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Mating in the red-sided garter snake, *Thamnophis sirtalis parietalis*: differential effects on male and female sexual behavior

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Summary. Female red-sided garter snakes, *Thamnophis sirtalis parietalis,* become unattractive to most males after mating in the field and in the laboratory. Male red-sided garter snakes vary in their latencies to court attractive females following copulation, with courtship resuming in minutes to hours. Unsuccessful males in mating balls disperse from mating pairs, but are not residually inhibited from courting attractive females. These patterns of behavior indicate that males have evolved mechanisms to maximize opportunities for copulation with several females, while females mate only once per season.

Introduction

In many species of animals the act of mating results in profound behavioral and physiological changes (Crews 1980; Dewsbury 1981). Such alterations in behavior and underlying physiological systems represent the fabric upon which selection acts to produce mating systems. In this paper, we report ongoing field and laboratory studies of the mating system of the red-sided garter snake (*Thamnophis sirtalis parietalis*), an animal which breeds in the largest aggregations of any terrestrial vertebrate in North America (Crews and Garstka 1982).

In the Canadian red-sided garter snake, large, male-biased aggregations occur during the spring breeding season (Garstka et al. 1982). Males emerge from underground hibernacula and remain in the vicinity for approximately 3–4 weeks. Females emerge singly or in small groups and are actively courted by a large number of males, forming mating balls (Crews and Garstka 1982). Most females mate with a single male upon emergence and then disperse, while the males remain in the den. During copulation, males deposit a copulatory plug in the females' cloaca (Devine 1975, 1977; Ross and Crews 1977, 1978). Mating stimuli also initiate a transient surge in the circulating concentration of estradiol that functions both to stimulate transport of the recently deposited sperm and to evacuate sperm stored in the female's oviduct from the previous year (Halpert et al. 1982).

The pattern of emergence of male and female red-sided garter snakes results in an overwhelming bias in the numbers of males during the breeding season. Male red-sided garter snakes do not engage in territorial disputes or establish dominance hierarchies during the breeding season, as is seen in other species of aggregating snakes (Prestt 1971; Lowe and Norris 1950). Thus, males compete with many other males for opportunities to mate, while females, if receptive, are assured of mating.

In these studies, we present evidence that males maximize opportunities to mate, but that most females, once mated, are both unattractive and unreceptive to males. A difference in the response of males and females to the act of mating may indicate differential selective pressures acting to produce variation in behavioral responses between the two sexes.

Materials and methods

Field studies

Study site. All studies were conducted during the breeding season, May-June, 1983, using animals that were collected at a winter hibernaculum (W. Den in Garstka et al. 1982) in the Narcisse Community Pasture, near Chatfield, Manitoba, Canada.

Experimental animals. Recently mated females (with copulatory plugs) and unmated females (without copulatory plugs) were

Table 1. Graduated scale of male courtship in the garter snake, *Thamnophis sirtalis parietalis*

Courtship score	Behavioral description
0.0	Male fails to approach female
0.5	Male approaches female, leaves, and returns for further investigation
1.0	Male follows female or is in contact with chin
1.5	Male follows female slowly while covering her body with his chin
2.0	Male aligns along female's back with rapid and repeated traverses along the length of the female's body
2.5	More intense following, traverses and caudo-cephalic waves with attempts at cloacal apposition
3.0	Successful intromission

collected at the dens. Unmated males (males that had not mated for 12 h) were selected from naturally occurring mating balls at the den site. Mated males (males that had mated with a female at most 12 h previously) were obtained at the den during copulation, or from mating pairs that occurred during other experiments. Animals were marked individually with indelible ink, segregated by sex in cloth bags and held at ambient temperatures until behavioral testing was initiated.

Behavior measures and testing conditions. Male courtship behavior was assessed by a a scale from 0 to 3 (Table 1) routinely used in field and laboratory studies with garter snakes. Female attractivity was determined by the frequency and intensity of courtship a female received after 5 min exposure to ten sexually active males. Female receptivity refers to the incidence of mating.

