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Embryonic Origin of Mate Choice in a Lizard With Temperature-Dependent Sex Determination

ABSTRACT: Individual differences in the adult sexual behavior of vertebrates are rooted in the fetal environment. In the leopard gecko (*Eublepharis macularius*), a species with temperature-dependent sex determination (TSD), hatchling sex ratios differ between incubation temperatures, as does sexuality in same-sex animals. This variation can primarily be ascribed to the temperature having direct organizing actions on the brain. Here we demonstrate that embryonic temperature can affect adult mate choice in the leopard gecko. Given the simultaneous choice between two females from different incubation temperatures (30.0 and 34.0°C), males from one incubation temperature (30.0°C) preferred the female from 34.0°C, while males from another incubation temperature (32.5°C) preferred the female from 30.0°C. We suggest that this difference in mate choice is due to an environmental influence on brain development leading to differential perception of opposite-sex individuals. This previously unrecognized modulator of adult mate choice lends further support to the view that mate choice is best understood in the context of an individual's entire life-history. Thus, sexual selection results from a combination of the female's as well as the male's life history. Female attractiveness and male choice therefore are complementary. © 2005 Wiley Periodicals, Inc. *Dev Psychobiol* 48: 29–38, 2006.

Keywords: mate-choice; embryonic environmental imprinting; male choice; leopard gecko

Mate choice behavior is a contextual phenomenon influenced by a variety of factors such as population density, predator presence, health status of potential mates, and the genotype and reproductive state of the choosing animal or the possible mate (Andersson, 1994; Berglund, 1993; Kelso & Verrell, 2002; Rosenqvist & Johansson, 1995). Experiences usually arising from social interactions during early life and adulthood are another source of variation (Galef & White, 2000). Prominent

examples of such sociosexual modulators are sexual imprinting in a variety of birds (Immelmann, 1975) and mate copying in poeciliid fish (Schlupp, Marler, & Ryan, 1994). Whether nonsocial embryonic experiences exerted by environmental factors can also affect adult mate choice is altogether unclear.

Studies in a variety of vertebrates have long identified the embryonic environment as an early nonsocial modulator of adult sociosexual behavior (Crews, 2003). Exogenous factors during embryogenesis that affect an individual's adult behavior include both biochemical modifiers, such as steroid hormones, but also physicochemical agents such as humidity (in oviparous land vertebrates), temperature, or light. For instance, variation in exposure to exogenous steroid hormones originating from the mother or, in litter bearing species, from siblings neighboring the developing embryo in utero,

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plays a significant part in shaping individual adult behavior. In mammals, maternal hormone production during pregnancy varies with duration of the pregnancy, genetic factors, and stress (Crews & Groothuis, 2005). Androgens, corticosterone, and cortisol produced by the mother reach the embryo via placental transmission, thereby exerting modulating influences on the developing embryonic brain and affecting future behavior. This is perhaps most remarkably exemplified in case of the spotted hyena, where female dominance is maintained by high androgen serum levels, even throughout pregnancy. It is believed that high and variable maternal testosterone levels are largely responsible for masculinization and early aggression of female offspring (Goyman, East, & Hofer, 2001). Another example illustrating the importance of the maternal hormonal state during pregnancy on offspring behavioral development in life is stress. Pups of rodent dams that experienced stress during pregnancy (e.g., handling or exposure to unstable social environments) are severely affected in their social behavior and cognitive functions (Weinstock, 1997) and, in the case of female pups, masculinized (Kaiser, Kruijver, Swaab, & Sachser, 2003). Diffusion of sex steroid hormones across the fetal amniotic and chorionic membranes of adjacent embryos has been shown to modify the developmental trajectory of brain nuclei that are involved in controlling behavior, thereby producing distinct behavioral phenotypes (Crews & Groothuis, 2005; Ryan & Vandenberg, 2002). In gerbils, this intrauterine position phenomenon leads to significant differences in adult aggressive behavior of females that developed between two male siblings as opposed to females that developed between two female siblings (Clark & Galef, 1995).

Work on the leopard gecko (*Eublepharis macularius*), a lizard with temperature-dependent sex determination (TSD), suggests that the embryonic environment shapes an individual's perception of its surroundings, which may include how it perceives potential mates. In this species, the ambient temperature during embryogenesis accounts not only for an individual's gonadal sex, but also for much of the observed within-sex variation in adult sociosexual behavior (Crews, Sakata, & Rhen, 1998; Rhen & Crews, 2000, 2002). For example, females and males are highly aggressive when hatched from eggs that were incubated at a temperature producing primarily male hatchlings (32.5°C) (Flores, Tousignant, & Crews, 1994). While males from a female-biased incubation temperature (30.0°C) are less aggressive than males from 32.5°C, they show greater sexual activity (Rhen & Crews, 1999). These behavioral phenotypes (Fig. 1a) are the effect of an incubation-temperature dependent developmental organization of limbic brain nuclei as the substrates for sexual behavior (Coomber, Crews,

