OVERCOMING STATISTICAL BIAS TO ESTIMATE GENETIC MATING SYSTEMS IN OPEN POPULATIONS: A COMPARISON OF BATEMAN’S PRINCIPLES BETWEEN THE SEXES IN A SEX-ROLE-REVERSED PIPEFISH

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Received February 1, 2012
Accepted September 18, 2012

The genetic mating system is a key component of the sexual selection process, yet methods for the quantification of mating systems remain controversial. One approach involves metrics derived from Bateman’s principles, which are based on variances in mating and reproductive success and the relationship between them. However, these measures are extremely difficult to measure for both sexes in open populations, because missing data can result in biased estimates. Here, we develop a novel approach for the estimation of mating system metrics based on Bateman’s principles and apply it to a microsatellite-based parentage analysis of a natural population of the dusky pipefish, Syngnathus floridae. Our results show that both male and female dusky pipefish have significantly positive Bateman gradients. However, females exhibit larger values of the opportunity for sexual selection and the opportunity for selection compared to males. These differences translate into a maximum intensity of sexual selection ($s_{max}$) for females three times larger than that for males. Overall, this study identifies a critical source of bias that affects studies of mating systems in open populations, presents a novel method for overcoming this bias, and applies this method for the first time in a sex-role-reversed pipefish.

KEY WORDS: Bateman gradient, mating system, microsatellites, operational sex ratio, opportunity for sexual selection, parentage analysis.

The quantification of genetic mating systems in the context of sexual selection remains a controversial topic (Jones 2009; Klug et al. 2010; Krakauer et al. 2011). This situation is unfortunate, because it stymies attempts to understand mating system evolution in a comparative context and to examine the effects of variation in mating systems on the sexual selection process. One approach to characterizing mating systems involves the use of metrics based on Bateman’s principles, which are related to three key observations from A. J. Bateman’s classic paper on parentage in Drosophila melanogaster (Bateman 1948). Bateman observed that male flies, compared to female flies, show (1) higher variance in number of offspring, (2) higher variance in number of mates, and (3) a stronger correlation between number of mates and number of offspring. Over the last several decades, Bateman’s observations have been explicitly formalized in the context of sexual selection theory (Shuster and Wade 2003; Jones 2009). The modern measures
of mating systems based on Bateman’s principles include the opportunity for selection, the opportunity for sexual selection, and the Bateman gradient (Crow 1958; Wade 1979; Arnold and Duvall 1994; Andersson and Iwasa 1996). The opportunity for selection (I) and opportunity for sexual selection (I_s) are defined as the variances in relative reproductive success and relative mating success, respectively (Crow 1958; Wade 1979; Wade and Arnold 1980; Arnold and Wade 1984). Mating success is defined as the number of individuals with whom the focal individual mates during the time period of interest, and reproductive success refers to the total number of offspring produced by an individual. The Bateman gradient (\( \beta_{ss} \)) is the least-squares regression of reproductive success on mating success (Arnold and Duvall 1994; Jones 2009).

If we restrict our attention to precopulatory sexual selection, we can see that the three measures based on Bateman’s principles are interrelated. At the heart of the sexual selection process, competition for access to mates produces variance in mating success (Darwin 1871; Jones and Ratterman 2009), but reproductive success is the ultimate measure of Darwinian fitness. Thus, the Bateman gradient plays a role in converting the variance in mating success into variance in reproductive success. The realization that these measures are related to one another leads to a simple solution for the maximum strength of sexual selection that can act on any trait. In units of phenotypic standard deviations, \( s_{\max}' \), which is equal to the product of the Bateman gradient and the square root of the opportunity for sexual selection, represents the largest absolute magnitude of a selection differential possible for any trait due to precopulatory sexual selection (Jones 2009). The utility of \( s_{\max}' \) is that it serves as a single quantity that encapsulates the idea that both the opportunity for sexual selection and the Bateman gradient must be positive for precopulatory sexual selection to operate.

However, metrics based on Bateman’s principles have their share of shortcomings. One of the most important problems, which we address here, is that Bateman’s principles can be extremely difficult to measure in natural populations of organisms (Parker and Tang-Martinez 2005). This difficulty arises from the fact that it can be very difficult to locate and sample all of the progeny from any particular parent. Often this problem affects one sex more than the other, especially when a species exhibits extended parental care. For instance, in a species like a guppy (Poecilia reticulata), a female will carry her entire complement of progeny within her body, so her total mating success and reproductive success may be relatively easy to infer with molecular markers (Kelly et al. 1999; Neff et al. 2008; Coleman and Jones 2011). However, if some females elude capture, then the males who mated with the unsampled females will appear to have both reduced mating success and reduced reproductive success relative to their true values for these variables (Table S1). This scenario can result in biases in estimates of any mating system metrics that rely on accurate estimates of variances in mating success and reproductive success. Furthermore, the biological setting for this bias is not restricted to guppies and their close relatives. Rather, it appears to apply to virtually every group of organisms in which uniparental care occurs, making it a pervasive problem for the measurement and comparison of natural mating systems. How studies should correct for this bias is not immediately obvious a priori. One of the goals of the present study is to investigate this issue and provide an approach that overcomes this problem.

