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The lexicon of love: what cues cause size-assortative courtship by male garter snakes?

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Abstract Cues that females use to select potential mates have attracted substantial research effort, but the criteria for male mate choice remain very poorly known. Red-sided garter snakes (*Thamnophis sirtalis parietalis*) court and mate in large aggregations around overwintering dens in southern Manitoba, Canada. Both courtship and mating are size-assortative: small male snakes court small as well as large females, whereas larger males court only large females. This system provides a unique opportunity to assess the cues that males use in selecting mates, and in particular the mechanisms that generate a size-related shift in mate preference. Experiments in which we manipulated body sizes and scents showed that both vision and scent (sex pheromones) were important. Large males directed intense courtship only when the stimulus provided both visual and chemical (skin lipid) evidence of large body size. Small males were much less discriminating in both respects. Thus, size-assortative mating in this system is generated not by larger males excluding their smaller rivals from the largest females (as has been reported in other reptile species), but by a size-related shift in the visual and pheromonal cues that elicit courtship. Males of some species may thus show complex patterns of mate choice, with the cues that stimulate courtship differing even among males within a single population based on traits such as age or body size.

Keywords Body size · Pheromone · *Thamnophis sirtalis parietalis* · Male mate choice

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Introduction

Darwinian orthodoxy says that females are choosy about mate selection whereas males are not, but (like most generalizations in evolutionary biology) there are many exceptions to the rule (Trivers 1972; Andersson 1994). If males contribute significant parental care, or are limited in the number of females with which they can copulate, selection may favor careful mate choice by males (Dewsbury 1982; Sargent et al. 1986; Schwagmeyer and Parker 1990; Olsson 1993). Nonetheless, studies on the cues that influence mate selection by males have attracted very little research compared to the voluminous literature devoted to mate selection by females (e.g. Andersson 1994). As a result, there is a general perception that males do not display the kinds of subtle, sophisticated mate-selection “choices” so well-documented in females. Is this a real difference between the sexes or an artifact of insufficient study of males? Information on mechanisms of mate choice by males may answer this question as well as providing a broader context within which to evaluate mate choice in general. Thus, we need a better understanding of the processes that determine which potential mate is selected by a male; and especially, why males within the same species may sometimes differ in the attributes of females that they select as mates.

Male red-sided garter snakes (*Thamnophis sirtalis parietalis*) provide an ideal opportunity in this respect. Perhaps because they have only a limited supply of sperm and mating-plug secretions, males are highly selective with respect to mate choice (Shine et al. 2000b). Not only do they tend to actively prefer large rather than small females (Hawley and Aleksyuk 1976; Shine and Mason 2001), but the degree of this mate preference shifts with male body size. Smaller (younger) males court and mate small as well as large females, but larger males restrict their courtship and mating almost entirely to larger females (Shine et al. 2001b). Because these animals tolerate close approach and disturbance by human observers, we can conduct simple experiments not only to determine which female cues elicit the most intensive

courtship, but also to compare responses by size classes of males that differ in mate choice.

Because female body size affects courtship intensity of red-sided garter snakes, males must somehow assess the body sizes of females. Vision offers the most obvious cue to female body size, but visual information may often not be available because most unmated females at a communal den are submerged beneath a writhing “mating ball” of dozens to hundreds of amorous males (Gregory 1974; Mason 1993). Experimental studies show that males can assess female body size with the vomeronasal system, by tongue-flicking lipids on the female’s skin (LeMaster and Mason 2002). Do such pheromonal cues over-ride visual cues entirely, or do snakes use both kinds of information to assess female body size? Also, does the proximate basis for size-assortative courtship in this system rely upon ontogenetic shifts in the degree of reliance on different sensory systems? Alternatively, size-assortative mating may have nothing to do with shifts in the cues that stimulate reproductive behavior. Instead, males of all sizes may prefer large females, with large males monopolizing matings with these more fecund females. Smaller males would thus be forced to court and mate smaller females, so that size-assortative mating would result from male-male not male-female interactions. This situation generates size-assortative mating in two lizard species (Olsson 1993; Cooper and Vitt 1997). If the same situation applies in garter snakes, we would not expect to see ontogenetic differences in the cues that elicit male courtship.