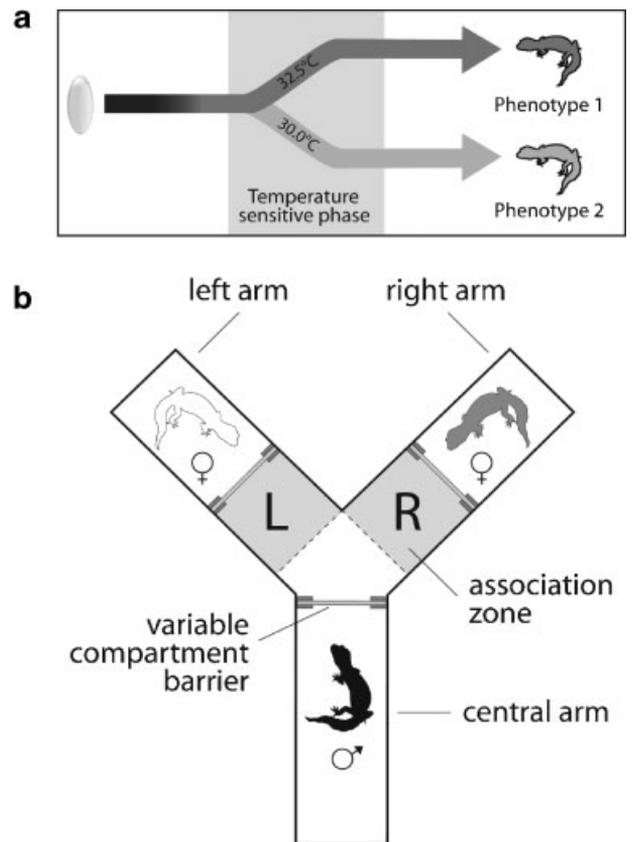


FIGURE 1 (a) Production of temperature phenotypes by incubating at 30 and 32.5°C. (b) Y-maze used in the experiments. Experimental males were placed in the central arm of the maze, while stimulus females were placed in the left and right arm, respectively. Compartments holding stimulus females were separated from the central arm by either plastic mesh screen (Experiment 1, All Senses), Plexiglas barrier (Experiment 2, Visual Only), or opaque Plexiglas barrier with 5 mm-high slit (Experiment 3, Olfactory Only). For further details see text.

& Gonzalez-Lima, 1997; Crews, Coomber, & Gonzalez-Lima, 1997). Together, these findings suggest the hypothesis that adult mate choice is likewise shaped by the embryonic environment in general, and embryonic temperature in particular.

Here we test this hypothesis in adult leopard geckos by presenting males from different incubation temperatures with females in the simultaneous choice paradigm of a Y-maze. In particular, we examine the choices made by males with different temperature phenotypes when presented with two females from different incubation temperatures. Males from 32.5°C successively presented with females of different temperature phenotypes were observed to court “colder” females (30.0°C) more than “warmer” females (32.5°C) (Flores et al., 1994), suggesting that when given a simultaneous choice they

would prefer colder females. According to our hypothesis that mate choice behavior in males depends on the temperature experienced during in ovo development, males from 30.0°C should choose the female from the warmer incubation temperature instead.

MATERIALS AND METHODS

Animals

All animals were hatched from eggs of known parentage collected on the day of laying. Eggs were incubated individually in moist vermiculite (1.5 water: 1 vermiculite, v/v) at constant temperatures ($\pm 1^\circ\text{C}$) that produce either 75% female and 25% male hatchlings (30.0°C), 25% female and 75% male hatchlings (32.5°C), or over 95% female and under 5% male hatchlings (34.0°C). Incubation temperatures were accordingly referred to as $T_{30.0}$ (30.0°C), $T_{32.5}$ (32.5°C), and $T_{34.0}$ (34.0°C). After hatching, animals were raised in isolation in propylene containers (30 × 12 × 6 cm) provided with a water dish and shelter, and fed live vitamin supplement-dusted crickets daily. During weeks 1–10 posthatching, juveniles were raised in environmental chambers set to a constant temperature of 30°C and a light-dark cycle of 14:10 L:D. Thereafter, chambers were maintained on a daily thermal cycle of 30°:18°C (14:10 hr) and 14:10 L:D photic cycle, with the humidity of 70%. Diet consisted of water and mealworms dusted with vitamin supplements given only three times a week. Adult animals (17–24 months) were introduced to new polypropylene solid-bottom home cages (20 × 24 × 45 cm) lined with a clean paper towel, and containing a water dish and plastic shelter. They were allowed to acclimate to their new environment for 1 week before behavior experiments commenced. All test animals were controlled for size and body weight in order to avoid possible size preferences as confounding parameters.

