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Effect of Hormonal Manipulation on Sociosexual Behavior in Adult Female Leopard Geckos (*Eublepharis macularius*), a Species with Temperature-Dependent Sex Determination

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Aggressive and sexual behavior in the adult leopard gecko (*Eublepharis macularius*), a species with temperature-dependent sex determination (TSD), is influenced by the temperature experienced as an egg, as well as by prenatal and perinatal hormones. This study focused on the effects of hormonal manipulation of adult female leopard geckos from different incubation temperatures. Following ovariectomy, females from both all-female (26°C) and male-biased (32.5°C) incubation temperatures exhibited a significant decrease in high-posture (HP) aggression toward male and female stimulus animals. Testosterone treatment attenuated this decrease in HP aggression toward female but not toward male stimulus animals. Ovariectomy also resulted in a loss in attractiveness in both groups of females. Following treatment with testosterone, over 50% of the females were attacked by male stimulus animals, suggesting a change in the pheromonal cues normally secreted by females. Unmanipulated females never exhibit tail vibrations, a male-typical courtship behavior. However, following ovariectomy with testosterone treatment, half of the females from both incubation temperatures exhibited this behavior, indicating an activational effect of testosterone. An effect of incubation temperature on aggression was evident with females from the male-biased incubation temperature exhibiting a greater likelihood of aggression compared to females from the all-female incubation temperature. This effect continued to be detected after hormone manipulation. Ovariectomized females from the all-female incubation temperature were less aggressive even with testosterone treatment toward males, whereas females from the male-biased incubation temperature showed no significant decline in aggression following testosterone treatment, suggesting that individuals from different incubation temperatures may have different sensitivities to hormones.

Sexually dimorphic behaviors are known to be modulated by steroid hormones, such as androgens and estrogens. In mammals, hormones from the mother, from the gonads of the fetus itself, or even from the neighboring fetuses can profoundly influence an individual's physiology and behavior as an adult. However, the extent to which these features can be separated from

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the genetic sex of the individual is problematic. Gonadal sex in many vertebrates is determined at fertilization by specific chromosomes inherited from the parents, a process known as genotypic sex determination (GSD). We now know that in mammals, a gene on the Y chromosome, designated SRY, channels gonadal development to result in the formation of testes and a male-typical phenotype. Because males and females differ genetically, the relative contribution of the embryonic environment cannot be distinguished easily from the genetic environment.

Many reptiles do not have heteromorphic sex chromosomes. Instead, gonadal sex is determined after fertilization by the incubation temperature of the egg, a process known as temperature-dependent sex determination (TSD) (reviewed by Bull, 1980; Raynaud and Pieau, 1985; Ewert and Nelson, 1991; Janzen and Paukstis, 1991; Crews, Bergeron, Flores, Bull, Skipper, Tousignant, and Wibbels, 1994). Reptiles with TSD exhibit various relationships between temperature and sex ratio. Low temperatures produce females and high temperatures produce males in lizards and the alligator, whereas this pattern is reversed in most turtles. A mixture of these patterns is evident in the leopard gecko, the snapping turtle, and in crocodiles wherein extreme incubation temperatures produce females and intermediate incubation temperatures produce varying ratios of males and females. Thus, in a species with TSD, males and females do not differ genetically, making it possible to study the effects of the embryonic environment without the effects of a sex-specific genetic environment.

In a previous study, we found that the behavior exhibited by adult leopard geckos was influenced by the incubation temperature of the egg as well as by prenatal and perinatal hormone manipulation (Flores, Tousignant, and Crews, 1994). The purpose of the present experiment was to determine the effects of adult hormone manipulation on aggressive and courtship behavior in females from an all-female incubation temperature compared to females from a male-biased incubation temperature.

METHODS

Animals

All animals were hatched from eggs obtained from controlled matings in the laboratory. Eggs were incubated individually in covered plastic cups containing moist vermiculite (1.5:1, water:vermiculite) at a constant temperature (±0.1°C) of 26°C ($n = 8$), which produces only female hatchlings, or 32.5°C ($n = 10$), which produces a male-biased (75:25) sex ratio (Viets, Tousignant, Ewert, Nelson, and Crews, 1993).