Methods

We studied garter snakes at a communal den near Inwood, southern Manitoba (50°32'N 97°30'W) in May 2001. These small [males up to 60 cm snout-vent length (SVL), females up to 75 cm SVL] non-venomous snakes gather in large numbers at overwintering dens, and mate before dispersing in early spring (Gregory 1974; Gregory and Stewart 1975). Males pursue newly-emerged females and virtually ignore human interference. Thus, we could assess the intensity of male courtship by holding female snakes by the tail and presenting them to males on the rocky slopes surrounding the den. We used a four-point scale to quantify intensity of the male’s courtship (0=no interest; 1=increased tongue-flick rate; 2=chin pressed against the female’s body; 3=body aligned with the female’s body). Chin-pressing and body-alignment are very distinctive and easily-scored behaviors, and are not seen in any context other than male courtship (Whittier et al. 1985). We scored the responses of the first five males from each of two size classes (small <45 cm SVL; large >45 cm SVL) whose heads came into close proximity (<2 cm) with the middle part of the female’s body. Because the den contains >10,000 snakes, and males move about constantly, the probability that any single male was recorded more than once is trivially small.

To evaluate the relative significance of vision and scent, we used dead garter snakes as our stimuli. These were five large (62–67 cm SVL) and five small (48–54 cm SVL) females, and five males similar in size to the small females (47–53 cm SVL). All were found dying after attack by predators (Shine et al. 2001a), and were humanely killed by interperitoneal injection of Brevital Sodium. These animals were soaked overnight in hexane to extract superficial lipids from the skin; the resultant solutions were evaporated down to provide samples of lipids from either small

females or large females. The soaking process did not change the animal’s superficial appearance, and the hexane itself rapidly evaporated. These hexane-soaked snakes should thus provide a “normal” visual stimulus, with little or no pheromonal contribution. We first tested the attractiveness of these animals by scoring responses of both large (>45 cm SVL) and small (<45 cm SVL) male snakes. We then painted the “target” animals with lipids extracted from either small or large females, and re-tested them. All of the targets were hexane-washed between successive trials to eliminate lipids from earlier treatments.

We obtained responses from 10 male snakes (5 small, 5 large) to each of five replicate animals within each of nine treatments (male, large female or small female treated with hexane, lipids from large females or lipids from small females). We analyzed courtship responses using three-factor ANOVA (with size class of the courting male, target type, and size class of the female from which lipid had been extracted as factors; and mean male response to each target as the dependent variable).

Results

A randomly collected subsample of the courting males showed a significant difference in mean body sizes between small snakes ($n=30$, mean=39.5 cm, range 34.5–43.1 cm) and large snakes ($n=30$, mean=49.6 cm, range 45.4–56.5 cm; $F_{1,58}=198.34$, $P<0.0001$). Because many males did not show any courtship response to many stimuli, the data-set on response intensities was highly non-normal in distribution. We overcame this problem by calculating mean intensities of response per target animal in each treatment. After arcsine transformation, the resulting distribution did not deviate significantly from normality and hence we could apply parametric statistical tests.

Three-factor ANOVA (with size class of the courting male, target type, and source of lipid as factors; and mean male response to each target as the dependent variable) yielded a significant three-way interaction term ($F_{4,72}=3.14$, $P<0.02$). To clarify this situation, we examined responses separately for large and small males. Small males responded more intensely to female targets than to male targets and to female lipids rather than hexane (control) scent, but with a significant interaction between target sex and lipid treatment (Fig. 1; $F_{4,36}=6.89$, $P<0.0003$). This interaction term reflects the lack of response of small males to hexane-painted males (Fig. 1). In contrast, large males responded much more intensely to large females painted with large-female lipids than to any other stimulus, but with a significant interaction term because (as for small males) hexane-soaked males attracted no courtship (Fig. 1; interaction, $F_{4,36}=3.39$, $P<0.02$).

