

Molecular Parentage Analysis in Experimental Newt Populations: The Response of Mating System Measures to Variation in the Operational Sex Ratio

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Submitted November 26, 2003; Accepted June 8, 2004;
Electronically published August 19, 2004

ABSTRACT: Molecular studies of parentage have been extremely influential in the study of sexual selection in the last decade, but a consensus statistical method for the characterization of genetic mating systems has not yet emerged. Here we study the utility of alternative mating system measures by experimentally altering the intensity of sexual selection in laboratory-based breeding populations of the rough-skinned newt. Our experiment involved skewed sex ratio (high sexual selection) and even sex ratio (low sexual selection) treatments, and we assessed the mating system by assigning parentage with microsatellite markers. Our results show that mating system measures based on Bateman's principles accurately reflect the intensity of sexual selection. One key component of this way of quantifying mating systems is the Bateman gradient, which is currently underutilized in the study of genetic mating systems. We also compare inferences based on Bateman's principles with those obtained using two other mating system measures that have been advocated recently (Morisita's index and the index of resource monopolization), and our results produce no justification for the use of these alternative measures. Overall, our results show that Bateman's principles provide the best available method for the statistical characterization of mating systems in nature.

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Keywords: Bateman gradient, index of resource monopolization, microsatellites, Morisita index, parentage, sexual selection.

Despite its origins in the nineteenth century (Darwin 1859, 1871), the theory of sexual selection has been an active area of research in evolutionary biology for only a few decades (Andersson 1994). Consequently, it should not be a surprise that considerable disagreement still exists with respect to some fundamental topics in sexual selection. One such topic is the quantification of genetic mating systems in the context of sexual selection (Payne 1979; Wade 1979; Wade and Arnold 1980; Arnold and Duvall 1994; Shuster and Wade 2003). In fact, recently the waters of mating system quantification have become noticeably muddier. Several new measures of mating systems have been proposed in the last decade (Clutton-Brock and Parker 1992; Kvarnemo and Ahnesjö 1996; Kokko et al. 1999; Fairbairn and Wilby 2001), but many of these have poorly defined connections to selection theory. Traditional measures of the mating system, such as the opportunity for sexual selection (Wade 1979; Wade and Arnold 1980), have been heavily criticized (Clutton-Brock 1983; Sutherland 1985a, 1985b; Grafen 1987), and some authors have suggested they be replaced by new measures, such as indices of resource monopolization (Kokko et al. 1999; Fairbairn and Wilby 2001) or measures based on the potential reproductive rates of the sexes (Clutton-Brock and Vincent 1991; Clutton-Brock and Parker 1992; Kvarnemo and Ahnesjö 1996). Our view is that none of these measures (including the opportunity for sexual selection) has been tested rigorously enough in empirical studies for any strong conclusions to be drawn with respect to how mating systems should be quantified. We need more empirical studies that test how these measures of the mating system respond to changes in the actual intensity of sexual selection.

In this study, we test empirically the validity of mating

system measures, with a particular emphasis on those derived from Bateman's principles (Bateman 1948), by manipulating the expected intensity of sexual selection in experimental populations. Bateman (1948) pointed out that the variance in mating success (i.e., number of mates), the variance in reproductive success (i.e., number of offspring), and the relationship between mating success and reproductive success are all closely tied to the process of sexual selection. Such a perspective makes intuitive sense. Because sexual selection by definition results mainly from competition for access to mates (Darwin 1871; Andersson 1994), fitness in the context of sexual selection arises from success in mating. For this success in mating to translate into evolutionary change, there must be a positive relationship between mating success and reproductive success so that success in mating can result in an increase in fitness (Arnold and Duvall 1994). Thus, we can easily see that variance in mating success, variance in reproductive success, and a positive relationship between mating success and reproductive success are all necessary for sexual selection to operate. This view admittedly does not fully accommodate sperm competition, cryptic female choice, and variance in mate quality, factors that also can affect the intensity of sexual selection (Andersson 1994). Nevertheless, it serves as a useful first approximation.

One major advantage of the view of sexual selection based on Bateman's principles is that these ideas have been formalized into quantitative measures of mating systems with strong ties to selection theory. The opportunity for selection (I) and the opportunity for sexual selection (I_s) are the variances in reproductive success and mating success, respectively, divided by the squares of their means (Wade 1979; Wade and Arnold 1980; Shuster and Wade 2003). These measures are called opportunities because they represent upper bounds on the magnitude of directional and other kinds of selection (Crow 1958; Arnold 1986). The relationship between mating success and reproductive success can be characterized by a least squares regression of reproductive success on mating success (Arnold and Duvall 1994). The slope of this regression is called the sexual selection gradient or Bateman gradient (Andersson and Iwasa 1996). It reflects the extent to which success in mating translates into success in progeny production (Arnold and Duvall 1994). Clearly, a steeper Bateman gradient indicates a higher potential for sexual selection. Disillusionment with approaches to the mating system based on the opportunity for sexual selection began in the 1980s, many years before the introduction of the Bateman gradient as a formal concept. It now seems clear that knowledge of the Bateman gradient is necessary for proper interpretation of the implications of variance in mating and reproductive success. Thus, a new approach to the quantification of mating systems, which simulta-

neously considers all three of these measures of the mating system, is emerging (Jones et al. 2000, 2002*b*; Lorch 2002; Woolfenden et al. 2002). However, very few studies have measured all three of these mating system descriptors, so we are in dire need of additional empirical research along these lines.

One way to test the utility of measures of the mating system based on Bateman's principles is to manipulate the expected intensity of sexual selection in experimental populations and see whether these mating system descriptors respond as predicted. No studies have yet taken this potentially informative approach, so our goal was to provide the first such rigorous test of Bateman's principles. The most straightforward method to manipulate the intensity of sexual selection is to alter the operational sex ratio, which is widely regarded as a primary ecological factor affecting the strength of sexual selection (Emlen and Oring 1977; Kvarnemo and Ahnesjö 1996). Shuster and Wade (2003) suggest that mean crowding is a more important factor affecting the intensity of sexual selection, but in experimental breeding populations, which usually involve a small spatial scale, changes in the operational sex ratio result in proportional changes in mean crowding. In a population with a surplus of males ready to mate, we expect strong sexual selection on males, whereas in a population with an excess of receptive females, we expect sexual selection to act more strongly on females. If Bateman's principles provide useful techniques by which to characterize genetic mating systems with respect to sexual selection, then they should respond in a predictable, quantitative fashion to changes in the sex ratio.