In a first phase of the experiment, all here-to-fore sexually naïve males were given a single mating experience with a female of known incubation temperature (either $T_{30.0}$ or $T_{34.0}$) in order to determine whether first sexual experiences will bias subsequent male mate preferences. Only males that successfully mated (i.e., complete mounting, copulation, and ejaculation) were recruited for subsequent mate choice tests. This resulted in seven $T_{30.0}$ males and seven $T_{32.5}$ males in the second phase of the experiment. Here, males were presented with a $T_{30.0}$ female and a $T_{34.0}$ female in the simultaneous choice paradigm of a Y-maze in order to test the hypothesis of incubation temperature-dependent mate preference in adulthood. No females of the rather scarce $T_{32.5}$ phenotype were available to us, so that $T_{34.0}$ females had to be used instead. Stimulus females could be the same as used in the first phase of the experiment, but males were never tested with the same female they encountered during their first sexual experience. A total of 19 females (8 $T_{30.0}$ and 11 $T_{34.0}$) were used as stimulus animals. The same female could serve as a stimulus female more than once, and in some cases different males were tested with the same pair of stimulus females. The reproductive status of each female was monitored following Rhen, Sakata, Zeller, and Crews (2000) prior to the experiments, and only

females that had reached late vitellogenesis were used. In female leopard geckos, reproductive status can be determined visually, since follicles and eggs are visible through the semi-translucent abdominal wall. Nonvitellogenic females respond aggressively towards courting males (Rhen, Sakata, & Crews, 1998) and would have introduced a bias in the male's choice.

Behavior Experiments and the Test Arena

Males were tested in a Y-maze testing arena within 1 week of their mating experience (Fig. 1b). The test male was placed into the central arm of the clear Plexiglas Y-maze (30.5 cm × 12.5 cm × 15.0 cm), while a $T_{30.0}$ and a $T_{34.0}$ stimulus female were placed into respective compartments at the ends of the left and right arm (32.5 cm × 12.5 cm × 15.0 cm) of the maze. The maze was then covered with a Plexiglas lid that was perforated thrice (each hole 2.5 cm in diameter) at the end of the central arm furthest away from the right and left arms of the Y. Female compartments were separated from the central arm holding the test male by divisions that could be varied to permit visual and olfactory stimuli together or separately; acoustic stimuli were present in all conditions. Males were tested under three sensory conditions sequentially:

1. Experiment 1. All senses: Compartments were separated by plastic mesh screen and allowed for the male to receive visual and olfactory information about the stimulus females.
2. Experiment 2. Visual only: Compartments were separated by Plexiglas barrier so that males were only able to see but not smell females. Spectrophotometric analysis of the Plexiglas used revealed ultraviolet (UV) blocking properties (90% transmission $T(\lambda)_{90} = 402 \text{ nm}$).
3. Experiment 3. Olfactory only: Compartments were separated by a black nontransparent Plexiglas screen with a 5 mm-high slit along the bottom length allowing for test males to smell but not see stimulus females.

Stimulus females were unable to see one another. Female placement was randomized and the maze washed thoroughly between trials with lipid-dissolving cleaning detergent (Alconox, Alconox, Inc., White Plains, NY) to remove possible scent markings left by previous test animals.

Prior to their first behavior test in the Y-maze, each experimental male was given three acclimatization sessions, consisting of a 15–20 min habituation period followed by 5 min with receptive stimulus females present in the stimulus compartments at the end of the long arms of the maze. As a result, males were familiar with being placed into the maze and associated it with the presence of females. Lining the center compartment with filter paper from the males' home cages during testing prevented the animals from perceiving the maze as new territory that had to be scent marked. Consequently, it was possible to reduce the length of the habituation period during the actual experiments to 10 min after which the choice stimuli were introduced and males were tested for 15 min. The interval between the three sensory paradigms was 1 week.

