

Effects of Testosterone on Sexual Behavior and Morphology in Adult Female Leopard Geckos, *Eublepharis macularius*

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The leopard gecko, *Eublepharis macularius*, is a species in which testosterone (T) is the primary circulating sex hormone in adults of both sexes. There are, however, sex differences in T physiology. Whereas males have prolonged periods with high T levels, T levels cycle in accord with follicular development in females. Specifically, T concentration increases during vitellogenesis, drops after ovulation, and then remains at previtellogenic levels until eggs are laid and the next follicular cycle begins. To determine the function of T in females, we manipulated both the level and the duration of T elevation using Silastic implants in intact, adult female leopard geckos. Females had low (~1 ng/ml), medium (~100 ng/ml), or high (~200 ng/ml) T levels for either a short (8 days) or a long (35 days) duration. Behavior tests with males were conducted on days 1–5 in the short-duration group or on days 29–33 in the long-duration group. For both short- and long-duration groups, T treatment decreased attractivity in females with medium and high T levels compared to females with low T levels. In contrast, females with a medium T level were more receptive than females with a low T level in the short-duration group. Females in the long-duration group were unreceptive regardless of T level. Females treated for a long duration also displayed more aggression toward and evoked more aggression from males than short duration females. Short-duration T treatment had no masculinizing effect on female morphology, whereas medium and high T levels for a long duration induced development of hemipenes. Overall, these results suggest that T can both increase and decrease sexual behaviors in the female leopard gecko. © 1999 Academic Press

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Adult female and male vertebrates often have distinct hormone profiles that regulate sex-typical reproductive behaviors (reviewed in Pfaff, Schwartz-Giblin, McCarthy, and Kow, 1994; Meisel and Sachs, 1994).

Estrogens (and progestins), for instance, are the predominant plasma sex steroids in many female vertebrates, whereas androgens are the most abundant steroids in males. Accordingly, estrogens (and progestins) control female-typical sexual receptivity while androgens control male-typical courtship and copulatory behavior. Yet, estrogens and androgens do not always regulate sex-typical behavior in such a congruent manner. It has been known for some time that estradiol (E2), via aromatization of testosterone (T) within the brain, is required for expression of the full repertoire of male sex behavior in certain species (Sachs and Meisel, 1988; Balthazart and Foidart, 1993). More recent work suggests that progesterone also facilitates male sexual behavior in a variety of vertebrates (Phelps, Lydon, O'Malley, and Crews, 1998). Furthermore, androgens can modulate many aspects of female sex behavior (Rissman, 1995; Staub and De Beer, 1997).

It has been found that T, like E2, can induce receptivity in ovariectomized rats (De Jonge, Eerland, and Van De Poll, 1986; Satou and Yamanouchi, 1998). In addition, progesterone increases T-induced receptive and proceptive behaviors (de Jonge *et al.*, 1986; Fernandez-Guasti, Vega-Matuszczyk, and Larsson, 1991). Although some data suggest that T also influences female preference for an opposite-sex vs a same-sex partner, the direction of this preference appears to depend upon sexual experience (de Jonge *et al.*, 1986; Koos Slob, de Klerk, and Brand, 1987). While these studies indicate that androgens may affect certain aspects of female sexuality, it is not clear that T is the primary hormone controlling such behaviors in intact, cycling female rats.

In contrast, the musk shrew is a species in which T levels greatly surpass E2 levels in females (Rissman and Crews, 1988). A series of experiments has

demonstrated a clear functional role for circulating T in female reproduction in this species. Specifically, T, but not E2, administered at physiological doses to ovariectomized females activates receptive behavior (Rissman and Bronson, 1987; Rissman, Cendenon, and Krohmer, 1990). Moreover, T must be aromatized within the brain to trigger sexual behavior in female musk shrews (Sharma and Rissman, 1994; Rissman, Harada, and Roselli, 1996; Freeman and Rissman, 1996). A recent review by Staub and De Beer (1997) indicates that levels of androgens, androgen receptors, and androgen-metabolizing enzymes are also relatively high in females of many other vertebrates. Since androgen effects are not sex-limited, these authors infer that male-typical steroids may play a much more significant role in normal female development and reproduction than previously realized.

