



## Social experience affects territorial and reproductive behaviours in male leopard geckos, *Eublepharis macularius*

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Social interactions have lasting effects on behaviour and physiology in a variety of organisms. In the leopard gecko, *Eublepharis macularius*, social experience alters neural metabolism and elevates circulating concentrations of androgens. In this study, we assessed the effects of social experience (housing with females versus housing in isolation) on the expression of social behaviours in male geckos (1) when gonadally intact, (2) following castration and (3) following testosterone administration. Given the neural and endocrine changes following social experience, we hypothesized that social experience would increase the capacity to display territorial and courtship behaviour in male leopard geckos. We found that intact males previously housed with females (experienced males) displayed more territorial marking and more activity when exposed to a neutral test arena relative to males housed in isolation (naïve males). Experienced males continued to show more marking and activity in the testing arena relative to naïve males following castration. However, the courtship behaviour of castrated naïve and experienced males did not differ significantly. Following testosterone administration, experienced males again showed more activity in the empty test arena and tended to show more courtship behaviour. In summary, we found support for the hypothesis that social experience leads to changes in territorial and courtship behaviours and, moreover, found that male leopard geckos share some degree of commonality with other vertebrates in behavioural plasticity following social experience.

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While much of the research in behavioural biology has focused on the persistent effects of early experiences on adult behaviour (Larsson 1978; Meisel & Sachs 1994; Sandnabba 1996), it has become increasingly apparent that experiences in adulthood, particularly social interactions, significantly affect phenotype. For example, aggressive experience and social environment alter the neuroendocrine system and the probability of displaying sexual and aggressive behaviours in mammals (reviewed in de Jonge & van de Poll 1984; Pendergrass et al. 1989; Sandnabba 1996; Cant 2000; French & Schaffner 2000; Ginther et al. 2001), lizards (e.g. Greenberg & Crews 1990; Stamps & Krishnan 1999), birds (e.g. Rundfeldt & Wingfield 1985; Wingfield et al. 1991; Mougeot 2000) and fish (e.g. Francis et al. 1993). Social status also affects the neurochemical modulation of escape behaviour in crayfish (Yeh et al. 1996). In particular, the presence of, and interaction with, individuals of the opposite sex

dramatically affects subsequent behaviour. For example, the presence of courting males facilitates ovarian growth in lizards and birds (Crews & Silver 1985; Ball & Bentley 2000). Interactions with females affect the propensity to display aggression in males of a variety of species (e.g. reviewed in de Jonge & van de Poll 1984; Zucker 1994), and courtship experience in adulthood significantly affects sexual preferences in male zebra finches, *Taeniopygia guttata* (Immelmann et al. 1991) and gynogenetic fish (Marler et al. 1997). Finally, sexual experience in adulthood increases the capacity to display copulatory behaviour in the absence of androgens in male rats, hamsters and cats (Larsson 1978; Meisel & Sachs 1994) and increases the sensitivity to the activational effects of androgens on the reinstatement of copulatory behaviour in male rats (Larsson 1978; Retana-Marquez & Velazquez-Moctezuma 1997).

Both species and strain within a species alter the effects of social and sexual experience on subsequent behaviour. For example, in male rats but not in rhesus monkeys, *Macaca mulatta*, mating experience can reverse the detrimental effects of social isolation on copulatory behaviour in adulthood (reviewed in Larsson 1978). Furthermore, in

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mice, early social experience facilitates the expression of both aggressive and copulatory behaviours in adulthood, but males selectively bred for aggressiveness show a greater facilitation than males selectively bred for non-aggressiveness (reviewed in Sandnabba 1996). Across strains of mice and guinea pigs there is also significant variation in experience-dependent changes in copulatory behaviour of intact and castrated males (Valenstein et al. 1955; McGill 1978). Species and strain differences in behavioural plasticity in response to social experience are likely to be correlated with differences in neural plasticity in brain regions modulating social behaviours.