Males were placed in a clear plastic test box (38 × 27 × 8 cm) in groups of ten or singly. Females were then introduced into the box singly. After a period of 5 min, the number of males courting or the courtship score of each male was recorded and the female was removed. The trials to measure courthip behavior were designed to be of short duration to avoid confounding effects of mating on male behavior. At least 2 min were allowed between introductions of females. Mated and unmated females were presented in the following order to control for sequential effects: unmated females, mated females, and unmated females. Frequency of male courtship did not differ from the beginning to the end of the experiment (Runs test, day 1: $P<0.95$; day 2: $P<0.63$; Spearman's rho rank correlation of order of trial vs number of males courting, day 1 = −0.01; day 2 = 0.32).

Laboratory studies

Experimental animals. All animals were collected near Narcisse Pasture, Manitoba, September 17–20, 1983, segregated by sex, and transported to the laboratory. Upon arrival, animals were housed in single sex groups on 14L:10D photoregimen and 23 °C. Animals were then placed under artificial hibernation conditions for 17 weeks (4 °C, 0L:24D) in cloth bags with moistened sponges. Upon emergence from artificial hibernation, animals were placed on a 14L:10D photoregimen and a 28:18 °C temperature regimen (14 h of 28 °C corresponding to lights on). These conditions stimulate male courtship behavior and female attractivity and receptivity in newly emerged garter snakes (Camazine et al. 1980).

Behavioral measures and testing. The same measures of male and female sexual behavior were applied in the laboratory as in the field. In the laboratory, males ($n=49$) were housed 5 per side in divided wire mesh cages (51 × 31 × 47 cm). Males were not moved from their home cages for the duration of the experiment. Females were housed ten per wire mesh cage (51 × 31 × 47 cm) in the same room but in separate environmental chambers.

Detailed information on the mating behavior of this species was recorded during mating trials. Females ($n=44$) were introduced individually into cages containing males (10 groups of 5 males), and left for 2 h or until a mating occurred. If a male mated, the remaining males in the cage were temporarily removed. Both the male and the female from mated pairs were then used in subsequent experiments. All laboratory matings resulted in the deposition of a copulatory plug in the female's cloaca.

Experimental cages were continuously monitored for mating activity after introduction of each female. The number of males courting and the intensity of male courtship was recorded at 0.5, 1, and 2 h. If mating occurred within 0.5 h, the number of males remaining aligned to the female during the initial period of copulation was recorded. Latency to mate and an index of the amount of courtship (number of males courting × time courting in min) a female received before mating were calculated. The proportion of females that mated yielded an index of female receptivity. The frequency of males mating, the incidence of multiple matings of males with more than one female as well as the duration of copulation was noted.

The effect of mating on female attractivity was tested immediately after mating occurred. Four groups of five males were used as test males; each female was tested with a single group of males. Behavioral scores of individual males were recorded 2 min after the introduction of a female. At least 2 min elapsed between each trial.

The effect of mating on subsequent male sexual behavior with a new stimulus female was also examined. When a male ceased copulation with a female during the mating trials, the mated female was immediately removed from the cage and the male's cagemates reintroduced. Then an unmated female was introduced into the cage. Males that had mated ($n=14$) were observed continuously for 1 h or until a maximal courtship score of 2.5 (without intromission) was exhibited. The courtship behavior of males was also tested 24 h after mating.

The effect of mating on the subsequent sexual behavior of the males that did not mate was also observed in the laboratory. Cagemates of the mating male were allowed to remain in the cage for the duration of the mating sequence. The behavior of unsuccessful males ($n=9$) was observed during three separate matings. In a second set of observations, once the mating pair separated, the mated female was removed and an unmated female introduced. The behavior of unsuccessful males ($n=4$) from two matings was monitored for 15 min or until courtship behavior was maximal (a score of 2.5) for all the males. To control for the effects of disturbing the males by removing and introducing females, the same manipulations were performed with two control cages of males ($n=7$) in which no mating occurred.