In this study, we examine the effects of T on female leopard geckos, *Eublepharis macularius*, an egg-laying species in which plasma T concentration varies through the reproductive cycle. Tousignant, Viets, Flores, and Crews (1995) first reported that T levels are significantly higher in vitellogenic females than in previtellogenic females or females after oviposition. However, dihydrotestosterone (DHT), E2, and corticosterone levels did not differ between these groups. We have replicated these basic results in an experiment designed to more precisely document hormonal and behavioral changes during the female cycle (Rhen, Sakata, and Crews, 1998). Using repeated measurements on females sampled through a single cycle, we found that plasma T levels are low when females are previtellogenic, increase slightly during early vitellogenesis, increase dramatically during late vitellogenesis, and then decrease to previtellogenic levels after ovulation (i.e., prior to oviposition). Moreover, we determined that females are not receptive when previtellogenic, become somewhat receptive during early vitellogenesis (receptive in ~20% of tests), are most receptive during late vitellogenesis when T levels are highest (receptive in ~65% of tests), and are again unreceptive after ovulation. Although these data suggest that T regulates female sexual behavior, the evidence is correlational. Here we directly investigate the hypothesis that T controls sexual receptivity in female leopard geckos. We also examine T effects on female attractivity, aggression, and morphology.

METHODS

Animals

All animals used in this study were intact females of various ages from our breeding colony at the University of Texas, Austin. Although a small number ($n = 4$) of animals used in the following study were obtained from other sources and had undocumented life histories, most ($n = 36$) were produced and raised in our laboratory under the following conditions. Animals were hatched from eggs incubated in moist vermiculite (1.5:1, water:vermiculite) at controlled temperatures of 26 ($n = 11$), 30 ($n = 14$), 32.5 ($n = 4$), or 34°C ($n = 7$) $\pm 0.1^\circ\text{C}$ in the laboratory. An incubation temperature of 26°C produces all females, 30°C produces a female-biased sex ratio (~1 male:3 females), 32.5°C produces a male-biased sex ratio (~3 males:1 female), and 34°C produces a female-biased sex ratio (~1 male:19 females) (Viets, Tousignant, Ewert, Nelson, and Crews, 1993).

Hatchlings were maintained individually in plastic shoeboxes (30 \times 12.5 \times 6 cm) containing a water dish and shelter and were fed live crickets on a daily basis. Hatchlings were exposed to a 14:10 L:D photic cycle and held at a constant temperature of 30°C in a single reach-in chamber until 10 weeks of age. At this time, juveniles were moved to another reach-in chamber where they experienced the same light cycle but were fed mealworms twice a week and neonatal mice once a week and were subjected to a 30:18°C daily temperature cycle. This light and temperature regime approximates summer conditions in Pakistan where leopard geckos are native. At sexual maturity (45 to 50 weeks of age), females in our colony are placed into breeding cages (60 \times 60 \times 44 cm) with one male and one to four other females. Each breeding cage contains a sand-filled shoebox for oviposition, a water bottle, and a small Plexiglas box for food. The feeding, light, and temperature regimes for these animals are similar to those described for juveniles.

Hormone Treatment

Forty randomly selected females were removed from their breeding cages for this study. The only criteria used to select animals was that they had reproduced successfully (i.e., produced viable hatchlings) and that they were previtellogenic. Previous work indicates that previtellogenic females are sexually unreceptive (Rhen *et al.*, 1998). This group of animals was then randomly assigned to 1 of 10 treat-

ment groups in a two-way design: one of five T doses for a short (8 days) or long (35 days) duration.

Surgery was performed using hypothermia anesthesia. Animals received a subcutaneous, 10-mm Silastic implant (i.d. 1.47 mm, o.d. 1.95 mm) filled with T and cholesterol mixed in varying proportions by weight: implants included mixtures of 0% T:100% cholesterol; 25% T:75% cholesterol; 50% T:50% cholesterol; 75% T:25% cholesterol; or 100% T:0% cholesterol. These implants were soaked for 24 h in reptilian Ringers at 4°C before implantation. As a 20-mm Silastic implant with pure T produced circulating levels within the physiological limits of intact, sexually active males (Flores and Crews, 1995), our manipulations of tubing length and T content were intended to produce plasma T concentrations ranging from female- to male-typical levels. However, actual plasma levels of T revealed no dose-response effect among the groups receiving 25, 50, 75, and 100% T. Consequently, plasma T levels were used to generate new groupings of subjects in which to analyze dosage effects. Subjects were placed into one of three groups: the pure cholesterol group with low T levels, a medium T level group, and a high T level group (see Table 1). Following surgery, females were isolated in polycarbonate cages (43 × 22 × 20 cm) with food, shelter, and water.

