



The transvestite serpent: why do male garter snakes court (some) other males?

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In large mating aggregations of red-sided garter snakes, *Thamnophis sirtalis parietalis*, in Manitoba, male courtship is directed not only to females, but also to other males with female-like skin lipids ('she-males'). We show that 'she-maleness' is an intrinsic property of a male rather than an artefact of lipid transfer from females, and that male–male courtship is very common in the field. She-males were distinctive in terms of appearance (they were heavier than other males and more often covered with mud), behaviour (they were inactive and rarely courted females) and performance (they were slow crawlers, ineffective courtiers and easily outcompeted by other males in mating trials). 'She-maleness' was not a characteristic of a particular subset of males, as envisaged in previous work; instead, it was a transitory phase that most (perhaps all) male snakes passed through soon after they first emerged from the winter den. Recently emerged males spent their first day or two relatively inactive, while restoring physiological functions (including locomotor performance and courtship ability). Experimental application of female skin lipids on to males dramatically decreased courtship levels of the recipient snakes. Thus, recently emerged males may derive two kinds of benefit from mimicking female skin lipids. First, female mimicry 'switches off' the male's own (energetically expensive) courtship at a time when that courtship would be unproductive. Second, it may disadvantage his rivals by distracting them from females, and increasing their energy expenditure.

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Female mimicry, whereby a male takes on a female's appearance, is a rare but widely publicized trait in human societies. Remarkably, parallels can be seen in other animal species, with some males within the population resembling females in traits such as size, colour, scent or behaviour (e.g. Burley 1982). Female mimicry has been reported in a diverse array of taxa, including insects (Forsyth & Alcock 1990; Peschke 1990), crustaceans (Laufer et al. 1994; Laufer & Ahl 1995), fish (Willmott & Foster 1995; Gonçalves et al. 1996), amphibians (Sparreboom 1996; Howard et al. 1997), birds (Hakkarainen et al. 1993; Saetre & Slagsvold 1996) and mammals (Le Bouef 1974). Males may benefit in various ways from their resemblance to females; for example, they may be less vulnerable to attack by rivals, or more capable of winning combat bouts, or obtaining 'sneaky' fertilizations (e.g. Kodric-Brown 1986; Saetre & Slagsvold

1996). For these reasons, mimicry of females might plausibly enhance a male's mating success, and several authors have interpreted female mimicry as an adaptive strategy in these terms (e.g. Rohwer et al. 1980; Burley 1982; Gonçalves et al. 1996).

Although female mimicry has evolved independently in several different phylogenetic lineages, it is rare in reptiles. The only well-studied example of female mimicry in this group comes from Mason & Crews (1985, 1986), who reported that a subset of male red-sided garter snakes, *Thamnophis sirtalis parietalis*, acted as female mimics. Males in this species use skin lipids as pheromonal cues for sex recognition (Mason et al. 1989, 1990; Mason 1992). Male and female lipid profiles differ considerably, except that a small proportion of males resemble females rather than other males in this respect (Mason et al. 1987). These 'she-males' attract intense courtship from other males (henceforth, 'he-males'); experiments using hexane extracts show that this effect is due to the skin lipids of she-males (Mason et al. 1989). The resultant confusion among their rivals may provide a mating advantage to she-males (Mason & Crews 1985,

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1986; Mason 1993). We report a more extensive study of 'she-maleness' and focus on the following questions.

(1) Is she-maleness a real and biologically significant phenomenon? If she-males are only marginally more attractive than other males, or are attractive only because of physical transfer of pheromones from females, or are rare or occur in only a few populations, then she-maleness may be of little real significance in the reproductive biology of garter snakes.

(2) If she-males are 'real', do they differ from he-males in aspects other than sexual attractiveness (e.g. morphology, behaviour, locomotor performance)? And, are the males that court she-males different from those that court females?

(3) Does a she-male show other modifications of competition and courtship behaviour?

(4) Although previous studies have interpreted she-maleness as a permanent attribute of a subset of animals, there is little evidence on this question. Alternatively, could she-maleness be a transitory stage for most or all males within this population?

METHODS

Site and Study Species

Red-sided garter snakes are small (average snout-vent length (SVL) of adult males 45 cm, adult females 56 cm) nonvenomous colubrid snakes that are widely distributed across North America (e.g. Rossman et al. 1996). Near the northern edge of their range in south-central Manitoba (Chatfield Community Pasture: see Mason & Crews 1985; Mason et al. 1987 for further information on this site), these snakes gather in massive aggregations in autumn and spring, around limestone sinkholes that serve as communal hibernacula (e.g. Gregory 1974, 1977; Gregory & Stewart 1975). The snakes mate soon after emerging in spring, and before dispersing to their summer ranges. Because males remain near the den longer than females, the operational sex ratio is highly male biased (often greater than 10:1) throughout the mating season (Gregory 1974). Most courtship and mating occur in small groups ('mating balls') consisting of one animal being courted plus several suitors. All of the males within such a ball attempt to align their body with that of the female, and vigorously attempt to position their tailbase close to hers. The vast numbers of snakes in some dens (>20 000 per den: R. Mason, unpublished data), combined with their tolerance to human presence, make the system ideal for research on reproductive biology. The Manitoba garter snake dens have provided a model system for unravelling the complexities of pheromonal communication in snakes (e.g. Mason & Crews 1985; Whittier et al. 1985; Mason et al. 1987, 1989, 1990).

Methods

We visited Manitoba in May 1997 and May 1998 to study snakes in the field (in the den itself, and in the surrounding meadows and aspen thickets) and in outdoor

arenas that we erected close to the den. The arenas allowed us to control the composition of courting groups. The specific techniques that we used were as follows.

Quantification of 'attractiveness'

Clearings adjacent to the den often contained several hundred male garter snakes engaged either in courtship (in 'mating balls') or in mate searching (moving around the area, frequently tongue flicking). We sat down in the grass and presented a 'target' animal (held by the tail) so that other males would encounter it. In each case, we placed the target snake in the path of another male, such that the head of the male being tested would encounter the midbody of the 'target' in close proximity (<1 cm). The courtship behaviours of the males tested were scored 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; henceforth referred to as the 'Whittier scale'). Two social contexts were tested: the targets were either introduced into a group of courting males, or placed in front of solitary (mate-searching) males. We used the Whittier scale to score responses of three solitary males. For courting groups, we tested responses of three males per trial, and repeated the trials three times (so that we obtained responses of nine group males). In each group trial we recorded the numbers of males with positive responses, and the maximum response intensity (on the Whittier scale) of males within each group.

