

Reproductive and Mating Success in the Small-Mouthed Salamander (*Ambystoma texanum*) Estimated via Microsatellite Parentage Analysis

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Abstract Bateman's principles of sexual selection predict that the sex with "cheaper" gametes may maximize reproductive efforts by mating multiply and so display greater positive covariance between reproductive and mating success. We conducted a semi-controlled breeding experiment to genetically quantify sexual selection in adult *Ambystoma texanum*, a sexually monomorphic salamander with simple courtship behaviors. We used four polymorphic microsatellite loci to genotype 57 adults enclosed in a breeding wetland and compared their multilocus profiles to that of 862 embryos collected from the enclosure. The molecular data were used to assign parentage, investigate the mating system, and measure sexual selection intensity. Parentage analyses indicated 36% of dams and 93% of sires were genetically sampled via their gametes but physically unsampled, suggesting that a large number of breeders over-wintered within the enclosure and/or some females released into the enclosure were already inseminated. We used the genetic data to generate estimates of individual reproductive and mating success and we interpret these in light of salamander behavior and sexual selection theory. The incidence of multiple mating in females (86%) was considerably higher than in males (32%) and the standardized variance in mating success was significantly greater in females. The correlations between reproductive and mating success were significant and of similar magnitude between the sexes, indicating that both

sexes increased reproductive success through increased mating success. This pattern may be a function of differential opportunities for mating success between the sexes.

Keywords Bateman gradient · Mating success · Reproductive success · Polygyny

Introduction

Sexual selection can occur when reproductive fitness within a sex is positively correlated with mating success (Bateman 1948). According to Bateman, differences in intensity of sexual selection are associated with differences in reproductive investment between sexes. When investment involves low fitness costs (e.g., sperm production in male *Drosophila*), individual fecundity is increased by mating with multiple partners. Consequently, intra-sexual competition for access to mates occurs, the strength of which is largely dependent on the sex ratio. In contrast, fecundity is not enhanced by multiple mating for the sex with high fitness investment in reproduction (e.g., egg producing females). Bateman's principles therefore predict that the intensity of sexual selection can be measured as the rate of increase of fecundity with mating success and that the sex under the more intense sexual selection regime will compete for access to high fitness mates (Arnold 1994).

Sexual selection intensity can be quantified by the slope of the regression line between fecundity and mating success (Bateman 1948; Arnold and Duvall 1994) as well as by the use of standardized variances associated with reproductive and mating successes (Wade 1979; Arnold and Wade 1984). The use of molecular genetic techniques in parentage analyses has provided a means by which to critically examine Bateman's principles where estimates of

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mating and reproductive success can be directly quantified. Molecular genetic parentage analyses appear to have validated Bateman's principles, demonstrating predictable differences in sexual selection intensity when sex ratios are manipulated (Jones et al. 2004), when usual levels of reproductive investment are reversed among sexes (Jones et al. 2005), and when mating effort of one sex is unitary and terminal (Andrade and Kasumovic 2005). When sexual selection is intense in one sex, the evolution of sexual dimorphisms may occur (Jones et al. 2002; Woolfenden et al. 2002).

Sexual dimorphisms are often pronounced in species with elaborate courtship rituals. In many salamanders, courtship varies from simple to complex among species (Petranka 1998; Houck and Arnold 2003). Often, complex courtship occurs in dense breeding aggregations where sex ratios are skewed towards males, and sexual selection intensities differ between the sexes in accordance with Bateman's expectations (Jones et al. 2002; Gopurenko et al. 2006). However, no study has examined the intensity of sexual selection in aggregate breeding salamanders where there is little courtship and little or no sexual dimorphism. For these species, sexual selection intensities may still be high because of mate competition as opposed to mate choice. Furthermore, salamanders are unusual among vertebrates because male gametes (sperm) are packaged into relatively few spermatophores that are expensive to produce (Harris and Lucas 2002), which might be important in mating system evolution.

