

R. Shine · R.T. Mason

Courting male garter snakes (*Thamnophis sirtalis parietalis*) use multiple cues to identify potential mates

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Abstract Mating aggregations of red-sided garter snakes (*Thamnophis sirtalis parietalis*) in Manitoba provide a unique opportunity to identify the cues that attract a male snake and induce him to court. The snakes are abundant, tolerate human presence, and males direct courtship to a subset of other males (“she-males”) as well as to females. Previous work has emphasised the role of pheromonal cues (skin lipid profiles) as sexual attractants in snakes. However, pheromones are so widely distributed throughout the den area that these chemical cues may be difficult for males to use to localise females. Our field studies show that males and females differ in several other attributes such as size (females are larger than males), body temperature (very cold snakes are often females), muddiness (females are often covered in mud) and whether or not they are solitary (most females are surrounded by courting males). Experiments show that males use all of these cues to identify possible sexual partners. Visual and thermal cues are particularly important for solitary mate-searching males; but after a mating ball forms around a female, pheromones may be most important. Our study also reveals substantial variation among individuals: females vary in attractiveness, and males vary in their intensity of courtship as well as in the magnitude of their preference for one potential partner versus another. Thus, a male snake’s “decision” whom to court depends not only on visual and thermal as well as chemical cues, but also on the male’s own preferences and on subtle differences among potential sexual “targets”.

Keywords Colubridae · Mate choice · Reproduction · Snake · *Thamnophis sirtalis parietalis*

Introduction

In any sexually reproducing species, the cues used by an individual for mate selection (i.e. to identify another individual as a potential sexual partner) are important from several perspectives, including sexual selection. Many mating systems impose strong selection on males for the ability to locate receptive females, and to direct effective courtship towards such individuals (Andersson 1994). Thus, males in such species should evolve the ability to respond to attributes that identify the sex or reproductive status of a conspecific animal.

Extensive research has documented a considerable diversity in the kinds of traits that animals use to determine whether or not another individual is a potential sexual partner. Perhaps the most obvious are visual cues, such as colours that vary according to sex and reproductive status, and highly ritualised displays that convey sexual information (e.g. Andersson 1994). Many species also use auditory cues to advertise their sexual status (e.g. Ryan et al. 1983). Chemical cues are also important: sex pheromones are known to play a role in the mating systems of many taxa (e.g. Andersson 1994). Although snakes have attracted less research than many other types of terrestrial vertebrates, there is strong evidence that pheromonal cues play a prominent role in the courtship of several species (e.g. Ford and Low 1984; Mason 1992). Pioneering studies by Noble (1937) suggested a key role for the vomeronasal system in this respect, and more recent work has clarified the exact nature of those chemical cues (e.g. Halpern and Kubie 1984; Mason et al. 1989, 1990; Mason 1992). However, other potential indicators of sex (and hence, potential cues for courtship) in snakes have attracted less attention. Pheromonal cues certainly play a role in snake courtship, but male snakes plausibly use other cues as well, perhaps in specific components of the courtship se-

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R. Shine (✉)
School of Biological Sciences A08, University of Sydney,
NSW 2006, Australia
e-mail: rics@mail.bio.usyd.edu.au
Tel.: +61-2-93513772, Fax: +61-2-93515609

R.T. Mason
Zoology Department, Oregon State University, Cordley Hall 3029,
Corvallis, OR 97331-2914, USA

quence. For example, several authors have speculated that male snakes may rely upon visual cues for the initial location of potential mates (Pisani 1976; Joy and Crews 1985; Gillingham 1987).

The scarcity of research on this topic undoubtedly reflects logistical constraints: most snakes are too scarce, and too easily disturbed, for quantitative studies on courtship behaviour. Mating aggregations of red-sided garter snakes (*Thamnophis sirtalis parietalis*) in southern Manitoba offer an exception to this situation (Gregory 1974, 1977; Gregory and Stewart 1975), and hence provide a unique opportunity to evaluate the mechanistic basis of mate attraction. This system has been extensively used to investigate the pheromonal aspects of courtship (Mason 1993). In the present paper, we look more broadly at the range of cues available to male garter snakes to distinguish conspecific females from males. The Manitoba garter snakes offer two additional advantages for such a study. First, a subset of males (“she-males”) act as female mimics (Mason and Crews 1985). These animals have skin lipid profiles similar to those of females, and may thereby benefit by confusing rival males in mating balls (Mason and Crews 1985; Mason et al. 1987; Shine et al. 2000a, 2000b). The presence of two types of “courtship targets” in this system provides additional opportunities for us to clarify the basis of sexual attractivity. Second, the huge numbers of snakes (>20,000 per den: unpublished data) must result in the ground surface near the den becoming criss-crossed with pheromonal trails, reducing the ability of male snakes to localise any particular female. Under such circumstances, we might expect males to use other cues, as well as scent, to locate potential mates.