During the 15 min test period, we quantified the time males spent in association with stimulus females. Association time is a commonly used measure for mate choice in a

variety of vertebrate models including teleosts and birds (Amundsen, Forsgren, & Hansen, 1997; Cummings, Rosenthal, & Ryan, 2002; Hansen, Amundsen, & Forsgren, 1999; Ruscio & Adkins-Regan, 2003; Ryan & Wagner, 1987; Schlupp et al., 1994). A male was considered to associate with a stimulus female when it had crossed the line marking the entrance into the association area in front of the female compartment and faced the female. Throughout the time males spent in association with the females the number of air-licks, and the frequency and duration of tail vibrations, the test male exhibited in front of either stimulus female were recorded and analyzed. Both are stereotypical components of the normal courtship repertoire displayed by male leopard geckos (Rhen & Crews, 2000), and therefore indicative of the degree of an animal's motivation to approach a particular female. During a sexual encounter, the male approaches the female and samples for female pheromones (Mason & Gutzke, 1990) by licking both the substrate or air (i.e., air licks), followed by licking the female. The male will then vibrate his tail against the substrate, thereby producing an audible buzz and a tactile vibration of the substrate (i.e., tail vibration). While a receptive female responds to the tail vibration by remaining static and occasionally by lifting her tail to signal readiness, a nonreceptive female usually avoids the male, or shows an aggressive display composed of stiling (pushing the body off the ground and arching the back), and waving the tail in an undulating fashion. Unless the female behaves aggressively, the courting male will follow his tail vibration with body gripping and mounting. The test was terminated either after 15 min, or if the test male did not enter the association area of any stimulus female once within 10 min of the test. In order to control for female behavioral responses to male courtship, which could influence the male's decision, all females were video taped during the behavior experiments. Tests in which one or both females exhibited an aggressive display toward the experimental male were excluded from the analysis.

Since leopard geckos are nocturnal (Anderson, 1963; Daniel, 1983), animals were tested between 17 and 20 h under red light in a darkened room. In addition to providing more natural conditions, this also minimized disturbance posed by the investigator's presence (Steele & Cooper, 1997).

Statistics

All measured parameters were analyzed using the Wilcoxon matched-pairs signed-ranks test. Association time was analyzed both as absolute time (s) and as relative time (% of total association time with both females). Differences were accepted as statistically significant when $p < .05$. All values are expressed as means \pm SEM unless stated otherwise, with indication of number (n) of separated determinations corresponding to individual numbers.

RESULTS

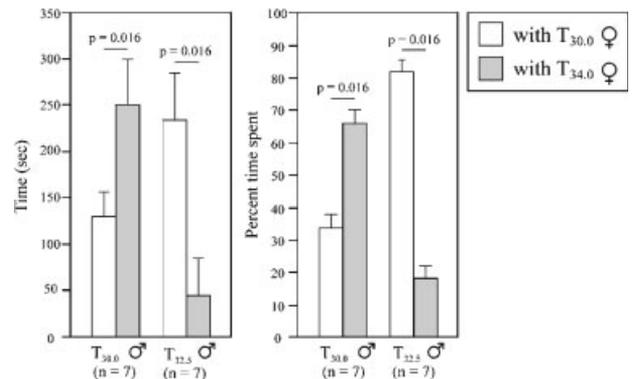
Mate Preferences of Males under the All Senses Paradigm

Both male temperature phenotypes exhibited a clear mate choice under the all senses paradigm (Fig. 2a). Yet,

while $T_{30.0}$ males spent more time (both absolute and relative) with $T_{34.0}$ females than with $T_{30.0}$ females (absolute: $p = .016$; relative: $p = .016$), $T_{32.5}$ males associated longer with $T_{30.0}$ females (absolute: $p = .016$; relative: $p = .016$). In both male phenotypes, relative association times showed little variation, revealing a very robust effect of embryonic environment on adult mate preference (Fig. 2b). These data also showed that males did not prefer the female phenotype they encountered during their first sexual experience, but rather chose unanimously one phenotype over the other. The air-licking frequency of $T_{30.0}$ males approached a significantly higher level for $T_{30.0}$ females than $T_{34.0}$ females ($p = .062$, Tab. 1). In $T_{32.5}$ males, this difference was

All Senses

a Comparison of absolute and relative association time



b Relative association time by individual male

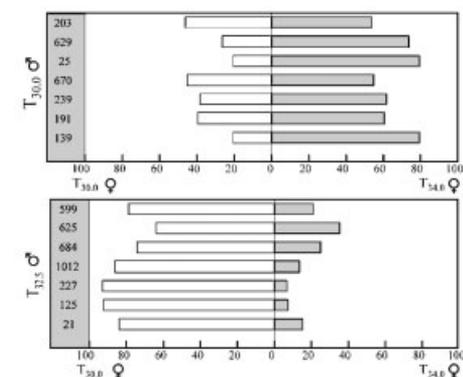


FIGURE 2 Results Experiment 1: All Senses. (a) $T_{30.0}$ males spent more time associated with $T_{34.0}$ females ($p = .016$), while $T_{32.5}$ males associated longer with $T_{30.0}$ females ($p = .016$). (b) The analysis by male shows little variation, revealing a very robust effect of the embryonic environment on adult mate preference. (All values mean \pm SEM, except for individual values in panel b, open bars = values measured with $T_{30.0}$ female, gray bars = values measured with $T_{34.0}$ female.)

