Behav Ecol Sociobiol (2012) 66:691–695
DOI 10.1007/s00265-012-1317-4

ORIGINAL PAPER

Facultative pheromonal mimicry in snakes: "she-males" attract courtship only when it is useful

R. Shine · T. Langkilde · R. T. Mason

Received: 12 October 2011 / Revised: 12 December 2011 / Accepted: 6 January 2012 / Published online: 26 January 2012

Abstract Males of many animal species mimic females, and thereby deceive rival males. Facultative shifts in posture, color, or movement allow a male using visually-based mimicry to adopt and terminate mimicry rapidly. Pheromonal mimicry is rare in vertebrates perhaps because it is difficult to redeploy pheromones rapidly enough to adjust male tactics to local conditions. In Manitoba garter snakes (*Thamnophis sirtalis parietalis*), female mimicry benefits males immediately after they have emerged from hibernation. While the snakes are cold and slow, courtship warms them and protects them against predatory crows. This benefit disappears as soon as the snakes are warm. We show that (unlike females) she-male garter snakes attract courting males only when they are cold. Low temperatures may suppress volatility of "less attractive" components of the pheromones (saturated methyl ketones) that she-males use to attract courtship, allowing male snakes to function as transvestites only when this tactic is beneficial.

Keywords Chemical communication · Reproductive tactics · Reptile sociality · Sexual mimicry

Communicated by T. Breithaupt

R. Shine (✉) · T. Langkilde
School of Biological Sciences A08, University of Sydney,
Sydney, NSW 2006, Australia
e-mail: rick.shine@sydney.edu.au

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

Present Address:
T. Langkilde
Department of Biology, Pennsylvania State University,
University Park, PA 16802, USA

Introduction

Intense competition among males has resulted in the evolution of a diverse array of tactics that function to deceive rivals (Andersson 1994). Mimicry of females is one such tactic, whereby a male that exhibits signals typical of conspecific females (such as color patterns, displays, or postures) thereby gains some fitness benefit (such as reduced aggression from rival males or access to food resources or females: Brower 1988; Vereecken and McNeil 2010). Males mimic females through pheromonal deception in many insects (Peschke 1987; Cardé and Bel 1995), but this tactic is rare in vertebrates, although pheromonally based sex recognition is common (Whiting et al. 2009; Vereecken and McNeil 2010). That scarcity may reflect the inflexibility of pheromonally based mimicry. Visual signals can be adjusted quickly: for example, male fish and cuttlefish can rapidly switch from male-like to female-like color patterns and postures, thereby enhancing mating success (Hanlon et al. 2005; Korzan et al. 2008). The longer timescales required to deploy chemical cues may preclude a male from facultatively adjusting his mimicry tactics.

Pheromonal mimicry of females occurs in red-sided garter snakes (*Thamnophis sirtalis parietalis*) in Manitoba (Mason and Crews 1985; Shine et al. 2000a, b). These small snakes overwinter in communal dens and court and mate in large springtime aggregations before dispersing to their summer ranges (Gregory and Stewart 1975; Shine et al. 2001b, 2006). Males emerging after 8 months underground are cold, slow, and lethargic and vulnerable to attack by predatory crows (Shine et al. 2000c, 2001c). During the day or so that a male takes to recover, he produces female-like skin lipids that attract courtship by other males; this female mimicry confers a benefit because the "she-male" is warmed and protected by his suitors (Shine et al. 2000a, b; LeMaster et al. 2008). After the animal has recovered from hibernation, he ceases producing female-like pheromones and begins to invest his efforts into courting females (and inevitably, she-males). However, the she-male tactic confers risks as well as benefits: courtship by other males impedes the courted snake's respiration (Shine et al. 2003a) and in extreme cases, the courted male may be forcibly copulated (Pfrender et al. 2001) or suffocated (Shine et al. 2001a). Because courtship imposes costs as well as benefits, a she-male snake could benefit by flexibly adjusting his mimicry tactics to his body temperature. Female mimicry is advantageous to a cold she-male but not to a hot one because a hot snake is able to detect predators and flee from them rapidly (Shine et al. 2000c, 2005).