The paper is organised as follows. First, we examine the characteristics of a random sample of snakes collected around the dens, to identify traits that differ consistently between males and females (and “she-males”). Any such trait could potentially offer useful information to a male snake concerning the sex of another individual. Then we use simple experiments, either in the field or in outdoor arenas, to investigate whether or not male garter snakes actually use these potential cues. Our field experiments tested male snakes in two situations: either when they were solitary (searching for mates) or when they were part of “mating balls” centred around a female (and hence, were actively courting). Plausibly, the cues that a male uses may differ in these two circumstances. Last, we ask more detailed questions about the nature of individual variation within both females (are all adult females equally attractive to males?) and males [are all males equally vigorous in courtship, and do they all prefer the same kind of sexual “target” (e.g. female versus “she-male”)?).

Methods

Species and study area

We studied red-sided garter snakes at large communal hibernacula in southern Manitoba (Chatfield Community Pasture and Inwood

quarry; see Mason and Crews 1985; Mason 1993; Shine et al. 2000a, 2000b for details). The snakes emerge from their overwinter dens in spring (April–May), and courtship and mating occur over this period before the snakes disperse to their summer ranges (e.g. Gregory 1974, 1977). Adult male snakes in this population average 45 cm snout-vent length (SVL), whereas adult females average 60 cm SVL. Males form large “mating balls” around the newly emerged females, with copulation occurring both within the den itself (a rock-lined depression) and on the surrounding grassland. Data in the present paper were gathered at the dens in May 1997, 1998 and 1999.

Attributes of he-males, she-males and females

To compare attributes of females, she-males and other males (hereafter referred to as “he-males”) that might serve as cues for courtship, we surveyed snakes in courting groups both inside and outside the den. Groups were chosen randomly, but we attempted not to resample the same snakes. Given the numbers of snakes in this system, any such inadvertent resampling would have been rare. The snake’s body temperature was determined with a quick-reading cloacal thermometer, and the snake was then sexed (by tail shape and/or manual eversion of the hemipenes), measured (SVL), and weighed. She-males were recognised by their attractiveness to other males; we found these snakes by examining “mating balls” that did not contain females. Patterns for all behavioural variables were virtually identical for each year, so we have combined the data sets for most analyses.

Identification of cues that initiate courtship

To test whether or not males use particular cues to initiate courtship, we assessed attractivity of “target” snakes in a number of situations. The simplest technique took advantage of the fact that we could sit down in the grassland beside the den with dozens of courting snakes within arms length. Holding a snake by the tail, we slowly introduced it into one of these courting groups, or in front of a solitary male. Each “target” snake was tested six times in succession: three times against solitary males, and three times within a courting group. The order of testing was randomised. In each of the “courting group” trials, we assessed responses of three males within that group. Our criterion for including a male’s response was that his head was close enough, while stationary, for him to have a reasonable opportunity to detect any cues emanating from the “target” snake. In practice, almost all males that encountered the “target” snake fulfilled this criterion, since most males stop and tongue-flick repeatedly as they move about in search of mates.

For each of the solitary males that encountered our “target” animal, we scored his intensity of courtship towards this animal on a four-point scale (modified from Whittier et al. 1985: 0=no interest, 1=elevated tongue-flick rate, 2=address chin, 3=align body with that of the target snake). In the case of the males in courting groups, we scored whether or not each male showed any interest (i.e. score>0) and the highest courtship intensity score exhibited by any of the three males within that trial. The work was done blind; the tester was not told which group the “target” snake belonged to.

We used this technique to assess the relative attractiveness of three types of potential “targets”: females, she-males and other males. We also examined the effects of two variables that we could manipulate experimentally:

1. Body temperature. We collected he-males from courting groups in the morning. One group was cooled on ice to a mean body temperature (at the time of testing later that day) of $5.6 \pm 3.3^\circ\text{C}$; the other group was not cooled, and averaged $29.8 \pm 2.7^\circ\text{C}$. Snakes from the two groups were offered in random order as potential “targets” for courtship. The “targets” were held immobile during the trials, so that greater movement by warmer snakes could not confound our results.

2. Influence of mud on the body surface. To determine if male courtship was affected by whether the “target” was clean or muddy, we cleaned freshly collected snakes (by washing them in water, in a plastic bag) or dirtied them (by placing them inside a plastic bag with thick mud). In each case, the snake was dried with paper towel after it was washed or dirtied. Snakes that had been muddied had substantial mud adhering to their rugose dorsal scales after the treatment, and looked very much like the “naturally” muddy animals.

