

REDUCED COURTSHIP SUCCESS BETWEEN PARAPATRIC POPULATIONS OF THE PLETHODONTID SALAMANDER *GYRINOPHILUS PORPHYRITICUS*.—Courtship in plethodontid salamanders is a complex ritual of physical contact, culminating in the “tail-straddle walk,” the precursor to the sperm-transfer stage. During this tail-straddle walk, the male moves forward so that the female’s chin rests on the back of his tail (i.e., the female straddles the male’s tail). If the female does not move away during the tail-straddling walk, the male will stop, deposit a spermatophore, and then walk forward with the female behind him, whereupon the female will lower her cloaca over the spermatophore and pick up the apical sperm cap in her cloaca (Arnold, 1976; Verrell and Arnold, 1989).

In the plethodontid *Desmognathus ochrophaeus*, large males are competitively superior in courtship to small males (Houck, 1988), and large females are mated preferentially over small females (Verrell, 1989a). Verrell (1991) suggested that, even if smaller males court successfully, they may still be at a disadvantage

because they produce fewer spermatophores with fewer sperm than larger males. In addition, recent observations have suggested that, although a small female can elicit spermatophore deposition by a larger male, the distance moved by the male after deposition of the spermatophore may be influenced by his own body size (Arnold et al., 1993). The result is that the female's cloaca may not be positioned above the spermatophore and the sperm cap is not retrieved.

Based on the extensive studies of *D. ochrophaeus* courtship (e.g., Houck et al., 1988; Verrell, 1989a, 1989b), Bernardo (1991) suggested a hypothesis that links speciation in plethodontids with life-history evolution. Differences in adult body size (evolved in allopatry via a change in age at maturation) can result in assortative mating (in terms of courtship and insemination) upon secondary contact of populations diverging in adult body size. This can result in reduced gene flow, thus enhancing divergence between populations.

The spring salamander (*Gyrinophilus porphyriticus*) is ideally suited to test this hypothesis. Populations of this plethodontid in the Piedmont, Cowee Mountains, Nantahala Mountains, and Balsam Mountains of North Carolina exhibit profound variation in age and size at maturation and median adult body size (Bruce, 1972, 1978). In the Cowee Mountains, there are two life-history variants that are parapatrically distributed. Individuals northwest of Watauga (or Cowee) Gap delay maturation and are larger at sexual maturity than individuals southeast of Watauga Gap. This variation in adult size may result in the reproductive isolation of these populations (Bruce, 1978).

Materials and methods.—I collected adult *G. porphyriticus* from four localities from 10 Sept. to 24 Nov. 1992. In the Cowee Mountains southeast of Watauga Gap, I collected animals from three sites: (1) Sargent Spring, a small spring adjacent to the grounds of the Highlands Biological Station, Macon County, NC; (2) Wolf Creek, a series of springs and streams descending from Cullowhee Mountain, Jackson County, NC; and (3) Kinley Mountain, a series of streams descending from Kinley Mountain, Macon County, NC, approximately 7.2 km south on Rt. 106 from Scaly Mountain, NC. These animals will be referred to as SE individuals. I collected animals northwest of Watauga Gap in the Cowee Mountains at one site: Alarka Creek, a system of streams and seeps between Leatherman and Blazed Gaps, 1 km west of Cowee Bald,

Swain County, NC. These animals will be referred to as NW individuals.

Upon collection, animals were placed in individual containers and transported to the Highlands Biological Station. Animals were anesthetized in a 1% solution of MS-222, sexed, and then measured for snout-vent length to the nearest 0.1 mm. Identification of sex was based on one or two characters: females were identified by the presence of large, yolky ova and/or folds in the cloaca, and males by the presence of coiled, black vasa deferentia and/or papillae in the cloaca. Vasa deferentia were observed by shining a high intensity beam of light through the animal's body.