Our second goal is to apply our new estimation procedure to produce the first complete appraisal of mating system metrics for both sexes of a sex-role-reversed species in the wild. A key piece of evidence supporting Bateman’s original assertions regarding the cause of sexual selection in males of species with conventional sex roles, such as flies and newts (Bateman 1948; Jones et al. 2002, 2004), comes from the observation that Bateman’s principles are reversed in a pipefish species in which sexual selection acts more strongly on females than on males (Jones et al. 2000, 2005). However, the only study that has quantified Bateman’s principles in both sexes of any sex-role-reversed species used small, captive breeding populations of the broad-nosed pipefish (Syngnathus typhle) and showed that the values of mating system metrics depended in part on the adult sex ratio (Jones et al. 2000, 2005). Although a strong case can be made that the operational sex ratio in \( S. typhle \) is usually biased toward an excess of females in the wild (Vincent et al. 1994), direct estimates of Bateman’s principles from a natural population of sex-role-reversed pipefish would provide independent support that the laboratory studies are being interpreted correctly.

The present study involves several parts. First, we use a simulation approach to show that estimates of Bateman’s principles are biased when some clutches are missing from the sample subjected to parentage analysis. Second, we develop a technique to produce unbiased estimates of the opportunity for sexual selection, opportunity for selection, Bateman gradient and \( s_{\max}' \) from samples with missing offspring and parents. And third, we conduct a microsatellite-based parentage analysis for a natural population of the sex-role-reversed dusky pipefish, \( S. floridana \). Overall, our study provides a methodological advance that should be useful in a wide range of future studies, and it also definitively addresses for the first time the sex differences in Bateman’s principles in a sex-role-reversed species in the wild.

**Methods**

**SAMPLE COLLECTION**

We sampled dusky pipefish (\( S. floridana \)) from the Gulf of Mexico near Port Aransas, TX (N27°52.947’, W97°06.076’) by pulling a seine with a 2 mm mesh through shallow seagrass on 21 July.
2006, during the height of the summer breeding season. We estimated population density by using stakes to demarcate two patches of seagrass, which totaled 190.5 m², and sampling intensively within these areas. We sampled the site until three consecutive full sweeps failed to yield additional adult specimens. We also collected a few additional fish from a patch of seagrass just outside the marked area to obtain a sufficient sample size for parentage analysis. All adult males and females were humanely sacrificed and preserved in 95% ethanol. We dissected pregnant males and counted the number of embryos in their brood pouches.

**MICROSATELLITE-BASED PARENTAGE ANALYSIS**

We extracted DNA from adult fin clips by using a Gentra PureGene™ cell and tissue kit. For pregnant males, we divided the brood pouch into 14 equal sections, with one division down the midline and six divisions along the length of the pouch, and removed three embryos from each section for microsatellite analysis. Because embryos are clumped by maternity within the pouch, this procedure allows us to estimate the total number of eggs contributed by each female (Jones and Avise 1997b). We used a 5% Chelex/Proteinase K protocol to extract DNA from individual embryos (Miller and Kapuscinski 1996; Mobley and Jones 2007, 2009).

All adults and the 42 mapped embryos per pregnant male were genotyped at three highly polymorphic microsatellite loci, *micro11.1, micro22.3*, and *micro25.22*, which originally were isolated from the Gulf pipefish, *S. scovelli* (Jones and Avise 1997a; Jones et al. 1999). Polymerase chain reaction conditions followed previous papers (Mobley and Jones 2007, 2009). One primer from each primer pair was labeled with a fluorescent dye and fragments were sized on an ABI 3730 Genetic Analyzer (Applied Biosystems). Microsatellite alleles were identified and scored through the use of GENOTYPER and GENEMAPPER software (Applied Biosystems). We tested for deviations from Hardy–Weinberg equilibrium and linkage equilibrium by using Fisher’s exact tests, as implemented in the program GENEPOP 4.0.10 (Raymond and Rousset 1995).

