Endocrine Mediation of Vertebrate Male Alternative Reproductive Tactics: The Next Generation of Studies

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SYNOPSIS. In many species of animals, males may achieve reproductive success via one of several alternative reproductive tactics. Over the past decade or so, there has been a concerted effort to investigate endocrine mechanisms that underlie such discrete behavioral (and often morphological) variation. In vertebrates, the first generation of studies focused on potential organizational or activational effects of steroid hormones (Moore, 1991; Moore et al., 1998). Some of these studies have made it clear that, in addition to circulating hormone levels, one must also consider other aspects of the endocrine system, including hormone receptors, binding globulins and potential interactions among endocrine axes. In this paper, I review recent work on endocrine mechanisms and suggest possibilities for future investigation. I highlight how individual variation in sensitivity to environmental conditions, particularly with respect to various stressors, may account for the existence of alternative male reproductive phenotypes. Along these lines, I briefly explain the logic behind our work with male phenotypes of longear sunfish (Lepomis megalotis) that is aimed at determining the tissue-specific distribution and activity of two enzymes that are common to androgen and glucocorticoid metabolism. A major goal of our work is to examine the potential role of steroidogenic enzymes in the transduction of environmental information to influence the expression of alternative male reproductive phenotypes.

INTRODUCTION

The “problem” and general approach

One of the major questions in biology is how an organism’s genotype and/or environment is translated into its phenotype. Today, our understanding of the mechanisms underlying this translation is no longer the complete “black box” that it once was. We now know that translation occurs via molecules such as transcription factors that affect morphological development and various neuroendocrine messengers that influence the expression of behavior. However, much still remains to be clarified, in particular the mechanisms underlying individual variation in behavior and morphology.

Because the generally continuous nature of variation among individuals makes such studies difficult, researchers have often focused on traits where expression differs in a sex-specific manner. For example, in many songbirds, males sing but females do not. Studying such sexual dimorphisms has facilitated rapid progress in our understanding of the mechanisms underlying such traits (Arnold and Gorski, 1984; Arnold and Breedlove, 1985; Kelley, 1988; Cooke et al., 1998; Arnold, 2000; Crews and Sakata, 2000) as compared to investigating the bases of more continuously varying traits. Studies comparing different rodent strains have also increased our understanding of the bases of phenotypic variation (Shire, 1979; Lephart et al., 1998; Mathias et al., 1999). However, intra-sexual comparisons within a species or strain allow the large genetic differences that exist across sexes or genetic strains to be “factored out.”

Within-sex variation in behavior and morphology

One area in which intra-sexual comparisons hold great promise is in the study of male reproductive behavior. In addition to the more typical intersexual dimorphisms, in a number of species a significant subset of sexually mature males regularly fail to express sex-typical morphology and/or behavior, resulting in largely non-overlapping intra-sexual polymorphisms. The intra-sexual polymorphism among males frequently takes the form of two phenotypes (“morphs”): one morph that exhibits the entire suite of sexually dimorphic characters typically considered male-typical and one morph that is female-like in the expression of some secondary sex characters. This second type of male is fully functional in terms of sperm production, but reproduces by using alternative behavioral tactics. These species have received much attention in the behavioral ecology literature (Austad and Howard, 1984; Gross, 1991, 1996; Sinervo and Lively, 1996; Brockmann, 2001), but our knowledge about the physiological mechanisms contributing to their intra-sexual variation lags far behind (Moore, 1991; Brantley et al., 1993a, b; Hews et al., 1994; Moore et al., 1998; Rhen and Crews, 2002).

This is surprising because species with multiple male morphs offer potentially powerful model systems for examining the physiological, and particularly neuroendocrine, bases of individual variation in behavior and morphology because variation among males is discrete. The reproductive behaviors of these species are often very stereotyped, which also adds to their usefulness as model systems. Hence, a growing number of investigators have turned their efforts toward study-
ing species where 1) two or more distinct types of males exist, 2) variation in behavior is correlated with morphological variation, and 3) all male types are reproductively competent.

