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BRIEF COMMUNICATION

The Role of Scent Marks in Female Choice of Territories and Refuges in a Lizard (*Podarcis hispanica*)

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Female mate choice based on male phenotypic traits is controversial in lizards, particularly in territorial species. In this study, we examine female choice of male scent marks in a territorial lacertid lizard (*Podarcis hispanica*) in which scent marks have been shown to signal male size (i.e., an important determinant of competitive ability in this species). Females were simultaneously exposed to three naturalized 4 m^2 choice areas bearing: (1) no scent marks (i.e., control), (2) scent marks of large males, and (3) scent marks of small males. Although female lizards preferentially associated with scent marked choice areas, we found no evidence that females chose territories marked by large males. Furthermore, in response to experimentally induced dusk at the end of choice trials, females preferentially took shelter in refuges scent marked by small males. Our results suggest that, like males in this species, females are able to use male scent marks to assess the body size of resident territorial males, but do not show a preference for territories occupied by large males.

Keywords: female choice, sexual selection, scent marks, chemical communication, lizards

The processes that bring together males and females for reproduction have long fascinated biologists and psychologists. Female mate choice is widespread in vertebrates (Andersson, 1994), but there is still considerable controversy about its existence in lizards. Many lizards are sexually dimorphic, show exaggerated secondary sexual traits, and exhibit polygynandrous mating systems where male reproductive success is subject to greater variability than female reproductive success (Stamps, 1983). However, and despite numerous investigations into lizard mating systems, conclusive evidence for female choice is scarce, particularly in territorial species (e.g., Cooper & Vitt, 1993; Olsson & Madsen, 1995, 1998; Tokarz, 1995; Sullivan & Kwiatkowski, 2007).

Several theoretical reasons have been put forward to explain why female mate choice may be less frequent in territorial lizards than in other vertebrates. Direct female benefits of mate choice are probably low because parental care is generally rare in lizards and virtually nonexistent in territorial lizards (Pianka & Vitt, 2003). In addition, lizard territoriality may hinder female sampling of males because male territories are typically much larger than female home ranges, and territorial males tend to exclude other males from their territories (Pianka & Vitt, 2003). Therefore, females will typically have access to few males (Stamps, 1983), or will have to incur considerable costs in terms of energy expenditure, sexual harassment, and predation risk to sample males far from their own territory (Censky, 1997; Perry & Garland, 2002; Tokarz, 1995). Traditional interpretations have therefore suggested that, at least in lizards that exhibit resource based territoriality, females may be better off selecting mates based on the quality of their territories' resources (food, hiding, or nesting sites, etc.), rather than on their "good genes' (Hews, 1990a, 1990b, 1993; Tokarz, 1995). By selecting high quality territories, females would be acquiring important resources and, at the same time, indirectly mating with high quality males while bypassing the elevated costs of mate sampling.

However, recent studies have shown that females of several territorial lacertid species are capable of using male scent marks to indirectly assess subtle aspects of male phenotypic quality (e.g., body condition, fluctuating asymmetry, parasite load, social dominance) and genetic compatibility (López & Martín, 2004; Mason & Parker, 2010). In addition to demonstrating the existence of extraordinary discrimination abilities in female lacertid lizards, these studies implicitly suggest that females may be using the information contained in scent marks to settle in territories held by high quality males, a strategy that may avoid the costs of direct male sampling.

P. hispanica is a lacertid lizard that exhibits typical resource based territoriality, and in which large dominant territorial males often subdue females and copulate with them without much prior courtship (Pérez-Mellado, 1997; E. Font, unpublished data).

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Hence, in this species female opportunities to exert direct precopulatory mate choice seem limited, and are probably constrained by the costs of male sampling. However, scent marks mediate important aspects of male territorial and intrasexual behavior in this species, including male assessment of a rival's body size and individual identity (Carazo, Font, & Desfilis, 2007, 2008). Male size is probably the most important determinant of male competitive ability in this and in other lizard species and is, according to available evidence, the male phenotypic character more commonly found under female mate choice in lizards (e.g., Cooper & Vitt, 1993; Perry & Garland, 2002). Our aim in this study was to address the possibility that *P. hispanica* females may use male scent marks to assess male size and indirectly associate with large (high quality) male's territories.