Table 1. Air-Licks Per Second and Tail Vibrations Per Second as Displayed by $T_{30.0}$ and $T_{32.5}$ Males in the Y-Maze under the all Senses, Visual Only, or Olfactory Only Paradigms

	$T_{30.0}$ males				$T_{32.5}$ males			
	<i>n</i>	$T_{30.0}$ females	$T_{34.0}$ females	<i>p</i>	<i>n</i>	$T_{30.0}$ females	$T_{34.0}$ females	<i>p</i>
Air-licks per second								
All senses	7	.141 ± .053	.097 ± .0323	.062	7	.099 ± .0190	.039 ± .0224	.031 ^a
Visual only	7	.193 ± .0618	.107 ± .0432	.031 ^a	7	.140 ± .0271	.203 ± .0300	.109
Olfactory only	6	.206 ± .0423	.209 ± .0299	.844	6	.179 ± .0459	.249 ± .0490	.813
Tail vibrations per second								
All senses	4	.006 ± .0023	.005 ± .0013	.875	4	.007 ± .0018	—	—
Visual only	2	.010 ± .0060	—	—	5	.005 ± .0019	.003 ± .0017	.625
Olfactory only	1	.020	—	—	1	—	.003	—

All values = mean ± SEM.

^aSignificant ($p < .05$).

significant ($p = .018$). Appetitive behavior (i.e., tail-vibrating) was not displayed by every male tested (four of seven males for each phenotype). $T_{32.5}$ males only tail-vibrated at the preferred $T_{30.0}$ females, whereas $T_{30.0}$ males did not differ in the frequency of tail-vibration exhibited to the two types of stimulus females ($p = .793$).

Mate Preferences of Males under the Visual Only Paradigm

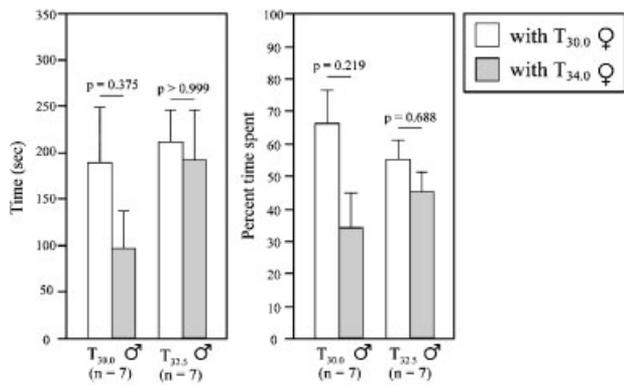
When restricted to the Vision Only paradigm, neither $T_{30.0}$ males nor $T_{32.5}$ males showed a preference for either phenotype of stimulus females ($T_{30.0}$ males relative time: $p = .375$, absolute time: $p > .999$; $T_{32.5}$ males relative time: $p = .219$, absolute time: $p = .109$; Fig. 3a). Overall, the individual decisions of males varied more within each phenotype than under the all senses condition (Fig. 3b). $T_{32.5}$ males showed a greater air-licking frequency when associated with $T_{30.0}$ females than with $T_{34.0}$ females ($p = .031$; Tab. 1). That was not the case for $T_{32.5}$ males ($p = .109$). As before in the all senses paradigm, not all males tail-vibrated. In fact, fewer $T_{30.0}$ males (two of seven) and more $T_{32.5}$ males (five of seven) than before displayed appetitive behavior. Both of the $T_{30.0}$ males that tail-vibrated displayed to $T_{30.0}$ females only, while $T_{32.5}$ males exhibited tail-vibration to both stimulus females. There was no significant difference in the frequency of tail-vibrations with which $T_{32.5}$ males courted $T_{30.0}$ females or $T_{34.0}$ females ($p = .625$).

Mate Preferences of Males under the Olfactory Only Paradigm

In this third experimental design, both groups of males consisted of only six animals, due to the death of one $T_{30.0}$ male and removal of a sick $T_{32.5}$ male. Permitting males only olfactory access to stimulus females was sufficient for the majority of $T_{30.0}$ males to exhibit a clear

Visual only

a Comparison of absolute and relative association time



b Relative association time by individual male

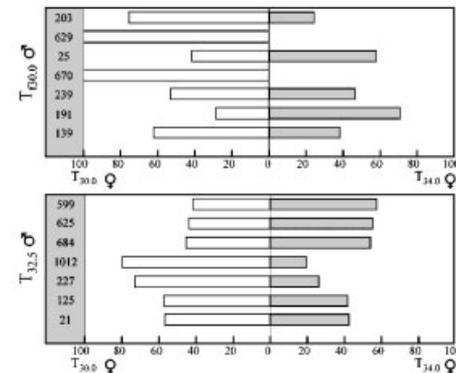


FIGURE 3 Results Experiment 2: Visual Only. Neither $T_{30.0}$ males nor $T_{32.5}$ males exhibited any significant preference for one of the two stimulus female phenotypes. (All values mean ± SEM, except for individual values in panel b; open bars = values measured with $T_{30.0}$ female, gray bars = values measured with $T_{34.0}$ female.)

