Behav Ecol Sociobiol (2003) 54:162–166
DOI 10.1007/s00265-003-0620-5

ORIGINAL ARTICLE

**R. Shine · B. Phillips · H. Waye · M. LeMaster · R. T. Mason**

# Chemosensory cues allow courting male garter snakes to assess body length and body condition of potential mates

Received: 3 August 2002 / Revised: 1 April 2003 / Accepted: 3 April 2003 / Published online: 14 May 2003


**Abstract** When choosing between two potential mates, a male may benefit by picking a larger (longer and/or more heavy-bodied) female because she is likely to produce more or larger offspring. Males of many species use visual cues to evaluate the sizes of their mates, but in some situations (at night or in a crowded mating swarm), vision may be useless. Potentially, males may be able to use chemical cues that convey information about female body size. We manipulated cues available to free-ranging male garter snakes (*Thamnophis sirtalis parietalis*) in large courting aggregations near communal dens in Manitoba, Canada. Males not only directed disproportionate courtship to longer and heavier-bodied females, but also courted most vigorously in response to lipids extracted from the skins of such females. Our data show that with a flick of his tongue, a male garter snake can identify not only a female's body length, but also her body condition.

**Keywords** Courtship · Garter snake · Lipids · Pheromones · *Thamnophis sirtalis parietalis*

## Introduction

Although evolutionary biologists have generally emphasized mate choice in females more than in males, there are numerous circumstances in which males are expected to discriminate carefully among alternative potential mates (Brown 1990, 1993; Schwagmeyer and Parker 1990; Olsson 1993; Andersson 1994). For example, male mate choice can occur if males allocate substantial parental care, or if they are capable of only a limited number of copulations within the mating season (Dewsbury 1982; Clutton-Brock 1991; Shine et al. 2001a). In some systems, males may need to select mates carefully to avoid unproductive interspecific copulations (Galvani and Johnstone 1998; Panhuis et al. 2001). Given that males exert mate choice, what characteristics of a female are likely to predict her subsequent fecundity (and hence, the fitness benefit likely to accrue from a mating)? In species with a wide range of adult body sizes, a female's length and body condition (mass relative to length) may provide two of the most reliable cues to predict her subsequent reproductive output (Fitch 1970; Seigel and Ford 1987).

Litter sizes in snakes typically depend upon maternal body size and condition (an index of energy stores; Naulleau and Bonnet 1996). Males of captive garter snakes (Hawley and Aleksiuk 1976; Shine and Mason 2001) and sea-snakes (Shetty and Shine 2002) direct disproportionate courtship towards large (and in garter snakes, heavy-bodied) females. Mate choice in this system may reflect a limit on the number of times a male can copulate. Male garter snakes insert large gelatinous mating plugs into the female's cloaca after mating. These plugs preclude remating by females, at least until the plug is expelled (Shine et al. 2000a). This substantial male investment in plug material may limit the number of times that a male can copulate effectively within a single breeding season.

Previous experimental studies that detected disproportionate courtship by male garter snakes towards larger, more heavy-bodied females were based on animals maintained in enclosures (Hawley and Aleksiuk 1976; Shine and Mason 2001). Under these conditions, the small numbers of snakes per arena meant that visual cues to female size and shape were available to males. But does the same male choice occur in nature, especially in areas around a communal den where most females are surrounded by (and often, submerged beneath) writhing

Communicated by W.E. Cooper

R. Shine (✉) · B. Phillips
School of Biological Sciences A08,
University of Sydney,
NSW 2006 Sydney, Australia
e-mail: rics@bio.usyd.edu.au
Tel.: +61-2-9351-3772, Fax: +61-2-9351-5609

H. Waye · M. LeMaster · R. T. Mason
Zoology Department,
Oregon State University,
Cordley Hall 3029, Corvallis, OR 97331-2914, USA

balls of males (Gregory 1974)? If male garter snakes do exert mate choice in such situations, as is suggested by the composition of courting groups in the field (Shine et al. 2001b), how can they identify larger or more heavy-bodied females in the midst of "mating balls"?