Method

Lizards were caught at the beginning of the reproductive season in Valencia, Spain (February-March 2006). We collected a total of 15 adult females (SVL, $\bar{x} \pm CI = 54.7 \pm 1.8$ mm) and 19 adult males (SVL, $\bar{x} \pm CI = 52.2 \pm 2.0$ mm). Females were carefully inspected by abdominal palpation to detect follicle development (Bleay & Sinervo, 2007), and gravid females were released back to the field. Scent mark donors were sorted in pairs (minimum SVL difference of 5 mm) that were selected from locations far apart (>1 km) to the associated experimental female. All trials took place in a $4 \times 1.7 \times 2$ m observation chamber with a one-way frontal glass and a substrate made of ground stone mixed with nontoxic resin that was shaped irregularly to mimic natural rock, resulting in a naturalized habitat including pot holes that were filled with water or soil with natural vegetation. The chamber can be divided by plastic sliding divisions in three 1.3×1.7 m visually identical areas (i.e., choice areas). Each area contains one approximately semicircular boulder (ca. 80 Ø and 60 cm height) terraced in two levels of decreasing area (i.e., 60 and 30 cm Ø), providing a total surface area ca. 4 m^2. We suspended a 100 W incandescent lamp above each boulder as a source of heat. Ambient lighting was provided by 12 fluorescent tubes fixed to the ceiling of the chamber (i.e., six Sylvania Reptistar alternated with six Sylvania T5 fluorescent tubes) that provided full-spectrum lighting. Finally, we also added construction bricks (three in each area) for lizards to use as refuges. A programmable system allowed us to adjust thermal and humidity profiles to mimic natural conditions in the field. The temperature gradient was closely monitored with the aid of iButton temperature sensors installed inside refuges, immediately below the basking lamp, and on the chamber's floor, far away from the basking site. Lighting was also programmed to mimic the natural day-night cycle, with incandescent lamps turning on at 9:30 and off at 17:30, and fluorescent tubes gradually turning on from 9:00 or off from 18:00 (6 every 15 min) to increase/decrease lighting and heat at experimentally induced dawn (beginning at 9:00) and dusk (beginning at 18:00).

We observed female spatial association to the three choice areas, corresponding to three different treatments: (1) scent-marked by a small male, (2) scent-marked by a large male (at least 5 mm SVL difference), and (3) not scent-marked (control). Except for their location inside the observation chamber (i.e., center, right, or left), choice areas were indistinguishable to a human observer. However, allocation of treatments to choice areas was counterbalanced across trials. Forty-eight hours before trials odor donor males were released into assigned choice areas and left to mark for ca. 36 hours (i.e., from 20:00–21:00 to 9:00, 2 days later). During male scent marking, we provided water ad libitum and released 3–4 mealworm beetle (*Tenebrio molitor*) larvae inside each compartment (including the control, to ensure that prey scents were present in all three areas). All male lizards were observed to behave naturally: after an initial extensive exploration of the area (characterized by frequent tongue-flicking and locomotion), they subsequently basked, drank water, hunted down prey, patrolled the area, and used refuges on a regular basis. Early the morning immediately preceding trials (before experimentally induced dawn) males were taken out of the observation chamber, the plastic sliding panels dividing the chamber were removed, and females were introduced into one of the refuges in the control, nonscented area. All trials took place between 9:30 and 18:30, when lizards are usually active in the field, during which time we recorded the position of experimental females with instantaneous scans (every 10 min). We collected a total of 52 scans for each female, but statistical analyses only considered scans recorded after females had visited the three choice areas. All observations were conducted by the same observer, who was blind to the position of small versus large scent-treated areas and refuges. In 10 out of 15 trials, females responded to experimentally induced dusk by entering into one of the refuges. After each trial, the chamber was thoroughly cleaned twice (first with deionized water and then with 70° ethanol), sprayed with heated water vapor using a standard steam machine, and left to dry for 12 h.