One other kind of test was conducted in the field. We took two large freshly dead female snakes (ca 70 cm SVL; cause of death unknown) that were found near the den. Such females attract courtship for at least 3 days after their death (personal observation). Each of these females was laid out dorsal surface uppermost approximately 50 cm from the nearest mate-searching male, and we recorded the number of males courting the dead female 15 s later. The trial was then terminated, and the female lifted up and placed elsewhere. In half the trials, the female was alone when placed on the ground. In the other half, she was accompanied by a single courting male (intentionally attracted to her, by placing the female very close to him). We could thus compare the effect of this male’s presence on the number of other males that joined the courting aggregation within 15 s. The two sets of trials used different females, and were conducted at different places at different times.

We also used open-topped outdoor arenas constructed of nylon fabric, 1.0×1.0 m wide×0.8 m high, to quantify influences on a “target” snake’s attractiveness to males. The first of these tests involved the role of female body size. Two females (one large, one small) were placed in each arena, and then 20 males were added. At seven 15-min intervals, we recorded the number of males courting each female at that point in time. To analyse the data, we calculated the mean difference (across all seven time periods) for the number of males courting small versus large females for each enclosure, and compared this to an expected value of 0 under the null hypothesis of equal courtship regardless of female body size.

In a second set of trials, we used the arenas to quantify the intensity of attractiveness of different “target” snakes to different courting males, as measured by the intensity of the male’s courtship response. Thus, we could determine whether the relative attractiveness of different females (or of females versus she-males) differed among individual males. To our knowledge, variation at this level has never been investigated among reptiles. To gather

these data, we placed a single “target” snake (a he-male, a she-male, or a female with her cloaca taped to prevent copulation) in each of 24 arenas. Of the 12 he-males used, 6 were vigorously rubbed against females in an effort to transfer skin lipids (as might occur in the course of normal activities). Because this manipulation proved to affect neither a snake’s behaviour nor his attractiveness to other males (Shine et al. 2000a), it does not affect any of the analyses in the current paper. We then added 6 males, one of which (the focal male) was paint-marked for recognition. We then scored the intensity of courtship by the focal male towards the “target” animal at 4-min intervals. The other males within the enclosure were not scored; they were simply present to ensure that the number of snakes was similar to those in many natural courting groups. After three replicate observations (i.e. 12 min), the “target” snake was moved to the next enclosure and the procedure repeated. Each “target” snake was used in all 24 arenas. We thus obtained a symmetrical data set whereby we could compare the intensity of courtship by 24 male snakes to 24 target stimuli.

We analyse these data both in terms of individual snakes (using identification numbers of each “test” and each “target” as factors in a two-way ANOVA), or in terms of general groupings (using “test” identification number and “target” category as factors). Thus, we can determine (1) whether or not individual males differ in their preferences (i.e. intensity of courtship) for different individuals within a given “target” group (either males or females), and (2) by treating individual “targets” as replicates within their sex, we can determine whether or not individual males differ in their relative intensity of courtship to males versus females. To examine this question we deleted the data for “rubbed males”, because this is an artificial group.

Data in the text are reported as the mean±SD.

Results

Attributes of he-males, she-males and females

Our survey data show that males and females differ in several respects (Table 1). For example, female garter snakes are much larger than males (in both length and mass), and are more frequently muddy. Post hoc Scheffé tests of the data in Table 1 show that males, females and

Table 1 Characteristics of male and female gartersnakes (*Thamnophis sirtalis parietalis*) and female mimics (“she-males”), at communal dens in southern Manitoba. The table provides mean values and associated SDs (in parentheses) for continuous variables, and proportions for categorical variables. See text for definitions. Tests for differences among the three types of snakes are contingency table analyses for categorical variables, and ANOVAs

for continuous variables. ANOVAs are one-factor except for temperature, for which data were available from 2 years. The ANOVA for this trait thus includes year as well as sex (male/female) as factors (she-males not included because no thermal data were available for this group in 1997); the interaction term was not significant so only the main effect of sex is shown. See text for results of post hoc comparisons

Trait	Males	“She-males”	Females	Test
Mean snout-vent length (cm)	45.3 (4.0) (n=899)	47.1 (3.9) (n=143)	55.9 (7.4) (n=91)	$F_{2,1130}=249.4, P<0.0001$
Mean body mass (g)	30.8 (8.2) (n=896)	31.7 (9.3) (n=143)	76.5 (33.5) (n=91)	$F_{2,1127}=361.7, P<0.0001$
Mean body temperature (°C)				
1997	16.9 (4.6)	–	16.4 (5.0)	
1999	18.7 (5.9) (n=878)	16.3 (3.0) (n=43)	17.7 (5.4) (n=243)	$F_{1,1117}=3.99, P=0.046$
Proportion of mud-covered snakes	0.17 (n=587)	0.90 (n=52)	0.75 (n=44)	$\chi^2=192.1, 1 \text{ df}, P<0.0001$
Number of other snakes courting the focal snake in a 5-min observation period	0.41 (1.5) (n=17)	16.5 (13.0) (n=22)	26.8 (7.4) (n=14)	$F_{2,50}=32.2, P<0.0001$