Species with discrete male morphological and/or behavioral polymorphisms occur among invertebrates (see Nijhout, 2003; Emlen, 2004) and vertebrates, especially teleosts (Taborsky, 1994). Among the best studied vertebrates in terms of alternative mating behavior and physiology are various anuran amphibians (Mendonça et al., 1985; Marler et al., 1995a; Leary et al., 2004), bluehead wrasse (Thalassoma bifasciatum, Warner, 1984; Godwin et al., 2000), longear and bluegill sunfish (Lepomis megalotis and L. macrochirus, Dominey, 1980; Gross and Charnov, 1980; Jennings and Philipp, 1992), plainfin midshipman (Pomichthys notatus, Bass, 1996), side-blotched lizards (Uta stansburiana, Sinervo et al., 2000), and tree lizards (Urosaurus ornatus, Moore et al., 1998). These species factor prominently in the review that follows.

**Hormones as key molecules influencing polymorphisms: steroid hormones and male reproduction**

Examination of intra-sexual polymorphisms should allow us to more easily identify endocrine differences related specifically to behavioral variation because background “noise” from differences related to gonadal or genetic sex would be eliminated. For example, there are discrete differences in the expression of secondary sexual characters and reproductive behaviors by the male morphs, despite the presence of functional testes in both types of males (e.g., Bass, 1996). Such polymorphic species may be particularly useful for examining how interaction between glucocorticoids and androgens may mediate reproductive behavior and function in response to stressful environmental stimuli (Knapp and Moore, 1996, 1997; Knapp et al., 2003). Androgens often have profound effects on reproductive behavior, morphology and physiology in both sexes (e.g., Phoenix et al., 1959) and levels of androgens and their receptors are often significantly suppressed by glucocorticoids in a variety of vertebrates (Kerr et al., 1995; Knapp and Moore, 1997). In turn, androgens can influence circulating levels of glucocorticoids (Pottinger et al., 1996; Young et al., 1996; Klukowski et al., 1997) or their receptors (Kerr et al., 1996). In addition, androgen and glucocorticoid synthesis and metabolism have several enzymatic pathways in common (Rumsby, 1997; see below). Differences in circulating levels of androgens and/or glucocorticoids have been documented in several polymorphic species (Kindler et al., 1989; Brantley et al., 1993a, b; Moore et al., 1998) and, rarely, neonates (Hews et al., 1994).

The conceptual framework for these studies was made explicit in the Relative Plasticity Hypothesis (Moore, 1991; Thompson and Moore, 1992). Briefly, this hypothesis posits that activation of steroid hormones should be more important in species in which males are “plastic” and can switch between or among phenotypes in adulthood, whereas organizational effects of steroid hormones should be more important in species with alternative male phenotypes that are “fixed” once adulthood is reached. This generalization was more recently modified (Moore et al., 1998) to make explicit the expectations of effects of social and/or abiotic factors on circulating hormone levels in adults, regardless of whether the species is “fixed” or “plastic.” These “first generation” studies have been summarized elsewhere (Brantley et al., 1993b; Moore et al., 1998).

**The “Second Generation” of Studies**

The “first generation” of studies on endocrine mediation of male ARTs logically focused on androgens because of their well-documented role of influencing male reproductive behavior and morphology in “typical” vertebrate species. However, components of the neuroendocrine system besides steroid hormones could be important. Here I discuss 1) some other molecules that have received less attention than the steroid hormones themselves and 2) the increased attention to energetic and environmental factors that could influence the expression of male ARTs via endocrine mechanisms.

**Peptide hormones**

Several peptide hormones are important in mediating reproductive behavior and male aggression. In many species, the appropriate display of aggressive behavior is important for successful reproduction. Two peptide hormones that have been examined with respect to male ARTs are gonadotropin releasing hormone (GnRH) and arginine vasotocin (AVT, the non-mammalian homolog of mammalian arginine vasopressin, AVP). For both peptides, the literature for morph differences in the number and size of peptide-
containing neurons in species with male ARTs was recently summarized by Foran and Bass (1999).

**Gonadotropin releasing hormone.** GnRH is central to the functioning of the male reproductive endocrine axis by ultimately controlling the secretion of androgens. Most studies of species with male ARTs have revealed correlations between male tactic and the size of preoptic area-anterior hypothalamic GnRH neurons. Across species, displaying males generally have more or larger GnRH cells than non-displaying males and females, but not always (Foran and Bass, 1999, and references therein). Data from the cichlid fish *Haplochromis burtoni* may help explain some of the inconsistencies across species with ARTs. This cichlid fish does not have alternative male morphs, but the presence of reproductively active territorial males and reproductively inactive non-territorial males has provided a good model system for studying neuroendocrine mediation of male reproductive behavior (review: Hofmann and Fernald, 2001). First, a male’s social environment (e.g., presence of other males) has profound influence on GnRH levels (White et al., 2002). Second, the three forms of GnRH in this species differ in whether or not levels of their mRNA change with changes in social environment or male social status (White et al., 2002). Some of the diversity in patterns of GnRH cell size and number among species with ARTs may be due to differences in the recent social experience of the individuals used for the studies. Similarly, the likely existence of multiple forms of GnRH in these species could account for additional variation given the unknown specificity of the antibodies used in some studies. Clearly, the data in the studies reviewed by Foran and Bass (1999) need to be reevaluated, but such a reevaluation is beyond the scope of the present paper.