The rough-skinned newt *Taricha granulosa* provides an excellent model system in which to test Bateman's principles. Sexual selection appears to act most strongly in males of this sexually dimorphic species (Janzen and Brodie 1989; Jones et al. 2002*b*), and males possess several secondary sexual traits that are absent in females, such as a large tail crest, nuptial pads, and greater physical size. In Oregon, newts migrate to ponds to breed in the winter. Males arrive earlier at the breeding ponds than females, which typically migrate in large numbers during rainy nights in late January and February (Pimentel 1960). Most of the females collected during these migrations are unmated and are suitable for use in breeding studies (Jones et al. 2002*a*). Females appear to mate relatively soon after entering the ponds, frequently with multiple males (Jones et al. 2002*a*, 2002*b*), and after the mating period they become unreceptive (Propper 1991). Mating starts with a prolonged period of amplexus, during which the male holds himself against the female's dorsal surface, periodically rubs his chin on her nares (Houck and Arnold 2003), and transports her away from rivals (Arnold 1977). After several hours of amplexus, the male releases the female

and deposits a spermatophore on the substrate in front of her. She may opt to pick up a sperm mass from the spermatophore. The male then clasps the female for a prolonged period of postmating amplexus (Davis and Twitty 1964; Propper 1991). After insemination, females lay eggs singly in vegetation over the course of several weeks (Twitty 1966). Finally, microsatellite markers have been cloned and characterized for this species, allowing for the rapid assessment of parentage in newt breeding aggregations (Jones et al. 2001). This combination of available techniques makes the rough-skinned newt a very useful model for the study of mating systems and sexual selection.

Our overarching hypothesis is that measures of the mating system based on Bateman's principles will respond to changes in the sex ratio in experimental breeding aggregations. Thus, in this article, we address three major questions. First, do quantitative mating system measures respond as predicted to changes in the operational sex ratio? Second, does direct measurement of the intensity of sexual selection on particular sexually dimorphic traits support the idea that Bateman's principles accurately capture the essence of the sexual selection process in this system? And third, do other proposed measures of the mating system perform as well as the measures based on Bateman's principles in capturing the intensity of sexual selection?

Methods

The Experimental Breeding Assemblages

We collected female newts on February 6 and 9, 1999, by walking on rainy nights along the Midge Cramer Path near the Benton County Fairgrounds (44°33'56"N, 123°19'17"W) in Corvallis, Oregon. Migrating females were collected by hand as they crossed the path and were transported to the lab. Males were collected by partially submerging plastic minnow traps in flooded areas along the path. At the time of collection, the males were already in breeding condition, with well-developed tail crests, nuptial pads, smooth skin, and a bloated appearance. Newts were individually marked with passive integrated transponder tags and held in single-sex tanks until the start of the experiment. We set up the breeding assemblages in 12 cylindrical cattle tanks (~1.7 m in diameter), each of which was filled with approximately 1,000 L of water. Throughout the experiment, water flowed as a continuous trickle into each tank, and standpipe drains kept the water level about 25 cm below the rim of each tank. Lids were not necessary, because rough-skinned newts are not accomplished climbers. The tanks were housed inside a building at Oregon State University's Oak Creek Laboratory of Biology. The building's temperature was not controlled, and the light regime approximated natural con-

ditions. The water temperature inside the tanks ranged from 9.0°C to 13.5°C. Once the cattle tanks were completely filled with water, we introduced eight males to each tank. On February 22, we added the females to the tanks. Throughout the experiment, newts were fed wax worms (*Galleria mellonella* larvae) at a rate of three per week per newt.

We were interested in the effects of the operational sex ratio and the distribution of male quality on the mating system, so we used a balanced two-factor experimental design. Our two sex ratio treatments were even, with eight males and eight females per tank, and skewed, with eight males and three females per tank. For the male quality treatments, we used male body mass as an indication of quality, because previous work has shown that positive sexual selection appears to be acting on male body size in natural populations (Jones et al. 2002b). Our two male quality treatments used either six large males with two small males (6L : 2S) or two large males with six small males (2L : 6S). The mean mass of males in our source population was 21.7 ± 3.7 g (mean \pm SD). For the tank experiment, our large males ranged in mass from 22.2 g to 31.4 g (mean 25.0 ± 1.9), whereas our small males ranged in mass from 12.6 g to 20.7 g (mean 17.6 ± 1.9). All males were sexually mature, as evidenced by well-developed secondary sexual traits, including a tail crest, nuptial pads, and cloacal swelling. This assessment of sexual maturity was corroborated by our results; we found that the two smallest males in the experiment successfully fertilized eggs. Each pairwise combination of treatments was replicated three times, resulting in a total of 12 tanks.

Mating activity commenced immediately on the introduction of females, and we allowed the newts to mate freely until the males were removed on May 6, 1999. Despite the introduction of suitable egg-laying substrate (including sticks, aquatic plants, and strips of artificial turf) into the tanks, negligible levels of egg laying occurred. Hence, on May 19, 1999, each female was isolated in a water-filled plastic shoebox equipped with strips of artificial turf as an egg-laying substrate. To induce egg laying, we gave each female an injection into the body cavity of 10 μ L of a 0.5 mg/mL solution of a luteinizing hormone-releasing hormone analog, des-Gly¹⁰-[D-His(Bzl)⁶]LH-RH ethylamide. Eggs were removed with forceps from the artificial turf, counted, and placed in a single layer under-water in mother-specific shoeboxes. The developing embryos were monitored daily for the presence of fungus, and affected eggs were quickly removed. Excluding those females that laid apparently unfertilized eggs ($N = 4$), only about 12% of eggs succumbed to fungus. Either hatched larvae or well-developed embryos were stored at -80°C for microsatellite genotyping. At the conclusion of the experiment, we also clipped (and stored at -80°C)

small tissue samples from the tails of the adult males and females for DNA analysis.

