

## TERRESTRIAL COURTSHIP AFFECTS MATING LOCATIONS IN *AMBYSTOMA OPACUM*

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**ABSTRACT:** The incidence of insemination in migrating female *Ambystoma opacum* was studied in a breeding population near a Carolina bay in South Carolina. Of 77 females that were hand-captured substantial distances from the nesting area and then held in isolation, 24-38 (31-49%) produced fertile clutches. The capture of females during migration that had been inseminated before reaching the pond basin suggests that the timing and location of mating is more variable in this species as compared to other ambystomatids. The evolution of terrestrial courtship, coupled with the potential for high mate competition among males at the nesting area, may have provided an opportunity for sexual selection of an alternate male mating strategy.

**Key words:** Caudata; *Ambystoma opacum*; Terrestrial breeding; Sexual selection; Mating location.

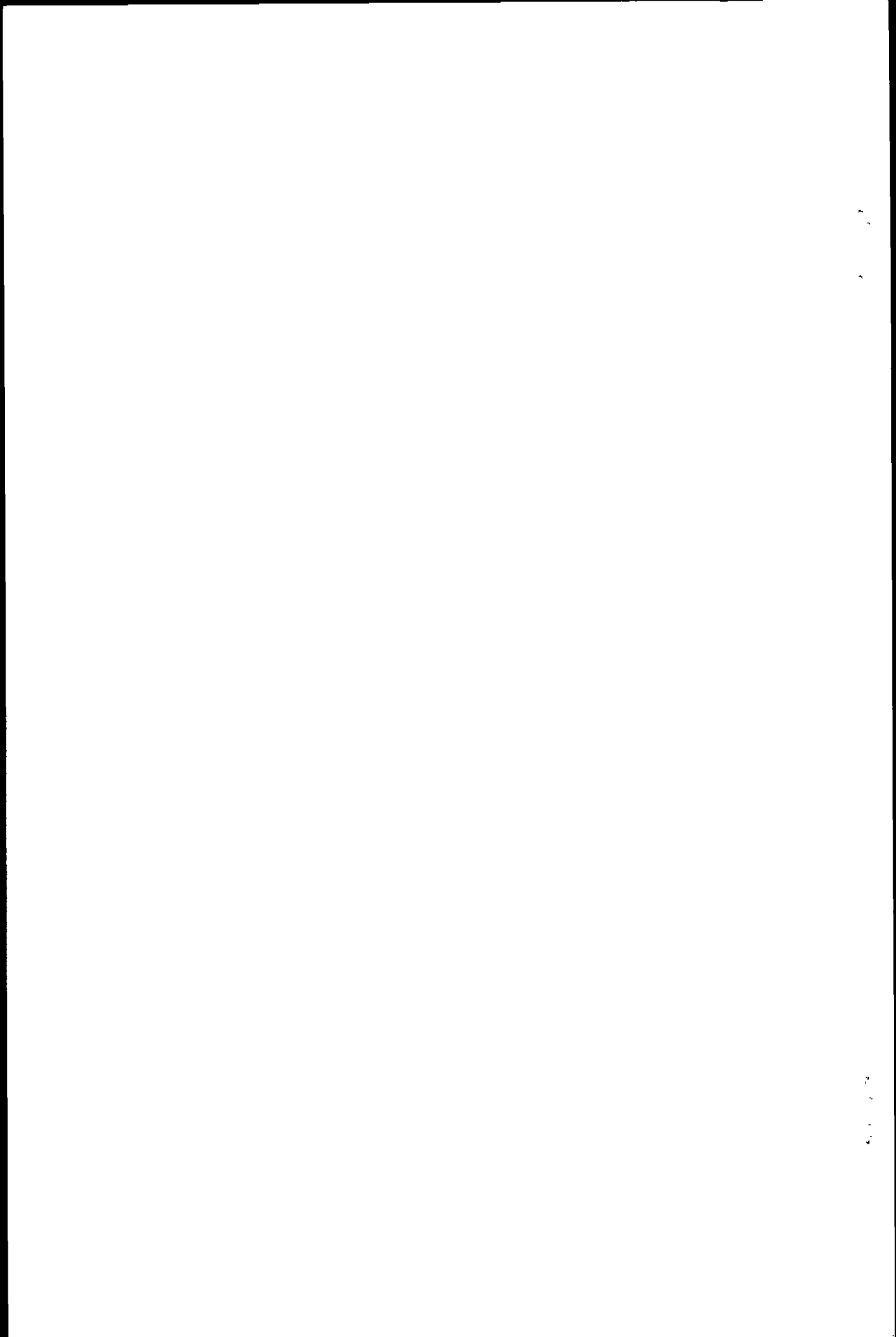
SEXUAL selection is the primary force that molds sexual strategies (sensu Halliday, 1980) of males and females. In species where the operational sex ratio (OSR) is highly skewed, the intensity of and opportunity for sexual selection may increase (Emlen and Oring, 1977). If the numbers of one sex are limited for the other, intrasexual competition can be expected to occur, resulting in high variance in mating success for the limited sex (Darwin, 1871). In a polygynous species, a skewed OSR and male-male competition may lead to monopolization of many females by only a few males (Emlen and Oring, 1977; Trivers, 1972). The result is high variance in male mating success, with some males accruing many matings, and some none (Trivers, 1972).

