulated in sneakers suggesting that the expression of this tactic might be particularly demanding at the level of cognition (Aubin-Horth *et al.* 2005). This approach not only allows us to confirm predictions of differential gene expression between alternative phenotypes, in processes that are a priori expected to differ between alternative morphs (e.g., reproduction vs. growth), but it enables the detection of differences in gene expression between morphs in unsuspected biological processes (e.g., neural plasticity).

7.8   DIFFERENTIAL COSTS IN ENDOCRINE-MEDIATED ARTs

The study of the physiological mechanisms underlying the expression of ARTs may also shed light on the evolutionary mechanisms involved, since from a functional point of view, the potential benefits of high androgen levels for the fitness of the individuals adopting the bourgeois tactic have to outweigh the costs associated with keeping those levels high for long periods. Androgens facilitate the physiology and behavior related to high intra- and intersexual competition typical of the bourgeois tactic. The required extra energetic resources needed for the expression of exaggerated secondary sexual characters and agonistic behavior patterns might have consequences for the allocation of energy to other functions. Especially when animals are constrained in their opportunities to increase energy uptake or when gains in reproduction are high, it may pay to evolve a mechanism that facilitates the expression of sexual traits, while downregulating other energetically expensive functions. This trade-off might explain why, in many species, androgens seem to suppress immunity (Folstad and Karter 1992, Wedekind and Folstad 1994). There is evidence indicating that humoral and cellular immunocompetence are costly (e.g., Martin *et al.* 2003) and trade off with reproduction (Sheldon and Verhulst 1996, Deerenberg *et al.* 1997, Norris and Evans 2000, Cichoń *et al.* 2001).

Few studies have addressed the differential costs in immunocompetence for alternative morphs due to different hormonal profiles of alternative tactics. In the corkwing wrasse (*Symphodus melops*), despite the fact that sneaker males differ from nest-holders in androgen levels (Uglem *et al.* 2002), no relationship has been found between male

reproductive tactics and leukocyte count (Uglem *et al.* 2001). In ruffs, there are no differences among morphs in humoral immunity but territorial males have higher cell-mediated immunity than satellites (Lozano and Lank 2004).

We have recently started to address this issue using the rock-pool blenny (*Parablennius parvicornis*) and the peacock blenny (*Salaria pavo*). In both species, bourgeois males exhibit both parental and territorial behavior, which does not allow them to forage far from their nest sites. In contrast, parasitic males do not have such constraints on energy uptake during the breeding season, and, as a result, nest-holder males of both species suffer a dramatic decrease in body condition not experienced by parasitic males (Gonçalves and Almada 1997). We therefore tested whether the expression of alternative male tactics has consequences at the level of immunocompetence in these two blennies. In salmonids, androgen treatment decreases antibody production by lymphocytes and may even kill them by apoptosis (Slater *et al.* 1995, Slater and Schreck 1997). Interestingly, a specific androgen receptor has been detected in these leukocytes (rainbow trout, *Oncorhynchus mykiss*, and chinook salmon, *Oncorhynchus tshawytsha*: Slater *et al.* 1995, Slater and Schreck 1998). We therefore focused our studies on the relative number of lymphocytes (i.e., leukocytes responsible for the production of specific antibodies)

and on antibody production in response to a challenge with a nonpathogenic antigen. In accordance with expectation, lymphocyte count (in both species) and antibody responsiveness (in the rock-pool blenny) were found to be higher in parasitic males than in bourgeois males (Ros *et al.* 2006; A. F. H. Ros and R. F. Oliveira, unpublished data) (Figure 7.5). This suggests that alternative morphs differ in their capacity to mount "specific" immune responses. Moreover, since lymphocyte numbers are negatively correlated to body size (Figure 7.5), and since competitive ability of the males increases with body size (Oliveira *et al.* 2000), it is plausible that in larger animals, relatively more energy is traded off with immunity than in smaller animals.