**Arginine vasotocin.** AVT has been shown to mediate the expression of male aggressive and reproductive behavior in a variety of species (e.g., Ferris et al., 1986; Boyd, 1994). In several species with male ARTs, correlations exist between male tactic and the size of AVT neurons in the preoptic area, although there is species variation in the direction of difference (reviewed in Foran and Bass, 1999). Calling male cricket frogs had smaller and fewer AVT neurons and overall coverage with immunoreactive product than non-calling satellite males in one of the four brain nuclei examined (Marler et al., 1999), a finding the authors interpreted as consistent with calling males releasing more AVT than non-callers and that this AVT elicits calling behavior. Although acoustically courting Type I plainfin midshipman males also had fewer AVT neurons (relative to body mass) than non-courting Type II males, these AVT-immunoreactive cells were larger (Foran and Bass, 1998). The opposite trend was found in bluehead wrasse. Terminal phase males, the displaying morph, have more AVT mRNA-containing magnocellular preoptic neurons than initial phase males, and each cell contains more mRNA (Godwin et al., 2000). More recently, AVT-ir magnocellular preoptic area cells in the two male morphs of the peacock blenny (*Salaria pavo*) were found to be similar in size and number, but these cells were fewer and smaller in the males than in females (Grober et al., 2002). In this species, sex-role reversal occurs such that females and sneaker males court nest-guarding males who defend limited nest sites. Sneaker males, however, had larger parvocellular AVT-immunoreactive cells than nest-holding males or females and had more of these cells than females but similar numbers to nest-holding males. Based on *in situ* hybridization for AVT mRNA, there were no differences in AVT cell size or cell number, but females and sneaker males had approximately twice the density of silver grains than nest-holding males. These results suggest that mRNA levels are correlated with the expression of courtship behavior rather than reproductive morph (Grober et al., 2002).

In contrast to the studies correlating ARTs with AVT cell size and number, a few studies have actually manipulated AVT and revealed tactic-specific effects. Semsar et al. (2001) found that AVT injections in bluehead wrasse increased courtship behavior in both territorial and non-territorial terminal phase males. In territorial males, AVT tended to decrease the number of chases toward other individuals, whereas the opposite was true in non-territorial males. AVT also induced territorial behavior in non-territorial males, whereas an AVP-receptor antagonist had opposite effects, decreasing courtship and territorial defense. Because the entire suite of behaviors related to territoriality was affected by AVT, Semsar et al. (2001) concluded that the AVT system may play a key role in motivation of behaviors related to mating. They based this conclusion on findings from several species where the effects of AVT/AVP manipulations reflected the animals’ natural levels of aggression and social organization. For example, in several songbirds that do not exhibit ARTs, AVT decreased aggression in two territorial species (Goodson, 1998a, b) but increased aggressive behaviors in a colonial species (Goodson and Adkins-Regan, 1999). In voles (Young et al., 1997) and deer mice (Bester-Meredith et al., 1999), neural patterns and levels of AVP receptor distribution similarly depend on the species’ social system.

Results from another study manipulating AVT are particularly interesting. AVT and isotocin (the teleost oxytocin-like peptide) injections directly into the hypothalamus in plainfin midshipman had differential effects on vocal physiology among the two male morphs and females (Goodson and Bass, 2000). AVT inhibited vocal-motor activity in courting Type I males but not in females and Type II males, where isotocin was the peptide with inhibitory effects on vocal physiology. The mechanistic basis for this similarity between Type II males and females, and divergence from Type I males, is currently unknown but is a fruitful avenue for future investigation. The factors determining the direction of the relationship between AVT/AVP and courtship behavior across species clearly warrants ad-
Understanding of the contributions of these other molecules to male morph differentiation.