In this study, we investigated the effects of extensive social experience on the behavioural phenotype of the leopard gecko, *Eublepharis macularius*. In a previous study, the effects of extensive social experience on neural metabolism and sex steroid hormone concentrations were investigated (Crews et al. 1997). One objective of the present study was to assess the behavioural correlates of these neural and hormonal changes. The leopard gecko is a medium-sized lizard indigenous to western India, Pakistan and Turkey, and is an interesting model system in which to study the biological basis of individual differences in behaviour. Early experience, namely embryonic incubation temperature, not only determines gonadal sex in this species, but also has profound effects on adult neuroendocrine and behavioural phenotypes (reviewed in Crews et al. 1998). For example, males hatched from eggs incubated at a temperature that produces primarily males (32.5 °C) have elevated concentrations of androgens and depressed concentrations of oestrogens relative to males from eggs incubated at a temperature that produces primarily females (30 °C) (Tousignant & Crews 1995; Coomber et al. 1997). Furthermore, males and females hatched from eggs incubated at warmer incubation temperatures are more aggressive in adulthood than individuals from cooler temperatures (Flores et al. 1994). In the current experiment we investigated the effects of social experiences in adulthood on behavioural phenotype by comparing the behaviours of socially experienced and naïve male geckos when gonadally intact, following castration and following androgen replacement. We hypothesized that, relative to socially naïve males, experienced males would be (1) more territorial, (2) more likely to display courtship behaviour following castration and (3) more sensitive to the activational effects of androgens on courtship behaviour.

## METHODS

### Animals and Procedures

We reared male leopard geckos in isolation until sexual maturity. All males were hatched from eggs incubated at 32.5 °C. We only used males hatched from eggs incubated at 32.5 °C because the effects of social experience on brain metabolism have been previously investigated in males from this incubation temperature (Crews et al. 1997) and because an aim of this study was to assess the behavioural correlates of these neural and hormonal changes. We

housed all geckos individually in polypropylene containers (30 × 12 × 6 cm) after hatching. For the first 10 weeks, we maintained all individuals on a 14:10 h light:dark cycle, at 30 °C, and provided water and crickets 5 days a week. From then on, we maintained individuals on an LD 14:10 h cycle during which temperature ranged from 18 °C at night to 30 °C during the day. We provided water and mealworms three times a week. Crickets and mealworms were dusted with vitamin supplements. After reaching sexual maturity, we placed males (1–1.5 years of age) either in an isolate cage (45 × 25 × 20 cm), or in a breeding cage with three to four intact, cycling female geckos (60 × 60 × 45 cm). Both groups of males were housed in the same environmental chamber (LD 14:10 h cycle; temperatures ranged from 18 °C at night to 30 °C during the day). Males were left in their respective housing conditions for 1–2 years and then tested for social behaviours.

### Behavioural Testing

The experimental design is summarized in Table 1. First, we investigated behavioural differences between gonadally intact males housed in isolation (i.e. naïve males,  $N=11$ ) versus males housed with intact females (i.e. experienced males,  $N=10$ ). We placed males in a neutral testing arena (45 × 25 × 20 cm) lined with a fresh paper towel, and observed activity and scent-marking behaviours for 5 min. We recorded the duration of active movement and scent marking. We defined activity as movement of the body and limbs in any direction (i.e. ambulation). Scent marking is a characteristic territorial behaviour in which the preanal pores are pressed down onto the substrate and swiped laterally. Because we considered scent marking and activity as separate behaviours, our measures of activity duration do not include scent-marking duration. If we did not observe the behaviour of interest, we assigned a duration of zero. We tested each male twice (day 1 and 4), separated by 3 days.

Thereafter, we obtained a rough estimate of the sexual vigour of the experienced males by testing them in the neutral arena with a receptive female. We did not test naïve males to preclude any social experience with females prior to castration. In these tests, we placed the males in a test arena freshly lined with paper towels and allowed them to habituate for 5 min. Thereafter, we placed a receptive female in the arena and observed courtship behaviour. Courtship behaviour in this species has previously been described (Crews et al. 1998). We first screened females with sexually vigorous males and classified only females that remained motionless and did not bite back in response to courtship as receptive. We recorded whether the male body-gripped the stimulus female within 5 min after her introduction. We tested each male three times, each occasion separated by 5 days. All experienced males in this experiment courted females on at least one of the three tests. We also placed naïve males in the test arenas three times for the same duration (i.e. 10 min) to equalize the amount of exposure to the test chamber and to ensure that differences in behaviour

**Table 1.** Experimental design and statistical analysis

	Intact	Castrated	T implant
Behavioural protocol	Males were tested twice in the empty test arena, and tests were separated by 3 days.	Males were tested 20 times with a female in the test arena. Males were first observed for 5 min in the empty test arena before the introduction of the female. Testing began 1 week after castration and tests were separated by 4 days.	Males were tested with a female in the test arena (8–10 tests). Testing began 3 days after implantation, and tests were separated by 4 days. Four days after the last test, all subjects were killed and checked for residual testes and implants.
Statistical analyses	Group differences in the percentage of males showing activity and scent-marking behaviour and in average duration of activity were analysed.	Average duration of activity as well as the percentage of tests in which scent-marking, body-gripping and mounting behaviours were displayed were calculated for each male within each phase (i.e. following castration and following testosterone implantation). Both experimental phases and all four behaviours were analysed in one two-way MANOVA (independent variables: group and experimental phase; dependent variable: behaviour).	

following castration would not be due to differences in the novelty of the test arena.