Behavior

Half of the animals ($n = 20$ total) were behavior tested with stimulus males beginning 1 day after surgery (i.e., short-duration treatment), whereas the other half ($n = 20$ total) were tested with the same males beginning 4 weeks after surgery (i.e., long-duration treatment). Geckos in our colony breed year round. Moreover, the stimulus males were sexually active, experienced breeders. Thus, it is unlikely that seasonal effects or sexual experience in males would have a large influence on our results even though short- and long-duration groups were tested at different times. Stimulus males from an incubation temperature of 32.5°C were rotated such that females were tested for 5 min each on 5 consecutive days (i.e., each female interacted with a given male only once). Experimental females were first placed into a neutral cage (43 × 22 × 20 cm) with a clean paper towel as a liner. Subject and stimulus animal behavior was recorded using a keypad timer (Witt/Timer program courtesy of Diane Witt, NIH, Bethesda, MD). Tests were ended after 5 min or if an attack or attempted copulation occurred.

In a sexual encounter, a male slowly approaches a female, first licking the substrate or the air with his

tongue and then licking the female. An attractivity pheromone in the skin of females (Mason and Gutzke, 1990) elicits a male-typical tail-vibration that creates an audible buzz and a tactile vibration of the substrate. During these encounters males may also drag their preanal pores on the substrate, presumably to deposit pheromones in a male-typical, scent-marking behavior. Males then body grip the female's skin with their jaws during courtship and mounting. Body grips are a major component of male copulatory behavior as they always accompany intromission. Thus, our measures of male sexual behaviors included marking duration, tail-vibration duration, and the frequency of tests in which a body grip occurred. Overall, these behaviors are an index of female attractivity.

Female sexual receptivity to male body grips was recorded as a composite index where females were considered receptive if they either displayed a tail lift (exposing the cloaca for intromission) or remained immobile when gripped by a male. Females were considered sexually unreceptive if they did not tail lift and fled from or attacked the courting male.

We measured the duration a female assumed a high posture (an aggressive display) as an index of female aggressive behavior. Although males normally display extreme intrasexual aggression, they rarely display such behavior toward females. Thus, we also considered the duration a male held a high posture as an index of female masculinization. Females were returned to their original breeding cages 7 to 10 days after hormone implants were removed. Two weeks later, attacks by resident males were quantified by counting the number of lacerations on the females' bodies.

Morphology

Although females normally do not have hemipenes, we previously reported that sexually mature, ovariectomized females develop fully evertable male genitalia when given DHT or T implants for 4 weeks (Rhen and Crews, 1996). To characterize the development of these male-typical structures more precisely, we measured the size of both hemipenes on the day of surgery and at weekly intervals for 4 weeks thereafter. Gentle pressure was applied at the base of the hemipenis and the length and width of each everted hemipenis were measured using a dial caliper (accurate to ± 0.1 mm).

Radioimmunoassay

A blood sample was drawn from each experimental animal by cardiocentesis using a heparinized 1-cc sy-

ringe with a 25-gauge needle. All samples were collected 2–3 days after behavior tests were completed (i.e., on day 8 for the short-duration group and on day 35 for the long-duration group). Blood was centrifuged at 3000 rpm for 10 min at 4°C and plasma was stored in plastic microfuge tubes at –80°C until assayed for levels of DHT, E2, and T using hormone-specific radioimmunoassays. Implants were then removed from all experimental animals while under hypothermia anesthesia. The antibodies used for RIA were DT3-351 for DHT, E26-47 for E2, and T3-125 for T (Endocrine Sciences, Calabasas Hills, CA). Briefly, plasma samples were mixed with approximately 500 cpm each of tritiated DHT, E2, and T. After a 1-h equilibration time at 25°C, steroids were extracted with 3 ml of ether. Extracts were then dried under a stream of nitrogen in a dry bath at 37°C. Steroids were resuspended in 500 μ l of isoctane saturated with ethylene glycol and separated on celite columns as described in Tousignant *et al.* (1995). The eluted steroids were dried under a stream of nitrogen in a dry bath at 37°C and resuspended overnight at 4°C in 330 μ l of phosphate-buffered saline. A single 100- μ l aliquot of each sample was used to determine individual recoveries while duplicate 100- μ l aliquots were used for radioimmunoassay with approximately 2000 cpm of the appropriate labeled steroid and the corresponding antibody. Assay sensitivity was 122 pg DHT/ml plasma, 195 pg E2/ml plasma, and 50 pg T/ml plasma. Intra-assay coefficients of variation were 9.7, 7.6, and 14.7% for DHT, E2, and T, respectively. Inter-assay variation was not calculated as all samples were run in a single assay.