Chemical cues offer a potential source of such information. Snakes use their vomeronasal systems to obtain many kinds of information about their environment, protruding the tongue-tips to collect molecules that are then analyzed in vomeronasal organs in the roof of the mouth (Halpern and Kubie 1984). Using this ability, male garter snakes (*Thamnophis sirtalis parietalis*) can identify the sex of another snake (Mason 1993), and whether or not a female has recently mated (Devine 1977; Shine et al. 2000a). We hypothesized that male garter snakes might also be able to assess a female's body size and body condition based on pheromonal cues. We conducted simple field experiments to test this idea.

## Methods

Red-sided garter snakes are small nonvenomous colubrid snakes that occur widely through North America (Rossman et al. 1996). In the Interlake region of south-central Manitoba, this species overwinters in large communal hibernacula (Gregory 1974, 1977; Gregory and Stewart 1975). Many thousands of snakes emerge from underground within a period of a few weeks in spring, and vigorous courtship and mating occur in the vicinity of these dens. Males remain near the den's entrance for about 2 weeks after emerging, whereas females disperse to their summer feeding ranges within a day or two of emergence (Shine et al. 2001a). This sex difference in residency generates a hugely biased sex ratio, such that any emerging female is immediately surrounded by many (sometimes, hundreds) of male suitors (Mason 1993; Shine et al. 2001b). Courting male garter snakes virtually ignore human observers, facilitating behavioral trials on free-ranging snakes.

We examined the ability of male garter snakes to discern the body size and shape of females at a large communal den near Inwood, Manitoba (see Shine et al. 2001a for locality information). To evaluate mate choice, we conducted a series of trials on the responses by free-ranging male snakes to visual and chemical cues of female conspecifics. This work took advantage of the males' tolerance to observers, allowing us to sit on the ground close to the center of the den and present alternative stimuli by laying them out on the ground in a cleared area. The trials were conducted in clear weather between 1000 and 1500 hours in early May, a time of high activity at the den. We generated the cleared area by gently displacing existing courting groups. The large numbers of males, and their frequent movements around the den, meant that such an open area was soon filled with more snakes, and thus any stimulus that we laid out was soon encountered by many mate-searching males. In each case the stimuli were held immobile, and the investigator (R.S.) sat still beside the testing area to collect data.

We used two types of stimuli to assess male courtship responses: (1) live female target snakes were held by the tail, and (2) paper towels soaked in hexane solutions (with and without lipids added; see Body size). Towels were fastened to wire frames (20 cm long, 5 cm wide) by folding the paper towel around a wire coat hanger that had been fashioned into a rectangular shape and taping the towel back onto itself on the underside of the wire frame (i.e., such that the tape was not exposed to the test snakes). The frame was then placed on the ground. We scored the responses of large adult males (>50 cm SVL) that approached the stimulus closely enough that their heads were directly above it and hence, each test male was in a position such that it could have tongue-flicked the stimulus. Because a male garter snake's courtship responses depend on his body size (Shine et al. 2001b), we restricted attention to large males only. Their responses were scored on a four-point scale (0=no interest; 1=tongue-flick; 2=press chin against the female or paper towel; 3=align body with the female or paper towel). Chin-pressing and alignment are seen only in courting males (Whittier et al. 1985). Each male's response was scored as a separate data point; given the >20,000 animals present at the Inwood den, it is unlikely that we inadvertently scored any given male more than once. If the number of males arriving at the stimulus was so high that we could no longer clearly see responses by individual animals, we gently displaced excess animals to ensure a clear view.

### Body size

To examine whether a female's body size affected her attractiveness, we tested responses of 22 large males to small (45–<55 cm SVL) females and a further 22 large males to large (>55–65 cm SVL) females. We used four females within each of these size classes as target snakes. To determine whether chemical cues played a role in any such discrimination, we soaked eight dead females (four large, four small) for 12 h in hexane to extract their skin lipids (Shine et al. 2000b). All eight of these females were found dying after being attacked by crows; they were humanely euthanized before the soaking procedure. Larger females have a greater absolute quantity of skin lipids as well as qualitatively different skin lipids (Shine et al. 2000b; LeMaster and Mason 2003); either of these attributes might offer cues to size. Therefore to distinguish between these possibilities, we painted either small or large quantities of lipids onto the paper towels, using one and five lengthwise brushstrokes per paper towel, respectively. These numbers of brushstrokes were chosen to generate a substantial difference in pheromone concentration on the paper towels. Each brushstroke covered the entire upper surface of the towel. This procedure produced three replicates of each of four types of stimuli (either small or large quantities of lipids from either small or large female garter snakes). These towels (in their wire frames) were placed on the ground as previously described. We scored responses of the first 15 large males to encounter each type of stimulus (i.e., 15 males responding to each of 4 stimulus types equals 60 males tested in total). The order of data collection was randomized among treatments.