To investigate possible temporal shifts in attractiveness, we tested the attractiveness of males, she-males and females as described above, then retested the same animals after they had been kept in a cool (12°C) room for 24 or 48 h in cloth bags. We also tested the attractiveness of males immediately after their initial emergence from the den (by taking only animals that we saw emerging from holes inside the den).

Surveys of courting groups

We walked around the den area to record attributes of 'mating balls'. By moving in simultaneously, two or three people could scoop all members of a given 'ball' into a plastic rubbish bin. The snakes were then extracted from the bin one by one, and we recorded their sexes, body sizes (SVL, mass) and body temperatures (the latter with a Schultheis quick-registering cloacal thermometer).

Most of the mating balls were centred on females, but some were centred on males (i.e. she-males). We defined snakes as she-males by the fact that they were attracting courtship from other males. To quantify the propensity of courting males to leave the target snake when disturbed (a trait that might plausibly differ between she-male-centred versus female-centred groups), we suddenly swept an arm over the courting group. At this stimulus, a proportion of the courting males left rapidly. We counted these snakes, and those that remained.

Locomotor performance

To measure maximal crawl speeds of different types of snakes, we captured animals around midday and kept

them in cloth bags, in the shade, until 1700 hours. By this time their body temperatures had equilibrated to air temperature, as verified with a quick-registering cloacal thermometer (body temperature range 24–26°C). We marked out a 5-m circle on a flat open grassy area near the den, and released each snake (in random order) on to the exact centre of that circle. The snake was then harassed with an aspen switch to encourage it to move rapidly out of the circle, in whichever direction it chose. We timed the interval between the snake's release and its reaching the edge of the circle. Each animal was tested only once.

Focal observations of snake behaviour

We marked individual snakes with a paint spot and observed them for 5-min periods on the same or subsequent days. The observer followed the snake throughout the period, recording its behaviour at 10-s intervals. We scored aspects such as whether the focal snake was being courted by another snake, and the posture of the focal animal. For example, mate-searching males typically spent much of their time in a distinctive 'head-up' surveillance pose, with the head and forebody raised well clear of the substrate. At the end of the 5-min period we recorded the total distance travelled, and then captured the snake and recorded its body temperature. Some of the snakes monitored in this way were males that we had captured on their initial emergence from hibernation, and marked at that time. Thus, we could assess the ways in which a male's behaviour shifted in the days after his initial emergence.

Arena trials

We erected 24 rectangular, open-topped, nylon arenas (1.0 × 1.2 m and 0.8 m high) in open areas less than 20 m from the den, or in a similar habitat 10 km away. Snakes were captured at the dens, and transferred to the arenas after sexing, weighing and measuring. We carried out a series of trials in which different numbers of she-males and he-males (plus one female in each case) were added to an arena. Because weather conditions can influence the duration of courtship needed to stimulate female receptivity (unpublished data), our comparisons rely upon experimental treatments that were run simultaneously. We matched 'competing' males in terms of body sizes, because this trait can also influence courtship success (unpublished data).

In some of these arena trials, we placed one male with a female to quantify the time a male required to achieve copulation in the absence of any interference from other males. For these trials, half of the arenas had he-males, and half she-males. One female was placed into each enclosure. We recorded mating in 42 of these trials (20 with she-males, 22 with he-males). We also ran trials with multiple males plus one female in each arena. Trials with two males (one she-male plus one he-male; $N=27$ matings recorded) were used to compare mating success in simple one-on-one competition. Trials with five males (one she-male, four he-males; $N=44$ matings recorded) were used to provide a closer approximation of the usual situation in the field. Finally, trials with 10 males (five she-males,

five he-males; $N=29$ matings recorded) were run for comparison with Mason & Crews's (1985) experiments.

We also carried out a single largescale trial, using all 24 arenas simultaneously, to quantify attractiveness of a sample of females, she-males and he-males, and the responses (courtship intensity on the Whittier scale) of a sample of males. Unlike all of the other trials reported here (which were conducted in the field, or on snakes that were tested within 1 h of being collected), the snakes used in this experiment were collected the day before the trial was conducted. We placed six male snakes (one of which, the focal male, was identified by yellow paint) in each arena, and then introduced a target snake (a he-male, a she-male, or a female with her cloaca taped; pheromones associated with mating change female attractiveness and so we had to preclude mating in these trials) to each arena. Of the 12 he-males used as targets, we vigorously rubbed six against females in an effort to transfer skin lipids (as might occur in the course of normal activities; see below). The other target snakes comprised six females and six she-males. We then scored the intensity of courtship (on the Whittier scale) by the focal male towards the target animal every 4 min. After three replicate observations, the target snake was moved to the next enclosure and the procedure repeated. We thus obtained a symmetrical data set whereby we could compare the intensity of courtship by 24 male snakes to 24 target stimuli.

Pheromonal manipulations

To test the possibility that she-males are simply normal males that have received lipids when they have rubbed against females, we vigorously rubbed three reproductive female snakes over each of six he-males. We confirmed the attractiveness of the females by testing them in the field (see above) immediately prior to the rubbing. We used different females for each he-male. The attractiveness of the 'rubbed' males was compared with that of he-males rubbed against other he-males and he-males that were not rubbed against any other snake. We also incorporated a 'rubbed male' treatment in our single largescale arena trial (see above).

Previous work has suggested that in order for a male garter snake to be attractive to another male, the target snake must lack male-specific pheromones as well as displaying female-specific pheromones (Mason et al. 1989). Thus, simply rubbing males against females may not be enough to create 'artificial she-males'. To clarify this possibility, we painted the dorsal surfaces of he-males with hexane to remove existing lipids. After the initial coat of hexane had dried, half of the males were repainted with hexane and the other half were painted with the hexane-based extract from the skins of 12 females. We then placed one male of each treatment group (control and experimental) together in an arena with a female, and recorded which males actively courted females, and which mated. As an additional control, we repeated the procedure using cooking oil dissolved in hexane, instead of lipids from the skins of female snakes. This control allowed us to check whether any effects of female skin lipids on male behaviour or mating success were a

function of lipids per se, rather than the specific lipids produced by female snakes.