Statistical Analyses

Data from behavior tests on two females (50 and 75% T-treated, long duration) were excluded from the analysis because they developed follicles during the experiment. A third animal (75% T-treated, short duration) died. All other data were analyzed using a three-way experimental design with T dosage, treatment duration, and test day as main effects. Male marking, tail vibration, and high posture duration and female high posture duration were analyzed with a univariate repeated-measures ANOVA. We present the overall means (neglecting time as a factor) for dependent variables when the analyses indicated that day effects were not significant (i.e., $P > 0.05$). Otherwise, data are also presented as a function of time. The frequency of tests in which a male body gripped the female and female receptivity to male body grips were

analyzed using a nominal logistic (log-linear) model fitted with a maximum likelihood procedure (SAS Institute, 1989). The logistic model is most appropriate for binary dependent variables (i.e., body gripped or not, receptive or unreceptive) and is analogous to the linear model for continuous variables in ANOVA (Chatterjee and Price, 1978; Sokal and Rohlf, 1981). Again T dosage, treatment duration, and day of testing were main effects in three-way analyses. Hormone concentrations were log-transformed and analyzed using a two-way ANOVA with dose and duration as factors. Hemipenis growth was analyzed with a multivariate repeated-measures ANCOVA using time as the within-subjects factor. The number of bites received by females upon their return to the home cage was analyzed with an ANOVA. Continuous variables are presented as least-squares means ± 1 standard error, whereas binomial data are presented as percentages. *Post hoc* comparisons were made using the Dunn–Sidak method to provide a significance level of $\alpha' = 1 - (1 - 0.05)^{1/k}$ where k = the number individual comparisons with an experimentwise error rate of $\alpha = 0.05$ (Sokal and Rohlf, 1981).

RESULTS

Hormone Levels

Females given T implants had elevated T levels relative to females that received pure cholesterol implants [$F(2, 29) = 33.7, P < 0.0001$; Table 1]. However, there was no detectable difference in T levels between short- and long-duration groups [$F(1, 29) = 1.4, P = 0.25$] and neither was there an interaction between level and duration of treatment [$F(2, 29) = 0.3, P = 0.76$]. Accordingly, comparisons among all six groups indicated that there were no differences between long- and short-treatment groups within each T level group and that each T level group was significantly different from all other T level groups ($k = 15$ comparisons, $\alpha' = 0.003$; Table 1).

Although T treatment also influenced the levels of DHT [$F(2, 29) = 6.9, P = 0.004$; Table 1], multiple comparisons revealed that DHT levels were elevated only in the high T level, short-duration group compared to the low T level, short- and long-duration groups ($k = 15$ comparisons, $\alpha' = 0.003$; Table 1). Overall, treatment duration had a marginal influence on DHT levels such that DHT tended to be higher in the short-duration than in the long-duration T-treated group [$F(1, 25) = 3.7, P = 0.06$; Table 1]. There was no

TABLE 1
Concentration of Dihydrotestosterone (DHT), Estradiol (E2), and Testosterone (T) in Female Leopard Geckos after Receiving Silastic Implants Filled with T and Cholesterol Mixed in Varying Proportions for Either a Short or a Long Duration

Treatment groups		Hormones			Sample size
Duration	Dose	DHT	E2	T	
Short	Low	0.29 ± 0.04 ^a	3.3 ± 1.0 ^a	1.1 ± 0.5 ^a	(n = 4)
	Medium	9.4 ± 2.7 ^{a,b}	2.6 ± 0.4 ^a	128 ± 8 ^b	(n = 5)
	High	11.4 ± 2.1 ^b	3.2 ± 0.8 ^a	208 ± 9 ^c	(n = 10)
Long	Low	0.2 ± 0.06 ^a	1.9 ± 0.1 ^a	0.8 ± 0.3 ^a	(n = 3)
	Medium	4.0 ± 1.7 ^a	3.0 ± 0.9 ^a	93 ± 26 ^b	(n = 6)
	High	6.2 ± 1.3 ^{a,b}	2.4 ± 0.23 ^a	206 ± 35 ^c	(n = 7)

Note. Subjects were grouped according to plasma levels of T to form low-, medium-, and high-dose categories. Mean hormone levels are shown in ng/ml plasma ± 1 standard error. Numbers of samples assayed are shown in parentheses. For each hormone, groups with different superscripts are significantly different from each other using *post hoc* comparisons.

detectable interaction between T level and duration of treatment [$F(2, 29) = 0.76, P = 0.48$].

Neither T level [$F(2, 29) = 0.03, P = 0.97$] nor duration [$F(1, 29) = 0.93, P = 0.34$] affected plasma E2 levels (Table 1). Likewise, there was no detectable interaction between these factors for plasma E2 levels [$F(2, 29) = 0.64, P = 0.53$].