We can focus on the roles of vision and scent in eliciting courtship by holding each of these factors constant in further analyses. In trials using large females as the visual stimulus, large and small males differed in their response to pheromone application (interaction between male body size and treatment, ANOVA, $F_{2,24}=10.08$, $P<0.0007$): small males responded almost equally to lipids from either small or large females, whereas large males responded much more intensely to the scent of large females than small females (Fig. 1). The same pattern was seen in trials using a male as the visual stimulus (interaction, $F_{2,24}=25.88$, $P<0.0001$). Small

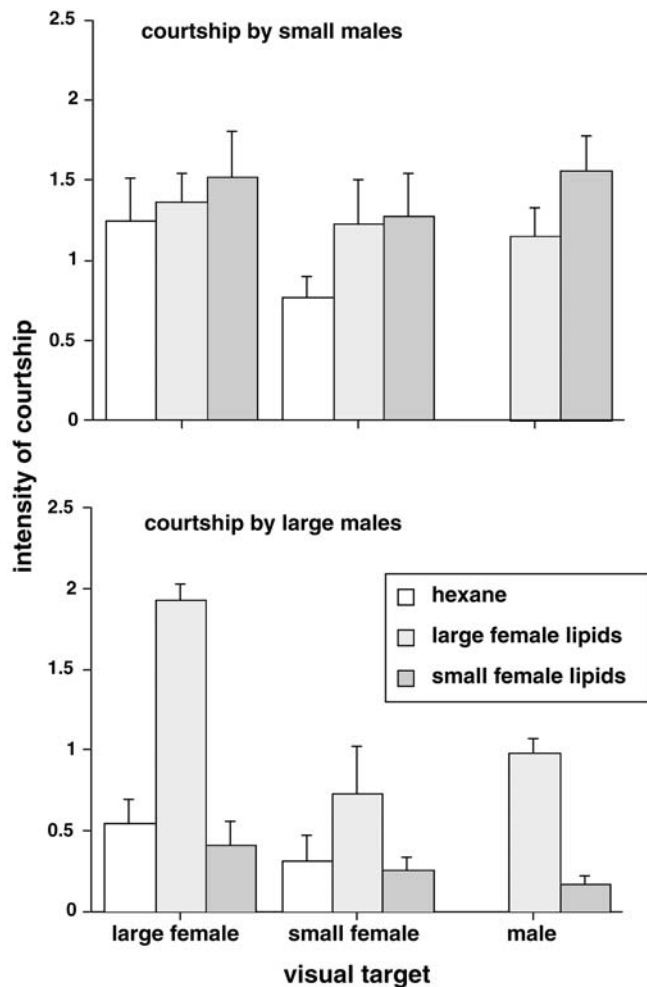


Fig. 1 The intensity of courtship (scored on a four-point scale; see text for details) that free-ranging male garter snakes (*Thamnophis sirtalis parietalis*) directed towards various stimuli in trials conducted at a den near Inwood, Manitoba. The stimuli comprised recently killed snakes (males, small females or large females) that had been soaked in hexane overnight to remove the skin lipids that serve as sex pheromones in this species. The snakes were then presented to mate-searching males either without further treatment (hexane) or after being painted with lipids extracted from large females or small females. Histograms show mean values, and bars show one standard error on either side of the mean. Data are shown separately for courtship by small (snout-vent length <50 cm) and large (SVL >50 cm) males; $n=5$ males for each trial, so a total of 90 males were tested. See text for statistical analyses

males courted small females more intensely than did large males, regardless of scent ($F_{1,24}=15.16$, $P<0.0007$; interaction, $F_{2,24}=0.59$, $P=0.56$).

We can examine the role of visual stimuli in the same way, by analyzing responses when pheromonal cues were held constant. In trials with the control (hexane) treatment, males of both size classes courted females but not males (Fig. 1), suggesting that either our hexane-soaked targets retained some slight pheromonal cues, or that females differ from males visually in some subtle way. Small males courted these hexane-soaked females more intensely than did large males (ANOVA, $F_{1,24}=10.89$, $P<0.003$) and large females attracted more interest than

did small females ($F_{2,24}=32.47$, $P<0.0001$; post-hoc tests show that courtship intensity to all three target groups differed at $P<0.05$). Trials in which we applied lipids from small females generated intense courtship from small males but not large males ($F_{1,24}=63.94$, $P<0.0001$), regardless of the visual stimulus (other terms have $P>0.56$). Lastly, trials in which we applied lipids from large females provoked intense courtship, with large males responding more strongly to the large female visual stimulus in this trial than to other stimuli whereas small males responded to all treatments bearing this scent (interaction, $F_{2,24}=3.84$, $P<0.04$).