We reconstructed maternal genotypes from the genotypes of embryos within each male’s pouch by using the computer program GERUD2.0 (Jones 2001, 2005). The reconstruction of maternal genotypes was further facilitated by the fact that embryos are arranged by maternity within the brood pouches of pipefish (Jones and Avise 1997b), and the results of GERUD2.0 invariably agreed with the expected maternal clumping of embryos. These reconstructed genotypes were then compared with the genotypes of field-collected females in an attempt to identify the mothers of the sampled broods. Each three-locus microsatellite profile observed in this study was unique to a particular adult, so cases in which genotypes inferred from progeny arrays matched genotypes of sampled females almost certainly represented instances of true parentage by the female in question. Assuming Hardy–Weinberg equilibrium and linkage equilibrium, the average expected frequency of the multilocus genotypes observed in our sampled adults was $2.8 \times 10^{-6}$. These frequencies are sufficiently small that no spurious matches would be expected in a dataset of the size we collected. In addition, these markers have been used successfully to reconstruct maternal genotypes and assign parentage unambiguously in previous studies involving both Gulf and dusky pipefish across several distinct populations of each species (Jones and Avise 1997a,b; Jones and Avise 2001; Jones et al. 2001; Mobley and Jones 2007, 2009).

**ESTIMATION OF MATING SYSTEM VARIABLES FOR MALES**

The estimation of mating system variables for males was straightforward, because each pregnant male carried his full complement of offspring in his brood pouch during the pregnancy. Thus, we calculated number of mates (i.e., mating success) and number of offspring (i.e., reproductive success) for the current pregnancy directly from the parentage data for each male. We estimated means and variances in mating and reproductive success across assayed males. We also calculated the opportunity for selection ($I$), the opportunity for sexual selection ($I_s$), and the Bateman gradient. The unstandardized Bateman gradient ($\beta_{ss}$) was estimated as the least-squares regression of absolute reproductive success on absolute mating success, whereas the standardized Bateman gradient ($\beta_{ss}'$) was the least-squares regression of relative reproductive success on relative mating success (Jones 2009). Relative mating and reproductive success were calculated by dividing each individual’s value of mating and reproductive success by the corresponding mean. Finally, we calculated the statistic $s_{\text{max}}'$, defined as $\beta_{ss}' \sqrt{T}$, which provides a measure of the maximum selection differential (in units of phenotypic standard deviations) that can arise as a consequence of precopulatory sexual selection (Jones 2009). We bootstrapped (1000 replicates) across male broods to determine 90% and 95% confidence intervals for all estimated mating system variables in males.

**ESTIMATION OF MATING SYSTEM VARIABLES FOR FEMALES**

*Simulation-based illustration of bias*

We conducted simulations to demonstrate that incomplete sampling of clutches from a population can result in biased estimates of the opportunity for sexual selection, the opportunity for selection, and the Bateman gradient. We focused on two cases. In the first case, we simulated breeding populations with low variances in mating and reproductive success and shallow Bateman gradients. In the second case, we simulated populations with high variances in mating and reproductive success and steep Bateman gradients.
Each simulated population contained a total of 100 pregnant males and 100 adult females. We were interested in the mating success and reproductive success of females, so we simulated female mating success by drawing a random number from a Gaussian distribution with a specified mean and standard deviation. In both cases investigated here, we used a mean of four. We used standard deviations in mating success of one and six for the low and high variance examples, respectively. Any negative values were converted to zeros. This procedure potentially changes the mean, so we adjusted it back to the desired value (i.e., four) by multiplying all mating success values by a constant. The values were then rounded to the nearest integer to produce an intelligible value for each individual’s mating success.

Reproductive success and Bateman gradients were simulated by specifying a mean reproductive success, the amount that reproductive success increases per mating, and the standard deviation in reproductive success. The standard deviation in reproductive success was assumed to be constant across mating success categories. This way of specifying the mean, slope, and standard deviation is convenient because it allows us to calculate an expected reproductive success for any given value of mating success. It also allows us to simulate variation in reproductive success by drawing an individual’s number of offspring from a Gaussian distribution with a mean equal to the expected reproductive success for that individual’s mating success value and any desired standard deviation. In both cases explored here, we used a mean reproductive success of 300 and a standard deviation in reproductive success within each category of mating success of 50. We assumed an increase in reproductive success per mating of zero for the low variance, shallow slope example and 75 for the high variance, steep slope example.

We calculated the true opportunities for selection, opportunities for sexual selection, and Bateman gradients from each simulated population, averaged across 30 replicates. These values were identical to those that would have been available to a researcher who had managed to sample the entire population of breeding males. We then took samples of between 10% and 90% of the breeding males and calculated values of the variable of interest for the females, assuming perfect parentage data and averaging across 30 replicates.

