

## Ontogenetic and Social Factors Affect the Endocrinology and Timing of Reproduction in the Female Leopard Gecko (*Eublepharis macularius*)

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Successful reproduction requires the coordination of physiological and behavioral processes with changing environmental conditions such that the offspring will survive to maturity. This coordination is achieved through the integration of external and internal stimuli by the brain which in turn modulates neuroendocrinological mechanisms supporting reproduction (Crews and Gans, 1992). Most environments fluctuate in a manner that leads to seasonal or cyclical reproduction (Crews, 1987; Negus and Berger, 1987; Wingfield, Hahn, Levin, and Honey, 1992). The seasonal rhythms in the environment are complemented by rhythms of sensitivity to the environment (Wingfield, 1985; Crews, 1987; Wingfield *et al.*, 1992). In species which have multiple reproductive periods per season, this coincidence of environmental change and sensitivity to the environment can be an important factor in the sexual maturation of females. In the mountain vole (*Microtus montanus*), females from cohorts born late in the season increase their reproductive success by delaying reproduction until the following year (Negus and Berger, 1986).

There is great variation in the physiological control of reproduction both within and between species (Bronson, 1989). Much of this variation between adults of the same species arises from factors experienced during embryonic or neonatal life (Adkins-Regan, 1983; Dörner, 1983; vom Saal, 1983, 1991). For example, steroid hormones present during these early periods "organize" the neuroendocrine mechanisms which will be "activated" by hormones secreted later in life as adults (Reinisch and Sanders, 1987; Moore, 1991; Ward, 1992). Indeed, the hormonal environment in the uterus of many mammals is affected by both maternal (Ward, 1992) and intrauterine sibling influences (Houtsmuller and Slob, 1990; Clark, Crews, and Galef, 1991). The prenatal physical environment also plays an important role in the process of sex determination in many oviparous

reptiles. In some species the temperature of the incubating egg determines the sex of the individual, a process known as temperature-dependent sex determination (TSD) (for review see Bull, 1983; Deeming and Ferguson, 1988; Ewert and Nelson, 1991). Species with TSD provide a unique opportunity to dissociate the effects of the embryonic environment and gonadal sex on the development of morphological and physiological traits. Such separation is not possible in species with genotypic sex determination (GSD) (Crews, 1993). This experiment was conducted to assess the effects of social experience, ovarian state, and incubation temperature on the endocrinology and timing of reproduction in captive-reared leopard geckos.

### METHODS

*Animals.* All animals were hatched from eggs of known parentage collected on the day of laying. Eggs at the University of Texas (UT) were incubated individually in covered plastic cups containing moist vermiculite (1.5:1, water:vermiculite by weight) at constant temperatures ( $\pm 0.2^\circ\text{C}$ ) of  $26^\circ\text{C}$  ( $n = 37$ ) which produces all females, or  $32.5^\circ\text{C}$  ( $n = 25$ ) which produces approximately 25% females, throughout the entire incubation period in upright Precision Scientific incubators. Egg incubation at  $34^\circ\text{C}$ , producing 95% females, occurred at Indiana University (IU) and differed in that eggs were incubated collectively in airtight plastic Rubbermaid containers ( $38 \times 26 \times 13$  cm) and flushed with air daily. To minimize mortality associated with the stress of high temperatures on developing embryos, eggs at IU were incubated at  $32^\circ\text{C}$  ( $\pm 0.2^\circ\text{C}$ ) for 1 day, switched to  $34^\circ\text{C}$  for the bulk of the incubation period, and then switched back to  $32^\circ\text{C}$  one to three days prior to hatching (see Viets, Tousignant, Ewert, Nelson, and Crews, 1993). Animals from this high incubation temperature of  $34^\circ\text{C}$  ( $n = 24$ ) were shipped to UT approximately 3 weeks after hatching from IU.

*Housing and maintenance.* All cages were cleaned weekly at which time the females were visually inspected for the presence and number of vitellogenic follicles. This is easily accomplished in the leopard gecko as one can readily see vitellogenic follicles or eggs through the skin of the abdominal region. The age of each female was noted at the time of appearance of the first vitellogenic follicles (reproductive maturity).

