

Sex Differences in the Nervous System of Reptiles

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SUMMARY

1. The study of sex differences in the brain and behavior of reptiles presents an excellent opportunity both to discern general principles of sexual differentiation in the nervous system and to explore the evolutionary history of this process in amniote vertebrates.

2. Findings in several reptiles suggest that some sex differences found in mammals and birds are conserved while others are not. Conserved features include areas in the limbic forebrain involved in the regulation of social and sexual behaviors. As in mammals and birds, it is rare to find differences in the distribution of sex steroid concentrating neurons in reptiles but common to find differences in the distribution of the various steroid hormone receptors and in their regulation.

3. This research has revealed that differences in social and sexual behavior are reflected better by the activity, not by the size, of hormone-sensitive limbic areas.

4. Finally, species differences in plasma levels of sex hormones are paralleled by differences in behavioral sensitivity to these hormones as well as by differences in the regulation of genes coding for steroid hormone receptors.

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INTRODUCTION

Amniote vertebrates originated approximately 300 million years ago. This origin was shortly followed by a divergence into the Synapsida, the stem group of mammals, and the Sauropsida, the lineage leading to modern reptiles including birds (Fig. 1). In order to address the evolution of sex differences in the nervous system of amniotes, we would ideally like to know the ancestral state of these differences. Since the fossil record will not provide this information, it must be inferred from patterns observed in living representatives. Sex differences in the nervous system are best characterized in mammals and birds. However, living members of both of these groups share conserved genotypic sex determination mechanisms and relatively conserved patterns of sexual differentiation. This conservation hampers our ability to infer the ancestral state of these mechanisms in the mammal and bird lineages and therefore the evolutionary history of mechanisms differentiating sexual function and behavior. In contrast, the reptiles exhibit much broader diversity in sexual determination and differentiation mechanisms (Crews and Gans, 1992).

The extant reptiles are represented by approximately 6500 species divided primarily among three major groups: the turtles, crocodilians, and squamates (the lizards and snakes); the fourth major group, *Sphenodon*, consists of just two species of tuatara. Divergence between turtles and the lineage leading to modern archosaurs (crocodilians and birds) and squamates occurred near the base of the amniote family tree about 300 million years ago. Within and between these reptilian groups, a variety of sex determination, sexual differentiation, and reproductive patterns is seen. This diversity includes (i) sex determination by either sex-limited chromosomes or environmental cues, (ii) a lack of males in species which reproduce parthenogenetically, (iii) distinct within-sex alternate mating phenotypes, and (iv) both oviparous and viviparous reproduction.

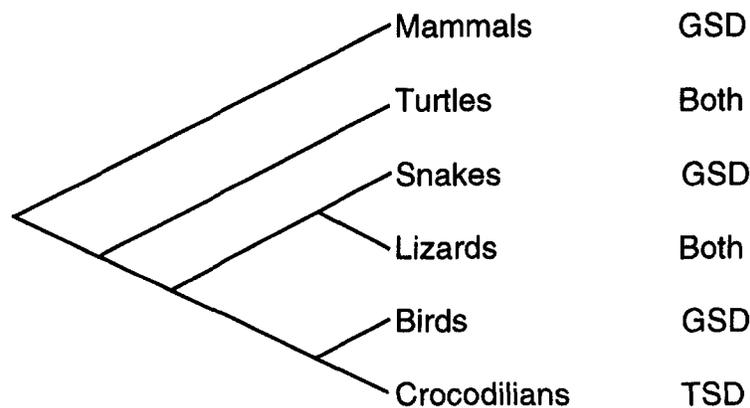


Fig. 1. Phylogeny of sex determining mechanisms in amniote vertebrates. In lizards and turtles both genotypic sex determination (GSD) and temperature-dependent sex determination (TSD) have been documented. From Crews (1994).

We contend that the utility of reptiles for addressing general questions about the causes and consequences of sex differences in the amniote nervous system lies in this diversity and the comparative approaches it allows. A comparative approach should allow the determination of what behavioral mechanisms are conserved through vertebrate history and which are more labile. Aside from intrinsic interest, this knowledge would have direct relevance to basic and applied human biology. Features which are conserved across the vertebrates can be more confidently extrapolated to humans than those which show evolutionary changes in response to life history, reproductive pattern, mating system, or a host of other selective forces.

The control of both male- and female-typical sexual behaviors in reptiles and the role of sex steroid hormones in this control have been reviewed recently (Moore and Lindzey, 1992; Whittier and Tokarz, 1992). We begin by briefly summarizing information on the neural bases of sociosexual behavior and supplementing these reviews with more recent information. Then we point out questions which are difficult to approach with mammalian and avian model systems and focus on reptilian systems which are, in contrast, particularly well suited to addressing these issues. Finally, we suggest areas where potentially useful information is lacking, research directions which should be productive, and how a perspective of behavioral neuroendocrinology based on natural diversity is useful in considering how mechanisms subserving sociosexual behaviors evolve.

NEUROANATOMICAL AND NEUROCHEMICAL SUBSTRATES OF SEXUAL BEHAVIOR IN REPTILES

The neural control of sexual and other sociosexual behaviors is remarkably conserved in vertebrates. The primary integrative centers for these behaviors in reptiles and other tetrapods are in the limbic system. Based on these homologies of limbic system structure and function across tetrapods and the lack of a well-developed neocortex in reptiles, Greenberg (1977) suggested that reptiles are relatively "pure" limbic preparations and therefore superior models for studying the function of these brain areas. Two areas of the limbic system are of special interest with respect to sexual behaviors: the preoptic area–anterior hypothalamus (POAH) and the ventromedial hypothalamus (VMH). The POAH exerts primary control over male-typical mounting and intromission behavior. This has been established for several species by experiments in which lesions of this area abolish male-typical sexual behavior, while intracranial implantation of androgens in the POAH reinstates this behavior in castrated males (e.g., Kingston and Crews, 1994; other reports are reviewed by Moore and Lindzey, 1992). The critical importance of the VMH for female-typical receptive behavior has been shown in similar studies where lesioning abolishes receptivity while intracranial implantation of estradiol-17 β restores it (e.g., Kendrick *et al.*, 1995; other reports are reviewed by Whittier and Tokarz, 1992).