Estimation of distributions of mating success, reproductive success, and the Bateman gradient

We developed a novel maximum-likelihood approach for the estimation of mating system variables in open populations. The details of the methods are included in the Appendix. Briefly, we started by estimating the mean mating and reproductive success for females by using knowledge of the male mating patterns and the sex ratio. We then estimated the standard deviation in mating success for females by using an explicit maximum-likelihood framework (Appendix). Our approach stepped through hypothetical mating success distributions and chose the one that maximized the probability of obtaining the observed parentage data, given the sampling regime used by our study. With the distribution of female mating success in hand, we proceeded to estimate the standard deviation in reproductive success and the slope of the Bateman gradient jointly by using an approach similar to approximate Bayesian computation (Beaumont et al. 2002). In short, this approach involved simulating hypothetical breeding populations, sampling from them according to the sampling scheme of the empirical study, and calculating summary statistics from the simulated sample. The hypothetical population with summary statistics most similar to the observed data provided our maximum-likelihood estimates of the standard deviation in reproductive success and Bateman gradient for females (see Appendix for more details). Our implementation of this procedure does not require an estimate of the number of females with zero mating success, because the proportion of unmated females emerges from the fact that as some females have high mating success other females must be excluded from mating (for a given sex ratio). Thus, our approach uses data from all females (collected or inferred) with offspring represented in the molecular parentage analysis (i.e., mating success of one or greater). We then used a bootstrapping approach to estimate confidence intervals for the variables of interest.

Results

We collected a total of 30 adult males and 32 adult females from our sample site. All but two of the males were pregnant, and 26 of these pregnant males had broods that were sufficiently developed for the embryos to be genotyped. Within the 190.5 m² area we demarcated for the estimation of population density, we collected 23 adult males and 25 adult females, leading to an estimate of 0.25 adults per m². The adult sex ratio in our population was very close to unity, as 48.4% of the adults were male and 51.6% were female.

Our markers proved to be highly polymorphic in our study population, with between 18 and 30 alleles per locus. We also detected no departures from Hardy–Weinberg equilibrium or linkage equilibrium (two-tailed Fisher’s exact test, $P > 0.05$). A low-frequency null allele was detected at locus Micro11.1 in two reconstructed female genotypes in two separate progeny arrays. However, null alleles are easily detected in parentage analyses based on progeny arrays (Jones and Ardren 2003; Jones et al. 2010), and this null allele was sufficiently infrequent (0.02) that it did not compromise the reliability of our results. Before we return to the results of the parentage analysis, we first consider the statistical issues associated with the estimation of mating system variables in open populations.
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Figure 1. Results of simulated mating systems with either a low variance in mating success and a shallow Bateman gradient (left) or a high variance in mating success and a steep Bateman gradient. In each case, we simulated populations with 200 breeding adult pipefish and an equal sex ratio. We assumed a mean mating success of four and a mean reproductive success of 300, resulting in a total of 400 simulated clutches. We then sampled a portion of these clutches ranging from 10% to 90% at random and conducted a simulated parentage analysis. The results of the parentage analysis produced an observed mating success and reproductive success for each simulated female in the dataset. We calculated $I_s$ and $\beta_{ss}$ directly from these data, either excluding (squares) or including (circles) females in the sample with zero observed mating success. We also calculated the true values given perfect knowledge of the population (diamonds). The results of our estimation procedure applied to the simulated samples are shown as the triangles. Each symbol represents a mean across thirty replicate simulations, and the error bars show one standard deviation.

BIAS IN BATEMAN’S PRINCIPLES IN OPEN POPULATIONS

Our simulations of hypothetical breeding populations show that the study of parentage in open populations can produce biased values for mating system metrics based on Bateman’s principles. In pipefish, the sampling issues that occur in open populations do not result in biased estimates for males, because each male carries his full complement of mating and reproductive success for a given pregnancy within his brood pouch. Thus, sampling fewer males will result in less precise estimates of means and variances in mating and reproductive success but will not result in any kind of systematic bias compared to larger samples. However, if some pregnant males are missing from the sample, then inferred values of mating and reproductive success for females might be biased, and we address this issue with our simulation results.

Our results show that the magnitude and direction of bias depends on the true values of mating system metrics in the population (Fig. 1). For example, when the true opportunity for sexual selection is near zero (Fig. 1, upper left), the opportunity for sexual selection for females calculated from the simulated parentage data almost always produces an underestimate, whether the females with zero mating success are included in the calculation or not. In contrast, when the true opportunity for sexual selection is relatively large, we find that the simulated parentage analysis results in an underestimate (Fig. 1, upper right). We find similar results for the opportunity for selection (not shown). Bias in the Bateman gradient shows a slightly different pattern. When the true slope is near zero, then the simulated parentage analysis almost always overestimates the female Bateman gradient (Fig. 1, bottom left). However, when the true slope is steep, then the simulated parentage data actually provide a good estimate (Fig. 1, bottom right). Thus, regardless of the true slope, when some pregnant males are missing from the sample, the Bateman gradient of females estimated from the raw parentage data will almost always be steep. This result makes sense, because when a male is missing from the sample, any female who had placed eggs in the missing male’s pouch will appear to the researcher to have both reduced mating success and reduced reproductive success relative to her actual
values. When the true slope is steep, these incorrect points will tend to fall along a line that passes through the origin, so the slope will appear steep whether or not some males are missing from the sample. When the true slope is shallow, however, this source of bias could seriously affect inferences that rely on the Bateman gradient. The standard deviations of the values calculated from the simulated samples compared to the true values (Fig. 1) demonstrate that these incorrect estimates represent true cases of bias rather than ordinary statistical error. The other important point apparent from Figure 1 is that the bias occurs even when samples are exceptionally complete. For instance, when 80–90% of the population is sampled, both the opportunity for sexual selection and the Bateman gradient can be quite far from their true values (Fig. 1, left panels).