In small-mouthed salamanders (*Ambystoma texanum*), adults mate annually at communal breeding sites and employ simple courtship behaviors. Courtship is initiated by limited bouts of mutual nudging, which triggers males to deposit spermatophores. Typically, the number of spermatophores deposited ranges from 20 to 80 per breeding male (Garton 1972; McWilliams 1992; Harris and Lucas 2002). Apart from sperm capping (whereby spermatophores are placed directly on those previously deposited), there is limited evidence of interference (i.e., competition) among males courting females (Licht and Bogart 1990; Petranka 1998). Females often choose from a large spermatophore field where gametes have been deposited by many males. Whether females randomly induct spermatophores or actively choose them based on some assessment is unknown. In accord with their limited courtship activities, there is little sexual dimorphism in *A. texanum* (Williams et al. in review), suggesting that female mate choice may be weak or absent with respect to male morphology. However, if females are choosy, then sexual selection intensity should be greater among males.

We used molecular parentage analyses to genetically quantify and assess sexual selection intensity in a wild *A. texanum* breeding aggregation. Adults migrating to a

wetland breeding area were intercepted and genetically sampled prior to being admitted to an enclosure within the wetland. Egg masses deposited within the enclosure were sampled and their parentage was genetically determined using a suite of highly polymorphic microsatellite loci. By these means we were able to quantify mating and reproductive success among breeders. We used this information to characterize the mating system and to estimate the intensity of sexual selection occurring in each sex.

Methods

Sampling Regime

The experimental enclosure (Fig. 1) was located within an ephemeral wetland (swale) in Tippecanoe County, Indiana, USA (UTM 505948.947/4472511.832). Construction occurred in early February 2003 during the dry season and two months prior to the start of the breeding migration; both the ground and the water were frozen solid to a depth of >30 cm during construction. The enclosure consisted of aluminum flashing (40 cm above and 25 cm below ground) and gauze netting (122 cm above, 25 cm below ground) designed to exclude all breeding adults other than those released but still allowing water flow with the remainder of the wetland. The size of the netting (6 mm²) was chosen to act as a barrier to both adults and eggs; furthermore, 60 cm of smooth plastic sheeting was draped over and taped to the netting to prevent salamanders from climbing over it. Pitfall traps were installed and spaced 10 m apart, both inside and outside of the fence perimeter. Traps were checked twice daily over the duration of the experiment (February

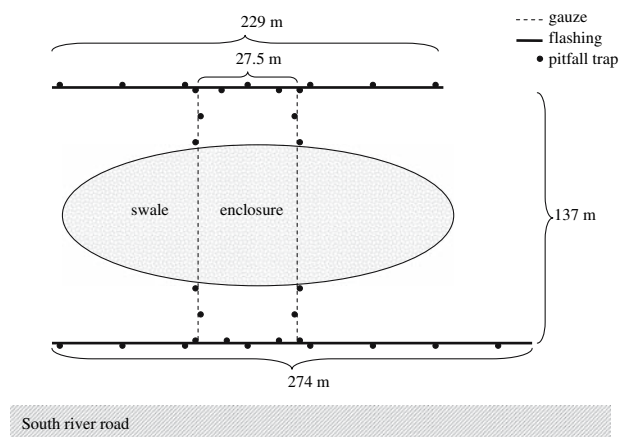


Fig. 1 Aerial representation of the enclosure. This seasonally ephemeral swale lies adjacent to South river road outside of West Lafayette, Indiana USA. Layout not drawn to scale; enclosure flashing, gauze netting and pitfall trap positions and numbers are approximate

15–June 15). Salamanders within the enclosure at the time of construction were sampled via the interior pitfall traps.

Adults were toe clipped to provide individual identification (Twitty 1966) and tissue for genetic analysis, then transferred to separate sex containers. Sex, weight, total length, snout-vent length (SVL), tail height and general condition of individuals were recorded (Williams et al. in review) before adults were introduced to the enclosure. Most salamanders were subsequently recaptured upon emigration roughly 30 days after oviposition ceased. Additional salamanders trapped while trying to immigrate into the enclosure after this introduction were excluded from the enclosure, but tagged for identity and sampled for tissue and morphometric data as described above. All known egg masses within the enclosure were collected for analyses.

