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A STATISTICAL STUDY OF MATE CHOICE: SEXUAL SELECTION IN A PLETHODONTID SALAMANDER (*DESMOGNATHUS OCHROPHAEUS*)

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Summary.—Our experiment revealed the existence of significant variation in mating success in a salamander species in which males do not provide courtship feeding, nest sites, or parental care. Differences in mating success were based on natural variation among adult males and females, rather than on traits of an artificially selected set of potential mates. Courtship encounters deliberately involved only one male and one female, thus eliminating the potentially confounding effects of male-male competition and variations in mate encounter rate. Even after eliminating these effects and random error, some females were more likely than others to elicit spermatophore deposition by a male, and some males were more likely than others to inseminate a female. Such variation among individuals represents an opportunity for sexual selection to act on phenotypic characters that affect mating success.

We advocate the use of a factorial experimental design to analyze sexual selection. This approach permits the statistical evaluation of separate male and female effects, interaction between these effects, and random effects. Designs which combine the evaluations of mating success and courtship behaviors could estimate the force of sexual selection on behavior.

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In recent years field workers have had much success demonstrating sexual selection. One type of demonstration is to measure the variance in male mating success (Howard, 1979; Kluge, 1981; Clutton-Brock et al., 1982; Fincke, 1982). Variance among surviving males in relative numbers of mates bearing progeny can be taken as an index of the opportunity for sexual selection (Crow, 1958; Wade, 1979; Wade and Arnold, 1980; Arnold and Wade, 1984*a*, 1984*b*). This index can be viewed as an opportunity for selection since it gives an upper bound on the amount that any phenotypic trait can be affected by directional sexual selection.

Despite their strengths, field demonstrations of variance in mating success are plagued by a potentially important ambiguity. Does the variance reflect the differences among males in mating ability, or does it reflect random variation arising from the sample of potential mates encountered, the size of that sample, differences in sexual compatibilities of pairs, and other sources?

We conducted a laboratory experiment with a plethodontid salamander, *Desmognathus ochrophaeus*, that separated intrinsic and random sources of variance in mating success. We focused on the statistical effects of mate choice in our experiment rather than on the actual behavioral mode of choice. We asked whether females caused variation in the mating success of males, and vice versa, and left further analysis of the behavioral nature of choice for a future study. The rationale for our focus is that variation in mating success is a necessary condition for sexual selection. The demonstration of significant variation is therefore the first step towards estimating the effects of sexual selection. By giving individuals replicated sequences of mating partners, we were able to demonstrate significant among-male and among-female components of variance in mating success. Our study encourages field studies of sexual selection by showing that intrinsic individual variation in mating success does exist.

Mating behavior has been studied in a great variety of salamander species (Salthe, 1967; Arnold, 1977; Halliday,

1977). Most salamanders transfer sperm by means of a spermatophore that is placed on the ground in front of the female during courtship. Usually, males perform elaborate courtship displays before spermatophore deposition and during sperm transfer. Once the female retrieves sperm from the spermatophore, she stores it for hours, weeks, or even months (depending on the species) until ova are fertilized (internally) just before oviposition (Boisseau and Joly, 1975). The most incisive experimental studies of salamander mate choice have been made by Halliday (1974, 1975, 1976, 1977) and Verrell (1982*a*, 1982*b*) working with aquatic newts. Halliday showed that females usually mate with males having greater spermatophore supplies. Verrell showed that males adjust their courtship to the sexual responsiveness of the female and that males preferentially court larger, more fecund females.

We studied mating success in a plethodontid salamander (*Desmognathus ochrophaeus*) that inhabits the Appalachian highlands of eastern North America. The terrestrial courtship of this species has two phases. During the preliminary phase (spermatophoreplay) the male approaches the female and abrades her with his teeth. (Teeth are sexually dimorphic in this species.) During the sperm transfer phase, the female follows the male in a tail-straddling walk (Fig. 1a) and may retrieve sperm from his spermatophore (Organ, 1961; Arnold and Houck, 1982). Strong circumstantial evidence indicates that the male introduces courtship pheromones from a gland on his chin into the female's circulatory system by abrading her skin with his premaxillary teeth. This courtship pheromone may enhance female response to courtship, although there is no direct experimental evidence which demonstrates that pheromone delivery affects insemination success (Arnold and Houck, 1982).

In plethodontid salamanders, the female governs the course of courtship (Organ and Organ, 1968; Arnold, 1976). Cooperation by the female is an essential prerequisite to spermatophore deposition. The male deposits a spermatophore only if the female follows him in a tail-straddling walk. During sperm transfer the female responds to the spermatophore directly, tactually orienting to it as she slides over it; she ignores spermatophores, however, unless she is actively following the male. Thus the fate of the spermatophore is determined during several seconds immediately after its deposition. During sperm pickup, as the female rests with the spermatophore inserted in her cloaca, the male repeatedly thrusts his tail base upward under her chin (Fig. 1b). This male action may stabilize the female's position over the spermatophore (Houck, 1982) and possibly facilitates the final stage of sperm transfer. In our experiment we considered both spermatophore deposition and actual sperm transfer to be male and female attributes since both sexes are active participants at each stage.

The courtship season of *D. ochrophaeus* usually lasts from at least September to June, although activity is interrupted during cold winter weather. Under laboratory conditions of relatively constant temperature, however, individual males and females are sexually active during the entire interval. Females are inseminated repeatedly throughout the courtship season, and sperm is stored in a specialized organ, the spermatheca, until ova are fertilized just prior to oviposition in June or July. The brood of a particular female may have multiple sires (Tilley and Hausman, 1976; Labanick, 1983), and laboratory experiments using allozyme markers indicate a system of mixed paternity (Houck et al., 1985). Females brood their eggs for about two months and then abandon the larvae shortly after hatching. There is no paternal care of offspring (Forester, 1979). Males and females reach sexual maturity at an age of 3 to 5 years and then commonly survive for at least several more years (Tilley, 1977, 1980). The social system has not been intensively studied in the field, but males are aggressive, par-