Receptivity

Duration of T treatment affected female receptivity to male body grips [likelihood ratio χ^2 (LR χ^2) (1 df) = 8.8, $P = 0.0029$; Fig. 1]. Overall, females treated for a short duration were more receptive than females treated for a long duration. *Post hoc* comparisons in-

dicated that there was no difference in receptivity in females treated for a short vs long duration with low or high T levels but that females with medium T levels were significantly more receptive when treated for a short duration compared to females treated for a long duration ($k = 3$ comparisons, $\alpha' = 0.017$; Fig. 1). Finally, neither test day nor its interaction with any other independent variable affected receptivity (P 's > 0.05).

Treatment duration significantly influenced female aggression (i.e., male-typical high postures) [$F(1, 147) = 6.3, P = 0.013$]. Females treated for a long duration displayed more high postures than short-duration females (2.2 ± 0.5 s vs 0.3 ± 0.5 s, respectively). No other independent variables affected female high postures (P 's > 0.05).

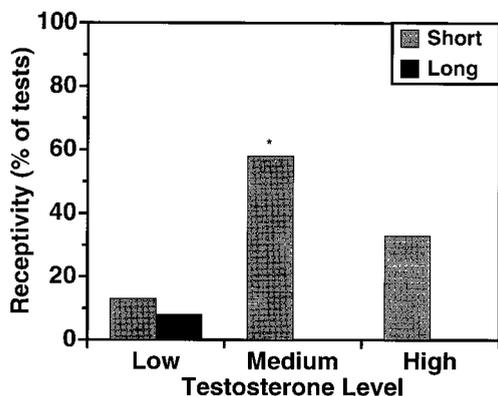


FIG. 1. Receptivity of female leopard geckos with low, medium, or high levels of circulating T for either a short or a long duration. Receptivity was measured as the percentage of behavior tests in which females displayed a tail lift or remained stationary when mounted by a male. Short- and long-duration groups differed only when females had medium T levels (indicated by asterisk).

Attractivity

Male marking in response to T treated females was significantly affected by the duration of treatment [$F(1, 147) = 35.6, P < 0.0001$]. Overall, males marked more in response to short-duration than long-duration T-treated females (67 ± 5 s vs 31 ± 5 s, respectively). However, there was also significant variation in marking behavior due to an interaction between test day and treatment duration [$F(4, 147) = 6.9, P < 0.0001$]. Comparisons of females treated for a short vs long duration show that there were significant differences on days 1–3 but not on days 4 and 5 ($k = 5$ comparisons, $\alpha' = 0.01$; Fig. 2). No other independent variables affected male marking behavior (P 's > 0.05).

Male courtship (tail-vibration duration) directed toward T-treated females was influenced by T level [$F(2, 147) = 6.3, P = 0.002$] but not the duration of treatment

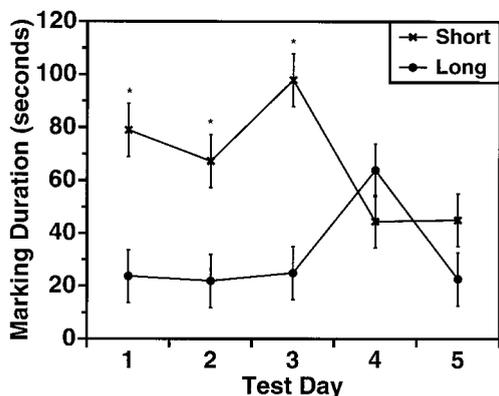


FIG. 2. Marking behavior displayed by male leopard geckos in response to female leopard geckos treated with T for either a short or a long duration. Data are presented for each of 5 consecutive days of testing. Short- and long-duration groups differed only on the first 3 days of testing (indicated by asterisks).

[$F(1, 147) = 0.09, P = 0.8$]. Females with high T levels were courted significantly less than females with low T levels ($k = 3$ comparisons, $\alpha' = 0.017$; Fig. 3). Females with medium T levels were intermediate in attractivity and were not different from females with high or low T levels. No other independent variables affected tail vibrations (P 's > 0.05).

Both the level of T [$LR \chi^2 (2 \text{ df}) = 30.5, P < 0.0001$] and the duration of T treatment [$LR \chi^2 (1 \text{ df}) = 8.2, P = 0.004$] influenced the frequency of tests in which a female was body gripped by the stimulus male. However, there was no detectable interaction between the T level and the duration of T treatment [$LR \chi^2 (2 \text{ df}) =$

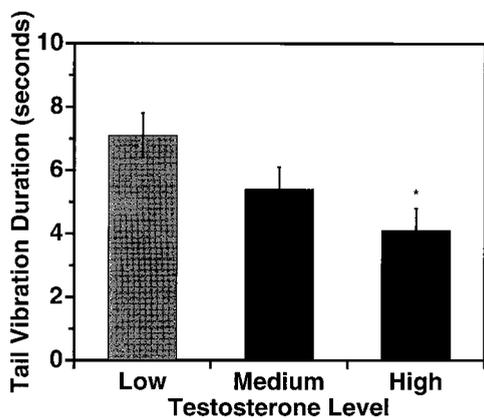


FIG. 3. Courtship behavior displayed by male leopard geckos in response to female leopard geckos with low, medium, or high levels of circulating T. Females with high levels of T elicited significantly less courtship behavior than females with low levels of T (indicated by asterisk).

