Tracing the Evolution of Brain and Behavior Using Two Related Species of Whiptail Lizards: *Cnemidophorus uniparens* and *Cnemidophorus inornatus*

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Abstract

Cnemidophorus whiptail lizards offer a unique opportunity to study behavioral and neural evolution because unlike most genera, ancestral and descendant species are still extant, and comparisons between species provide a window into correlated changes in biological organization through speciation. This review focuses on the all-female or parthenogenetic species *Cnemidophorus uniparens* (descendant species), which evolved through several hybridization events involving the sexually reproducing species *Cnemidophorus inornatus* (ancestral species). Data compiled over more than 2 decades include behavioral, endocrine, and neural differences between these two related species of whiptail lizards. For example, unlike females of the ancestral species, individuals of the descendant species display male-like mounting behavior (pseudocopulatory behavior) after ovulation. Pseudocopulatory behavior in the parthenogen is triggered by the progesterone surge after ovulation, and the behavioral capacity to respond to progesterone appears to be an ancestral trait that was inherited from *C. inornatus* males through the hybridization events. Interestingly, the regulation of sex steroid hormone receptor mRNA in brain areas critical for the expression of sociosexual behaviors differs between females of the two species and suggests that evolutionary changes in the regulation of gene expression could be a proximate mechanism that underlies the evolution of a novel social behavior in the parthenogen. Finally, because the sexual species is diploid, whereas the parthenogen is triploid, differences between the species could directly assess the effect of ploidy. The behavioral and neuroendocrinological data are pertinent for considering this possibility.

Key Words: dopamine; estrogen receptor; evolution; lizard; progesterone receptor; sexual behavior; steroid hormone

Whiptail Lizards as a Model System for the Study of Behavioral Evolution

Comparative models systems are advantageous to the study of behavior as well as the neural substrates underlying behavior because they lend insight into the evolutionary history of, and the general rules governing, a particular phenotype. For example, traits or characteristics shared by many different taxa are often evolutionarily more ancient and hence more fundamental compared with traits that are unique to a species and evolutionarily more recent and derived (Gould 1977). In addition, comparative systems often include species with novel adaptations or traits that can be considered alternative solutions to evolutionary problems. The study of such systems often requires us to reconsider standard approaches or perspectives to understand and incorporate features of particular species. Reptiles in particular are useful taxa in the study of behavioral evolution because they are the present-day representatives of ancestors of mammals and birds.

We study the neural mechanisms underlying species differences in behavior in two related species of whiptail lizard. Whiptail lizards (genus *Cnemidophorus*) afford a particularly good opportunity to investigate the evolution of social and sexual behaviors and their neural mechanisms because a direct ancestor-descendant phylogeny is present. Approximately one third of extant whiptail lizard species are all-female (parthenogenetic) species that resulted from hybrid unions of sexual species (Wright 1993). For example, the parthenogenetic desert-grasslands whiptail (*Cnemidophorus uniparens*) descended from an initial hybridization event between two sexually reproducing species, the rusty rumped whiptail (*Cnemidophorus burti*) and the little striped whiptail (*Cnemidophorus inornatus*) and a subsequent back-crossing of the diploid parthenoform with *C. inornatus* (Wright 1993; Figure 1). Consequently, two thirds of the triploid genome of the descendant parthenogenetic species is derived from *C. inornatus*, the maternal ancestral species. This ancestor-descendant relationship of these two extant species affords a window into the evolution of behavior and the brain.

Parthenogenetic *Cnemidophorus* lizards also provide a unique system for studying reproductive behavior because many of these species, including *C. uniparens*, display both male- and female-typical sexual behavior. Thus the neural substrates for the display of masculine and feminine reproductive behaviors can be investigated within the same individual. Because females of the ancestral, sexual species naturally display only female-typical sexual behaviors, the