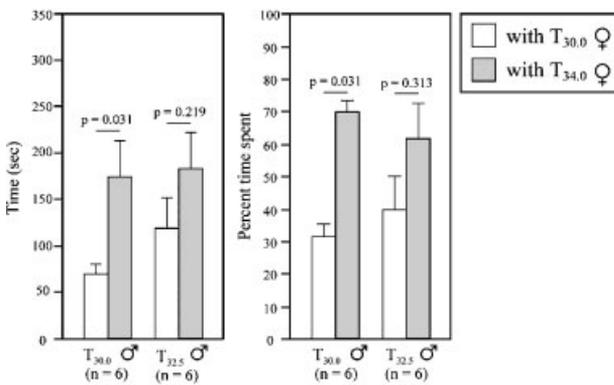
mate choice, but not for $T_{32.5}$ males (Fig. 4a and b). $T_{30.0}$ males associated significantly longer with $T_{34.0}$ females ($p = .031$). Neither of the two male phenotypes displayed a greater frequency of air-licks during association with stimulus females ($T_{30.0}$ males: $p = .844$; $T_{32.5}$ males: $p = .813$, Tab. 1). Only one male of each phenotype tail-vibrated; while the $T_{30.0}$ male tail-vibrated at the $T_{30.0}$ female, the $T_{32.5}$ male tail-vibrated at the $T_{34.0}$ female.

DISCUSSION

Sociosexual experience early in life has long been identified as a source of variation in adult mate choice

Olfactory only

a Comparison of absolute and relative association time



b Relative association time by individual male

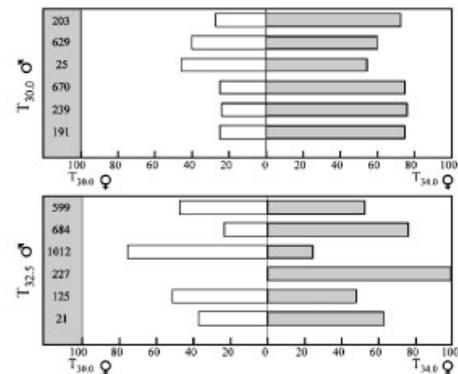


FIGURE 4 Results Experiment 2: Olfactory Only. $T_{30.0}$ males exhibited a clear mate preference for $T_{34.0}$ females ($p = .031$), whereas $T_{32.5}$ males did not show a preference for any of the two female phenotypes ($p = .219$ absolute; $p = .313$ relative). (All values mean \pm SEM, except for individual values in panel b; open bars = values measured with $T_{30.0}$ female, gray bars = values measured with $T_{34.0}$ female).

behavior. In vertebrates, this is exemplified above all by sexual imprinting, a process first described in birds in which a young animal learns to prefer distinct parental traits in future prospective sexual partners (Immelmann, 1975). Other aspects of adult sexual behavior are also affected by early experiences, and studies both in oviparous and viviparous species have demonstrated that the embryonic environment as a nonsocial experience can wield the necessary modulating influences (Crews, 2003; Lehmann, Stöhr, & Feldon, 2000; Sakata & Crews, 2003). Although together these studies might suggest a correlation of embryonic environment and adult mate preference, there is little experimental evidence in support of this hypothesis. Here we report that in male leopard geckos adult mate choice is shaped by the embryonic temperature. Given the simultaneous choice between two phenotypes of females under the All Senses paradigm, male leopard geckos from different incubation temperatures exhibited opposite mate preferences. To our knowledge, the present study is the first to identify a nonsocial, epigenetic factor that exerts its influence during embryonic development and thus shapes future mate preference in adulthood.