**Receptors.** One class of molecules that could influence the differential actions of steroid hormones in male morphs are hormone receptors, especially for androgens and glucocorticoids. One hypothesis to explain the observed differences in reproductive behavior among male morphs is that one or more brain nuclei in non-courting morphs are insensitive to the hormonal signals that normally facilitate such behavior in courting males. This hypothesis predicts that areas such as the preoptic area, hypothalamus, and amygdaloid and septal regions in the brains of non-courting males should contain no or fewer androgen receptors than these same structures in courting males. These brain areas have been shown in vertebrates without ARTs to be involved in the expression of sexual or aggressive behavior, and often to be sexually dimorphic in size and/or presence of steroid hormone receptors (reviewed in Baum, 1992).

Similarly, glucocorticoid hormones are known to have important effects on both behavior and androgen levels in several vertebrate species and often increase in response to a variety of stressors (Sapolsky, 1992; Knapp and Moore, 1997; Sapolsky et al., 2000). In species with alternative male morphs, stressors that can raise glucocorticoid levels may play an important role in influencing into which morph a male develops or the behavior displayed by a morph (Knapp et al., 2003). For this reason, it will also be important and interesting to know whether the male morphs differ in the pattern of glucocorticoid receptor distribution, especially in the hypothalamus and limbic system.

Unfortunately, nothing is currently known about the distribution and relative densities of any steroid hormone receptor in the brains and other potential target tissues of alternative male morphs in any vertebrate species! One reason for this current gap in our knowledge is the unpredictability of cross-reactivity of antibodies against mammalian hormone receptors in the fish, anuran and reptile species that contain the majority of vertebrate examples of male ARTs. Thus, methods such as *in situ* hybridization that rely on species-specific probes are likely to be more efficient. However, this approach also has associated drawbacks, as partial sequences of the receptors must be determined before specific probes can be developed. Given that several steroid hormones are now known to have several receptor subtypes (e.g., Hawkins et al., 2000; Greenwood et al., 2003), the sequencing itself is no small undertaking. Several labs, including our own, are currently working to remedy this gap in our knowledge.

**Binding globulins.** Another class of molecules that could influence differential availability of steroid hormones between male morphs are binding globulins. These molecules are hypothesized to act as a reservoir for circulating steroid hormones, affecting the levels of hormones that the target tissues "see" (reviewed in

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**Steroid hormones.** Steroid hormones featured prominently in the first generation of studies examining the neuroendocrine bases of male ARTs. However, these studies focused almost exclusively on morph differences or similarities in circulating hormone levels or on the effects of manipulating hormone levels. These early investigations focused on components of the neuroendocrine system that are relatively easy to measure and manipulate. As patterns for the role of steroids in the development and expression of male ARTs emerged, the potential roles of other molecules involved in steroid hormone mechanisms of action have begun to receive more attention.

Besides the steroid hormones themselves, several other molecules are logical candidates for mediating male morph differences in behavior and morphology (Fig. 1). Circulating levels of steroid hormones are regulated via steroidogenic enzymes that help synthesize the hormones from cholesterol or that inactivate the steroids. Once the steroids are released into the blood, binding globulins can regulate hormone availability to target tissues or protection from metabolism. Finally, the presence of a specific receptor in the target tissue is required for a hormone to have its effect. Most commonly, the steroids affect DNA transcription and the production of other molecules that are more immediately responsible for influencing the activity of the target cell. Here, I summarize our current understanding of the contributions of these other molecules to male morph differentiation.

**Steroid hormone** is necessary for a steroid hormone to have its effects on cell activity.

**Binding globulins.** An additional study, but the importance of a species' mating and social systems should not be underestimated (cf., Goodson and Bass, 2001).

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Breuner and Orchinik, 2002). Radioimmunoassays and enzyme immunoassays measure total, rather than free (unbound), circulating levels of the hormone. Thus the potentially “active” free portion of the circulating hormones is unknown and similar total hormone levels between morphs could mask a potentially important mechanism for endocrine mediation of behavior or morphology (cf., Breuner and Orchinik, 2001; Deviche et al., 2001; Lynn et al., 2003).