Tissue Collection and Genetic Procedures

Adult toe clips were preserved in 1 ml of lysis buffer prior to DNA extraction. In addition to the adults used in the experimental breeding enclosure, we also analyzed toe clippings from salamanders captured at the swale but not included in the breeding experiment. Individual egg masses sampled from the enclosure were collected in separate plastic bags; embryos from each egg mass were dissected and collectively stored in 100% ethanol according to their original egg mass designation. DNA extraction for both tissue types followed a standard proteinase K/phenol-chloroform/isoamyl alcohol procedure. From a library of ten polymorphic microsatellite loci developed for *A. texanum* (Williams and DeWoody 2004), four tetramer loci were end-labeled with fluorescent dye and used for paternity analyses (AT141, AT102, AT65 and AT49). Pilot tests ($n = 22$) indicated these four loci were highly polymorphic (number of alleles per locus >12), were estimated to have low frequency of null allele occurrence (<5%) and were conducive to multiplex genotyping. PCR conditions followed Williams and DeWoody (2004) although specific pairs of loci were co-amplified as duplexes (AT141 and AT102; AT65 and AT49). All PCR annealing temperatures were originally set to 54°C but lowered to 52°C for reruns. Paired duplex PCR's for each sample were run concurrently on an ABI 377 sequencer (Applied Biosystems) and fluorescently labeled alleles were detected and scored for size using associated GENESCAN 3.1.2 and GENOTYP-ER 2.5.2 software. To minimize genotyping errors, each adult was independently rerun (and re-genotyped), and adults with homozygous loci were rerun using relaxed PCR annealing temperatures. All embryos with ambiguous genotypes were rerun, and up to 30% of all embryos were randomly selected and rerun at two or more loci to ensure

that systematic errors in genotyping had not occurred over the course of the analyses.

Parentage Analyses

Parentage analyses employed the program PEDIGREE 2.2 (Herbinger et al. 2006; available at <http://herbinger.biology.dal.ca:5080/Pedigree>) to reconstruct probable parental genotypes directly from the embryo dataset. Reconstructed parental genotypes were compared to the sampled adult genotypes to identify parents. We also compared the PEDIGREE algorithm to our novel method, Iterative Family Network Construction (IFNC) that is conceptually similar to PEDIGREE but uses a fundamentally different computational approach. Detailed descriptions of procedures, assumptions and run conditions used by PEDIGREE and IFNC, as well as results of both methods, are available as Electronic Supplementary Material.

Analyses of Mating Patterns and Sexual Selection

Mating and reproductive success of parents (i.e., number of partners and number of offspring) were quantified genetically and measures of sexual selection based on Bateman's principles (Arnold and Duvall 1994) were used to characterize the general mating system. Means and variances of mating and reproductive success were calculated for each sex. Absolute variances were standardized (variance/mean²) in each sex to determine the opportunity for selection (I_{rs}) and the opportunity for sexual selection (I_{ms}); standardized variance estimates were tested for between sex differences using F -ratio tests. Sexual selection gradients (Bateman curves) for each sex were determined by simple linear regression of reproductive success against mating success. Sex differences in Bateman gradients were tested using two-tailed F -tests as described by Zar (1999).

Results

Sampling Regime

The first rains of April 2003 marked the commencement of salamander migration to the breeding wetland with the majority of captures occurring from the 13th–27th of that month. Eighty *A. texanum* adults (23 ♀ and 57 ♂) were captured by internal and external pitfall traps circumscribing the enclosure. All 23 females and a random subset of 22 males were released into the enclosure at the end of April. Twelve adult *A. texanum* (9 ♀ and 3 ♂) that were not previously sampled were detected inside the enclosure towards the end of the experiment. These individuals were

included in the parentage analyses as potential breeders; in all, the total number of known adults in the enclosure was 57 (32 ♀ and 25 ♂). A total of 65 egg masses containing 962 embryos were collected from the enclosure in May 2003. The number of embryos per egg mass varied from 2 to 79 with a mean of 14.8 embryos/egg mass.