Molecular Parentage Analysis

All adult newts were genotyped using six *Taricha granulosa* tetranucleotide loci (*Tgr01*, *Tgr02*, *Tgr04*, *Tgr06*, *Tgr10*, and *Tgr14*). The primer sequences, DNA extraction protocols, and polymerase chain reaction conditions are described elsewhere (Jones et al. 2001, 2002a, 2002b). We also genotyped on average 23.5 embryos or larvae from each female that laid fertilized eggs (a total of 1,362 embryos from 58 females). Initially, all embryos were genotyped at three loci (usually *Tgr06*, *Tgr10*, and *Tgr14*). The other three loci were used to resolve issues of ambiguous paternity.

Paternity assignment was performed by complete exclusion. Because females were isolated from one another while laying eggs, we knew the maternity of each offspring a priori. Each tank contained eight males, so it was a simple matter to assign offspring to one of the eight males by excluding the other seven. We used the computer program CERVUS (Marshall et al. 1998) to verify our exclusions.

Quantitative Analysis of the Mating System and Sexual Selection

From the results of the molecular parentage analysis, we were able to estimate the number of genetic mates (mating success) and genetic offspring (reproductive success) for each adult in the sample. From these data, the quantitative measures of the mating system based on Bateman's principles can easily be calculated. We calculated the opportunity for selection (I) and the opportunity for sexual selection (I_s ; see first section of this article; Wade 1979). We estimated the Bateman gradient (β_{ss}) as the least squares regression of reproductive success on mating success. We also calculated for comparison the index of resource monopolization (Ruzzante et al. 1996) and the Morisita index (Morisita 1962), which have been suggested as alternative measures of the potential for sexual selection (Kokko et al. 1999; Fairbairn and Wilby 2001). The index of resource monopolization is defined as

$$Q = \frac{(s^2 - \bar{x})}{(n\bar{x}^2 - \bar{x})}, \quad (1)$$

and the Morisita index is

$$I_s = n \left[\frac{\sum_{i=1}^n x_i^2 - n\bar{x}}{(n\bar{x})^2 - n\bar{x}} \right], \quad (2)$$

where n is the number of individuals, x_i is the value of the resource (e.g., mating success or reproductive success) for individual i , \bar{x} is the mean value across the n individuals, and s^2 is the variance.

We estimated the intensity of sexual selection on specific phenotypic traits by using the techniques outlined by Lande and Arnold (1983). We measured adult snout-vent lengths (SVL), tail lengths (TL), tail heights (TH), and body masses. Tail height is measured as the distance from the ventral to the dorsal edge of the tail at its deepest point. Males were measured about halfway through the experiment on April 1–3, and females were measured at the end of the experiment on June 11 and 19. To permit comparisons across studies, we focus on SVL, TL, and TH, the three traits that were analyzed in the previous study of sexual selection in a natural population of *T. granulosa* (Jones et al. 2002b). All traits were log transformed and standardized to have a mean of 0 and a variance of 1. Mating success and reproductive success were divided by their mean to produce measures of relative fitness (transformed means = 1). We performed two selection analyses, one with mating success as the measure of fitness and a second with reproductive success as the measure of fitness. The interpretations of these distinct analyses are slightly different with respect to sexual selection. We estimated standardized selection differentials as the covariance between each trait and relative fitness (Lande and Arnold 1983). Standardized selection gradients, which correct for selection on correlated traits, were estimated from multiple linear regressions of relative fitness on the traits.

All quantitative measures of the mating system and the intensity of sexual selection were calculated in two different ways. First, they were calculated within each tank and averaged across replicate tanks within each of the four treatments (i.e., even 6L : 2S, even 2L : 6S, skewed 6L : 2S, and skewed 2L : 6S). Second, they were calculated by pooling observations across tanks within a treatment. These different approaches to the calculation of measures of sexual selection resulted in small quantitative changes but did not affect the major conclusions of the study. In the tables, we present the analysis of the pooled observations for selection differentials and gradients, because we never observed a significant effect of tank as a factor in the multiple regression analysis ($.16 < P < .89$). The lack of a significant tank effect suggests that pooling across tanks is appropriate. For opportunities of sexual selection and the measures of inequality, we present the values that were calculated for each individual tank and then averaged within a treatment. Values calculated by pooling across tanks within a treatment did not deviate substantially from the values that we present in the tables.

Results

Assessment of Mating Patterns

We used a total of 66 females in this experiment. Three of the females laid no eggs, and one female laid only four eggs, so these females were excluded from further analysis. Of the remaining 62 females, four laid eggs that showed no signs of development and quickly succumbed to fungus, indicating that they probably had not been fertilized. These females were retained for all analyses as females that failed to obtain mates and consequently had zero fitness. One female's eggs were fertilized entirely by males that were not in our tanks, indicating that she had carried stored sperm into the experiment. We never observed her in amplexus and hence removed her from the analysis as well. One female used previously stored sperm as well as sperm from a male with whom she mated during the experiment, so she was retained in the analysis with only that fraction of her progeny fertilized by our experimental male contributing to his fitness. Thus, a total of 61 of the 66 females were used in all of our analyses. Fifty-seven of these 61 females (93%) mated successfully, whereas four (7%) did not. All males ($N = 96$), a large fraction of whom fathered no offspring, were included in the analysis.

The parentage assessment was very successful. We unambiguously assigned parentage for 1,356 of the 1,362 offspring (99.6%). We were not able to exclude all males for six offspring (from two females), but in all cases, these progeny were compatible with a male that fathered other offspring in their family. Thus, we assigned them to the same father as the other progeny from the clutch. Our data set also contained some mutations ($N = 12$), which typically resulted in an allele that did not appear in the other progeny from the same family. For those offspring suspected of containing a paternal mutation, we assayed additional loci until all exclusions were verified by at least two loci. Hence, these few mutations were not a major difficulty in this analysis. A high-frequency null allele also segregates at one of the loci (*Tgr04*), so we considered exclusions valid for this locus only if they involved heterozygous genotypes.