Hatchling geckos were offered a controlled amount of crickets (Fluker's Cricket Farm) and "giant" mealworms (Rainbow Mealworms) three times weekly. Upon reaching 10 weeks of age and throughout the duration of the experiments, individuals were also offered one neonatal mouse per week. Water was available *ad libitum*. All food items were dusted with a pulverized mixture of vitamin-mineral powder (Petco Animal Supplies) and calcium diphosphate (Texas Gulf Supplies).

In Experiment One the majority of hatchling geckos were housed in-

dividually in plastic boxes ( $12 \times 30 \times 6$  cm). Each box contained a paper towel substrate, a petri dish for water, and a plastic shelter. A subset of females ( $n = 8$ ) from the  $26^\circ\text{C}$  incubation temperature were housed communally in a large polycarbonate container ( $43 \times 22 \times 20$  cm). Both types of containers were housed in controlled environmental conditions throughout the experiment. During the first 10 weeks of age the chambers were continuously maintained at  $30^\circ\text{C}$  and had a 14:10 LD photic cycle. At 10 weeks of age all individuals were switched to daily photothermal cycles of  $30^\circ\text{C}:18^\circ\text{C}$  (14:10, day:night) and corresponding LD periods. Relative humidity was not regulated and ranged from 30–60%.

In Experiment Two some females ( $n = 11$  for  $26^\circ\text{C}$  and  $n = 10$  for  $32.5^\circ\text{C}$ ) were transferred from the socially isolated conditions at 50 weeks of age and paired with a reproductively active adult male. Other females ( $n = 11$  for  $26^\circ\text{C}$  and  $n = 10$  for  $32.5^\circ\text{C}$ ) were transferred from the socially isolated conditions at 65 weeks of age and paired with a reproductively active adult male. All pairs were housed in polycarbonate cages ( $43 \times 22 \times 20$  cm) and maintained under similar environmental conditions.

*Blood sampling protocol.* Blood samples were taken from all animals at 17:00 hr ( $\pm 30$  min) and each individual was bled within 30 sec of being taken from its cage. Approximately 200  $\mu\text{l}$  of whole blood were collected through cardiocentesis using a sterile heparinized 25-gauge needle and tuberculin syringe. Individual blood samples were then transferred to microhematocrit tubes and stored on ice until centrifugation. The whole blood was centrifuged at 2000 revolutions per minute for 12 min in a refrigerated centrifuge maintained at  $10^\circ\text{C}$ . The separated plasma was then aliquoted into plastic microcentrifuge tubes and stored at  $-20^\circ\text{C}$  until assayed.

*Blood sampling schedule.* In Experiment One individuals were bled once upon reaching 50 weeks of age. In Experiment two individuals were bled immediately prior to being paired with an adult reproductively active male. Each female was then bled again at 5 and 10 weeks after pairing unless they had ovulated and were gravid (eggs in the oviduct). All gravid females were bled at 17:00 hr on the afternoon following oviposition. Females which did not become gravid were maintained under the same conditions through the next reproductive season and then bled following oviposition.

*Hormone determination.* Plasma levels of progesterone (P), dihydrotestosterone (DHT), testosterone (T), estradiol (E2), and corticosterone (B) were determined using a radioimmunoassay following chromatographic separation of individual hormones according to the methods of Whittier, Mason, and Crews, (1987) with some modifications. Briefly, plasma samples were mixed with approximately 1000 counts per minute (cpm) each of the five tritiated steroids and allowed to equilibrate for a

minimum of 4 hr. Each sample was then extracted with ether (3 ml) and the extracts were dried in a dry bath set at 37°C under a stream of nitrogen. Individual extracts were resuspended in 500  $\mu$ l isooctane saturated with ethylene glycol and separated on celite columns. Each column consisted of a 1.5-cc "glycol trap" (Celite:ethylene glycol:propylene glycol, 6:1.5:1.5, w:v:v) and a 0.5-cc "water trap" (Celite:water, 3:1, w:v) rinsed with pure isooctane. The samples were applied to the columns and fractions were eluted with ethyl acetate in isooctane as follows: neutral lipids, pure isooctane (3 ml); P-1% ethyl acetate (3.6 ml); DHT-10% (4.5 ml); T-20% (4.5 ml); E2-40% (4.0 ml); and B-50% (4.0 ml). Overlap in adjacent fractions with these columns averaged 3.2%. The eluates were dried in the dry bath and resuspended in 300  $\mu$ l phosphate-buffered saline solution. Sample aliquots (100  $\mu$ l) were assayed in duplicate for each hormone. A 50- $\mu$ l aliquot from each sample was placed directly into scintillant to determine recovery of hormone. The hormone values from radioimmunoassays were corrected for individual recoveries which averaged 67.6% for P, 65.8% for DHT, 78.0% for T, 69.2% for E2, and 60.5% for B. The level of detectability was established as two standard errors below the binding observed in blank assay tubes. Samples were run in multiple sets. Mean intra-assay variation and inter-assay variation were as follows: P-12.3, 26.0; DHT-8.2, 20.9; T-7.9, 23.0; E2-9.0, 19.7; and B-11.4, 24.8.