Can she-males adjust their attractivity to other males (and hence, the intensity of courtship to which they are subjected) based upon their own body temperature? To test this idea, we quantified courtship responses of male garter snakes to experimentally manipulated she-males during the mating season at a large communal den in Manitoba. Based on the temperature-dependent benefits of female mimicry (above), we predicted that the intensity of courtship directed to a she-male would shift with the target snake's body temperature. Under this hypothesis, a she-male that experiences variable weather conditions (and thus, shifts between warm and cool body temperatures) would cease to attract courtship from other males as he warms up but would regain his attractiveness as he cools down.

Methods

Thermal effects on sexual attractiveness

We captured snakes at a den near Inwood, Manitoba (50°7′N 97°9′W) during May 2003; May is the peak time of post-winter emergence and mating in this population (Shine et al. 2006). The den contains more than 30,000 snakes, mostly males that roam around the den searching for females (Shine et al. 2006). We collected 10 unmated females (reproductive status determined by absence of a mating plug: Shine et al. 2000d), 10 “he-males” (males that were collected while actively courting females), and 10 she-males (males found while they were being courted by other males). We placed each animal in an individual cloth bag, half of which were kept cool (in an insulated cool box, at temperatures similar to those exhibited soon after emergence: mean body temperature 16.67±0.49°C), and the other half allowed to warm to the usual body temperatures of active snakes (29.48±0.35°C; Shine et al. 2000c) by placing them in a sun-exposed position. These small snakes heat and cool rapidly and always achieved the target temperatures (above) in less than 15 min.

During the mating season, male garter snakes in this population virtually ignore humans (Moore and Mason 2001; Shine et al. 2003a). Thus, the attractiveness of any given snake can be quantified by holding the snake by the tail within the den area and recording the courtship responses of the first five males to encounter the body of the “target” snake. We scored courtship intensity of each male on a range from 0 (no interest) to 3 (vigorous courtship, including chin rubbing and body alignment [Whittier et al. 1985]: see Shine et al. 2003b for details of this method). Thus, the possible courtship intensity scores to a given target snake ranged from 0 (no interest by any of the five he-males) to 15 (intense responses by all five he-males). High densities of courting males meant that we were generally able to score the responses of five he-males in less than 1 min—and thus, before the target animal's temperature had changed significantly from the level induced by our treatments.

Facultative shifts in attractiveness

Even if newly emerging she-males are attractive only when cold, this might reflect a one-way switch—that is, a she-male loses his attractiveness to other males as soon as he heats up and thereafter functions as a he-male. Alternatively, a warm she-male may regain his female mimicry if he cools down again. To test this idea, we collected 25 she-males from the den (as above) and exposed them to 15-min cycles of heating followed by 15 min of cooling (same temperature ranges as above). Each snake was tested only once (by exposing it to five mate-searching males). One group of five she-males was tested immediately after collection, whereas all other snakes were heated up for 15 min. Five of these animals were then tested while warm, and all of the rest cooled down for 15 min. Five of these animals were then tested while cool, and the remainder heated up again. Lastly, the remaining five snakes were cooled down and tested. The experiment simulated fluctuating thermal conditions, common in the Manitoba spring (Shine et al. 2006). For both experiments, data on courtship scores (sum total of response intensities [rated 0 to 3, see above] by the five first he-males to contact the target snake) were normally distributed and analyzed using one-factor ANOVA with treatment as the factor.

