

## On the Organization of Individual Differences in Sexual Behavior<sup>1</sup>

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**SYNOPSIS.** Research on the sexual differentiation of mating behavior has impeded progress in our understanding of the proximate bases of individual differences in two ways. First, by viewing variation as categorical rather than continuous, and second, by not placing sufficient emphasis on the fact that males and females differ genetically in many vertebrates, environmental contributions to individual differences cannot be distinguished from genetic contributions. Just as there are two levels of organization of sex, so, too, are there two levels of organization of the sexual phenotype. Primary organization is the process of sexual differentiation that follows gonadal determination and is manifest as the morphological, physiological and behavioral aspects of the sexual phenotype. There is a second, and subsequent, level of organization, however, that is directly related to primary organization and is the basis of individual variation in sexually dimorphic behaviors. This level can be termed secondary organization. Because individual variation is the substance of evolutionary change, understanding its organization will require both new paradigms and alternative animal model systems that allow separation of the effects of genes and hormones from environmental and experiential stimuli. The plasticity of the sexual phenotype and how each individual emerges from its own unique circumstances integrates these different levels of organization. As might be predicted, recent research suggests that the relationship between primary and secondary organization and the development of individual differences in sociosexual behaviors involves more than just sex steroid hormones.

### INTRODUCTION

Understanding how the individual emerges requires a perspective that integrates evolutionary outcomes and causal mechanisms. While much has been learned about the consequences of individual variation in the evolution of traits, the same cannot be said about its proximate causes. Just as there are two levels of organization of sex, so too are there two levels of organization of the sexual phenotype. Primary organization is the process of sexual differentiation that follows determination of the gonad and is manifest as the morphological, physiological and behavioral aspects of the sexual phenotype. There is a subsequent level of organization, however, that is directly related to primary organi-

zation and is the basis of individual variation in sexually dimorphic behaviors. This can be termed secondary organization.

Behavioral endocrinologists have concentrated on the problem of sexual differentiation and virtually ignored the problem of individual differences. Persistence in this approach will not contribute to our understanding of individual differences. There are several reasons for this. First and foremost, in species with sex chromosomes, genetic sex and gonadal sex are inextricably linked, making it difficult to distinguish environmental from genetic contributions to individual differences. Second, sexuality resides in the brain, not in the gonads, and what dictates gonadal sex need not dictate sexuality. Third, sex and sexuality are often confused, yet are conceptually distinct; gonadal sex is a discrete character that categorizes the individual, whereas sexuality is a suite of continuously variable traits that is unique to each individual. Fourth, even as adults each individual is capable of displaying the behaviors characteristic of the op-

<sup>1</sup> From the Symposium *Animal Behavior: Integration of Ultimate and Proximate Causation* presented at the Annual Meeting of the Society for Integrative and Comparative Biology, 26–30 December 1996, at Albuquerque, New Mexico.

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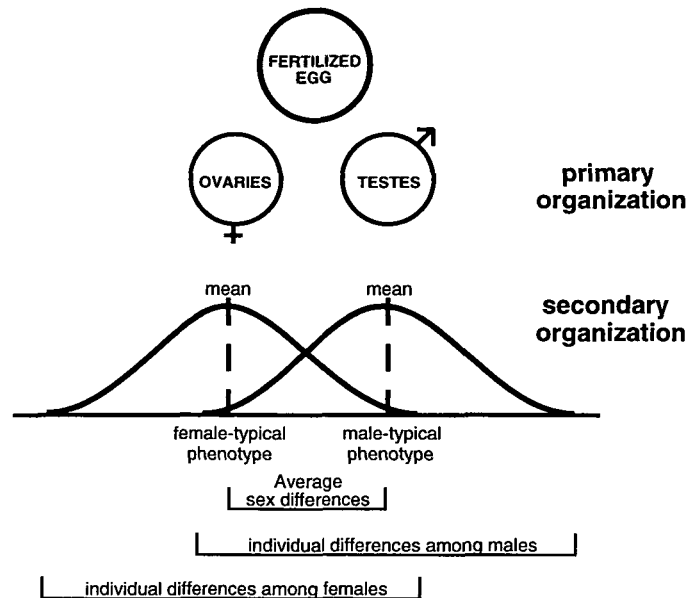


FIG. 1. Levels in the organization of sexual behavior. Beginning with a single cell, the zygote, how does variation between the sexes, as well as within each sex, emerge? In mammals and other vertebrates with sex chromosomes, primary organization arises indirectly from the genetic constitution of the individual. The steroid hormones secreted by the gonads organize the differences between the sexes. The process of secondary organization that follows establishes the differences between individuals. Variation within a sex often is greater than the difference between the "average" male and female. The next frontier in behavioral neuroendocrinology will address the relationship between these levels, thereby increasing our understanding of the proximate bases of individual variability.

posite sex; indeed, it is rare for the potential to express any behavior to be sex-specific. The result is that variation among individuals within a sex is usually greater than the difference between the averages for each of the sexes. Thus, although the sexes differ in the average value of many traits, this often is a statistical phenomenon (Fig. 1). Even studies of sexual selection, which are founded on the concept of variation among individuals in sexual behavior, usually are limited to within-sex variation and do not incorporate the continuous and often overlapping variation in the same behavior between the sexes.