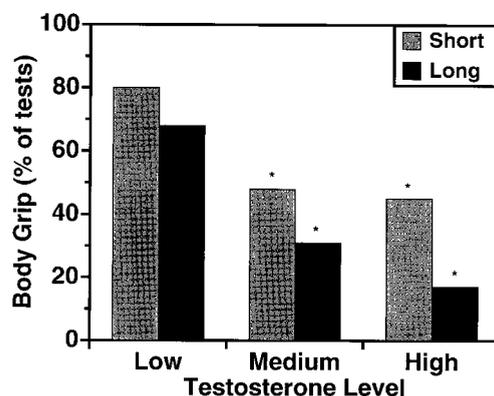


FIG. 4. Body grip frequency by male leopard geckos in response to female leopard geckos with low, medium, or high levels of circulating T for either a short or a long duration. Females with medium and high levels of T were mounted less than females with low levels of T within the same treatment duration group (indicated by asterisks).

3.2, $P = 0.20$]. Within the short-duration group, females with high T levels received fewer body grips than females with low T levels ($k = 5$ comparisons, $\alpha' = 0.01$; Fig. 4). Females with medium T level were also more likely to receive fewer body grips than females with low T levels within the short-duration group. In the long-duration group, females with both medium and high T levels received significantly fewer body grips than females with low T levels ($k = 5$ comparisons, $\alpha' = 0.01$; Fig. 4). There was no difference in body grip frequency between females with low T levels treated for a short duration and those treated for a long duration. However, a low frequency of body grips occurred on day 4 of testing in the long-duration group; only 1 of 18 females was body gripped. This caused a significant duration \times day interaction [$LR \chi^2 (4 \text{ df}) = 17.6, P = 0.002$] and accounts for both a significant day effect [$LR \chi^2 (4 \text{ df}) = 12.6, P = 0.013$] and the significant duration effect noted above. In support of this interpretation, the day and duration effects and their interaction become nonsignificant if day 4 data are excluded from the analysis (results not shown). However, the effects of circulating T level described above remain highly significant even when day 4 is excluded. Finally, there was no interaction between T level and day or among T level, duration, and day (P 's > 0.05).

Females treated for a long duration elicited more male aggressive displays (i.e., high postures) than females treated for a short duration ($43 \pm 6 \text{ s vs } 26 \pm 6 \text{ s}$, respectively) [$F(1, 147) = 7.4, P = 0.007$]. No other independent variables affected male high posture du-

ration (P 's > 0.05). Long-duration, T-treated females also provoked more overt male aggression even after hormone implants were removed (T levels drop within a day after implants are removed; T. Rhen, unpublished data). Both the circulating level of T [$F(2, 30) = 5.8, P = 0.008$] and the duration of T treatment [$F(1, 30) = 24.7, P < 0.0001$] influenced the number of bites received by females during the first 2 weeks in their home cages. There was also a significant T level \times duration interaction [$F(2, 30) = 5.8, P = 0.008$]. Females with low T levels in the short- and long-duration groups were not attacked at all. Similarly, there were no attacks on females with medium and high T levels in the short-duration group. In contrast, females with medium and high T levels within the long-duration group were the only females attacked ($k = 5$ comparisons, $\alpha' = 0.01$; 1.5 ± 0.25 and 1.9 ± 0.25 bite marks, respectively).

Hemipenis Development

The pattern of growth was the same for hemipenis length and width and was similar between the left and the right sides: females with medium and high T levels treated for a long duration developed fully evertable hemipenes by the second week of treatment. However, there was no difference in hemipenis growth between females with medium and high T levels for a long duration (results not shown). All between-subjects and within-subject effects were highly significant ($P < 0.0001$) in a multivariate repeated-measures ANOVA. Since long-duration T treatment, but not cholesterol or short-duration T treatment, induced hemipenis growth, there was also a highly significant time \times T level \times T duration interaction [approximate $F(8, 64) = 8.4, P < 0.0001$].