Thereafter, we gonadectomized all males under cold anaesthesia, returned them to an isolate cage, and allowed them 1 week to recover from surgery. We returned naïve males to their original home cage, and housed experienced males in the isolate cages in which they had been housed for 2 days prior to castration. Therefore, following castration, both groups of males were housed in cages in which they were familiar. Testing began 1 week after castration, and tests were separated by 4 days. We conducted 20 postcastration tests. We placed males in a neutral testing arena (45 × 25 × 20 cm) lined with a fresh paper towel for 5 min (habituation period) and recorded durations of activity and scent marking. Thereafter, we placed a receptive female in the arena and observed males for courtship behaviour. If the male failed to body-grip the female within 5 min, we terminated the test. However, if the male did body-grip the female within 5 min, we continued the test for up to 10 min to allow for mounting. We stopped testing immediately if and when a male mounted. All males stopped courting by the 20th test.

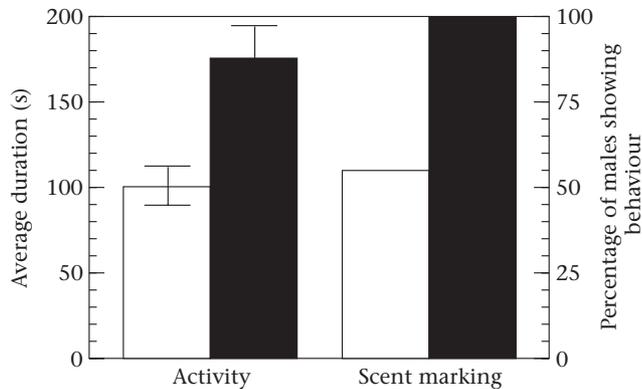
After the postcastration tests, we implanted males subcutaneously under cold anaesthesia with a Silastic testosterone (T) implant (1.96 outer diameter × 1.47 interior diameter × 20 mm). This implant size is known to approximate physiological levels of T in adult males and effectively elicits courtship behaviour in castrated males (Rhen & Crews 1999). After 3 days of recovery, we tested all males every 4 days with a receptive female in the testing arena using the paradigm described above (range 8–10 tests/male). We initially placed implants on the back (tests 1–5) but, because of frequent wound breaks, we moved the implants to an area just behind the shoulders after the fifth test. If implants were missing from the male, the male was not tested and was reimplanted immediately with the same implant. Testing resumed 4 days after reimplantation (i.e. at the time of the next scheduled test). Five males were not tested for at least one

test (one test was missing for four males, and two tests, for one male). Behavioural scores for males who needed reimplantation did not differ significantly from those who did not need reimplantation, and, therefore, this was ignored in the analysis. Four days after their final test, we killed the males by rapid decapitation and checked them for intact implants and for evidence of testicular growth. We excluded one experienced male from the experiment following castration because he had residual testes (i.e. experienced males,  $N=9$ ).

### Statistical Analyses

First, we analysed group differences in the proportion of males that showed activity or scent marking in the empty test arena when gonadally intact. We categorized males as 'active' if they were active on at least one of the two tests, and as 'inactive' if they were inactive on both tests. We used the same categorization for scent-marking behaviour. We analysed group differences using a likelihood ratio test. Furthermore, we analysed group differences in the average duration of activity (across days 1 and 4) using a univariate analysis of variance (ANOVA). The distribution of average activity durations did not deviate significantly from normality (Shapiro–Wilk  $W$  test:  $W=0.95$ ,  $N=21$ ,  $P=0.144$ ).

