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COURTSHIP BEHAVIOR OF THE SMALL-MOUTHED SALAMANDER (AMBYSTOMA TEXANUM): THE EFFECTS OF CONSPECIFIC MALES ON MALE MATING TACTICS

by

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(With 2 Figures)
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Summary

1. The courtship behavior of *A. texanum* consisted of a rapid nudging period followed by males producing many spermatophores, some of which were picked up by the female. Neither amplexus or leading by the male were integral components of courtship. Consequently, proposed geographic variation in *A. texanum* courtship remains unsubstantiated.
2. Courtship behavior of *A. texanum* and *A. barbouri* (formerly pond and stream form *A. texanum*, respectively) is very similar; only the location of courtship and perhaps the frequency of sexual interference tactics are different for these two sibling species.
3. *A. texanum* courtship is rapid, males produce large numbers of spermatophores per courtship and invest little courtship time per spermatophore, and intermale competition is extreme.
4. Male *A. texanum* promote their sexual success using sexual interference behavior (*e.g.* covering other spermatophores with their own) and to a lesser degree sexual defense behavior (*e.g.* forcefully nudging rival males).
5. Male sexual success is primarily enhanced directly — *A. texanum* males increase the number of spermatophores produced when at least two other males are courting the same female. The temporal allocation of these additional spermatophores is adaptive only if males are maximizing the number of ejaculates per female or breeding typically occurs in polygamous aggregations.

Introduction

Intrasexual selection results when individuals of the same sex can increase their fitness by competing for more or better mates (DARWIN, 1871).

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Because it is the relative fitness of an individual which is important in natural and sexual selection, increases in individual fitness can occur when the absolute reproductive success of an individual is increased or when a competitor's success is decreased. Males can promote their reproductive success by interfering with rival males (ARNOLD, 1972; VERRELL, 1989), defending receptive females (ARNOLD, 1972; VERRELL, 1989), or by adopting alternative strategies of sexual resource allocation (PARTRIDGE & HALLIDAY, 1984).

Quantification of sexual resources and their allocation in ambystomatid salamanders is simplified compared to many other vertebrates because males and females provide no parental care, typically leaving breeding ponds soon after mating and egg laying (DUELLMAN & TRUEB, 1986). In addition, all *Ambystoma* have internal fertilization but transfer sperm indirectly via spermatophores. Consequently, allocation of the majority of a male's sexual resources is readily quantifiable by counting the number of spermatophores and recording when they are deposited.

The primary objective of this study was to experimentally investigate how numbers of rival males affect sexual resource allocation strategies of the small-mouthed salamander (*Ambystoma texanum*). Salamander sexual interference tactics include placing their own spermatophores on top of rival males producing "multiple spermatophores" and mimicking female behavior so that rival males deposit their spermatophores in locations where females have little chance of locating them (ARNOLD, 1976; HALLIDAY, 1990). Salamander sexual defense tactics include actively defending females by biting and chasing rival males or isolating a female by moving her away from rival males. The sexual resources of male salamanders are limited within a breeding season (ADAMS, 1940; ARNOLD, 1976; HALLIDAY, 1976). Thus, the distribution of male sexual resources during a breeding season is an important determinant of male reproductive success.

The second objective of this study involved resolving a controversy concerning the type of courtship behavior characteristics of *A. texanum*. *A. texanum* reportedly exhibits intraspecific variation in courtship behavior (ARNOLD, 1977) and also alternative mating and life history strategies between different ecological morphs with similar courtship behavior (PETRANKA, 1982a, 1984). Five descriptions of *A. texanum* courtship have been published. WYMAN (1971), studying populations from north-central Illinois, found males performed amplexus and leading behavior and extensively nudged females during courtship. In contrast, GARTON (1972), and LABANICK & DAVIS (1978), studying populations from southern Illinois, found no amplexus or leading. Instead, pairs performed prolonged bouts of mutual nudging during courtship. ARNOLD (1972) and PETRANKA (1982a), studying populations from southern Michigan and cental Kentucky, respectively, observed courtship behavior similar to that observed by GARTON (1972).