Genotyping

Fifty-seven adults and 862 embryos were successfully genotyped at all four loci. Genotype reruns of all adults were identical to original scores in nearly all (>99%) cases. Similarly, nearly all (~90%) embryo genotypes were consistent across reruns. There was no evidence of linkage disequilibrium among pairs of loci, but departures from Hardy–Weinberg equilibrium were apparent at loci AT141 and AT65 ($P < 0.05$, Table 1). Estimates of null allele frequency were <0.04 at leach locus and thus did not confound parentage analysis (Summers and Amos 1997). Allelic diversity per locus was high, ranging from 15 to 22 alleles per locus, as were exclusion probabilities (Table 1). Each adult had a unique multilocus genotype, and only 49 embryos (out of 371,091 pairwise comparisons) shared multilocus genotypes.

Parentage Analyses

Patterns of mendelian segregation suggested two egg masses consisted of full siblings, 20 consisted of half siblings and 34 contained embryos which were related as siblings to some but not all other embryos in the mass. This latter category probably consisted of admixed egg masses, limiting the use of known cohorts in downstream parentage analyses (Gopurenko et al. 2006). Parentage assignments using both PEDIGREE and the IFNC approach were remarkably concordant with one another. In total, 730 embryos were confidently allocated to crosses between 14 dams and 28 sires (see Electronic Supplementary Material).

Nine of the parental genotypes reconstructed from embryo genotypes were directly identified as captured females but only three of the 28 sires could be similarly assigned. The remaining reconstructed parent genotypes were not observed among the sampled adults (i.e., those physically captured). Two maternal families each contained full sibling progeny and 12 maternal families consisted of half sibling cohorts (see Appendix). In contrast, the majority of paternal families consisted of full sibling rather than half sibling progeny (25 vs. 10 families).

Estimates of Mating and Reproductive Success

Estimates of mating and reproductive success were predicated upon the genetic parentage analyses and include adults that successfully produced offspring as well as adults in the enclosure that did not contribute gametes to the offspring pool. Therefore, standardized variances in reproductive and mating success presented here may be overestimated if sampling was incomplete (Arnold and Duval 1994).

The frequency of multiple mating among females (86%) was greater than in males (32%). In addition, there was a tendency for females to mate with more partners than males (Fig. 2). The frequency distribution of mating success was highly skewed to single partners for males, whereas females tended to mate with 2–3 partners (Fig. 2). Mean mating success for females (1.03) was roughly 1.4 times that of males (0.75) whereas the variance among females (2.42) was almost four times greater than in males (0.63). Standardized variance of mating success (I_{ms}) among females was twice that of males (Table 2) and this difference was significant ($P < 0.05$).

The greatest reproductive success attributable to a single female was 126 embryos versus 107 for a single male. Mean female reproductive success was 19.7 and 14.3 for males. Overall, the distribution of reproductive success differed between the sexes (Fig. 3). Variance in reproductive

Table 1 Number of alleles (N_a), observed (HO) and expected (HE) heterozygosity, null allele frequency (Null), exclusion probability with one parent ($P_{Ex 1}$) and with two parents ($P_{Ex 2}$) and polymorphic information content (PIC) at four microsatellite loci among 57 adult *Ambystoma texanum*

Locus	N_a	HO	HE	Null	$P_{Ex 1}$	$P_{Ex 2}$	PIC
AT141*	22 (17)	0.870	0.934	+0.030	0.738	0.849	0.921
AT102	16 (16)	0.923	0.915	−0.011	0.683	0.812	0.899
AT65*	20 (19)	0.870	0.927	+0.028	0.716	0.835	0.912
AT49	15 (14)	0.833	0.906	+0.039	0.660	0.795	0.890
Mean	18.3 (16.5)	–	0.921	–	–	–	0.905
Total	–	–	–	–	0.992	0.999	–

Parentheses indicate numbers of alleles seen among embryos

* Significant deviation from HWE ($P < 0.05$) due to heterozygote deficiency

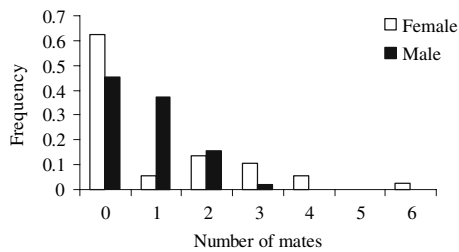


Fig. 2 Distribution of mating success. Total frequency of adults of each sex mated with 1–6 mates

