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BEHAVIOURAL INTERACTIONS BETWEEN A RARE MALE PHENOTYPE AND FEMALE UNISEXUAL *LEPIDODACTYLUS LUGUBRIS*

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A rare male phenotype of the unisexual gecko, *Lepidodactylus lugubris*, was captured on the University of Hawaii, Hilo campus. The male was housed with females in different stages of their reproductive cycles. Observations were made of interactions between the male and individual females. The male approached females with either no evidence of egg development or small, probably ovarian, eggs more often than females with larger, probably oviductal, eggs; and was observed neck-biting and moving on top of females although no intromissions or copulations were observed. Female geckos were more aggressive than the male; the male was less likely to approach females that reacted to his approaches aggressively. Although it seemed that the male was attempting to court the female geckos, we do not know if courtship attempts failed because of the male or female behaviour. Sperm were present in the testes and epididymis. However, all motile sperm appeared to be headless, suggesting that the male was infertile.

INTRODUCTION

Unisexual species have been documented in many lizard genera (*Cnemidophorus*, *Lacerta*, *Lepidophyma*, *Hemidactylus* and *Lepidodactylus*) and in one species of snake (*Ramphotyphlops braminus*; Nussbaum, 1980). Most, if not all, of the unisexual reptiles arise from the hybridization of two sexual ancestors (Cole, 1990) and subsequently reproduce through premeiotic doubling of their chromosomes, which results in a full chromosome complement after meiosis (Cole, 1975). The genotypes of the unisexual hybrids are thus maintained intact except for later mutations. It is surprising, then, when one encounters a phenotypic male individual in the midst of a unisexual clone (for example see Ota *et al.*, 1993).

Male phenotypes are found in unisexual lizard species under a variety of conditions. When the range of a unisexual species overlaps with the range of a congeneric sexual species, the unisexuals are often found with bite marks on their abdomens indicating that mating has occurred (Darevsky & Danielyan, 1968). In some cases of unisexual-bisexual mating, hybrid male young are produced by the unisexual female. For example, male hybrids resulted from the mating of the unisexual whiptailed lizard, *Cnemidophorus uniparens*, with the sexual species, *C. inornatus*; from the unisexual *C. neomexicanus* with the bisexual *C. inornatus* (Cuellar & McKinney, 1976); and from the unisexual *C. laredoensis* with the bisexual *C. gularis* (Walker *et al.*, 1989). Hybrid males have also been reported in the unisexual *Lacerta* (as cited in Darevsky *et al.*, 1978).

Phenotypic males have also been found among offspring of unmated unisexual lizards. For example, Darevsky *et al.* (1985) reported a number of lethal abnormalities in the offspring of unmated unisexual *Lacerta*. Most of the abnormal embryos of the unisexual species (*L. armeniaca, L. dahli* and *L. rostombekovi*) had hemipenes. The majority of the male foetuses died during embryogenesis, and foetuses that were extracted from eggs died within a few days. Adult males have been found in collections of unisexuals obtained in areas where the range of the unisexual did not overlap with the range of a congeneric bisexual species, indicating that some non-hybridized unisexual males survived to adulthood. Males were found in three unisexual species of *Cnemidophorus (C. tesselaus, C. exsanguis* and *C. velox*; Taylor *et al.*, 1967), in *Lacerta (L. armeniaca*; Darevsky *et al.*, 1978) and in a unisexual gecko (*Lepidodactylus lugubris*; Cuellar & Kluge, 1972). To the best of our knowledge, the behaviour of a phenotypic male unisexual has yet to be described.

The courtship and copulatory behaviour of geckos is not well documented. Courtship behaviour in the diurnal banded gecko consists of tail waving, licking and bites to the female's body, followed by a strutting walk in which the male pushes the female forward. During copulation, the male grasps the female by the neck and then intromits (Porter, 1972). Mating behaviour is rarely observed in nocturnal geckos. Church (1962) observed only four matings between *Hemidactylus frenatus* pairs during a year of study and six years of living with the geckos. Male *H. frenatus* apparently engage in little or no courtship behaviour. The males stealthily approach the female, rush at her and then copulate (Marcellini, 1977*a*). No data on mating and copulatory behaviour in sexual *Lepidodactylus* are available. Despite the paucity of data on mating behaviour in nocturnal geckos, the present study was designed to (1) document and describe any mating attempts between a phenotypic male and female *L. lugubris;* and (2) compare the interactions between

male and female *L. lugubris* with the interactions previously described between female *L. lugubris* (Brown *et al.*, 1991).

