Male flat lizards prefer females with novel scents

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In lizards, pheromonal cues are important for mate recognition and are expected to diverge during the speciation process. We tested for divergence in pheromonal mate and species recognition between male flat lizards (*Platysaurus broadleyi*) belonging to the same population, a different population, and a sister species. Males were given a choice between two refuges, an odourless control and one of the following: a conspecific female from the same population (sympatric), a conspecific female from a distant population (allopatric), and a female from their sister species (heterospecific), *P. capensis.* Males chose refuges treated with the scent of a heterospecific over an odourless control refuge but showed no preference for refuges that were scented with female conspecific (allopatric or sympatric) cues over the control. These results were consistent in the breeding and non-breeding seasons. In a second experiment males were offered a choice of female scents between sympatric and allopatric conspecifics; an allopatric conspecific and a heterospecific; and a sympatric conspecific and a heterospecific. Males showed a trend of preferring heterospecific over conspecific refuges and allopatric over sympatric conspecific-scented refuges, but these results were not significant at alpha <0.05. Contrary to our original expectations, these experiments do not provide any evidence for a pheromonal pre-mating isolation mechanism within this species complex. However, our results suggest a preference for novel female scents by males, consistent with selection for genetic diversity.

Key words: lizard, pheromones, novel mate preference.

INTRODUCTION

Signals important in species recognition may diverge between closely related taxa because of the fitness benefits associated with the correct identification of conspecific signals (Ryan & Getz 2000). As such, mate recognition systems have an important role in maintaining the integrity of species (Ryan 1990). Ideally, each species is most responsive to conspecific signals (Cooper & Vitt 1986a) and should be able to differentiate between populations of conspecifics based on these signals, because of the potential negative costs associated with out-breeding depression (Endler & Houde 1995). These species recognition signals may or may not conflict with the signals used for the assessment of conspecific mate quality (Wymann & Whiting 2003). Considerable empirical evidence has been gathered concerning female mate choice for conspecific males and between population differentiation (Wong *et al.* 2003), but fewer studies have considered male mate discrimination between species and between populations (Wymann & Whiting 2003). In allopatric populations signal divergence occurs predominately through random genetic drift (Cooper & Vitt 1987), sexual selection and differences in natural selection pressures (Langerhans *et al.* 2004). Signals may be chemical, visual or behavioural in nature, and one sensory modality may influence the extent of communication in another (Hews & Benard 2001). Although squamate reptiles have multiple sensory modalities, chemoreception plays a pivotal role in the mediation of a variety of behaviours (e.g. Cooper 1997; Cooper *et al.*, 1999; Labra & Niemeyer 1999), particularly in close quarters.

Lizards use chemical communication to determine the presence or absence of prey or predators (Cooper 1998; Downes & Shine 1998a,b), and in a social context, in mate (Olson & Shine 1998), kin (Cooper & Vitt 1984a,b) and rival recognition (Cooper & Vitt 1987; Lopez & Martin 2002) and for the assessment of reproductive status (Cooper & Vitt 1986b). Pheromones used for intraspecific communication are produced by the femoral glands, cloacal glands, generation glands and the ventral skin (Cooper *et al.* 1999). Femoral glands of one lizard group, the Cordylidae, vary seasonally in males, generally becoming enlarged during the reproductive season (Cooper *et al.* 1999). Their ventral position allows for pheromone deposition onto the rocky substrates on which most cordylid

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lizards live (Cooper *et al.* 1999). While the importance of such communication is suspected in several lizard families, experimental evidence is lacking, particularly for the Cordylidae (Cooper *et al.* 1999). The cordylid genus *Platysaurus* (flat lizards) presents a good model to test pheromonal recognition of potential mates because the clades in this genus are well defined (Scott *et al.* 2004).