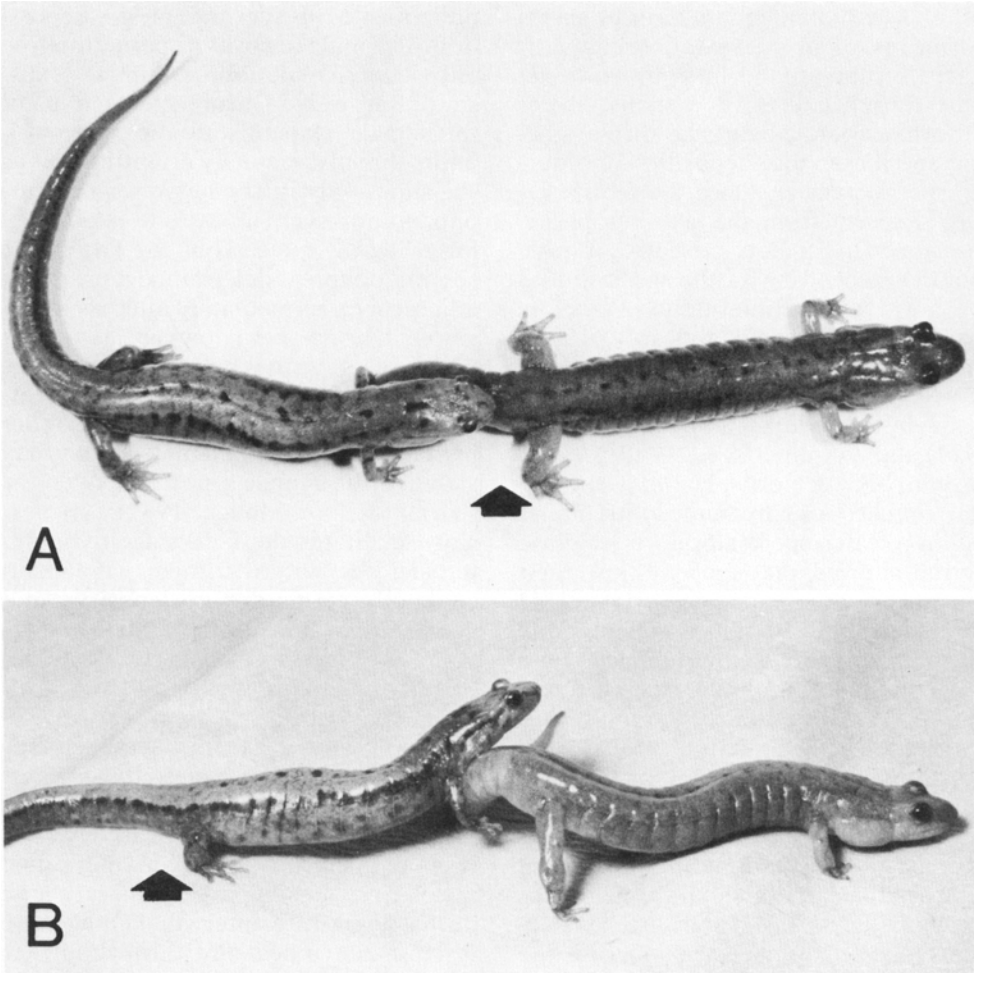

FIG. 1. Two phases of the tail-straddling walk in *D. ochrophaeus*. The arrow shows position of the spermatophore. A. Spermatophore Deposition: the male (right) has led the female forward, and now stops and deposits a spermatophore while the female's chin rests on the base of his tail. B. Sperm Transfer: after spermatophore deposition, the male has moved forward approximately one body length and the sperm mass is inserted in the female's cloaca. The male extends his hind legs and thrusts upward with his tail base under the female's head.

ticularly during the courtship season (Arnold, 1977).

We designed our experiment so that it would have several features which facilitate the analysis of mate choice: (1) Animals were exposed to natural variation in mate morphology and behavior. We sampled sexually mature animals at random from a natural population so that we could determine whether there was natural variation in mating success. (2) Animals had the same opportunity to encounter mates. In field studies, differences in mating success can arise simply from differences in access to mates. Some males may have poorly situated territories, for example, or have low rank in a dominance hierarchy and consequently have poor mating success. We controlled access to mates in our experiment so that we could determine whether attributes of the individual, rather than its social or

physical setting, affected mating success. (3) Each animal encountered a comparable sample of mates. In practice, one could make samples of mates exactly comparable by pairing every male with every female. This complete design would require such a large number of pairings that only a small sample of each sex can be tested in a single breeding season. We opted instead for a fractional design that made samples of mates statistically rather than exactly comparable. (4) Repeated trials were conducted with each animal. Individual difference in mating success was the issue of primary interest so we needed repeated trials to evaluate the magnitude and statistical significance of individual variation. Each animal encountered each mate on two occasions. (5) A single male and a single female were paired at each trial. We were interested in the effects of individual attributes on mating success so we avoided a mating design in which multiple males and females are paired at each trial. Consequently, aggressive interactions between males and communal effects of mass courtship do not confound our results. (6) Actual insemination was used as a measure of mating success. The simple tendency to associate with potential mates is sometimes used as an indicator of mate choice rather than copulation or insemination. Our experiment focused on a late stage of the mating process—the interval between mate encounter and insemination—and this feature enables us to relate our results directly to sexual selection theory.

Materials and Methods

Subjects

We collected *D. ochrophaeus* in August 1980 from a single site in Macon Co., North Carolina (approx. 1,300 m elev.). From a sample of 200 sexually mature adults, 31 males and 31 females were chosen randomly to be used as experimental animals. Two weeks before the experiment began, each animal was briefly anesthetized in a 2% aqueous solution of ether, and its sex and maturity were verified by cloacal examination.

Maintenance

Animals were maintained at a temperature of 15–16°C on a natural (Chicago) photoperiod. Experimental trials were conducted under these same conditions. Animals were individually housed in plastic boxes (9 × 17 × 31 cm) with a damp paper towel substrate and a crumpled, damp paper towel that served as a retreat. Each animal was continuously supplied with active *Drosophila* culture vials so that they could feed ad libitum on larval and adult flies.

Courtship Trials

During courtship trials, a single male was paired with a single female in a clear plastic box (9 × 17 × 31 cm) with a flat, damp paper towel substrate that offered no retreats. Each pair was visually isolated from other pairs. Pairs were placed in the trial boxes 30–60 min before sunset, and the results were scored the next morning, 15–17 hr later. If the female is inseminated, the sperm mass remains clearly visible in her cloaca for approximately 24 hr following insemination. The animals were immediately returned to their own maintenance boxes after scoring.

Despite the simplicity of the test container, females could reject mates. An uninterested female simply remained motionless or ran away, and the male would eventually cease courtship. During the experiment, each animal was paired with a prospective mate on every fourth night. Preliminary experiments showed that both sexes could sustain sexual activity if there were three days of solitude between courtship trials. Males are unable to sustain spermatophore production if they repeatedly encounter females every night or every other night (D. Roulston, pers. comm.).

Scheduling of Courtship Trials

Every male and every female was paired with a prospective mate on each

of 36 nights. These 36 courtship trials were grouped into six blocks, so that a single block consisted of six trials. Within each block, animals were assigned mates using a Youden square design (Cochran and Cox, 1957; Cox, 1958; Kirk, 1968). A particular feature of this design is that, within each block of six trials, every possible combination of two different males had at least one female mate in common (Table 1). Conversely, any two females had at least one male in common in each block. This design feature permitted statistical comparisons between males and females within each block.