Ethical Note

We do not believe that our experimental or observational studies induced significant stress or suffering to our study animals. Maintaining snakes in enclosures did not result in any aggression; these are extraordinarily docile animals, and there are no published reports of intraspecific aggression within the entire genus (Rossman et al. 1996). Work on the same population has shown that handling, and maintenance in bags for some hours, does not result in any detectable rise in the snakes' corticosteroid levels (I. Moore, unpublished data). Overt behaviour is unaffected by handling; for example, male snakes recommence courting immediately even when picked up by the midbody and placed close to a female. All of the snakes were released at the capture site after each experiment. The research was conducted under the authority of the Oregon State University Institutional Animal Care and Use Committee and 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'.

RESULTS AND DISCUSSION

The Biological Significance of She-maleness

She-males attracted consistently high levels of courtship from other males (Fig. 1). We analysed these data using two-factor ANOVAs (with factors being group versus lone, and target type). She-males were less attractive than females, but much more attractive than other males (for proportion of males responding to the target snake: effect of target type: $F_{2,90}=52.18$, $P<0.0001$; for the mean intensity of response: effect of target type: $F_{2,90}=76.85$, $P<0.0001$; in each case including those below, post hoc Tukey-Kramer tests show that she-males differed significantly from other groups). The proportion of males responding to any test animal was higher for solitary than group males (Fig. 1a; for proportion of males responding to the target snake: effect of solitary versus group: $F_{1,90}=7.70$, $P<0.01$), but she-males did not differ from other targets (females or he-males) in this respect (interaction with target type: $F_{2,90}=1.37$, $P=0.26$). However, the intensity of response by males showed a different pattern: males within mating balls tended to respond more vigorously than did solitary males (Fig. 1b; for the mean intensity of response on the Whittier scale: effect of solitary versus group: $F_{1,90}=5.14$, $P<0.03$) and this was particularly true for the response to she-males (interaction with target type: $F_{2,90}=5.21$, $P<0.01$). The trend for males to respond more strongly to she-males within courting groups than when they were solitary (Fig. 1) may reflect a shift in the kinds of cues (pheromonal versus visual and thermal) used by mate-searching males in different contexts (R. Shine, I. T. Moore & R. T. Mason, unpublished data).

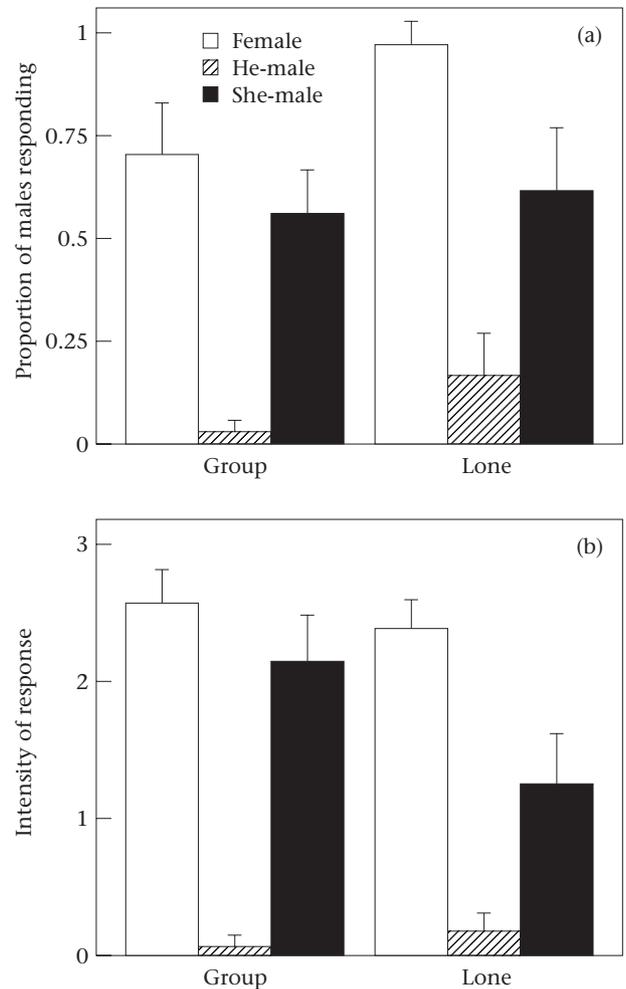


Figure 1. The relative attractiveness of females, he-males and she-males to free-ranging male garter snakes. Attractiveness was assessed by measuring the proportion of males responding to the 'target' snake, and the mean intensity of courtship of males on a four-point (Whittier) scale (see text). Males were tested either when they were part of a 'mating ball' (group) or when they were solitary (lone). Means are shown \pm SE.

Does 'she-maleness' result simply from the accidental transfer of sex-specific skin lipids from females to males? Males that were vigorously rubbed against females did not increase in attractiveness. They remained similar in this respect to nonrubbed males, or males rubbed against other males, rather than resembling she-males (Fig. 2). A two-factor ANOVA (with the factors being group versus lone, and target type) gave the following results: proportion of males responding to the target snake: effect of target type: $F_{3,76}=24.71$, $P<0.0001$; effect of solitary versus group: $F_{1,76}=1.74$, $P=0.20$; interaction: $F_{3,76}=0.15$, $P=0.93$; for the mean intensity of response: effect of target type: $F_{3,76}=35.22$, $P<0.0001$; effect of solitary versus group: $F_{1,76}=0.87$, $P=0.35$; interaction: $F_{3,76}=3.35$, $P<0.03$. In each case, post hoc Tukey-Kramer tests show that the only significant comparisons involved she-males versus other groups.