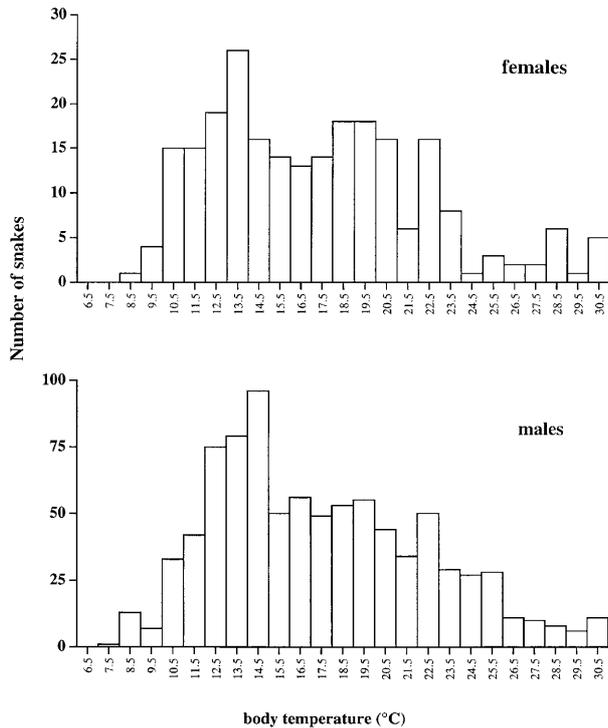


Fig. 1 Body (cloacal) temperatures of free-ranging garter snakes. Mean body temperatures did not differ between the sexes (see text), but the frequency distributions show that a higher proportion of very cool snakes were females

she-males differed significantly from each other in average SVL ($P < 0.001$ in each comparison) and that females were heavier than either males or she-males ($P < 0.001$). She-males and he-males did not differ significantly from each other in mean body mass (Scheffé $P > 0.05$). The proportion of snakes that were covered in mud was lower for he-males than for either she-males ($\chi^2 = 140.99$, 1 *df*, $P < 0.0001$) or females ($\chi^2 = 79.23$, 1 *df*, $P < 0.0001$), but the proportion of mud-covered animals was similar in she-males and females ($\chi^2 = 3.03$, 1 *df*, $P = 0.082$). Females are also the target of intense courtship by large numbers of males. Most females are constantly surrounded by a writhing ball of suitors, whereas males were not. She-males were intermediate in this respect (Table 1; Scheffé post hoc tests show that each group differed from the other two at $P < 0.003$). Females exhibited slightly lower mean body temperatures than did males (note that the ANOVA reported in Table 1 is based only on he-males and females, because no thermal data for she-males were available in 1 of the 2 years). Many of the coldest animals were females (Fig. 1). Thus, low temperature may give a cue as to the snake's gender.

Our data are more limited with respect to she-males, mainly because these animals are morphologically similar to other males (Mason and Crews 1985), and thus can only be identified on the basis of their sexual attractiveness to other males. Our data confirm the similarity between she-males and he-males in overall body size, but

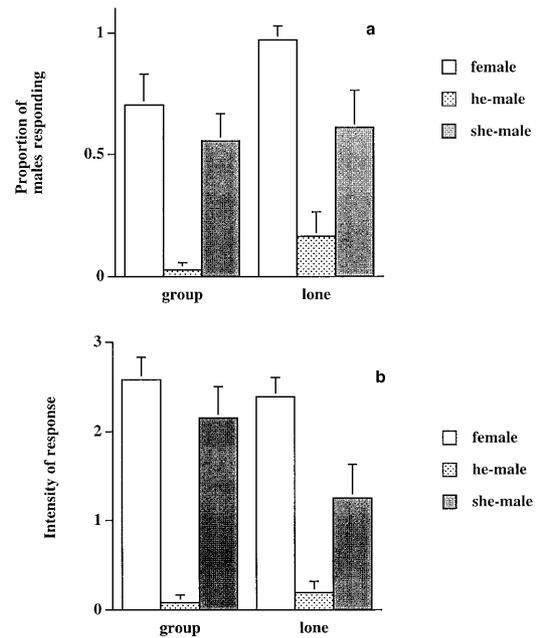


Fig. 2a,b Responses of free-ranging male garter snakes to “target” snakes of different types. We measured responses of males either when they were part of courting groups (*group*) or when they were solitary (*lone*). These animals were presented either with a female snake, a she-male (i.e. a male with female-like skin lipids) or a he-male (a male with male-specific skin lipids). The response of the males depended upon the type of stimulus and the context in which it was presented. Histograms show means and associated SDs. **a** The proportion of tested males that responded to the stimulus. **b** The mean response intensity on a four-point scale (see text for further explanation)

also show that she-males were consistently muddier than other males (Table 1).