To test for overall treatment effects on female spatial association (i.e., number of scans in each area) we used Friedman's test for matched samples (Siegel & Castellan, 1989). Simple main effects (control vs. small, control vs. large, and small vs. large) were calculated according to Siegel and Castellan's (1989) planned procedure and reanalyzed post hoc by conducting three Wilcoxon's paired tests that were conservatively protected against an increase in experiment-wise error rate with Bonferroni's sequential correction (Holm, 1979). As Bonferroni corrections have recently been criticized because they can result in a considerable loss of power (e.g., Moran, 2003), we provide both corrected and uncorrected p values. Finally, to test for differences in the refuges occupied by females at the end of trials, we conducted a goodness-of-fit chi-square test. Because the chi-square test can be inaccurate when expected counts are small (<5), we accompany this with a randomization test based on 10 Monte Carlo simulations (20,000 random samples each) on a multinomial sampling distribution (http://faculty.vassar.edu/lowry/webtext.html). All reported p values are two-tailed. Alpha-level was set at 0.05 for statistical tests and confidence intervals.

Results

Friedman's test confirmed the existence of significant treatment effects ($n = 15$, $\chi^2 = 12.259$, $df = 2$, $p = .02$) on female spatial association (i.e., number of scans in each area). Planned comparisons detected a significant difference between the number of scans in small versus control (rank difference = 12.57, critical value = 2.56) and large versus control (rank difference = 14.73, critical value = 2.56) areas, but not between small versus large (rank difference = 2.17, critical value = 2.56) areas. Simple main

effects were also tested using Wilcoxon's test for paired samples, which confirmed that, as suggested by descriptive statistics, females spent more time in areas scent marked by large males ($N = 15$, $Z = -3.112$, $p = .002$; after Bonferroni correction, $p < .01$) and small males ($N = 15$, $Z = -2.277$, $p = .023$; after Bonferroni correction, $p < .05$) than in control areas, but we did not find a significant treatment effect when comparing the time spent in areas scent marked by large versus small males ($N = 15$, $Z = -0.175$, $p = .861$). Because Wilcoxon's test for paired samples is less powerful than its parametric equivalent, we also performed a post hoc paired t test on the continuous variable 'ratio of time spent in each area' (i.e., number of scans in each area/total number of scans) to ensure that we were not missing a significant difference because of the loss of power. To deal with skewness of the data, we arcsine transformed the ratio variable before conducting the paired t test, that did not reveal any significant differences ($t = -0.480$, $df = 14$, $p = .639$). Because of the relatively small sample size of our experiment ($n = 15$), we performed power calculations (paired t test, $n = 15$, $\alpha = .05$, $\sigma = 0.39$) for small ($\delta = 0.2$, power = 0.44), medium ($\delta = 0.3$, power = 0.79), and large ($\delta = 0.4$, power = 0.96) size differences between population means (Cohen, 1992). These analyses indicate a high chance of detecting medium to large significant differences given our sample size and the nature of our data. In short, our results suggest that females do not preferentially associate with areas scent marked by large versus small males, and thus fail to exhibit a preference toward potentially more competitive males (Carazo, Font, & Desfilis, 2007; Table 1).

Out of the 10 trials in which females responded to experimentally induced dusk by entering a refuge, seven females entered into refuges in the area scent marked by small males, two into refuges in the area scent marked by large males, and only one into a refuge in the control area. The chi-square test indicated a marginally significant departure from expected frequencies ($\chi^2 = 6.21$, $df = 2$, $p = .048$). Monte Carlo simulations also detected a significant effect (i.e., all $p < 0.025$), yielding a cumulative $p = .022$.