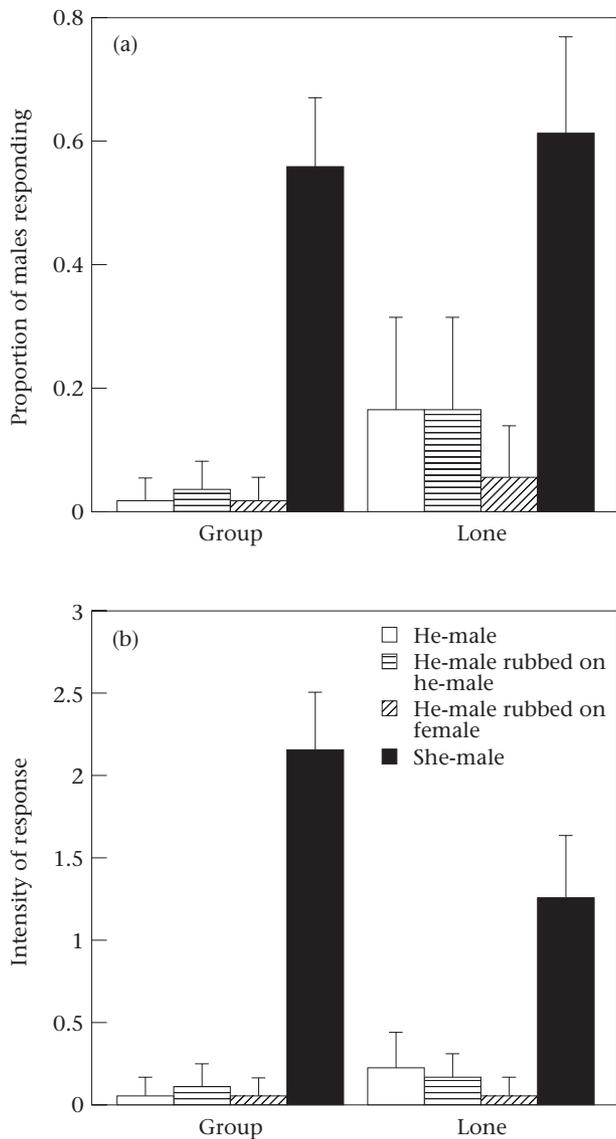


Figure 2. Effects of vigorously rubbing female garter snakes against males on the attractiveness of those recipient males to other courting snakes. The response of other males to these snakes was compared with the response to she-males. We tested the responses of both group and lone males (see Fig. 1). Means are shown \pm SE.

Even if she-males are attractive (above), they might attract little courtship because of the abundance of females, or the relative behaviours (movements, habitat selection, etc.) of the two types of sexual targets. Our data falsified this possibility: we detected high levels of male-directed courtship in the field. We recorded 400 courting groups, 107 of which (27%) were focused on she-males rather than females. On some sampling occasions, the majority of the courting groups that we found were focused on she-males rather than females.

In keeping with this result, the focal animal sampling detected high levels of courtship to she-males (comparing she-males and he-males in terms of the proportion of time being courted: $F_{2,50}=44.84$, $P<0.0001$; see Fig. 3). Indeed, she-males attracted more suitors than did

females, but the difference fell short of statistical significance (for group size: $F_{1,277}=2.94$, $P=0.08$).

She-maleness is not restricted to the few huge dens where we worked. We have seen she-males attracting courtship at many garter snake dens in Manitoba, including small dens (<100 snakes). Also, Noble (1937) reported she-males among a small sample of garter snakes from New York (and also in a viperid species in captivity).

Attributes of She-males

She-males did not differ from he-males in mean SVL (means 47.2 versus 46.4 cm; $F_{1,184}=1.77$, $P=0.19$), but were significantly heavier (one-factor ANCOVA with male 'type' as the factor, SVL as the covariate, and ln mass as the dependent variable: slopes: $F_{1,182}=0.45$, $P=0.50$; intercepts: $F_{1,183}=7.68$, $P<0.01$).

The majority of the snakes in the grassland around the den were clean, but some were covered in mud. A higher proportion of she-males than he-males were covered in mud (90% of 52 she-males versus 17% of 587 he-males: $\chi^2_1=140.99$, $P<0.0001$). Females were intermediate in this respect (75% of 44 females were covered in mud).

Maximum crawling speeds of she-males (and females) were significantly lower than those of he-males (mean time to cover 5 m \pm SD = 13.09 ± 0.99 s for she-males, 13.28 ± 0.94 s for females, 10.58 ± 1.13 s for he-males; one-factor ANOVA with snake type as the factor: $F_{2,56}=8.63$, $P<0.001$; Tukey–Kramer tests confirm that he-males differed from the other two groups).

Focal animal sampling showed that she-males were intermediate between males and females in the distances they moved during the 5-min observation period (Fig. 3; one-factor ANOVA, with type of focal animal as the factor: $F_{2,47}=18.45$, $P<0.0001$), and the proportion of time they spent in an alert 'head-up' surveillance posture ($F_{2,50}=7.18$, $P<0.002$). Body temperatures of he- and she-males were similar and lower than those of females ($F_{2,46}=3.43$, $P=0.04$). Tukey–Kramer tests show that she-males were significantly cooler than females but that he-males and she-males did not differ ($P=0.07$).

Although she-males were relatively inactive in the field, the largescale arena trial on sexual preferences yielded the opposite trend. The snakes that we had captured as she-males the day before were more vigorous courtiers than were other males (on the Whittier scale, mean intensity of courtship directed to females \pm SD = 1.79 ± 1.16 for she-males, 1.38 ± 1.15 for he-males; $F_{1,550}=6.17$, $P<0.025$).

The males that courted she-males did not differ in consistent ways from the males that courted females, in traits such as distance from the den (Fig. 4; one-factor ANOVA with the sex of the focal animal as the factor: $F_{1,275}=1.71$, $P=0.19$), body size ($F_{1,277}=0.05$, $P=0.82$) or body temperature ($F_{1,85}=0.24$, $P=0.63$). Trials in which we suddenly swept an arm over the courting group showed that the proportion of snakes leaving (versus staying with) the group did not differ between groups centred on females ($\bar{X} \pm$ SD = $64 \pm 27\%$) versus those centred on she-males ($81 \pm 22\%$; $F_{1,39}=3.69$, $P=0.06$).