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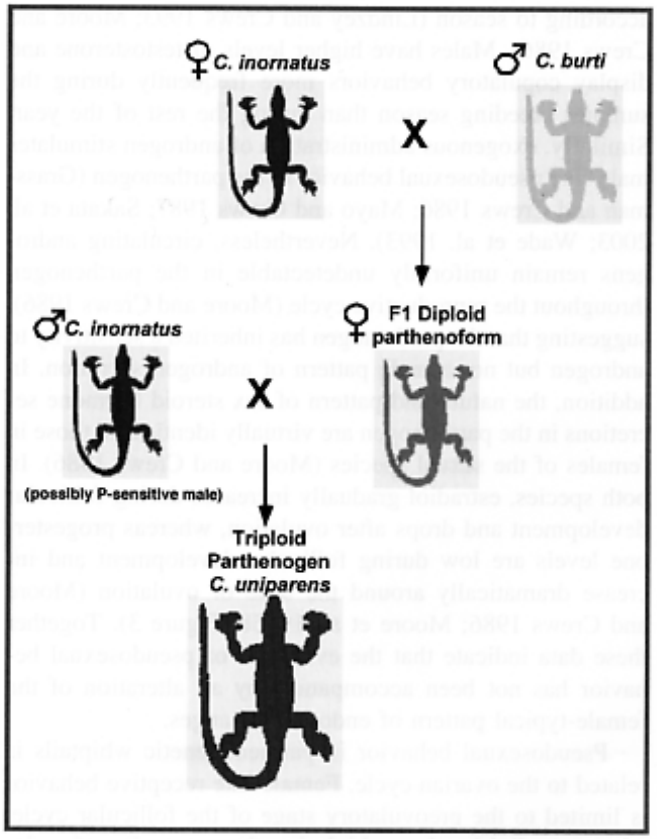

Figure 1 Proposed evolution of the parthenogen, *Cnemidophorus uniparens*. A female *Cnemidophorus inornatus* hybridized with a male *Cnemidophorus burti* to form a diploid parthenoform (F1), which then hybridized with a male *C. inornatus* to form the parthenogen. Because progesterone can reinstate sexual behavior, it is possible that a progesterone (P)-sensitive male was involved in the hybridization process, thereby enabling the display of male-like pseudosexual behavior after ovulation in the parthenogen. Adapted from Crews D. 1998. Evolutionary antecedents to love. Psychoneuroendocrinology 23:751-764.

comparison between the ancestral species and the parthenogen enables us to learn how endocrine and neural systems changed during speciation to allow for the display of male-typical as well as female-typical behaviors.

Finally, because the sexual species is diploid, whereas the parthenogen is triploid, this system provides the opportunity to study the effects of ploidy on phenotype. Moreover, unlike any mammal, the parthenogen naturally reproduces clonally, which makes possible the direct study of environmental influences without the major confound of genetic differences. Recently we have been able to create gonadal males in this naturally all-female species by blocking the synthesis of estrogens during embryonic development (Wennstrom and Crews 1995). Thus we can study sex differences in genetically identical individuals.

Natural History and Husbandry

Cnemidophorus lizards live throughout southwestern North America, Central America, and South America. The two species discussed herein (*C. uniparens* and *C. inornatus*) are found in the Chihuahuan desert and adjacent areas in the southwestern United States and northern Mexico. *C. uniparens* live in northern Mexico, southern Arizona, and New Mexico; and *C. inornatus* are distributed through southwest Texas and New Mexico, with a smaller population in Arizona (Wright 1993).

Adult *C. uniparens* are 60 to 90 mm long from snout to vent, with tails as long as two thirds of their body length. Individuals have six longitudinal light yellow stripes and a pale underside that sometimes has a bluish tint (Figure 2). They inhabit lowland desert and grassland areas, particularly mesquite grasslands and foothill slopes. Adult *C. inornatus* are 50 to 70 mm from snout to vent, and body size and coloration are sexually dimorphic. Both males and females have six longitudinal light yellow stripes, similar to those of *C. uniparens* individuals. Females are smaller and are pale blue or gray, whereas males tend to be larger and have bright blue ventral scales (Figure 2). *C. inornatus* inhabit grassland areas as well as desert shrublands and appear to avoid steep or rocky areas.

Both species are diurnal insectivores that are rarely seen in pairs or groups unless engaged in courtship. To catch these lizards, we use a drift fence technique, which entails driving individuals into a movable, standing net. This approach has been very successful with these agile and fast animals.

In the laboratory, we house individuals either in groups or as isolates in glass aquaria with a 2- to 3-inch layer of

Figure 2 (A) *Cnemidophorus inornatus* male photographed in western Texas. (B) *Cnemidophorus uniparens* individual in a breeding cage in the laboratory at the University of Texas at Austin.

sand at the bottom. Group cages (75 × 32 × 32 cm) accommodate four to five size-matched *C. uniparens,* or two to four females and one male *C. inornatus*. These limits are appropriate for the number of individuals per group cage because more individuals would lead to crowding and deficits in reproductive capacity. Isolate cages contain one individual and measure 25 × 32 × 32 cm. Cage lids are made of wire mesh. All cages are provided with a 60-watt bulb with a reflector to provide a thermal gradient, overhead ultraviolet lights (Vitalite; Duro-Test, Honolulu, HI), and wood blocks for retreat from the light. Water is provided ad libitum, and individuals receive two to three crickets or mealworms three times per week (e.g., Wade and Crews 1991a,b).