For the analysis of behavioural differences between naïve and experienced males when castrated and following T replacement, we analysed four behavioural parameters (average duration of activity in the empty test chamber, the percentage of tests in which the male scent-marked in the empty test chamber, the percentage of tests in which the male body-gripped the female and the percentage of tests in which the male mounted the female) across both experimental phases using a two-way repeated measures multivariate ANOVA. The between-subject variables were group (naïve versus experienced) and experimental phase (castrated versus T-implanted) and the within-subject variable was behaviour (i.e. the four behavioural parameters). We also included the



**Figure 1.** Relative to gonadally intact naïve males ( $N=11$ ; □), intact experienced males ( $N=10$ ; ■) showed more activity (longer average duration) in the empty test arena, and a significantly greater percentage of experienced males scent-marked in the arena.

identity of the male as a random variable nested within group; adding this factor eliminates the variability among subjects due to individual differences from the error term (Sokal & Rohlf 1995; Stevens 1996). All proportion data were arcsine square-root transformed to improve normality. We used Pillai's trace as our multivariate statistic because it is the most robust to deviations from multivariate normality and homogeneity of variance-covariance matrices (Olson 1974). There was a significant main effect of behaviour in all analyses because we used different scales of measurement for the behavioural scores; these statistics are not reported.

For all analyses, we set  $\alpha=0.05$ . All statistics were done using JMP version 3.1 (SAS Institute 1995). The experimental design and statistical approach are outlined in Table 1.

## RESULTS

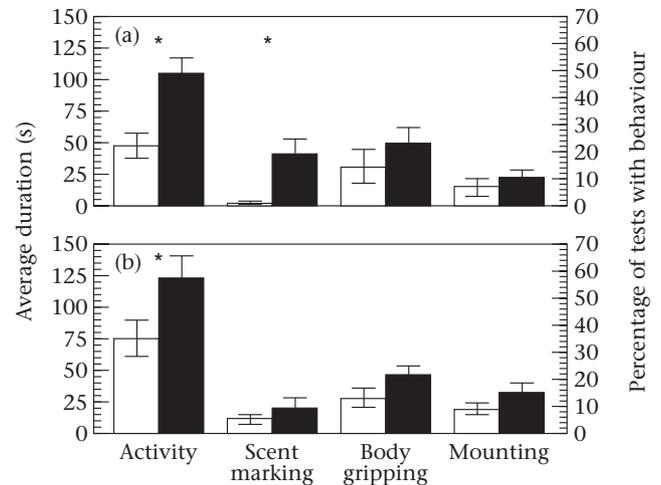
### Behavioural Differences between Experienced and Naïve Males

#### Gonadally intact

Whereas all naïve and experienced males were active when placed in the test chamber, a significantly greater percentage of experienced males displayed scent-marking behaviour (chi-square test:  $\chi^2_1=10.0$ ,  $P=0.002$ ; Fig. 1). Furthermore, although the percentage of active males was equal across the groups, the average activity duration was significantly higher in experienced males (ANOVA:  $F_{1,19}=9.4$ ,  $P=0.006$ ).

#### Following castration and following testosterone administration

There were significant main effects of group (MANOVA:  $F_{1,17}=62.0$ ,  $P<0.001$ ) and experimental phase ( $F_{1,17}=21.7$ ,  $P<0.001$ ). Overall, experienced males had elevated scores, and scores following T implantation were significantly higher than those following castration. More importantly, there were a number of significant



**Figure 2.** (a) Following castration, experienced males ( $N=9$ ; ■) showed more activity (longer average duration) and displayed scent-marking behaviour in the empty test chamber on a greater percentage of tests than naïve males ( $N=11$ ; □). However, there were no differences in the percentage of tests in which males body-gripped or mounted stimulus females. (b) Following testosterone implantation, experienced males showed increased duration of activity in the empty test chamber, and there was a trend for experienced males to show more body-gripping and mounting behaviour. \*Significant group differences.

interactions: group\*behaviour ( $F_{3,15}=10.3$ ,  $P=0.001$ ), experimental phase\*behaviour ( $F_{3,15}=4.6$ ,  $P=0.018$ ) and group\*experimental phase\*behaviour ( $F_{3,15}=3.5$ ,  $P=0.043$ ). Because we were particularly interested in group differences within each experimental phase, we ran separate MANOVAs for each phase.

In the analysis of group differences following castration, there was a significant effect of group (MANOVA:  $F_{1,18}=10.0$ ,  $P=0.005$ ), and overall, experienced males had elevated scores. More importantly, there was a significant group\*behaviour interaction ( $F_{3,16}=4.8$ ,  $P=0.014$ ). Consequently, we ran four separate univariate ANOVAs with group as the sole independent variable. Average activity duration ( $F_{1,18}=11.7$ ,  $P=0.003$ ) and the percentage of tests with scent marking ( $F_{1,18}=13.3$ ,  $P=0.002$ ) were significantly greater in experienced males, whereas there were no significant differences between the groups in the percentage of tests with body grips ( $F_{1,18}=1.8$ ,  $P=0.193$ ) or mounts ( $F_{1,18}=1.3$ ,  $P=0.278$ ; Fig. 2a).