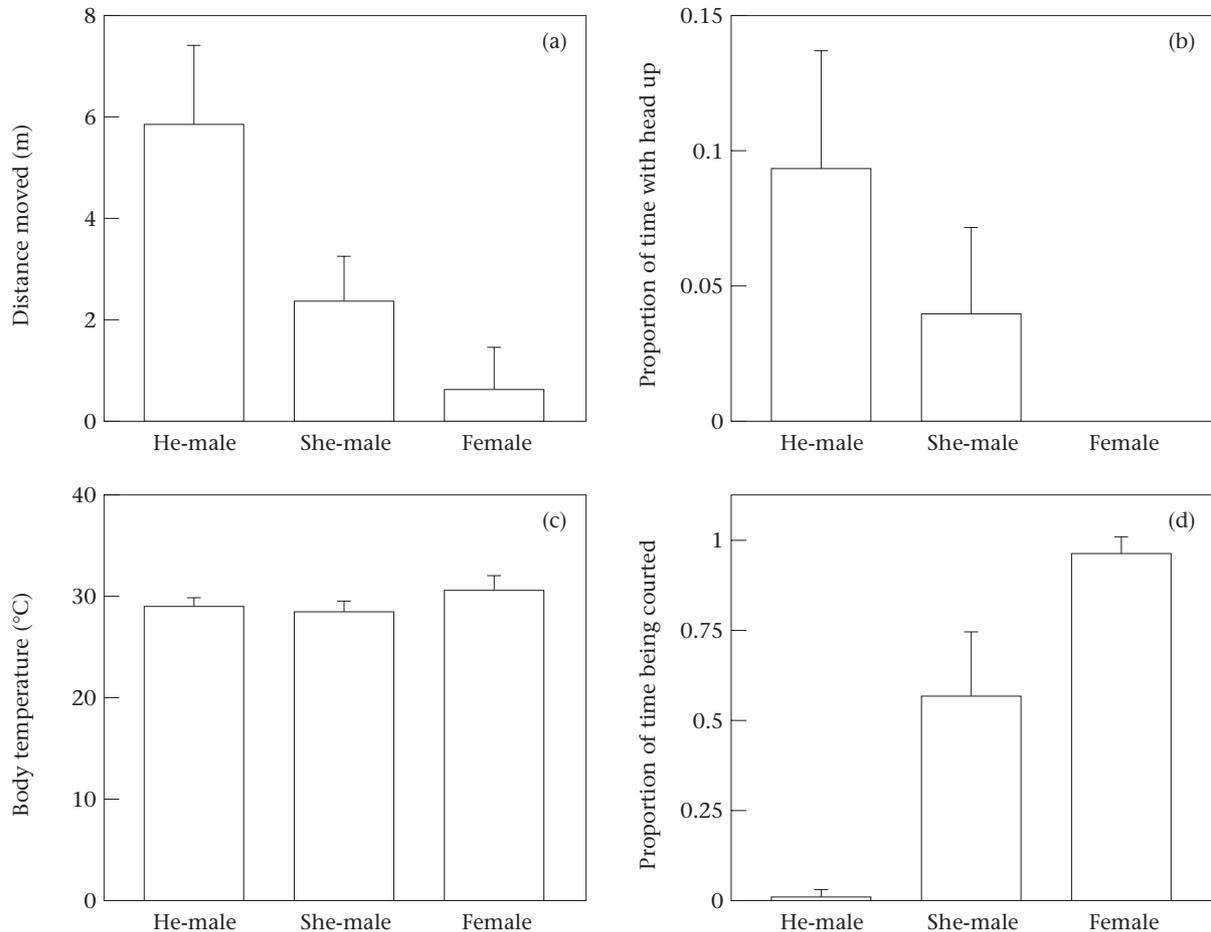


Figure 3. Focal animal sampling of free-ranging garter snakes to compare she-males, he-males and females in terms of (a) the distances they moved during the 5-min observation period; (b) the proportion of time they spent in an alert 'head-up' surveillance posture; (c) their mean body temperatures; and (d) the amount of courtship they attracted from other males. Means are shown \pm SE.

Results of Arena Trials

In arena trials with one male per female, she-males required much longer ($\bar{X} \pm \text{SD} = 34.0 \pm 19.1$ min of courtship) to achieve copulation than did he-males tested at the same time (21.5 ± 13.9 min; $F_{1,40} = 5.86$, $P < 0.021$).

This disadvantage to she-maleness was also evident in multiple-male trials. Regardless of whether the trial involved two males (one she-male plus one he-male), five males (one she-male, four he-males) or 10 males (five she-males, five he-males), she-males obtained fewer matings than he-males. She-males obtained only seven of 27 matings in the two-male trials (26% success, versus null of 50%, $\chi^2_1 = 6.26$, $P < 0.02$), two of 44 matings in the five-male trials (<5% success, versus null of 20%, $\chi^2_1 = 6.84$, $P < 0.01$), and five of 29 matings in the 10-male trials (17% success, versus null of 50%, $\chi^2_1 = 12.45$, $P < 0.001$).

The arena trial where we quantified male courtship intensity to different 'targets' allows us to test whether she-males prefer to court other she-males rather than females. In this trial, the snakes that we classified as she-males courted more intensely than he-males (using the Whittier scale; see above). Although she-males attracted relatively little interest from the majority of the

males that we tested, the relative intensity of courtship to female versus male targets differed between the two types of males. He-males displayed an average of 115 times as much courtship to females as to males, whereas for she-males the mean difference was 63 times. Using a nested three-factor ANOVA with factors of focal male type (he-male versus she-male), target type (male or female), and individual male nested within male type, we detected a significant interaction between target type and male type ($F_{1,550} = 9.63$, $P < 0.002$). That is, both types of males preferred females over she-males, but this preference was stronger in courting he-males than in courting she-males.

Temporal Variation in She-maleness

When retested after 24 or 48 h, a she-male's attractiveness to other males declined rapidly, both when tested against group males and against solitary males (Fig. 5). Statistical analysis confirms the significance of this decline; a two-factor ANOVA (with the factors being group versus lone, and time since initial test) gave the following results. The proportion of males responding to