During the summer, we maintain individuals on a 14:10 light:dark light cycle, with temperatures that fluctuate from 33°C during the day to 23°C at night. In November, we acclimate all individuals to a photothermal cycle that resembles the conditions that induce hibernation in the field by decreasing photoperiod and temperature on a weekly basis. During hibernation, we keep individuals on an 8:16 light:dark light cycle, with temperatures that fluctuate from 12.5°C during the day and 10°C at night. After the animals have been in hibernation for 10 wk, we increase photoperiod and temperature on a weekly basis until they return to the summer photothermal levels (e.g., Sakata et al. 2002). We maintain humidity at 40 to 50% throughout the year (Wade and Crews 1991b).

Both species reproduce well in our laboratory, and we regularly collect and incubate eggs and raise individuals hatched from eggs. Females and parthenogens produce eggs during the summer, and we add more water to the sand at one end of the cage to provide adequate moisture for egg laying. We palpate the abdomens of the adult females weekly to determine the degree of ovarian development, checking those with eggs in the oviduct daily for oviposition. We collect eggs within 24 hr of oviposition and place each clutch in a 30-mL plastic cup that contains a 1:1 vermiculite:water mixture. We seal the cups with plastic sandwich bags that are secured by a rubber band and place them in an incubator at 28.5°C. At this temperature, the hatchlings emerge in an average of 57 days and mortality rates are low (e.g., Wennstrom and Crews 1995).

We house hatchlings, up to 10 per cage, in 10-gallon aquaria with 3 to 4 cm of sand, wood blocks, and shallow trays with water. We feed the individuals with vestigial-winged fruit flies, which are unable to fly, twice a day for the first few weeks. As individuals increase in size, we feed them small wax worms and 2-wk-old crickets (Wennstrom and Crews 1995; C. Gill and D. Crews, unpublished observations).

Hormonal Regulation of Sexual Behavior in the Sexual and Parthenogenetic Whiptail Lizards

Sexual activity in males of the ancestral species depends on testicular androgens (Lindzey and Crews 1986), which vary according to season (Lindzey and Crews 1993; Moore and Crews 1986). Males have higher levels of testosterone and display copulatory behaviors more frequently during the summer breeding season than during the rest of the year. Similarly, exogenous administration of androgen stimulates male-like pseudosexual behavior in the parthenogen (Grassman and Crews 1986; Mayo and Crews 1987; Sakata et al. 2003; Wade et al. 1993). Nevertheless, circulating androgens remain uniformly undetectable in the parthenogen throughout the reproductive cycle (Moore and Crews 1986), suggesting that the parthenogen has inherited a sensitivity to androgen but not a male pattern of androgen secretion. In addition, the nature and pattern of sex steroid hormone secretions in the parthenogen are virtually identical to those in females of the sexual species (Moore and Crews 1986). In both species, estradiol gradually increases during follicular development and drops after ovulation, whereas progesterone levels are low during follicular development and increase dramatically around the time of ovulation (Moore and Crews 1986; Moore et al. 1985b; Figure 3). Together these data indicate that the evolution of pseudosexual behavior has not been accompanied by an alteration of the female-typical pattern of endocrine changes.

Pseudosexual behavior in parthenogenetic whiptails is related to the ovarian cycle. Female-like receptive behavior is limited to the preovulatory stage of the follicular cycle, when estradiol levels are high; but the expression of male-