What is the relative attractiveness of he-males, she-males and females?

Males responded strongly and reliably to females, at intermediate intensity to she-males, and virtually not at all to he-males (Fig. 2). Interestingly, the responses of solitary males differed from those of males in courting groups: solitary males were more likely to respond (albeit at a low level of intensity) to other males, but less likely to respond vigorously to she-males. Statistical analysis using two-factor ANOVA confirmed the significance of these patterns. For the proportion of males responding to the “target” snake, the ANOVA revealed a significant effect of “target” type ($F_{2,90} = 52.18$, $P < 0.0001$) and a difference in response between solitary versus grouped snakes ($F_{1,90} = 7.70$, $P < 0.01$). There was no significant interaction between these two effects ($F_{2,90} = 1.37$, $P = 0.26$). Post hoc Scheffé tests confirmed that the proportion of males responding to the stimulus differed significantly ($P < 0.0003$) in all comparisons (females elicited more responses than either she-males or he-males, and she-males elicited more responses than he-males).

The mean intensity of response was also affected by the “target” type ($F_{2,90}=76.85$, $P<0.0001$) and whether the responding males were solitary versus in a group ($F_{1,90}=5.14$, $P<0.03$). However, the interaction between these two factors was also significant ($F_{2,90}=5.21$, $P<0.01$; see Fig. 2). Lone males and group males responded with similar intensity to females ($F_{1,22}=1.41$, $P=0.25$) and he-males ($F_{1,22}=2.05$, $P=0.17$), but group males responded more intensely to she-males ($F_{1,22}=12.33$, $P=0.001$). Thus, although the group males responded less intensely to he-males than to either females or she-males (Scheffé test, $P<0.0001$), their responses to females and she-males were not significantly different (Scheffé test, $P=0.19$). In contrast, all post hoc comparisons were significant for the analysis on response intensity of solitary males: females attracted more intense responses than did either she-males or he-males, and she-males attracted more intense responses than did he-males (all $P<0.0001$ with Scheffé tests). These analyses show that a male garter snake can consistently distinguish between he-males, she-males and females, but his relative intensity of courtship towards each of these types of “target” depends on whether he is mate-searching alone, or is part of a courting group.

Are males attracted to colder snakes?

Because the coldest snakes are often females (Fig. 1, Table 1), males might use the body temperature of a snake as a cue to its sex. To test this proposition, we compared responses of courting males to warm versus experimentally cooled he-males. This treatment strongly affected the responses of solitary males, and exerted a smaller (but still significant) effect on males in courting groups. Males directed more courtship towards cold males, as predicted (Fig. 3; for the proportion of males responding to the “target” snake: effect of “target” type $F_{1,50}=18.801$, $P<0.0001$; effect of solitary versus group $F_{1,50}=13.99$, $P<0.001$; interaction $F_{1,50}=9.23$, $P<0.005$; for the mean intensity of response: effect of “target” type $F_{1,50}=11.61$, $P<0.002$; effect of solitary versus group $F_{1,50}=1.00$, $P=0.33$; interaction $F_{1,50}=1.00$, $P=0.33$). Cooler “targets” attracted a significantly higher proportion of responses from lone males ($F_{1,25}=15.66$, $P<0.001$). However, the proportion of group males that responded was not significantly greater to “cool” than to “warm” targets ($F_{1,25}=3.20$, $P=0.09$).

Are males attracted to dirtier snakes?

The presence of mud strongly influenced the amount of courtship directed towards the “target” animals, in the predicted direction. The attractiveness of females, she-males and he-males was increased by mud (Fig. 4).

We analysed these data separately for each type of “target” snake, using a two-factor ANOVA in each case. These analyses revealed significant effects of treatment