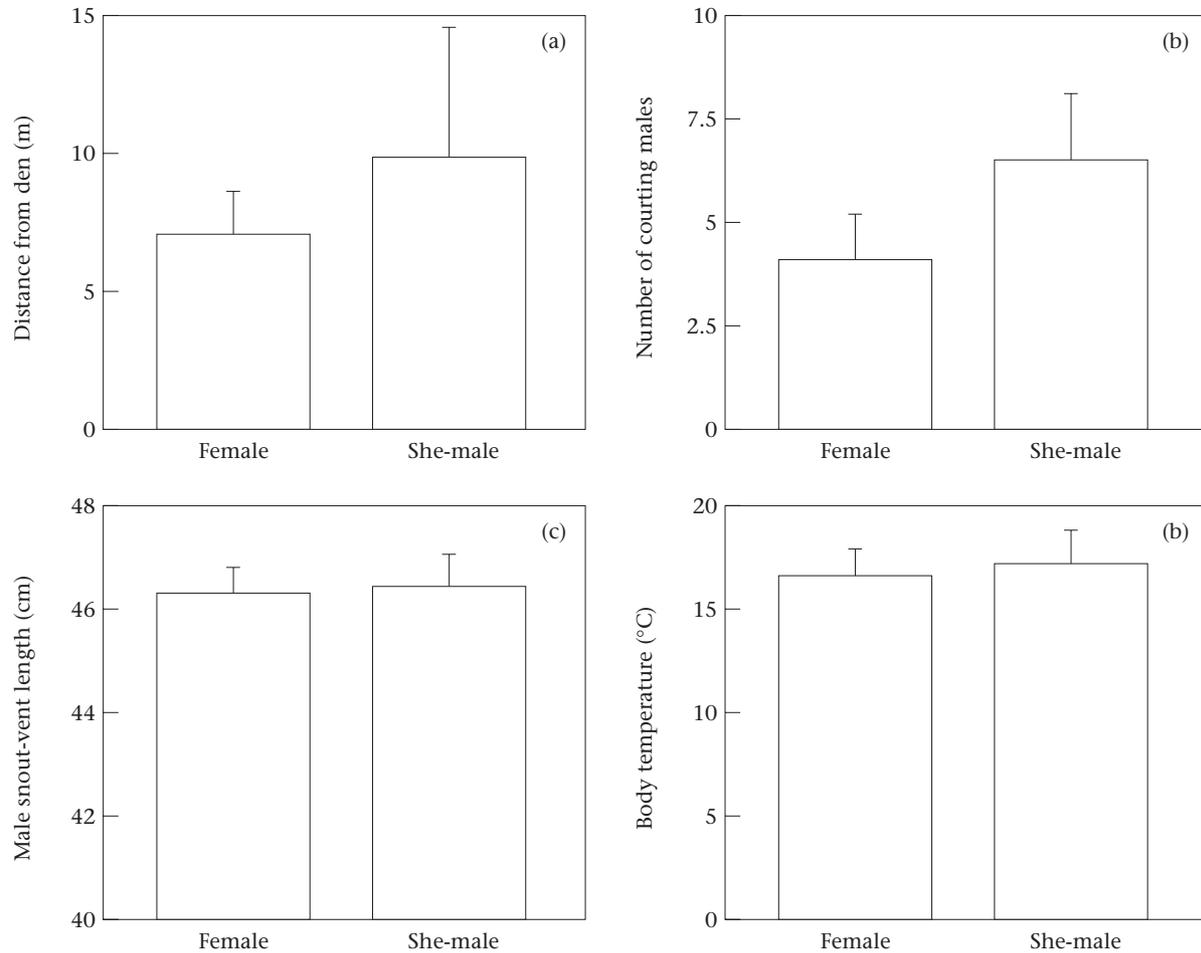


Figure 4. Characteristics of groups of males found courting she-males compared with those found courting females, in terms of (a) distance from the den; (b) group size; (c) body size; and (d) body temperature. Means are shown \pm SE.

the she-male declined with time (effect of time since initial test: $F_{2,102}=16.26$, $P<0.0001$), especially for males already in a mating ball (effect of solitary versus group: $F_{1,102}=6.37$, $P<0.02$; interaction: $F_{2,102}=0.92$, $P=0.40$). The mean intensity of response also declined with time (effect of time since initial test: $F_{2,102}=28.78$, $P<0.0001$), to a similar degree in solitary versus group males ($F_{1,102}=2.04$, $P=0.16$), but with an earlier decrease in group than in solitary males (interaction: $F_{2,102}=5.20$, $P<0.01$). This difference probably reflects the use of different kinds of cues for mate recognition by solitary versus group males (R. Shine, unpublished data). Neither females nor he-males showed any change in 'attractiveness' when retested after similar periods (all $P>0.50$).

The 38 males captured as they first emerged from the den attracted very intense courtship, among the strongest that we ever saw directed to she-males (tested against group males only; mean courtship intensity \pm SD = 2.62 ± 0.55 ; mean proportion of males responding = 0.68 ± 0.19 ; see mean scores in Figures to see how these numbers compare with other samples). This result suggests that all (or virtually all) males are she-males at emergence.

Males that we collected as they first emerged, and marked to indicate the day of emergence, showed temporal shifts both in activity levels and in female mimicry. On their first day after emerging, the males were lethargic, did not court other snakes, and were frequently courted (Fig. 6). This behaviour changed rapidly, however, and by their second day postemergence the males moved about much more, actively courted females, and were almost never courted themselves (Fig. 6; for mean distances moved: $F_{3,17}=5.25$, $P<0.01$; for proportion of time alone: $F_{3,17}=3.62$, $P<0.04$; for proportion of time being courted: $F_{3,17}=5.83$, $P<0.01$; for proportion of time courting: $F_{3,17}=5.55$, $P<0.01$). These results provide compelling evidence that she-maleness is a transitory phase that most or all male garter snakes pass through shortly after emerging from the den.

The Consequences of 'Artificial' She-maleness

Our 'artificial' she-males (with male lipids removed and female ones added) attracted significant courtship from other males. However, the application of the female lipids also modified the behaviour of the male to which the