SEXUAL DIMORPHISMS IN BRAIN AREAS

Size differences in brain nuclei important for the control of certain behaviors are often proposed or implied to be causally linked to differences in the frequency or amplitude with which those behaviors are displayed. Rather than individual behavioral patterns, these nucleus size differences are more commonly associated with suites of behaviors constituting a certain behavioral phenotype such as male-typical copulatory behaviors and female-typical receptive behaviors. Correlations between overall brain nucleus size or the number or size of neurons within these nuclei and behavioral phenotype are known from a variety of taxa. These include examples from fish varying in the display of male-typical sexual and aggressive behavior (e.g., Bass, 1992; Grober and Bass, 1991; Grober *et al.*, 1994; Davis and Fernald, 1990; Francis *et al.*, 1993) and sexual dimorphisms in the POAH in both mammals and birds [e.g., the sexually dimorphic nucleus of the POA in rats (Gorski, 1984), and gerbils (Yahr, 1995), the preoptic area in quail (Panzica *et al.*, 1987); and the song control nuclei of passerine birds (Nottebohm, 1989)]. Aside from the difficulties of proving causation from correlational relationships (in this case, difference in nucleus size = difference in behavior), we might ask how strong this relationship between morphometric differences in the central nervous system and differing behavioral phenotypes is. Given a natural or experimentally induced behavioral similarity, should we expect morphometric similarities in important brain nuclei between different sexes or experimental groups?

This laboratory has approached these questions using several reptile species. The whiptail lizards (genus *Cnemidophorus*, Teiidae) consist of 45 species, of which 15 are all-female and obligately parthenogenetic. The parthenogen, *C. uniparens*, is descended from a hybridization event between two bisexual, sexually reproducing species. Molecular analyses have established *C. inornatus* as the maternal ancestral species (Wright, 1993).

Despite consisting entirely of female individuals, *C. uniparens* regularly exhibits both male-like and female-like pseudosexual behaviors which are indistinguishable from those shown by male and female *C. inornatus*, respectively (Crews and Fitzgerald, 1980). This display of male-like pseudosexual behavior by *C. uniparens* is not associated with a change in the nature and pattern of circulating sex steroid hormones compared to those observed in females of the sexual ancestor *C. inornatus* (reviewed by Crews and Moore, 1993). Male-typical sexual behavior is controlled primarily by androgens in males of the ancestral *C. inornatus*, but androgens are uniformly undetectable by radioimmunoassay in plasma of *C. uniparens*, including during the postovulatory period of the ovarian cycle, when male-like behavior is most commonly observed (Moore *et al.*, 1985). The weight of evidence instead suggests that male-like pseudosexual behavior in *C. uniparens* is stimulated by the postovulatory rise in circulating progesterone (Grassman and Crews, 1986). The evolutionary precursor of this novel neuroendocrine mechanism is seen in male *C. inornatus*, where mounting and intromission behavior can be reinstated in a significant proportion of castrated males with progesterone (reviewed by Crews, 1989).

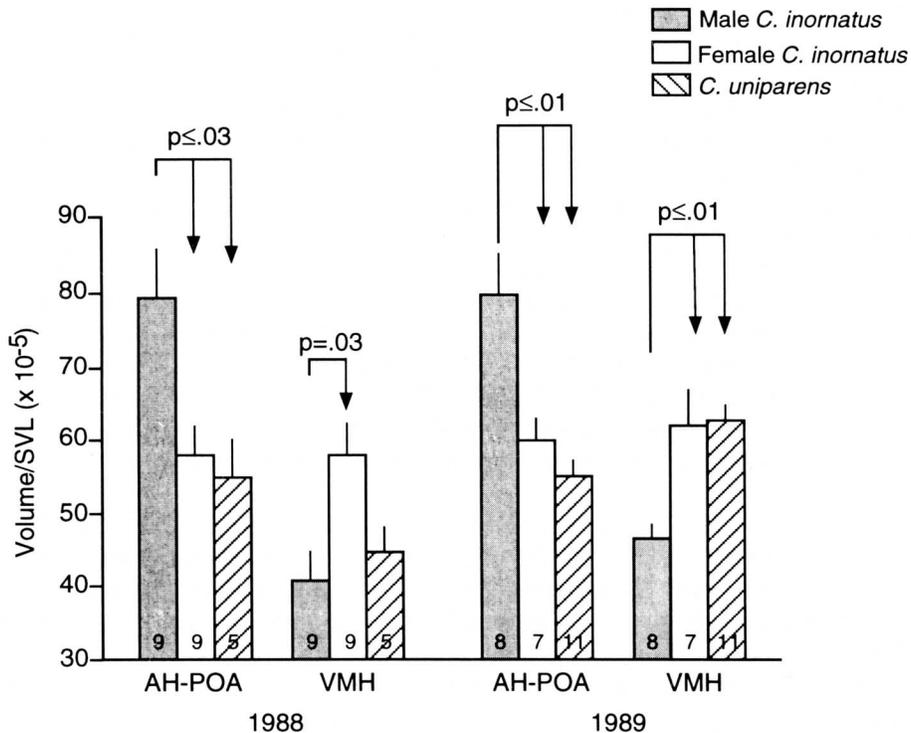


Fig. 2. Sexually dimorphic brain areas in whiptail lizards. Volumes relative to body size of two sexually dimorphic areas in the brain of the sexual ancestral species and the parthenogenetic descendant species of whiptail (*Cnemidophorus*) lizard. Significant differences are marked. Sample sizes are indicated at the bottom of the histograms. Vertical bars indicate ± 1 SE. From Crews *et al.* (1990).

There are sex differences in the overall sizes of both the POAH and the VMH as well as in individual neuronal soma sizes within these brain areas in the sexual ancestral species. Male *C. inornatus* have significantly larger POAH volumes than do females and greater neuronal soma sizes within this area (Crews *et al.*, 1990; Wade and Crews, 1991) (Fig. 2). In contrast, female *C. inornatus* have significantly larger VMH volumes and greater neuronal soma sizes in the VMH than do males. These data are in agreement with reported sex differences in brain nucleus size of other vertebrates. However, if behavior is held constant and sex is instead varied, will a similarity in brain nucleus size be observed across sexes? In *Cnemidophorus*, the answer appears to be no.

Despite regularly displaying male-like courtship, mounting, and copulatory behavior, *C. uniparens* have a POAH that is female-like in volume (Crews *et al.*, 1990). Moreover, treatment with exogenous testosterone in adulthood, which strongly stimulates male-like pseudosexual behavior and expression of external secondary sexual characters such as ventral blue coloration and enlarged secretory femoral pores, does not produce a discernible change in the size of the

POAH (Wade *et al.*, 1993). Treatment with either estrogen or dihydrotestosterone is similarly ineffective in changing POAH size. A similar lack of correspondence exists between the size of the POAH and strong- and weak-courting males of the sexual ancestral species (Wade *et al.*, 1993).