METHODS

SUBJECTS

Seven *L. lugubris* were collected in the grounds of the University of Hawaii, Hilo campus. One gecko was a male phenotype captured on 22 May 1986 (confirmed *L. lugubris* in Radtkey *et al.*, 1995). The male phenotype had well-developed hemipenal sacs and femoral pores. When the male was dissected (12 June 1990), he was at least five years old, as he was captured as an adult and it requires 9-12 months for a gecko to reach maturity (Brown & O'Brien, 1993). The contents of the epididymis and vas deferens were squeezed out and placed in amphibian ringers solution. Sperm were examined for evidence of motility using a phase-contrast microscope. The testes were removed and fixed in Steives fixative (Humason, 1979) for 24 hr. The samples were then washed in 50% ethanol for 30 min, post-treated in iodine-alcohol solution for 6 hr, and were embedded in Paraplast-Plus. Sections of 7-10μ in thickness were stained with either Delafield's Hematoxylin and Eosin Y (H&E, regressive method) or Feulgen's technique (Humason, 1979) and examined for histological information regarding sperm formation and maturation.

All six females had produced at least one egg clutch prior to placement with the male. The females were in different phases of their reproductive cycles when individually housed with the male. Reproductive phase was determined in two ways: (1) time elapsed since oviposition, and (2) visible presence of ovarian or oviductal eggs. Eggs were measured by placing the gecko in a narrow plexiglas box and examining the gecko's translucent ventral surface. Eggs greater than 1 mm in size can be observed in this manner. Two of the females had no visible signs of egg development; one was housed with the male immediately after oviposition (F-OVI) and the other a week after oviposition (F-OVI-1WK). The remaining four geckos had visible eggs measuring 3 mm (F-3MM), 5 mm (F-5MM), 7 mm (F-7MM) or 9 mm (F-9MM). F-9MM laid eggs while housed with the male.

PROCEDURE

The study was conducted from 28 October 1986 to 5 March 1987. Each female was individually housed with the male for five days in a 32 cm x 28 cm x 31 cm plexiglas enclosure. Enclosures contained a wooden platform suitable for hiding, a plant, and an inch of pebbles covered with sphagnum moss. An interval of at least 10 days occurred between each time the male was housed with a female. Observations began 4 hr after pairing and were made during the morning (0600 - 1200 hr) and afternoon (1200 - 2100 hr). A total of 51.25 hr of observations were made, 27.75 hr in the morning and 23.5 hr in the afternoon. All behaviour that occurred between the male and each female was recorded in a series of 15 min serial records. At least six serial records were obtained (three in the morning and three in the afternoon) daily. The total numbers of serial records collected per pair was as follows: F-OVI = 30; F-OVI-1WK = 41; F-3MM = 33; F-5MM = 34; F-7MM = 33; F-9MM = 34. The following interactive behaviour patterns were recorded during the observations: *orient to, face to, approach, orient away, face away, move away, click, push-up, lunge, bite, touch, in proximity* as defined in Brown *et al.* (1991), and *follow, head-bob, hit, on top of, chirp and multiple chirp,* all behaviours which we had not previously observed in *L. lugubris* and which are defined in Table 1.

ANALYSIS OF RESULTS

Interactive behaviour displayed by the male and females were grouped as follows: behaviour associated with contacting included *orient to, face to, approach* and *follow*, and behaviour associated with withdrawal included *orient away, face away* and *move away*. Aggressive behaviour was divided into three categories: *clicks, head-bobs/pushups* and *lunges/bites/rurrs*. Mean frequencies per 15 min were obtained for each male/female pairing, and grand means were then computed across all pairings. Wilcoxon tests for related samples were used to compare male and female behaviour.

To analyse changes in interactive behaviour across time, behaviours were compared across the first four 24 hr intervals of collected observations. Friedman tests for dependent samples were used to analyse behavioural changes over time.

TABLE 1. Definitions of previously undescribed interactive behaviours observed between the male and female geckos.

Follow: Gecko moves after another as it moves away.
Head-bob: Gecko moves head up and down; the rest of its body remains stationary.
Hit : Gecko contacts another with front feet.
On top of : One gecko climbs onto another, either remaining there or immediately moving off.
Chirp: Soft slow vocalization only produced by one female.
Multiple chirp: A long extended vocalization only produced by the male.