Distributions of male and female mating success are shown in figure 1. The major differences in these distributions among tanks were between the even and skewed sex ratio treatments, so we separated these treatments to compile these histograms. The most striking results are that many more males than females failed to mate and that the number of males with no mating success increased dramatically in the skewed sex ratio treatment. In the even sex ratio treatment, the male mating success histogram is statistically different from the female histogram but only barely so (contingency χ^2 test, $df = 3$, $P = .027$). However, male and female histograms are very different from

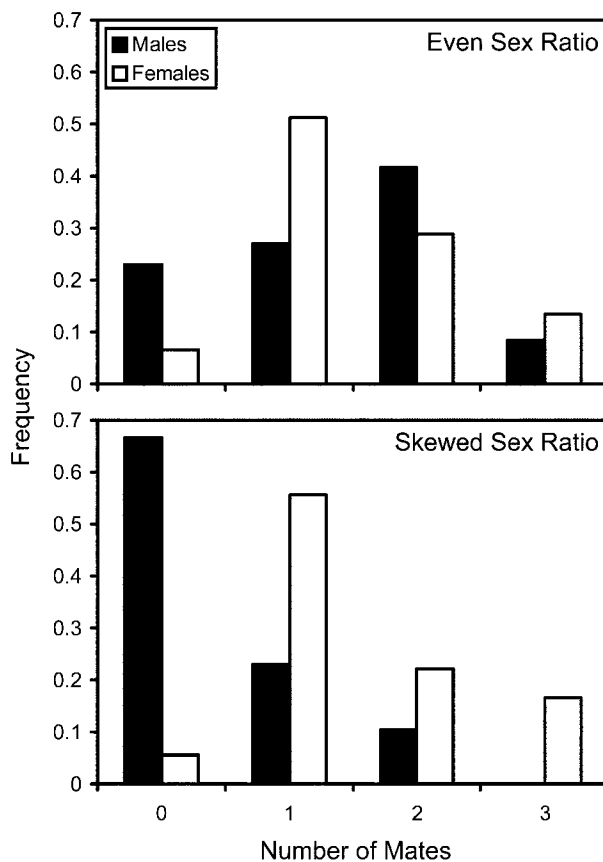


Figure 1: Mating success histograms for males (solid bars) and females (open bars) in the even (top) and skewed (bottom) sex ratio treatments.

one another in the skewed sex ratio treatment (contingency χ^2 test, with two- and three-mate categories lumped, $df = 2$, $P < .001$). In no case did a single male mate more than three times, indicating that no single male could monopolize access to a large number of females in a tank. Female mating success histograms are remarkably similar in the even and skewed sex ratio treatments (contingency χ^2 test, $df = 3$, $P = .95$).

The Intensity of Sexual Selection

Our results provide strong evidence that sexual selection on males usually acts on tail height (table 1). In three of the four treatment combinations, we found significant selection favoring taller tails. The same results were obtained regardless of whether we used mating success or reproductive success as our measure of fitness and whether we looked at selection differentials or selection gradients (table 1), despite positive correlations among the traits. Linear regressions showed positive correlations in both sexes be-

Table 1: Selection coefficients in the experimental newt populations

Sex ratio, male size treatment, and trait	Sex	Fitness = mating success				Fitness = reproductive success			
		s'	P	β'	P	s'	P	β'	P
		Even:							
6L : 2S:									
SVL	Males	.02	.841	.18	.303	.03	.854	.08	.763
TL	Males	-.07	.545	-.33	.077	-.00	.990	-.27	.345
TH	Males	.19	.112	.28	.037	.36	.040	.47	.027
SVL	Females	.13	.241	.15	.384	.02	.822	-.04	.811
TL	Females	.06	.569	-.07	.671	.08	.468	.14	.406
TH	Females	.16	.157	.14	.262	-.09	.400	-.11	.340
2L : 6S:									
SVL	Males	-.27	.092	-.52	.011	-.17	.392	-.38	.124
TL	Males	-.01	.964	.21	.262	-.02	.908	.06	.804
TH	Males	.24	.146	.35	.035	.41	.033	.53	.013
SVL	Females	-.13	.283	-.09	.629	.06	.659	-.07	.741
TL	Females	-.09	.456	-.00	.988	.19	.162	.29	.139
TH	Females	-.13	.274	-.10	.499	-.10	.470	-.16	.299
Skewed:									
6L : 2S:									
SVL	Males	-.58	.090	.15	.708	-.47	.242	.38	.422
TL	Males	-1.01	.002	-1.10	.010	-1.04	.006	-1.27	.013
TH	Males	-.41	.242	-.28	.364	-.45	.269	-.34	.362
SVL	Females	.08	.574	.05	.809	.34	.139	.43	.259
TL	Females	.11	.437	.13	.555	.06	.829	-.09	.795
TH	Females	.10	.522	.13	.538	-.01	.955	-.02	.938
2L : 6S:									
SVL	Males	-.45	.122	-.48	.150	-.14	.697	-.14	.704
TL	Males	-.26	.387	-.16	.641	-.15	.676	-.33	.398
TH	Males	.47	.103	.61	.045	.91	.006	1.05	.004
SVL	Females	-.03	.889	-.03	.933	.14	.458	.17	.457
TL	Females	-.03	.891	-.03	.934	.13	.496	.17	.450
TH	Females	.00	.987	.02	.953	-.22	.232	-.34	.150

Note: The standardized selection differential (s') is the covariance between the standardized trait and relative fitness, whereas the standardized selection gradient (β') is estimated by the partial regression of relative fitness on the traits. In these analyses, we use either mating success (number of genetic mates) or reproductive success (number of offspring) as our measures of fitness. The two sex ratio treatments involved either eight males with eight females per tank (even) or eight males with three females per tank (skewed). The male size treatments used either six large males and two small males per tank (6L : 2S) or two large males and six small males per tank (2L : 6S). SVL, snout-vent length; TL, tail length; TH, tail height. Boldface values are significant at $\alpha = 0.05$.

tween snout-vent length and tail length (males: $N = 96$, $r^2 = 0.41$, $P < .001$; females: $N = 61$, $r^2 = 0.39$, $P < .001$) as well as between snout-vent length and tail height (males: $N = 96$, $r^2 = 0.07$, $P = .008$; females: $N = 61$, $r^2 = .08$, $P = .03$). One surprising result from our selection analysis is that in the skewed sex ratio 6L : 2S tanks, we found strong negative selection on tail length. A closer inspection of the data shows that in two of these tanks, the smallest male in each enjoyed higher mating and reproductive success than all of the other males combined. Because these were skewed sex ratio tanks, a total of only nine females were present in the three tanks, and these results consequently should be viewed with caution. We

are not prepared to rule out that an interesting biological phenomenon was at work in these tanks, but the most likely explanation is that these are atypical results due to two unusually lucky males. In no case did we find evidence for significant sexual selection on females with respect to either mating success or reproductive success (table 1). In summary, our analysis of selection on the phenotype indicates that strong sexual selection is operating on males, but we found no evidence for sexual selection on females.