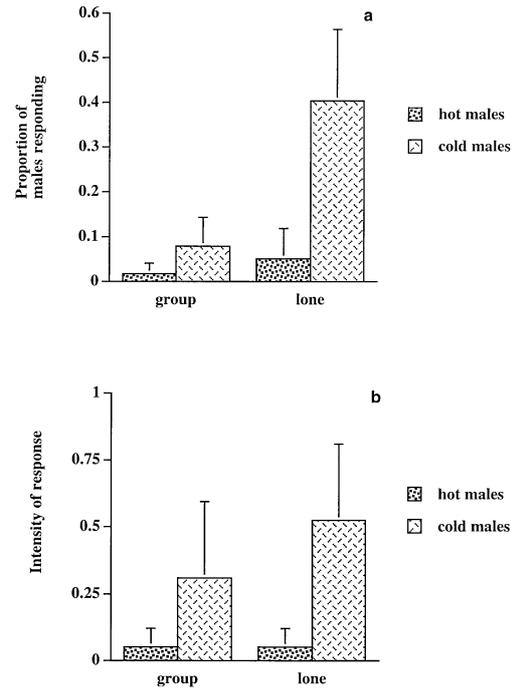


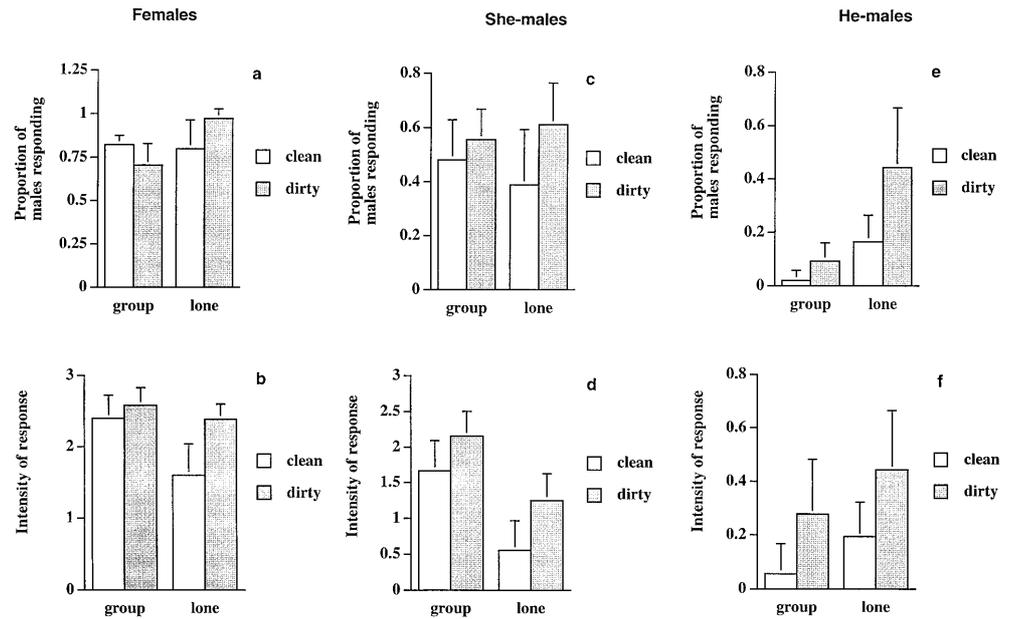
Fig. 3a,b Responses of free-ranging male garter snakes to other males that had been either cooled or kept hot. Males responded more strongly to cold “targets”, especially if the snakes being tested were not already part of a courting group. Histograms show means and associated SDs

on the attractiveness of female snakes (for the proportion of males responding to the “target” snake: effect of clean versus dirty $F_{1,30}=0.20$, $P=0.66$; effect of solitary versus group $F_{1,30}=4.14$, $P=0.051$; interaction $F_{1,30}=5.76$, $P<0.03$; for the mean intensity of response: effect of clean versus dirty $F_{1,30}=9.90$, $P<0.005$; effect of solitary versus group $F_{1,30}=10.35$, $P<0.005$; interaction $F_{1,30}=3.84$, $P=0.059$; see Fig. 4a,b). To clarify these effects, we conducted separate one-factor ANOVAs on data for group versus lone males. A muddy “target” female was more attractive than a clean “target” for lone males (for proportion of males responding $F_{1,15}=6.68$, $P<0.03$; for intensity of response $F_{1,15}=13.20$, $P<0.003$). In contrast, the responses of group males were not significantly affected by the muddiness of the “target” (for proportion of males responding $F_{1,15}=1.37$, $P=0.26$; for intensity of response $F_{1,15}=0.70$, $P=0.42$).

The proportion of she-males courted (Fig. 4c) did not differ for muddy versus clean “targets” (effect of clean versus dirty $F_{1,56}=2.16$, $P=0.15$; effect of solitary versus group $F_{1,56}=0.03$, $P=0.86$; interaction $F_{1,56}=0.54$, $P=0.47$). However, dirty she-males were courted more intensely than were clean ones (Fig. 4d; $F_{1,56}=4.79$, $P<0.04$) and group males courted them more intensely than did solitary males ($F_{1,56}=13.95$, $P<0.005$). The interaction term was not significant ($F_{1,56}=0.15$, $P=0.70$).