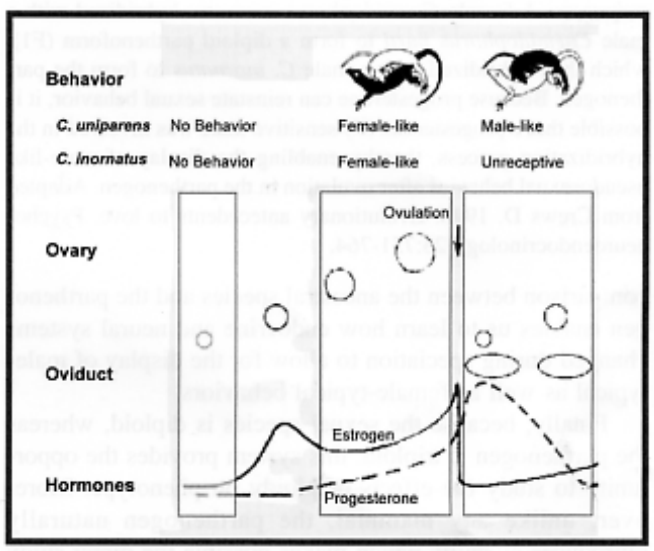

Figure 3 Changes in sex steroid hormones and behavior across the reproductive cycle in the parthenogen and female *Cnemidophorus inornatus*. During follicular development, when estradiol is the dominant hormone, both species display female-like receptive behaviors, whereas after ovulation, when progesterone is the dominant hormone, female *C. inornatus* display unreceptive behavior and the parthenogen displays male-like mounting behavior. Adapted from Crews D. 1989. Unisexual organisms as model systems for research in the behavioral neurosciences. In: Dawley RM, Bogart JP, eds. Evolution and Ecology of Unisexual Vertebrates. New York: New York State Museum. p 132-143.

like mounting behavior occurs most frequently during the postovulatory stages of the cycle, when progesterone levels are highest (Moore et al. 1985a). The transition from female- to male-like pseudosexual behavior occurs at ovulation, when there is a parallel transition from estradiol dominance to progesterone dominance. The time of this occurrence suggests that changes in hormone levels could underlie the changes in behavior. Exogenous administration of progesterone to ovariectomized animals elicits pseudosexual behavior, whereas estradiol elicits female-typical receptive behavior (Grassman and Crews 1986). Thus it appears that the postovulatory surge in progesterone has been exploited as the hormonal cue, which triggers male-like pseudosexual behaviors in the parthenogen.

The display of pseudosexual behavior in the parthenogen appears to be functionally significant. Whereas individuals housed in isolation ovulate eventually, those housed with other reproductively active individuals ovulate sooner. Likewise, intact parthenogens housed with a hormonally stimulated individual displaying only male-like behaviors ovulate more frequently and produce an average of 2.5 clutches per season, whereas isolated individuals produce an average of only 0.8 clutches per season (Crews et al. 1986). This disparity is analogous to the fact that females of the sexual species housed in isolation ovulate considerably less frequently than those housed with sexually active males (Crews et al. 1986). Thus the display of male-like pseudosexual behavior in the parthenogen is evolutionarily relevant.

Whereas progesterone levels are uniformly low in *C. inornatus* males (Lindzey and Crews 1986), copulatory behavior is sensitive to progesterone in approximately two thirds of sexually active males. For example, sexual behavior in castrated males can be reinstated with androgen and, in a subset of males, with progesterone; such individuals are referred to as progesterone-sensitive males (Lindzey and Crews 1988, 1992, 1993). These individual differences in progesterone sensitivity appear to be consistent across repeated administrations of progesterone (Lindzey and Crews 1988; J. T. Sakata and D. Crews, unpublished data) and are correlated with individual differences in androgen sensitivity (Lindzey and Crews 1992). The decline of sexual behavior late in the reproductive season can also be delayed with progesterone administration (Lindzey and Crews 1986). With regard to the evolution of pseudosexual behavior in the parthenogen, it is possible that a progesterone-sensitive male was involved in the hybridization events leading to the formation of *C. uniparens* (Figure 1). Thus the sensitivity of male sexual behavior to progesterone may have been co-opted in the parthenogen, enabling progesterone to become the primary hormonal trigger inducing male-like pseudosexual behavior (Crews 1989).

In light of these findings, we tested the generality of the facilitatory role of progesterone across other species such as rats and mice. Whereas previous studies highlighted the inhibitory role of a supraphysiological dose of progesterone (e.g., Erickson et al. 1967; Erpino 1973; Morin 1977), we found that physiological doses of progesterone can facilitate the expression of mounting behavior in male rats (Witt et al. 1995). Moreover, work on progesterone receptor knockout mice indicates that progesterone stimulation is important not only in the display of mounting behavior while intact but also in experience-dependent plasticity (Phelps et al. 1998). The latter finding is interesting because preliminary studies have found differences in experience-dependent plasticity between progesterone-sensitive and -insensitive male *C. inornatus* (J. T. Sakata and D. Crews, unpublished data). Thus studies originally performed on a parthenogenetic species eventually led to a re-evaluation and new understanding of the role of progesterone in the display of copulatory behavior in male vertebrates.