In each of the first three blocks, each male and each female encountered six different mates. A particular mate could be encountered more than once in separate blocks. At the end of the three blocks, each animal had encountered as many as 18 different mates. Thus, because of the Youden square design, during the first three blocks each and every pair of males had up to three different mates in common (one common mate in each block) and likewise for females. This feature—that random pairs shared as many as three mates that were encountered in the same sequence (by blocks)—permitted statistical tests for sequence effects. These sequence effects describe trends in average courtship success within a season.

The first three blocks were scheduled in the fall (October 8–December 19), and the last three blocks were scheduled in the spring (March 15–May 22). The same three Youden squares used for mate assignment in the first three blocks were used in the last three blocks, but in reverse order. Thus each animal encountered the same sample of 18 mates in the fall and in the spring. Because blocks were scheduled in reverse order in the spring, we were able to test for statistical interaction between mate sequence and season effects.

The entire experiment consisted of 1,116 mating trials (31 pairs × 36 nights). Errors in pairing were few and had a trivial effect on the overall balance of the experiment. On one night during the first block two pairs were mismatched. Neither pair deposited spermatophores, so a makeup, correction trial was scheduled 16 nights later, and results in that trial were substituted for results in the mismatched trial. One pair of animals escaped during the third block, and another pair died during the interlude between the fall and spring trials. Substitutes were chosen at random from the original field sample, and these were immediately inserted into the mating design without missing any trials.

Scoring of Mating Success

Two components of mating success were scored at each courtship trial: spermatophore deposition and sperm transfer. We determined the number of spermatophores deposited by counting the number of spermatophore bases remaining on the substrate (see Arnold, 1976 for a diagram of a plethodontid spermatophore). For statistical analyses we simply used spermatophore presence or absence to characterize spermatophore deposition, rather than the actual count. Sperm transfer was scored by examining the female's cloaca for the presence of a sperm mass. Presence or absence of cloacal sperm is an unambiguous score because the sperm form a coherent mass. We did not determine whether the female retrieved more than one sperm mass in a night, but other experimental observations suggest that she retrieves no more than one sperm mass in an evening.

Statistical Analyses

Analyses of variance were computed with the general linear models (GLM) procedure of SAS (SAS User's Guide, 1979). In the analyses the identities of males and females were construed as samples from larger populations (random effects), while season and blocks within seasons (sequence) were construed as experimental treatments (fixed effects). Type IV sums of squares are reported in Tables 4–6.

The balance of the experiment was

TABLE 1. Youden squares used to assign mating partners. Each entry in the square gives the identity of the male assigned as a mate to a particular female (columns) on a particular night (rows). Each rectangular block of six nights is called a Youden square.

	Female																												
Night	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	27	30	3	16	10	24	4	19	5	26	23	7	11	28	2	22	12	18	14	6	13	25	1	29	31	15	9	17	8	20	21
2	30	26	22	17	12	16	5	18	29	4	31	20	8	25	27	2	28	7	1	24	19	21	10	14	11	9	3	13	6	15	23
3	17	13	6	10	3	1	18	25	7	19	26	23	5	2	16	24	22	21	15	14	28	27	9	20	4	11	8	12	29	31	30
4	11	8	21	4	18	26	24	14	16	6	3	10	2	20	31	23	7	1	13	30	29	15	19	17	22	28	25	5	27	12	9
5	3	22	20	8	29	11	27	16	30	2	28	13	21	1	9	15	14	17	4	31	24	10	5	26	25	18	7	6	23	19	12
6	19	18	16	28	2	12	20	23	15	7	5	11	14	30	13	17	27	31	3	10	21	26	22	9	29	6	24	25	1	8	4
7	4	9	21	10	15	19	5	28	14	7	17	2	22	25	18	6	8	23	3	31	30	24	11	26	27	12	20	13	29	16	1
8	26	21	22	2	31	6	4	13	16	1	28	24	17	30	8	25	14	11	20	9	3	23	15	19	12	10	27	29	7	5	18
9	16	15	31	27	23	4	14	22	18	13	21	12	9	19	7	26	1	2	25	11	6	10	24	5	3	20	30	17	28	8	29
10	7	10	2	6	27	18	29	11	28	9	23	25	24	14	22	8	17	3	5	12	16	30	20	1	26	19	4	15	31	13	21
11	17	3	20	5	25	13	22	10	9	23	12	4	27	7	15	29	31	19	18	30	1	26	6	28	14	16	8	2	24	21	11
12	11	4	26	7	16	31	23	25	2	20	6	1	19	21	12	9	10	8	17	5	22	18	14	15	13	29	28	30	3	24	27
13	26	4	19	11	24	7	8	2	31	25	21	22	9	15	13	18	29	30	12	14	6	16	27	23	20	17	5	28	10	3	1
14	17	2	5	4	7	28	3	21	20	26	14	16	11	29	25	31	22	6	23	12	13	18	1	24	8	15	30	27	9	10	19
15	24	29	4	15	8	3	13	22	30	23	16	1	17	28	12	5	27	21	31	18	14	19	9	20	6	7	2	10	26	25	11
16	2	13	8	6	22	16	1	25	28	4	26	23	30	14	11	7	12	10	15	17	9	24	31	29	27	21	3	18	5	19	20
17	28	16	21	22	10	9	26	18	13	7	31	5	29	1	24	6	19	12	8	20	23	30	4	3	25	27	14	11	15	17	2
18	6	10	28	3	14	12	18	9	22	30	11	17	8	25	5	29	26	1	2	4	19	15	24	21	16	13	27	23	20	31	7

slightly perturbed by the missing animals, and this complicated the calculations. Since exact computations on the unbalanced data were prohibitively expensive, two alternatives were tried: first, the slightly unbalanced data were treated as balanced data; and second, data were made to appear balanced by treating missing subjects and their replacements as one animal. Approximate computation on the correct data set (Tables 4–6) and correct computation on the approximate data set gave similar results: magnitudes of main and interaction effects differed only slightly. Thus the reported results are taken as adequate approximations to exact computations.

The deletion of animals could have induced experimental interaction in the design. This possibility complicates the interpretation of male × season and female × season interaction effects since some animals were present for only part of one season. Fortunately, the slight imbalance due to missing animals appears not to have affected the interaction terms. Computations using all animals (Tables 4–6) and computations based only on animals present for the entire study gave similar significance levels for interactions.

A separate analysis was performed to evaluate the statistical interaction between male and female effects. The same computational checks described above were used in this analysis.