Housing and Maintenance

Newly hatched geckos were housed individually in a 12 × 30 × 6 cm plastic shoebox. The box contained a water dish and a plastic shelter. After

65 weeks, each animal was transferred into a larger polycarbonate cage measuring 43 × 22 × 20 cm containing a water dish, a large plastic shelter, and a brick. A 60-W incandescent light positioned directly above the individual cages cycled on and off 1 hr after and 1 hr before the fluorescent lights. This allowed for individual thermoregulation.

During the first 10 weeks of age, individuals were exposed to a controlled environment (14:10 LD photic cycle, 30°C constant temperature). From 10 weeks of age, individuals were exposed to the same photic cycle and a corresponding 30:18°C daily thermal cycle. This variable thermal cycle reflects natural conditions, but the cooler night temperature decreases survival of young animals.

Hatchling leopard geckos were maintained on live crickets and mealworms. The diet of juvenile (>10 weeks) and adult geckos was supplemented with neonatal mice once a week. Animals were fed three times a week and water was available ad libitum. The food was supplemented with vitamin-mineral powder (Petco Animal Supplies) and calcium diphosphate (Texas Gulf Supplies).

Ovariectomy and Hormone Manipulation

Animals from both incubation temperatures were gonadectomized as adults (after 65 weeks of age). One-half of the individuals from each incubation temperature were immediately implanted with either testosterone (TESTO) (Sigma) or cholesterol (CHOL) (Sigma) filled Silastic pellets (20 × 1.47 × 1.95 mm) (Dow Corning). This size capsule results in circulating concentrations of testosterone within the physiological limits observed in intact, sexually active male leopard geckos (Tousignant and Crews, 1995). Animals were allowed to recover for 3.5 weeks after implantation before behavior testing. After testing, the animals' implants were removed and the steroid treatment reversed. The counterbalanced administration of either TESTO or CHOL controlled for a treatment effect and, at the same time, allowed comparisons of the extent of masculinization and defeminization within the same individual (Fig. 1). Further, the sequential design allowed the individuals to serve as their own control. Surgery was performed using hypothermia anesthesia.

Radioimmunoassay

Each animal was bled by cardiocentesis at 1800 hr (±1 hr) 1 day before each of the four phases of behavior testing. Approximately 250 μl of whole blood was collected using a heparinized 25-gauge needle and 10-cc syringe. Blood samples were then transferred to microhematocrit tubes and centrifuged at 2000 rpm for 12 min at 10°C. The plasma was stored in plastic microcentrifuge tubes at −20°C until radioimmunoassay analysis (Tousignant, Viets, Flores, and Crews, 1995). Hormone values were corrected for individual recoveries, which averaged 90% for androgens and 87% for estrogen. The level of detectability was established as two standard deviations below the

binding observed in blank assay tubes. Samples were run in duplicate sets. Mean intraassay variation and interassay coefficient of variation were as follows: total androgens (13.5, 12.5) and estrogens (9.6, 8.0).

Behavior Testing

All animals were tested on 24 occasions over a 4-month period for heterotypical sexual behavior. Aggressive behaviors were also quantified. Animals were tested with a female stimulus animal from the same incubation temperature and a male from the 32.5°C incubation temperature on each test day. All stimulus animals were in breeding condition and of approximately the same age. The phases included (a) Phase 1 (pretesting)—before ovariectomy and implantation, (b) Phase 2—after ovariectomy and immediate implantation, (c) Phase 3—after removal of first implant and simultaneous exchange with second implant, and (d) Phase 4 (post-testing)—after removal of implant and recovery (Fig. 1). The time interval between Phases 1 and 2 and between Phases 2 and 3 was 3.5 weeks. The time interval between Phases 3 and 4 was 2 weeks.

The female stimulus animal was always presented first. This sequence of presentation was necessary as male stimulus animals tend to be aggressive and could attack the experimental female, affecting her subsequent behavior. Each animal was tested in three trials with two different types of stimulus animals (male or female) for a total of six tests in each phase.

Each test consisted of observing a subject/stimulus pair for 5 min following the introduction of the stimulus animal into the experimental animal's home cage. There was at least a 1-hr time delay between presentation of the male and female stimulus animals. In addition, the paper lining the test cage was replaced so that pheromone exchange between stimulus animals would be minimized.