All species of *Platysaurus* are restricted to rocky outcrops (Scott *et al.* 2004) and this substrate specificity may result in a greater potential for isolation than in other taxa. The two *Platysaurus* species selected for this study are sister species (Scott *et al.* 2004). *Platysaurus broadleyi* is endemic to the Northern Cape Province along the Orange River from Augrabies Falls National Park in the east to Klein Pella in the west, while *P. capensis* ranges from northern Namaqualand to the Richtersveld and southern Namibia (Branch & Whiting 1997; Scott *et al.* 2004). Our experiment deals with two populations of *Platysaurus broadleyi* and a single population of *P. capensis*, which is allopatric to all *P. broadleyi.* The three populations are separated along an east-west gradient; *P. capensis* occurs in the west and the two populations of *P. broadleyi* are separated by *c.* 100 km to the east. *Platysaurus broadleyi* were from Augrabies Falls National Park (28°35′S, 20°20′E) and Onseepkans (28°45′S, 19°16′), while *Platysaurus capensis* were from the immediate vicinity of Kamieskroon (30°11′S, 17°55′E). Although females and juveniles of both species appear very similar, the males are markedly different (Branch & Whiting 1997). Reproductive cycles are synchronized between the sexes, with mating occurring in both species during the spring (Van Wyk & Mouton 1996).

As the two species *P. broadleyi* and *P. capensis* have recently diverged, but are allopatric in distribution, they may share traits used in mate recognition (Wymann & Whiting 2003). Previous studies of *P. broadleyi* found that large female body size overrides conspecific visual cues in determining male mate preference (Wymann & Whiting 2003), and interspecific aggression in other species of *Platysaurus* suggests poor species recognition based solely on visual cues (Korner *et al.* 2000). Different responses to pheromonal cues in sister species would provide strong support for a pre-mating isolation mechanism (Cooper & Vitt 1986a). Therefore, we set out to determine the importance of pheromones, in the absence of visual, auditory or behavioral stimuli, in species and mate recognition. Our aim was also to examine levels of divergence in species recognition in this complex. Using this system, we specifically tested whether male *P. broadleyi* from the Augrabies population (eastern most population) are able to differentiate between the scents of female conspecifics from the sympatric population, conspecifics from a distant, allopatric population (midway between Augrabies and the sister species), and their sister species, *P. capensis.* Given that the three populations occur along an east–west gradient with the Augrabies population in the east and the sister species in the west, we predicted that divergence in species recognition would be a function of distance (a proxy of relatedness) such that the Augrabies population would have a preference in the order: sympatric population > allopatric population > sister species.

In order to test discrimination we used a refuge choice test to identify male preference between refuges scented with the chemical cues of females from these populations and a control. Refuge tests provide a good indication of pheromone preferences (Cooper 1998) and have been used in numerous studies (e.g. Downes & Shine 1998a,b; Martin & Lopez 2000).

MATERIALS & METHODS

Lizard maintenance

The lizards used in the experiments were housed in an animal unit with semi-controlled temperature and a 12 hour light/dark cycle. Most lizards were from an existing, long-term captive population normally housed in glass terraria (60 × 30 × 40(h) cm) with a paper substrate and terra cotta tiles. Some lizards were caught more recently (several months prior to trials) to supplement sample sizes; all lizards were caught as adults from the three sites described above and had been in captivity for several years. Lizards were fed Whiskers® tinned cat food twice a week and live mealworms once a week. Water was available at all times. Male and female lizards were housed separately so that individual female scents would be similarly novel to the receiver. Husbandry procedures and experiments were approved by the University of the Witwatersrand Animal Ethics Committee (certificate number 2003 31 2a).

Scent trials

Single females of each of the three groups (conspecific sympatric population; conspecific allopatric population; heterospecific) were randomly selected and used for scent transferral. To obtain female scent a refuge was placed into the female's enclosure 48 hours before the trials were run. Refuges consisted of two ceramic tiles (20 × 20 cm), one above the other, separated by a small (1 cm^3) block of wood at the entrance, providing a sloping refuge that closed at the rear. This narrow opening ensured that lizards made contact with both surfaces of the tiles and thus maximized scent transfer. Ten *P. broadleyi* females from the sympatric population, three *P. broadleyi* females from the allopatric population during the non-breeding season and ten during the breeding season, and six *P. capensis* females were used to scent tiles. Each tile was scented by a single female.

Soiled tiles were discarded. Used tiles were either discarded or washed, following the procedure outlined in Downes & Shine (1998a) in which tiles are soaked for a minimum of two hours in hot soapy water, using a pine-scented detergent, scrubbed, soaked for 30 min in hot water, rinsed and air-dried. In addition, we then wiped the tiles with 70% ethyl alcohol. The trial terraria were also washed with pine disinfectant and wiped with alcohol before each trial.