APPLYING OUR ESTIMATION PROCEDURE TO SIMULATED DATA

Figure 1 also shows the results of our estimation procedure, as applied to the simulated datasets. Our approach appears to do a very good job of estimating the opportunity for sexual selection, except when 20% or less of the population appears in the sample (Fig. 1, top row). In the cases in which the estimates are imprecise, the lack of confidence will result in wide bootstrap confidence intervals, so even under these circumstances our approach is an improvement over calculating the biased measures directly from the raw data. The results in Figure 1 indicate that the Bateman gradient is more difficult to estimate than the opportunity for sexual selection, especially when the slope is shallow. Even when a large proportion of the population is sampled, the standard deviations of the estimates tend to be quite large. Thus, if the true slope is shallow, we expect our procedure to produce a small point estimate for the Bateman gradient with wide bootstrap confidence intervals (Fig. 1, bottom left). However, when the true Bateman gradient is steep, our procedure produces very accurate estimates with extremely narrow confidence limits (Fig. 1, bottom right).

MATING PATTERNS OF MALES

The results of our microsatellite-based parentage analysis demonstrated that pregnant males in our sample had mated from one to three times per pregnancy (Table S2). The mean mating success of males, including the two nonpregnant males, was 1.79 (Table 1). The brood sizes of pregnant males ranged from 115 to 607, yielding a mean reproductive success, including the two nonpregnant males, of 330.5 (Table 1). Male standard length ranged from 117 to 192 with a mean of 148.7 ± 4.0 (mean ± standard error of the mean). Other studies have found a positive relationship between body length and brood size in dusky pipefish (Jones and Avise 1997b; Mobley and Jones 2007, 2009), but this relationship, while positive, was not statistically significant in the present study ($N = 28$, $r = 0.26$, $P = 0.18$).

We calculated mating system measures based on Bateman’s principles for males directly from the parentage data and bootstrapped across males to produce 90% and 95% confidence intervals (Table 1). The opportunities for selection and sexual selection in male dusky pipefish are relatively small but significantly larger than zero (Table 1), and the male dusky pipefish Bateman gradient is surprisingly steep. On average males carried an additional 129.4 offspring for each additional mating event (Table 1), and this slope differed significantly from zero at $\alpha = 0.05$. The maximum strength of precopulatory sexual selection, $s'_{\text{max}}$, for male dusky pipefish in our study population was 0.31, and this value was significantly larger than zero (Table 1).