Quantitative Measures of the Mating System

Table 2 shows the quantitative measures of the mating system based on Bateman's principles for the experimental

Table 2: Quantitative characterization of the mating systems in the experimental newt populations

Sex and sex ratio	\bar{X}_{rs}	σ_{rs}^2	I (SE)	\bar{X}_{ms}	σ_{ms}^2	I_s (SE)	β_{ss} (SE)
Male:							
Even	153.2	21,100	1.02 (.22)	1.35	.91	.67 (.29)	114.1 (18.4)
Skewed	59.2	12,600	3.33 (.23)	.44	.51	2.75 (.30)	136.8 (15.9)
Female:							
Even	164.5	7,990	.30 (.04)	1.49	.68	.30 (.05)	21.7 (21.2)
Skewed	175.4	9,540	.27 (.13)	1.33	.67	.36 (.19)	-18.7 (44.4)

Note: The opportunity for selection (I) is the variance in reproductive success (σ_{rs}^2) divided by the square of the mean reproductive success (\bar{X}_{rs}), and the opportunity for sexual selection (I_s) is the variance in mating success (σ_{ms}^2) divided by the square of mean mating success (\bar{X}_{ms}). The Bateman gradient (β_{ss}) is estimated by the least squares regression of reproductive success on mating success. Each of these values was calculated separately for each tank, and here we show the mean across tanks within each sex ratio treatment. SEs of interest are shown in parentheses. Note that β_{ss} was calculated differently here than in figure 2.

newt populations. The male size distribution treatments had little or no effect on these measures of the mating system, so we present only the comparison of skewed versus even sex ratios in these tables. Clearly, the sex ratio treatment had a pronounced effect on the values of these mating system measures. In the even sex ratio treatments, the opportunity for selection (I , based on reproductive success) in males is about three times larger than the opportunity for selection in females. The opportunity for sexual selection (I_s , based on mating success) is only about twice as high in males than in females (table 2) in the equal sex ratio treatments. In the skewed sex ratio treatments, on the other hand, I and I_s are approximately eight to 12 times higher in males than in females. Formal statistical analysis shows that I and I_s for males are significantly higher on average in the skewed sex ratio treatments than in the even sex ratio treatments (ANOVA, all $P < .001$). No such pattern was evident in females (ANOVA, all $P > .05$). Regardless of the sex ratio, the male Bateman gradient is steep and significantly different from 0 (table 2; fig. 2), and the female Bateman gradient is shallow and not significantly different from 0 (fig. 2).

Alternate measures of the potential for sexual selection, the index of resource monopolization (Q) and the Morisita index (I_b), are shown in comparison to I and I_s in figure 3. The Morisita index actually displays a pattern very similar to that shown by I and I_s . The major differences are that the discrepancy between males and females is less extreme, as is the disparity between the skewed and even sex ratio treatments. The index of resource monopolization, on the other hand, departs from the other two measures when mating success is used as the measure of fitness. Females in the skewed sex ratio treatment have a striking negative value of Q_{ms} , and the value of Q_{ms} for males is not significantly greater than 0. However, the rank order of values for Q_{rs} and Q_{ms} across treatments and sexes are

approximately the same as those for the other measures of inequality.

The Effects of the Operational Sex Ratio and Male Size Treatments

Overall, the mating behavior of the rough-skinned newt appeared to be extremely resistant to changes in the operational sex ratio and the size distribution of males. The only detectable changes occurred as a consequence of our manipulation of the sex ratio. In the tanks with an excess of males, we saw increases in I , I_s , I_{b-rs} , and Q_{rs} for males relative to the even sex ratio treatment. We also saw a small, nonsignificant increase in the slope of the Bateman gradient (β_{ss}). These patterns agreed well with our estimation of selection coefficients, since the absolute magnitude of the selection differentials and selection gradients on male traits also tended to increase in response to the skewed sex ratio (table 1).

The male size distribution treatments had no clear effect on any of the variables that we measured in this experiment. The only obvious difference among the 2L : 6S and the 6L : 2S treatments was the unusual pattern of sexual selection in the 6L : 2S treatment with the skewed sex ratio. However, without additional supporting evidence, the significance of this observation should not be overstated.

Surprisingly, other aspects of the mating behavior of females were also extremely robust to our treatments. For example, we found no significant effects of either of our treatments on time in amplexus, number of amplexus partners, number of observed inseminations, realized reproductive success, or realized mating success (ANOVAs, all $P > .05$). Aspects of male mating behavior did change but only as a strict numerical consequence of the change in the sex ratio. For example, if the number of females decreases, then mean male mating success will necessarily