Variance components were estimated by fitting a linear model for the success rate per trial (Tables 4–6). These variance components were then used to calculate the variance of success per 36 trials (Table 7). Thus the male variance for the average of n trials is approximately $\sigma_\alpha^2 + (m^2/n^2)\ \sigma_\beta^2 + (1/n)\ \sigma^2$, where σ_α^2 is the among-male component of variance, σ_β^2 is the among-female component of variance, m is the average number of different mating partners encountered in n trials (for $n = 36$ trials in our experiment, $m = 11.83$), and σ^2 is the error variance. The male variance for the sum of successes in n trials is $n^2\sigma_\alpha^2 + m^2\sigma_\beta^2 + n\sigma^2$, and the comparable female variance is $n^2\sigma_\beta^2 + m^2\sigma_\alpha^2 + n\sigma^2$.

The ratios of estimated variance components (Table 8) do not have F-distributions, since the numerators and denominators are not independent. In the balanced case, the expected mean squares for males and females for success at a single trial would be $k\sigma_\alpha^2 + \sigma^2$ and $k\sigma_\beta^2 + \sigma^2$, respectively, where k is the number of males or females. In this case, under the null hypothesis of equal gender variance components, the ratio (male mean square/female mean square) has an F distribution with $(k - 1, k - 1)$ degrees of freedom. Due to the loss of two salamander pairs and their subsequent replacement, our experiment was not perfectly balanced. The statistical significance levels for variance component ratios reported in Table 8 are based on the F-statistic described above and are thus approximate rather than exact owing to the small imbalance.

Results

We used three variables to describe mating success. *Spermatophore success* is the deposition of a spermatophore(s), while *transfer success* is the transferal of a sperm mass to the female, given that a spermatophore has been deposited. *Insemination success* is the product of these two variables. Spermatophores were deposited in 727 of 1,116 courtship trials yielding an average spermatophore success of 65%. Of 727 trials with spermatophore depositions, 603 resulted in insemination, yielding an average transfer success of 83% and an average insemination success of 54%.

Distributions of spermatophore and insemination success are shown in Table 2. The female proclivity for multiple insemination can be appreciated from the fact that the average female picked up sperm during 19 different courtships. Success was variable in both sexes: males, for example, ranged from only one to as many as 29 inseminations in 36 trials.

TABLE 2. Distributions of spermatophore, transfer and insemination success for the samples of 29 males and 29 females with complete data for 36 trials.[1]

	Spermatophore success (number of spermatophores)																			Mean ($\bar{x}$)	Variance (I')	Relative variance ($I'/\bar{x}^2$)	Expected binomial variance (E.B.V.)
	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31				
Males		1				1	2	1	3	3	3	1	6	1	3		1	1	2	23.793	15.670	0.028	8.068‡
Females		1		2		3	1	1		4	1	2	2	2	2	5	1	2		23.517	21.901	0.040	8.155‡

	Transfer success (proportion of courtships with sperm transfer)																		
	≥0.05	0.575	0.675	0.70	0.725	0.75	0.775	0.80	0.825	0.85	0.875	0.90	0.925	0.95	0.975	1.0	$\bar{x}$	I'	$I'/\bar{x}^2$	E.B.V.
Males	1		1		2	2	2	3	3	4	3	5	2	1			0.833	0.017	0.025	0.0058†
Females		1	1		3	2	1	5	4	3	6		1	1		1	0.824	0.007	0.010	0.0062†

	Insemination success (number of inseminations)																					$\bar{x}$	I'	$I'/\bar{x}^2$	E.B.V.
	1	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30				
Males	1				1		1	2	4	2	3	2	3		4	2	1			2	1		19.827	29.219	0.074	8.907‡
Females		1				1	4	3	2	1	2	1	3	4	3	1	2	1					19.379	15.887	0.042	8.947‡

[1] Means and variances for spermatophore and insemination success are based on raw counts, but mean and variance for transfer success rate are computed using spermatophore success as a weight.
‡ Variance = $n\bar{p}\bar{q}$, where $\bar{p} = \bar{x}/36$, $\bar{q} = 1 - \bar{p}$, $n = 36$.
† Variance = $\bar{p}\bar{q}/n$, where n = average spermatophore success, $\bar{p} = \bar{x}$, $\bar{q} = 1 - \bar{p}$.

TABLE 3. Sources of relative variance in insemination success in *Desmognathus ochrophaeus* using Arnold and Wade's (1984*a*) analysis of fitness variance. The analysis is based on the sample of 29 males and 29 females with complete data.

Source of variance in insemination success:	Contribution to total opportunity for selection:		
	Symbol	Males	Females
Spermatophore success (number of spermatophores, w_1)	I_1	0.028	0.040
Transfer success (proportion of spermatophores transferred, w_2)	I_2	0.025	0.010
Covariance between spermatophore success (w_1) and transfer success (w_2):			
Unweighted*	$COI(1,2)$	0.019 (0.59)	−0.005 (−0.22)
Weighted by spermatophore success*	$COI(1,2\|1)$	0.014 (0.58)	−0.004 (−0.21)
Change in covariance between insemination success (w_1w_2) and transfer success (w_2) caused by selection at the spermatophore deposition phase:	$COI(12,2\|1) - COI(12,2)$	−0.012	0.001
Insemination success (number of inseminations, w_1w_2)	I	0.074	0.042

* Product moment correlations are shown in parentheses.

Analyses of Variance

The relationships between variances in spermatophore, transfer and insemination success are given in Table 3. The variance in insemination success is composed of variances due to spermatophore and transfer success and a series of covariance terms, using the fact that insemination success is the product of spermatophore and transfer success (Arnold and Wade, 1984*a*, 1984*b*). Thus a sizable fraction of male variance in insemination success is due to a correlation between spermatophore and transfer success ($r = 0.59$, $P < 0.001$). Usually such an unweighted correlation between multiplicative fitness components must be viewed with caution. Zero spermatophore success means no transfer success, and this can contribute to covariance between the two components. In the present case, however, the unweighted covariance is as large as the covariance with terms weighted by the spermatophore success, indicating that the correlation is not a spurious consequence of some males having zero or low spermatophore success. The corresponding female correlation is not statistically significant ($r = -0.22$, $P > 0.05$).

The variances shown in Tables 2 and 3 are based on the subsamples of 29 males and 29 females with complete data so that connections to the raw data can be visualized. The calculations in Tables 4–8 are based on the entire data set.