Two hypotheses have been proposed concerning why WYMAN (1971) may have seen different courtship behavior: 1) introgression of *A. texanum* and *A. laterale* is known to occur (DOWNS, 1978; BOGART *et al.*, 1987) and the individuals WYMAN (1971) observed courting may have been hybrids of these two species (GARTON, 1972; ARNOLD, 1977; PETRANKA, 1982a); 2) intraspecific geographic variation in breeding habits may occur in *A. texanum* (ARNOLD, 1977).

In this paper I report quantitative descriptions of *A. texanum* courtship using animals collected from southern Iowa, the northwestern limit of *A. texanum's* geographic range (CONANT, 1975). If large scale geographic variation in courtship behavior occurs, *A. texanum* from this part of the range should have amplexus courtship behavior like that observed by WYMAN (1971).

The quantitative descriptions reported here will also enable comparisons of courtship behavior between two ecological morphs of *A. texanum* which differ in morphology, life history, and reproductive biology (PETRANKA, 1982a, 1984; KRAUS & PETRANKA, 1989). PETRANKA (1982b) categorized the population variation into two ecological forms — a stream form and a pond form. More recently, KRAUS & PETRANKA (1989) have argued that these population differences warrant sibling species status for the stream form and named this form *A. barbouri*. The salamanders used in this study are from a pond form (*A. texanum*) population.

Interspecific differences in duration of breeding season have often been invoked as primary selective forces shaping amphibian mating behavior (ARNOLD, 1976; WELLS, 1977; ARAK, 1983; VERRELL, 1989). *A. texanum* is an explosive breeder (BURGER, 1950), with immigration to breeding ponds, courtship and egg laying, and emigration occurring over a relatively short period of time (MCWILLIAMS & BACHMANN, 1988). *A. barbouri* is a nonexplosive breeder (PETRANKA, 1984) and lays larger but fewer eggs than pond form breeders (PETRANKA, 1982b). Because these two species are so recently diverged (KRAUS & PETRANKA, 1989), a comparison of these two sibling species' courtship behavior may offer insights into the rates at which different components of courtship evolve.

Methods

Salamander capture.

All 45 salamanders used in this experiment were captured on 3 or 4 February 1985 while migrating to breeding ponds at Flaming Prairie Preserve, Louisa Co., Iowa. The dates of capture, adult sex ratio, and estimated size of the entire breeding population using these flooded woodland ponds have been published elsewhere (McWilliams & Bachmann, 1988). Briefly, *Ambystoma texanum* is a synchronous migrator with males generally migrating earlier than females. The sex differences in migration make it likely that variable numbers of males and females encounter each other during courtship.

Salamanders were captured using drift fences. Only *A. texanum* salamanders were captured. Since only one other *Ambystoma* species (*A. tigrinum*) occurs in southern Iowa (Christiansen, 1981; Camper, 1988), and since sympatric populations of *A. tigrinum* and *A. texanum* do not occur at my study area, I assume the study animals are not hybrids. When a salamander was captured, its sex and weight were recorded. Then each animal was placed in its own one gallon plastic container lined with moist sand and leaf litter. On 4 February 1985, all animals were placed in an environmental chamber with a 12:12 L:D cycle and a 15°C constant temperature. All trials were completed under these conditions within 20 days after capture. The 45 salamanders used in this study and the resulting 15 clutches of eggs were returned to Flaming Prairie Preserve upon completion of the experiment.

Experimental design.

Individual females were placed in a tank with either one, two, or three males. Treatment (*i.e.*, number of males) order and subjects for each treatment were randomly selected. The resulting completely randomized design included five replicates of each treatment totalling 15 trials. Each animal was used only once so that 30 males and 15 females were used in this experiment.

Two 10 gallon aquaria with gravel substrate and 10-12 cm of dechlorinated water were used for all 15 trials. Gravel was thoroughly scrubbed and rinsed between each trial. Fresh dechlorinated water was used for each trial.

The appropriate number of males and females were then randomly selected and each salamander was weighed. When two or three males were used the tail fin of one or two males, respectively, was clipped so individuals could be identified. A red light source placed 5 m from the aquaria was the only illumination used during a trial. Red light conditions are thought to simulate night-time conditions since salamanders apparently have low sensitivity to red light (Grusser-Cornehls & Himstedt, 1976). Earlier trials revealed that no courtship occurred when fluorescent lights were used.