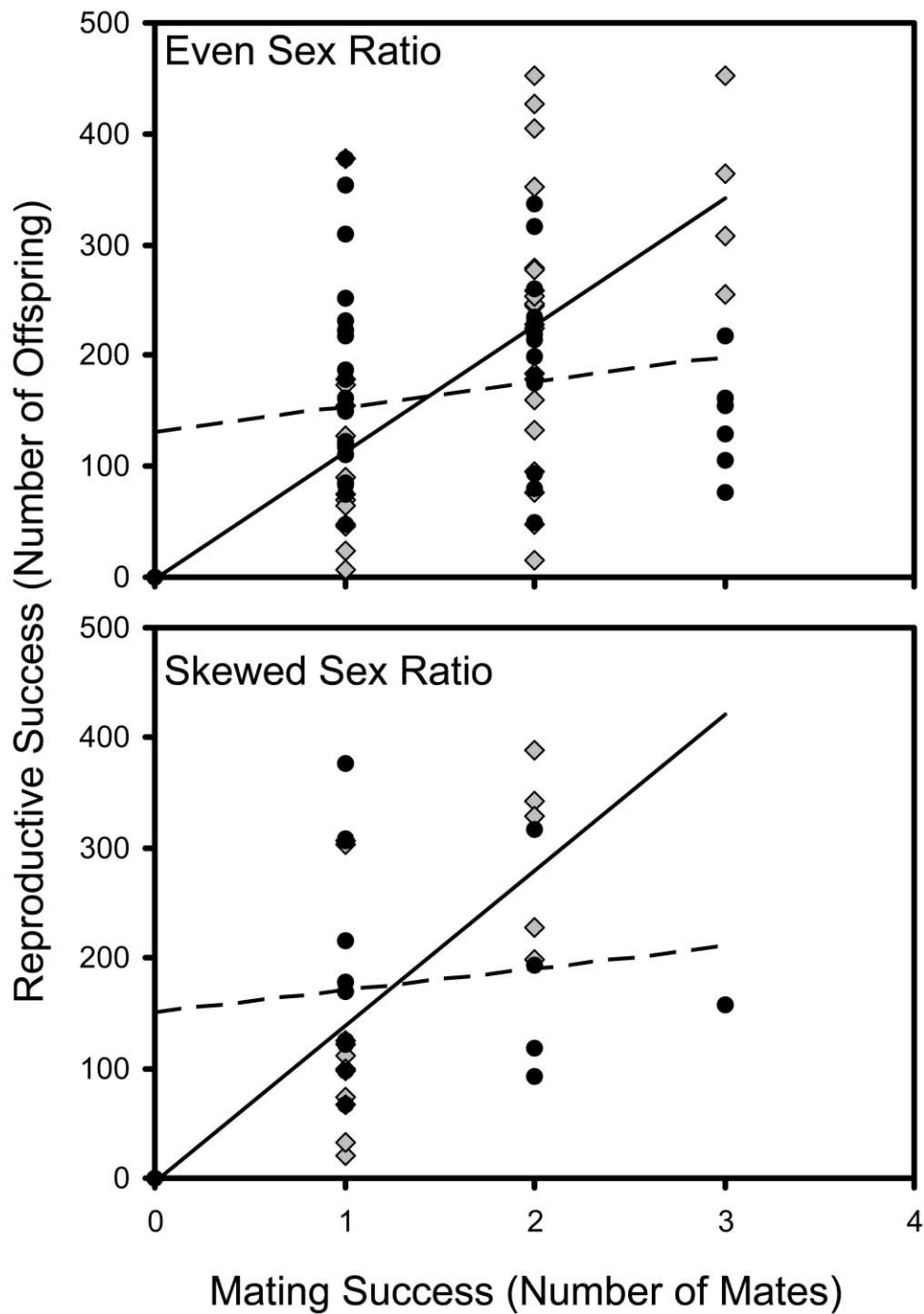


Figure 2: Bateman gradients (sexual selection gradients) for males and females in the even and skewed sex ratio treatments. In both panels, diamonds represent males, and solid circles represent females. The male gradient is shown as a solid line and the female gradient is a dashed line. These Bateman gradients are calculated from data pooled across tanks within each sex ratio treatment. Males and females with zero mating success and zero reproductive success are included in the analysis. In the even sex ratio treatment, the male and female Bateman gradients are given by $y = 114.6x - 1.9$ and $y = 22.4x + 131.5$, respectively. In the skewed sex ratio treatment, the male and female gradients are $y = 141.5x - 2.7$ and $y = 20.0x + 151.5$, respectively. In both cases, the male gradients are significantly different from 0 (regression, $P < .001$) and significantly steeper than the female gradients (ANCOVA, $P < .001$). The slopes of the female gradients do not differ significantly from 0 (even, $P = .20$; skewed, $P = .62$).