Although both testosterone and progesterone can facilitate the display of male sexual behavior in a number of species, their relative potencies have not been compared. Whiptail lizards are a useful model in which to study the effects of testosterone and progesterone because both hormones elicit the full repertoire of courtship behavior in both *C. inornatus* males and *C. uniparens* individuals. We recently assessed differences in the capacity of exogenous testosterone and progesterone to induce male-typical courtship behavior in gonadectomized whiptail lizards (Sakata et al. 2003). In both species, individuals implanted with testosterone showed more frequent courtship behavior than those implanted with progesterone or cholesterol.

We also examined whether testosterone and progesterone differentially affected the retention of courtship behavior after implant removal. We administered behavior tests to the animals after they had received hormone implants and until all individuals were displaying similar levels of behavior. We then removed the implants and administered additional behavior tests: 10 to *C. inornatus,* or 20 to *C. uniparens*. In both species, individuals previously implanted with testosterone retained the expression of courtship behavior longer after implant removal than those previously given progesterone. Therefore the hormone that was more effective at activating courtship behavior was also more effective at maintaining courtship behavior after implant removal. Although both hormones have the capacity to elicit identical sexual behaviors in both species, testosterone has a greater and more lasting effect on courtship behavior and possibly on the neural circuits underlying courtship behavior.

Neural Correlates of Evolutionary Changes in Behavior

Studies of sexual behavior in mammals have traditionally focused on nuclei within the preoptic area and hypothalamus. We know that across species, the medial preoptic area is critical to the regulation of male sexual behavior, and the

ventromedial hypothalamus (VMH[1]) is involved in the display of female receptive behaviors. Studies on whiptail lizards have indicated that these brain areas are also involved in the regulation of sexual behavior in *C. inornatus* and *C. uniparens*. Intracranial administration of androgens into the anterior hypothalamus-medial preoptic area (AH/POA[1]) of male *C. inornatus* and *C. uniparens* induces the display of male-like copulatory behaviors in gonadectomized animals (Mayo and Crews 1987; Rozendaal and Crews 1989), and lesions of these nuclei impair the display of male-like copulatory behaviors in gonadally intact individuals (Kingston and Crews 1994). Similarly, implantation of estradiol into the VMH increases the display of receptive behaviors in *C. uniparens* individuals and *C. inornatus* females (Wade and Crews 1991b), and lesions of the VMH abolish the display of receptive behaviors (Kendrick et al. 1995).

[1]Abbreviations used in this article: AH/POA, anterior hypothalamus/preoptic area; AR, androgen receptor; ER, estrogen receptor; PR, progesterone receptor; PvPOA, periventricular preoptic area; VMH, ventromedial hypothalamus.

Species Differences in Steroid Hormone Receptor Expression

There is considerable evolutionary conservation in the distribution of steroid hormone receptors across species. Using polymerase chain reaction, we cloned fragments of the progesterone receptor (PR[1]), androgen receptor (AR[1]), and estrogen receptor (ER[1]) genes of whiptail lizards; we used these clones to synthesize probes for use in in situ hybridization assays (Young et al. 1994). The neuroanatomical distribution of these receptors in the brains of parthenogenetic and sexual whiptail lizards are similar to each other and, moreover, to the distribution in other species, with receptor-containing cells concentrated in septal, amygdaloid, cortical, preoptic, and hypothalamic nuclei (Young et al. 1994; Figure 4). Yet both species and sex differences exist in the regulation and amount of receptor expression.

As in females of other species, circulating concentrations of gonadal steroid hormones and reproductive behavior vary as a function of ovarian state in whiptail lizards. Although the pattern of circulation of steroid hormones is similar between *C. inornatus* females and *C. uniparens* individuals (Moore et al. 1985b), estradiol concentrations in