The factors contributing to differences in the sexual phenotype involve more than gonadal sex steroid hormones. They may include, but are not limited to, (i) the embryonic environment, (ii) age and experience, and (iii) the psychophysiological condition of the mother. All have been shown to play important roles in the differentiation of the neural basis of sexually dimorphic behav-

iors, as well as in individual differences within a sex. By conducting experiments with species that remove the confound of sex-limited genes, a new perspective of sexuality and its underlying mechanisms is obtained.

#### THE PRIMARY ORGANIZATION OF THE SEXUAL PHENOTYPE

Most concepts of sex determination and sexual differentiation arise from studies of mammals. In mammals and many other vertebrates, gonadal sex is determined at fertilization by the inheritance of specific chromosomes from the parents, a process referred to as genotypic sex determination (GSD). In vertebrates the individual initially is bipotential, and primary and accessory sex structures, the gonads and the duct systems and associated structures that convey the gametes, are organized sequentially by separate mechanisms. For example, in mammals, the Y chromosome-linked *SRY* gene initiates the genetic cascade leading to

testicular development, thereby causing individuals to develop a male-typical phenotype. Female development is generally believed to be a default mode, resulting from the absence of this gene. Thus, in species with GSD (whether male or female heterogamety), the sexes differ in genetic constitution. Studies indicate that the genetic basis for male-typical sexual behavior is distinct and separate from that for female-typical sexual behavior (Goy and Jakway, 1962), and the genetic mechanisms of sex determination may influence the brain directly (Beyer *et al.*, 1992; Lahr *et al.*, 1995; Tordjman *et al.*, 1995; van Abeelen *et al.*, 1989). While this genetic difference between the male and female facilitates studies of sex differences, it is an impediment to understanding the proximate bases of individual differences in sexual behaviors of males *versus* females.

#### THE SECONDARY ORGANIZATION OF THE SEXUAL PHENOTYPE

From an evolutionary perspective, reproduction is predicated on individuals preferentially mating with other individuals. Traditionally, behavioral neuroendocrinologists have focused on how gonadal sex hormones organize differences between the sexes and have treated individual variation in sexual behavior within a sex as a problem to be accommodated by large sample sizes, culling out individuals that do not share the desired trait, and/or statistics. Indeed, the fact that variation in any number of traits is as great in inbred, homozygous strains as in naturally-occurring outbred species has never been explained satisfactorily (Shire, 1966; Crews *et al.*, 1985). Studies of natural polymorphisms and inbred strains have revealed much about the genetic basis of behavior, but such systems or methods have rarely been exploited by behavioral endocrinologists, although recent attempts to determine the physiological bases of social experience have met with some success (*e.g.*, Francis *et al.*, 1993; Yeh *et al.*, 1996).

#### INDIVIDUAL DIFFERENCES IN SEXUAL BEHAVIOR

The most obvious mechanism that might account for individual differences in sexual

behavior, namely circulating levels of sex hormones, bears no systematic relation to the frequency or intensity of an individual's sexual behavior (Feder, 1984). A similar phenomenon is found in reptiles. In the little striped whiptail lizard (*Cnemidophorus inornatus*), males can exhibit low or high levels of sexual behavior. There are no significant differences in the circulating concentrations of androgens between these two groups of males (Lindzey and Crews, 1992), but they do differ in sensitivity to exogenous hormones (Lindzey and Crews, 1993) and in receptor regulation (Crews *et al.*, 1996b).

Whatever the mechanism(s), pronounced within-sex variation has been described in strongly sexually dimorphic vertebrate species. Alternative mating patterns exist when males display different morphological and/or behavioral phenotypes (Dominey, 1984; Gross, 1996). In all of these forms, the morphs may be distinct or intergrade. In one form of this phenomenon, some males exhibit a "satellite" mating tactic, patrolling the periphery of a larger male's territory and attempting to "sneak" matings while the resident male is distracted. Another mating pattern occurs when some males in the population resemble or "mimic" females, enabling them to gain access to females in the resident male's territory (*e.g.*, Warner *et al.*, 1975; Dominey, 1980; Gross and Charnov, 1980; Howard, 1984; Mason and Crews, 1985; Knapp and Moore, 1996; Rand, 1991). In still other species, males may exhibit different color patterns that accompany the behavioral differences (*e.g.*, Hews *et al.*, 1994; Thompson and Moore, 1991; Sinervo and Lively, 1996). In some species the different mating types are associated with genetic polymorphisms, or the presence of certain alleles; in this instance we refer to the phenotypes as strategies (*e.g.*, Lank *et al.*, 1995; O'Riain *et al.*, 1996; Ryan *et al.*, 1992). In other species the different mating phenotypes are an opportunistic response to the physical or social environment early in development or later in adulthood; this conditional flexibility of the phenotype is referred to as tactics. (I have chosen to exclude group mating systems observed in