The potential contribution of binding globulins to male morph differences in behavior is currently limited to a single study. In tree lizards, the male morphs are represented by differences in the coloration of the dewlap (throat fan) used in social communication. Males with a central blue spot on an orange background are more aggressive and territorial, whereas males with solid orange dewlaps are less aggressive and non-territorial (review: Moore et al., 1998). Morph differences in binding capacity of an androgen-glucocorticoid binding globulin have recently been reported (Jennings et al., 2000). Orange-blue males have a higher glucocorticoid binding capacity than orange males, which suggests that higher levels of free corticosterone exist in orange males. This finding supports our earlier demonstration that orange males are more resistant to suppression of total testosterone (T) levels in the face of elevated total corticosterone levels (Knapp and Moore, 1996, 1997). Binding globulins thus represent potentially important molecules, especially for mediating environmental influences on morph behavioral variation. In particular, orange-blue males may be more buffered from environmental stressors because of higher levels of binding globulins that reduce free levels of glucocorticoids, which in turn may affect circulating levels of yet other hormones, especially androgens.

Steroidogenic enzymes. Circulating levels of steroid hormones are the product of the net actions of enzymes involved in synthesizing hormones from cholesterol or metabolizing steroids to inactive molecules (Bentley, 1998). For example, the synthesis of T involves a number of enzymes. Similarly, T can be converted to inactive metabolites via any of several enzymes. To date, the role of only one steroidogenic enzyme in male morph divergence has been reported in the literature (Schlinger et al., 1999).

The enzyme aromatase converts androgens such as androstenedione or T to estrogens such as estriol and estradiol, respectively. Schlinger et al. (1999) found that the activity of aromatase in the hindbrain was higher in plainfin midshipman females and sneaker Type II males than in acoustically courting Type I males. The hindbrain contains the brain nuclei that control the swimbladder muscles responsible for the courtship “hum” produced by Type I males but not Type II males or females (reviewed in Bass, 1992). Recently, brain cells containing aromatase were more specifically located via in situ hybridization studies and antibodies generated against midshipman aromatase (Forlano et al., 2001). Most interestingly, aromatase in the sonic motor nucleus was found not in neurons, but in glial cells. The authors speculate that the consistent ventricular position of aromatase-ir cells and in situ hybridization silver grains and the location and pattern of projections seem ideal for systemic testosterone to be aromatized to bathe the brain in estradiol. This estradiol could then modulate multiple neuronal functions (Forlano et al., 2001).

Two other enzymes, 11β-hydroxylase and 11β-hydroxysteroid dehydrogenase, hold particular promise for understanding phenotypic variation due to their commonality in androgen and glucocorticoid metabolic pathways. In the teleost fishes studied to date, the male morphs differ in circulating androgen levels (Brantley et al., 1993b). Displaying males have significantly higher levels of 11-ketotestosterone (11KT) than do non-displaying sneaker males, although both male morphs generally have similar levels of T. 11KT is a non-aromatizable metabolite of T that has behavioral and spermatogenic effects in a number of teleost fish (Borg, 1987, 1994; Kindler et al., 1991). In blennies, 11KT manipulation influences the expression of morph-specific behavior and morphology (see Oliveira et al., 2001). 11KT is produced from T in two steps involving the enzymes 11β-hydroxylase (11β-H) and 11β-hydroxysteroid dehydrogenase (11β-HSD) (Fig. 2) (Bentley, 1998). These two enzymes are also involved in cortisol/corticosterone synthesis (11β-H) and inactivation or reactivation (11β-HSD) (White, 1997). The potential significance of the same enzyme being used for both androgen and glucocorticoid regulation in fish has been suggested before (Kime, 1987), but has not received direct attention. We recently highlighted the potential role of these enzymes in mediating transduction of environmental information with respect to adoption of male ARTs in sunfish (Knapp et al., 2002). A potential role of these enzymes in sex change in bluehead wrasse has also recently been proposed (Perry and Grober, 2003).

Our hypothesis of a role for 11β-H and 11β-HSD in male ARTs in teleosts is based on data from species without distinct male morphs. In vertebrates that do not produce 11KT, such as mammals, a relationship still exists among androgen and glucocorticoid levels, these enzymes and behavioral variation. First, changes in 11β-H activity affect the availability of precursors for androgen synthesis, particularly in the adrenal glands. For example, disorders involving 11β-H are a cause of congenital adrenal hyperplasia and are responsible for virilization of affected human females (White et al., 1994). Secondly, the presence of 11β-H and 11β-HSD in the testes can mediate glucocorticoid effects on reproductive function (Michael and Cooke, 1994; Monder et al., 1994). The 11β-HSD inhibitor glycyrrhetinic acid decreases T production by rat gonads in vitro (Sakamoto and Wakabayashi, 1988; Monder et al., 1994). 11β-HSD activity was higher in testes of dominant male rats compared with the testes of subordinates (Monder et al., 1994), a finding that has direct implications for our current line of research. In
rodents, 11β-HSD has been found in several tissues including brain, liver, kidney and gonads (Lakshmi et al., 1991; Michael and Cooke, 1994; Monder et al., 1994), again pointing to a role for this enzyme in mediating responses to environmental stimuli and reproductive function, likely through its effects on circulating levels of glucocorticoids.