*Statistical analysis.* All parametric results were analyzed using single or double classification analysis of variance (ANOVA) or repeated measures analysis of variance where appropriate. Results from radioimmunoassays were log-transformed to stabilize variance. Non-parametric data for number of follicles, number of females ovulating, and overall egg laying success were analyzed using Fisher's exact, Kruskal-Wallis and Spearman rank-order correlation tests where appropriate.

## RESULTS

*Experiment one.* There was no significant difference between females raised in isolation and those raised in groups either for age at reproductive maturity ( $F(1, 27) = 1.89, P \geq .179$ ) or in the distribution of females in each category of follicle number ( $Z(26) = -0.582, P \geq .278$ ) and thus these two data sets were combined.

There was a significant effect of incubation temperature on the distribution of females having zero, two, or four vitellogenic follicles at 50 weeks of age with more females from cooler incubation temperatures having four vitellogenic follicles (Kruskal-Wallis ( $df = 2$ ),  $P \leq .01$ ) (Fig. 1). This effect was also evident in the age at which females reached sexual maturity, with females from cooler incubation temperatures beginning vitellogenesis at an earlier age ( $F(2, 57) = 4.16, P \leq .02$ ) (Fig. 2).

There was a significant correlation between the number of follicles which

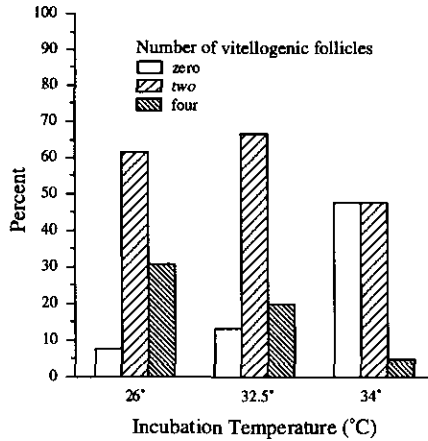


FIG. 1. The proportion of female leopard geckos (*Eublepharis macularius*) from a 26°C, 32.5°C, or 34°C incubation temperature having zero, two, or four vitellogenic follicles. Determination of number of vitellogenic follicles was made by visual inspection through the ventral abdominal region.

a female exhibited and her circulating concentration of T ( $Z(54) = 3.698$ ,  $P \leq .001$ ) (Fig. 3). A weak but significant correlation ( $r = .305$ ,  $P \leq .023$ ,  $df = 54$ ) was observed between T and E2 levels when all data were pooled, but not when different ovarian categories were examined separately. Determination of the role of ovarian state in endocrine physiology was restricted to those females from an incubation temperature of 34°C

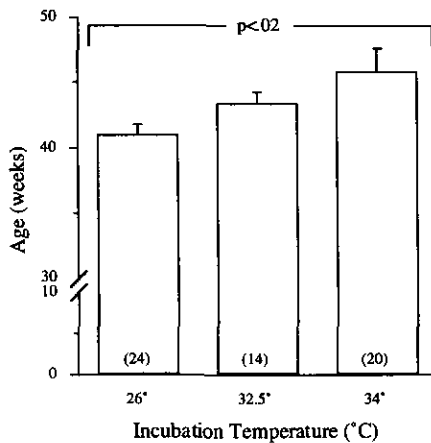


FIG. 2. The age in weeks ( $\pm 1$  standard error) at sexual maturity (development of first vitellogenic follicles) of female leopard geckos (*Eublepharis macularius*) from incubation temperatures of 26°C, 32.5°C, or 34°C. Sample sizes are indicated in parentheses.