some birds and mammals in which only a single male and female reproduce. Such systems are unlike alternative mating tactics in that the nonbreeding individuals are subordinate to the breeding pair, often are progeny of the pair and hence genetically related, and usually do not reproduce [this lack of breeding does not necessarily mean there is a hormonal or gonadal suppression, however].)

The behavioral endocrinology of alternate reproductive patterns is still in its infancy, but as yet there is no consistent evidence these alternative strategies/tactics are related to differences in the circulating levels of steroid hormones (Brantley *et al.*, 1993; Godwin *et al.*, 1996; Moore and Thompson, 1990). While it is true that plasma hormone levels in some species of birds, reptiles, amphibians, and fishes are different in the alternate phenotypes, these differences may not be involved in the behavioral and morphological differences that characterize the different phenotypes (Brantley *et al.*, 1993; Godwin *et al.*, 1996). However, there is some evidence that male morphs differ in their sensitivity to hormones (Brantley *et al.*, 1993; Crews *et al.*, 1996b).

Investigations of the neuroendocrine mechanisms underlying alternative mating strategies/tactics are limited. The gerbil has been mentioned already, but the most thorough exploration has been conducted by Bass (1996, 1997) with the plainfin midshipman fish. This small fish appears to be an example of a fixed mating tactic, as rearing fish in high densities significantly increases the number of one of the types of male. Type I males establish breeding territories and emit a prolonged "hum" produced by a pair of skeletal muscles attached to the swimbladder to attract females. Type II males resemble females and, like them, do not vocalize and have greatly reduced sonic muscles. The neural control of vocalization involves three brain nuclei: motoneurons in the caudal brainstem that innervate the sonic muscles, pacemaker neurons that establish the rhythmic firing frequency of the motoneurons, and ventral medullary neurons link the pacemaker-motoneuron circuit bilaterally. As is the case with the

sonic muscles, the neurons in each nucleus are largest in Type I males, and smaller in both Type II males and females. There are comparable size differences in the preoptic area, which contain neurons that concentrate androgen as well as gonadotropin-releasing hormone.

Can we regard homosexuality and transsexuality in humans as alternative mating tactics even though they do not result in offspring? There is no compelling evidence that homosexual behavior in humans is organized before or after birth by steroid hormones (Beach, 1979; Byne and Parsons, 1993), although this idea has been vigorously championed (Collaer and Hines, 1995; Dörner, 1978). Studies of monozygotic and dizygotic twins and of genealogies suggest that homosexuality has a genetic component (Bailey and Pillard, 1991; Bailey *et al.*, 1993; Hamer *et al.*, 1993) and may reflect neuroendocrine differences (Gladue *et al.*, 1984; but see Gooren, 1986a, b) that, in turn, are correlated with brain differences between heterosexuals and homosexuals (LeVay, 1993). There is no evidence to date of a genetic predisposition for transsexuality. Psychosexual tests clearly indicate a discordance between gender identity and gonadal sex in transsexuals (Hertoft and Sørensen, 1978), and recently a neural correlate has been described (Zhou *et al.*, 1995). Finally, it has been noted that gonadal females are better able to cope with the new sex role than are gonadal males (Hertoft and Sørensen, 1978). This is consistent with the hypothesis that the female is the ancestral sex, and the male sex is derived from females (Crews, 1982, 1987, 1993). Since evolutionarily more ancient traits, in this case the female sex, are more fundamental than more recently derived traits, it would follow that females would be more resilient than males.

Moore (1991) has argued that understanding how alternative mating patterns within a sex arise and are expressed may provide insight into the bases of continuous individual variation and individual differences generally. I myself am cautious. It seems to me that alternative mating patterns are unlike individual differences in several fundamental aspects. First, to date descrip-

tions of alternative mating tactics appear to be restricted to males, whereas variation occurs in both sexes. Lyon's (1961) discovery of X inactivation led Vandenberg *et al.* (1962) to propose that females are more variable than are males. This has been confirmed in studies of humans on various morphological, physiological, and behavioral indices in monozygotic male and female twins, dizygotic twins of the same sex and opposite sex, and siblings (McFadden, 1993, 1997) as well as in animals (Thiessen, 1994). This fact has enormous implications not only for our understanding of behavioral neuroendocrinology, but also for evolutionary biology. For example, most research in sexual selection has used strongly dimorphic species in which the female chooses among various males. Typically these studies focus on the variation among males and ignore the female. Not only is our understanding of the behavioral and neuroendocrine mechanisms underlying mate choice limited by this emphasis on exaggerated traits (Barlow, 1997; see also Tregenza and Wedell, 1997), but it may be that the emphasis on the male is misplaced. Phenomena such as sperm storage, monogamy, etc. would seem to make the female the more important sex.