In amphibians, several conditions may promote strong sexual selection. The absolute sex ratio is often male-biased, due to the earlier age at maturity of males (Berven, 1981; Howard and Kluge, 1985; Husting, 1965). In addition, the OSR usually is biased toward males (e.g., Sexton et al., 1990), because males arrive at breeding sites prior to females (Beneski et al., 1986; Douglas, 1979; Howard and Kluge, 1985; Semlitsch, 1983, 1985; Spotila and Beumer, 1970) and remain longer. Male-male competition for females occurs and mating

success often is size-related; larger males acquire more matings (Berven, 1981; Houck, 1988; Howard, 1979, 1980).

Although most studies of variance in male mating success in amphibians have been conducted with anuran species (e.g., Howard, 1983; Wells, 1977), several have examined salamanders (Halliday, 1976; Houck, 1988; Houck et al., 1985; reviewed by Verrell, 1989). These studies have documented variance in male spermatophore production, courtship success, and mating success. Given the high variance in mating success among males, Houck et al. (1985) showed that the opportunity for sexual selection was much greater in males than in females. Such selection should act to increase reproductive success in males by affecting traits that increase competitive ability in physical encounters (such as body size), or by favoring alternative mating behaviors (Howard, 1981).

In most ambystomatid salamanders, the typical reproductive behavior involves aquatic courtship, spermatophore deposition and transfer, and egg-laying (Duellman and Trueb, 1986). Arnold (1976) noted several factors that produce extreme competition among males in *Ambystoma maculatum* and *A. tigrinum*: a male-biased sex ratio, spatial and temporal clumping of both males and females, and sexual



interference by spermatophore covering. He described the setting for courtship as "a polyandrous frenzy".

The reproductive behavior in the marbled salamander, *Ambystoma opacum*, does not follow the typical ambystomatid pattern. In *A. opacum*, breeding adults have terrestrial courtship, spermatophore deposition, and oviposition (Gibbons and Semlitsch, 1991; Noble and Brady, 1933). Adults migrate towards a dry or partially filled pond basin in autumn, breed and nest on land, and females brood their clutches for several weeks or until the nest is inundated (Jackson et al., 1989; Petranka, 1990; Petranka and Petranka, 1981; Petranka et al., 1982). The OSR usually is highly male-biased due to earlier arrival by males (Graham, 1971; D. Scott, unpublished data). The sex ratio of the entire breeding population often is biased towards males (Graham, 1971; Stenhouse, 1987), possibly because males mature at an earlier age (D. Scott, unpublished data). Male courtship behaviors in *A. opacum* are similar to those exhibited by several other ambystomatids (Arnold, 1972; Noble and Brady, 1933), and variance in male mating success is probably high.