Males were placed in the aquaria and allowed a 30 minute acclimation period. The female was then introduced and behavior recording initiated. Using a Datamyte 1000 recorder (DataMyte Corp., Minnetonka, Minnesota) specific courtship events and the corresponding time were recorded. Four events were recorded: 1) when the female was added to the aquarium, 2) first contact between a male and the female, 3) first and subsequent spermatophores deposited and picked up by each male and the female, and 4) intermale displacements and chases. Since the Datamyte recorded the time at which each event occurred, courtship behavior could then be quantified using the phase distinctions of Salthe (1967) and Garton (1972) (Fig. 1). In addition, the number of spermatophores observed deposited on top of other spermatophores was recorded. Behavior recording ended when the last spermatophore was deposited. Observations ended 30 minutes after sexual activity had stopped.

Statistical analysis.

Because a single aquarium represented the experimental unit, data taken on individual males within the same aquaria represented repeated measures taken on the same experimental unit. Consequently, a completely randomized design, split-plot analysis of variance (ANOVA) with conservative degrees of freedom (Snedecor & Cochran, 1980) was used to analyze these aspects of the experiment. Otherwise, a standard ANOVA model for a completely randomized design was used (Snedecor & Cochran, 1980). All values used in the ANOVA were tested for normality and homogeneity of variance and found to conform to the assumptions of ANOVA. The Waller & Duncan (1969) test was used for multiple means comparisons when significant main effects were found. Pearson product-moment correlations were used for all tests of association (SAS Institute Inc., 1985).

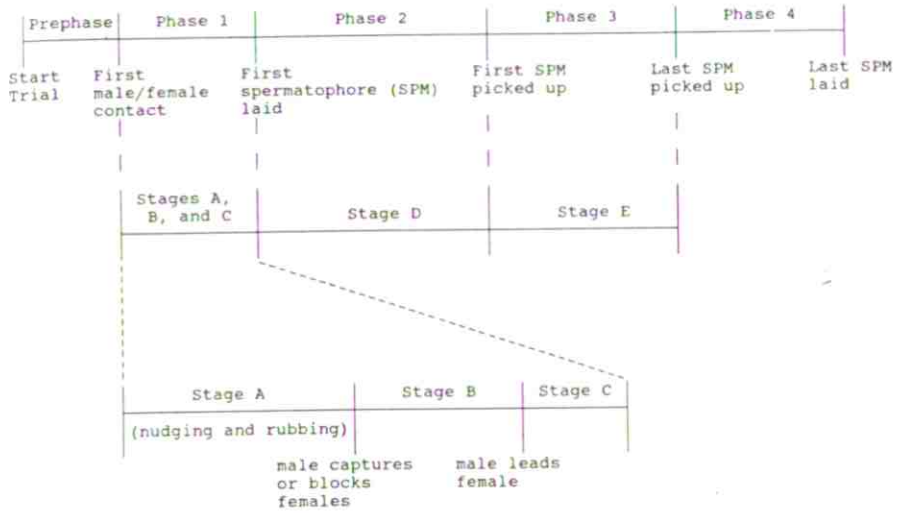

Fig. 1. Salamander courtship and its partitioning into distinct Phases or Stages of courtship. Phases and Stages are as defined by Garton (1972) and Salthe (1967), respectively. Stages D and E are not exactly comparable to Phases 2 and 3 when many spermatophores are laid and picked up during a single courtship.

Results

Three trials were excluded from the analysis because at least one individual exhibited no sexual activity. Additionally, one trial was excluded because sexual activity began after the initial observation period, making counts of spermatophores deposited and picked up incomplete. Consequently, only four replicates for the one and two male treatments, and three replicates for the three male treatment were used in the statistical analysis.

A. texanum courtship behavior.

Since courtship behavior in my study was similar to that described by Arnold (1972), Garton (1972) and Petranka (1982a), no specific descriptions of courtship behavior are provided here. Generally,

A. texanum courtship proceeded as follows: males never deposited spermatophores or exhibited any other sexual activity prior to female introduction. Initially, inter- and intrasexual contacts were nudges primarily directed towards the cloacal and head regions. After initial contact with a female, male(s) began moving quickly around the aquarium, resulting in more contact and nudges with conspecifics. Interactions between males and females often included intertwining and mutual nudging. Males often used a stiff-legged, waddling motion (called vent-shuffling by ARNOLD, 1972) just before and during spermatophore deposition. Amplexus using the forelimbs was never observed. Males led females and oriented them to specific spermatophores twice out of 1550 spermatophores observed deposited.