But consider an alternative perspective. Mating is more than the possession of bigger, louder, more colorful etc. traits. It results from a complementary interaction that encompasses performance and preference in *both* sexes. That is, evolution selects for breeding success, not for the success of one sex (Adkins-Regan, 1997; Eberhard, 1996; Travis, 1988). Thus, studies of the developmental and physiological history of both the male and female, and how their perception and preference for a suitable mate, are needed.

Another important question that needs to be addressed is why alternative mating patterns appear to be restricted to males. Is it because we have not looked, or because they simply do not occur in females? Lastly, it needs to be determined if alternative mating strategies exist in monomorphic species with little or no sexual dimorphisms (Barlow, 1997) and in species exhibiting sex-role reversal (*e.g.*, Phalaropes and Ja-

canas in which females compete for mates and spotted hyenas in which females are dominant).

A final, but equally important question that, to the best of my knowledge, has yet to be addressed is the issue of variation within a tactic. Specifically, is the variation within a tactic equal to, or less than, the difference between the averages of the different tactics?

Second, although by no means exhaustive, a review of the literature suggests there may be a relationship between the mechanisms that determine gonadal sex and type of alternative mating tactic displayed. That is, species with fixed tactics seem to display GSD, whereas species with plastic tactics can exhibit either GSD or environmental sex determination (ESD) (Table 1). Thus, while there is no absolute dichotomy (*e.g.*, the frequency of the two forms of males in the midshipman is influenced by social conditions rather than arising from a genetic polymorphism), the absence of any fixed tactic species displaying ESD raises a number of questions. The first to come to mind emerges from the proposal that ESD is the evolutionary precursor to GSD (Bull, 1983; Janzen and Paukstis, 1991). Only a phylogenetic analysis might resolve this issue. Second, if this trend continues to be supported, then essentially the same problem exists in studying fixed polymorphisms as with sex chromosomes. Namely, although strictly sex-based differences are avoided, there still is a potential confounding of genotypic *vs.* environmental effects.

Clearly, what is needed is an animal model system that is gonochoristic (separate sexes in separate individuals) and exhibits continuous within-sex variation in both sexes, yet does not have the complications arising from GSD.

#### A NEW ANIMAL MODEL SYSTEM FOR THE STUDY OF INDIVIDUAL DIFFERENCES

The developmental effects of steroid hormones traditionally have been associated with viviparity. The shelled egg, however, is not immune from maternal or uterine influences known to affect the mammalian embryo (Crews and Bull, 1987). In the leopard gecko (*Eublepharis macularius*),

TABLE 1. Relationship between alternative mating tactics and mode of sex determination in vertebrates.\*

Species	Tactic	SDM	Authority
<b>Mammals</b>			
Rodent spp.	Plastic	GSD	Clark and Galef, 1995
Naked mole-rat	Fixed	GSD	O'Riain <i>et al.</i> , 1996
Red deer	Plastic	GSD	Clutton-Brock, 1991
<b>Birds</b>			
Ruff	Fixed	GSD	Lank <i>et al.</i> , 1995
<b>Reptiles</b>			
Green iguana	Plastic	GSD	Dugan, 1983
Red-lipped plateau lizard	Fixed	GSD	Rand, 1991
Red-sided garter snake	Fixed	GSD	Mason and Crews, 1985
Side-blotched lizard	Fixed	GSD	Sinervo and Lively, 1996
Tree lizard	Fixed	GSD	Thompson and Moore, 1991, 1992
<b>Amphibians</b>			
Bullfrog	Plastic	GSD	Howard, 1984
Toad	Plastic	GSD	Arak, 1988; Høglund and Robertson, 1988
<b>Fishes</b>			
Angelfish spp.	Plastic	ESD	Lutnesky, 1994
Bluegill sunfish	Fixed	GSD	Dominey, 1980; Gross and Charnov, 1980
Coho salmon	Fixed	GSD	Gross, 1984
Goby spp.	Plastic	ESD	Sunobe and Nakazono, 1993; Nakashima <i>et al.</i> , 1996
Guppy	Fixed	GSD	Reynolds <i>et al.</i> , 1993
Midshipman	Fixed	GSD	Brantley <i>et al.</i> , 1993
Pacific-salmon	Fixed	GSD	Jones and King, 1952
Parrotfish spp.	Plastic	ESD	Robertson and Warner, 1978
Pupfish	Plastic	GSD	Kodric-Brown, 1986
Sailfin Molly	Plastic	GSD	Reynolds <i>et al.</i> , 1993
Sea basses	Plastic	GSD	Petersen, 1991
Swordtail	Fixed	GSD	Ryan <i>et al.</i> , 1992
Wrasse spp.	Plastic	ESD	Warner and Robertson, 1978