The variation apparent in Table 2 confounds contributions from three distinct sources. Thus the variance in male insemination success could arise from intrinsic differences among males in success rate, from differences in the sample of mating partners and from random error. Using a binomial model, we can estimate the expected error variance as $n\bar{p}\bar{q}$, where $n = 36$ is the number of trials, $\bar{p}$ is the average success rate and $\bar{q} = 1 - \bar{p}$. This is the variance expected if all males had identical success rates and encountered identical samples of mates. The observed variances exceed binomial expectations by a considerable amount in most cases (Table 2), so random error clearly is not the only source of variance in success. In order to separate the contributions from intrinsic differences and mate

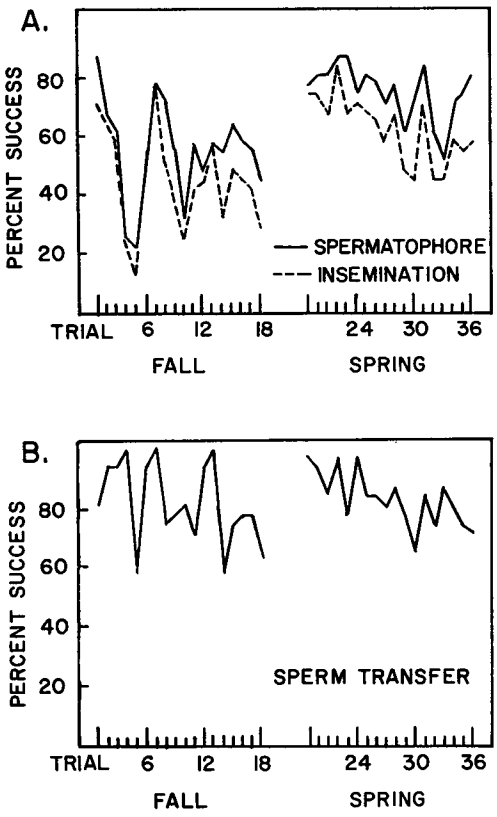

FIG. 2. A. The percent success of spermatophore deposition and of insemination for 31 male-female pairs of *D. ochrophaeus*. B. Sperm transfer success, given that a spermatophore has been deposited. Each trial represents one night of courtship opportunity. See text for further explanation.

samples, we report below the results of fitting linear models to the data. These analyses also test for seasonal and sequence effects. The variances reported in Tables 4–6 are on a per trial basis, in contrast to the 36 trial variances for spermatophore and insemination success reported in Tables 2 and 3.

Spermatophore Success.—Spermatophore success varied significantly among males, among females, and with season (Table 4). Males were no more variable than females (Table 8). The seasonal effect was due to a higher success in the spring (Fig. 2a). The apparent tendency for spermatophore success to decrease within each season was not statistically significant (see sequence main effect and season × sequence interaction in Table 4). Females varied significantly in seasonal effect on spermatophore success, but there was no such demonstrable variation among males.

Transfer Success.—Transfer success varied significantly among males but not among females (Table 5). Transfer success varied within each season (sequence main effect), but there was no seasonal difference in average transfer success nor in the rate of decline (Table 5, Fig. 2b). Neither sex showed significant variation in seasonal effects.

Insemination Success.—Both sexes showed significant variation in insemination success, but males were three times more variable than females (Tables 6 and 8). Insemination success was higher in the spring, but decreased in each season at the same rate (Fig. 2a). The decline in insemination success within each season can be attributed to falling rates of transfer success (sequence effects in Tables 5 and 6). Neither sex showed statistically significant variation in seasonal effects.

Interaction Between the Sexes.—Separate analyses of variance were used to test for a statistical interaction between males and females as well as for the main effects of season, sequence, male and female. These analyses gave no evidence of interaction in any of the three mating success variables.

Components of Variance

The components of male and female variance for 36 trials are displayed in Table 7. Intrinsic variation among individuals, as revealed by the among-male and among-female components of variance, was generally greater than variation arising from random errors in success. In males, error variance was appreciably less than intrinsic variance among males in all three measures of courtship success. By contrast, random error was the leading source of variation in female transfer success. Mating partners generally made

TABLE 4. Analysis of variance in spermatophore success. Note that the total variance for the experiment, 0.23, is the binomial variance for single trials, $n\bar{p}\bar{q}$, where $\bar{p} = 0.651$ is the average spermatophore success, $\bar{q} = 1 - \bar{p}$, and $n = 1$.

Source of variation	Degrees of freedom	Sums of squares*	Mean square	F	P
Season	1	6.46	6.46	33.23	0.0001
Sequence	2	0.30	0.15	0.78	0.4602
Season × sequence	2	0.99	0.50	2.55	0.0787
Male	32	15.10	0.47‡	2.43	0.0001
Female	32	18.17	0.57†	2.92	0.0001
Male × season	29	5.63	0.19	1.00	0.4682
Female × season	29	8.89	0.31	1.58	0.0274
Model	127	61.42	0.48	2.49	0.0001
Error	988	191.97	0.19		
Total	1,115	253.41	0.23		

* Sums of squares given here (SAS Type III) reflect adjustment for other factors in the model. Due to the slight imbalance caused by loss of two salamander pairs, the model SS does not exactly equal the sum of the individual components.
‡ $32.29\ \sigma_{\alpha\alpha}^2 + \sigma^2$; where $\sigma_{\alpha\alpha}^2 = 0.0086$ and $\sigma^2 = 0.1943$.
† $32.26\ \sigma_{\beta}^2 + \sigma^2$; where $\sigma_{\beta}^2 = 0.0116$.

only small contributions to male and female variance, accounting for only 2–15% of the total.

The percentages of total variance represented by the among-male and among-female components of variances (Table 7) are intraclass correlations or repeatabilities of success. Thus the repeatability of male insemination success is 74% while female repeatability is only 42%. These values also estimate the interclass correlation between success in successive blocks of 36 trials. Thus the correlation between male insemination success from one season of 36 trials to the next is 0.74, assuming that males encounter different samples of females and that all other conditions are identical between seasons. In addition, the repeatabilities place an upper bound on heritability (Falconer, 1981). Thus the heritability of spermatophore success in males could not be greater than 56%.

Opportunity for Selection

We found striking sexual differences in variance in mating success, expressed in Table 8 as opportunities for selection. Males are twenty times more variable than females in transfer success and three times more variable in insemination success. Apparent opportunities for selec-

TABLE 5. Analysis of variance in transfer success.

Source of variation	Degrees of freedom	Sums of squares*	Mean square	F	P
Season	1	0.00	0.00	0.00	0.9520
Sequence	2	1.91	0.95	7.70	0.0005
Season × sequence	2	0.08	0.04	0.31	0.7371
Male	32	8.85	0.28‡	2.23	0.0002
Female	32	5.00	0.16†	1.26	0.1571
Male × season	29	5.18	0.18	1.44	0.0648
Female × season	29	3.53	0.12	0.98	0.4963
Model	127	28.60	0.23	1.82	0.0001
Error	599	74.25	0.12		
Total	726	102.85	0.14		

* SAS Type III.
‡ $20.64\ \sigma_{\alpha\alpha}^2 + \sigma^2$; where $\sigma_{\alpha\alpha}^2 = 0.0074$, and $\sigma^2 = 0.1240$.
† $20.63\ \sigma_{\beta}^2 + \sigma^2$; where $\sigma_{\beta}^2 = 0.0016$.