ESTIMATING MATING PATTERNS OF FEMALES

The application of our estimation procedure required an estimate of the local breeding population size. We obtained such an estimate by using our parentage data in a mark-recapture framework (Jones and Avise 1997b; Pearse et al. 2001). Because pipefish breeding populations are dynamic groups spread over extensive patches of seagrass (Vincent et al. 1994), this estimate of population size is probably best interpreted as an approximation of the number of adults in the nearby vicinity that had a reasonable probability of being involved in the mating events that produced the observed broods. The 26 pregnant males in our sample mated with just under two females each on average, so their pouches contained a total of 50 groups of full siblings. We reconstructed the maternal genotypes for each of these full-sibling groups, and found a total of 31 distinct multilocus female genotypes, indicating that some females had mated with more than one pregnant male in our sample (Table S3). The genotypes of these females were considered the marks in our mark-recapture experiment. We collected a total of 32 adult females from our population and genotyped them. All of these females had different multilocus microsatellite genotypes from one another, and 13 of them matched maternal genotypes inferred from the progeny arrays of pregnant males, indicating that we collected 42% of the females that mated with the males in our sample. Thus, our dataset contained 31 female marks, we collected a second sample of 32 females, and we found that 13 of them were marked. By applying standard Lincoln–Petersen equations (Pollock et al. 1990; Jones and Avise 1997b), we obtained a point estimate of the local adult female breeding population size of 74.4 individuals with a 95% confidence interval ranging from 52.7 to 96.1. This analysis clearly shows that our sample was missing both males and females. Given our sex ratio near unity, the point estimate for the number of males was approximately 70, and we collected 30 adult males. As noted above, this situation did not present a problem for the estimation of mating system variables for males, because each male carried his full mating and reproductive success in his pouch. However, some females must have mated with males that were not in our sample, so the
Table 1. Mating system estimates for male and female dusky pipefish. We present results for males based on the microsatellite-determined parentage of their broods. For females, we produce estimates based on the raw data, either excluding (second set of rows) or including (third set of rows) females in our collection assigned zero mating success, for comparison with the results of our estimation procedure, which are shown in the last three sets of rows. We show results from our procedure based on three different estimates of the number of breeding females, which correspond to the point estimate (N = 74), the lower 95% confidence limit (N = 53), and the upper 95% confidence limit for population size (N = 96). As we change the number of females, we also change the number of males to maintain the observed sex ratio, which is very near unity. The sample size remains constant, so the proportion of the population sampled varies as the population size changes. The values presented in this table are the means for mating success (Xms), reproductive success (Xrs), the corresponding standard deviations (σms and σrs), the opportunity for sexual selection (I), the opportunity for selection (I′), the absolute and standardized Bateman gradients (βss and β′ss), and the maximum intensity of precopulatory sexual selection (s′max). We also present bootstrap estimates of 95% and 90% confidence intervals for each variable. Point estimates are shown in boldface type, and confidence intervals are in normal type.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Xms</th>
<th>Xrs</th>
<th>σms</th>
<th>σrs</th>
<th>I</th>
<th>I′</th>
<th>βss</th>
<th>β′ss</th>
<th>s′max</th>
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<tbody>
<tr>
<td>Males</td>
<td>1.8</td>
<td>330.5</td>
<td>0.79</td>
<td>162.9</td>
<td>0.19</td>
<td>0.24</td>
<td>129.4</td>
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<td>95% CI</td>
<td>1.5–2.1</td>
<td>267–387</td>
<td>0.56–0.98</td>
<td>122–197</td>
<td>0.09–0.38</td>
<td>0.11–0.44</td>
<td>46–184</td>
<td>0.26–0.97</td>
<td>0.09–0.53</td>
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<tr>
<td>90% CI</td>
<td>1.5–2.0</td>
<td>277–380</td>
<td>0.59–0.95</td>
<td>127–189</td>
<td>0.10–0.34</td>
<td>0.13–0.41</td>
<td>62–178</td>
<td>0.34–0.93</td>
<td>0.13–0.48</td>
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<tr>
<td>Females</td>
<td></td>
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<td>0.88</td>
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<td>155.3</td>
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<td>241–370</td>
<td>0.62–1.09</td>
<td>118–234</td>
<td>0.20–0.38</td>
<td>0.18–0.56</td>
<td>89–205</td>
<td>0.49–1.12</td>
<td>0.23–0.64</td>
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<tr>
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<td>253–355</td>
<td>0.67–1.05</td>
<td>125–224</td>
<td>0.21–0.37</td>
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<td>0.82–1.25</td>
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<tr>
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<td>1.38–2.03</td>
<td>216–396</td>
<td>0.68–1.47</td>
<td>0.49–1.64</td>
<td>125–192</td>
<td>0.68–1.04</td>
<td>0.60–1.21</td>
</tr>
<tr>
<td>90% CI</td>
<td>1.4–1.9</td>
<td>260–356</td>
<td>1.46–2.03</td>
<td>233–389</td>
<td>0.76–1.45</td>
<td>0.57–1.59</td>
<td>130–191</td>
<td>0.70–1.03</td>
<td>0.66–1.19</td>
</tr>
<tr>
<td>N = 53</td>
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<tr>
<td>Females</td>
<td>1.7</td>
<td>309.9</td>
<td>1.41</td>
<td>258.4</td>
<td>0.71</td>
<td>0.72</td>
<td>165.5</td>
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<tr>
<td>95% CI</td>
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<td>1.05–1.62</td>
<td>165–323</td>
<td>0.39–0.94</td>
<td>0.29–1.10</td>
<td>118–195</td>
<td>0.63–1.05</td>
<td>0.44–0.97</td>
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<tr>
<td>90% CI</td>
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<td>260–356</td>
<td>1.15–1.60</td>
<td>187–314</td>
<td>0.47–0.91</td>
<td>0.37–1.04</td>
<td>126–193</td>
<td>0.68–1.04</td>
<td>0.52–0.94</td>
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<td>N = 96</td>
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<tr>
<td>Females</td>
<td>1.7</td>
<td>309.9</td>
<td>2.01</td>
<td>356.2</td>
<td>1.44</td>
<td>1.35</td>
<td>166.9</td>
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<tr>
<td>95% CI</td>
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<td>1.68–2.05</td>
<td>270–412</td>
<td>1.01–1.51</td>
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<td>0.71–1.03</td>
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<td>260–356</td>
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<td>288–409</td>
<td>1.18–1.50</td>
<td>0.87–1.75</td>
<td>137–190</td>
<td>0.74–1.02</td>
<td>0.85–1.25</td>
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</table>

Mating and reproductive successes of individual females implied from the parentage analysis were almost certainly biased (Table S1). One additional interesting observation from Table S3 is that the adult females we collected in our sample appear to be drawn from a different distribution of mating success compared to the inferred females. This observation suggests that the demographics and movement patterns of dusky pipefish during the breeding season may be complex and are worthy topics of study. Given these considerations, our estimates of the local breeding population size should be considered provisional, pending the results of detailed studies of dusky pipefish reproductive ecology.