Results

Thermal effects on sexual attractiveness

Females attracted the most intense courtship and he-males the least; she-males were intermediate (group effect $F_{2,24}$= 146.60, P<0.0001; Fig. 1a), and temperature affected

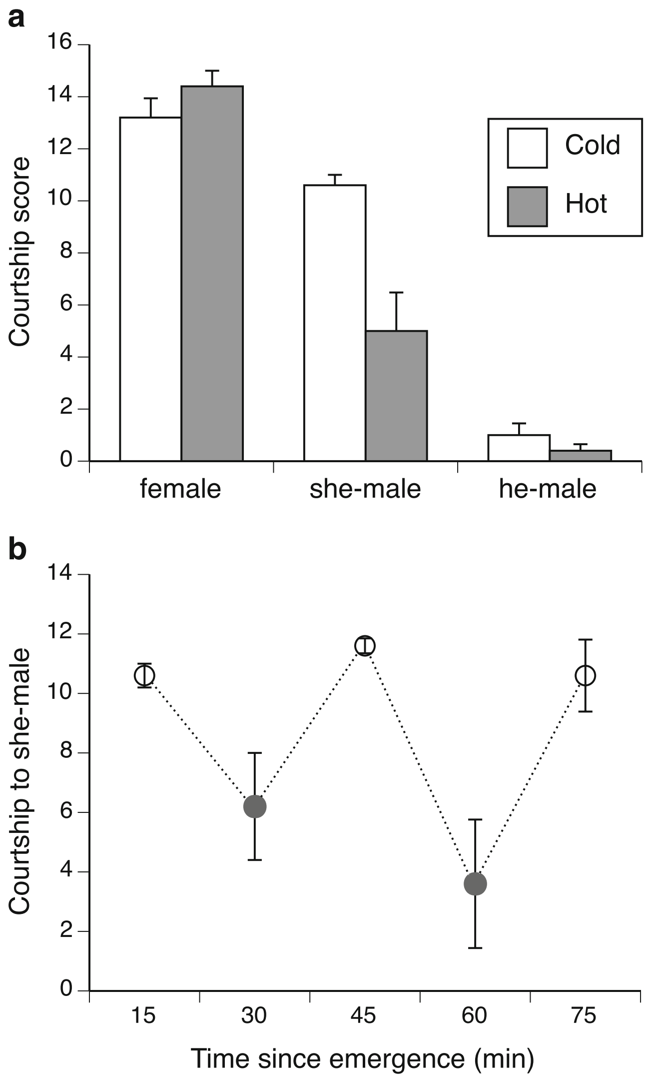

Fig. 1 The effect of experimentally manipulating a garter snake's body temperature (to means of 16.7°C vs. 29.5°C) on the intensity with which it is courted. **a** Effects of body temperature on intensity of courtship to females, female-mimicking males (she-males), and he-males. The dependent variable is the sum of courtship scores (0 to 3, see text) of the first five male snakes to encounter a "target" snake held by the tail within the communal den area (N=10 target snakes per category). **b** Intensity of courtship to she-males after alternating exposure to hot and cold conditions. These males were captured soon after emergence from hibernation and subjected to cycles of cooling and warming, after which we measured the intensity of courtship that they attracted from free-ranging male snakes. The graphs show mean values and standard errors. The dependent variable is the sum of courtship scores (0 to 3, see text) of the first five male snakes to encounter a target snake held by the tail within the communal den area (N=5 target snakes per category). See text for statistical tests of these patterns

courtship intensity to she-males but not to females or he-males (interaction $F_{2,24}$=10.58, P<0.001; see Fig. 1a). As predicted, she-males attracted courtship only when they were cold (unpaired t test on courtship levels to hot vs. cold she-males, t_{18}=3.84, P< 0.002).

Facultative shifts in attractiveness

Female mimicry disappeared when the she-males were heated but reappeared when they cooled (Fig. 1b). Courtship intensity differed among the groups ($F_{4,20}$=6.23, P<0.003); post hoc Fishers' PLSD tests show that all "cold" treatments attracted more courtship than did all "hot" treatments (P<0.05 in all comparisons). Thus, male snakes in this population flexibly switch in and out of she-male mode, adopting female mimicry only when they are cold (i.e., when they can benefit from this tactic).