The red-sided garter snake (*Thamnophis sirtalis parietalis*) is of interest in this context because mating behavior is not coupled with gonadal activity (Crews *et al.*, 1984). In this species, sexual behavior is dramatically dimorphic, but the sizes of the POA and VMH are monomorphic, even with treatment of exogenous hormones (Crews *et al.*, 1993) (Fig. 3). The single exception is seen during hibernation, when males have larger POA volumes than females. There is a sex difference in seasonal fluctuations of POA and VMH volumes, with females showing changes while males do not (Crews *et al.*, 1993). This difference may relate to the dependence of female sexual behavior in this species on elevated estrogen levels (Mendonça and Crews, 1996), while male-typical sexual behavior is independent of the activation by androgens (reviewed by Crews, 1991; Crews *et al.*, 1993).

Another species in which behavior is sexually dimorphic, but the size of the POAH and VMH is not, is the leopard gecko (*Eublepharis macularius*) (Coomber *et al.*, 1996). The leopard gecko shows temperature-dependent sex determination and is discussed at length under *Alternative Forms of Sexual Differentiation* below.

If the sex differences in nucleus size in *C. inornatus* result from sex steroids organizing an undifferentiated brain in the perinatal or early postnatal period as in laboratory rodents, androgen treatment in adulthood might not be expected to produce measurable effects. To separate the two remaining potential sources of variation in POAH size, organizational effects during development and strict genetic influences, studies are under way comparing normal female *C. uniparens* to phenotypically male individuals produced experimentally with an aromatase inhibitor (Wibbels and Crews, 1994; Wennstrom and Crews, 1995). Altering the steroid hormone environment during the perinatal and early postnatal periods through castration/replacement experiments alters growth and expression of external secondary sexual characteristics in garter snakes (Crews, 1985; Shine and Crews, 1988) and in the tree lizard [*Urosaurus ornatus* (Hews and Moore, 1996; Hews *et al.*, 1994)], but brain characters have not yet been examined. However, while sex differences in brain area size may play a role in sexually differentiated behaviors, the unisexual whiptail lizards, garter snakes, and leopard geckos show that such differences are not a requirement.

SEXUAL DIMORPHISM IN METABOLIC ACTIVITY OF BRAIN AREAS

Sex differences in the activity of brain areas may be more important for sexually differentiating behavioral function than differences in the size of brain areas. Functional metabolic mapping of 2-deoxyglucose utilization in *C. uniparens* shows that individuals displaying male-like behavior have greater activity in the

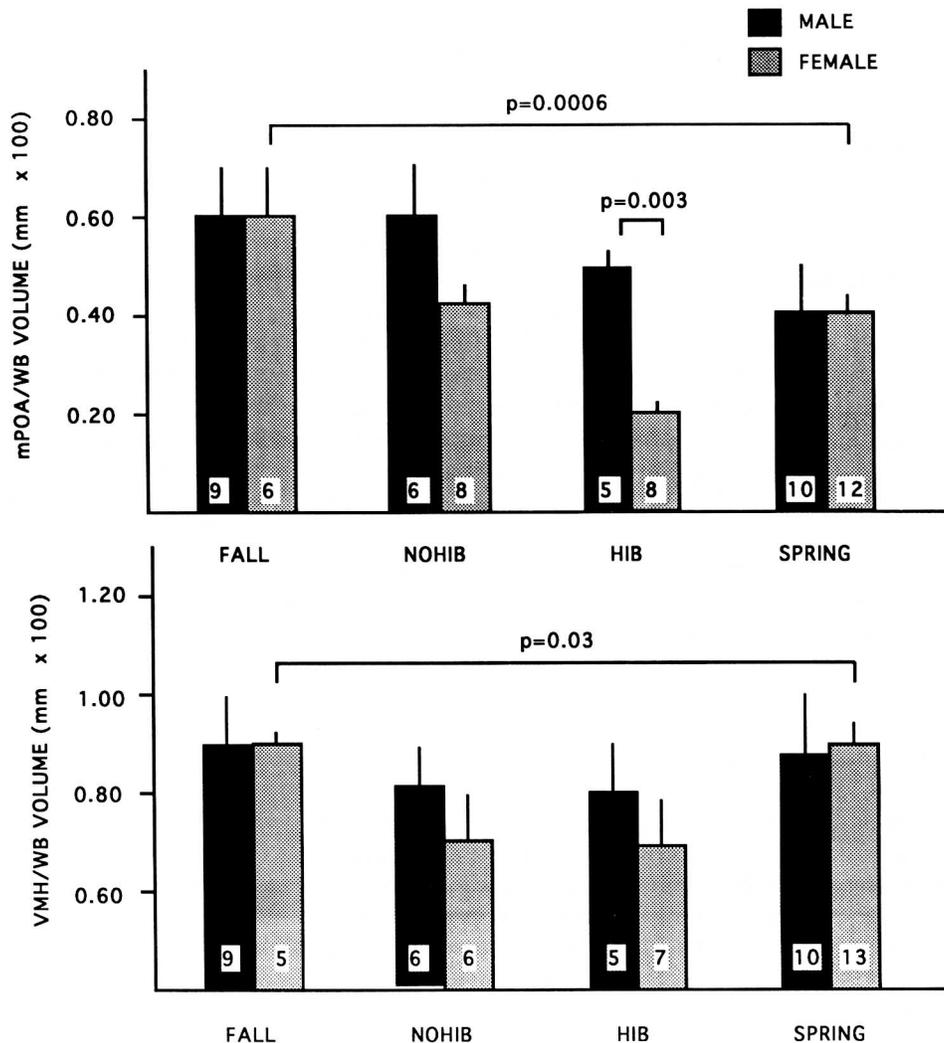


Fig. 3. Average volume of the preoptic area (top) and ventromedial hypothalamus (bottom) of male and female red-sided garter snakes (*Thamnophis sirtalis parietalis*) at different times of year (before entering hibernation, FALL; during hibernation, HIB; and on emergence from hibernation, SPRING; animals were not allowed to enter hibernation, NOHIB). Significant differences are marked. Sample sizes are indicated at the bottom of the histograms. Vertical bars indicate ± 1 SE. From Crews *et al.* (1993).