DISCUSSION

In this study we found that both the circulating concentration of T and the duration of T treatment influenced a number of social behaviors in female leopard geckos exposed to male stimulus animals. Foremost, a medium level of T, administered for a short duration, induced female-typical receptivity, whereas long-duration treatment with T did not. The lack of receptivity in females treated for a long duration may be related to the finding that these females displayed more aggression (i.e., high postures) than females treated for a short duration. Female attractiveness was also influenced by T treatment. However,

plasma T levels and duration of T treatment had differential effects on distinct measures of female attractiveness. For example, the amount of time that males spent scent marking was primarily influenced by the duration of treatment; males marked more with short-duration than with long-duration females. In contrast, male courtship (tail vibration) and mounting (body grip) behaviors were affected mainly by the circulating T levels in females. Females having a high T level, regardless of treatment duration, elicited fewer tail vibrations than females with low T levels while females with medium T levels were intermediate in attractiveness. Females with medium and high T levels were mounted less often than females with low T levels in both the long- and the short-duration groups. Finally, females having medium and high T levels for a long duration were apparently masculinized in their pheromone phenotype because they evoked more male aggressive displays (i.e., high postures) and overt attacks than females with low T levels or females having any T level for a short duration. These long-duration T-treated females also developed male-typical genital morphology. In sum, T treatment increased one measure of female sexual behavior, had little effect on others, and decreased still others.

These seemingly paradoxical effects make sense when we relate the circulating T levels and the duration of T treatment in our study to sexual dimorphism in T physiology. As expected, females with low T levels were unreceptive and had T concentrations (i.e., 1.1 and 0.8 ng/ml) within the normal range for previtellogenic females (i.e., mean levels of 0.25–1.0 ng/ml in previous studies; Tousignant and Crews, 1995; Tousignant *et al.*, 1995; Rhen *et al.*, 1998). However, the medium and high levels of T (i.e., 93–208 ng/ml) in our study were atypical for vitellogenic females (i.e., mean levels of 4–5 ng/ml in previous studies; Tousignant *et al.*, 1995; Rhen *et al.*, 1998) but within the normal range of adult males (i.e., mean levels of 70–156 ng/ml in previous studies; Tousignant and Crews, 1995; Sakata, Rhen, and Crews, 1998). Nonetheless, the finding that male-typical levels of T induced receptivity in females treated for a short duration generally supports our conjecture that increasing T during late vitellogenesis governs female sexual behavior in the leopard gecko. Indeed, our short-duration (i.e., 8 day) treatment is similar to the normal time period during which T is elevated in cycling females (Rhen *et al.*, 1998). Another caveat is that T levels may have varied from day 1 to day 8 postimplantation (when hormone levels were first mea-

sured), even though hormone levels did not differ between day 8 and day 35.

Studies in other reptiles also suggest that T may regulate female sexual behavior. Exogenous T induces receptive behavior in ovariectomized female lizards, *Anolis carolinensis* (Noble and Greenberg, 1940; Mason and Kocher Adkins, 1976). More circumstantial evidence comes from other species. For example, the concentration of T increases above basal levels during vitellogenesis in Kemp's ridley sea turtle (Rostal, Owens, Grumbles, MacKenzie, and Amoss, 1998). In this species, T levels are approximately 10-fold higher than E2 levels throughout the year but increase to 20-fold above E2 at the time of mating. A large increase in T concentration at the time of mating is also found in female American alligators (Guillette, Woodward, Crain, Masson, Palmer, Cox, You-Xiang, and Orlando, 1997). Yet the functional significance of T remains unclear because both T and E2 increase in a correlated manner in the sea turtle and alligator.

With the current data we cannot distinguish whether T is directly inducing receptivity in female leopard geckos or whether T is first aromatized to E2 within the brain. The latter hypothesis is the most likely explanation, considering that exogenous E2 also induces receptive behavior in ovariectomized leopard geckos (T. Rhen and D. Crews, unpublished data) and that circulating E2 levels (i.e., 1.9–3.3 ng/ml) in our study were not affected by T manipulation (i.e., mean levels of 1–3 ng/ml in previous studies; Tousignant and Crews, 1995; Tousignant *et al.*, 1995; Rhen *et al.*, 1998). Another possibility is that exogenous T may have synergistic effects with endogenous circulating E2 in intact females to induce receptivity. This hypothesis, however, needs to be addressed in ovariectomized animals given precisely controlled doses of T and E2. Finally, the aromatization of T is essential for the normal display of receptive behavior in female musk shrews, another species in which T is the primary circulating steroid in females (Freeman and Rissman, 1996). T upregulates aromatase expression in female shrews and doves, just as it does in males of these species (Hutchison, Wozniak, and Hutchison, 1992; Dellovade, Harada, and Rissman, 1994). In contrast to T activation of receptivity in leopard geckos treated for a short duration, females treated for a long duration were unreceptive regardless of T dosage. This extended treatment is equivalent to the average time it takes for females to move from the early vitellogenic stage to oviposition, encompassing nearly an entire reproductive cycle. Particularly interesting is the mechanism by which T may first activate and then