7.9   CONCLUSIONS AND PROSPECTS FOR FUTURE RESEARCH

We have summarized the effects on ARTs of different hormones (mainly androgens and glucocorticoids) at both the organizational and the activational levels. However, these effects vary from species to species in a fashion that is not consistent with the type of ART expressed, as predicted by the relative plasticity hypothesis. In particular, in the case of sex steroids, it is conceivable that the expression of a given tactic requires that androgens reach a threshold level for the expression of the bourgeois traits. But, above that threshold, further variations in androgen levels are not associated with the expression of the tactic and may merely reflect the social environment faced by individuals following different tactics. We have also shown that androgens are more relevant for the differentiation of morphological traits than of behavioral traits, which implies that differences in androgen levels between alternative tactics are more likely when the ART involves an intrasexual dimorphism. This difference between ARTs with and without associated variation in the expression of morphological traits is thus a point that should not be neglected in future studies. Another point that needs to be stressed here is that in order to understand the mechanisms of ARTs more research effort is needed focusing on the processes of hormone action at the target tissues, since they may vary between alternative tactics. Most of the work conducted so far is based on correlations of circulating levels of hormones in individuals following alternative tactics and on hormone manipulations in different adult morphs.

At the conceptual level, the views on the role that hormones play in the control of behavior have been changing with time. Two major changes have occurred in
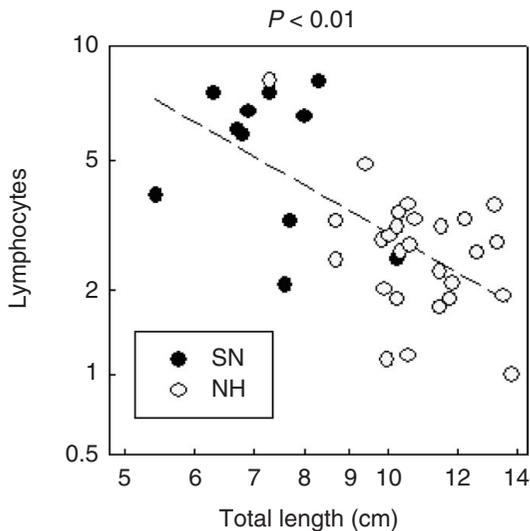

Figure 7.5 Preliminary results on the relationship between lymphocyte counts, total length, and alternative reproductive tactics in the peacock blenny. SN, sneaker males; NH, nest-holder males. (A. F. H. Ros, unpublished data.)

recent years. Hormones have been seen classically as causal agents of behavior of the type one-hormone-one-behavior relationship. This view has been supported mainly by studies of castration and hormone-replacement therapy that showed that a behavior was abolished by castration and restored by exogenous administration of androgens (Nelson 2005). Currently a probabilistic approach to the effects of hormones on behavior has been adopted and hormones are seen as facilitators of behavior rather than as determinant factors (Simon 2002). Accordingly, hormones may increase or decrease the probability of the expression of a given behavior by acting as neuromodulators on the neural pathways underlying that behavioral pattern. Second, there has been a recognition that the social environment feeds back to influence hormone levels (Wingfield *et al*. 1990, Oliveira 2004), which is seen as an adaptive mechanism through which individuals may adjust their motivation according to the social context they are facing. This indicates a two-way type of interaction between hormones and behavior. Accordingly, hormones (e.g., androgens) are viewed as playing a key role as endocrine mediators of the effects of social context on the expression of social behavior. These new views of the role hormones play in the control of behavior should be incorporated in future studies on the endocrine basis of ARTs.

Finally, the strengths of the comparative approach in understanding the proximate mechanisms of intrasexual variation in reproductive behavior should be stressed. It is a valuable tool for various reasons. First, it promotes the development of a conceptual framework to explain these phenomena that is not species centered. One major problem in this area is that a lot of research effort has been invested in only a reduced number of species, so that the information available for these few species has great detail but tends to be extrapolated as valid to the vertebrates as a whole. Therefore, the collection of data on different species exhibiting alternative tactics contributes to the awareness that similar functional phenomena may have different underlying mechanisms and promotes the search for commonalities among species. In turn, these prompt the generation of hypotheses that organize the observed variation and thus contribute to the development of a framework that explains the evolution of proximate mechanisms underlying alternative tactics.

Acknowledgments

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