\* Mating tactics (TACTIC) are considered fixed if there is no change in the morphology or behavioral tactic during development or adulthood; plastic refers to a change in morphology or behavior in response to changes in the hormonal, social, or physical environments during development or adulthood. Genotypic sex determination (GSD) refers to gonadal sex being determined at fertilization by specific chromosomes inherited from the parents. Environmental sex determination (ESD) refers to gonadal sex being determined after fertilization by environmental factors. The sex-changing tropical fish totals several hundred species (spp.). SDM = sex-determining mechanism. AUTHORITY refers to description of the mating tactic, not the sex-determining mechanism. See text for details.

incubation temperature, rather than sex chromosomes, determines sex, a process known as temperature-dependent sex determination (TSD). However, incubation temperature does more than establish the gonadal sex of the individual. Indeed, the temperature experienced during embryogeny accounts for much of the individual and sexual variation observed in adult morphology, endocrine physiology, and sociosexual behavior. Further, temperature-induced behavioral differences both between and within the sexes are reflected in differences in the size and metabolic activity of specific brain areas involved in the regula-

tion of aggressive and sexual behaviors. Other factors, such as sexual experience and age are also important in brain organization and the development of an individual's sexuality.

#### *Environmental effects during development*

In reptiles with TSD, the physical stimulus of temperature is transduced in the mid-trimester of development to modulate expression of the genes coding for steroidogenic enzymes and sex steroid hormone receptors (Crews, 1996; Crews *et al.*, 1994). This, in turn, alters the hormonal milieu, and the temperature-specific sex-determin-

ing cascades appropriate for that temperature are stimulated and inhibited such that individuals become gonadal males or females. At intermediate incubation temperatures, intersexes are not formed but the sex ratio varies. Thus, TSD species do not have sex chromosomes and each individual has an equal potential to become a male or a female. Rather than the presence or absence of a particular gene(s), incubation temperature serves as the trigger activating and suppressing the physiological cascades that lead to the development of testes or ovaries.

In the leopard gecko, incubation of eggs at 26°C produces only female hatchlings, whereas incubation at 30°C produces a female-biased sex ratio, and 32.5°C produces a male-biased sex ratio; incubation of 34–35°C again produces virtually all females (Viets *et al.*, 1993; D. Crews, unpublished data). Hence, females from eggs incubated at 26°C are referred to as low-temperature females, whereas those females from eggs incubated at 34°C are referred to as high-temperature females; the two intermediate incubation temperatures are referred to as female-biased (30°C) and male-biased (32.5°C) temperatures. Since the effects of incubation temperature and gonadal sex covary, any difference between individuals could be due to the incubation temperature of the egg, the gonadal sex of the individual, or both factors combined. To assess the contribution of each, these factors must be dissociated. Studying same-sex animals from different incubation temperatures reveals the effects of temperature, whereas comparing males and females from the same incubation temperature reveals the effects of gonadal sex. In recent studies of the leopard gecko we have discovered that incubation temperature has profound organizational effects on individual variation.

*Morphology.*—Adult leopard geckos are sexually dimorphic, with males having open secretory pores anterior to the cloaca. In low-temperature females these pores are closed, whereas in females from a male-biased temperature they are open (Crews, 1988). Head size is also sexually dimorphic, with males having wider heads than females, yet females from a male-biased

temperature have a wider head than low-temperature females (Crews, 1988).

*Growth.*—Although males are the larger sex, incubation temperature has a marked effect on growth within a sex. Females from the male-biased temperature grow faster and larger than do females from the female-biased temperature, and become as large as males from the female-biased temperature (Tousignant and Crews, 1995).

*Endocrinology.*—Circulating concentrations of androgens in both newborn and adult males are approximately 10–70 times higher than in females (Gutzke and Crews, 1988; Tousignant and Crews, 1995). However, the endocrine physiology of the adult varies in part due to the temperature experienced during incubation (Coomber *et al.*, 1997; Tousignant *et al.*, 1995). For example, plasma estrogen levels are significantly higher in males from the female-biased temperature compared to males from the male-biased temperature (Coomber *et al.*, 1997). Among females, circulating estrogen levels are significantly higher, and androgen levels significantly lower, in low-temperature females compared to females from the male-biased temperature (Gutzke and Crews, 1988; D. Crews, unpublished data). Whether this also is the case in hatchlings from different incubation temperatures is not yet known.