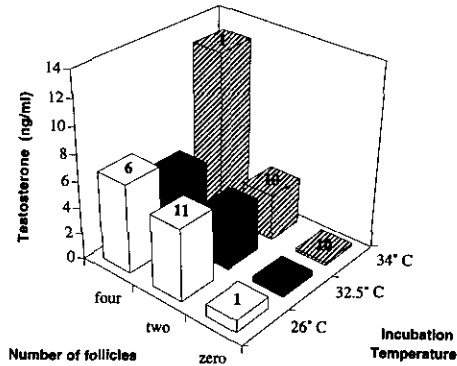


FIG. 3. Mean circulating concentrations of testosterone in female leopard geckos (*Eublepharis macularius*) from a 26°C, 32.5°C, or 34°C incubation temperature and having zero, two, or four vitellogenic follicles. Sample sizes for each group are at the top of individual columns.

and having either two vitellogenic follicles or no vitellogenic follicles (= non-vitellogenic). Females having vitellogenic follicles had significantly higher levels of T than those females not having vitellogenic follicles ( $F(1, 18) = 5.10, P \leq .037$ ) (Fig. 3). There were no significant differences between the two groups for DHT, E2, or B. Determination of the role of incubation temperatures in endocrine physiology was restricted to females having two vitellogenic follicles. With ovarian state held constant, females incubated at 26, 32.5, and 34°C differed significantly in their plasma levels of B ( $F(2, 26) = 3.47, P \leq .046$ ), but not in their levels

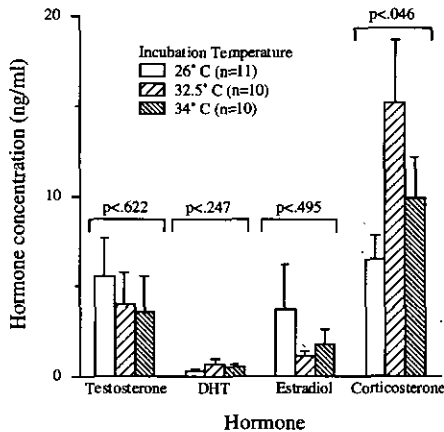


FIG. 4. Mean ( $\pm 1$  standard error) circulating concentrations (ng/ml) of testosterone, dihydrotestosterone, estradiol, and corticosterone for female leopard geckos (*Eublepharis macularius*) from a 26°C, 32.5°C, or 34°C incubation temperature. Data for each group are restricted to animals having two vitellogenic follicles.

of T, DHT, or E2 ( $F(2, 26) = 0.482, 1.48, 0.721$  respectively,  $P \geq .25$  in each case) (Fig. 4). In all groups the number of females having detectable levels of P was too few to warrant comparison. There were no significant differences in the levels of hormones measured between isolated and socialized females from an incubation temperature of 26°C.

*Experiment two.* Significantly more females from both incubation temperatures ovulated and became gravid (had eggs in the oviduct) in the season during which they were paired if pairing occurred at 50 weeks of age compared to 65 weeks of age (Fisher's exact  $P \leq .001$ ). This effect was not due to a permanent infertility in the animals paired at 65 weeks as there were no significant differences in the proportion of females who eventually laid eggs (including the subsequent breeding season) in any of the groups ( $\chi^2 = 0.897, df = 3, P \leq .83$ ). However, the lack of ovulation in the females paired at 65 weeks of age resulted in a significant increase in the amount of time following pairing for reproduction (oviposition of fertile eggs) to occur even though an adult male was present continuously ( $\bar{X} = 84.5$  days for 50 weeks vs  $\bar{X} = 309.1$  days for 65 weeks,  $F(1, 27) = 54.36, P \leq .001$ ).

At the start of the experiment, females bled and then paired at 50 weeks of age had significantly higher levels of T ( $F(1, 19) = 7.48, P \leq .01$ ), but not DHT, E2, or B ( $F = 0.009, 0.097, 1.25$  respectively;  $P \geq .28$  for all cases) than females bled and then paired at 65 weeks of age. There were no significant differences in the levels of T, DHT, E2, or B between females from different incubation temperatures ( $F(1, 19) = 0.0003, 0.009, 1.55, 0.657$  respectively;  $P \geq .23$  for all cases). Again, there were too few animals having detectable levels of P for a valid statistical test.