TABLE 2. Summary of male and female interactive behaviour.

Behaviour	Female		Male			
	Grand mean	SD	Grand mean	SD	*U*	*P*
Contact	0.6	0.3	0.7	0.6	7.5	NS
Withdrawal	0.4	0.2	0.6	0.4	5.0	NS
Click	3.2	2.1	0.0	--	0.0	<.05
Head-bob/ Push-up	0.4	0.4	0.1	0.1	0.0	<.10
Lunge/Bite/ Rurr	0.5	0.3	0.1	0.1	0.0	<.05

RESULTS

MALE BEHAVIOUR

In his interactions with the females, the male displayed more contact and withdrawal behaviour than aggressive behaviour (Table 2). The male *L. lugubris* was more likely to approach females with no sign of egg development or small eggs than females with large eggs (r = -0.65; Fig. 1) and to withdraw from these females after the approach (r = -0.78; Fig. 1). The male was observed biting females five times. The majority of the time (3/5), the male approached the female, bit her on the neck and then moved away without further interaction. The male was observed on top of a female three times during the study. On two occasions the male was lying on top of F-OVI at the beginning of the 15 min serial record. Once the male remained on F-OVI throughout the record, and once, when the experimenter disturbed the cage, the male moved away. During the above observations, the male neither bit the female nor curled around her. The third time the male moved on top of a female occurred with F-5MM after she approached him while clicking. Once the male moved on top of her, the female became quiet, and the male moved off of her and away.

The male *L. lugubris* vocalized a multiple chirp (MC) call four times (once in the morning and three times in the evening after 1830 hr) with three different females, F-OVI-1WK (n = 2), F-5MM (n = 1) and F-9MM (n = 1) during our observations. While calling, the male was located either in (n = 3) or behind the platform (n = 1). No interactions between the male and females were in progress at the time of the MC calls. After the MC calls, the females did not approach the male. F-OVI-1WK remained out of the experimenter's sight (n = 2), F-9MM continued moving about the enclosure without approaching the male, and F-5MM continued eating a mealworm and chirped softly.

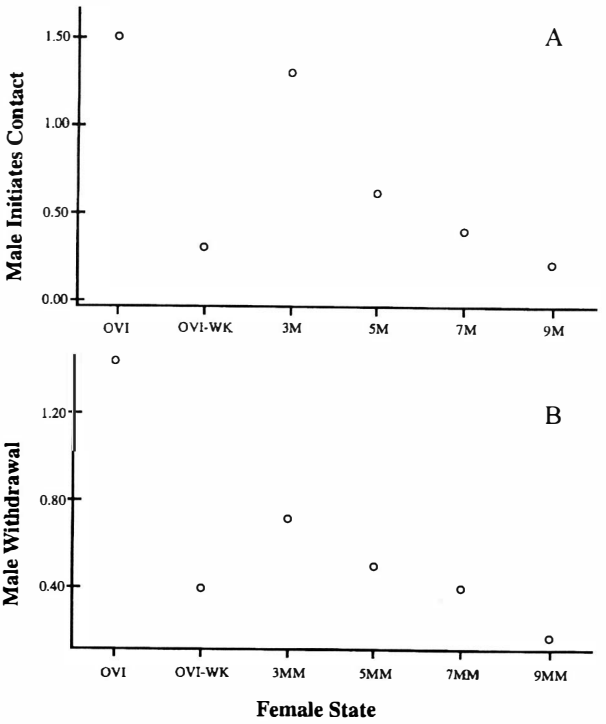

FIG. 1. The relationship between female reproductive state and (A) the male's attempts at contacting the female; and (B) the male's withdrawals from the female. See methods for interpretation of abbreviations for female state.

FEMALE BEHAVIOUR

Females exhibited higher frequencies of aggressive behaviour (*clicks, head-bobs/push-ups,* and *lunges/ bites/rurrs*) than the male throughout the observations; the differences were statistically significant (see Table 2). Females responded to male approaches in a variety of ways: by intense aggression (16/67), by moderate aggression (7/67), by moving away (7/67), by orienting to or away from the male, and by not reacting (37/67). Females with small eggs were more likely to react to the male's approach aggressively than females with large eggs (r = -0.55). Female bites, unlike male bites, always occurred during prolonged aggressive interactions. F-3MM and F-5MM moved on top of the male during the observations. Usually (7/9 times) the female climbed over or onto the male during an aggressive interaction; twice the female rurred while on top of the male. On two occasions the female climbed over the male and moved to another part of the enclosure.