Individuals were considered to exhibit high-intensity aggression if they responded with a high posture (score of +1 or more) on two or more of the three tests or if they attacked (+2) the stimulus animals on any trial. They were considered to exhibit heterotypical sexual behavior if they displayed a tail vibration (+3) or any other courtship behaviors on at least one out of the three trials in any given phase (see Flores *et al.*, 1994, for details). Tests were terminated at the end of 5 min or if an attack or attempted copulation occurred. Tests were performed during the late afternoon coinciding with the onset of daily activity cycles as observed in communal breeding cages.

Behavioral Measures

The following measures of aggression and courtship displayed by the experimental animal toward the male and female stimulus animals were recorded using an event recorder program for a Macintosh computer (Witt/Timer Program courtesy of Diane Witt, NIH, Bethesda, MD): the frequency of attacks, high postures, tail vibrations, and tail grip/headshakes. The following mea-

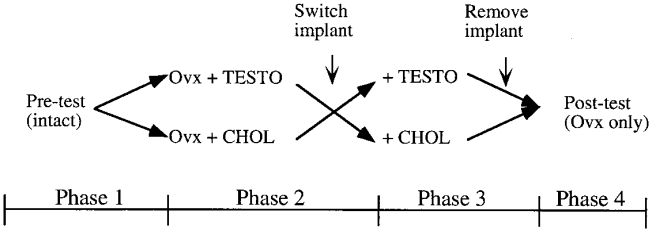

FIG. 1. Schematic of the experimental protocol used with a sequential, counterbalanced administration of either testosterone or cholesterol, controlling for treatment effect. Ovx, ovariectomy; TESTO, testosterone implant; CHOL, cholesterol implant; NT, no treatment (no implant).

sures of aggressive and courtship displayed by male stimulus animals directed toward the experimental animals were also recorded: the frequency of attacks, high postures, licks, tail vibrations, tail grip/headshakes, neckgrips, and the position for copulation.

Statistics

Statistical analysis consisted of the likelihood ratio χ^2 test and the two-tailed Fisher's exact test for nonparametric behavioral data. Following tests for heterogeneity of variance, hormone values were log transformed and two-way analysis of variance (ANOVA) and Tukey post hoc tests (Systat program for Macintosh) were used to analyze hormone measures. Prior to pairwise comparisons, overall treatment effects were analyzed.

RESULTS

To determine if Phases 2 and 3 could be combined (Fig. 1), the behavioral scores for high-posture (HP) aggression, attack, attractivity, and heterotypical sexual behavior were analyzed for order effects using Fisher's exact test for differences. There were no statistically significant differences for the order of administration of TESTO or CHOL. Therefore the data for Phases 2 and 3 were combined. Results are summarized as follows: (i) the overall effects of hormone manipulation (not including incubation temperature effects) on aggressiveness, attractiveness, and the display of heterotypical sexual behavior, (ii) the effects of incubation temperature on aggressive behavior, and (iii) hormone analysis.

Effect of Ovariectomy and Hormone Treatment

Aggression. The likelihood of HP aggression exhibited toward a male or a female stimulus animal differed significantly as a function of hormone treatment. There was a significant decrease in the likelihood of HP aggression toward both male ($P = 0.01$; χ^2 test) and female ($P = 0.01$) stimulus animals

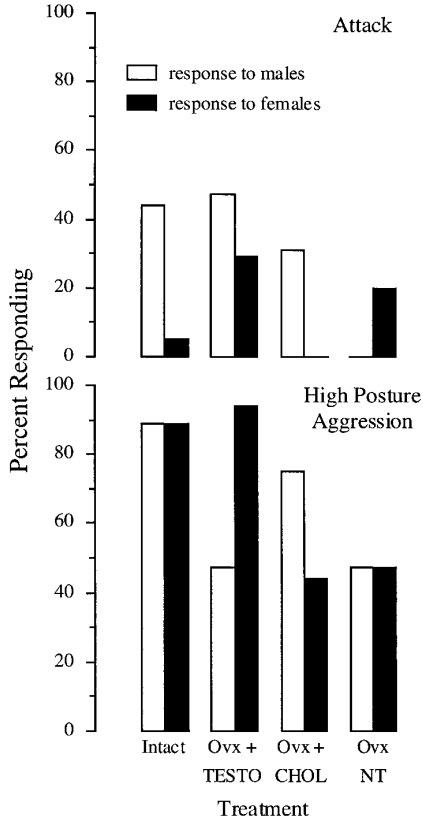

FIG. 2. Hormone-induced aggressive behavior in the female leopard gecko toward male and female stimulus animals (combined 26°C and 32.5°C female responses). Abbreviations as in Fig. 1.