*Aggressive behavior.*—Incubation temperature is also a major determinant of the nature and frequency of an adult animal's behavior. For example, females usually respond aggressively only if attacked, whereas males will posture and then attack other males, but rarely females (Gutzke and Crews, 1988; Flores *et al.*, 1994). However, males from the female-biased temperature are less aggressive than males from the higher, male-biased temperature and, although not as aggressive as males from that same incubation temperature, females from the male-biased temperature are significantly more aggressive toward males than are females from the low or female-biased temperatures. These same females show the male-typical pattern of offensive aggression. Incubation temperature also influences the ability of exogenous testosterone to restore aggression. Following ovariectomy

and testosterone treatment, low-temperature females do not exhibit increased levels of aggression toward male stimulus animals, whereas females from male-biased temperatures return to the high levels exhibited while gonadally intact (Flores and Crews, 1995). This suggests that incubation temperature influences responsiveness to steroid hormones in adulthood in males.

*Sexual behavior.*—Courtship is a male-typical behavior. In a sexual encounter, the male will slowly approach the female, touching the substrate or licking the air with his tongue. Males also display a characteristic tail vibration, creating a buzzing sound, when they detect a female. Intact females have never been observed to exhibit this tail-vibration behavior, regardless of their incubation temperature. However, if ovariectomized females from low and male-biased temperatures are treated with testosterone, they begin to tail-vibrate toward female, but not male, stimulus animals; males appear to regard such females as male because the females are attacked (Flores and Crews, 1995). Thus, incubation temperature influences the perception of individuals as well as how they are perceived by conspecifics.

*Volume and metabolic activity of specific brain areas.*—There is no statistically significant sexual dimorphism in the volume of the preoptic area (POA) and ventromedial hypothalamus (VMH) between males and females from the same incubation temperature (Coomber *et al.*, 1997). There are, however, consistent differences across incubation temperatures, suggesting that incubation temperature of the embryo has a direct effect on brain organization independent of gonadal sex (Coomber *et al.*, 1997). The POA is larger in both males and females from the male-biased temperature compared to animals from the female-biased temperature. Similarly, the volume of the VMH is larger in low-temperature females compared to females from the male-biased temperature.

In terms of metabolic differences, males on average have greater cytochrome oxidase (C.O.) activity in the POA, whereas females on average have greater C.O. activity in the VMH (Coomber *et al.*, 1997).

Again, incubation temperature is an important determinant, but so, too, is gonadal sex (*i.e.*, hormones). Males and females from the male-biased temperature have greater C.O. activity in the POA compared to animals from the other incubation temperatures, whereas females from the female-biased temperature have greater C.O. activity in the VMH compared to females from the male-biased temperature.

As mentioned, there is a significant increase in aggression in females with increasing incubation temperature (Flores *et al.*, 1994). In reptiles, the external amygdala is homologous to the mammalian amygdala and, as in mammals, is involved in the control of aggression (Greenberg *et al.*, 1984). Analysis of females from different incubation temperatures reveals that C.O. activity increases in this and other brain areas as a function of incubation temperature in a manner that parallels differences in aggression within females (Coomber *et al.*, 1997).

Are these differences in the volume and C.O. activity of brain nuclei a consequence of a direct action of temperature, or an indirect result of temperature's sex-determining function? This has been tested using the classic gonadectomy and hormone replacement therapy approach (Crews *et al.*, 1996a). In both males and females from the male-biased temperature, the volume of the POA decreases, and the VMH increases following gonadectomy. Low-temperature females, however, show no change in the volume of the POA or VMH. Treatment with testosterone causes the volume of the POA to increase, and the VMH to decrease, in both sexes from the male-biased temperature. However, testosterone treatment of low-temperature females has no effect on the volume of the POA or VMH. This latter result is similar to previous findings that females from different incubation temperatures have different sensitivities to exogenous testosterone (Flores and Crews, 1995) which, in other lizards, reflects steroid hormone receptor abundance (Crews *et al.*, 1996b; Young and Crews, 1995). Thus, it appears that males and females from the same incubation temperature respond to sex steroids in the same way, but that, within a



sex, geckos from different incubation temperatures respond to sex steroid hormone manipulation differently. These behavioral and brain volumetric/metabolic differences indicate that temperature is the primary differentiating cue.

Although we do not know at this time if hormones differ in the yolk, the embryo, or in the neonate in same-sex individuals from different incubation temperatures, steroid hormones do not explain the fact that the level and intensity of aggressive behavior, as well as growth, is a function of incubation temperature, not gonadal hormones. Administration of estrogen to eggs incubating at a male-producing temperature has no effect on either aggressive behavior or growth of these females; that is, hormone-determined females respond according to their incubation temperature, not their gonadal sex. Further, if ovariectomized at birth, females from a 26°C incubation temperature grow at a rate characteristic of males from a 32.5°C incubation temperature, whereas females from a 32.5°C incubation temperature do not show an acceleration of growth or an increase in aggression. Together, these studies of gonadectomy and hormone treatment of embryos, hatchling and adult animals suggest that incubation temperature has a direct organizing effect on an individual's response to hormones and to gonadectomy.