### Body condition

To determine if males could detect a female's body condition, we tested the attractiveness of 30 females within a small range of body lengths (55–60 cm SVL) but a wide range in mass (47.2–92.8 g) and thus, body condition. These females comprised the 15 fattest and 15 thinnest animals (based on residual scores from a general linear regression of ln mass to ln SVL) from a sample of 89 females within this SVL range that we collected at the den. Subsequent dissection of 16 of these animals confirmed that the fatter snakes had larger energy stores (for fat-body mass, means=4.7 vs 1.7 g, $F_{1,14}$=17.81, $P$<0.001; for liver mass, means=3.4 vs 2.2 g, $F_{1,14}$=24.52, $P$<0.001) and hence, were more likely to reproduce and to produce larger litters when they did so (Naulleau and Bonnet 1996; Bonnet et al. 2001). First, we tested courtship responses of six large male garter snakes to each of these animals (total sample, 6×30=180 males), using the method previously outlined. Then, we selected four "fat" and four "thin" females, wiped hexane-soaked paper towels along their dorsal surfaces, and presented these paper towels (on wire frames) to male snakes in the den. We scored responses by five large males to each of these stimuli, yielding responses by 20 males to lipids from fat females and by an additional 20 males to lipids from thin females. Again, order of testing was randomized. Because our courtship scores provide ordinal data only, we used non-parametric ranking tests to analyze these data.

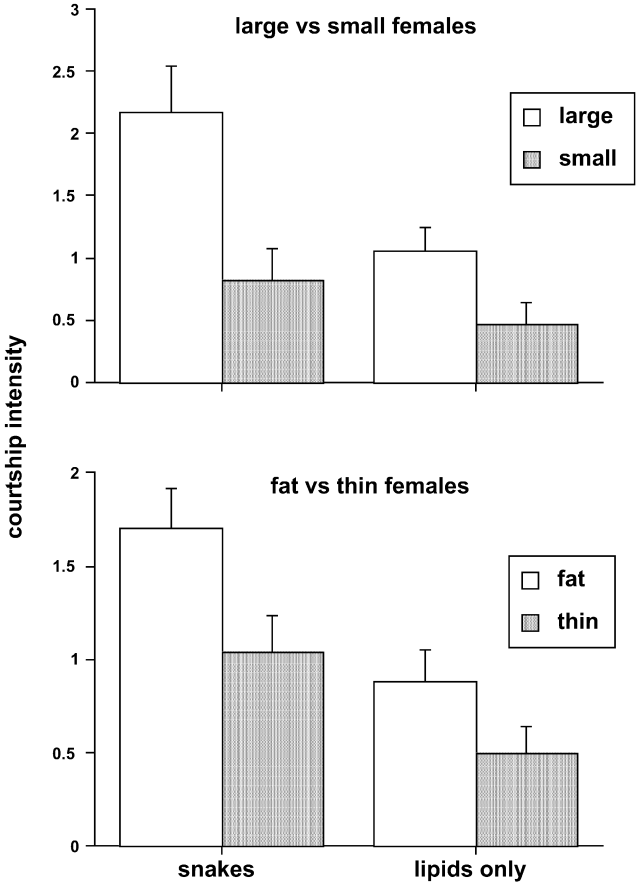


**Fig. 1** Courtship intensity of male red-sided garter snakes to females of various body sizes (*upper graph*) and body condition scores (*lower graph*). We rated male courtship intensity on a four-point scale (see text) in response to each class of female (*left-hand side*) and to hexane-soaked paper towels containing skin lipids from each type of female (*right-hand side*). For the comparison of lipids from large versus small females, data are based on trials using one brushstroke of hexane solution only. Histograms show mean values, and error bars show 1 SE on either side of the mean. Sample sizes (numbers of males tested) were as follows: for large versus small females, $n$=22 males to each size class; for lipids from large versus small females, $n$=15 males to each size class; for fat versus thin females, $n$=6 males to each of 30 females; for lipids from fat versus thin females, $n$=20 males to each type