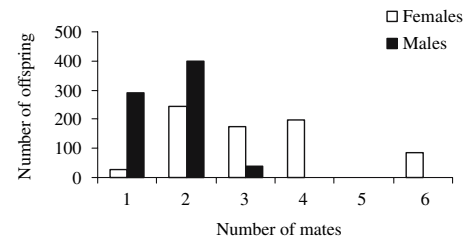


Fig. 3 Distribution of reproductive success across parents grouped by mate success. Total number of offspring produced by each sex mated with 1–6 mates

success was 2.4 times greater in females (1216.7) than males (507.8); however standardized variance of reproductive success (I_{rs}) was only marginally greater in females (Table 2) and this difference was not significant ($P > 0.05$).

Analysis of Bateman's gradients (Table 2, Fig. 4) indicated that relative reproductive success (RS) was positively correlated to mating success (MS) for both females ($RS = 18.342 MS + 0.892$; adjusted $r^2 = 0.668$; $P < 0.0001$) and males ($RS = 21.053 MS - 1.373$; adjusted $r^2 = 0.544$; $P < 0.0001$). The slopes of the two regression lines did not significantly differ ($F = 0.554$; $df (1, 84)$; $P = 0.459$) and 95% confidence intervals of the regression coefficients for each sex (♀: 0.07–0.80; ♂: 0.38–0.80) overlapped.

Discussion

Sampling Regime

Our parentage results were surprising in part because of an apparently large number of unsampled breeders. Alternatively, most adults that we introduced into the enclosure did not appear to breed. The integrity of the enclosure was never in doubt and at no time did water levels exceed the height of the enclosure fencing. We find it implausible that adults or their gametes could overcome the fencing, but there are several alternative explanations. First, the introduced adults may not have bred because of our manipulations, because they were unable to find a mate, or because they never intended to breed. If the latter, perhaps

they were captured not while migrating to the breeding pond but while simply foraging. Second, we may not have exhaustively sampled embryos from the breeding pond. This is quite possible, but the pond was shallow (<0.5 m) and was searched in its entirety multiple times. By inference, the mean clutch size among females we sampled was 52, compared to 500–700 ova generally observed in this species (Petranka 1998). This disparity may reflect incomplete sampling, incomplete egg deposition by females (other *Ambystoma* salamanders are known to partition their clutches; Gopurenko et al. 2006), egg/embryo mortality, or some combination thereof. Our field observations indicated many individual eggs failed to differentiate because they were unfertilized or succumbed to infections. Third, rampant genotyping errors could have caused problems with the parentage analyses, but each sampled adult (and a subset of embryos) was genotyped twice and these results indicate that our error rates are very low. Finally, the literature suggests adults emigrate from the breeding pond shortly after mating and immigrate again the following spring. However, if we introduced 57 adults into an enclosure that already contained scores of salamanders, then it would not be surprising that we physically sampled so few breeders.

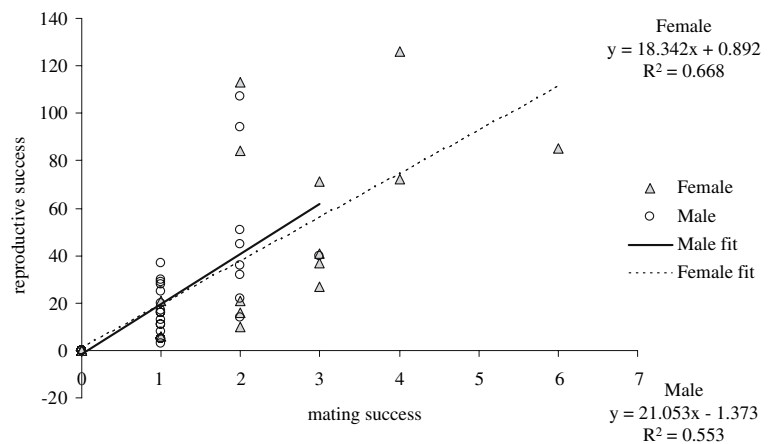
We think the latter explanation, that adults over-wintered within the site prior to enclosure construction, is most likely. Adult *Ambystomid* salamanders generally emigrate following the reproductive season and over-winter >50 m from the breeding pond (Semlitsch 1998; Regosin et al. 2005), but adult *A. texanum* may be an