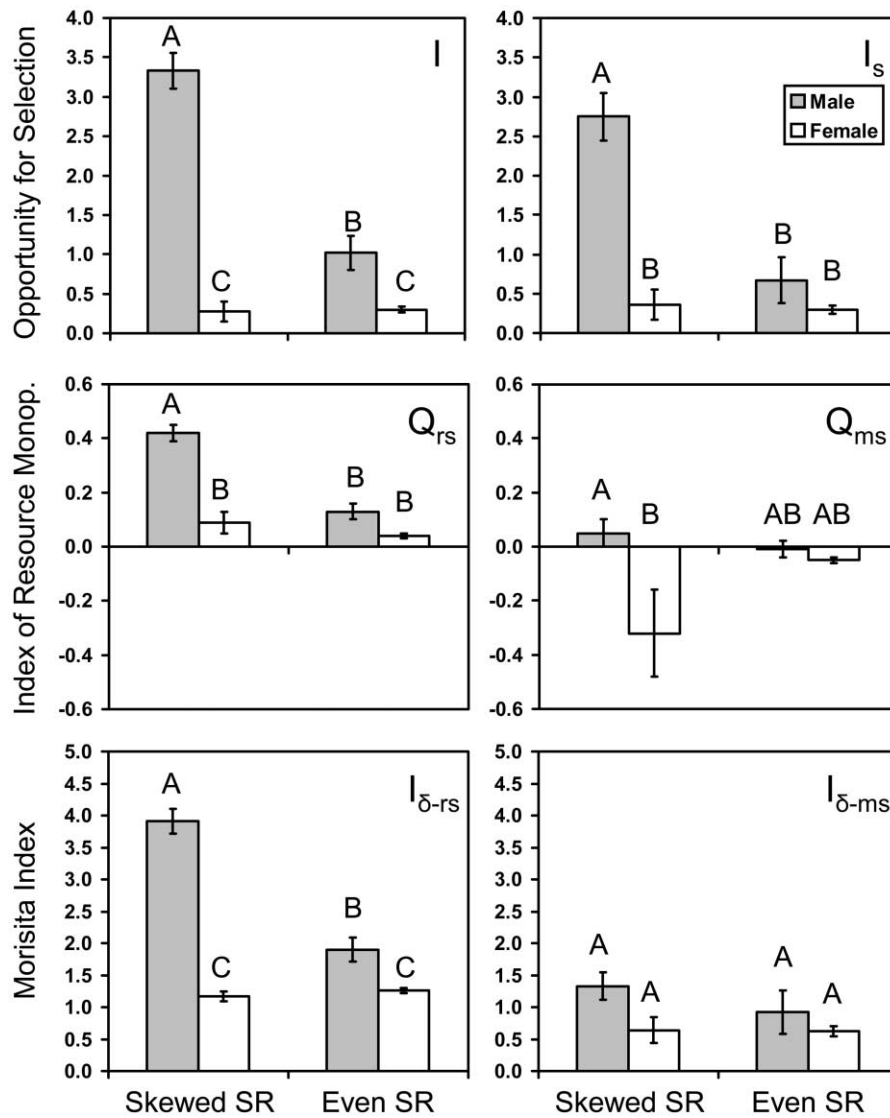


Figure 3: Measures of inequality that have been proposed as measures of the intensity of sexual selection. Each panel shows the values calculated for males and females in our two sex ratio treatments (skewed vs. even). All values were calculated for each breeding assemblage, and means across tanks within a treatment are shown here. Error bars show 1 SE of the mean. Means indicated by bars that share the same letter were not significantly different from one another at $\alpha = 0.05$ (Tukey's method for multiple comparison of means).

decrease, assuming female behavior remains unchanged. These results indicate that female mating behavior was extremely resistant to the changes that we imposed on the demographics of the populations.

Discussion

The Quantitative Characterization of Mating Systems

The major goal of this study was to test empirically the validity of mating system measures based on Bateman's

principles by experimentally manipulating the expected intensity of sexual selection in artificial breeding aggregations of newts. Our major finding is that measures of the mating system based on Bateman's principles (the opportunity for selection, the opportunity for sexual selection, and the Bateman gradient) respond as predicted to changes in the operational sex ratio. A change from an even sex ratio to an excess of males is expected to result in an increase in the intensity of sexual selection on males (Emlen and Oring 1977; Clutton-Brock and Parker 1992; Kvarnemo and Ahnesjö 1996). In our experiment, just

such a change in the sex ratio resulted in substantial increases in the opportunities for total selection and sexual selection. Our sex ratio manipulation also resulted in a nonsignificant increase in the Bateman gradient in the expected direction.

Our second major result is that direct estimation of the intensity of sexual selection on particular phenotypic traits generally agrees with the indirect measures of sexual selection strength based on Bateman's principles. Thus, the absolute values of selection coefficients also increased in response to a more male-biased operational sex ratio. Ours is the first experiment to experimentally manipulate the intensity of sexual selection in order to test the utility of Bateman's principles in the study of sexual selection. Overall, our results show that measures based on Bateman's principles respond correctly to changes in the strength of sexual selection, and they closely parallel direct measures of the intensity of sexual selection on particular traits. Hence, the measures of the genetic mating system based on Bateman's principles provide valuable techniques for the characterization of mating systems with respect to sexual selection.

An important consideration is that a robust quantitative characterization of the mating system requires knowledge of all three of Bateman's principles. Measures based on variances in mating success and reproductive success (Bateman's first two principles), proposed in the 1970s and 1980s, have been heavily criticized (Clutton-Brock 1983; Sutherland 1985a, 1985b, 1987; Koenig and Albano 1986; Grafen 1987, 1988; Hubbell and Johnson 1987). However, most of the criticism preceded the introduction of the Bateman gradient (Arnold and Duvall 1994), which appears to be critical to interpretation of variance-based measures. With data on Bateman gradients, we can see clearly whether there is a functional relationship between variance in mating success and variance in reproductive success. Because sexual selection is driven by competition for access to mates, sexual selection cannot occur unless there is a positive relationship between mating success and reproductive success in addition to variance in mating success and reproductive success (Arnold and Duvall 1994). Thus, all three of the measures based on Bateman's principles must be positive for sexual selection to operate.

Consideration of all three of Bateman's principles in newts leads to the conclusion that sexual selection operates on males but not on females in these breeding assemblages. They also indicate that the intensity of sexual selection on males is higher in the skewed sex ratio treatment than in the even sex ratio treatment. Even though the variances in mating success and reproductive success in males are low in the even sex ratio treatment, the very steep Bateman gradient indicates that success in mating competition does result in higher fitness, so a large fraction of the observed

variance in mating success is of significance to sexual selection. Females also display some variance in mating success, but their flat Bateman gradient suggests that this variance is unrelated to the sexual selection process. These considerations demonstrate the importance of the Bateman gradient in the interpretation of variances in mating and reproductive success. Thus, in males we find evidence for sexual selection, with a higher opportunity for sexual selection in the skewed sex ratio treatment, whereas we find no evidence that sexual selection operates on female newts.

Do we need alternative methods for the characterization of mating systems in natural populations? We believe the answer to this question is a clear no. In this study, we calculated the index of resource monopolization (Q) and the Morisita index (I_δ), because these particular indices seem to be the two alternative metrics that are gaining the most support in the sexual selection literature (Kokko et al. 1999; Fairbairn and Wilby 2001). These indices provided results that were superficially consistent with the results based on I and I_s . However, they display some important differences. Our a priori expectations for this experiment were that sexual selection would be strong in males and weak or absent in females in both sex ratio treatments and that sexual selection would be stronger on males in the skewed sex ratio treatment than in the even sex ratio treatment. The patterns displayed by I and I_s come closest to fulfilling these predictions. The Morisita index also is consistent with expectations, except that it indicates a greater than expected potential for sexual selection on females, and $I_{\delta-ms}$ does not yield any statistically significant insights. The index of resource monopolization is far more difficult to interpret. The relative values for Q_{rs} are similar to those for I and $I_{\delta-rs}$ but with reduced statistical significance. However, the values of Q_{ms} display a very different pattern. In the skewed sex ratio treatment, Q_{ms} for females is negative and significantly smaller than Q_{ms} for males, so the relative values of Q_{ms} in the sexes are consistent with expectations. But how should we interpret the negative values in females and the fact that the confidence limits for males overlap 0? In the even sex ratio treatment, the values of Q_{ms} for males and females are significantly different neither from each other nor from 0.