POA than individuals displaying female-like behavior (Rand and Crews, 1994). Conversely, individuals displaying female-like behavior show much greater activity in the VMH than those displaying male-like behavior. Courting male red-sided garter snakes show greater activity in the POA than do noncourting males (Allen and Crews, 1992), and a similar difference is seen between sexually receptive and nonreceptive females in this species (Mendonça *et al.*, 1996). Within- and between-sex differences in metabolic activity are also seen in leopard

geckos with cytochrome oxidase histochemistry (Coomber *et al.*, 1996). The leopard gecko work is discussed at length in a later section.

SEXUAL DIMORPHISMS IN THE REGULATION OF STEROID HORMONE RECEPTORS

Steroid hormones act on limbic and telencephalic structures primarily by binding intracellular receptors (Pfaff *et al.*, 1994). These receptors are transcription factors which alter target gene expression. An understanding of how steroid hormones regulate and interact with receptors in discrete areas of the brain should provide important insights into how the functions of these areas are differentiated sexually and otherwise. Recent technical advances have allowed the cloning and characterization of messenger RNAs for sex steroid hormone receptors and their mapping and quantification in the brain and peripheral tissues of *Cnemidophorus* (reviewed by Young and Crews, 1995) and the red-eared slider turtle (*Trachemys scripta*) (Crews, 1996). The neuroanatomical distribution of sex steroid receptors in the brain is strongly conserved between the sexes and throughout the vertebrates (reviewed by Pfaff *et al.*, 1994), but there is variation in the regulation of these receptors between nuclei within the brain, between the sexes, and across species (reviewed by Young and Crews, 1995).

Sex differences in steroid receptor mRNA regulation in the brain of *Cnemidophorus* appear to parallel those described in rats. In females of both rodents and two lizard genera, estrogen increases the abundance of progesterone receptor (PR) mRNA or protein in the mediobasal hypothalamus (reviewed by Young and Crews, 1995). Estrogen produces a similar increase in abundance of estrogen receptor (ER) mRNA in female *Cnemidophorus* (Young *et al.*, 1995a), but a decrease in both ER protein and ER mRNA in female rats. Progesterone causes a decrease in both PR and ER protein and/or mRNA in both female *Cnemidophorus* (Godwin *et al.*, 1996) and rodents (Blaustein and Turcotte, 1990; Brown and MacLusky, 1994). In contrast to this estrogenic upregulation of ER- and PR-mRNAs in the VMH of females, neither male *C. inornatus* nor rats show discernible responses to the same dosages of estrogen in this brain area (Fig. 4). The estrogen dosage used in the *Cnemidophorus* study effectively stimulates receptive behavior in females but not in males (Young *et al.*, 1995b; J. Godwin and D. Crews, unpublished data). Male *C. inornatus* do respond to this estrogen dose with changes in ER-mRNA levels in other brain areas in ways similar to females, demonstrating that the sex difference in the VMH is not due to a general lack of responsiveness to estrogen. Preliminary results indicate a reversed sex difference for androgenic effects in the POA, with males strongly decreasing AR-mRNA abundance in response to androgen while females regulate weakly or not at all (J. Godwin *et al.*, unpublished data).

Inter- and intrasexual differences in neural activity might also be reflected in more subtle gene regulation differences than a presence or absence of differences as described above. A good illustration of this is seen in a within-female difference in the display of male-typical behavior. Heterotypical mounting and

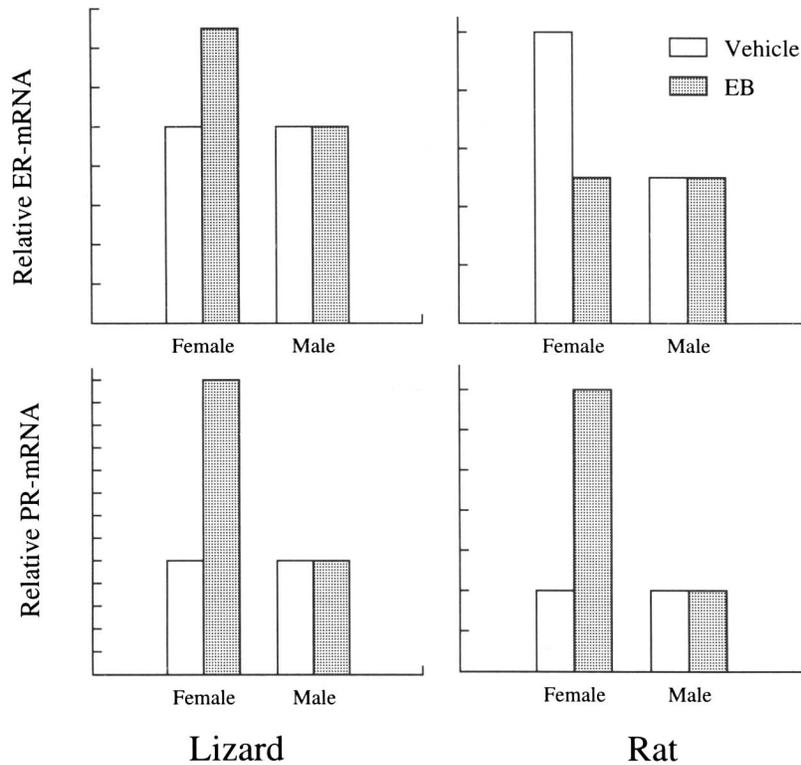


Fig. 4. Sex differences in the regulation of estrogen receptor (ER) mRNA and progesterone receptor (PR) mRNA expression by estradiol in the ventromedial nucleus of the hypothalamus of the laboratory rat and whiptail lizards. Note that while the patterns of regulation of PR-mRNA expression are similar between the species, the regulation of ER-mRNA expression is in the opposite direction. In both instances, however, the sexual dimorphism remains. From Young and Crews (1995).

copulatory behavior is common in the unisexual descendant whiptails but rare in females of the sexual ancestral species. *Cnemidophorus uniparens* have greater abundances of ER- and PR-mRNA in the POA than *C. inornatus* females both in an ovariectomized condition and over the course of the ovarian cycle (Young *et al.*, 1995c). *Cnemidophorus uniparens* also show a greater increase in both ER- and PR-mRNA abundances in the VMH in response to estrogen than do *C. inornatus* females and the display of receptive behavior is strongly correlated with the PR-mRNA response (Young *et al.*, 1995a,b). Assuming that greater abundances of ER- and PR-mRNAs reflect higher receptor protein levels, the POA in *C. uniparens* is more sensitive to circulating estrogen and progesterone than that in *C. inornatus* females (Fig. 5). Since progesterone can reinstate male-typical behavior in both male *C. inornatus* and *C. uniparens*, it is reasonable to suggest that a differential sensitivity in the POA to postovulatory progesterone levels accounts for the display of male-like pseudosexual behavior in *C. uniparens* but not in females of its direct sexual ancestor, *C. inornatus*.