arrest female receptive behavior in the leopard gecko. Androgens have similar time-dependent effects on sexual receptivity in rats: various androgens can induce receptivity when given in acute doses (De Jonge *et al.*, 1986; Frye, Van Keuren, and Erskine, 1996; Satou and Yamanouchi, 1998) or inhibit receptivity when treatment is prolonged (Blasberg and Clark, 1997; Blasberg, Robinson, Henderson, and Clark, 1998; Clark, Blasberg, and Brandling Bennett, 1998). It has been suggested that, in rats, androgens may reduce receptive behavior by downregulating the expression of estrogen receptors in the ventromedial nucleus of the hypothalamus (Brown, Sherz, Hochberg, and MacLusky, 1996). Thus, it is possible that T similarly inhibits the expression of critical genes involved in the control of receptivity in the leopard gecko and that these effects take longer to develop than T-induced receptivity (Pfaff *et al.*, 1994; Blasberg *et al.*, 1998). Indeed, the development of other sexual behaviors also varied with time in our study.

Testosterone influenced most measures of female attractivity, but in different ways for different traits. Females exposed to medium and high T levels for a long duration elicited less male marking behavior than females treated for a short duration. However, male marking elicited by the short-duration group declined on test days 4 and 5 to levels observed in the long-duration group. In contrast, the inhibitory effects of medium and high T levels on other measures of female attractivity (i.e., male courtship and mounting behavior) were immediate and did not differ between long- and short-duration groups. These T effects on attractivity are probably mediated by decreased levels of female-specific semiochemicals that presumably elicit male sexual behavior (Greenberg, 1943; Mason and Gutzke, 1990). Likewise, the increased aggression that males directed toward long-duration, T-treated females may be related to increased levels of male-specific semiochemicals (Mason and Gutzke, 1990). Although we have no direct data indicating that such male-specific skin lipids are under androgenic control, males that have been castrated for over a year no longer provoke offensive aggression from intact males. In this species, extreme intermale aggression often leads to severe lacerations of the type that these females received after they had their T implants removed and were returned to their home cages. If sex-specific expression of pheromones is indeed controlled by T, our data would suggest that different semiochemicals display different developmental dynamics, with female attractivity pheromones responding rapidly and male pheromones changing relatively

slowly. Overall, the effect of long-duration T treatment on female attractivity and male aggression in our study is comparable to that reported by Flores and Crews (1995) for ovariectomized female leopard geckos. Similar changes in sex-typical pheromones were hypothesized to mediate the increased aggression directed toward estrogen receptor knockout female mice, which have substantially elevated T levels (Rissman, Wersinger, Taylor, and Lubahn, 1997; Ogawa, Eng, Taylor, Lubahn, Korach, and Pfaff, 1998).

Whereas we can only infer that pheromone profiles were influenced by T treatment, T exposure clearly masculinized female morphology in the leopard gecko. Females developed fully evertable hemipenes within 2 weeks of receiving long-duration T implants. We originally hypothesized that such male-typical morphology (and reduced attractiveness) may interfere with normal reproduction, as it does in the spotted hyena (Frank, 1997). However, T treatment did not affect any measure of subsequent reproduction (we recorded reproductive performance for 16 months after implants were removed and females were returned to their home cage; results not shown). Briefly, the proportion of infertile eggs produced by T treated females was not different than cholesterol-treated females. Likewise, fecundity (i.e., the number of eggs produced per female per month) was unaffected by T treatment. And finally, egg viability was not influenced by T treatments. Overall, these measures of reproductive success were similar to those found in our breeding colony as a whole, which suggests that a single treatment with T did not have any long-term consequences. Thus, T effects in female leopard geckos appear to be activational in nature. Indeed, hemipenes have completely regressed and females no longer have bite marks indicative of male aggression.

In conclusion, T influenced an entire suite of sexual behaviors in the female leopard gecko. Whereas T activated receptive behavior to levels that we observe in females during late vitellogenesis, it also decreased female attractivity, elicited aggression from males, and induced development of male-typical genital morphology. These seemingly contradictory findings can be explained by the fact that the T concentration in the medium and high T level groups were in the normal range of intact males. Although our results generally suggest that T may be involved in female leopard gecko reproduction, additional experiments will be required to test this hypothesis in more detail. For example, we plan to manipulate hormone metabolism (e.g., using aromatase inhibitors) and hormone action (e.g., using estrogen and androgen antagonists)

in intact females and in ovariectomized females given physiological doses of T.

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