Repeated measures analysis of hormone levels revealed a significant interaction between reproductive state and the animal's age at the time of pairing for T, E2, and B ( $P \geq .03, .04, .03$ , respectively), but no effect of incubation temperature. There were no significant differences for DHT regardless of incubation temperature or age at pairing. Post-hoc separate analyses for each pair time showed that T and E2 levels significantly decreased after oviposition compared to the levels at the time of pairing for animals socialized at 50 weeks of age ( $F(1, 11) = 19.71, 11.30$ ;  $P \leq .001, .006$  respectively) (Fig. 5), while B levels were not significantly different ( $F = 2.66, P \leq .131$ ). Analysis for the animals paired at 65 weeks of age showed no significant differences for T, E2, or B when hormone levels were compared between reproductive states ( $F(1, 12) = 0, 0.133, 2.51$ ;  $P \geq .993, .722, .139$ , respectively).

## DISCUSSION

Incubation temperature rather than sex chromosomes determines sex in the leopard gecko and many other oviparous reptiles (Bull, 1980, 1983). The present results provide evidence that incubation temperature also

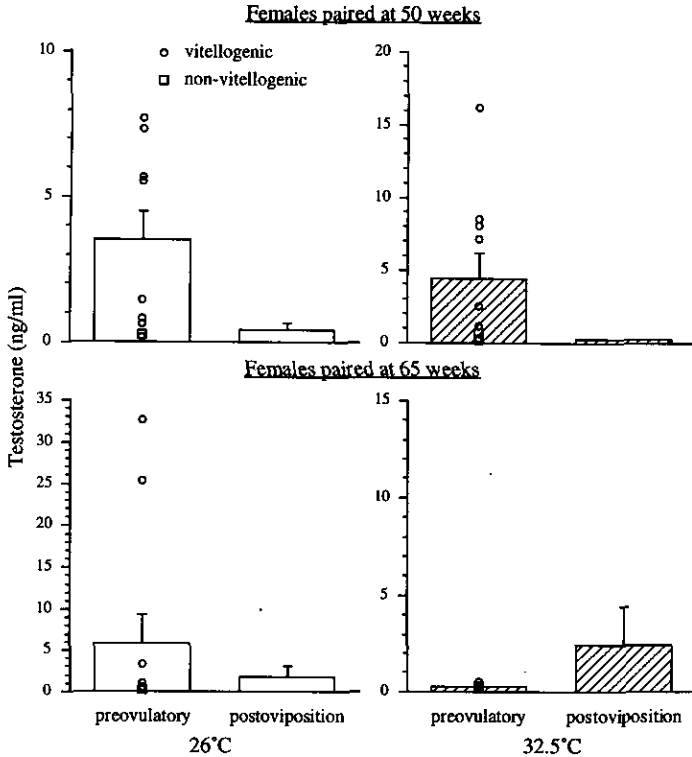


FIG. 5. The effect of ovarian state on the endocrine physiology of female leopard geckos (*Eublepharis macularius*) from incubation temperatures of 26°C and 32.5°C. Illustrated are the mean ( $\pm 1$  standard error) circulating concentrations (ng/ml) of testosterone for females prior to ovulation and after oviposition and paired with an adult breeding male at 50 weeks (top panel) or at 65 weeks of age (bottom panel). Individual data points for preovulatory females indicate whether they were vitellogenic (O) or not ( $\square$ ). Females did not have visible vitellogenic follicles immediately after oviposition and thus individual data points are omitted.

affects the timing and physiology of reproduction in the leopard gecko. Females from lower incubation temperatures reach sexual maturation at an earlier age and are more likely to develop multiple pairs of vitellogenic follicles than are females from higher incubation temperatures. Gutzke and Crews (1988) obtained similar results, reporting that females from a male-biased incubation temperature (32°C) were masculinized in their physiology and behavior relative to females from lower incubation temperatures (26 and 29°C) which result in all-female and female-biased sex ratios, respectively. Female attractiveness as measured by the courtship response of an adult male also decreases as incubation temperature increases (Flores, Tousignant, and Crews, 1994). Attractiveness in this species appears to be mediated by "skin-derived semiochemicals" and male



courtship is a powerful indicator of the availability of these potential pheromones (Mason and Gutzke, 1990). Thus, incubation temperature may have an effect on the production or release of these semiochemicals in the adult female.