Discussion

Our results show that females preferentially associate with areas that have been marked by conspecific male lizards. The presence of conspecifics in a territory may be a cue to resource availability (e.g., mates, refuges, food, basking sites), or absence of predators (e.g., Stamps, 1988), and it has been proposed that scent marks may function as social attractors for conspecifics (e.g., López & Martín, 2004). Hence, the fact that females were attracted to scent marked areas is in accordance with theoretical expectations. However, we did not find evidence that females preferentially associate with areas scent marked by larger males. Our study of scent mark function in *P. hispanica* hence argues against the notion that females may be choosing territories on the basis of the resident male's size. Although we did not find evidence of female choice of male territories, females in our study did exhibit a preference when selecting a refuge in which to spend the night. That females select refuges with male scent marks is expected because marked refuges will usually be indicative of safe refuges in which to spend the night. However, females in our study preferentially selected refuges in areas scent marked by small males, which is interesting on several fronts. First, female choice of refuges marked by small males could be a female strategy to avoid forced copulas by large males (Kokko & Rankin, 2006). Because females may be able to avoid forced copula attempts by small males, but not by large males, choosing refuges marked by small males could reduce the overall costs of sexual harassment. Second, our findings strongly

Table 1
Time Each Experimental Female Spent in: (1) The Area Scent Marked by 'Small' Males, (2) the Area Scent Marked by 'Large' Males, and (3) the Control Area

		Treatment areas		
Female	Choice of refuge	'Small' male scents	'Large' male scents	Control
1	'Large' male	17 (0.61)	7 (0.25)	4 (0.14)
2	'Small' male	30 (0.75)	9 (0.23)	1 (0.03)
3	—	4 (0.17)	15 (0.65)	4 (0.17)
4	—	18 (0.44)	12 (0.29)	11 (0.27)
5	'Small' male	9 (0.47)	7 (0.37)	3 (0.16)
6	Control	3 (0.08)	17 (0.47)	16 (0.44)
7	'Small' male	7 (0.35)	12 (0.60)	1 (0.05)
8	'Small' male	17 (0.47)	12 (0.33)	7 (0.19)
9	—	18 (0.40)	16 (0.36)	11 (0.24)
10	'Small' male	9 (0.33)	9 (0.33)	9 (0.33)
11	—	20 (0.56)	7 (0.19)	9 (0.25)
12	'Small' male	3 (0.09)	25 (0.78)	4 (0.13)
13	'Small' male	14 (0.40)	18 (0.51)	3 (0.09)
14	—	13 (0.39)	13 (0.39)	7 (0.21)
15	'Large' male	5 (0.21)	15 (0.63)	4 (0.17)
$\bar{x}$		12.47 (0.38)	12.92 (0.43)	6.24 (0.19)
SD		7.70 (0.19)	4.96 (0.17)	4.25 (0.11)
CI		3.90 (0.09)	2.51 (0.09)	2.15 (0.05)

Note. Data represent raw number of scans (associated proportions are given in brackets). Descriptive statistics include means ($\bar{x}$), confidence intervals (CI), and standard deviations (*SD*).

suggest that females of *P. hispanica* are capable of discriminating among males of different body size using scent marks (for a demonstration of a similar ability in males see Carazo, Font, & Desfilis, 2007). This result agrees with recent reports of sophisticated female chemosensory assessment of male quality and condition in other lacertids (e.g., López & Martín, 2004; Mason & Parker, 2010). Overall, however, our results do not agree with previous findings suggesting that female lacertids may be using male scent marks as a way to indirectly choose their mates. Although male size is related to male competitive quality in this species, this discrepancy could simply mean that females attend to phenotypic traits other than size in their assessment of male phenotypic quality (e.g., López & Martín, 2004; Mason & Parker, 2010).

In any event, the uncomfortable truth is that information about how female lacertids choose their territories in the wild, and about the link between female preferences in scent choice trials, their acceptance or rejection of copulas with associated males, and subsequent male reproductive success is very scarce. For example, rather than exerting precopulatory mate choice based on male phenotypic traits, females could be using the information contained in scent marks to bias fertilizations in favor of selected males by cryptic female choice (e.g., Calsbeek & Sinervo, 2002; Calsbeek & Bonneaud, 2008; Olsson & Madsen, 2001). The latter possibility is particularly interesting (Calsbeek & Bonneaud, 2008), and would reconcile the seemingly widespread existence of female preferences toward certain male scents in scent choice trials with the relative scarcity of evidence in support of precopulatory female choice in territorial lacertid lizards.

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