Indices like I_δ and Q have been advocated because they use a random distribution of resources as their null models (Kokko et al. 1999; Fairbairn and Wilby 2001). However, even the advocates of these measures are quick to point out that they do not possess the close ties to formal selection theory that I and I_s possess (Kokko et al. 1999; Fairbairn and Wilby 2001). The alternative indices also have no clear connection to the Bateman gradient. This lack of connection to selection theory is a severe drawback for I_δ and Q . In addition, we see no advantage for the

measure to be standardized relative to a random distribution of resources. Rather, it is preferable for them to reflect the maximum strength of sexual selection that could operate in a population (or sex) because, all else being equal, the realized strength of sexual selection will be a constant fraction of the maximum. Until theory relating I_s and Q to the intensity of sexual selection is developed, the use of these measures to quantify mating systems with respect to sexual selection cannot be justified.

Thus, our view is that we need fewer—not more—measures of the mating system. A more standard approach to mating systems would facilitate comparative studies of mating system evolution and sexual selection, and we believe that the measures of the mating system based on Bateman's principles provide the most promising approach. Very few studies have applied this approach to natural populations, but those that have been conducted so far tend to indicate that Bateman's principles do accurately reflect the intensity of sexual selection in natural and experimental populations (Arnold and Duvall 1994; Jones et al. 2000, 2002*b*; Garant et al. 2001; Woolfenden et al. 2002). We have no doubt that there will be some exceptional cases in which Bateman's principles are misleading, because they cannot guarantee any particular strength of sexual selection. However, this caveat will apply to any indirect measure of the intensity of sexual selection based on the mating system. Broad comparative studies of mating systems using Bateman's principles should allow us to identify these exceptional cases, because they may well be the most interesting and informative systems with respect to the study of sexual selection and mating system evolution.

The view of sexual selection that we present here admittedly ignores the roles of mate quality and offspring quality in the sexual selection process. However, these issues are somewhat separate from the characterization of genetic mating systems. In species such as newts, which do not provide paternal care, most of the variance in fitness among males (the sexually selected sex) is probably due to differential mating success rather than differences in the quality of their mates. Nevertheless, it should be noted that the importance of mate and offspring quality has not been investigated in newts to such an extent that we can be sure that these topics are unimportant. If these aspects of the sexual selection process are important, additional studies other than appraisals of genetic mating patterns will be necessary for a comprehensive understanding of the selective pressures acting on sexually selected traits.

The Natural Mating System of the Rough-Skinned Newt

Our results are of interest also because they extend previous research on the natural mating system of the rough-

skinned newt. Jones et al. (2002*b*) used a microsatellite-based assessment of parentage to characterize sexual selection and mating system measures in an unmanipulated, natural population of *Taricha granulosa*. The results of this study are largely consistent with the results of that field study. The distribution of mating success in the field was very similar to the distribution that we observed in our skewed sex ratio treatment, suggesting that the operational sex ratio is similarly skewed in nature. The values of I and I_s from the field study bolster this conclusion. For males, I and I_s were 4.48 and 3.06 in the field population, respectively (Jones et al. 2002*b*). For females, I and I_s were 0.23 and 0.44 (Jones et al. 2002*b*). These values are very close to those observed in the skewed sex ratio treatments of this study. The field study also found a significantly positive slope for the male Bateman gradient and a nonsignificant slope for the female gradient.

One surprising implication of the similarity between the skewed sex ratio treatment and the natural mating patterns is that the operational sex ratio in the field may be only about two or three males per female. The focal population in the field study contained 96 adult males and 42 adult females. The females enter the population slowly over the course of several weeks, with only a few arriving per night (Pimentel 1960). Additionally, females probably mate within a few days and then become unreceptive (Twitty 1966; Propper 1991). Given this information, we might expect the operational sex ratio to be approximately five to 10 males per female. However, at any particular pond entry point, only a fraction of the males will be close enough to detect the female. Because of this spatial structuring, the effective operational sex ratio may be considerably smaller than the absolute operational sex ratio estimated as a simple ratio of receptive males to receptive females in the entire population.

One very striking result in our study is that female mating behavior remained essentially unchanged in the different treatments of this study. We did not observe any changes in the time spent in amplexus, the number of mating partners, mating success, or reproductive success in our different treatments. It is particularly surprising that females did not respond in any way to the fairly dramatic change in the operational sex ratio. One possible explanation is that female mating behavior is not very plastic or not easily influenced by male competition. Alternatively, our treatments did not differ drastically enough from one another to cause a major change in female behavior.

Conclusions

This study set out to test a new approach to characterize mating systems using Bateman's three principles: the opportunity for selection, the opportunity for sexual selec-

tion, and the Bateman gradient. Very few studies have calculated all three of these measures simultaneously, and none have tested them by experimentally manipulating the intensity of sexual selection. Our results show that the measures based on Bateman's principles do accurately reflect the intensity of sexual selection in our experimental populations by responding as expected to manipulation of the sex ratio. We find no justification for replacing these measures with other "measures of inequality" that possess no clear relationship to selection theory. Furthermore, widespread adoption of measures based on Bateman's principles would facilitate additional robust comparative studies of mating system evolution and sexual selection.

Acknowledgments

We would like to thank E. Adams, M. Manier, C. Palmer, M. Pfrender, J. Sapp, and M. Westphal for help with various aspects of this project. We thank W. Liss and the Department of Fisheries and Wildlife at Oregon State University for the use of facilities at the Oak Creek Laboratory of Biology. We are grateful to several anonymous reviewers, J. Graves, T. Halliday, and T. Tregenza for valuable comments on the manuscript. This work was supported by grants from the National Science Foundation (A.G.J., S.J.A.) and funds from Oregon State University (S.J.A.).

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Associate Editor: Allen J. Moore