These findings may be analogous to the finding in mammals that embryonic hormone experience can influence the development of behavioral and physiological traits expressed in the adult (vom Saal, 1991; Ward, 1992). In mammals sexual differentiation is channeled into male or female development according to the type of gonad established at fertilization by the pairing of sex chromosomes (Jost, 1947). However, in some polytocous mammals the age of reproductive maturation and the endocrine physiology of females is affected by their embryonic exposure to androgens produced by male siblings in utero (vom Saal, 1983; vom Saal and Bronson, 1978, 1980; Clark and Galef, 1988; Houtsmuller and Slob, 1990). Typically, females that develop next to males are masculinized in their physiology and show decreased levels of female-typical behavior and increased levels of male-typical behavior. In female mongolian gerbils (*Meriones unguiculatus*), this phenomenon is manifested in a variety of characteristics including elevated levels of T and DHT and decreased levels of E2 in females with neighboring male fetuses compared to females that lack male neighbors (Clark, Crews, and Galef, 1991).

In this experiment, the number of vitellogenic follicles which female leopard geckos exhibited correlated with their incubation temperature. This indicator of the level of ovarian activity is positively correlated with the circulating level of T in the female. Controlling for the number of vitellogenic follicles shows that most of the variation observed in the circulating concentration of T, but not DHT or E2, in reproductively mature female leopard geckos is due to ovarian activity and not the incubation temperature experienced during embryogenesis. Although different methods were used for egg incubation at 34°C to decrease embryonic mortality prior to hatching, the temperatures experienced by the embryos during most of their development, including the sex-determining period, were held constant. Females incubated at 34°C showed the same pattern of circulating concentrations of testosterone in relation to ovarian activity as did females incubated at other temperatures.

The lack of a correlation in circulating concentrations of testosterone with incubation temperature in adult female leopard geckos is in contrast to the results obtained by Gutzke and Crews, (1988) who reported that females from male-biased incubation temperatures had elevated levels of T and decreased levels of E2 compared to females from female-biased incubation temperatures. However, this study differed in protocol. In the previous study, blood samples were taken after behavioral testing was performed and the females had attained 25 g of body weight, whereas in the present study individuals were sampled prior to any behavioral tests

and at a constant age (50 weeks) following sexual maturation. Tousignant and Crews (1995) have shown that at 25 g of body weight, most females will be approximately 25–30 weeks of age and will not have reached sexual maturity. Thus, the hormone levels reported in the study by Gutzke and Crews may reflect the physiology underlying somatic growth rather than reproduction.

Tousignant and Crews, (1995) observed a significant difference in E2 levels between animals from different incubation temperatures sampled at 50 weeks of age; in both males and females, individuals from a 30°C incubation temperature had the highest levels. Although females from a 30°C incubation temperature were not included in this experiment, the levels of E in the present groups of females were lower overall than those observed for the females from a 30°C incubation temperature in the Tousignant and Crews study and similar to those levels observed for females from the 26 and 32.5°C incubation temperatures in that study. Thus, an incubation temperature of 30°C may be optimal for the organization of the neuroendocrine system responsible for the control of E2 secretion.

Reproduction is the ultimate test of the masculinizing effects of environmental variables. In the gerbil, early masculinization due to the androgenic influence of fetal male siblings leads to a difference in the reproductive capacity of females (Clark and Galef, 1988; Clark, Spencer and Galef, 1986). In the mouse early androgenization is apparent in some characteristics, but this does not lead to differences in reproductive capacity (vom Saal, 1981). In the leopard gecko, Gutzke and Crews, (1988) observed a lack of reproduction in "hot" females from a male-biased (32°C) incubation temperature during the study. However, the results of the present study and that of Viets *et al.* (1993), when taken together, demonstrate that regardless of their incubation temperature, all females eventually have the potential to reproduce. In the present study, females from 26, and 32.5°C incubation temperatures followed for two reproductive seasons (even under constant stimulatory conditions these captive-bred females demonstrate distinct reproductive bouts or seasons which are relatively constant within individuals from year to year) have not shown a significant difference in either the number of eggs laid or in the number of offspring hatching.