Given the great potential for glucocorticoids to mediate behavioral differences among individuals (Sapolsky, 1985, 1991; Knapp and Moore, 1996), it is surprising that none of the field studies of fish species with ARTs published to date report cortisol levels in both male morphs. We recently measured cortisol levels in field-collected longear sunfish to determine whether the male morphs differ in this regard (Fig. 3).

Our data are consistent with findings from a Texas population of bluegill sunfish, where cortisol levels tended to be higher in non-parental males than parental males on the day of breeding (Thompson, 1998). High levels of 11KT coincident with low cortisol levels and vice versa are in turn consistent with predicted morph differences in 11β-HSD activity. Our plasma hormone data suggest that investigation of morph differences in other neuroendocrine components related to androgen-glucocorticoid interactions holds great promise for a better understanding of the neuroendocrine mechanisms underlying the expression of this intrasexual variation in reproductive behavior.

The effects of 11β-HSD on cortisol metabolism may be particularly important in parental males of polymorphic fish species as these males have high levels of 11KT (see Brantley et al., 1993b for review). In addition, differences in 11KT levels between parental and sneaker males predict differential expression of 11β-H and 11β-HSD in the testes. Our lab is currently working to test the hypothesis that the reproductive phenotypes in longear sunfish and other polymorphic species differ in the expression and possibly distribution of one or both of these enzymes using enzyme activity assays and in situ hybridization. The localization of 11β-H and 11β-HSD will also be important, particularly in the brain where the distribution of 11β-HSD has been found to vary across nuclei in rodents (Lakshmi et al., 1991).

Energetic/environment considerations

The relative energetic costs of different male ARTs and social context can influence what behavior males will exhibit. Considerations include the actual metabolic or time expense of displaying versus non-displaying, as in vocalizations by anuran amphibians (Wells, 2001), territorial behavior by lizards (Marler et al., 1995b), or the social or abiotic environment (discussed in Knapp et al., 2003). Another set of "second generation" studies has begun to investigate the role of energetic and environmental factors on the expression of male ARTs. In contrast to the neurobiological

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**Fig. 2.** Common steroidogenic enzymes in the metabolism of androgens and cortisol in teleosts. 11β-H, 11β-hydroxylase; 11β-HSD, 11β-hydroxysteroid dehydrogenase.

**Fig. 3.** Mean plasma (± SEM) androgen (A) and cortisol (B) levels in reproductively active parental male, sneaker male, and female longear sunfish (*Lepomis megalotis*). Blood samples were collected from animals in the field immediately after capture by seine. Sample sizes are given in parentheses. R. Knapp and T. S. Jessop, unpublished.
work described above that has focused mainly on teleosts, most of these studies have focused on reptiles and amphibians.

In the tree lizard, non-territorial orange males appear to behave as sedentary satellites on the territories of orange-blue males in relatively wet years but behave as nomadic sneaker males in relatively dry years (Knapp et al., 2003). Correlated with yearly differences in behavior are morph differences in corticosterone and T levels. We have hypothesized that differences in population densities caused by yearly variation in suitable habitat are the driving force behind this hormonal and behavioral variation, mainly through differences in the frequency of male-male interactions (Moore et al., 1998; Knapp et al., 2003). Additional studies are required to test this hypothesis and are planned for the future.

Another example of likely feedback of social interactions onto circulating hormone levels comes from Galapagos marine iguanas (Amblyrhynchus cristatus). In this species, the body size at which males can be successful territorial males varies with population density and previous environmental perturbations such as El Niño (Wikelski and Trillmich, 1997). Environmental manipulation can be mimicked by androgen manipulation. Manipulation of T levels by injections successfully changed male behavior among the three ontogenetic reproducible tactics of territorial, satellite and sneaker males (Wikelski et al., 2004). When T was blocked pharmacologically, territorial males decreased territorial behavior and attracted fewer females relative to controls. When injected with T, satellite male marine iguanas started to defend territories and sneaker males began to court females, behaviors these males normally would not exhibit until they reached a larger body size (Wikelski et al., 1996). However, T-manipulation decreased mating success for satellite and sneaker males because they engaged in damaging male-male fights or spent energy on head-bobs without attracting females ( Wikelski et al., 2004).