TABLE 6. Analysis of variance in insemination success.

Source of variation	Degrees of freedom	Sums of squares**	Mean square	F	P
Season	1	4.60	4.60	21.63	0.0001
Sequence	2	2.49	1.24	5.84	0.0030
Season × sequence	2	1.15	0.57	2.69	0.0683
Male	32	25.67	0.80*	3.77	0.0001
Female	32	12.67	0.40*	1.86	0.0027
Male × season	29	8.66	0.30	1.40	0.0769
Female × season	29	6.86	0.24	1.11	0.3141
Model	127	66.90	0.53		
Error	988	210.28	0.21		
Total	1,115	277.19	0.25		

* Expected mean squares as in Table 4: $\sigma_{\alpha\gamma}^2 = 0.0183$, $\sigma_\alpha^2 = 0.0057$, $\sigma^2 = 0.2123$.
** SAS Type III.

tion which do not correct for error variance underestimate both of these sexual differences. There is no sexual difference in variation in spermatophore success.

Variation in components of mating success provide modest opportunities for selection (Table 8). Considering the insemination component of sexual selection on males, the most that the mean of a phenotypic trait could be shifted by is one quarter of a standard deviation, $\sqrt{0.063}$. If we had used the total variance, rather than the among-male component, we would have overestimated the maximum selection intensity to be 29% of a standard deviation, $\sqrt{0.085}$.

DISCUSSION

Our experiment revealed variation in mating success both among males and among females. In the sperm transfer phase of courtship, variance among males was greatest: some males were much more successful at inseminating females. Also, some females were more likely than others to elicit spermatophore deposition by their mates. There was no demonstrable variation among females at the sperm transfer phase.

The evolutionary significance of this intrasexual variation in insemination success is that it represents an opportu-

TABLE 7. Sources of male and female variances in spermatophore transfer and insemination success. The total variances reported here are comparable to the variances for subsamples in Table 2 and are synthesized from components of variances reported in Tables 4–6.

	Males					
	Spermatophore success[1]		Transfer success[2]		Insemination success[1]	
Components of variance:						
Among males	11.15	56%	0.0074	67%	23.72	74%
Mating partners	1.62	8%	0.0002	2%	0.80	2%
Error	6.99	35%	0.0034	31%	7.64	24%
Total	19.76	100%	0.0110	100%	32.16	100%
			Females			
Among females	15.03	65%	0.0016	28%	7.39	42%
Mating partners	1.20	5%	0.0008	14%	2.56	15%
Error	6.99	30%	0.0034	59%	7.64	43%
Total	23.23	100%	0.0058	100%	17.59	100%
Means	23.44		0.8333		19.44	

[1] Variances and mean are for the sum of successes in 36 trials.
[2] Variances and mean are for the average success over 36 trials.

nity for sexual selection in each sex (Crow, 1958; Wade and Arnold, 1980). By taking the square roots of the actual selection opportunities reported in Table 8, we can calculate the extent to which a phenotypic character could be affected by variation in insemination success (Arnold and Wade, 1984*a*, 1984*b*). In our experiment, the most that the mean of a male character affecting insemination success could be shifted is 25% of a phenotypic standard deviation. The comparable figure for females is 14%. These are relatively large opportunities for sexual selection, considering that we experimentally controlled other factors likely to increase these opportunities, including seasonal effects, access to mates, and male combat. Comparable measurements from single-season field studies show that the means of male characters can be shifted by as much as half a standard deviation by the directional force of sexual selection (Arnold and Wade, 1984*a*, 1984*b*).

How do the components of mating success variance calculated from our results compare with variances commonly calculated from field data? In a single-season field study, only one estimate of mating success is usually available for each mate, and estimates for all individuals are used to compute the variance in mating success. Some of this variance may be due to true differences in mating success, but some variance also might arise from (1) differences in proceptivity or receptivity of the sets of females encountered by each male; (2) differences in the numbers of potential mates encountered by each male; (3) differences among the females in how they react to particular males and (4) random fluctuations in success. Field data over multiple seasons offer some improvement over single-season data, since one could test for year-to-year consistency in mating success. Nevertheless, the among-male variance component calculated from such data is still likely to confound variations from sources 1, 2, and 3, above. Our results are unique because we were able to separate the part of the variance due to true differences among males from these other four sources as a result of our experimental design.

TABLE 8. Apparent and actual opportunities for selection in males and females, calculated from the variances and means reported in Table 7. Opportunities for selection are equivalent to variances in relative success (mean = 1) and to squared coefficients of variation.

	Spermatophore success	Transfer success	Insemination success
Apparent opportunity for selection[1]:			
Male	0.036	0.016	0.085
Female	0.042	0.008	0.047
Ratio	0.86 n.s.	2.00*	1.81 n.s.
Actual opportunity for selection[2]:			
Male	0.020	0.011	0.063
Female	0.027	0.002	0.020
Ratio	0.74 n.s.	5.50**	3.15**

[1] The total male or female variance divided by the squared mean.
[2] The among-male or among-female component of variance divided by the the squared mean.
n.s. = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; see text.

The present results are complementary to, but not a substitute for, field results. Indeed, two critical kinds of field data are needed to determine whether the estimates of selection opportunity are representative of events in nature. Viewing the estimates as conditional on encounter between potential sexual partners, we still need to know whether sexual behavior in the laboratory mimics events in nature. In our case, the laboratory probably does reflect natural courtship interactions since the sexual behavior of plethodontid salamanders is highly stereotyped and since field observations of courtship agree with laboratory results (Arnold, 1976). Nevertheless, estimates of selection opportunity from field observations would be a valuable supplement. Secondly, since the opportunity for selection will vary with the rate of sexual encounter, we need to know the average rate of courtship in nature. Although field data on this point are needed, we can make a rough approximation of the maximal rate from laboratory results, and this can be used to estimate the minimal selection opportunity that would be realized in nature. From the courtship rate of pairs kept in

residence in the laboratory and from field data on the length of the courtship season, 36 courtships is a reasonable estimate of the average number of courtships per season. This is the courtship rate that was used in the present experiment.