He-males that were muddy were also more attractive than were clean he-males (for the proportion of males responding to the “target” snake: effect of clean versus dirty $F_{1,26}=7.50$, $P<0.02$; effect of solitary versus group

Fig. 4a–f Responses of free-ranging male garter snakes to conspecifics that we experimentally cleaned or dirtied (i.e. covered in mud). Because females and she-males (i.e. female-mimics) were often mud covered already, the manipulation involved cleaning them. He-males (i.e. other males) were usually not muddy, so the manipulation involved dirtying them. We quantified responses of both males in courting groups, and solitary males. Histograms show means and associated SDs



$F_{1,26}=14.91$, $P<0.001$; interaction $F_{1,26}=2.53$, $P=0.12$; for the mean intensity of response: effect of clean versus dirty $F_{1,26}=7.50$, $P<0.02$; effect of solitary versus group $F_{1,26}=3.14$, $P=0.09$; interaction $F_{1,26}=0.03$, $P=0.87$; see Fig. 4e,f).

Are males attracted to courtship by other males?

In the first set of trials, the number of additional males courting the female after 15 s averaged 1.71 (SD=1.74, $n=21$) for the “solitary female” treatment, and 3.06 (SD=1.63, $n=18$) for the “courted female” treatment; this difference was statistically significant ($F_{1,37}=6.13$, $P<0.02$). In the second set of trials (with a different female), the analogous values were 0.47 (SD=0.64, $n=15$) versus 1.40 (SD=1.06, $n=15$; $F_{1,28}=8.58$, $P<0.007$). Thus, males use the courtship activity of other males as a cue to the presence of females.

Are males attracted to larger snakes?

The “large” females in our arenas were substantially bigger than their counterparts (means±SD SVLs of 68.5 ± 4.80 vs 53.7 ± 3.66 cm; masses of 119.4 ± 22.91 vs 56.7 ± 10.09 g). Larger females attracted more courtship, with 2 to 4 more males (of the total of 20 per enclosure) usually courting the larger female than the smaller one. The bias towards more courtship to the larger female was highly significant (based on mean numbers per enclosure to avoid pseudoreplication, mean±SD of difference in numbers of males courting small vs large female = 2.80 ± 1.58 , against a null of 0; 21 df, $t=8.31$, $P<0.0001$).

Are all females (or males) equally attractive to courting males?

Individual males differed in the relative intensity of their courtship response to different “targets”. First, we use two-factor ANOVA where the factors are the identification number of the courting male and of the “target”, and restrict analysis to female “targets” only. Individual males differed significantly in their overall intensity of courtship ($F_{23,288}=6.86$, $P<0.0001$), females differed significantly in their overall attractiveness ($F_{5,288}=21.24$, $P<0.0001$), and males differed in terms of which females they found most attractive (interaction $F_{115,288}=2.73$, $P<0.0001$). The same analysis restricted to responses to male “targets” yielded the same general result: again, males differed in their intensity of courtship ($F_{23,864}=2.19$, $P<0.002$), “target” males differed in their attractiveness ($F_{17,864}=1.76$, $P<0.03$), and males differed in terms of which other males they found most attractive (interaction $F_{391,864}=1.93$, $P<0.0001$). The same result held true if we deleted the “rubbed males” from the data set. These analyses thus indicate that there is substantial individual variation in attractiveness, courtship intensity, and the courting snake’s “preferences” for particular “target” individuals.

Do courting males differ in their “sexual preferences”?

A two-factor ANOVA with “test” snake identification number and “target” sex as factors reveals highly significant individual variation both in courtship intensity and in sexual preference. Unsurprisingly, females attracted more intense courtship than males ($F_{1,384}=560.59$, $P<0.0001$) and the males differed significantly in their overall intensity of courtship ($F_{23,384}=4.21$, $P<0.0001$). More interesting, however, is the significant interaction

term ($F_{23,384}=4.08$, $P<0.0001$), showing that the degree to which males preferred female rather than male “targets” differed significantly among the courting males.

Discussion

Male red-sided garter snakes use a complex array of cues to assess the sex and reproductive status of other snakes that they encounter during the mating season. Although previous research has focussed strongly on pheromonal cues, our study suggests that these chemical traits are only one component of the information that males use to locate potential mates. Importantly, context determines the relative importance of different cues, as shown by significant differences between lone and grouped males in their courtship responses to cues such as the sex, body temperature and muddiness of “target” individuals. Our data also reveal considerable individual variation in both attractiveness and preference within the superficially chaotic mating assemblages of these small snakes.

Several of the patterns that we have documented fit well with other data on this species’ ecology. For example, females attain much larger body sizes than conspecific males in most or all garter snake populations (e.g. Fitch 1981; Rossman et al. 1996). The sex differences in muddiness and temperature (Table 1, Fig. 1) reflect the fact that females disperse from the dens soon after emergence, whereas males tend to remain in the den vicinity for much longer (e.g. Gregory 1974; Gregory and Stewart 1975; Shine et al., 2001). Both sexes are typically dirty when they first emerge from hibernation (personal observation); their bodies are coated in mud from the burrows in which they have spent the winter. The mud on the males’ bodies brushes away as they move through dew-soaked grass.