## Results

### Body size

Despite the scarcity of visual cues (often, only a small proportion of the target female was visible), male snakes directed more intense courtship to large rather than small females (Mann-Whitney $U$=127.5, $n$=22 large, 22 small females, $P$<0.006). Importantly, the males also exhibited more intense courtship to paper towels containing lipids from large rather than small females (comparing responses to large vs small females: 1 brushstroke on a paper towel, Mann-Whitney $U$=61.5, $n$=15 large, 15 small, $P$<0.035; 5 brushstrokes, $U$= 65.0, $P$<0.035: see Fig. 1). The quantity of concentrate painted onto the paper towels (either one or five brushstrokes) did not affect the intensity of courtship (comparing 1 vs 5 strokes: lipids from large females, $U$=101.5, $n$=15, 15, $P$=0.65; lipids from small females, $U$=102.5, $n$=15, 15, $P$=0.62). Thus, male garter snakes responded to the type of lipid, not its quantity (at least over the range used in this experiment).

### Body condition

When tested at the den, the fatter snakes attracted more intense courtship. Linear regression showed that female body condition (residual score from the general linear regression of ln mass vs ln SVL) was related to the number of large male snakes (out of 6 tested per female) that tongue-flicked the stimulus ($n$=30 females, $r$=0.37, $P$=0.045) or chin-rubbed the stimulus ($n$=30, $r$=0.37, $P$=0.045). Lipids from more heavy-bodied females also stimulated more intense courtship by males. Hexane-soaked paper towels rubbed against fat females attracted more courtship than did towels rubbed against thin females ($U$=159.5, $n$=22 fat, 22 thin, $P$<0.04; Fig. 1).

## Discussion

Our trials show that free-ranging male garter snakes at a communal den can assess both the body size and the body condition of females (or some traits correlated with these variables), and thus devote disproportionate courtship to larger, heavier-bodied females. The response to paper towels containing female lipids shows that visual cues are not necessary for this discrimination: male garter snakes are capable of assessing a female's body size and condition from chemical cues alone.

Remarkably, male snakes typically required only one or two tongue-flicks before initiating vigorous courtship (chin-pressing) to the paper towels. It was our strong impression (although unquantified) that many males performed only a single tongue-flick before chin-pressing the stimulus. Thus, the chemoreceptive system of these animals provides rapid and sophisticated information about attributes of potential partners likely to predict reproductive output. With only a few tongue-flicks, a male garter snake is able to determine not only the sex of another animal (Mason 1993) and its recent mating history (Devine 1977; Shine et al. 2000a), but also its body size and body shape. Such sensitivity has presumably been a strong target of sexual selection, in that males with better chemosensory discrimination are likely to benefit by selecting the best mates (Andersson 1994).

Although rapid discrimination among potential mates that differ in probable fecundity is likely to be favored under many circumstances, exact details of the mating system will influence the degree to which such an ability enhances male reproductive success. Several aspects of the mating system of red-sided garter snakes may provide a particularly high fitness benefit to mate evaluation by males:

1. These animals court and mate in very large aggregations, such that a mate-searching male has potential access to many females and hence, the time required to find another potential mate is trivial compared to the time required for courtship prior to obtaining a copulation. Discriminating among alternative potential mates would be less important in males of species that must search for widely-dispersed females, especially where aspects of the habitat or the animal's sensory systems render mate location more difficult. In such a species, the time required for courtship may be trivial relative to that required to locate a female and hence, selection should favor active courting of any reproductive female that is encountered regardless of her probable fecundity. Prolonged mate-searching polygyny is widespread in snakes (e.g., rattlesnakes: Duvall et al. 1985, 1993) and should provide little fitness benefit to male ability to discriminate among alternative mates unless the male can detect fitness-relevant attributes of the female through substrate-deposited pheromonal trails rather than requiring direct physical contact. If males can recognize larger, more heavy-bodied females through pheromone trails, a discriminating male could benefit by trail-following the better female. In garter snakes, the female skin lipids that stimulate trail-following by males are the same as those that stimulate courtship (LeMaster et al. 2001), so this scenario warrants further testing.
2. Red-sided garter snakes mate in such large aggregations that visual cues to a female's size may often be hidden beneath dozens of rival males. Thus, chemical assessment of mate quality may be particularly significant in this system.
3. Male garter snakes produce large mating plugs and hence, may be able to perform only a limited number of effective copulations per season. Selection against courting smaller, thinner females will be less intense if such matings do not reduce the male's ability to later copulate with a larger female.

Because such aspects of the mating system will influence the fitness benefits of mate discrimination by males, it would be instructive to examine other species, or lower-density populations of red-sided garter snakes, to see whether this ability to evaluate female size from chemical cues is widespread in other snakes also.

Pheromonal cues are important in mate selection in many kinds of organisms (Tirindelli et al. 1998; Sandler et al. 2000), but most studies on this topic have emphasized the potential role of pheromonal cues in enabling mate-searching males to discriminate between suitable and unsuitable mates. That is, males have been tacitly assumed to interpret the pheromone as a dichotomous variable—for example, to determine whether the individual being tested is either a male or a female, or either a conspecific or a heterospecific (reviewed by Andersson 1994). Our data on garter snakes suggest that the information content of pheromonal cues is actually much greater than this simple "yes or no" scenario. We suspect that chemosensory discrimination among alternative mates will prove to be phylogenetically widespread, and that males of many taxa can derive complex information about potential mates based solely on pheromonal cues.

**Acknowledgements** We thank D. Roberts, A. Johnson, G. Johnson, and R. Nesbitt for help and encouragement. Financial support was provided by the Australian Research Council, the National Science Foundation, and the Whitehall Foundation (W95–04). Research was conducted under the authority of Oregon State University Institutional Animal Care and Use Committee Protocol No. LAR-1848B. All research was conducted in accord with the U.S. Public Health Service *Policy on Humane Care and Use of Laboratory Animals* and the National Institutes of Health *Guide to the Care and Use of Laboratory Animals*, and conforms to all current Canadian laws.

## References

Andersson M (1994) Sexual selection. Princeton University Press, Princeton, New Jersey

Bonnet X, Naulleau G, Shine R, Lourdais O (2001) Short-term versus long-term effects of food intake on reproductive output in a viviparous snake *Vipera aspis.* Oikos 92:297–308

Brown WD (1990) Size-assortative mating in the blister beetle *Lytta magister* (Coleoptera:Meloidae) is due to male and female preference for larger mates. Anim Behav 40:901–909

Brown WD (1993) The causes of size-assortative mating in the leaf beetle *Trirhabda canadensis* (Coleoptera: Chrysomelidae). Behav Ecol Sociobiol 33:151–157

Clutton-Brock TH (1991) The evolution of parental care. Princeton University Press, Princeton, New Jersey

Devine MC (1977) Copulatory plugs, restricted mating opportunities and reproductive competition among male garter snakes. Nature 267:345–346

Dewsbury DA (1982) Ejaculate cost and male choice. Am Nat 119:601–610

Duvall D, King MB, Gutzweiler KJ (1985) Behavioral ecology and ethology of the prairie rattlesnake. Natl Geogr Res 1:80–111

Duvall D, Schuett GW, Arnold SJ (1993) Ecology and evolution of snake mating systems. In: Seigel RA, Collins JT (eds) Snakes: ecology and behavior. McGraw-Hill, New York, pp 165–200

Fitch HS (1970) Reproductive cycles in lizards and snakes. Univ Kansas Mus Nat Hist Misc Publ 52:1–247

Galvani A, Johnstone R (1998) Sperm allocation in an uncertain world. Behav Ecol Sociobiol 44:161–168