Assuming that variance in mating success is high among males of *A. opacum*, strong sexual selection could promote an alternative male mating strategy. Because courtship and insemination occur on land, a male is not limited to the pond basin in his search for potential mates. We report evidence that a large proportion of a breeding population of female *A. opacum* is inseminated before females reach the wetland.

#### METHODS

A population of *A. opacum* was studied during their annual breeding migration to Ginger's Bay, a 1 ha Carolina bay on the Upper Coastal Plain in Aiken County, South Carolina (for habitat description, see Jackson et al., 1989; Scott, 1990). The Ginger's Bay population has been studied since 1986, when a drift fence was constructed to encircle the breeding site (Gibbons and Semlitsch, 1981). The drift fence/pitfall trap technique has allowed us to capture,

mark, and census virtually all adult *A. opacum* that enter the site, as well as adults and newly metamorphosed juveniles that exit (Pechmann et al., 1989). In June 1991, a new drift fence was constructed well beyond the wetland boundary, at an elevation above the known high-water mark. Known nest sites are most abundant nearer the center of the wetland and are four-fold less frequent at a radius of 40 m (Jackson et al., 1989). The new fence is approximately circular with a mean radius of 64 m (1 SD = 9.9 m).

Marbled salamanders collected for this study were captured by hand within 4 h of sunset on cool, rainy evenings during their breeding migration towards Ginger's Bay. Males were distinguished by swollen vents. Three to five persons searched for females in the area along and immediately adjacent to the drift fence, resulting in frequent passes (~10–15 min) at every point along the fence. Most females were captured before falling into a pitfall trap. A few females escaped hand-capture and fell into traps, but they were removed within 15 min and were not used in this study if a male *A. opacum* was present in the bucket. We saw no spermatophores deposited near the fence or in the traps. Females that were found in the pitfall traps during the morning trap-check were not included in this study.

Females were held individually for 15 days in two environmental chambers under a photoperiod and temperature cycle that approximated environmental conditions. Each female that did not oviposit during this period of solitary confinement was placed with 1–3 males and no other females in an outdoor terrarium for a mate choice experiment unrelated to the current study. At the end of the mating experiment, tissue samples (7-mm tail tips) were taken from the females that had produced clutches in the terraria, as well as the putative sires. Eggs were hatched in pond water, and whole-body homogenates were made of each of 10 hatchlings drawn at random from each clutch. Genotypes were determined at four polymorphic loci (malic enzyme, aspartate aminotransferase, glucosephosphate isomerase, and pepti-

dase) using standard horizontal starch-gel electrophoretic techniques (Selander et al., 1971). Independent mother-offspring genotype data confirmed inheritance of the alleles at each locus (J. Krenz, unpublished data).

Paternity exclusion analysis was performed using the genotype data for hatchlings, males, and females from the terraria. The four-locus genotype of the putative sire, coupled with that of the mother, generated an array of possible genotypes for the hatchling. If the hatchling's genotype did not match any in the array, then the male was excluded from paternity of that hatchling. This process could only disprove, but not demonstrate, paternity.

### RESULTS

During the 1991 breeding migration of *A. opacum*, males began to enter Ginger's Bay on 2 October ( $n = 302$ ). By 14 October, 208 additional males, yet only six females, had entered; the OSR on this date was decidedly male-biased (85:1). We began to hand-collect females ( $n = 36$ ) on 15 October, when 251 males and 120 females were captured at the drift fence en route to Ginger's Bay: the OSR was 6:1 at this time. An additional 34 females were collected during the night of 25 October, and seven more through 1 November, for a total of 77 females that we presumed were uninseminated. The total breeding population at Ginger's Bay in 1991 was 769 males and 175 females. The mean arrival date for females was nine days later than for males. Approximately 64% of the males had entered Ginger's Bay before any females entered. Almost 30% of the breeding females had not yet reached Ginger's Bay when ~99% of the males had entered; 44% of our hand-collected sample of females was collected outside the drift fence after 99% of the males had been put inside.