The repeatability of mating success in the field depends upon the number of sexual encounters during the courtship season and the fraction of those encounters that are with different partners. The sensitivity of repeatability to these two factors can be seen by bracketing the probable number of encounters and fraction of unique partners likely to be realized in the field. Repeatability increases with number of encounters and with mating partner fidelity. If the number of different partners equals about one third the total number of encounters, as in our experiment, the expected repeatability of male insemination success would be 60% for an 18 encounter season, 74% for a 36 encounter season, and 84% for a 72 encounter season. If every encounter were with a different partner, the corresponding repeatabilities would be 51%, 61%, and 68%, and if every encounter were with the same partner the figures would be 61%, 76%, and 82%. Of course these are maximum estimates since in the field there are bound to be additional sources of variability that affect the denominator of repeatability. Thus the upper bound for repeatability of male insemination success is in the range of 50–90%.

The variation in insemination success documented in our experiment probably has much different consequences for males than for females. Female mating success and its variance are commonly ignored because the total fitness of a female is not thought to be affected by her mating success. In contrast, the selection gradient for mating success may be much steeper in males since a male's fitness may increase with each successful insemination. We believe that female variance is a useful statistic and, by reporting variances for both sexes, we have chosen not to prejudge such sexual differences in *D. ochrophaeus*. It may be the case that a female can fertilize her entire clutch (average of 20 ova) from a single insemination and that additional inseminations have no impact on her fitness. On the other hand, the female might increment her fitness with each successful insemination. A non-zero selection gradient for female mating success could arise, for example, if long-stored sperm tended to be defective, so that there was a premium on replenishment, or if ejaculates were catabolized and used to nourish the female or her brood. Nourishment from salamander spermatophores has not been documented, but some female insects derive energy and nutrients from spermatophores or ejaculates (Boggs and Gilbert, 1979). In salamanders, as in most organisms, the fitness consequences of multiple insemination is an open field of inquiry. Perhaps the best course is a sexually symmetrical treatment of mating success and its statistics, leaving the selective significance of mating success as a separate issue. This is the route we have taken.

The opportunity for sexual selection was much greater in males than in females. This result has been anticipated by many authors, yet it is surprising how seldom this expectation actually has been tested (Bateman, 1948; Fincke, 1982; Clutton-Brock, 1983). In addition to the sexual differences in overall variance, males and females also showed opportunities for selection at different stages of courtship: females varied significantly only at the spermatophore deposition phase (Fig. 1a), but males varied at the sperm transfer phase (Fig. 1b) as well.

The actual causes of variance in mating success are only partially resolved by our experiment. The variation was not generated by differences in male combat ability, by differences in frequency of mate encounter, or by random differences in sexual success, since these factors were eliminated by experimental design and computations. The sexual difference occurred at the sperm transfer stage. Males execute a complex "leading and braking action" during sperm transfer (Fig. 1b).

It is possible that masculine differences in the technique for leading and stabilizing the female over the spermatophore are responsible for male variation in transfer success. Thus the present experiment identified sperm transfer as a crucial stage for focus in detailed behavioral studies.

Sexual motivation varied with season, and both sexes showed individual variation in seasonal trends. These individual differences in schedules of motivation contributed to sexual selection in *D. ochrophaeus*. This result strengthens Halliday's (1983) suggestion that causal analysis of mate choice should focus on motivation and its neuroendocrine basis.

Our statistical analysis provides a test for variation in female choice. Under the female choice hypothesis, females that concurred in their ranking or preference for males could produce male variance in mating success. If the females differed in their rankings of the males, however, then one expects an interaction between male and female effects (we are grateful to M. Bulmer for this insight). Although this interaction was not statistically significant in our experiment, the power of our test for interaction may be low since, for any same-sex pair, the number of mates in common is small (0 to 3) and since, for any male-female pair, the number of repeat matings also is small (0 to 6). The primary goal of our experiment was to estimate male and female components of variance. An experiment with relatively few pairs and many trials per pair might provide a more powerful test for sexual compatibility. The present results suggest that the contribution from compatibility differences is slight.

In addition to demonstrating an opportunity for sexual selection for a species in which males apparently contribute only genes to their offspring, our study illustrates the use of factorial experimental design to analyze sexual selection. Using this approach to analyze male-female courtship encounters, we can evaluate the importance of separate terms for male effects, females effects, and male-female interactions while controlling for variation associated with other factors such as sequence of mates or season. Similar symmetrical designs are used by quantitative geneticists to analyze pairwise crosses between a series of inbred strains (diallel crosses) and by ecologists and behavioralists to analyze competition or behavioral interactions between strains or species (competitional diallels) (Jinks, 1954; Hayman, 1954; Griffing, 1956; McGilchrist, 1965; Breese and Hill, 1973; Hay, 1974; Norrington-Davies, 1967; Durrant, 1965; McClintock and Adler, 1978). Crossley and McDonald (1979) used individuals from a single strain of *Drosophila* in a diallel design to study courtship behavior. They showed that one or both sexes showed stable variation in most elements of courtship behavior, but generally there were no nonadditive statistical interactions between the sexes. Because their experiment used a complete design, they could use only a few (5) individuals of each sex. In our experiment, we used an incomplete (but balanced) design so that a larger number of individuals ($N = 31$) of each sex could be tested. Also, we scored mating success so that we could assess the opportunity for sexual selection. One could combine these two approaches by scoring details of courtship behavior in addition to scoring mating success. The effects of sexual selection could then be assessed directly on behavioral traits (Arnold, 1983*a*, 1983*b*; Lande and Arnold, 1983).

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Literature Cited

Arnold, S. J. 1976. Sexual behavior, sexual interference and sexual defense in the salamanders

Ambystoma maculatum, Ambystoma tigrinum and *Plethodon jordani*. Zeit. für Tierpsychol. 42: 247–300.

———. 1977. The courtship behavior of North American salamanders with some comments on Old World salamandrids, pp. 141–183. *In* D. H. Taylor and S. I. Guttman (eds.), The Reproductive Biology of Amphibians. Plenum, N.Y.

———. 1983*a*. Morphology, performance and fitness. Amer. Zool. 23:347–361.

———. 1983*b*. Sexual selection: the interface of theory and empiricism, pp. 67–107. *In* P. Bateson (ed.), Mate Choice. Cambridge Univ. Press, Cambridge, U.K.

ARNOLD, S. J., AND L. D. HOUCK. 1982. Courtship pheromones: evolution by natural and sexual selection, pp. 173–211. *In* M. Nitecki (ed.), Biochemical Aspects of Evolutionary Biology. Univ. Chicago Press, Chicago.

ARNOLD, S. J., AND M. J. WADE. 1984*a*. On the measurement of natural and sexual selection: theory. Evolution 38:709–719.

———. 1984*b*. On the measurement of natural and sexual selection: applications. Evolution 38: 720–734.