Males assumed a characteristic posture while depositing spermatophores (described by ARNOLD, 1972, p. 180) and laterally fanned their tail slowly as a spermatophore was deposited. During spermatophore deposition, males grasped their cloaca with both hindlegs (as observed by GARTON (1972) and LABANICK & DAVIS (1978)), but also regularly grasped a piece of gravel on the substrate while depositing a spermatophore (as observed by UZZELL (1969)). Individual males deposited at least five spermatophores before the female began picking up spermatophores (Phase 2, Fig. 2). When picking up spermatophores, females assumed a posture like that of males depositing spermatophores. The number of spermatophores picked up by females varied among individuals (Table 1).

Once females stopped picking up spermatophores they became rigid and unresponsive to male nudging. Females often would gulp air and then remain at the water's surface. Males always continued to deposit spermatophores and nudge conspecifics after the female had stopped picking up spermatophores (Phase 4, Fig. 2).

A few unusual observations of *A. texanum* courtship require further comment. Three males performed "amplexus" using their hind feet to grasp the female. The amplexus posture was similar to that used by males when they grabbed gravel on the substrate and deposited a spermatophore. All three males subsequently deposited spermatophores on the back and head of the female. In each case the female seemed disturbed by the spermatophores and performed somersaults and rolls on the gravel substrate (apparently trying to dislodge the spermatophores).

Generally, males did not deposit spermatophores until after making direct contact with the female. However, in two of the three-male trials, a male which had not yet deposited spermatophores, and which had not yet encountered the female, began depositing spermatophores after encountering a male depositing a spermatophore. Thus, direct contact with a female was not a necessary stimulus for the initiation of male spermatophore deposition.

TABLE 1. Effects of conspecific males on success rates of spermatophores (SPM)

No. of males	Repl.	No. SPM deposited	No. SPM picked up	Spermatophore success rates (%) Overall[1]	Corrected[2]	Corrected max.[3]
1	1	49	24	41	71	71
	2	48	25	52	56	56
	3	44	17	39	59	59
	4	91	41	45	48	48
2	1	51, 59	16	15	18	40, 33
	2	102, 65	18	11	17	41, 29
	3	56, 52	17	16	20	40, 40
	4	23, 32	20	36	36	87, 63
3	1	106, 94, 73	19	7	16	48, 44, 58
	2	118, 127, 128	23	6	11	33, 39, 30
	3	104, 52, 76	67	29	35	78, 100, 100
ANOVA (testing for number of male effects):						
$F_{2,8} = 4.39$				9.45	16.46	0.46
P value = 0.05				0.008	0.002	0.65

[1] (no. SPM picked up/total no. SPM deposited) × 100.
[2] (no. SPM picked up/total no. SPM deposited during Phases 2 and 3) × 100.
[3] (no. SPM picked up/no. SPM deposited per male during Phases 2 and 3) × 100.

TABLE 2. Effects of varying numbers of conspecific males on courtship duration

No. of males	Duration (sec) of courtship periods (mean+SD) Pre-pha e	Phase 1	Phase 2	Phase 3	Phase 4	Total length of courtship
1	315±30	165±72	425±293	1695±1056	1306±815	4000±1084
2	216±246	1073±1191	841±806	1393±1012	2503±2165	5087±1242
3	51±47	943±839	1039±871	2196±1267	2123±957	6321±1663

Phases are defined in Fig. 1.