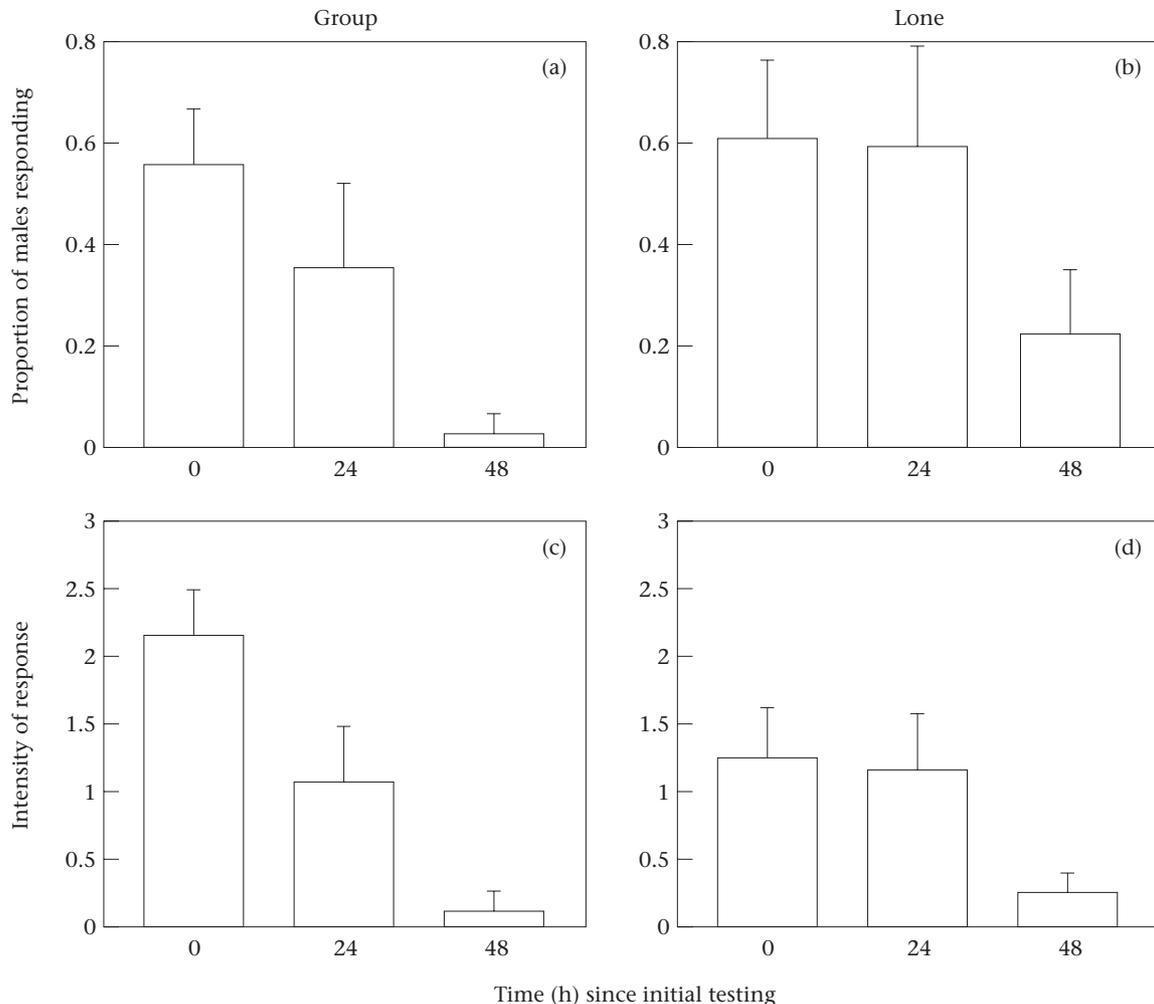


Figure 5. Attractiveness of she-males to other males as a function of the time between successive tests. We examined (a, b) the proportion of males responding and (c, d) the intensity of response of both solitary (lone) and group males, as explained in Fig. 1. Means are shown+SE.

lipids had been applied. The experimental procedure almost completely eliminated courtship behaviour by the recipient male; these 'artificial' she-males courted less frequently than their control counterparts in 30 of 34 trials (against a null of equal numbers courting per enclosure: $\chi^2_1=19.88$, $P<0.001$).

In consequence, the 'artificial' she-males experienced very low mating success in these trials. They obtained only five of 25 matings (20%, versus a null of 50%; $\chi^2_1=9.00$, $P<0.01$). The trials in which we used cooking oil rather than female skin lipids indicated that the oil had no effect on male courtship intensity or mating success: 'cooking oil' males obtained seven of 13 matings (54%, versus a null of 50%; $\chi^2_1=0.08$, $P=0.85$).

GENERAL DISCUSSION

Our data on female mimicry support and extend previous studies on this system, and suggest a new interpretation of the phenomenon. Our major results were as follows.

(1) She-maleness cannot be dismissed as rare or trivial; in the field, a high proportion of all courtship was directed to these animals.

(2) She-maleness was not an artefact of lipid transfer from females.

(3) Although she-males were similar to he-males in overall size, they were distinctive in several ways. She-males were fat, mud-covered and slow, and relatively poor at obtaining copulations (especially if in competition with other males).

(4) These correlates suggest that she-maleness is a transitory phenomenon in the postemergence life of most or all males. Males attracted courtship and were slow, inactive and mud covered for only a short period after they emerged from the den. After this time, they lost their attractiveness and increased the vigour of their own courtship.

(5) 'She-male' skin lipids not only made a male attractive to other males; they also made him reluctant to court females.

Although we advocate a very different interpretation of she-maleness than that of Mason & Crews (1985, 1986),

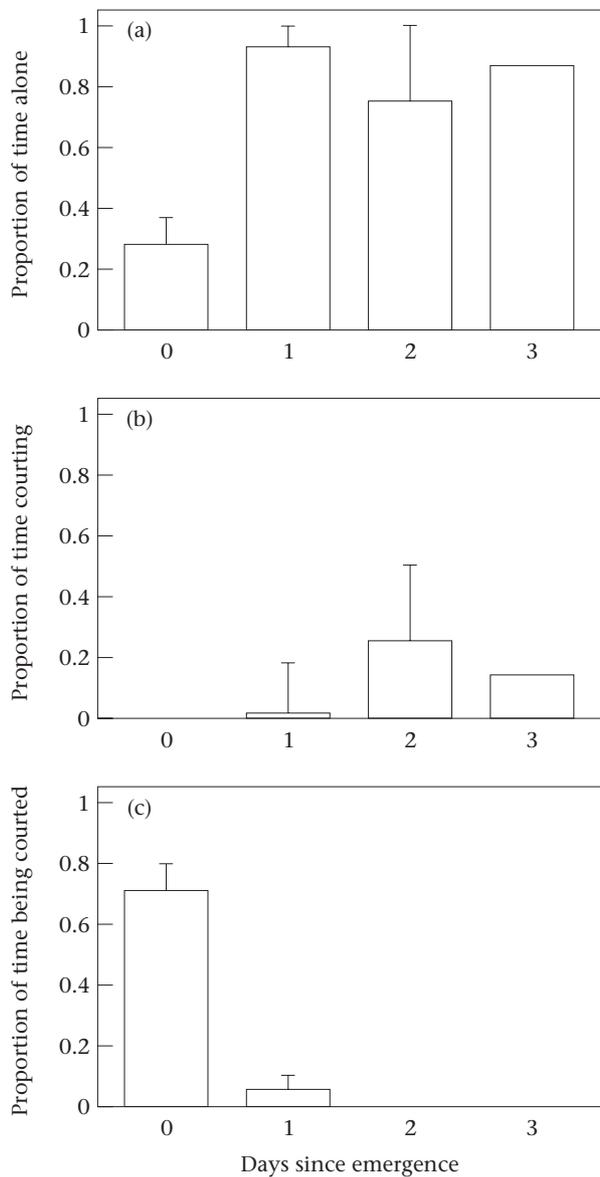


Figure 6. Temporal shifts in (a) proportion of time alone; (b) proportion of time courting; and (c) proportion of time being courted in male garter snakes, individually marked as they emerged from their overwintering dens. Means are shown \pm SE.

