

From: CHEMICAL SIGNALS IN VERTEBRATES 4

Edited by David Duvall, Dietland Muller-Schwarze  
and Robert M. Silverstein  
(Plenum Publishing Corporation, 1986)

PHEROMONE MIMICRY IN GARTER SNAKES

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Female mimicry is found in many diverse taxa including insects and most vertebrate classes (Weldon and Burghardt, 1984) and occurs in two forms. In several species, males assume a female-like morphology or color pattern (Dominey, 1980; Gross, 1979), whereas in others, males mimic female behavior patterns (Arnold, 1976; Thornhill, 1979). In some species males mimic both female morphology and behavior (Gross, 1983).

We describe here an unusual form of female mimicry in a reptile (Mason and Crews, 1985). This form of mimicry is unique to the animal kingdom in that it is expressed not as a behavioral or morphological feminization but as a physiological feminization, the production of what was previously believed to be a sex-limited attractiveness pheromone.

The red-sided garter snake (Thamnophis sirtalis parietalis) is the most northerly living reptile in North America. During the winter months these animals hibernate in large communal dens throughout much of their range (Aleksiuk and Stewart, 1971). In the spring, males synchronously emerge from the dens and aggregate in areas immediately surrounding the den opening. Aggregations are comprised entirely of males and can number into the thousands. Females emerge singly over the next three to four weeks.

Upon emergence, a female will usually be courted by 10-20, but sometimes as many as 100 males forming a "mating ball" (Aleksiuk and Gregory, 1974). Males recognize and begin to court females by detecting a pheromone released by the female (Crews, 1976; Noble, 1937). Rapid tongue-flicking by the male delivers the pheromone cues from the female's dorsal surface to the male's vomeronasal organ (Garstka and Crews, 1981). During courtship, the male presses his chin along the female's dorsum and traverses the length of her body, eventually coming to rest along the female's back with the cloacas aligned. Only one male will be successful intromitting one of his hemipenes.

During the course of a five week field season in the spring of 1983, we captured entire mating balls and censused the participants. Each animal in the ball was sexed by cloacal probing and the hemipenes extruded (Laszlo, 1975). In 33 of the 200 mating balls censused no females were found. Rather, in each of these 33 mating balls, a single male was being courted by the other males. Because these males could be

distinguished from other males by the fact that they elicited courtship, we referred to these males as she-males.

She-males are indistinguishable from normal males, each possessing hemipenes, testes, and accessory sex structures identical to those found in males. The mean snout-vent length and body weight of she-males does not differ significantly from that of other males. In addition, the weights of she-male livers, fat bodies, adrenals, and testes relative to body weight also do not differ significantly from those of other males. Histological examination of testes shows no difference in the stage of spermatogenesis between males and she-males. As is typical at this time of year, both she-males and males have regressed testes. Sperm is present in the ductuli deferentia in members of both groups, and the number of sperm and their motility and morphology do not appear to be different. Finally, blood samples have been taken from males ( $n = 2$ ), females ( $n = 2$ ), and she-males ( $n = 2$ ) and stained to detect heterochromatin. In the garter snake, the condensed W chromosome, which is found only in females, is composed largely of heterochromatin (Becak, 1983). Only blood samples from females stain positively for heterochromatin; thus, she-males are genotypically as well as phenotypically male.

The mating behavior of she-males was investigated to determine whether they exhibit a normal sexual preference and court females or whether they court males. She-males were placed in a 40 cm x 28 cm x 10 cm clear plastic box with a recently emerged, unmated female for ten minutes. All 33 she-males courted vigorously for the duration of the test. Further, 3 of the she-males mated with the stimulus female in the time allotted. Although she-males would not court males, they did court each other.

The attractiveness of the she-males is not due to prior physical contact with recently emerged females. Males rubbed against a recently emerged, attractive female were not courted by other males. This agrees with field and laboratory observations that recently mated males that have been in contact with females for up to one hour have never been observed to be courted. The attractiveness of the she-males appears to be permanent as is the case in sexually mature females (Bona-Gallo and Licht, 1983). All of the she-males tested ( $n = 33$ ) elicited courtship from sexually active males when tested at 3, 18, and 40 weeks following capture.

Both normal males and she-males prefer females to she-males. A simultaneous choice test was conducted in which a she-male and a female of similar size were placed in an arena with 10 males. After five minutes the number of males that courted either stimulus animal was recorded. A total of 12 simultaneous choice trials were run each having a duration of five minutes. Males showed a significant preference for females compared to she-males in all 12 simultaneous choice tests (binomial probability,  $p < .005$ ). Similar results were obtained when males were presented with females and she-males in a sequential choice paradigm.