after ovariectomy (Fig. 2, bottom). The decrease in HP aggression toward male stimulus animals was maintained in TESTO-treated females ($P = 0.009$) such that there was no significant difference from ovariectomized females with no treatment ($P = 0.63$). Treatment with TESTO attenuated this decrease in HP aggression (TESTO vs CHOL, $P = 0.002$). In contrast, HP aggression toward female stimulus animals was comparable in TESTO-treated females and intact females ($P = 0.52$) and significantly greater in TESTO-treated females than in ovariectomized females with no treatment or ovariectomized and treated with CHOL ($P = 0.01$, 0.006, respectively).

The likelihood of offensive attacks exhibited toward male and female stimulus animals differed significantly as a function of hormone manipulation (Fig. 2, top). Following ovariectomy, females displayed a significant decrease in this behavior toward males ($P = 0.003$), but not toward females ($P = 0.23$).

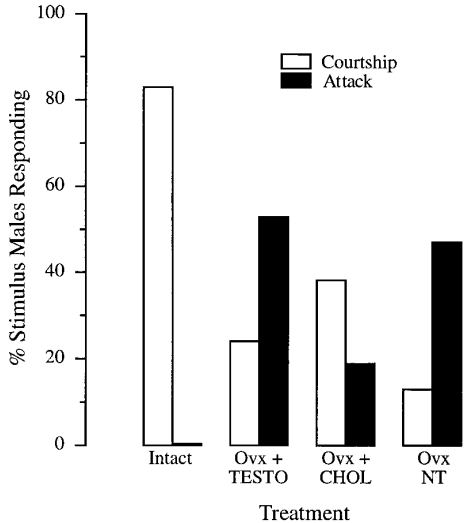

FIG. 3. Attractiveness of female leopard geckos as perceived by male stimulus animals before and after hormonal manipulation. Abbreviations as in Fig. 1.

In contrast, there were no differences in offensive attacks before and after ovariectomy with CHOL treatment toward both male and females stimulus animals ($P = 0.33$, 0.52, respectively). Also, females did not attack males more frequently following treatment with TESTO ($P = 0.57$), but there was a trend ($P = 0.07$) in the likelihood of offensive attacks directed toward female stimulus animals.

Attractiveness. Attractiveness of females differed significantly as a function of hormone manipulation. There was a significant decrease in the likelihood of a tail vibration (TV) exhibited by male stimulus animals toward females following ovariectomy ($P = 0.001$) (Fig. 3). Males were also less likely to exhibit TV to females treated with CHOL ($P = 0.007$) or with TESTO treatment ($P = 0.001$). Although TESTO treatment resulted in a lower percentage of males exhibiting TV (24%) compared with CHOL treatment (37%), the difference was not statistically significant ($P = 0.31$).

The likelihood that a male stimulus animal would attack females differed significantly as a function of hormone manipulation. Male stimulus animals never attacked unmanipulated females. There was a significant increase in the number of male stimulus animals attacking females following ovariectomy (47%) ($P = 0.001$ Fisher's exact test), TESTO treatment (53%) ($P = 0.001$), but not CHOL treatment (19%) ($P = 0.09$) (Fig. 3). Also, there was a significant decrease in the likelihood of an attack by a stimulus male animal toward a female with CHOL treatment compared to a female with TESTO treatment ($P = 0.04$).