Discussion

Male garter snakes locate females using visual, thermal, and chemical information, but only pheromonal cues (epidermal lipids) elicit intense courtship (Shine and Mason 2001; Mason and Parker 2010). Thus, the only way for newly emerged (slow, weak) male snakes to obtain the benefits of being courted (warming, protection against predators) is to mimic females pheromonally. Courtship is beneficial only while the snake is cold, and remarkably, male red-sided garter snakes exhibit temperature-dependent female mimicry.

Although many vertebrates (notably, mammals and reptiles) use pheromones for sexual identification, female mimicry by males usually is based on visual rather than chemical signals (Mason and Parker 2010). For example, in the lizard *Platysaurus broadleyi*, small males rely on visual signals to mimic females, and thus avoid aggression from larger males—but this system works only at a distance (Whiting et al. 2009). Although pheromonal mimicry could enhance the effectiveness of this female-mimicking tactic, it has not evolved—perhaps because a she-male lizard can change his posture to display otherwise-hidden ventral color cues (male identifiers) to a receptive female, but he cannot rapidly adjust the chemical structure of his epidermal lipids in response to the social context. In snakes, epidermal lipids likely evolved for functions such as waterproofing and were co-opted as sex pheromones because their structure is affected by circulating sex steroids (Maderson 1986; Mason and Parker 2010). Thus, a snake presumably is unable to shift the chemical composition of its skin lipids over a brief timescale.

How, then, can a she-male's attractiveness be affected by rapid cooling or heating (Fig. 1b)? In red-sided garter snakes, methyl ketones serve as sex pheromones. Monounsaturated methyl ketones are a more powerful stimulant for male courtship than are saturated methyl ketones, and the relative amounts of the two types of ketones affect the intensity of male response (LeMaster et al. 2008; Mason and Parker 2010) Because the ratio of saturated to unsaturated ketones is higher in she-males than females (LeMaster et al. 2008), she-males elicit less intense courtship (Fig. 1a). This pheromonal mix also may adjust a she-male's attractiveness to its body temperature. Unsaturated fats have double bonds between carbon molecules, enabling them to “slide” over each other and remain liquid at low temperatures, whereas saturated fats (that lack those double bonds) become solid (Gunstone 1996). This is why cooking oil (unsaturated) is liquid at room temperature, whereas margarine (saturated) is not. Thus, the only ketones likely to be volatile (and hence, detectable to a courting male) at low temperatures are the (highly attractive) unsaturated ones. At higher temperatures, courting males will detect a higher proportion of (less attractive) saturated to (more attractive) unsaturated ketones. The she-male's mix of pheromones hence will have more appeal to courting he-males when the female-mimicking snake is cold (and thus, when she-maleness is beneficial) than when the animal is hot (when she-maleness confers costs but no benefits).

This hypothesis could be tested by directly sampling odor headspace from cold versus warm snakes (ideally, females as well as she-males), to quantify the relative thermal volatility of saturated and unsaturated ketones. Alternative mechanisms are possible also: for example, a temperature-driven shift in physical properties of the skin lipids, in a way that influences attractiveness to courting males. Regardless of the mechanism, however, the end result is that male garter snakes can exploit the thermal lability of their pheromones to optimize their sexual tactics. Rather than female mimicry being a simple and uninterrupted phase in the post-emergence life of a male garter snake at these dens (as envisaged by previous research), the reality is more complex. A given male can shift in and out of female mimicry depending upon local weather conditions as well as his own stage of post-hibernation recovery. Remarkably, then, males can change their female-mimicry tactics over short time intervals even in a system where the primary sex-identifying signals are chemical rather than visual.

Acknowledgments We thank Mike Wall and the Johnson family for assistance and the Australian Research Council for funding.

Ethical standards All experiments reported in this paper comply with the current laws of the country in which they were performed.

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