In the analysis of group differences following T implantation, there was a significant main effect of group (MANOVA:  $F_{1,17}=5.2$ ,  $P=0.035$ ), and overall, experienced males had elevated scores. Although the group\*behaviour interaction did not reach significance ( $F_{3,15}=2.2$ ,  $P=0.132$ ), we analysed each behaviour separately using a univariate ANOVA to assess which behavioural parameters contributed most to this overall difference (Fig. 2b). We found that only the average duration of activity was significantly elevated in experienced males relative to naïve males ( $F_{1,17}=4.5$ ,  $P=0.050$ ). However, the difference in the percentage of tests with courtship behaviour approached significance (body grips:  $F_{1,17}=3.6$ ,  $P=0.074$ ; mounts:  $F_{1,17}=4.2$ ,  $P=0.057$ ).

## DISCUSSION

In this study, we analysed the behavioural differences between adult males given extensive social experience (i.e. males housed with females for 1–2 years: experienced males) and males housed in isolation for the same duration (naïve males). We analysed differences while gonadally intact, following castration and following testosterone (T) replacement (Table 1), and we investigated group differences in activity, scent-marking and courtship behaviours. We found that, relative to naïve males, experienced males displayed more territorial marking behaviour in the empty test arena when gonadally intact and following castration. Experienced males were also more active in the empty arena, and this difference was found under all hormonal conditions. There were no significant differences in courtship behaviour following castration between experienced and naïve males, but experienced males tended to show more courtship behaviour following T replacement.

Experienced males were more likely to display agonistic marking behaviour in the empty chamber than naïve males both when gonadally intact and following castration. Gonadally intact experienced males have elevated concentrations of androgens relative to naïve males (Crews et al. 1997), and because territorial marking is androgen dependent, it is possible that the difference in marking behaviour between experienced and naïve males when intact was due to this hormonal difference. However, that this behavioural difference persisted following castration suggests that the difference could be androgen independent. Aggression outside of the breeding season has been found independent of androgens in some birds (Wingfield 1994), and similar mechanisms might underlie the differences found here. Interestingly, the display of territorial behaviour during the nonbreeding season is independent of androgens in older male European starlings, *Sturnus vulgaris*, but not in young, less socially experienced males (Pinxten et al. 2000). Sexual experience increases aggressiveness in male rats in an androgen-independent manner (Albert et al. 1988), and it has been postulated that social experiences may be more critical in modulating the expression of agonistic behaviour than gonadal steroids (e.g. de Jonge & van de Poll 1984).

The amount of activity shown during the habituation period was the parameter most reliably affected by social experience. Experienced males were significantly more active when intact, following castration and following T administration, and this difference was not due to a difference in the proportion of tests in which males were active (Figs 1 and 2; data not shown). Naïve males tended to show little activity in the neutral test arena; this inactivity could be analogous to freezing behaviour in rodents during open-field tests, a behaviour that reflects the individuals' level of anxiety or fear (Dennenberg 1969). The relationship between open-field behaviour and anxiety, however, has not been investigated in reptiles. When gonadally intact, naïve males also showed more fleeing behaviour in the empty test arena (data not shown), and this is consistent with this interpretation.

Similarly, copulatory behaviour is less severely affected by novel environments in sexually experienced male rats than in naïve males, and a difference in anxiety in response to handling and novelty is thought to underlie this difference (Pfaus & Wilkins 1995).

Taken together, these data indicate that more active male geckos show more territorial behaviour. A similar phenomenon is found in mice. Male mice selected for aggressiveness show higher rates of ambulation than mice selected for nonaggressiveness (reviewed in Sandnabba 1996). Seasonal changes in activity are correlated with changes in territoriality in green anoles, *Anolis carolinensis* (Jenssen et al. 1995). One possible explanation for this parallel between agonistic behaviour and ambulation in the test chamber is that both are inversely related to the amount of fear or anxiety experienced by the individual. Males that are less stressed by handling and by placement into the test arena are more likely to explore and mark their territory. Interestingly, male rats are less aggressive in strange cages, and it is proposed that this is due to heightened fear or neophobia (Mink & Adams 1981).