Although sex recognition in squamates is thought to rely on both vision and chemoreception, it is the latter that is commonly assigned primary importance during courtship (Mason, 1992). Indeed, male leopard geckos generally court females with intact skin but respond with antagonistic behavior to females that are shedding. Mason and Gutzke (1990) suggest the presence of a skin-derived odoriferous chemical that is important for sex-recognition and is transiently undetectable during shedding. In addition to sex recognition, such pheromones, and particularly their composition, have been reported to determine mate preference in other squamates (Mason, 1992). When presented with a choice between skin lipid extracts from large and small females, male red-sided garter snakes (*Thamnophis sirtalis parietalis*) prefer extracts from large females, which contain higher concentrations of unsaturated methyl ketones (LeMaster & Mason, 2002). In light of these findings it is striking that $T_{32.5}$ male leopard geckos only displayed a preference when permitted multimodal sensory access to the stimulus females (All Senses paradigm), whereas $T_{30.0}$ males chose even in the Olfactory Only paradigm. While this seems to suggest that olfaction is of greater importance for $T_{30.0}$ than $T_{32.5}$ males, a closer look at air-licking behavior of the two male phenotypes renders such a conclusion premature. In both cases where $T_{30.0}$ males showed mate preference (i.e., All Senses and Olfactory Only) the air-licking frequencies with which males sampled both stimulus females did not differ significantly. In contrast, mate choice in $T_{32.5}$ males corresponded to the air-licking frequency under the All Senses condition;

males associated with the female phenotype they sampled at a greater frequency. In the two experimental settings where $T_{32.5}$ males did not exhibit clear mate preference (i.e., Visual Only and Olfactory Only), the air-licking frequencies for both stimulus females were virtually identical. Apparently a unimodal signal (i.e., scent) is sufficient for $T_{30.0}$ males to make a decision, whereas $T_{32.5}$ males require a multimodal signal (scent and vision). This hypothesis is supported by the fact that both male phenotypes did not associate longer with either of the stimulus females when allowed only visual access. Despite our results from the Visual Only experiment, it is conceivable that the visual signal alone is sufficient to elicit choice in either male phenotype, but only if the crucial part of the signal ranges in the UV spectrum that was blocked by the Plexiglas we used. UV sensitive receptors have been identified in the retinas of a number of squamates (Sillman, Johnson, & Loew, 2001), and UV vision plays a role in mate choice for numerous vertebrates (Cummings et al., 2002). Alternatively, specific female behavior patterns and not the use of UV cues may influence male mate choice. If so, they were missed by the experimenters both during the test and in later analysis of the video recordings of the stimulus females.

Receiver psychology has long pointed out that the informational value of a signal can depend on whether it comprises only one type of stimulus or is compounded of many (Couvillon & Bitterman, 1982; Guilford & Dawkins, 1993; Rowe, 1999). Moreover, experimental results clearly indicate that multimodal signals can be pivotal for mate choice decisions in vertebrates. In garter snakes large males only engage in intense courtship when both visual and olfactory cues indicate the presence of large females, whereas small males are much less discriminating in both respects (Shine, Phillips, Wayne, LeMaster, & Mason, 2003). Similarly, the two male phenotypes of leopard geckos in our study may reflect a difference in mate perception not unlike that observed in garter snakes. Since perception requires analysis of sensory information in higher neural circuits (Beach, 1983; Wagman & Miller, 2003), it seems plausible to propose a direct effect of the incubation temperature on the development of particular brain regions as the mechanism underlying this embryonic environmental imprinting. One possible target for such organizational influences could be the two olfactory systems that vertebrates evolved (Halpern & Martinez-Marcos, 2003). Anatomically distinct structures that differ significantly in their central neural projections, the systems have discrete functions. While the main olfactory system is primarily responsible for the detection of volatile odors, the accessory olfactory system facilitates detection of nonvolatile odoriferous chemicals (Firestein, 2001).

Which of the two systems is involved in mate recognition and preference is the subject of an ongoing controversial debate, but work in mice and humans suggests that both are of pivotal importance (Bakker, 2003). What is more, both systems appear to be either directly or indirectly affected by the embryonic environment. For one, there is evidence that the sensitivity of the main olfactory system to pheromones is embryonically organized by sex steroid hormones (Baum & Keverne, 2002). Further, projections from the accessory system relay olfactory information into the limbic brain, in particular the preoptic area (POA) and the hypothalamus (Scalia & Winans, 1975).

Unlike in mammals, the POA in leopard geckos is not sexually dimorphic, but varies in same-sex animals in relation to their incubation temperature (Coomber et al., 1997). In males, the POA is larger in $T_{32.5}$ than in $T_{30.0}$ animals. Similarly, the volume of the ventromedial nucleus of the hypothalamus (VMH) varies with the incubation temperature, being smaller in $T_{32.5}$ than in $T_{30.0}$ males. Besides the size of the POA and VMH, the neural activity of both nuclei in $T_{30.0}$ and $T_{32.5}$ males are subject to embryonic organization in response to incubation temperature. As studies measuring cytochrome oxidase (CO) activity, a rate-limiting enzyme in oxidative phosphorylation in nervous tissues, have shown, limbic brain nuclei such as the POA and VMH differ significantly in their metabolic capacities (Coomber et al., 1997; Crews et al., 1997). Functionally coupled neural regions are believed to exhibit covariant changes of CO activity proportional in magnitude to the strength of correlation between the nuclei (Sakata, Crews, & Gonzalez-Lima, 2004). The correlation patterns determined for limbic brain nuclei in $T_{30.0}$ and $T_{32.5}$ males differ significantly (Sakata, Coomber, & Gonzalez-Lima, 2000), again suggesting a causative link between incubation temperature and functional connectivity of limbic regions.