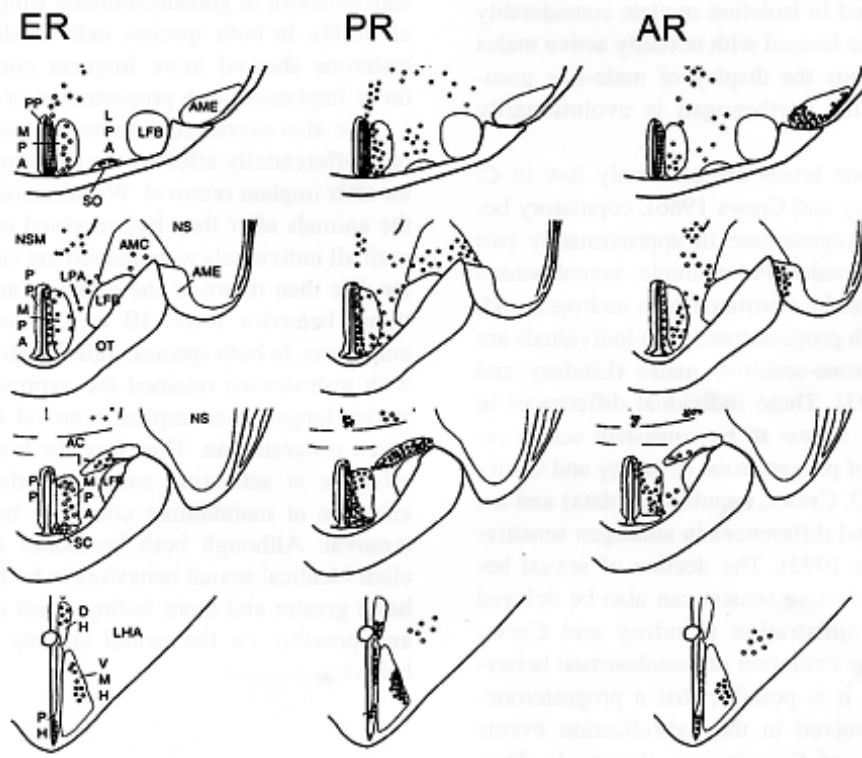

Figure 4 Distributions of estrogen receptor (ER), progesterone receptor (PR), and androgen receptor (AR) mRNA in *Cnemidophorus uniparens* and *Cnemidophorus inornatus*. Open circles indicate low levels of expression; closed circles indicate high levels of expression. AC, anterior commisure; AMC, nucleus centralis amygdalae; AME, nucleus externus amygdalae; DH, nucleus dorsalis hypothalami; LFB, lateral forebrain bundle; LHA, lateral hypothalamic area; LPA, lateral preoptic area; MPA, medial preoptic area; NS, nucleus sphericus; NSM, nucleus septalis medialis; OT, optic tract; PH, nucleus periventricularis hypothalami; PP, nucleus periventricularis preopticus; SC, nucleus suprachiasmaticus; SO, nucleus supraopticus; VMH, nucleus ventromedialis hypothalami. Adapted from Young LJ, Lopreato GF, Horan K, Crews D. 1994. Cloning and in situ hybridization analysis of estrogen receptor, progesterone receptor, and androgen receptor expression in the brain of whiptail lizards (*Cnemidophorus uniparens* and *C. inornatus*). J Comp Neurol 247:288-300.

the parthenogen are approximately five-fold lower than in female sexual whiptails. Yet the display of receptive behaviors does not differ between the species (Young et al. 1995a). Consistent with the difference in circulating concentrations of estradiol is the finding that receptive behavior is elicited at lower estradiol concentrations in ovariectomized parthenogens than in ovariectomized female *C. inornatus* (Young et al. 1995a). Because steroid hormone concentration in the periphery are governed by negative feedback, we postulate that the lower estradiol concentrations in the parthenogen could be due to heightened sensitivity to estradiol and increased negative feedback.

The regulation of ER and PR mRNA expression across the reproductive cycle varies between species and brain areas (Young et al. 1995b). In many brain areas, the parthenogen expresses higher levels of hormone receptor mRNA, an effect that is consistent with species differences in ploidy (Neaves and Gerald 1968); but the magnitude of the difference fluctuates with reproductive state, indicating that there might be additional differences between the species governing the expression of steroid hormone receptors. For example, the parthenogen expresses much higher levels of ER mRNA than *C. inornatus* females after ovulation in the periventricular preoptic area (PvPOA[1]), but in individuals with yolking follicles (vitellogenic individuals), the difference is relatively small. In the VMH, the level of ER mRNA in the parthenogen is lower in postovulatory individuals than in vitellogenic individuals, but this difference does not exist in females of the sexual species (Young et al. 1995b).