most of the patterns that they identified are consistent with our data. Thus, for example, our results agree in terms of the attractiveness of these animals, their overall similarity in body size to other males, and the fact that a male that rubs against a female does not thereby become attractive to other males. This latter result reflects the fact that males will not court a snake with male-identifying lipids (probably squalene), even in the presence of female-specific lipids (Mason et al. 1989). In our 'artificial she-male' trials we solved this problem by washing males with hexane prior to applying the female pheromone. Mason & Crews's (1985, 1986) data on hormone levels (high testosterone in she-males) also fit well with the observation that testosterone levels are highest at

emergence and fall rapidly thereafter (Krohmer et al. 1987). The fact that she-males are heavier than he-males (above), combined with the observation that mate-searching males lose mass rapidly after emergence (Shine et al., unpublished data), also fits the notion that she-males are newly emerged animals.

The primary conflicts between our study and the earlier work by Mason & Crews (1985, 1986) involve the temporal stability of she-maleness, and the ability of she-males to obtain copulations in arena trials. Our data suggest reasons for these apparent contradictions. First, the long-term stability of she-maleness from Mason & Crews's work (1985, 1986) may reflect the timing of retesting. If all males are she-males immediately after emergence in spring, then retesting the same male when it emerges the next year will lead to the conclusion that the animal continues to operate as a she-male. The second contradiction between the two studies concerns the ability of she-males to obtain matings in arena trials. She-males performed poorly in all of our trials, but out-competed he-males in the Mason & Crews (1985) study. Our work (both in the major arena trial and in the field) shows that these snakes shift very quickly from languid she-maleness to vigorous courtship. This shift provides a plausible reason why she-males might experience higher mating success if they were kept in captivity for even a few hours after they were originally collected (as she-males) in the field. Thus, there is little empirical disagreement between the two data sets.

Mason & Crews (1985, 1986) were the first to draw attention to this interesting phenomenon, and they accurately clarified some of its correlates. With the benefit of a much larger data set, however, their interpretation can be falsified. She-maleness is not an alternative mating strategy pursued by a small minority of males, as suggested by Mason & Crews (1985, 1986). Instead, it results from a transitory (condition-dependent?) shift in skin lipids shown by most (all?) male snakes at the time that they emerge from their overwinter dens. None the less, males may still vary in their reliance upon she-male 'tactics'. We do not know if some males continue to operate as she-males for a protracted period after emergence, whereas others rapidly adopt 'male' lipid profiles. Further studies are needed to resolve these issues.

The obvious question, then, is 'why do males become she-males at the time they emerge from hibernation?' This question can be asked at both proximate (mechanistic) and ultimate (evolutionary) levels, and our data provide insights into both of these issues. On a proximate level, our experiment to create 'artificial' she-males yielded a completely unexpected result: the application of female skin lipids modified not only the behaviour of other males (i.e. it elicited courtship from them), but also that of the male to which the lipids were applied (i.e. it stopped his own courtship). Thus, although she-maleness is associated with changes to both of these attributes (i.e. male passivity as well as attractiveness), a single mechanism may produce both of these effects. Males that produce female-like skin lipids thereby become she-males in behaviour as well as attractiveness.

The effects of female lipids on male behaviour deserve further investigation. Male garter snakes are notoriously amorous. For example, males continued to court even when held by the hindbody; and males released from holding bags recommenced courtship within a few seconds of release, as soon as they contacted a female. Thus, the suppression of courtship by application of skin lipids is a remarkable result. It would be interesting to know what receptors are involved, and the nature of the feedback system that switches off sexual behaviour. Strong parallels occur in other kinds of animals. In spider crabs, *Libinia emarginata*, small males use female mimicry, are relatively uninterested in courtship, and display distinctive hormone profiles (Laufer et al. 1994; Laufer & Ahl 1995). In other cases, the mimicry may serve other functions: for example, small or malnourished male rove beetles produce a female sex pheromone that discourages aggression from other males (Forsyth & Alcock 1990; Peschke 1990).

The other mechanistic implication of our results concerns the transitory nature of she-maleness, and in particular the rapid loss of attractiveness by she-males (see Fig. 5). Clearly, the lipids that render she-males attractive must emanate from some part of the body surface that is under direct (and rapid) control. The lipids themselves are stable; for example, dead female snakes can continue to attract courtship for several days (personal observation). Thus, the precipitous decline in attractiveness of the she-males suggests an active 'switching-off' of the appropriate lipids. For this to be possible over the time span of hours rather than days, the lipids that attract courtship must be under direct control rather than (for example) embedded in the inert epidermis. 'Hinge' areas of thin skin at the base of each scale offer a possible site of lipid release, which could be modified rapidly in response to levels circulating in the bloodstream.

Explanations concerning the 'ultimate' (evolutionary) significance of she-maleness are more difficult to test. The reason cannot involve a reduced probability of attack from other males (as occurs in other cases of female mimicry: e.g. Trivers 1976), because male garter snakes display no agonistic behaviour towards each other at any time (Rossman et al. 1996). Our other (unpublished) studies on this system suggest some possible advantages to she-maleness. Telemetric monitoring shows that males move about very actively during their tenure at the dens (sometimes moving over 1 km/day), and mark-recapture studies show that male snakes lose mass rapidly during this time (Shine et al., unpublished data). Presumably for this reason, males generally disperse from the dens after a relatively brief period (less than 2 weeks) of intense courtship activity. In combination, these data suggest that energy costs of courtship are substantial.

By producing female-like skin lipids, a male may enhance his reproductive success in four ways: (1) he may gain a mating advantage by confusing other males (as advocated by Mason & Crews 1985, 1986); (2) he does not waste energy on courtship at a time when that courtship will be ineffective (because of his poor locomotor performance); (3) he induces competing males to waste

time and energy in fruitless courtship, thus reducing the number of competitors that he will face when he commences his own courtship period; and (4) he distracts competing males from receptive females, potentially increasing the availability of unmated females a day or two later.

Such costs and benefits are difficult to quantify, but these ideas suggest directions for further studies on this remarkable phenomenon.

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