The present data also indicate that differences between the data sets of this study and that of Gutzke and Crews, (1988) may be due to a "window" of sensitivity for the initiation of ovulation by behavioral and physical factors involved in reproduction. In the present experiment females paired at 65 weeks of age had decreased levels of T and E2 compared to those paired at 50 weeks and did not ovulate; in fact they did not initiate reproduction until the following year. Thus, even though the leopard gecko has multiple clutches per year, if reproduction is not initiated within this critical period, the entire breeding season is forgone regardless

of the incubation temperature of the individual. This phenomenon does not appear to be related to environmental factors as these animals were continuously held in stimulatory conditions. Further, under these conditions, individual female leopard geckos appear to have an endogenous breeding cycle which is repeated in subsequent years (A. Tousignant and D. Crews, unpublished data). It is possible that the females from the male-biased incubation temperature in the Gutzke and Crews study were still refractory to reproductive stimuli at the termination of their experiment. In montane voles (*Microtus montanus*) females born in later cohorts often postpone breeding until the following season (Negus and Berger, 1986). By delaying reproduction these animals can increase their chances of successfully reproducing, possibly by investing more energy in growth. In red squirrels (*Sciurus vulgaris*) body mass is the best predictor of survival rate and reproductive success with heavier animals achieving greater success (Wauters and Dhondt, 1989). Further, individuals from "early nests" are more likely to be recruited into the local population than those from "late nests" although this is not necessarily due to differences in body mass (Wauters, Bijmens, and Dhondt, 1993). Preliminary data for the leopard gecko have not shown a significant difference in the production of eggs or offspring during the first active breeding season between females who breed during their first year of life and those who wait until their second year. This translates to a greater number of offspring at 2 years of age for females who start breeding during their first year (A. Tousignant and D. Crews, unpublished data). In the laboratory, the leopard gecko can live in excess of 10 years and thus the impact of this difference would be low. However, life expectancy in natural situations is likely much lower and postponing reproduction could result in a significant decrease in individual reproductive success.

Incubation temperature has profound effects on the sex ratio in the leopard gecko and correlates to morphological and physiological traits which affect the adult phenotype. The present data indicate that reproduction in laboratory situations may be postponed, but not inhibited, by the "organizational" influences of male-biased incubation temperatures. Further experimentation is warranted to determine whether the differences in behavior and physiology previously observed have relevance in natural situations where factors such as body size and aggressiveness may affect survival. Finally, these data demonstrate the importance of controlling for ovarian state in comparisons of hormone profiles for females of this species.

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## Role of the Nutritional Status of the Litter and Length and Frequency of Mother-Litter Contact Bouts in Prolonging Lactational Diestrus in Rats

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Food restricting lactating rat dams over the first 2 weeks of lactation results in a prolongation of the period of lactational diestrus. Such food restriction has not only a direct effect on the dam but also the pups are undernourished, and the pattern of dam-litter contact is also changed. In a series of studies, we investigated the effects of nursing undernourished pups and the change in dam-litter interaction on the prolongation of lactational diestrus. While nursing undernourished pups in the last 2 weeks of lactation is sufficient to extend lactational diestrus in *ad lib*-fed dams nursing well-nourished pups in the last 2 weeks of lactation is not necessary for the prolongation of lactational diestrus seen in food-restricted dams. Further, neither nursing underfed pups nor increased nest time in the first 2 weeks postpartum are necessary factors for the prolongation of lactational diestrus in food-restricted dams. © 1995 Academic Press, Inc.

It has been shown that restricting the food intake of female rats for the first 2 weeks of lactation to approximately 60% of the gram intake of *ad lib*-fed females affects the dams' reproductive rate by either delaying implantation if she has been impregnated at the postpartum estrus or prolonging the period of lactational diestrus if a postpartum mating has not occurred (Woodside, Wilson, Chee, and Leon, 1981; Woodside, Cohen, and Jans, 1987; Woodside, 1991). These results are consistent with those of other studies which have shown that the availability of metabolic fuels has a profound effect on reproductive function in a variety of mammalian species (Bronson, 1988; Bronson and Heideman, 1990; Loudon, McNeilly, and Milne, 1983; Schneider and Wade, 1989; Schneider, Friedenson, Hall, and Wade, 1993). Both the extended delay of implantation and the prolongation of lactational diestrus may be viewed as adaptive since they delay the point at which the dam has to partition resources between current and future reproductive efforts. (Daly and Wilson, 1984).

In addition to these effects on reproduction, food restricting the dam also results in maternal and litter malnourishment, as reflected in maternal