Males of the side-blotched lizard, Uta stansburiana, also show variation in phenotype that is sensitive to social environment. In this species, males exhibit one of three ARTs that are correlated with variation in dewlap color (Sinervo and Lively, 1996; Sinervo et al., 2000). Orange-throated males are very aggressive and defend large territories. Blue-throated males are less aggressive and defend smaller territories. Males with yellow throats are sneakers and are not territorial. Relative frequencies of the three morphs in a given year appear to determine each morph’s reproductive success, in part because of the differing number of neighboring males (Sinervo and Lively, 1996). The morph differences in behavior are reflected in physiological differences, some of which can be induced with hormone manipulations. Orange males have higher T levels, endurance, activity and home range size than blue or yellow males (Sinervo et al., 2000). Experimentally elevating T in the latter two morphs increased their endurance, activity, home range size and control over female territories similar to those seen in unmanipulated orange males (Sinervo et al., 2000). However, because orange males showed lower survival than the other morphs, the better performance of these males is not without costs.

Among anuran amphibians, male ARTs take the form of calling males and non-calling satellite males that attempt to achieve matings with females attracted to the calling males. Because anuran vocalizations are among the most metabolically expensive vertebrate signals studied to date (reviewed by Wells, 2001), it has been proposed that the energetic expense can mediate male calling behavior by directly influencing glucocorticoid levels that in turn influence androgen levels (Hormone-Energetics Vocalization Model; Emerson, 2001). Glucocorticoid levels rise as energetic expenditure increases, eventually to a level high enough to inhibit androgen secretion and, in turn, inhibit calling behavior. Thus, males are predicted to switch from a calling to a non-calling state until glucocorticoid levels return to levels low enough to allow increases in androgen levels to recur (Emerson, 2001). This model is supported by the findings of Mendonça et al. (1985), who found that calling male bullfrogs (Rana catesbiana) had higher corticosterone levels and lower androgen levels than non-calling satellite males.

A recent study of two non-territorial, explosively breeding toad species, Bufo woodhousii and B. cognatus, revealed that calling males had significantly higher levels of corticosterone than non-calling males (Leary et al., 2004). However, elevated corticosterone levels did not correlate with suppressed androgen levels in these species. We have proposed that calling behavior in these species is related to circulating levels of corticosterone, but unlike Emerson’s (2001) model, is independent of androgen levels (Leary et al., 2004). Instead, we propose that corticosterone plays a major role in regulating AVT secretion. Work is currently in progress to distinguish between the two pathways in these Bufo species: direct corticosterone-AVT mediated calling behavior or behavior mediated by corticosterone-androgen-AVT. The difference in pathways is likely to be evolutionarily significant as the two pathways give less or more importance to androgens, hormones that have important effects on other aspects of the male phenotype.

**Future Generations of Studies**

I believe the big questions for the future in understanding endocrine mediation of male ARTs are questions of the mechanisms underlying phenotypic plasticity. This is not a new idea, but one whose time has clearly come (Sinervo and Svensson, 1998; Rhen and Crews, 2002; Hofmann, 2003; Nijhout, 2003; Shuster and Wade, 2003; West-Eberhard, 2003). Approaching this question from behavioral ecological and molecular mechanistic perspectives simultaneously will advance our understanding greatly, as an understanding of the ultimate and proximate mechanisms underlying such behavioral variation cannot arise from addressing each
level in isolation (Emerson, 2000; West-Eberhard, 2003, pg. 10).

To address these questions (outlined below), we must continue to use tried and true methods while adding tools from the ever-growing molecular tool kit. Behavior observations have always been important and will continue to be, even in relatively well-studied systems. For example, our discovery of the plasticity of behavior in non-territorial orange male tree lizards (Knapp et al., 2003) resulted from conducting relatively simple mark-recapture studies over several years under differing environmental conditions. The recent explanation for the existence of the long-enigmatic "she males" in garter snakes (Thamnophis sirtalis) was derived from careful observation. It was determined that "she male" is a temporal phase through which all males pass for natural selection (thermoregulatory) benefits rather than sexual selection benefits (Shine et al., 2001). Similarly, attention to details of habitat stability brought to light an important environmental variable mediating reproductive state in the cichlid fish H. burtoni (Hofmann et al., 1999).