We calculated estimates of mating system variables for females from our raw data and compared them to the results produced by our new estimation procedure (Table 1). The raw data included the mating and reproductive success of all females inferred to have mated with collected males (Table S3), and we calculated the variables by either excluding or including the 19 females with observed mating successes of zero. Some of the females with nonzero mating success actually existed in our collection, but some of them were females we inferred to exist by the presence of their genotypes (Table S3). In other words, we did not possess the actual female, but the fact that her genotype appeared in the progeny of a male meant that such a female must have existed in the population at the time the male mated. The Bateman gradient derived from the raw data agreed surprisingly well with the Bateman gradients we estimated, regardless of whether we included females with apparent mating success of zero (Table 1). Based on our simulation results above, this pattern is expected if the slope is steep. However, the variances in mating and reproductive success were substantially lower when calculated from
the raw data compared to those produced from the estimation procedure. These discrepancies were not merely statistical error, as reflected by the fact that the confidence intervals for values calculated from the raw data did not overlap the confidence intervals produced by our estimation procedure (Table 1). Furthermore, the values of \( s'_{\text{max}} \), \( I_s \), \( I_m \) and \( s'_{\text{max}} \) calculated from the raw data without zeros were much lower than those produced by our estimation procedure. When we included the females with apparent mating success of zero, \( I_s \), \( I_m \) and \( s'_{\text{max}} \) happened to fall very close to the values produced by our maximum-likelihood approach. This situation appears to have arisen by coincidence in this particular dataset. The standard deviations in mating and reproductive success appear to be severe underestimates, but the means are also underestimated to such a degree that the values of \( I_s \), \( I_m \), and \( s'_{\text{max}} \) are close to the maximum-likelihood estimates. However, the distributions of female mating and reproductive success are clearly incorrect, with means in mating and reproductive success that are far too small to be reconciled with our observed sex ratio and mating patterns of males (Table 1). Given our simulations and other analyses, we conclude that merely calculating \( I_s \), \( I_m \), and \( s'_{\text{max}} \) from the raw data including individuals with apparent mating success of zero will not generally correct the bias problem.

We also found that our estimation procedure was sensitive to the estimate of the local breeding population size. We produced estimates of mating system variables assuming the point estimate of population size from the mark-recapture data and compared them to estimates obtained from the lower and upper 95% confidence limits of population size (Table 1). Estimates of the Bateman gradient were almost unaffected by assumptions about breeding population size, but estimates of the variances in mating and reproductive success, as well as those of \( I_s \), \( I_m \), and \( s'_{\text{max}} \), were sensitive to assumptions regarding population size.

COMPARISON OF MALE AND FEMALE MATING PATTERNS

Dusky pipefish in our study population were characterized by a polygynandrous mating system, with successful males and females both showing a tendency to mate with multiple partners. However, male and female mating patterns did differ in some important ways. For instance, the distribution of male mating success showed a relatively low variance (Table 1), with a large proportion of males mating with two females (Fig. 2A) and a range of 0–3 mates per male. In the female case, the raw data showed a pattern of mating that also seemed to imply a relatively low variance (Fig. 2B). Because the raw data included genetically inferred mating events from an incompletely sampled population, the fact that we observed one female with four mates in our sample implied that some females likely mated with more than four males. This intuition was supported by our estimated distribution of mating success for females (Fig. 2B), which showed a large proportion of females that failed to mate and a small number of females with greater than four mates each. Potential reproductive rates of dusky pipefish have not been studied, but data from \( S. \) typhle, a related species with a similar mating system (Jones and Avise 1997b; Jones et al. 1999), show that large females can completely fill 2.7 males on average during the course of a male pregnancy (Berglund and Rosenqvist 1990). Because each mating typically fills only part of a male’s brood pouch in these polygynandrous pipefish, a maximum number of mates of seven for females, as implied by our estimate of the mating success distribution, appears reasonable.

Female dusky pipefish exhibited much higher variances in mating and reproductive success compared to males in our population (Table 1). Our best estimates of \( I_s \) and \( I_m \), based on the point estimate of adult female population size of 74, showed these values for females to be 6.5 and 4.8 times those of males, respectively. The nonoverlapping confidence intervals indicated that female values were significantly higher than those of males. Only in the case of the smallest population size (\( N = 53 \) breeding females) did the 90% confidence interval for \( I \) in females overlap that in males, and even then the overlap was slight. Regardless of assumptions regarding population size, the values of \( I \), for males and females had nonoverlapping 95% confidence intervals.