The sex difference in body temperature also reflects time since emergence; both sexes generally remain relatively cool for a day or two after emerging, by which time the females have begun to disperse (Shine et al. 2000a). She-males are also often recently emerged animals (Shine et al. 2000a, 2000b). A similarly left-skewed distribution of body temperatures in females, but not males, has been reported in a previous study of *T. sirtalis* (Gibson and Falls 1979).

Although our experiments demonstrate that several cues are important for mate location, we cannot be sure of which sensory modalities are involved in each case. For example, muddiness might attract males (Fig. 4) visually, or because the mud itself has a distinctive aroma, or because it masks the scent of other chemicals in the integument. Similarly, larger females might be more attractive either visually or because they produce more or different pheromones. Heightened attractiveness of actively courted females, and of larger more fecund females, has been documented in previous studies (e.g. Hawley and Aleksyuk 1975; Luiselli 1996). The same ambiguity about cues exists for attraction to male court-

ship (probably visual cues, but possibly airborne scents), or to colder snakes (probably thermal cues, but possibly mediated via scent also). We conclude that males use multiple cues to locate prospective mates. Indeed, male garter snakes likely use most or all of the available sex-specific cues (i.e. all traits that consistently distinguish males from females) in mate location. This reliance upon multiple cues may be particularly important in a large den, where the scent-trails of thousands of females may provide a confusing sensory environment for a mate-searching male. The sensory basis of mate discrimination in this situation remains to be demonstrated, but appears to involve visual and thermal, as well as pheromonal, cues.

Our experiments suggest the possibility that solitary males may rely to a significant degree upon visual and thermal cues (Figs. 2, 3 and 4), whereas males already in mating balls may be most highly attuned to pheromonal cues. We base this interpretation on the results that: (1) she-males (which mimic females pheromonally and in muddiness but not in terms of body size) tended to be more attractive to group males than to lone males (Fig. 2); (2) cold snakes attracted great interest from lone males, but less from group males (Fig. 3), and (3) changing a snake’s appearance (by cleaning or dirtying it) had more effect on its attractiveness to lone males than to group males (Fig. 4).

Although ours is the first empirical study on non-pheromonal cues for mate recognition in snakes, previous authors have speculated that male garter snakes might use visual as well as chemical cues to locate females (e.g. Pisani 1976; Joy and Crews 1985). Visually mediated mate location also fits with the observation that when few females are available, male garter snakes typically remain motionless, with their heads held well above the ground, and move rapidly towards any disturbance (personal observation). More generally, intuition suggests that mate-searching males could benefit by taking advantage of any cue that predicts the sex and reproductive status of another snake. Thus, we might expect intense sexual selection for the recognition and use of such cues. The same line of argument applies to any other situation in which animals benefit by accurate location or direction-finding. For example, studies on migratory animals frequently discover that such organisms take advantage of a diverse array of cues for effective navigation (Dingle 1980). This often involves considerable redundancy in the cues used (e.g. Mazeroll and Montgomery 1998). Similarly, natricine snakes rely on visual as well as chemical cues while foraging (Chizsar et al. 1981; Drummond 1985; Heinen 1994, 1995).

Males that use a variety of cues to locate mates may benefit not only from the additional information they can use, but also from the fact that different sensory modalities operate effectively over different distances. For example, snake sex pheromones are large molecules that do not disperse through the air (Mason 1992). Thus, they are useful only for short-range communication. Airborne odors and visual cues (such as a conspecific’s body size,

or the occurrence of courtship by other males) could enable a male to locate a female over a much greater distance. An additional advantage to using thermal cues might be that cooler female garter snakes may accept copulations after a briefer period of courtship (Gartska et al. 1982).

The alternative cues available to male snakes also differ in their reliability. For example, visual and thermal cues may often be unreliable: some large, cold snakes are actually males, and some of the actively courted animals are she-males. Pheromones provide the most reliable information as to sex and status, and this might cause them to assume greatest importance within mating balls.

Overall, one of the strongest results to emerge from our study is the subtlety and complexity of the system. At first sight, the massive mating aggregations of red-sided garter snakes in Manitoba appear to be extraordinarily chaotic, with males dashing about frantically and attempting to court any other snake that they encounter. The reality is far more complex. Not only do the males use a wide range of cues to locate females, but the emphasis they place on particular cues shifts as they move from initial location to active courtship. Similarly, the arena trials reveal significant differences among individual males in terms of their degree of preference for different sexes, and for individuals within those sexes. Part of this variation may also reflect differences in the non-target members of each courting group and/or be linked to the relative body sizes of the participants: courtship and mating tend to be size assortative in the Manitoba garter snakes (Shine et al. 2000b). Such differences among individuals in the cues that elicit courtship are probably widespread among animals, but have not been documented previously among reptiles.

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