There is considerable species variation in the effects of heterosexual social experience on the propensity to show sexual behaviour after castration and after hormone replacement. We found that socially experienced male geckos were not significantly more robust to castration than naïve males. Sexual experience also has negligible effects on postcastration sexual behaviour in dogs (Hart 1968), whereas in hamsters and mice, differences between experienced and naïve males persist up to 3–4 weeks after castration (Lisk & Heimann 1980; Phelps et al. 1998). Although the effect of sexual experience on rat copulatory behaviour following castration is less robust, significant differences between sexually experienced and naïve male rats have been documented (Larsson 1978; Retana-Marquez & Velazquez-Moctezuma 1997). However, socially experienced male geckos tend to show more courtship behaviour following T replacement relative to naïve males, and similar results have been found in rats (Larsson 1978; Retana-Marquez & Velazquez-Moctezuma 1997). Furthermore, gonadally intact male geckos that scent-mark in the empty test chamber are more likely to court stimulus females (J. T. Sakata, A. Gupta, C-P Chuang & D. Crews, unpublished data), and the fact that intact experienced males marked more than intact naïve males suggests that experienced males are more likely to court females than naïve males when gonadally intact. Similar differences in sexual behaviour between intact experienced and naïve male rodents have also been reported (e.g. Dewsbury 1969; Lumley & Hull 1999). Therefore, between geckos and rats, there appear to be differences in the effects of social experience on robustness to castration but conservation in its effects on sexual behaviour in the presence of androgens.

We hypothesize that the behavioural differences between experienced and naïve male geckos could be causally linked to experience-dependent alterations in metabolic activity in the key limbic brain areas. Gonadally intact experienced male geckos have elevated metabolic capacity in caudal hypothalamic areas such as the ventromedial hypothalamus (VMH) and anterior

hypothalamus (AH) relative to naïve males (Crews et al. 1997). Both areas have been implicated in the control of territorial and sexual behaviour in a number of species (Crews & Silver 1985; Nyby et al. 1992; Bernstein et al. 1993; Meisel & Sachs 1994; McGinnis et al. 1996). Because differences in metabolic capacity may reflect differences in baseline neural activity (Wong-Riley 1989; Gonzalez-Lima 1992), we propose that metabolic elevations in the AH and VMH represent an increased priming to display agonistic and sexual behaviours. These increases in metabolic capacity, however, do not seem to be sufficient to produce an increased robustness to castration, and it is possible that increases in other nuclei such as the preoptic area and/or amygdala are necessary for this (Sakata et al. 2001). Whether behavioural differences between gonadally intact experienced and naïve males are due to experience effects on brain metabolism, or whether the neural differences are due to the effects of social experience on behaviour remain questions for future research.

We do not know which specific experiential factors are central in producing these behavioural differences. In the present experiment, experienced males were not only allowed to copulate with receptive females, but were also allowed to interact with and smell females constantly. Thus, it is possible that simply housing males with females without allowing copulatory experience would be sufficient to produce the behavioural differences reported here. However, we propose that the sexual interactions with females were paramount in producing these behavioural differences given the similar effects that sexual interactions have in other species (Meisel & Sachs 1994).

Although the leopard gecko has a different mechanism of sex determination (temperature-dependent sex determination) than that of many other vertebrates (e.g. genotypic sex determination), we found that the hierarchical organization of courtship behaviour remains conserved across leopard geckos and other species. Courtship behaviour in the leopard gecko is hierarchically organized such that mounting behaviour is contingent upon body-gripping behaviour. Both following castration and following T administration, the percentage of tests in which mounting was displayed was less than the percentage of tests in which body gripping was observed. Upon closer investigation of the data, this difference was due to the fact that mounting was lost sooner following castration than body gripping and reinstated later following androgen replacement (data not shown). Similar patterns of loss and recovery have been found in a variety of species (reviewed in Hutchison 1978; Meisel & Sachs 1994). A tenet of behavioural endocrinology is that sexual behaviours are organized hierarchically according to their dependence on hormonal stimulation (Meisel & Sachs 1994): behaviours that are more reliant upon androgenic stimulation are lost sooner following castration and recovered later following androgen replacement. Therefore, we propose that mounting behaviour is more dependent on the presence of androgenic stimulation than body-gripping behaviour. Mounting could require more androgenic stimulation because more sexual motivation is needed to achieve or attempt mounting, or because the

motor and neural circuits that sustain mounting undergo attrition sooner following androgen withdrawal. Finally, that this hierarchy was seen in both experienced and naïve males suggests that the hierarchy is not socially plastic.

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