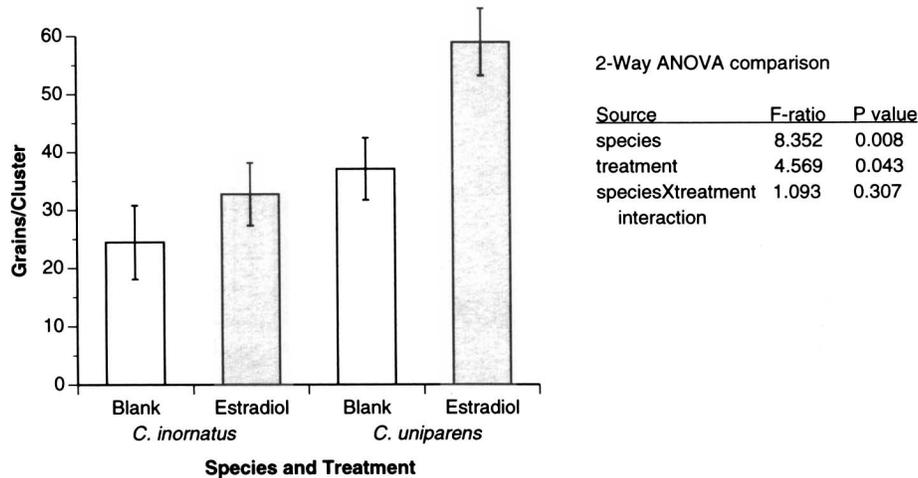


Fig. 5. Evolution of a novel neuroendocrine mechanism controlling male-typical mounting and copulatory behavior. Depicted is the abundance of progesterone receptor mRNA measured as the average number of silver grains per cluster in the preoptic area (POA) of the ancestral sexual (*Cnemidophorus inornatus*) and descendant parthenogenetic (*C. uniparens*) whiptail lizards. Statistical comparisons given on the right. From Crews (1996).

The possibility of sex differences in neuropeptides and neurotransmitters described in mammals and birds is as yet poorly characterized or completely unexplored in reptiles. In a finding similar to those in rodents, Jones and co-workers noted a strong dimorphism in cerebral cortex and apparently greater AVT immunoreactivity in the diencephalon in male than female green anole lizards (Popper *et al.*, 1992; see also Stoll and Voorn, 1981; Thepen *et al.*, 1987; Smeets *et al.*, 1990). There is also evidence that estrogen affects numbers of tyrosine hydroxylase-immunoreactive cells in hypothalamic nuclei of female *Cnemidophorus* (M. Rand and D. Crews, unpublished data), but sex differences have not been examined.

ALTERNATE FORMS OF SEXUAL DIFFERENTIATION

Gonadal sex is determined by sex chromosomes in mammals and birds. The ubiquity of male heterogamety in mammals and female heterogamety in birds indicates (i) at least one change from an ancestral amniote condition of male or female heterogamety in one of these groups or (ii) the derivation of chromosomal sex determination in both groups from a nonchromosomal system. Reptiles exhibit both male and female heterogamety as well as sex determination by an environmental cue (temperature-dependent sex determination; TSD). In TSD species, gonadal sex is determined by the incubation temperature the egg experiences prior to and during gonadal morphogenesis (reviewed by Crews,

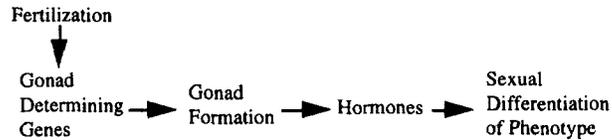
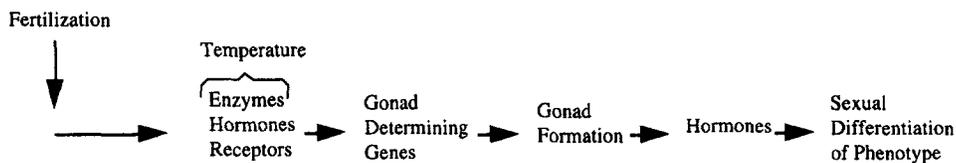
GENOTYPIC SEX DETERMINATION**TEMPERATURE-DEPENDENT SEX DETERMINATION**

Fig. 6. In the current model of vertebrate sex determination and sexual differentiation (top), gonadal sex is fixed at fertilization by specific chromosomes, a process known as genotypic sex determination. Only after the gonad is formed do hormones begin to exert an influence, modifying specific structures that eventually will differ between the sexes. Research on reptiles with temperature-dependent sex determination indicates that sex determination in these species is fundamentally different in at least one way (bottom). Gonadal sex is not irrevocably set by the genetic composition inherited at fertilization, but rather depends ultimately on which genes encoding for steroidogenic enzymes and hormone receptor are activated during development by temperature. Incubation temperature modifies the activity as well as the temporal and spatial sequence of enzymes and hormone receptors such that sex-specific hormone milieus, created in the urogenital system of the developing embryo, determine gonad type. From Crews (1993).

1996) (Fig. 6). TSD occurs in many species of turtles and lizards, but does not occur in snakes, and is the exclusive form of sex determination in tuatara (*Sphenodon*) and crocodylians; this is especially relevant since crocodylians are the closest living relatives to modern birds (these two groups diverged ~250 million years ago).

TSD may be the primitive form of sex determination in reptiles and perhaps amniotes generally (Bull, 1983). The nature and possibly primitive status of TSD raise two questions. First, given this possibly primitive status, might the ancestral state of sexual differentiation in this group be best approached through study of species exhibiting TSD? Second, following gonadal morphogenesis in mammals, birds, and at least some reptiles, sex steroid hormones from the gonads play a dominant role in sexually differentiating the somatic tissues of the body including the nervous system. If temperature is the critical organizing cue for gonadal differentiation, does this cue exert organizational effects on the nervous system independently of or in concert with the gonads? The answer to the first question will have to wait for studies in a variety of amniote lineages to be performed, but the answer to the second question appears to be 'yes' for at least the leopard gecko.