It remains to be determined the role, if any, of endogenous sex hormones in this differentiation process. Standard histochemical methods have assessed the activity of 3 $\beta$ - and 17 $\beta$ -HSD enzymes at the beginning, during, and after sex determination at both male- and female-producing incubation temperatures in the red-eared slider (Thomas *et al.*, 1992). Male- and female-producing incubation temperatures result in different patterns of HSD activity in the adrenal and mesonephros during development. Significantly, reaction product is not observed in the genital ridge at the beginning of the temperature-sensitive period at either incubation temperature, nor is it apparent in the differentiating gonads in embryos during temperature-sensitive period; the only activity detected in the gonad is observed after the temperature-sensitive pe-

riod. This suggests that in the slider, tissues proximate to the gonad or elsewhere, such as the brain, may produce steroid hormones at the beginning and during the sex-determining period. Indeed, Merchant-Larios and colleagues recently has found in embryos of the Olive Ridley sea turtle incubating at a female-producing temperature increasing levels of testosterone in the diencephalon during the temperature-sensitive window and decreasing levels of testosterone in gonads (Merchant-Larios *et al.*, 1998; Salame-Mendez *et al.*, 1998). Given that testosterone reduces to dihydrotestosterone via reductase, Merchant-Larios' observations are consistent with our finding that 5 $\alpha$ -reductase activity in the brain compared to the gonad is two-fold higher during, and five-fold higher after, the temperature-sensitive window (R. Vohra and D. Crews, unpublished data). In addition, estrogen receptor is present in the turtle brain (Mak *et al.*, 1982). These findings, coupled with the observation of neural innervation of the embryonic gonad (see also Crews, 1993), suggest that the brain may be the site of transduction of the temperature signal. Experiments combining administration of estrogen, dihydrotestosterone, and aromatase inhibitor *in ovo* with gonadectomy soon after hatching would resolve this issue.

#### *The importance of experience and age*

Experience is cumulative, depending upon preceding events and, at the same time, setting the stage for future experiences. Social deprivation, environmental complexity, and nutrition can alter the physiology and structure of the brain (*cf.* Bhide and Bedi, 1984; Kraemer *et al.*, 1984; Turner and Greenough, 1985). Although we have known for years that sociosexual experience and age influence an individual's sexual behavior, there has been only limited work extending these effects into the realm of brain morphology and activity (*cf.* Keverne *et al.*, 1993; Kollack-Walker and Newman, 1996). In relation to sexual behavior, the best information is on the telencephalic neural system that underlies song in birds, but only in terms of the development of the song system in juvenile birds (Brenowitz

and Kroodsmas, 1995; Brenowitz *et al.*, 1996).

Since organisms age as they gain sociosexual experience, but do not necessarily gain sociosexual experience as they age, it is important to separate the effects of age from those attributable to experience. By incubating leopard gecko eggs at temperatures that produce either females only or a male-biased sex ratio (26°C or 32.5°C, respectively), and then raising animals in isolation for at least one year before housing some lizards together in breeding groups, it was possible to assess the relative effects of age and sociosexual experience.

In general, males show more changes with age and sociosexual experience than do females (Crews *et al.*, 1997). Further, sexual experience has opposite effects on the volume and C.O. activity between the sexes. The volume of the POA increases with sexual experience in low-temperature females with sexual experience, but not in females from the male-biased temperature, whereas C.O. activity in the VMH increases in females from the male-biased temperature, but not in low-temperature females (Crews *et al.*, 1997). Age in males, on the other hand, causes the volumes of the POA and VMH to decrease, but C.O. activity in the POA to increase. That is, the POA becomes smaller and more active in males as the male ages. These results indicate that the volume and metabolic capacity of specific brain regions are dynamic in adulthood, changing as leopard geckos age and gain sociosexual experience, but the nature and degree of change is dependent upon prenatal events.

#### CONCLUSION

Can the information learned from these experiments on temperature-induced differences in leopard geckos be applied to animals in nature? For example, is there a mix of different phenotypes in natural populations and does this variation observed in behaviors such as aggression relate to mating success? Unfortunately, nothing is known of the behavioral ecology of leopard gecko in nature, but the present results identify issues worthy of investigation. Similarly, this research has implications for our current

understanding of GSD species. In general, much of the research on the proximate mechanisms underlying the establishment of the sexual phenotype (which obviously includes behavior patterns) has emphasized the role of gonadal sex hormones in the development of sexual dimorphisms. Yet the diversity in natural systems described herein suggests that factors other than sex chromosomes or the steroid hormones secreted following gonadal differentiation can play important roles in the development of within-sex differences in sexual behavior. A partial list of other possible factors would be (i) the embryonic environment (broadly defined to include hormones as well as physical factors such as temperature), (ii) the psychophysiological condition of the mother during pregnancy or egg-laying (*cf.* Pigliucci, 1996), (iii) the sociosexual experiences during growth and adulthood, and (iv) the aging process.