Discussion

Our results suggest that males use both visual and pheromonal cues to assess female body size, and that large and small males differ in their response to these cues. Large males directed intense courtship only when the target provided both visual and pheromonal evidence that it was a large female (Fig. 1). In contrast, small males courted intensely if the stimulus provided either visual or pheromonal evidence that it was a female, regardless of body size (Fig. 1). One caveat to this conclusion is that the hexane-treated animals may have retained slight traces of pheromones even after overnight soaking, so that some of the responses we interpret as visual might reflect very subtle vomeronasal cues instead.

Our data are consistent with earlier reports that male garter snakes use visual and pheromonal cues to assess a female's body size and allocate courtship effort accordingly (Shine and Mason 2001; LeMaster and Mason 2002). Figure 1 also reveals a marked disparity between small and large male snakes in terms of their reliance on these different kinds of cues. Larger males were highly selective courters (requiring both visual and pheromonal evidence of large female body size) whereas smaller males were less selective (Fig. 1). In particular, small males directed intense courtship to both the visual and chemical attributes of small females, whereas large males did not. Mark-recapture studies on Manitoba garter snakes suggest that all males grow rapidly and that most males within our small category when first measured will be large males within a year or two (Shine et al. 2001b). Thus, the shift in mate preference reflects an age-related change within the life of individual males rather than a behavioral polymorphism within the garter snake population.

This size-related shift in the cues that stimulate courtship supports earlier mate-choice trials (Shine et al. 2001b) in showing that size-assortative mating within red-sided garter snakes is not an indirect result of male-male competition for the largest and most fecund females. Even in the absence of larger rivals, small male garter snakes vigorously courted stimuli that did not attract interest from larger males (Fig. 1). Thus, size-assortative mating in this species reflects an obligate shift in male mate preferences, rather than a facultative redirection of courtship by males unable to compete with larger rivals. Nonetheless, such competition might have been an initial

selective pressure for the size-based divergence in mate recognition systems.

This size-based shift in the stimuli eliciting courtship has major consequences for the mating system of red-sided garter snakes. Not only does it generate strongly size-assortative courtship and mating (Shine et al. 2001b), but it also results in intense sexual conflict between juvenile females and small adult males (Shine et al. 2000a). Juvenile females often copulate, presumably because they cannot avoid doing so (Shine et al. 2000a). In turn, this sexual conflict may have moulded major patterns of den emergence behavior, and affected mortality schedules of different age and sex classes within den populations (Shine et al. 2000a). Our results thus support and extend earlier conclusions that the superficially chaotic mating assemblages of garter snakes involve diverse and complex male-female interactions, mediated by body sizes of the participants (e.g. Shine et al. 2000a, 2000b; Shine and Mason 2001).

The generality of these results is difficult to assess. There is a strong link between female body size and reproductive output in many kinds of organisms: larger females are likely to reproduce more often, and produce more (and often, larger) eggs per clutch (e.g. Fitch 1970; Seigel and Ford 1987; Clutton-Brock 1991; Andersson 1994). This link may favor a general male preference for large (and hence, fecund) females. Such a preference has been demonstrated in several species of lizards (e.g. Olsson 1993; Cooper and Vitt 1997; Whiting and Bateman 1999) and two species of snake (Aleksiuk and Gregory 1974; Hawley and Aleksiuk 1976; Gartska et al. 1982; Shetty and Shine 2002). In all these taxa, males must be able to assess the body sizes of potential partners. The proximate mechanisms by which they accomplish this task undoubtedly vary interspecifically, and our study indicates that intraspecific variation warrants examination as well. More generally, male mate choice may exhibit as much subtlety and complexity as the more intensively studied reproductive "decisions" of females. Accordingly, we will need a much better understanding of male mate choice before we can fully appreciate mating system diversity.

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