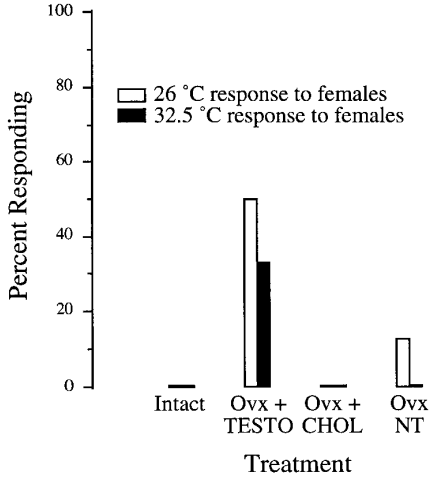

FIG. 4. Effects of exogenous testosterone on the display of heterotypical sexual behavior, specifically the display of tail vibrations, by female leopard geckos toward male and female stimulus animals. Abbreviations as in Fig. 1.

Heterotypical Sexual Behavior. Unmanipulated females never exhibited tail vibration when presented with a female stimulus animal. However, following hormone manipulation, ovariectomized females began to exhibit TV (Fig. 4). Half (4/8) of the females from the all-female incubation temperature and one-third (3/9) of the females from the male-biased incubation temperature exhibited TV (combined $P = 0.002$) following ovariectomy with TESTO treatment.

Effect of Incubation Temperature

Ovariectomy and treatment with TESTO resulted in fewer females from the all-female (26°C) incubation temperature exhibiting HP aggression toward male stimulus animals (6/8 while intact vs 1/8 following treatment) ($P = 0.02$) (Fig. 5, bottom). However, all (8/8) exhibited HP aggression toward female stimulus animals. All of the females from the male-biased (32.5°C) incubation temperature exhibited HP aggression toward male stimulus animals while intact, and 7/9 of the females continued to display HP aggression following ovariectomy with TESTO treatment ($P = 0.21$). Ovariectomy with no hormone treatment resulted in a significant decrease in the number of individuals exhibiting this behavior toward male and female stimulus animals (4/7 in each case) ($P = 0.05$). Following ovariectomy with CHOL treatment, the likelihood of HP aggression exhibited by females from both incubation temperatures toward male stimulus animals was comparable to that before manipulation ($P = 0.71, 0.18$, respectively). However, HP aggression toward female stimulus animals significantly decreased in females from the male-

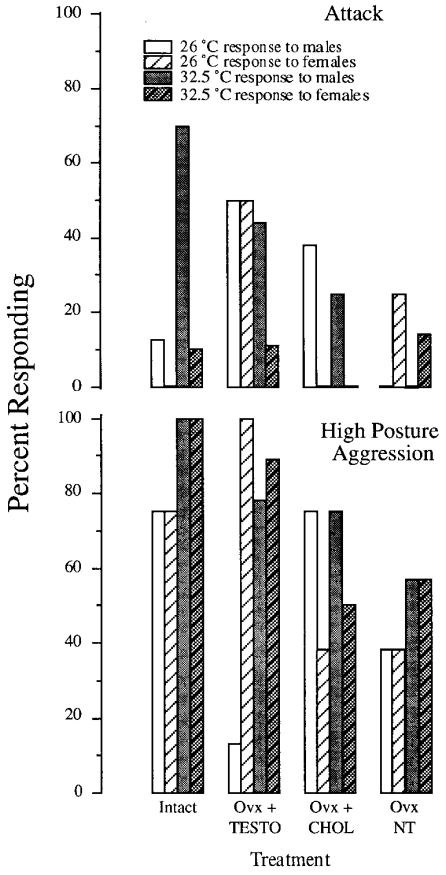

FIG. 5. Effect of hormonal manipulation on the aggressive behavior of female leopard geckos from an all-female (26°C) and a male-biased (32.5°C) incubation temperature, toward male and female stimulus animals. Abbreviations as in Fig. 1.

biased incubation temperature ($P = 0.02$), but not in females from the all-female incubation temperature ($P = 0.15$).