Table 2 Standardized variance measures based on Bateman's principles

Sex	Ave_{rs}	σ_{rs}^2	I_{rs}	Ave_{ms}	σ_{ms}^2	I_{ms}	$\beta_{ss} (s)$
♀	19.7	1216.7	3.12	1.027	2.416	2.29	18.34 (20.38)
♂	14.3	507.8	2.48	0.745	0.634	1.14	21.05 (15.22)

Opportunity for selection (I_{rs}) is the variance in reproductive success (σ_{rs}^2) divided by the square of mean reproductive success (Ave_{rs}); opportunity for sexual selection (I_{ms}) is mating success variance (σ_{ms}^2) divided by the square of mean mating success (Ave_{ms}). The Bateman gradient (β_{ss}) and associated residual standard deviation (s) were estimated as the least-squares regression of reproductive success on mating success

Fig. 4 Bateman's gradients for female (dashed line) and male (solid line) adults. Gradient equals the regression slope of reproductive success (number of offspring) fitted to mating success (number of matings)



exception. Post-mating migrations for *A. texanum* range from 0 m to 125 m and many adults are subsequently captured <50 m from the breeding sites (Williams 1973). Furthermore, males and females may not be distributed uniformly: upon prebreeding migrations, *A. maculatum* and *A. laterale* males are captured closer to the breeding pond than are females (Regosin et al. 2005). This pattern should lead to male-biased sex ratios early in the breeding season, and the sex ratios of the reconstructed (uncaptured) parents in our study were greatly skewed towards males (5 ♂: 1 ♀). The most parsimonious explanation for the observed skew is that more males over-wintered at our study site, as might be expected if males mate annually but females do not. An alternative explanation is that females utilized sperm stored from previous matings (Tennessen and Zamudio 2003).

Sexual Selection Intensity and *A. texanum* Mating System

Despite unsampled adults, parentage reconstruction provided a means to assign parentage to 85% of the offspring and allowed calculation of reproductive success, mating success, and their associated variances. The genetic data indicated both sexes engaged in multiple mating, and that it was almost three times more common among females than males. This threefold difference provides some evidence that females have greater access to male gametes than the reverse situation. Male *A. texanum* usually deposit a few dozen spermatophores per courtship, but annual sperm supplies are limited and thus there is a tradeoff with regard to future matings (McWilliams 1992), especially if the operational sex ratio is male-biased (Harris and Lucas 2002). In other words, a male's mating success should be proportional to his sperm allocation relative to the total number of spermatophores within the breeding arena—which likely contains several hundred spermatophores

(McWilliams 1992; Harris and Lucas 2002). Under this scenario, multiple mating by males should be infrequent if females randomly induct spermatophores. This may explain the greater incidence of multiple mating by females, with the effect accentuated as the operational sex ratio becomes more male biased. We explore this scenario in a comparative framework relative to Bateman's principles.

The opportunity for sexual selection arises when variance in both mating and reproductive success is high and the covariance between the two is positive and strong (Arnold and Duvall 1994). For salamanders and newts, standardized variances of both statistics are consistently greater in males than in females (Table 3) and this is especially apparent in male-biased populations (Jones et al. 2004). Furthermore, pronounced sexual dimorphisms associated with variance in mating success have been identified in some species (Janzen and Brodie 1989; Howard et al. 1997). Where investigated (Jones et al. 2002, 2004), male reproductive success was correlated with variation in particular morphological attributes, indicating that such characters were clearly under sexual selection. For those species listed in Table 3, the male bias towards greater standardized variances and covariance in both I_{rs} and I_{ms} is testimony to the hypothesis that variance in mating success is strongly related to overall fitness of males and this is manifested as a steeper slope of the Bateman gradient in males relative to females.