Of the 77 hand-collected females that we held in environmental chambers, 20 individuals spontaneously laid fertile clutches. Of the remaining 57 females that were used in the subsequent mate choice experiment, 39 did not oviposit and 18 laid fertile clutches. A sample of four of these 18 clutches was used in paternity exclusion

analysis. For each of the four clutches, there were some hatchlings which could not have been sired by any of the putative fathers (i.e., any of the males placed in the terrarium with the female); each of the four females produced some offspring using sperm acquired prior to capture. Because previous inseminations generated at least some of the offspring in each of the four cases examined, it is likely that many of the remaining 14 females also were inseminated before reaching Ginger's Bay. Thus, a minimum of 31% (24 of 77), and perhaps as much as 49%, of the hand-collected females were inseminated before reaching the wetland.

### DISCUSSION

A relatively high proportion of female *Ambystoma opacum* was inseminated before reaching the drift fence. It is unlikely that these females were inseminated when animals were stopped by the drift fence: collections were frequent, no spermatozoa were noted, and 44% of our sample was collected after 99% of the males had entered the pond basin. There are two possible explanations for our observation: females stored sperm from insemination in the previous year or males were courting and inseminating females before females entered the "breeding site".

Sperm storage from a previous year is unlikely for two reasons. First, many of the hand-collected females in this study had no opportunity to breed within the Ginger's Bay drift fence in a previous year. Of the 24 females known to be inseminated before reaching Ginger's Bay in 1991, 15 had known ages and migration histories. One of the 15 females was a 1-yr-old (metamorphosed in the spring of 1991) and could not have been inseminated previously. Nine were migrating to Ginger's Bay for the first time at age 2, and it is unlikely they had bred before at a different pond because *A. opacum* is relatively philopatric to the natal pond (Pechmann et al., 1991). One 3-yr-old was intercepted for the first time, and presumably was on its first breeding migration. Thus, 11 of the 15 known-age animals had not been intercepted at the drift fence during a pre-

vious migration, and presumably had no opportunity to breed prior to our observations in 1991. Second, although a female possesses spermathecae, these cloacal sperm storage structures are probably used to hold sperm only for the brief period between sperm transfer and fertilization (D. Sever, personal communication). In a terrestrial plethodontid salamander, *Eurycea cirrigera*, spermathecal sperm found in post-breeding females were degenerate (Sever, 1991, 1992). Similar observations for *Ambystoma opacum* have also shown sperm degeneration (D. Sever, personal communication), again indicating that sperm are probably not maintained within the spermathecae between years. These results contradict the suggestion made by Houck and Schwenk (1984) that the plethodontid *Desmognathus ochrophaeus* could maintain stored sperm between years. However, long-term sperm storage is known to occur in some salamander species (Boisseau and Joly, 1975; Massey, 1990; Pool and Hoage, 1973), and this mechanism cannot be ruled out entirely as the possible source of our observation.

A more likely explanation is that males are courting and inseminating females before they reach the pond basin. Most species of *Ambystoma* are restricted to aquatic breeding. The evolution of terrestrial breeding in *Ambystoma opacum* apparently provides an opportunity for greater variation in mating behavior compared to congeneric species. The observation that a large proportion of incoming females had been inseminated before arriving at the pond basin suggests that some males may not migrate as far towards the pond as other males do, and may breed earlier, resulting in a more prolonged mating season than in other ambystomatids. Although males near the center of the nesting area may have a higher probability of encountering a female, some matings occur outside the wetland where the density of potential male rivals is greatly reduced. If competition for females occurs, and insemination success is related to body size (Houck, 1988), then a male's breeding strategy may be related to body size. Further research may be directed at whether

variation among males in breeding location is related to phenotype or reproductive success.

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