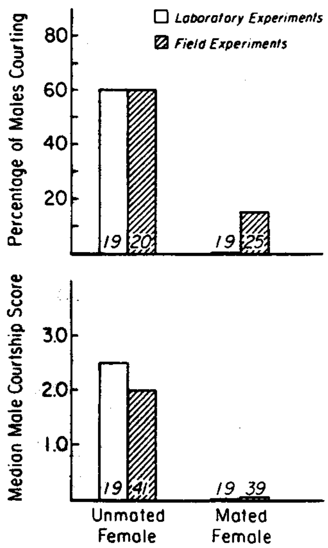

Fig. 1. Influence of mating on sexual attractivity of female *Thamnophis sirtalis parietalis* in the laboratory (*open bars*) and in the field (*shaded bars*). *Upper scale:* The percentage of males exhibiting a courtship score of 2.0 or greater is presented. *Lower scale:* The median male courtship score of males exposed to unmated and mated females. The number of trials is indicated on the lower portion of each bar

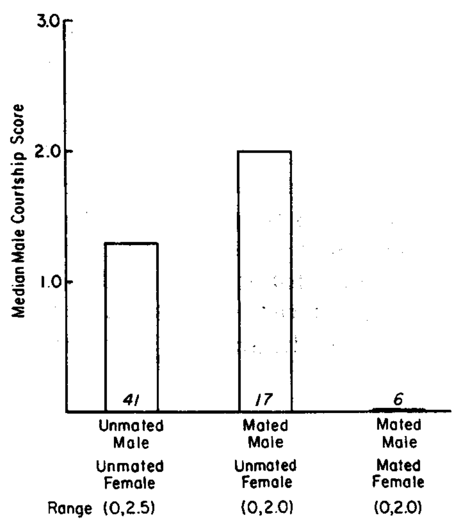

Fig. 2. Effect of mating and female status on courtship behavior of male *Thamnophis sirtalis parietalis* in the field

Results

Effect of mating on female attractivity in the field and in the laboratory

Females that mate become unattractive to most males and remain unattractive at least for 12 h after mating in the field and 24 h in the laboratory (Fig. 1, $P<0.01$). Fewer males court mated females and median intensity of courtship is less ($P<0.01$). In the field, 15% of the males tested (3/20), however, repeatedly courted mated females, even though these females were unreceptive. In addition, males that had mated previously exhibit a preference for unmated females (Fig. 2, $P<0.05$).

Effect of mating on subsequent male courtship behavior in the field and in the laboratory

In the field, all males that had mated exhibited courtship behavior when exposed to an unmated female within 12 h after mating (Fig. 2). Once males that mated exhibit renewed interest in courtship, their behavior is not significantly different from unmated male courtship ($P<0.50$, Fig. 2). In the lab, a more detailed study of the latency of males to court an unmated female after mating indicated there is variation among male responses. Half of the males tested ($n=14$) courted females within 1 h of mating whereas the other half of the males were unresponsive to females at least an hour after mating. All males tested courted an unmated female 24 h after mating.

Effect of exposure to a copulatory pair on male courtship behavior

When a male in a mating ball intromits, the unsuccessful males exhibit characteristic behaviors. Within a few minutes of intromission, the unsuccessful males leave the mating pair and the 'mating ball' disperses. No obvious visual cue or movement from the mating pair initiates this response by the other males. For the duration of the copulation, the unsuccessful males move about the cage, occasionally showing sporadic but brief interest in the copulating female. The inhibitory effect of the mating pair on the courtship behavior of unsuccessful cagemates was transitory, in that when a new unmated female is introduced at the end of the copulatory sequence, the unsuccessful males exhibit courtship at previous levels.

Observations on the mating behavior of T.s. parietalis in the laboratory

Thirty-four percent of the females (15/44) were sexually receptive, mating within 4 h in 2 days of testing (Fig. 3). Females tended to mate more frequently the second day out of hibernation (34.8% of females tested days 1 and 2 vs 19.4% of females tested day 1), although this trend is not statistically significant. The mean latency of females to mate was significantly affected by the time the females