Gregory PT (1974) Patterns of spring emergence of the red-sided garter snake (*Thamnophis sirtalis parietalis*) in the Interlake region of Manitoba. Can J Zool 52:1063–1069

Gregory PT (1977) Life-history parameters of the red-sided garter snake (*Thamnophis sirtalis parietalis*) in an extreme environment, the Interlake region of Manitoba. Nat Mus Can Publ Zool 13:1–44

Gregory PT, Stewart KW (1975) Long-distance dispersal and feeding strategy of the red-sided garter snake (*Thamnophis sirtalis parietalis*) in the Interlake of Manitoba. Can J Zool 53:238–245

Halpern M, Kubie JL (1984) The role of the ophidian vomeronasal system in species-typical behavior. Trends Neurosci 7:472–477

Hawley AWL, Aleksiuk M (1976) Sexual receptivity in the female red-sided garter snake (*Thamnophis sirtalis parietalis*). Copeia 1976:401–404

LeMaster MP, Mason RT (2003) Evidence for a sex pheromone mediating mate choice in the red-sided garter snake, *Thamnophis sirtalis parietalis*. J Chem Ecol (in press)

LeMaster MP, Moore IT, Mason RT (2001) Conspecific trailing behavior of red-sided garter snakes, *Thamnophis sirtalis parietalis*, in the natural environment. Anim Behav 61:827–833

Mason RT (1993) Chemical ecology of the red-sided garter snake, *Thamnophis sirtalis parietalis*. Brain Behav Evol 41:261–268

Naulleau G, Bonnet X (1996) Body condition threshold for breeding in a viviparous snake. Oecologia 107:301–306

Olsson M (1993) Male preference for large females and assortative mating for body size in the sand lizard. Behav Ecol Sociobiol 32:337–341

Panhuis TM, Butlin RK, Zuk M, Tregenza T (2001) Sexual selection and speciation. Trends Ecol Evol 16:364–371

Rossman DA, Ford NB, Seigel RA (1996) The garter snakes: evolution and ecology. University of Oklahoma Press, Norman, Oklahoma

Sandler B, Nikonova L, Leal W, Clardy J (2000) Sexual attraction in the silkworm moth: structure of the pheromone-binding-protein-bombykol complex. Chem Biol 7:143–151

Schwagmeyer PL, Parker GA (1990) Male mate choice as predicted by sperm competition in thirteen-lined ground squirrels. Nature 348:62–64

Seigel RA, Ford NB (1987) Reproductive ecology. In: Seigel RA, Collins JT, Novak SS (eds) Snakes: ecology and evolutionary biology. Macmillan, New York, pp 210–252

Shetty S, Shine R (2002) The mating system of yellow-lipped sea kraits (*Laticauda colubrina*, Laticaudinae). Herpetologica 58:170–180

Shine R, Mason RT (2001) Courting male garter snakes use multiple cues to identify potential mates. Behav Ecol Sociobiol 49:465–473

Shine R, Olsson MM, Mason RT (2000a) Chastity belts in gartersnakes: the functional significance of mating plugs. Biol J Linn Soc 70:377–390

Shine R, O'Connor D, Mason RT (2000b) Female mimicry in gartersnakes: behavioural tactics of "she-males" and the males that court them. Can J Zool 78:1391–1396

Shine R, Elphick MJ, Harlow PS, Moore IT, LeMaster MP, Mason RT (2001a) Movements, mating and dispersal of red-sided gartersnakes from a communal den in Manitoba. Copeia 2001:82–91

Shine R, O'Connor D, LeMaster MP, Mason RT (2001b) Pick on someone your own size: ontogenetic shifts in mate choice by male garter snakes result in size-assortative mating. Anim Behav 61:1133–1141

Tirindelli R, Mucignat-Caretta C, Ryba N (1998) Molecular aspects of pheromonal communication via the vomeronasal organ of mammals. Trends Neurosci 21:482–486.

Whittier JM, Mason RT, Crews D (1985) Mating in the red-sided gartersnake, *Thamnophis sirtalis parietalis*: differential effects on male and female sexual behavior. Behav Ecol Sociobiol 16:257–261