The likelihood of offensive attacks toward female stimulus animals significantly increased following TESTO treatment in females from the all-female incubation temperature compared to unmanipulated females ($P = 0.038$) (Fig. 5, top). This was not the case with females from the male-biased incubation temperature ($P = 0.7$). Unmanipulated females from the male-biased incubation temperature exhibited significantly different responses toward male or female stimulus animals, being less likely to attack a female (7/10 vs 1/10, respectively) ($P = 0.009$). Following ovariectomy, none of the females from the male-biased incubation temperature attacked male stimulus animals ($P =$

TABLE 1
Circulating Concentrations of Total Androgens and Estradiol (ng/ml) in Female Leopard Geckos (*Eublepharis macularius*) from an All-Female Incubation Temperature (26°C) and a Male-Biased Incubation Temperature (32.5°C) before and after Hormone Manipulation

	26°C Females		32.5°C Females	
	Total androgens	Estradiol	Total androgens	Estradiol
Intact	.41 (.07)	.56 (.07)	.41 (.06)	.81 (.11)
	$n = 6$	$n = 7$	$n = 9$	$n = 9$
Ovx + TESTO	197.70 (27.8)	.58 (.07)	146.63 (23.72)	.59 (.18)
	$n = 8$	$n = 8$	$n = 9$	$n = 8$
Ovx + CHOL	.24 (.02)	.26 (.05)	.32 (.07)	.38 (.14)
	$n = 5$	$n = 8$	$n = 5$	$n = 8$
Ovx NT	.23 (.02)	.20 (.02)	.29 (.05)	.27 (.05)
	$n = 7$	$n = 8$	$n = 5$	$n = 7$

Note. Means shown with standard errors in parentheses. Ovx, ovariectomy; TESTO, testosterone implant; CHOL, cholesterol implant; NT, no treatment (no implant).

0.001). Ovariectomy with CHOL treatment had no effect on the likelihood of attacks toward male and female stimulus animals by females from the all-female incubation temperature ($P = 0.28$). In contrast, females from the male-biased temperature with the same treatment did show a decreasing trend ($P = 0.07$) in the likelihood of an attack toward male stimulus animals, but not toward female stimulus animals ($P = 0.55$).

Hormones

Hormone values are presented in Table 1. Androgen and estrogen levels were not significantly different between females from the all-female (26°C) and male-biased (32.5°C) incubation temperatures (F ratio = 0.42, $P = 0.51$ and F ratio = 0.98, $P = 0.32$, respectively; ANOVA). Pairwise comparisons indicated that following ovariectomy and TESTO treatment, androgen levels were elevated ($P < 0.001$; Tukey post-hoc test). Estrogen levels were significantly lower after ovariectomy with CHOL ($P < 0.001$) and after removal of the implants ($P < 0.001$). Estrogen levels were unchanged following TESTO treatment ($P = 0.56$).

DISCUSSION

Both the incubation temperature of the egg and gonadal hormones modify adult sociosexual behavior in leopard geckos. Incubation temperature, however, appears to exert a greater influence on adult aggressive behavior than do hormones. As previously reported (Flores *et al.*, 1994; Gutzke and Crews, 1988), unmanipulated females from a male-biased incubation temperature (32.5°C) were more aggressive to males than were females from an all-female

incubation temperature (26°C). The presence of the ovaries is not unimportant, but the time at which they are removed appears to be crucial in determining the level of aggression exhibited by females. If ovariectomy is performed on the day of hatch, females from the all-female incubation temperature are more aggressive as adults (Flores *et al.,* 1994), but if performed on adults (present study), females become less aggressive. This suggests that in the female leopard gecko, ovarian hormones exert an organizational effect on aggressiveness after hatching but before adulthood.

In mammals the hormonal environment early in life shapes hormone-dependent sociosexual behaviors in the adult. Perinatal treatment with steroid hormones influences sensitivity to hormones in adulthood, but these behavioral responses vary according to the species, the time during development at which the hormone is administered, and the type and amount of hormone given. It has been noted that the intrauterine position effect in polytocus mammals is analogous to the effects of incubation temperature on adult heterotypical aggressive behavior in a TSD species (Crews and Bull, 1987). For example, in rats, females that develop *in utero* next to males (2M female) are more sensitive to testosterone as adults than are females that do not develop between males (0M). That is, treatment with testosterone during adulthood causes 2M, but not 0M, females to display enhanced male-typical sexual behavior (Clemens and Coniglio, 1971; Clemens, Gladue, and Coniglio, 1978). If 2M females are treated with anti-androgens *in utero,* they become relatively insensitive to testosterone treatment and exhibit less masculine sexual behavior (Clemens and Gladue, 1978). In guinea pigs, 2M females display enhanced levels of mounting relative to 0M females when ovariectomized and treated with testosterone for 6 weeks in adulthood (Gandelman, 1986). Similarly, the elevated titers of testosterone experienced by 2M females during fetal life lead to increased aggression in adulthood (vom Saal, 1981, 1991). As with rats, 2M female mice are more aggressive than 0M females when treated with testosterone (Rines and vom Saal, 1985).