In leopard geckos, all embryos develop as females at low and high incubation temperatures, while varying proportions of males and females are produced at intermediate temperatures (Fig. 7). If differentiation of the neural substrates underlying male- and female-typical sociosexual behavior depends only on sex steroid hormone profiles determined by the differentiated gonads, similar

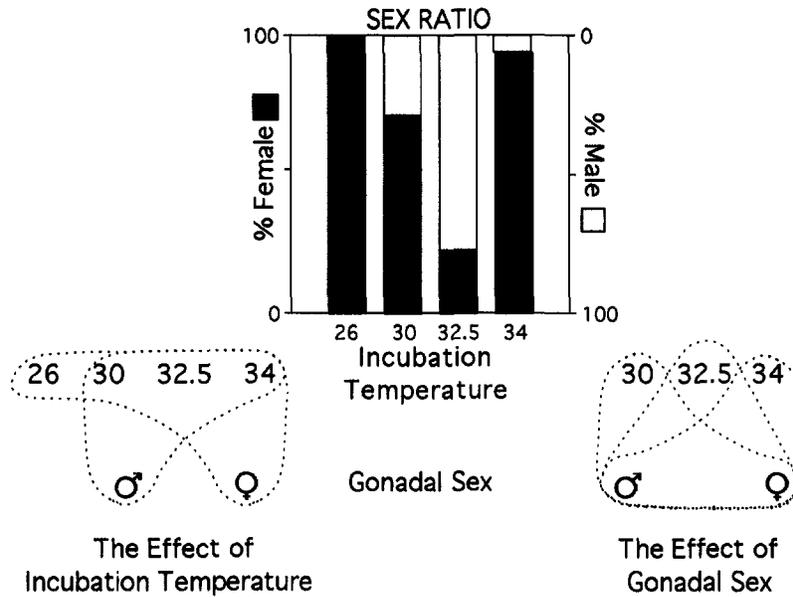


Fig. 7. Pattern of temperature-dependent sex determination in the leopard gecko (*Eublepharis macularius*) and the strategy for uncoupling gonadal (and hence hormonal) sex and environmental variables in the establishment of individual differences. The middle panel portrays the effect of incubation temperature on sex ratio: extreme temperatures produce females, whereas intermediate temperatures produce different ratios. 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, they must be dissociated. Studying same-sex animals that differ only in the incubation temperature experienced reveals the effects of temperature (left), whereas comparing males and females from the same incubation temperature reveals the effects of gonadal sex (right). Dotted lines are group comparisons made in each condition. From Coomber *et al.* (1996).

behavioral phenotypes should be observed within the sexes regardless of incubation temperature. This is not the case. Instead, individual levels of male-typical offensive aggression and female-typical sexual attractiveness covary with incubation temperature in leopard geckos (Table I) (Flores and Crews, 1995; Flores *et al.*, 1994). Females from male-biased incubation temperatures show increased levels of offensive aggression and decreased sexual attractiveness compared to females from female-biased and exclusively female incubation temperatures. Likewise, males from female-biased incubation temperatures are less aggressive than males from a male-biased incubation temperature.

These within-sex behavioral differences have parallels in the size and metabolic activity of nuclei in the limbic system. Indeed, despite differing greatly in circulating androgen and estrogen levels, several brain characters show a greater similarity between males and females from the same incubation temperature than between same-sex individuals who developed at different incubation temperatures (Coomber *et al.*, 1996). No statistically significant sexual dimorphisms were found in the volumes of the POA or VMH between males and females

Table I. Sociosexual Behavior in Leopard Geckos from Different Incubation Temperatures^a

| Sex | Type | Temp. (°C) | Aggression | Attractivity | Courtship |
|--------|------------------|------------|------------|--------------|-----------|
| Female | Low-temperature | 26 | * | *** | — |
| | Female-biased | 30 | ** | ** | — |
| | Male-biased | 32.5 | *** | * | — |
| | High-temperature | 35 | *** | *** | — |
| | Ovex + TESTO | 26 | — | — | * |
| Male | TESTO | 32.5 | *** | — | * |
| | Female-biased | 30 | **** | — | ** |
| | Male-biased | 32.5 | ***** | — | ** |

^a An asterisk indicates display of the behavior, and the number of asterisks indicates significant differences in relative frequency or intensity. Dashes indicate absence of the behavior. Ovex + TESTO, individuals ovariectomized as adults and treated with a Silastic capsule containing testosterone. Details given in text and by Flores and Crews (1995) and Flores *et al.* (1994).

from the same incubation temperature (Fig. 8). There were, however, consistently significant differences across incubation temperatures. The volume of the POA was 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 was larger in low temperature females compared to females from

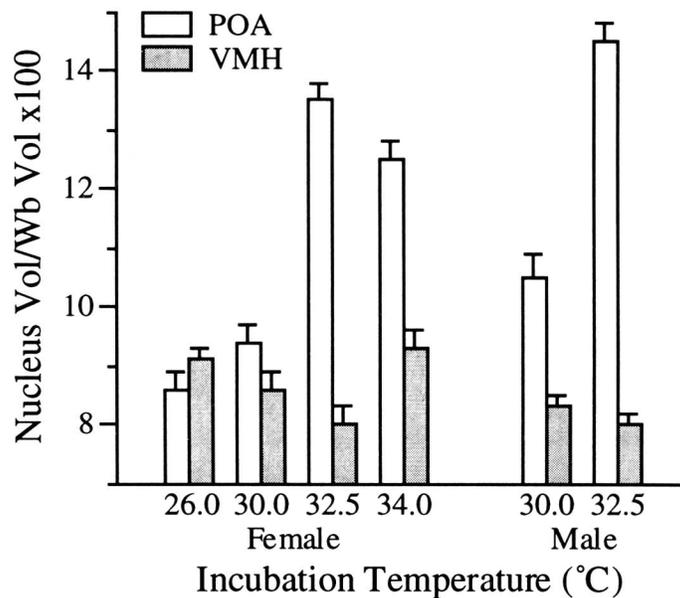


Fig. 8. Effect of incubation temperature on the volume of the POA and VMH in the leopard gecko. All animals were sexually mature but reproductively inexperienced. Mean ratio of nucleus volume divided by whole brain (Wb) volume $\times 100$ is presented, with vertical bars representing standard error. From Coomber *et al.* (1996).

the male-biased temperature. This supports the hypothesis that incubation temperature has a direct organizing effect on the volume of the POA and VMH.