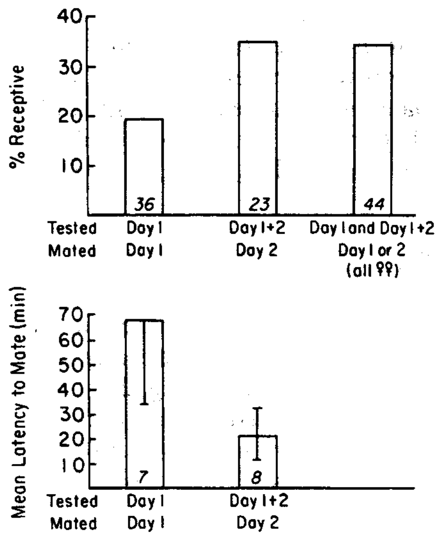

Fig. 3. *Upper portion:* Percentage of females that were sexually receptive to males on day 1, day 2, and days 1 or 2 (total) are represented. *Lower portion:* Mean latency to mate (min) of females tested on day 1 or, day 1 and 2

were out of hibernation (Fig. 3, $P < 0.05$). Females tested on day 1 exhibited a mean latency of 68 min (± 34 min, SD); females tested on days 1 and 2 that mated on day 2 exhibited a mean latency of 21 min (± 11 min, SD). The amount of courtship the females received was not correlated with either the frequency of mating or latency to mate as long as at least one male courted.

Twenty-nine percent (14/49) of the males exposed to females during the mating trials mated; half of the males that mated did so more than once (1 male mated 4 times, 2 males mated 3 times, and 4 males mated twice). Thus, 61% of the matings (17/28) resulted from males mating with more than one female on separate occasions. Mean duration of copulation for all matings ($n = 28$) was 17.6 min (± 5.49 min, SD).

Discussion

These experiments demonstrate the differential effect of mating on the sexual behavior of male and female *T.s. parietalis* in the field and in the laboratory. Once mated, females become sexually unattractive to most males and are unreceptive to those males that persist in courting. Since females in the field rapidly disperse from the den sites after mating to areas where males are not present (Gregory 1976; Devine 1977), these mating-induced behavioral changes in the female minimize further copulatory attempts by males and may reduce risks of females to predation (Garstka et al. 1982).

The specific copulatory stimuli that bring about changes in attractivity of females is not known in this species. In a related species, the plains garter snake (*T. radix*), the copulatory plug contains a pheromone that overrides the female attactiveness pheromone and renders females unattractive to males (Ross and Crews 1977, 1978).

The effect of copulation on subsequent sexual behavior of males was found to be variable. In the field, all males regained the capacity to court at previous intensities by 12 h. In the lab, where the latency of males to court a new stimulus female were more closely monitored, 50% of the males courted at maximal intensities within an hour of mating, and all males by 24 h of mating. The mechanism by which males are inhibited from courting is not known.

Although we have no field data on multiple copulatory success of individual males, in laboratory mating trials multiple copulations of individual males with more than one female are not uncommon. In our observations, males have mated with from 2 to 4 females in as many days and produced copulatory plugs with each mating. It appears that post-ejaculatory latency to court and mate with a new stimulus female may be an important variable in the mating success rate of a male.

The latencies for males to court after mating indicate that male *T.s. parietalis* can recover from copulation more quickly than the plains garter snake, *T. radix* (24 h latencies were reported for the latter species: Ross and Crews 1978). Selection for shorter latencies such as those exhibited by the red-sided garter snake may be the result of more intense male competition for mates found at the large dens that are characteristic of this subspecies. A more rigorous test could be achieved by comparing post-ejaculatory latencies to court of males from populations of *T. sirtalis* from large and small dens.

In the red-sided garter snake, unsuccessful males find a female unattractive shortly after intromission occurs, and disperse from the mating pair, as has been described previously in this and other garter snake species (Ross and Crews 1978; Devine 1977). However, there appear to be no residual effects of exposure to copulating pairs or mated females on the courtship behavior of males that have not mated, as in *T. radix* (Ross and Crews 1978).

We conclude from these experiments that the behavior of male and female garter snakes during courtship and after mating has evolved in response to different selective pressures. Female snakes do not compete for mates and if receptive are assured of finding a male to mate. Females have evolved mechanisms for preventing multiple matings, including evacuation of sperm from previous year's matings (Halpert et al. 1982) and a decline in sexual attractivity and receptivity in response to mating.

It is important to note in *T.s. parietalis*, males do not establish territories or dominance hierarchies. Further, a male must compete with many other males to mate. Under these conditions the inhibition of subsequent male sexual behavior by the act of mating has been minimized. Males exposed to copulating pairs rapidly lose interest in the mating female, but are not inhibited when presented with a new female. Thus males appear to be maximizing opportunities for mating with several females.

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