The male and the females were equally likely to exhibit contact (*orient to, face to, approach* and *follow*) and withdrawal behaviour (*orient away, face away* and *move away*) (Table 2). Behaviours associated with contacting, withdrawing from and maintaining proximity to another animal exhibited no statistical change across time. Neither did behaviour associated with aggression (*clicks, head-bobs* and *push-ups,* and *bites, lunges* and *rurrs*).

HISTOLOGICAL DATA

The male *L. lugubris* examined in the present study demonstrated small but apparently normal testes and

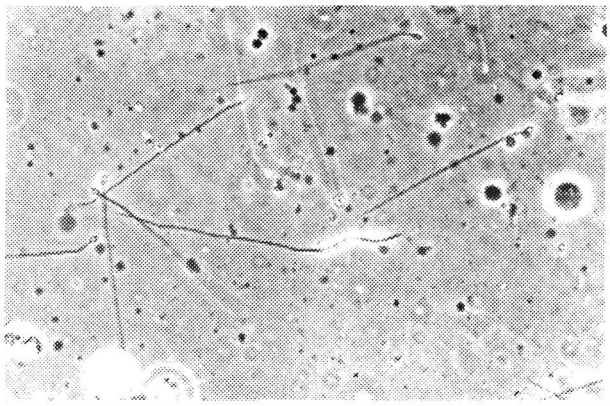

FIG. 2. Motile headless sperm and immotile sperm with head.

sperm ducts. There was an apparent lack of typical cell division events in the testes when compared to a normal adult male *Hemidactylus frenatus* (Murphy-Walker, personal observations). Histological and *in vitro* examination of testicular and epididymal sperm revealed that the majority of sperm present were headless and only headless sperm were motile (Fig. 2).

DISCUSSION

The male *L. lugubris* displayed behaviours, such as neck biting and positioning himself on top of a female, associated with gecko courtship (Porter, 1972). We did not, however, observe a completed intromission or a copulation. The male was also more likely to approach females that had recently laid eggs; however, these females responded to male approaches with behaviours most often observed in dominance contests between females (Brown *et al*., 1991). If the male's behaviours were indeed courtship, then it is possible that either the male and/or the female *L. lugubris* might not possess the behavioural repertoire needed to respond to and complete a normal courtship and mating sequence.

In our previous research on the behaviour of *L. lugubris* (Brown *et al*. 1991), the interactive behaviour between female dyads decreased over time, reflecting a stabilization of the dominance relationships between females. In contrast to our previous research, the frequencies of the interactive behaviours were maintained at high levels throughout the five days the male was housed with each of the females. High levels of interactive behaviours were most likely maintained between the male/female dyads because neither mating nor dominance status was resolved by the end of the five days of observation.

The *L. lugubris* male vocalized the multiple chirp call associated with many gecko species (Marcellini, 1977*b*). The MC call most likely functions as a territorial spacing mechanism among male geckos. Marcellini (1977*b*) showed that in the house gecko, *Hemidactylus frenatus*, the call repelled male but was ignored by female *H. frenatus*. Female *L. lugubris* also did not respond to the male's MC calls. The fact that the phenotypic male *L. lugubris* used the MC call indicates that he possessed the ability to display behaviour usually not associated with unisexual geckos.

Even though unisexual species occasionally produce phenotypic males, it is unlikely that the males are capable of reproduction due to abnormalities in spermatogenesis. Of the phenotypic male *L. lugubris* described by Cuellar & Kluge (1972), only one had apparently normal testes and sperm ducts. The male *L. armeniaca* described by Darevsky *et al*. (1978) lacked the typical cell order found in the testes of bisexual *Lacerta* and had few mature sperm. The majority of the male's sperm in the present study were headless and probably incapable of fertilization as there was no apparent nuclear material present for recombination events nor enzymes capable of penetrating egg membranes as evidenced by the lack of an acrosomal cap. Unfortunately the lack of sufficient samples did not allow for discrimination between mitotic, meiotic, or spermatogenic abnormalities as the causal agent of headless sperm.

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