Such examples of "hormonal inheritance" (Crews *et al.*, 1989) have parallels in rats, gerbils, mice, and even humans (*cf.* Clark and Galef, 1995; vom Saal, 1991). For example, a female fetus located between two males (a 2M female) is exposed to higher levels of androgen produced by the neighboring males compared to a female fetus located between two females (a 2F female). As adults, these 2M females have lower estrogen and higher testosterone levels, have a masculinized phenotype, are less attractive to males and more aggressive to females, and produce litters with significantly greater male-biased sex ratios relative to 2F females. Brain nuclei can also vary according to uterine position. In gerbils, the sexually dimorphic area of the preoptic area (SDA-POA) is responsible for copulatory behavior in males (Yahr, 1995) and, as females differ in their sexual behavior according to intrauterine position, the SDA-POA is likely to be involved in their behavior as well. Long-term changes are apparent in the C.O. activity of the SDA-POA, with 2M females having greater activity compared to 2F females (Jones *et al.*, 1997). Metabolic capacity also differs in the posterior anterior hypothalamus, an area replete with neurons containing gonadotropin releasing hormone, which may explain the

physiological differences between 2M and 2F females.

Such data suggest that it might be more profitable to view hormones and other factors during embryogenesis as allowing for the growth of neural connections. Later, participation in particular behaviors throughout life may establish and then maintain the unique functional integrity of the attendant neuroendocrine systems within each individual (Crews, 1987). For example, an individual's sociosexual experience not only can modify its mating behavior, but experience can change how it responds to sex steroid hormones. In guinea pigs, the amount of sexual activity experienced as a juvenile affects the level of sexual behavior displayed as an adult, as well as modifies the individual's response to castration and androgen replacement therapy (Valenstein and Young, 1955). In cats, males that have had sexual experience before castration persist in displaying sexual behavior, whereas males that were sexually naive prior to castration show an abrupt cessation in sexual behavior; conversely, castrated, sexually naive male cats given exogenous testosterone take longer to exhibit sexual behavior compared to castrates that have had sexual experience (Rosenblatt, 1965). In gulls, testosterone treatment of males reared in social groups induces sexual behaviors, but not in males reared in isolation; males reared in social groups until sexually mature and then subjected to social isolation for two weeks exhibit lower levels of sexual behavior in response to exogenous testosterone compared to similarly reared males exposed to unfamiliar conspecifics (Groothuis, 1995). If a female zebra finch is raised in a mixed sex group, she will prefer the company of males when she reaches adulthood (Adkins-Regan, 1997). However, if she is raised in an all-female group, she will associate more with females and, if also treated with exogenous estrogen, will preferentially pair with females. In whiptail lizards, the behavior of the partner can regulate the abundance of steroid receptors in the brain independently of the gonads (Hartman and Crews, 1996). One interpretation of these data is that sociosexual experience mediates the effects of

androgen by affecting the individual's sensitivity to androgen, perhaps by influencing receptor density and/or the metabolism of the hormone.

Thus, experience can have a permanent organizing effect on behavior and, hence, the brain. Lehrman (1962) noted that "the two sexes within the same species might differ with respect to the relative degree of dependence of their sexual behavior upon the presence of various hormones and upon various situation and experiential factors." This same logic can be extended to individual differences. Research on the leopard gecko illustrates how such nongenomic factors can affect the differentiation of the adult phenotype. In this species, the confound of genetically-based sex differences is avoided because sex is determined by incubation temperature, not by sex chromosomes. Incubation temperature accounts for much of the sexual and individual variation in morphology, endocrine physiology, and aggressive and sexual behavior. These behavioral differences between and within the sexes are reflected, in turn, in differences in the size and metabolic activity of specific brain areas known to be involved in the regulation of aggressive and sexual behaviors, revealing how the environment can influence brain plasticity.

#### ACKNOWLEDGMENTS

I thank E. A. Brenowitz, L. Drickamer, J. Godwin, S. B. Hrdy, D. McFadden, M. C. Moore, M. Rand, M. J. Ryan, J. Sakata, E. Vance, J. Wingfield and two anonymous reviewers for their comments on the manuscript. The support of NIMH Research Scientist Award 00135 is gratefully acknowledged.

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Corresponding Editor: Paul A. Verrell