In contrast to other salamanders, standardized variance in both mating and reproductive success for *A. texanum* was greater in females than males. Furthermore, significant Bateman gradients in both sexes suggest that reproductive success was enhanced by increased mating success. Curves for each sex were not significantly different from one another, indicating the intensity of sexual selection was roughly equal among the sexes. Given that male mate competition is an inevitable consequence of male biased operational sex ratios within salamander breeding aggregations, it could be expected that the potential for sexual

Table 3 Comparison of standardized variance of reproductive (I_{rs}) and mating success (I_{ms}) as well as Bateman's gradient (β_{ss}) for newts and salamanders. Sex ratio (M:F) as indicated

Species	(M:F)	I_{rs}		I_{ms}		β_{ss}		References
		♀	♂	♀	♂	♀	♂	
<i>Taricha granulosa</i>	(1:1)	0.30	1.02	0.30	0.67	21.7	114.1	Jones et al. (2004)
<i>Taricha granulosa</i>	(2.7:1)	0.27	3.33	0.36	2.75	-18.7	136.8	Jones et al. (2004)
<i>Taricha granulosa</i>	(2:1) ^b	0.23	4.48	0.44	3.06	22.2	74.6	Jones et al. (2002)
<i>Ambystoma tigrinum</i> ^a	N.a	0.38	0.68	0.28	0.37	N.a	N.a	Gopurenko et al. (2006)
<i>Ambystoma texanum</i>	(1.4:1)	3.12	2.48	2.29	1.14	18.3	21.1	This study

N.a: data not applicable to this analysis

^a Analyses do not include adults with zero reproductive success

^b Information not reported in reference but inferred from available data

selection would also be greater in male *A. texanum*. However, our finding that Bateman's gradient was significant for both sexes suggests that variance in mating success was potentially related to variance in fitness in both sexes. That is, both males and females had increased reproductive success from increased mating success and the effect was comparable between the sexes. As noted by Sutherland (1985), in order to infer sexual selection, a character associated with high mating success must be identified. Sexual dimorphism in *A. texanum* is apparent only as a trend for greater female SVL (Williams et al. in review) and this is most likely attributed to fecundity selection in females. Larger females produce more ova than smaller females in the plethodontid salamander *Desmognathus ochrophaeus* (Verrell 1989a), in many Ambystomatid salamanders (Petranka 1998), in anurans (Howard 1988) and in sand lizards *Lacerta agilis* (Verrell 1989b, 1995; Olsson 1993). Thus, males can maximize their reproductive success by choosing larger females and by mating with multiple females (Olsson 1993). The reverse logic can also be applied here; if female *A. texanum* induct spermatophores based on some qualitative fitness assessment of male phenotype, male I_{ms} would have been much higher. Therefore, it appears that neither sex has obvious fitness related morphological traits associated with mating success—despite the apparent opportunities for sexual selection.

In theory, the sex with the greatest reproductive investment (presumably, females) has the least opportunity for sexual selection. Exceptions to this theory have rarely been documented, although in dark eyed Juncos (Aves) both sexes have roughly equivalent correlations between reproductive and mating success (Ketterson et al. 1998). Potential explanations for this case include indirect maternal fitness increases associated with female promiscuity (Parker and Tang-Martinez 2005), but these explanations do not apply to *A. texanum* as adults do not acquire

any obvious benefit from courtship and there is a complete absence of parental care (Verrell 1989a; Trauth et al. 1990). An alternative explanation is that detection of multiple mating may be a sampling artifact associated with reproductive output; i.e., chances of detecting multiple mating by females increases as their reproductive output increases. Thus, the relationship between reproductive and mating success could be spurious (Fig. 4; Parker and Tang-Martinez 2005). Myers and Zamudio (2004) clearly demonstrated the problem of inferring multiple paternities when female clutch sizes are low, and when sperm from contributing males is unequally sampled. Unfortunately, there is no effective way to distinguish this artifactual effect from a true pattern of covariance between mating and reproductive success.