Species differences in neural response to exogenous estradiol administration (0.5 μg) also exist in ovariectomized

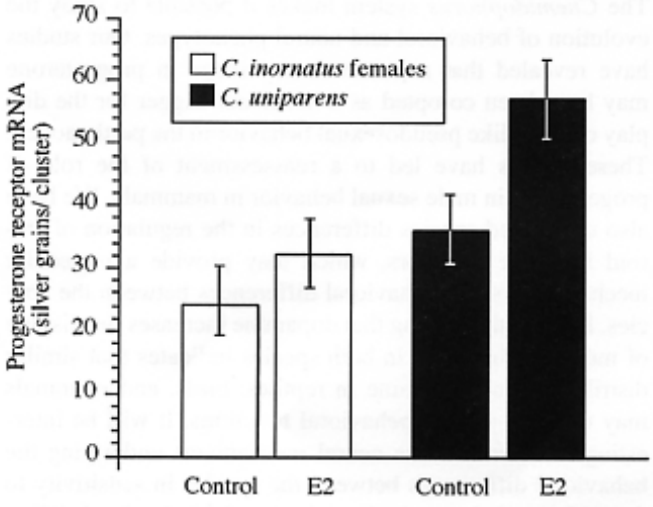

Figure 5 Species differences in the induction of progesterone receptor mRNA in the periventricular preoptic area. Estradiol (E2) significantly increases PR mRNA only in the parthenogen in ovariectomized animals. Adapted from Godwin J, Crews D. 1999. Hormonal regulation of progesterone receptor mRNA expression in the hypothalamus of whiptail lizards: Regional and species differences. J Neurobiol 39:287-293.

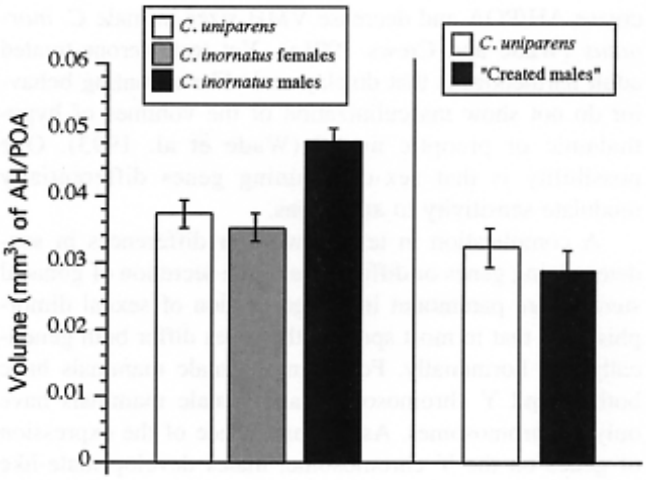

Figure 6 Species differences in sexual dimorphisms in the anterior hypothalamus/preoptic area (AH/POA) continuum. Male *Cnemidophorus inornatus* have larger AH/POA than female *C. inornatus*, but the size of the parthenogen does not appear to be different in "created males" and in females (see text). Adapted with data from Crews et al. 1990; Wade and Crews 1991a; Wennstrom et al. 1999.

individuals. For example, estradiol increases the abundance of ER mRNA in the VMH in females of the sexual species and the parthenogen, but the magnitude of the increase is greater in the parthenogen (Godwin and Crews 1995; Young et al. 1995a). Because of the evolutionarily conserved role of the VMH in the expression of female-like receptive behaviors, this species difference in ER mRNA expression may account for the increased sensitivity to estradiol in the parthenogen. Furthermore, estradiol treatment increases PR mRNA expression in the PvPOA of the parthenogen, but not in females of the sexual species (Godwin and Crews 1999; Figure 5). This finding suggests a possible proximate mechanism underlying species differences in behavior. In other words, estradiol during vitellogenesis increases PR expression in the PvPOA of the parthenogen, thereby sensitizing them to the postovulatory progesterone surge and potentially priming the display of male-like pseudosexual behavior. In females of the ancestral species, estradiol does not upregulate PR in the PvPOA during vitellogenesis, and *C. inornatus* females do not display male-like pseudosexual behavior in response to the surge of progesterone after ovulation.

Species Differences in Neural Sexual Dimorphisms

In *C. inornatus*, the volumes of the AH/POA continuum and the VMH are sexually dimorphic (Crews et al. 1990; Wade and Crews 1991a). The AH/POA is larger in males, whereas the VMH is larger in females (Figure 6). Parthenogenetic individuals have AH/POA and VMH volumes similar to females of the sexual species (Crews et al. 1990). Moreover, androgen increases associated with reproductive activity in-

crease AH/POA and decrease VMH sizes in male *C. inornatus* (Wade and Crews 1991a). Yet testosterone-treated adult parthenogens that display male-like mounting behavior do not show masculinization of the volumes of hypothalamic or preoptic nuclei (Wade et al. 1993). One possibility is that sex-determining genes differentially modulate sensitivity to androgens.