Differences in the behavior of animals from different incubation temperature may be explained by differential sensitivities to hormones and perhaps by differences in the quantity of steroid receptors in the brain. As noted, female leopard geckos from an all-female and a male-biased incubation temperature differ in their level of aggressive behavior. Both types of females exhibited a dramatic decrease in the likelihood of aggression after ovariectomy, but females from the male-biased incubation temperature were more sensitive to TESTO treatment than females from an all-female incubation temperature when tested with male stimulus animals. That is, all of the females from the male-biased incubation temperature continued to display HP aggression, whereas all but one female from the all-female incubation temperature ceased aggressive posturing. Further, exogenous TESTO attenuated the effects of ovariectomy in females from the male-biased incubation temperature, but could not override these effects in females from the all-female incubation

temperature. Interestingly, the likelihood of HP aggression directed toward female stimulus animals by animals from the all-female incubation temperature did not change significantly after ovariectomy and TESTO treatment, but if an ovariectomized female without hormone treatment did attack, it was always in response to a female stimulus animal. It is possible that these females are exhibiting hormone-dependent aggression toward one gender. Whalen and Johnson (1987) found that chronic testosterone treatment in adult, castrated male mice induced attacks toward olfactory-bulbectomized males, but not toward lactating female mice. Unmanipulated female leopard geckos from the male-biased incubation temperature also show a gender preference, being more likely to attack a male than a female.

Attractiveness of female leopard geckos is dependent on the presence of the ovaries in adulthood. Regardless of incubation temperature, males were less likely to court females after their ovaries were removed. This is similar to the effects of ovariectomy in adulthood on the attractivity of female mammals (Beach, 1948).

Normally male leopard geckos will attack other males and court females. The likelihood that a male stimulus animal would attack a female leopard gecko increased significantly after ovariectomy with TESTO treatment. Intact females were never attacked, whereas over 50% were attacked after ovariectomy and TESTO treatment. A previous study indicates that male and female leopard geckos have differences in the chemical composition of their skin-derived lipids, suggesting olfaction-based sex recognition (Mason and Gutzke, 1990). It is possible that testosterone changed the chemical composition of the females' skin lipids, perhaps inducing a male-typical pheromone, which could be responsible for eliciting the attacks by these males. One week after TESTO implants were removed, six of these females were attacked again by the same males, but since they had not shed their skin after removal of the implant, the male-typical pheromone could have still been present.

Tail vibration is a male-specific behavior seen only as a male courts a female. Normally female leopard geckos never court other females. Following ovariectomy and TESTO treatment approximately, half of the females exhibited TV to female stimulus animals, but never to male stimulus animals. One ovariectomized female from the all-female incubation temperature did tail vibrate in the absence of TESTO. However, this female had been implanted with TESTO in Phase 3 and may have been experiencing residual hormonal and testing effects. Parsons, MacLusky, Krieger, McEwen, and Pfaff (1979) found that ovariectomized females implanted with estrogen for 1 week, then removed and reimplanted 5 days later, showed enhanced sexual behavior compared with cholesterol-treated females. Almost half of a group of female mice exhibited male sexual behavior 2 weeks after removal of testosterone implants (Gandelman and Kozak, 1988), suggesting that steroids have long-term, reversible effects on behavior in adulthood (Arnold and Breedlove, 1985).