"Classic" endocrine methods such as hormone removal and replacement studies have also not outlived their usefulness. Hormone manipulations have recently been used to support genetic models of male phenotype heritability in the ruff (Philomachus pugnax) (Lank et al., 1995, 1999). As noted by these researchers, the use of endocrine manipulations to help distinguish between competing genetic hypotheses represents a potentially powerful methodology that could be used more widely with great success. Androgen treatment of adult female tree lizards has also revealed potential genotypes underlying dewlap color in this species (discussed briefly in Moore et al., 1998).

Combining "classic" methods with more detailed attention to variation in an individual's social and abiotic environment will contribute much to our understanding of endocrine mediation of alternative male reproductive behavior and morphology (see Sinervo et al., 2000; Semsar and Godwin, 2003). In addition, localization of hormone receptors and steroidogenic enzymes via immunocytochemistry and in situ hybridization have focused attention on particular neural structures deserving of further study.

Finally, genomic approaches such as microarrays and subtractive cloning methods hold great promise for examining regulation of endocrine genes, especially those involved in translating environmental information into behavior and morphology. With respect to individual variation in reproductive behavior, these methods are being pioneered in H. burtoni cichlids (Hofmann, 2003) and bluehead wrasse (J. Godwin, personal communication).

So what are some of the big questions or factors to consider? Two stand out as key: the role of the environment (both social and abiotic) and interactions among endocrine axes.

**Role of environment.** The role of the social or abiotic environment in mediating the expression of ARTs (both within and between the sexes) has already been documented in several species (Sinervo and Lively, 1996; Knapp et al., 2003). Individual variation in sensitivity to environmental conditions may evolve into discrete phenotypes by selection on switch points in endocrine regulatory mechanisms (see Nijhout, 2003; Shuster and Wade, 2003, p. 449; West-Eberhard, 2003, p. 149). For example, Moore et al. (1998, p. 147) proposed that we may be able to develop endocrine models to distinguish between whether the ARTs within a particular species are examples of evolutionarily stable strategies where the alternative phenotypes have the same level of fitness or whether the ART is an example of individuals making the "best of a bad situation" and one phenotype is restricted to using a less fit tactic. Although not explicitly stated in that paper, individual variation in sensitivity to environmental conditions, especially stressful stimuli, would be central to such models (see also Buchanan, 2000). It is this hypothesis of variation in sensitivity to environmental conditions that has motivated my research on glucocorticoids described above.

**Interaction among endocrine axes.** The potential for interactions among different endocrine axes to mediate the expression of ARTs and phenotypes promises to be a particularly fruitful area for future research. As outlined above, steroidogenic enzymes that link androgen and glucocorticoid metabolic pathways (e.g., Knapp et al., 2002; Perry and Grober, 2003) and glucocorticoid-AVT/AVP interactions (Perry and Grober, 2003; Leary et al., 2004) are two areas that deserve closer attention. Study of the cross-talk among endocrine axes, particularly those that involve glucocorticoids, are likely to provide a richer understanding of neuroendocrine mediation of male ARTs (see also Vialu, 2002). Some of the particular questions to be addressed in this area include: Does the nature of the relationship between glucocorticoids and AVT/AVP vary depending on the species studied, similar to the manner in which the effects of AVT/AVP on aggressive and courtship behavior vary (Goodson and Bass, 2001; Semsar et al., 2001)? Are the mechanisms for cross-talk between androgen-glucocorticoid axes conserved across species with male ARTs? What is the role of other hormones, such as growth hormone and thyroid hormones, that mediate overall metabolism and growth (e.g., Maggioni, 2000, 2002)? Do particular points of cross-talk between endocrine axes, such as at the hypothalamus versus target tissue level, predispose a species to display discrete male ARTs, i.e., more easily allow for the expression of discrete behavioral or morphological tactics and the evolution of correlated characters (see Sinervo and Svensson, 1998; Sinervo and Zamudio, 2001; Nijhout, 2003; Knapp et al., 2003)?

Much still remains to be learned about the physiological mechanisms underlying male ARTs in vertebrates. Consideration, or at least acknowledgment, of the multiple factors that can influence the expression.
of discrete alternative phenotypes in behavior or morphology will aid this endeavor (see Bass, 1998; West-Eberhard, 2003, Chapters 1 and 2). It has been 20 years since the Society’s last symposium highlighting male ARTs (Austad and Howard, 1984). Given the growing attention of researchers to such phenotypic variation, I predict that there will be enough new information to warrant the next symposium in much less than another 20 years.

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