In all these experiments, *P. broadleyi* males from the Augrabies population were given a choice of two refuge sites constructed from unglazed ceramic tiles in a neutral arena. The experimental set-up involved a terrarium (30 × 60 × 30 cm) with a refuge at each end. The test lizard was placed in the centre of the terrarium in the morning, when lizards are most active, and its overnight refuge preference noted the following morning. Two experiments were conducted, the first provided males with a choice of a scented and control tile during the breeding season and non-breeding season, the second gave males a choice between two tiles both scented with the chemical cues of females from different populations or species during the breeding season. In the wild, males typically defend territories from August until about February (or later). For the purposes of our study, we called this the breeding season, although females are only receptive for a much narrower window (M.J.W., pers. obs.). During the breeding season we conducted trials in the period August–December and in the non-breeding season, during April–May.

Male choice between control and scented refuges

In these two choice tests one refuge was scented with an odourless distilled water control and the other with one of the following: a conspecific female from the sympatric population, a conspecific female from the allopatric population, and a heterospecific female. Each male lizard was tested once ($n = 30$) per treatment in a randomized sequence to control for order effects. These experiments were conducted under identical conditions during breeding and non-breeding seasons. Male choice was analysed with a generalized linear mixed model (GLMM) of the binomial family using the statistical program R v. 2.1. A binomial model was used because the response variable was a choice of two options. Two random terms were included in the model, male nested in day was used to control for repeated testing of males in the breeding and nonbreeding season and female identity was to control for a possible female identity effect. The influence of treatment (sympatric conspecific, allopatric conspecific or heterospecific female) and experimental time (breeding season or non-breeding season) were analysed. Post hoc chi-squared tests were used to compare between treatments.

Male choice between two female scented refuges

Males were offered 1) a choice between a tile scented by a *P. broadleyi* from the sympatric population and a tile scented by a *P. capensis* female; 2) a choice between *P. broadleyi* from the sympatric population and *P. broadleyi* from the allopatric population; and 3) a choice between allopatric *P. broadleyi* and *P. capensis.* These results were analysed using chi-square contingency tables.

RESULTS

Male choice between control and scented refuges

Female species and population (treatment) influenced male retreat site selection (treatment $F_{1,92} = 4.79$, $P = 0.01$), but not time of season or the interaction term ($F_{1,92} = 0.54$, $P = 0.46$ and $F_{1,92} = 0.95$, $P = 0.39$; Fig. 1). In the breeding and non-breeding season, males consistently chose *P. capensis* over the control scented tiles ($\chi^2_1 = 8.06$, $P = 0.004$). Males however, did not prefer conspecifics over control scented trials (allopatric: $\chi^2_1 = 2.4$, $P = 0.12$; sympatric: $\chi^2_1 = 2.4$, $P = 0.12$).

Male choice between two female scented refuges

When males were offered a choice between refuges scented with two females, there was a

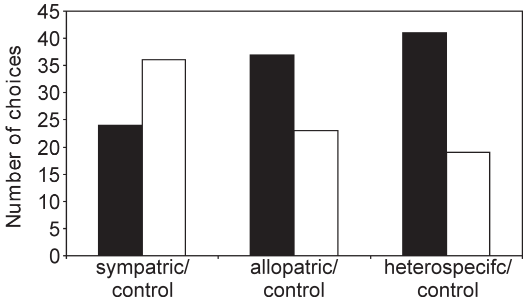

Fig. 1. Refuge scent choice trials with female scents versus control tiles using male *Platysaurus broadleyi* as test subjects. Consp = conspecific. The allopatric population was >100 km away and the sister species was *Platysaurus capensis.* Males significantly preferred the heterospecific scented refuges, but showed no significant preference for the sympatric or allopatric *P. broadleyi* over the control.