BATEMAN, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity 2:349–368.

BOGGS, C. L., AND L. E. GILBERT. 1979. Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. Science 206:83–84.

BOISSEAU, C., AND J. JOLY. 1975. Transport and survival of spermatozoa in female Amphibia, pp. 94–104. *In* E. S. E. Hafez and C. G. Thibault (eds.), The Biology of Spermatozoa. S. Karger AG, Basel, Switzerland.

BREESE, E. L., AND J. HILL. 1973. Regression analysis of interactions between competing species. Heredity 31:181–200.

CLUTTON-BROCK, T. H. 1983. Selection in relation to sex, pp. 457–481. *In* D. S. Bendall (ed.), Evolution from Molecules to Men. Cambridge Univ. Press, Cambridge, U.K.

CLUTTON-BROCK, T. H., F. E. GUINNESS, AND S. D. ALBON. 1982. Red Deer: Behavior and Ecology of Two Sexes. Univ. Chicago Press, Chicago.

COCHRAN, W. G., AND G. M. COX. 1957. Experimental Designs. Wiley, N.Y.

COX, D. R. 1958. Planning of Experiments. Wiley, N.Y.

CROSSLEY, S., AND J. MCDONALD. 1979. The stability of *Drosophila melanogaster* courtship across matings. Anim. Behav. 27:1041–1047.

CROW, J. F. 1958. Some possibilities for measuring selection intensities in man. Human Biol. 30:1–13.

DURRANT, A. 1965. Analysis of reciprocal differences in diallel crosses. Heredity 20:573–607.

FALCONER, D. S. 1981. Introduction to Quantitative Genetics, 2nd Ed. Longman, London.

FINCKE, O. M. 1982. Lifetime mating success in a natural population of the damselfly, *Enallagma hageni* (Walsh) (Odonata: Coenagrionidae). Behav. Ecol. Sociobiol. 10:293–302.

FORESTER, D. C. 1979. The adaptiveness of parental care in *Desmognathus ochrophaeus* Cope (Urodela: Plethodontidae). Copeia 1979:332–341.

GRIFFING, B. 1956. A generalized treatment of the use of diallel crosses in quantitative inheritance. Heredity 10:31–50.

HALLIDAY, T. R. 1974. Sexual behaviour of the smooth newt, *Triturus vulgaris* (Urodela: Salamandridae). J. Herpetol. 8:277–292.

———. 1975. An observational and experimental study of sexual behavior in the smooth newt, *Triturus vulgaris* (Amphibia: Salamandridae). Anim. Behav. 23:291–322.

———. 1976. The libidinous newt. An analysis of variations in the sexual behavior of the male smooth newt, *Triturus vulgaris*. Anim. Behav. 24:398–414.

———. 1977. The courtship of European newts: an evolutionary perspective, pp. 185–232. *In* D. H. Taylor and S. I. Guttman (eds.), Reproductive Biology of Amphibians. Plenum, N.Y.

———. 1983. The study of mate choice, pp. 3–32. *In* P. Bateson (ed), Mate Choice. Cambridge Univ. Press, Cambridge, U.K.

HAY, D. A. 1974. A quantitative method for analysing competition where individuals cannot be identified. Aust. J. Biol. Sci. 27:183–196.

HAYMAN, B. I. 1954. The theory and analysis of diallel cross. Genetics 39:789–809.

HOUCK, L. D. 1982. Male tail loss and courtship success in the plethodontid salamander *Desmognathus ochrophaeus*. J. Herpetol. 16:335–340.

HOUCK, L. D., S. G. TILLEY, AND S. J. ARNOLD. 1985. Sperm competition in a plethodontid salamander: preliminary results. J. Herpetol. *In press*.

HOWARD, R. P. 1979. Estimating reproductive success in natural populations. Amer. Natur. 114: 221–231.

JINKS, J. L. 1954. The analysis of continuous variation in a diallel cross of *Nicotiana rustica* varieties. Genetics 39:767–788.

KIRK, R. 1968. Experimental Design: Procedures for the Behavioral Sciences. Brooks/Cole, Belmont, CA.

KLUGE, A. G. 1981. The life history, social organization, and parental behavior of *Hyla rosenbergi* Boulenger, a nest-building gladiator frog. Misc. Publ. Mus. of Zool., Univ. of Michigan No. 160.

LABANICK, G. M. 1983. Inheritance of the red-leg and red-cheek traits in the salamander *Desmognathus ochrophaeus*. Herpetologica 39:114–120.

LANDE, R., AND S. ARNOLD. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

MCCLINTOCK, M., AND N. ADLER. 1978. The role of the female during copulation in the wild and

domestic Norway Rat (*Rattus norvegicus*). Behaviour 67:67–96.

McGILCHRIST, C. A. 1965. Analysis of competition experiments. Biometrics 21:975–985.

NORRINGTON-DAVIES, J. 1967. Application of diallel analysis to experiments in plant competition. Euphytica 16:391–406.

ORGAN, J. A. 1961. Studies of the local distribution, life history, and population dynamics of the salamander genus *Desmognathus* in Virginia. Ecol. Monogr. 31:189–220.

ORGAN, J. A., AND D. J. ORGAN. 1968. Courtship behavior of the red salamander *Pseudotrition ruber*. Copeia 1968:217–223.

SALTHE, S. N. 1967. Courtship patterns and phylogeny of the urodeles. Copeia 1967:100–117.

SAS USER'S GUIDE. 1979. J. P. Helwig and K. A. Council (eds.), SAS Institute Inc. Cary, NC.

TILLEY, S. G. 1977. Studies of life histories and reproduction in North American plethodontid salamanders, pp. 1–41. *In* D. H. Taylor and S. I. Guttman (eds.), The Reproductive Biology of Amphibians. Plenum, N.Y.

———. 1980. Life histories and comparative demography of two salamander populations. Copeia 1980:806–821.

TILLEY, S. G., AND J. HAUSMAN. 1976. Allozymic variation and occurrence of multiple inseminations in populations of the salamander *Desmognathus ochrophaeus*. Copeia 1976:734–741.

VERRELL, P. 1982*a*. The sexual behaviour of the red-spotted newt, *Notophthalmus viridescens* (Amphibia: Urodela: Salamandridae). Anim. Behav. 30:1224–1226.

———. 1982*b*. Male newts prefer large females as mates. Anim. Behav. 30:1254–1255.

WADE, M. J. 1979. Sexual selection and variance in reproductive success. Amer. Natur. 114:742–746.

WADE, M. J., AND S. J. ARNOLD. 1980. The intensity of sexual selection in relation to male sexual behaviour, female choice, and sperm precedence. Anim. Behav. 28:446–461.

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