The metabolic capacity of limbic system nuclei was measured in leopard geckos from various incubation temperatures using the cytochrome oxidase histochemistry technique (Gonzalez-Lima, 1992). Cytochrome oxidase (CO) plays a limiting role in oxidative metabolism and increased activity of neurons in a brain region leads to increased CO activity in their mitochondria. This technique therefore assesses the metabolic capacity of a brain area rather than the acute glucose utilization measured by the 2-deoxyglucose method. Studies from a variety of taxa show the promise of this method for investigating sexual differentiation of brain function [e.g., frogs (Marler *et al.*, 1992) and gerbils (Jones *et al.*, 1994, 1996)].

In leopard geckos, males have greater CO activity in the POA, whereas females have greater activity in the VMH (Coomber *et al.*, 1996). Incubation temperature is important, however. Males and females from the male-biased temperature had greater CO activity in the POA compared to animals from the other incubation temperatures, whereas females from the female-biased temperature had greater CO activity in the VMH compared to females from the male-biased temperature (Fig. 9).

ALTERNATE MATING PHENOTYPES

Male-like behavior in parthenogens and determination of sex and sexuality by temperature both provide natural experiments not available in mammals or birds. As described above, these are useful models for dissecting the structure-activity relationships underlying the display of sex-typical behaviors. Another promising group of reptiles for addressing this issue is those exhibiting alternate male mating phenotypes (Crews and Bull, 1987; Moore, 1991). In these species, males fall into two or more distinct categories distinguished by variation in morphological and behavioral characters. This type of discontinuous behavioral and morphological variation is not found in mammals and has been described from only one bird species (Lank *et al.*, 1996). The divergent male morphs typically differ in display of male-typical courtship, aggressive defense of breeding territory, or both [*Urosaurus* (Hover, 1985; Thompson and Moore, 1991); *Sceloporus undulatus* (Rand, 1991), and red-sided garter snake (Mason and Crews, 1985)]. Morphological differences are often associated with these behavioral morphs and can include differences in body size, coloration, and even skin-derived pheromones (reviewed by Cooper and Greenberg, 1992; Mason, 1992). In the red-sided garter snake, a small proportion of males is female-mimics ("she-males"). Mating in this species takes place on emergence from hibernation in spring. Large groups of males form mating balls in which individual males attempt to mate with females as they emerge from hibernacula. The she-males appear to confuse other males by appearing to be female and thereby enhance their own probability of copulation. These she-males do not differ from other males in gross morphology but produce the female attractivity pheromone and

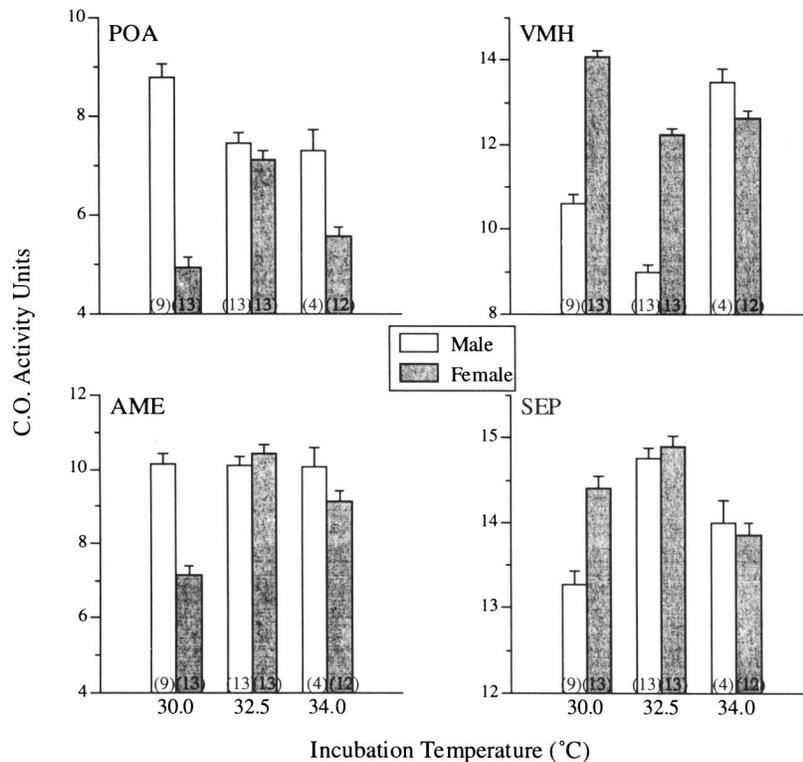


Fig. 9. Sex differences in cytochrome oxidase (C.O.) activity ($\mu\text{mol}/\text{min}/\text{g}$ tissue wet weight) in adult leopard geckos (*Eublepharis macularius*) from different incubation temperatures. Sample sizes in parentheses. Mean C.O. activity units depicted, with vertical bars the standard error. POA, preoptic area; VMH, ventromedial hypothalamus; SEP, septum; AME, medial amygdala. From Coomber *et al.* (1996).

lack the male identification pheromone (Mason *et al.*, 1989). She-males also have an unusual circulating steroid profile with plasma levels of testosterone that are higher than those of other males (Mason and Crews, 1985).

In the tree lizard, *Urosaurus ornatus*, and the red-lipped western fence lizard, *Sceloporus undulatus erythrocheilus*, the alternate male morphs differ in coloration and territorial behavior (Hover, 1985; Rand, 1991). Androgen manipulations in adulthood do not alter the expression of morph-specific external characters (Thompson and Moore, 1992; Rand, 1992) but early postnatal manipulations do strongly affect their frequency in *Urosaurus* (Hews *et al.*, 1994). Castration within 30 days of hatching in *Urosaurus* reduces the frequency of the orange-blue throat coloration (characteristic of the aggressive male morph) relative to sham-operated controls, while testosterone implants increase it. Further work has shown that this sensitivity to androgen effects on morph type has an early critical period which ends sometime between 30 and 60 days posthatch (Hews and Moore, 1996).