Effects of conspecific males on the duration of courtship phases. About 70% of the courtship period involved females picking up spermatophores (Phase 3) and males depositing spermatophores after the female had stopped picking up spermatophores (Phase 4, Table 2). Males began depositing spermatophores soon after contact with the female

TABLE 3. Effects of conspecific males on spermatophore investment (total courtship time/no. of spermatophores)

No. of males	Repl.	Overall courtship time per SPM[1])	Individual male courtship time per SPM[2])
1	1	63	63
	2	79	79
	3	61	61
	4	46	46
2	1	43	92, 80
	2	25	65, 42
	3	45	87, 94
	4	79	189, 136
3	1	14	45, 42, 42
	2	36	79, 159, 108
	3	20	51, 58, 74
ANOVA (testing for number of male effects):			
	$F_{2,8}$ = 4.48		1.10
	P value = 0.05		0.38

[1]) Courtship duration (sec)/total no. spermatophores (SPM) deposited.
[2]) Courtship duration (sec)/no. SPM deposited by individual males.

(Table 2, $\bar{x}$ = 734 s, SD = 909). Females began picking up spermatophores on average 752 s after the males began depositing spermatophores (Table 2). Increasing numbers of males did not cause a consistent change in the duration of courtship phases ($F_{2,9}$=0.35, p>0.05, original df = 8,36).

Courtship time and spermatophore deposition.

The total duration of courtship (Table 2) increased significantly as more conspecific males were present ($F_{2,8}$ = 7.35, p = 0.01). More conspecific males also resulted in more spermatophores being deposited per male (Table 1). Consequently, spermatophore investment (Table 3) did not change significantly in response to different numbers of males. Males depositing more spermatophores simply spent more time courting. From a female's perspective, however, more males resulted in a significantly faster rate of spermatophore production (Table 3, overall courtship time per SPM). A new spermatophore was deposited on average every 23.3 s when three males were present as opposed to every 62.3 s with only one male present (Table 3).

Temporal aspects of spermatophore deposition.

In general, males deposited significantly more spermatophores ($\bar{x}$ = 33.5, SD = 8.8) while the female was picking up spermatophores compared to

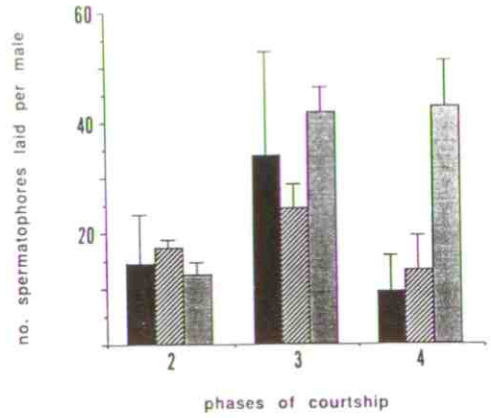

Fig. 2. Effects of conspecific males on temporal patterns of spermatophore laying (mean ± SE). Phases are defined in Fig. 1. black: one male; hatched: two males; spotted: three males.

other times during courtship (Fig. 2, $F_{1,23}$ = 12.4, p < 0.005, original df = 2,46). Significantly fewer spermatophores were deposited during Phase 2 ($\bar{x}$ = 14.8, SD = 2.3) compared to Phase 4 ($\bar{x}$ = 21.9, SD = 18.3). The change in numbers of spermatophores deposited by males during Phase 2, 3, and 4 was not the same across treatments (Fig. 2, $F_{2,23}$ = 5.46, p = 0.01). Males with two conspecific males continued to deposit large numbers of spermatophores after the female had stopped picking up spermatophores (Fig. 2, Phase 4). Numbers of spermatophores deposited by males alone ($\bar{x}$ = 58, SD = 22.1), and with one other male ($\bar{x}$ = 55, SD = 23.6) were significantly less than the number of spermatophores deposited by males with two other males present ($\bar{x}$ = 97.6, SD = 26.2) (Table 1).

Males deposited spermatophores at similar overall rates regardless of the number of conspecifics present (Table 4, $F_{2,8}$ = 0.06, p>0.8). Spermatophore deposition rates were highest before the female started picking up spermatophores, declined slightly while she picked up spermatophores, and then declined significantly once the female stopped picking up spermatophores (Table 4, $F_{1,8}$ = 16.6, p >0.005, original df = 2,16). As more males courted the female, the degree of decline in deposition rate was less (though not significantly so: $F_{2,8}$ = 1.95, p = 0.2, original df = 4,16).

Male weight ($\bar{x}$ = 9.7 g, SD = 2.2) was not significantly correlated with the total number of spermatophores produced during courtship (r = 0.02, p = 0.92) or with spermatophore deposition rate during the interval when