As mentioned, the consideration of nonbreeding females in our analyses may have inflated estimates of reproductive and mating success. The exclusion of nonbreeding females from the analyses resulted in a change at both I_{rs} and I_{ms} (as indicated by greater standardized variance estimates for males at both measures). Regardless, the Bateman gradient for breeding females in this reduced dataset (not shown) is similar to our original findings, i.e., the slope of the female gradient is significantly positive (RS = 15.906 MS + 8.961; adjusted $r^2 = 0.287$; $P < 0.05$) and not significantly different in slope from the male gradient ($F = 0.835$; df (1, 61); $P = 0.365$). Therefore, a positive female Bateman curve exists whether or not nonbreeding females are considered in our analyses.

This positive female gradient may be an artifact of stochastic differences between sexes in opportunity for mating success. In *A. texanum*, this would occur if females randomly induct spermatophores from the dozens generally deposited by courting males (Harris and Lucas 2002). For females, this random mating behavior may act as a genetic bet hedging strategy to increase genetic variation in offspring (Slatkin 1974; Stockley et al. 1993; Waser and

DeWoody 2006). Variance in mating success among females should be considerable in this scenario, limited mostly by the mean number of spermatophores inducted (females average 29 during single courtship events; McWilliams 1992). The limiting factor for male mating success under this scenario would be the allocation of finite spermatophores (Harris and Lucas 2002). One causal factor promoting differences in mating success is likely a male-biased skew in operational sex ratio within mating aggregations. Therefore, a negative relationship between allocations of limited sperm reserves relative to density of competing males would exist as a form of mate competition among males (Parker et al. 1996). Male mating success would be lower than in females and negatively related to the level of skew in male sex ratio.

It is commonly assumed that sperm production by males is “cheap”; however, annual sperm production is limited in salamanders (Verrell et al. 1986) and the cost of sperm may not be so cheap. If spermatophores are expensive, selection should favor behaviors whereby males judiciously allocate their gametes. Some evidence of this

phenomenon exists in *A. texanum*; Harris and Lucas (2002) found males staggered allocation of their limited supply of spermatophores according to both the density of males in the breeding arena and to the reserve of sperm held by the males. The genetic data presented here supports this idea by indicating that male mating success is more likely driven by a density function of mate competition rather than by female mate choice. The distribution of this phenomena among aggregate breeding salamander species is unknown, but our general prediction could be tested in other species where mate choice and mate competition are expected to be relaxed—typically, those which have simple courtship behaviors and a general absence of sexual dimorphism.

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Appendix

Matrix of inferred parental crosses and number of embryos designated to each cross (parent—offspring array)

Males ↓	Females →										F:A	?F:B	F:C	?F:D	F:E	F:F	F:G	?F:H	?F:I	(N)
	1697	1701	1710	1713	1714	1716	1719	1748	1752											
1683															5					5
1687									29											29
2465				5																5
M: A								30												30
M: B									5											5
M: C				24	21															45
M: D	11																			11
M: E	3																			3
M: F	13											19								32
M: G									95			12								107
M: H									18			20		2						40
?M: I											23							11		34
M: J										14					22					36
M: K		64													30					94
M: L												4		18						22
M: M										28										28
M: N																	48			51
M: O																	37			37
M: P																	25			25
M: Q								6		8										14
M: R		20																		20

continued

Males↓	Females →																	(N)	
	1697	1701	1710	1713	1714	1716	1719	1748	1752	F:A	?F:B	F:C	?F:D	F:E	F:F	F:G	?F:H		?F:I
?M: S																	4		4
M: T													17						17
M: U				8															8
M: V												13							13
M: W															16				16
?M: X																		8	8
?M: Y																		5	5
M: Z														16					16
M: AA				5															5
M: BB				11															11
?M: CC													9						9
?M: DD													6						6
?M: EE													4						4
M: FF								5											5
(N)	27	84	16	37	21	6	41	10	113	71	23	85	19	72	21	126	15	13	(800)

Parent types deduced from partitions of embryo dataset independently attained using PEDIGREE and IFNC. Parents identified among the adults tagged in field experiment have individual identity code # (e.g., “1697”); adults not identified among field caught adults given prefix “F” or “M” for females and males—followed by individual identity code letter (e.g., “F: A”). Parents at which sex is arbitrarily assigned are highlighted in bold and prefixed by “?”

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