Given that females from the male-biased incubation temperature are masculinized in other traits such as growth, endocrine physiology, and aggressive behavior (Crews and Bull, 1987; Crews, 1988; Gutzke and Crews, 1988; Flores *et al.*, 1994; Tousignant and Crews, 1995; Tousignant *et al.*, 1995), it is surprising that more of these females did not show heterotypical sexual behavior following TESTO treatment. This may be due to the fact that in the present study, each group of females was tested with peers (i.e., females from the male-biased incubation temperature were tested with stimulus females from the same incubation temperature). Indeed, in a previous study (Flores *et al.*, 1994), each experimental animal was tested with stimulus females from both incubation temperatures (i.e., from an all-female and a male-biased incubation temperature) and no differences were found in their response. This means that although females from different incubation temperatures respond differently to female stimuli, they do not distinguish between females from different incubation temperatures as do males. It has been established that females from male-biased temperatures are less attractive than females from a low, all-female temperature (Flores *et al.*, 1994; Gutzke and Crews, 1988). Neonatally androgen-treated female mice (both 0M and 2M) show augmented male-typical sexual behavior compared to unmanipulated females, but there are no differences in this enhanced heterotypical behavior between 0M and 2M females (Gandelman and Kozak, 1988); this appears similar to the results of female leopard geckos from the all-female incubation temperature compared to females from the male-biased temperature. Finally, 2M female mice are less responsive to pheromonal cues than 0M females (vom Saal, 1981). Female leopard geckos from the male-biased incubation temperature may be exhibiting a similar phenomenon.

It is important to note that only female stimulus animals elicited the TV behavior from ovariectomized females treated with TESTO; males were never courted by the experimental females. This indicates a gender preference, which may be mediated in part by pheromonal cues secreted by the stimulus animals. Female leopard geckos ovariectomized and treated with TESTO may actually have an enhanced olfactory sense, making them more aware of the two sexes. In golden hamsters, testosterone treatment increases chemosensory investigation in castrated males (Havens and Rose, 1992). Adult ovariectomized rats implanted with testosterone and exposed to the odor of urine collected from estrogen-implanted ovariectomized rats display more male-typical mounting behavior compared to female rats not exposed to urine (Boehm and Aron, 1988). This suggests that olfactory cues may be modulatory factors involved in heterotypical sexual behavior in female mammals and leopard geckos.

Our previous studies demonstrated that the differentiation of sexually dimorphic behaviors in the leopard geckos is affected by incubation temperature and hormone manipulation early in life (Flores *et al.*, 1994; Gutzke and Crews, 1988). The present study indicates that in female leopard geckos,

aggressive behaviors are dependent on incubation temperature and, to a lesser extent, ovarian hormones. Heterotypical sexual behavior and attractiveness, on the other hand, are more dependent on gonadal hormones. It is possible that these incubation temperature effects may be due to differences in the hormonal environment or to temperature-induced differences in hormone receptors in embryos (Crews *et al.*, 1994). In songbirds, the concentration of androgen in an egg increases with the order of laying, and the aggressive behavior of young from later laid eggs is greater than that of young from earlier laid eggs (Schwabl, 1993). Intact males typically average 75 ng/ml testosterone (range, 15–400 ng/ml) or 10- to 20-fold higher levels of androgens than vitellogenic females and 50- to 200-fold higher levels than nonvitellogenic females (Tousignant and Crews, 1995; Tousignant *et al.*, 1995). Although the circulating androgen levels between adult females from the all-female and male-biased incubation temperatures were equivalent in the present study, other experiments have found that females from male-biased temperatures have higher circulating levels of androgens than do females from the all-female incubation temperature (Gutzke and Crews, 1988; Tousignant *et al.*, 1995). However, in those studies blood was collected from juvenile prepubertal geckos, whereas the geckos in the present study were considerably older and had undergone one breeding season. In both groups of females, estrogen levels with TESTO treatment were comparable to levels while intact. This is likely due to aromatization of testosterone to estrogen. Comparison of TESTO with CHOL treatment revealed a significantly higher level of estrogen ($P < .003$) during TESTO treatment.

Whalen's orthogonal model (1974), based on mammalian research, characterizes masculinity and feminity as independent continua. That is, the degree to which an individual is masculine or feminine can vary independently. We have suggested that sexual differentiation in a TSD species may be unidimensional in which masculinization and femininization are at opposite ends of the same continuum (Crews, 1993; Flores *et al.*, 1994). In this unidimensional model, the extent to which an individual is masculinized is reflected in a corresponding degree of defeminization (and vice versa). For example, following ovariectomy and TESTO treatment, females from both the all-female and male-biased incubation temperatures began to exhibit male-typical courtship behaviors and were less attractive. Thus, the results of this study do not refute this unidimensional model.

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