Species with alternate mating morphs within a sex provide the same advantage for probing the nature of within- and between-sex differences that was

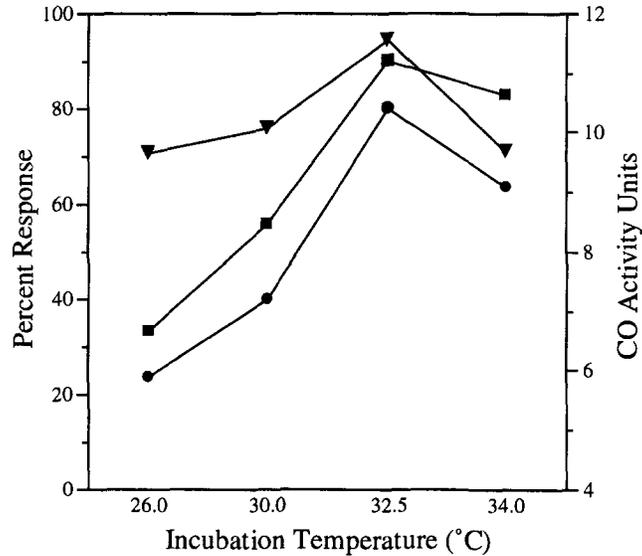


Fig. 10. Level of aggression (circles) in female leopard geckos from different incubation temperatures compared to mean cytochrome oxidase (C.O.) activity units ($\mu\text{mol}/\text{min}/\text{g}$ tissue wet weight) in the external amygdala (AME; squares) and nucleus sphericus (NS; inverted triangles) of females from the same incubation temperature. From Coomber *et al.* (1996).

discussed above for parthenogenetic and TSD species: the ability to examine behavioral variation without the confound of differing gonadal sex (Crews, 1988; Crews and Bull, 1985; Moore, 1991). The relevance of such within-sex differences to between-sex differences arises from the observation that one male morph in these systems is often more similar to females in morphological and/or behavioral characters than to the other male morph. Nothing is known of neural differences between alternate male morphs in reptiles, but several lines of evidence suggest that this will be a promising area of inquiry. First, neural differences between male morphs are suggested by behavioral differences (snakes, lizards) and differing neuroendocrine responses to social interactions (Knapp and Moore, 1995, 1996). Second, striking neural differences occur between alternative male morphs in fish in which the male morphs show behavioral and morphological differences similar to those observed in reptiles (Grober and Bass, 1991; Bass, 1992; Grober *et al.*, 1994; Davis and Fernald, 1990; Francis *et al.*, 1993). Finally, the evidence to date on early hormonal determinants of external male morph characters in garter snakes (Crews, 1985) and tree lizards (Hews and Moore, 1996; Hews *et al.*, 1994) has striking parallels to early organizational effects of steroid hormones on neural sex differences in well-studied rodent models (Goy and McEwen, 1980). This similarity between lizards and rodents suggests that alternate male morphs will provide another useful system in which the neural basis of differences in sexual behavior can be studied.

CONCLUSION

The study of sex differences in the nervous system began 30 years ago, yet the study of these differences in reptiles is just beginning. The findings so far in several reptile models suggest both conservation and diversity of basic mechanisms. It will be important to assess the degree of conservation of mechanisms and reasons for evolutionary lability. One important step in assessing conservation of mechanisms will be studies in turtles and crocodylians. Up to now studies of the reptilian nervous system have concentrated on the squamates, lizards and snakes. Squamates form the most speciose group of reptiles and its members display an amazing diversity of sex determination and differentiation patterns. Squamates are also generally the most suitable group of reptiles for nervous system studies for reasons of body size, husbandry, and relative ease of behavioral observation under laboratory conditions. However, the lineage leading to modern turtles diverged from the lineages giving rise to mammals and other reptiles close to the origin of the amniotes and major differences may have arisen between these groups. Information on crocodylians would be similarly useful since they are the only other living representatives of the lineage which gave rise to dinosaurs and birds but do not show the derived features of female heterogametic sex determination and homeothermy which all living birds share.

The diversity apparent even from the few model species for which information is available suggests that we need a broad approach to sex differences in the nervous system. This need is apparent from the examples in which behavioral differences are not reflected in size differences in nuclei controlling those behaviors in the CNS but are correlated with dissimilarities in the activity of these areas. We have discussed acute metabolic activity, metabolic capacity, and regulation of steroid hormone receptors. These are all promising methods for further exploring the sexual differentiation of function in reptilian nervous systems. For example, are the sex differences in steroid hormone receptor mRNA regulation seen in both rodents and lizards with male heterogametic, genotypic sex determination present in species with female heterogamety, TSD, or alternate male phenotypes? If so, in examples like male leopard geckos from female-biased incubation temperatures or she-male garter snakes, is the pattern of steroid receptor mRNA regulation in the VMH more female-like or male-like? There is also a substantial body of information for mammals and birds regarding sex differences in neuropeptides and other types of neurotransmitters for which there is little corresponding information in reptiles. Such neurochemical sex differences are attractive experimentally since these transmitters can be augmented or blocked using methods which have been successful in elucidating the roles of steroid hormones in behavioral function.

Another contribution emerging from reptile studies concerns the influences which determine nervous system differences within and between the sexes. Our current understanding of these differences centers around organizing actions of gonadal steroids. However, data from reptiles indicate that cues not originating from the gonads, such as incubation temperature, can be important in organizing the brain and behavior. It appears that incubation temperature may act

independently of gonadal sex in leopard geckos, as evidenced by volume differences in the POA and VMH, or in concert with gonadal sex, as suggested by differences in the metabolic capacity of these brain areas across sex and incubation history. Temperature can also be an important organizing and/or activating cue outside of TSD species. Incubation temperature differences produce variation in behavior and other characters in several snake species with genotypic sex determination (Burger, 1989, 1990). In red-sided garter snakes, temperature is the cue which activates mating behavior in males during the spring breeding season (reviewed by Crews, 1990). These temperature effects on the brains of the leopard gecko and snakes suggest the potential utility of reptiles for addressing thermal sensitivity in the mammalian brain. Several thalamic and hypothalamic areas in mammals are sensitive to temperature and the hierarchical organization of the neural mechanisms controlling homeostatic thermoregulation closely parallels that of sexual behavior (Craig *et al.*, 1994; Satinoff, 1983). It is tempting to speculate that this temperature sensitivity in mammals partly reflects a primitive condition that is now largely masked by homeothermy.

Finally, although individuals are separable into discrete classes based on gonadal sex, their sexuality is actually a suite of characters showing continuous variation. This is true of any taxonomic group including mammals and birds but is perhaps more apparent in reptiles with their diversity of sex determination and differentiation patterns. This perspective may better equip us to think about how sexuality evolves since evolutionary forces act on variation among individuals rather than between the sexes.

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