Production of the attractiveness pheromone may afford she-males an advantage in the mating ball. In observations of mating behavior in the field, she-males joining an established mating ball caused some males already courting the female to stop and begin to court the she-male, resulting in the she-male gaining a better position along the female's body. While in the field, a competitive mating test was run in which groups of 5 males and 5 she-males were selected at random and released into an arena (1 m x 1 m x 1 m) with an unmated, attractive female. The trials continued for one hour or until a mating occurred. Mating balls formed around the female in every trial. A total of 42 mating trials

were conducted. Of these, 29 resulted in a she-male mating with the female and 13 with a male mating, making she-males more than twice as successful as males in the mating competition ( $\chi^2 = 6.39$ ,  $p < .025$ ). The advantage to the she-male's attractiveness thus appears to lie in its ability to gain a better position in the mating ball and at the same time effectively blocking males from mating, in essence causing sexual interference (Arnold, 1976).

To investigate the physiological basis of the she-males' attractiveness, blood samples were taken from males, females, and she-males in the field and analyzed by radioimmunoassay (Camazine et al., 1980). Analysis of variance for each hormone revealed that females have significantly higher circulating levels of estradiol (E) than both males and she-males ( $p < 0.05$ , Student-Newman-Keuls Test). Conversely, she-males have significantly higher levels of testosterone (T) than both females and males. There were no significant differences in circulating levels of dihydrotestosterone (DHT) (Mason and Crews, 1985). Presumably, the higher levels of T in she-males are a result of either higher secretion or reduced clearance from the circulation.

The finding that a male animal with female-like aspects to its physiology has significantly higher levels of circulating T than do males lacking these qualities is intriguing. It is well known that T does not always exert its effect on target tissues as T directly but as one of two metabolites, DHT or E. We hypothesize that the high levels of T in the circulation of she-males may lead to physiologically significant levels of E in the skin. At this time, levels of E in the skin of she-males remain to be determined. However, in support of this hypothesis are those studies which have isolated aromatase, the enzyme responsible for converting T to E, in the skin and liver of both mammals and birds (George et al., 1981; Slaunwhite et al., 1965).

The female attractiveness pheromone may be related to vitellogenin and is transported in the blood and delivered through the skin (Crews, 1976). Further, exogenous estrogen injection will induce pheromone production in females and males (Kubie et al., 1978). Unlike females, estrogen-injected male garter snakes are never courted by males. However, serum from estrogen-injected males and females, when spread on the backs of control males, will elicit courtship from sexually active males (Crews, 1976). These observations suggest that the skin in the male and female differ in their transport of the attractiveness pheromone.

The pheromone produced by the she-males is the same as that produced by females. Samples obtained from the skin surface using the non-polar solvent toluene revealed that the skin of females and she-males, but not of males elicited courtship behavior from conspecific males at the hibernaculum (Camazine et al., 1980).

She-males may develop as a result of hormones experienced early in life. It is well-established that gonadal hormones organize morphological sex differences, including the brain, early in life, as well as activate sexual behavior in the adult (Feder, 1984; Goy and McEwen, 1980). It has been suggested further that the hormones associated with the heterogametic sex are responsible for determining the development of internal and external sex characters (vom Sall, 1983). In mammals in which the male is the heterogametic sex, T is the organizing hormone whereas in birds, in which the female is the heterogametic sex, it is the ovarian hormone estrogen that organizes sexual differentiation (Adkins-Regan, 1981). In those snakes that have sex chromosomes, the female is the heterogametic sex (Crews, 1985). Within this context of female heterogamety, it is significant that early estrogen treatment will induce production of the

attractiveness pheromone in neonatal female and male T. s. parietalis (Crews, 1985).

#### ACKNOWLEDGMENTS

We wish to thank Michael Moore, Jim Blank, Joan Whittier, Eric Pianka, Mark Grassman, Mike Ryan, and Jim Bull for their suggestions which greatly improved this manuscript. We also wish to thank David Hinkley, Jim Bull, and Fred Marsteller for their assistance with the statistical analyses. Marie Anne Diamond and Yuki Morris provided excellent technical assistance. Special thanks to Bill Koonz and Merlin Shoesmith of the Manitoba Department of Natural Resources for their invaluable help. Supported by National Institute of Health grant NICHD 16687 and by a National Institute of Mental Health Research Scientist Development Award to D. Crews.

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