Animals were housed for the duration of the experiment in clear plastic shoe boxes with sphagnum moss that was kept very moist with stream water. The animals were fed salamanders (*D. ochrophaeus* and *Eurycea wilderae*) ad libitum (their preferred food; this species is a salamander specialist; Bruce, 1979). Prey salamanders were not offered unless all previous prey had been consumed. Animals were kept in an environmental chamber at 11 C with a 12:12 L:D cycle.

The pairing design compared courtship success of dissimilar-sized adults taken from different populations with courtship success of dissimilar-sized adults taken from the same population. In addition, courtship success of similar-sized adults taken from the NW population was compared with that of the SE population. Three types of animals were distinguished for these mating trials: mature individuals from the southeast Cowee Mountains (designated SE), mature individuals from the northwest Cowee Mountains that were not larger than the largest SE individual (designated sNW), and mature individuals from the northwest Cowee Mountains that were larger than the largest SE individual (designated lNW). There were no individuals from the southeast Cowee Mountains that were designated as large.

The design consisted of pairing a single male with a single female in an individual chamber [either a clear plastic shoebox (9 × 17 × 31 cm) or a 10-gallon aquarium] lined with very damp paper towels. Each male was exposed to each female only once. Each male was eventually paired with every other female and vice versa. Pairings began on 24 Nov. 1992 and were completed on 3 March 1993, a period that encompasses the courtship season for *G. porphyriticus* (Bruce, 1972). A trial began by placing the male and female together at 2030 h, and was terminated the following morning at 0800 h, when

TABLE 1. RESULTS OF COURTSHIP PAIRINGS OF *Gyrinophilus porphyriticus* TAKEN FROM THE COWEE MOUNTAINS, NORTH CAROLINA. Codes refer to locality and size class of individuals: SE = southeast Cowees; sNW = northwest Cowees that are smaller than the largest SE individual; INW = northwest Cowees that are larger than the largest SE individual. Size range (in mm SVL) is listed below each code.

		Females		
		SE (n = 7) (75.4–86.1)	sNW (n = 1) (74.6)	INW (n = 2) (90.2–113.9)
SE (n = 3) (68.9–85.2)	Total no. of trials	18	3	5
	No. proceeding to male persuasion	11 (61.1%)	3 (100.0%)	4 (80.0%)
	No. proceeding to tail-straddle walk	4 (22.2%)	0 (0.0%)	3 (60.0%)
	No. resulting in insemination	3 (16.7%)	0 (0.0%)	0 (0.0%)
sNW (n = 4) (78.9–83.8)	Total no. of trials	25	4	8
	No. proceeding to male persuasion	1 (4.0%)	0 (0.0%)	2 (25.0%)
	No. proceeding to tail-straddle walk	0 (0.0%)	0 (0.0%)	1 (12.5%)
	No. resulting in insemination	0 (0.0%)	0 (0.0%)	1 (12.5%)
INW (n = 5) (86.7–101.6)	Total no. of trials	34	5	10
	No. proceeding to male persuasion	4 (11.8%)	1 (20.0%)	9 (90.0%)
	No. proceeding to tail-straddle walk	0 (0.0%)	0 (0.0%)	7 (70.0%)
	No. resulting in insemination	0 (0.0%)	0 (0.0%)	5 (50.0%)

the presence/absence of a spermatophore base on the substrate was noted and the female's cloaca was examined for insemination. From 2030–2400, the trials were conducted under red light to allow visual inspection of courtship progress every 15 min. After 2400, the red light was turned off.

The furthest stage of progress was recorded for each pairing as no contact observed between male and female; contact observed between male and female; male initiation of courtship, i.e., when the male continually slides his chin over the head and body of the female; female receptiveness, indicated by the tail-straddle walk and presence of a spermatophore the following morning (tail-straddle walk was always followed by deposition of a spermatophore); and completion of courtship, indicated by the presence of sperm in the cloaca of the female the following morning. Insemination was scored by pressing the female against a glass and noting the absence/presence of a milky white mass in her cloaca. Most of the successful courtships were completed before 2400 h.