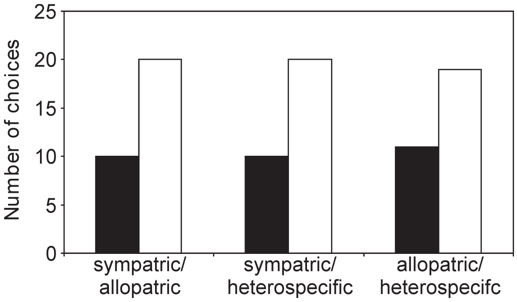

Fig. 2. Refuge scent choice trials comparing pair-wise female scents using male *Platysaurus broadleyi* as test subjects. Consp =conspecific. The allopatric population was >100 km away and the sister species was *Platysaurus capensis.* Males showed a preference for novel scents, preferring allopatric and heterospecific scented refuges.

trend for males to select refuges that had a more novel scent (Fig. 2): heterospecific vs sympatric ($\chi^2_1 = 3.3$, $P = 0.067$); heterospecific *vs* allopatric ($\chi^2_1 = 3.3$, $P = 0.067$); and allopatric *vs* sympatric ($\chi^2_1 = 2.1$, $P = 0.144$). The power estimated for a small effect size (15% variation) calculated post hoc for these trials was 0.29, which is comparable to other studies in this field (Jennions & Møller 2003). These results, although marginally non-significant, suggest an overall tendency for males to prefer the novel female scent.

DISCUSSION

Contrary to our expectations, in all experiments male *P. broadleyi* exhibited a trend in preferring scent cues for both allopatric conspecifics and heterospecifics, rather than sympatric conspecifics. Refuges scented with females from a greater geographic distance (a proxy for genetic distance; verified in Scott *et al.* 2004) appeared to be the most attractive to males. Males preferred heterospecific scented refuges to the control, in both the breeding and non-breeding season, suggesting that this result is not influenced by the reproductive status of the female or the male. Although this result seems unexpected, a similar result was found in a recent study on fish. Wong *et al.* (2005) found that when swordtail fish were given a choice between a conspecific and heterospecific, males preferred the odour cues of the heterospecific females. The similar pattern observed in this study may be due to a pre-existing sensory bias in the information processing system of the receiver for some component of the ancestral heterospecific scent (Basolo 1990). Sensory bias has been cited as the explanation for individuals preferring heterospecific signals either due to some pre-existing aspect of the sensory system and brain, or because individuals might be more sensitive to signals that deviate from the population mean (i.e. a preference for the novelty; Basolo 1990; Ryan 1990; Ryan & Rand 1993; Ryan & Wagner 1987; Ryan *et al.* 1990). Some component of the scent might have co-evolved with the preference in these *P. broadleyi* lizards, and later, this element may have been lost in *P. broadleyi* females (Ryan & Rand 1993). In the absence of an appropriate test of the sensory bias hypothesis, a more parsimonious explanation is simply that males prefer novel scents.

Novel scents could signal an individual with greater genetic difference. Selection for genetic diversity is thought to enhance an individual's ability to mount an immune response against parasites and pathogens. Therefore, by selecting a more genetically different mate, an individual could enhance his own fitness and also avoid the deleterious effects of inbreeding (Shykoff & Schmid-Hempel 1991).

A potential constraint of conducting pheromonal choice experiments during the breeding season is that female receptivity is difficult to assess and the duration of the receptive period may be short. A non-receptive female will likely have a scent of diminished attractiveness to male test subjects. During spring, when the trials were repeated, the results were the same. This level of preference suggests that female receptivity was not a significant constraint here. Males were strongly attracted to the conspecifics from the distant population and this is likely due to the combination of conspecific mate choice cues and the scent's novelty. Unexpectedly, male lizards appeared to select refuge sites at random when given a choice between a control tile and one scented by a conspecific of the same population. As, on average, individuals may be more attracted to individuals from their own population to avoid out-breeding depression and because mate preference criteria tend to vary from population to population (Endler & Houde 1995), this result is unusual but hints at the sensory importance of novel cues that still bear a core signature necessary for mate recognition.

Contrary to our original expectations, these experiments do not provide any evidence for a pheromonal pre-mating isolation mechanism within this complex. Because *P. broadleyi* and *P. capensis* are allopatric (Wymann & Whiting 2003), selective pressures driving divergence in pheromonal signalling may be weak or absent (Ryan & Getz 2000). As previous studies have shown that visual cues in *Platysaurus* do not prevent species misidentification (Korner *et al.* 2000; Wymann & Whiting 2003), it suggests that recent divergence has not promoted premating isolating mechanisms and that novelty, with the associated benefits of genetic diversity, is an intriguing alternative hypothesis warranting further investigation.

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