A complication in testing whether differences in sex-determining genes or differences in the secretion of gonadal steroids are paramount in the generation of sexual dimorphisms is that in most species, the sexes differ both genetically and hormonally. For example, male mammals have both X and Y chromosomes, and female mammals have only X chromosomes. As a consequence of the expression of genes on the Y chromosome, males develop male-like gonadal morphology as well as masculine patterns of hormone secretion. Thus the effects of sex-linked genes are intertwined with the effects of sex-linked patterns of hormone secretion.

An ideal system for the study of sexual dimorphisms would enable the dissociation of genetic and hormonal factors. Parthenogenetic lizards offer a tremendous opportunity to study this question. Estrogen is necessary during development in *C. uniparens* for the generation of female-like gonadal morphology. Treatment of *C. uniparens* eggs with an aromatase inhibitor during development prevents the conversion of testosterone to estrogen and results in the creation of males in the unisexual species. To produce males in the parthenogenetic species, we treat eggs on day five of incubation by pipetting 20 μg of the aromatase inhibitor fadrozole dissolved in 1μl of ethanol directly onto the eggshell (Wennstrom and Crews 1995; Wibbels and Crews 1994). "Created" males are genetically identical to parthenogenetic individuals, but they have fully developed male genitalia and motile sperm, and they display only male-like mounting behaviors. Interestingly, although created males exhibit male-like genitalia and behavior, the volumes of the AH/POA and VMH are not significantly different between created males and normal parthenogens (Wennstrom et al. 1999; Figure 6). In other words, created males have female-like AH-POA and VMH volumes. Thus because created males have a female genotype, it is possible that the capacity for androgens to alter neuromorphology and engender sex differences is linked to male-determining genes.

We have also noted sex differences in the regulation of ER and PR mRNA in limbic brain nuclei in the ancestral sexual species (Godwin and Crews 1995). For example, exogenous estradiol treatment of gonadectomized individuals increases ER and PR mRNA in the VMH of females but not of males. This difference in receptor regulation is paralleled by behavioral differences in response to estradiol because only females show receptive behavior after estradiol administration. Estradiol treatment also increases ER mRNA expression in the dorsal hypothalamus in females but not in males. Whether similar differences in regulation exist between created males and normal parthenogens has yet to be investigated.

Species Differences in the Modulation of Male Mounting Behavior by Catecholamines

Studies have revealed that catecholamines modulate the display of social and sexual behaviors in mammals and birds (Absil et al. 1994; Balthazart et al. 1997; Melis and Argiolas 1995). In addition, there is considerable homology in the expression of catecholamine synthesizing enzymes across taxa, including mammals, birds, reptiles, amphibians, and fish (Smeets 1994; Smeets and Gonzalez 2000). Nevertheless, whether the similar catecholamine populations found in reptiles underlie functions that are similar in birds and mammals is relatively unknown.

To determine whether dopamine modulates the display of courtship behavior in lizards, we tested a range of doses of a specific dopamine D1 receptor agonist on the display of mounting behavior in gonadectomized *C. inornatus* males and *C. uniparens* individuals (Woolley et al. 2001). In both species, the D1 agonist increased the proportion of individuals displaying mounting behavior, and it decreased the latency for individuals to display mounting behavior. Interestingly, the dose that was most effective differed between the species. *C. inornatus* males required a 10-fold higher dose of the agonist to elicit the same level of behavior as *C. uniparens* individuals. We hypothesize that this difference could be modulated by species differences in gonadal steroid exposure, sex determining genes, and/or ploidy. For example, it is plausible that the parthenogen has elevated levels of D1 receptor expression because it is triploid.

Conclusion

The *Cnemidophorus* system makes it possible to study the evolution of behavioral and neural phenotypes. Our studies have revealed that a postovulatory surge in progesterone may have been co-opted as a hormonal trigger for the display of male-like pseudosexual behavior in the parthenogen. These studies have led to a reassessment of the role of progesterone in male sexual behavior in mammals. We have also uncovered species differences in the regulation of steroid hormone receptors, which may provide a molecular mechanism for the behavioral differences between the species. Finally, the finding that dopamine increases the display of mounting behavior in both species indicates that similar distributions of dopamine in reptiles, birds, and mammals may underlie similar behavioral functions. It will be interesting to determine the neural mechanisms underlying the behavioral differences between the species in sensitivity to dopamine and, in particular, whether the behavioral differences are correlated with species differences in the regulation of dopamine receptor expression.

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