Because adult *G. porphyriticus* are difficult to collect in large numbers (and exceptionally difficult to collect in the Cowee Mountains), only 22 individuals were used. This necessitated the repeated use of individuals and so precluded a rigorous pairing design, equal sample sizes, and statistical analysis. However, given the difficulty in observing *G. porphyriticus* courtship (mine are the first published observations), some guarded interpretation is justified.

Results.—The results of the pairings are summarized in Table 1. The discrepancy between number of males and females and number of pairings is due to the loss of two SE individuals (one of each sex; one died and one escaped). The data are separated into three phases of courtship: male initiation of courtship; female receptivity (tail-straddle and spermatophore deposition); and insemination of female.

Male initiation of courtship: Whereas sNW males did not appear to be willing to court (despite the presence of mature gonads), SE males initiated courtship in nearly 70% (18 of 26) of the pairings in which they were involved. Large NW males showed some proclivity to court any females that they were exposed to, although they appeared to be more likely to court a INW female. When courting INW females, they were successful in insemination (50% of pairings), whereas they failed to engage sNW or SE females in tail-straddle walk.

Female receptivity: Females from the INW class were receptive to most males that initiated courtship (in 11 of 15 trials, or 73.3%), and they appeared not to discriminate on the basis of population (SE vs NW) or size. sNW females were rarely courted by males and did not respond to those that did court them. This may be related to the fact that most sNW females did not have large yolky eggs and perhaps were not ready to court and oviposit until a later age and larger body size.

In contrast to INW females, SE females were only receptive to SE males. In no pairings did

SE females engage in tail-straddle walk with NW males. However, they were responsive to SE males, engaging in tail-straddle walk in 36.4% (4 of 11) of pairings where courtship was initiated.

Insemination of female: Large NW females participated in tail-straddle walk with all three classes of males. However, their chances at insemination were highest when engaged in tail-straddle walk with INW males (five of seven, or 71.4% success), and decreased when with sNW and SE males (one of four, or 25% success).

Because sNW females never participated in tail-straddle walk, insemination was not a consideration. SE females were only engaged in tail-straddle walk with SE males, and insemination was usually successful (three of four or 75%).

In summary, 20% (nine of 45) of within-population pairings resulted in insemination, but none of 67 between-population pairings were successful. These data indicate a high level of sexual incompatibility between these parapatric populations.

Discussion.—In *G. porphyriticus*, changes in age at maturation are accompanied by shifts in body size (Bruce, 1972, 1978). These changes in body size appear to result in reduced courtship success between animals from different populations. My data suggest that sexual incompatibility between northwest and southeast Cowee Mountains populations of *G. porphyriticus* is not due to SE males (who were always ready to court). I suggest three nonmutually exclusive mechanisms for the incompatibility. First, SE females are not receptive to courtship by males from NW populations. Despite attempted courtship by both sNW males and INW males, SE females were only receptive to the advances of SE males. This mechanism appears to be behavioral.

Second, NW females cannot be inseminated by SE males because of differences in body size. While INW females were engaged in the tail-straddle walk by SE males, the female does not appear to be able to move forward enough to position her cloaca over the spermatophore. This mechanism appears to be morphological (i.e., based on body size).

Third, males prefer to court larger females. All males showed a higher propensity to court the INW females rather than sNW and SE females. The result of these three mechanisms is that INW females may only be inseminated by INW males and that SE females may only be inseminated by SE males.

The first mechanism is critical to sexual isolation, because, unlike the second mechanism, a SE female can stop whenever her cloaca is

over the spermatophore, even if the male is much larger than she. The second mechanism can only account for reduced courtship success when the male is the smaller individual.

Because the tail-straddle walk is a consistent feature throughout the family Plethodontidae, these three mechanisms could also explain reduced courtship success in any plethodontid for which intraspecific variation in body size exists. Additional work on more abundant plethodontids (e.g., the desmognathines in the southern Appalachians) that are more amenable to rigorous experimental design and statistical analysis is needed to further explore these mechanisms.

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