Previous work in leopard geckos and in rodents has hinted towards the possible importance of the embryonic experience for an individual's attractiveness. In the leopard gecko, females from an incubation temperature producing exclusively female hatchlings (26.0°C) receive more courtship than females from any other incubation temperature (Flores et al., 1994). Female mice that develop in intrauterine proximity to two female siblings (2F females) are more attractive to males than females that develop between two male neighbors (2M females) (vom Saal & Bronson, 1978). Complementarily, female gerbils respond more to scent markings of 2M males than markings by 2F males (Clark & Galef, 1994). However, none of these studies considered whether the embryonic experience of the choosing animal affected the observed choice. Our results demonstrate that in the leopard gecko male mate choice is incubation temperature dependent, but it further suggests that female attractiveness and

male mate choice behavior are closely related variables of a complementary system. That is, whether or not a particular female phenotype is more attractive than another may only be answered in the context of the choosing male. Likewise, a male's choice may depend as much on the embryonic environment experienced by the stimulus females as on his own embryonic experience.

The potential evolutionary ramifications of the embryonic environmental imprinting we observed in male leopard geckos seem complex and are not immediately apparent. Most obviously, it is surprising that male leopard geckos display mate choice at all. According to sexual selection theory, males choose between potential mates only if their investment in the offspring exceeds that of the females, or when female fecundity varies in relation to body size (Andersson, 1994; Savalli, 2001). For instance, in syngnathid fishes sex roles are reversed and males are responsible for egg care, which has led to male mate choice (Berglund, Rosenqvist, & Svensson, 1986; Berglund & Rosenqvist, 1993). Further, males in a variety of squamate species where the number of eggs per clutch increases with maternal body size favor larger over smaller females (LeMaster & Mason, 2002; Olsson et al., 2002). In leopard geckos, however, females invest more energy in the offspring than males, and fecundity is reduced to two eggs per clutch regardless of maternal body size. That males exhibit mate preference nonetheless would be less surprising, if they were to gain an apparent benefit from their preference.

One such adaptive function may be sib avoidance, especially given clutches of two where eggs are incubated at the same environmental temperature. Yet, since leopard geckos mature at about 1 year of age it seems not convincing to have such a mechanism in place unless siblings remained in close proximity throughout this first year of life or the species showed strong philopatry toward their natal breeding sites. Little is known about the natural habitat sizes of male leopard geckos or of their dispersal patterns during early life. Results of recent studies suggest natal philopatry in some lizards (Fuller, Bull, Murray, & Spencer 2005), but it is unclear whether this also applies to leopard geckos.

The other possible adaptive value of embryonic environmental imprinting would be if offspring quality differed in females from different incubation temperatures. Virtually nothing is known about the survival prospects of juvenile leopard geckos in the wild, let alone the dependence of survival rates on maternal temperature phenotype. However, an analysis of egg weights and sizes, as well as hatchling biometrics in captive animals did not reveal any particular correlation of maternal temperature phenotype and offspring quality (Putz unpublished).

It must not be underestimated that the animals in the present study were all incubated at a constant temperature throughout their entire in ovo development, which may have heightened the temperature effect on adult mate preference. For this embryonic imprinting to serve any of the discussed adaptive purposes, it would have to be pronounced enough even in case of variable nest temperatures as can be found in the wild. An alternative explanation may be that the observed effect is simply a by-product of the brain's ability to develop with great plasticity from earliest embryonic stages onwards. As such, embryonic environmental imprinting as described in the present study would not have any adaptive value per se, but rather arise from the physiological context of the developing brain (not unlike Gould and Lewontin's spandrels, Gould & Lewontin, 1979).

Regardless of its evolutionary significance, embryonic environmental imprinting certainly bears consequences for mate choice studies in general. For one, it implies that results from studies with individuals of which the embryonic history is unknown (e.g., field studies) have to be interpreted in light of a possible embryonic environmental imprinting. Assuming that neural substrates of sexual behavior are the target of such an imprinting, it may be helpful to combine mate choice studies with analysis of brain morphologies where circumstances permit. Studies with other species where the embryonic environment produces distinct adult phenotypes (e.g., intra-uterine position effect in rodents) would be informative. Furthermore, our results point out the importance of yet another epigenetic component driving mate choice and thus stress the evolutionary significance of the organism-environment continuum.

NOTES

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