The Bateman gradients in the two sexes of dusky pipefish proved to be surprisingly similar (Fig. 3). Males gained an average of 129.4 offspring per mating, whereas females gained an
average of 166 offspring per mating, according to our estimated values (Table 1). Our study provided no convincing evidence that the female Bateman gradient was significantly steeper than that of males. However, we did find that the maximum strength of precopulatory sexual selection, as measured by $s'_{\text{max}}$, was significantly higher for females than for males in our study population (Table 1). This difference was driven primarily by the larger magnitude of $I_s$ in females compared to males.

**Discussion**

This article makes three major contributions to the study of mating systems and sexual selection. First, we demonstrate a major problem with the estimation of Bateman’s principles from open populations. Our results suggest that a systematic over- or under-estimation of variances in mating and reproductive success, as well as the Bateman gradient, may occur whenever some groups of offspring are missing from the sample subjected to parentage analysis. In the case of pipefish, the female is the affected sex, because females can deposit eggs in the pouches of multiple males, some of which will almost certainly be missing from any study of a wild population. In other taxa, this problem could affect either or both sexes, depending on the pattern of parental care and the method of sampling for offspring and adults. Second, we suggest a solution to this problem of bias by developing a maximum-likelihood procedure to estimate the distributions of mating success and reproductive success, as well as the Bateman gradients, from samples with missing clutches. Third, we apply this novel approach to provide the first estimates of Bateman’s principles for both sexes from a natural population of a sex-role-reversed pipefish. Our results show that females have higher variances in mating and reproductive success compared to males, which translate into opportunities for sexual selection ($I_s$) and selection ($I$) in females that are at least fourfold higher than those of males. However, we did not find strong evidence that Bateman gradients ($\beta_{ss}$) were steeper in females than in males, emphasizing that all three of Bateman’s principles should be calculated and considered together in evaluating the role of the mating system in sexual selection. The maximum intensity of precopulatory sexual selection, as measured by $s'_{\text{max}}$, provides one way to combine $I_s$ and $\beta_{ss}$, and we found that $s'_{\text{max}}$ was about three times larger in female than in male dusky pipefish, supporting the conventional wisdom that the potential for sexual selection is greater in females of this species.

Bias of the type we have identified is potentially a major problem in the estimation of mating system variables from natural populations. Different types of organisms will be prone to different sorts of bias. For instance, in live-bearing fishes, such as guppies and swordtails, pregnant females can be collected, so males will be the sex subject to bias unless virtually the entire breeding population can be sampled (Taterenkov et al. 2008). In species with male parental care, such as pipefish or sea spiders (Barreto and Avise 2011, Mobley et al. 2011), males can be collected with their offspring, so females will be the sex subject to bias. In many species, however, neither parent can be collected with the offspring. In these cases, both sexes will be affected.
instance, in a study of the brood-parasitic brown-headed cowbird (Molothrus ater), Woolfenden et al. (2002) conducted intensive sampling for cowbird eggs in the nests of several host species over the course of six breeding seasons. Their sampling efforts were extremely successful considering they were dealing with an open population. Of 299 genotyped offspring, they identified the male parent in 227 cases (76%) and the female parent in 201 cases (67%). Thus, even in this exceptionally complete study, some parents certainly remained unsampled and an unknown number of offspring escaped detection. The results of the study showed significantly positive Bateman gradients and high opportunities for sexual selection in both sexes. Without further analysis, we have no way of knowing how close the values reported by Woolfenden et al. (2002) are to the true values, but our results suggest that missing offspring will result in steep estimates of the Bateman gradient, possibly for both sexes, regardless of the true slope. Missing offspring also could result in either over- or underestimates of the variances in mating and reproductive success, depending on the magnitudes of the true values. At the very least our study suggests a need for great caution in estimating and comparing any mating system measure based on the variance in mating success, the variance in reproductive success, or the relationship between mating and reproductive success in open populations.

Our estimation procedure provides one solution to the bias that potentially plagues studies of mating systems involving open populations. The first step in the procedure involves estimating the standard deviation in mating success, and we accomplish this goal by using explicit equations for the probability of the observed data, given a hypothesized distribution of mating success (Appendix). One major constraint in our approach is the choice of the hypothesized mating success distribution. We use a Gaussian distribution in which values are rounded to the nearest whole number and negative values are assigned a mating success of zero. This approach offers the advantage of allowing a single distribution to approximate a range of variances from near zero to an arbitrarily large value. The resulting distribution is similar to a binomial or negative binomial distribution, depending on whether the variance is smaller or larger than the mean, but it somewhat ameliorates the arbitrary limit on the maximum opportunity for sexual selection imposed by these discrete distributions. In the case of a binomial distribution, of course, the variance is always smaller than the mean, so the opportunity for sexual selection will be less than unity unless the mean mating success is very small, which will only happen when many individuals of both sexes fail to mate or when the sex ratio is extremely skewed. For a negative binomial distribution, the variance is always larger than the mean, but the maximum value of \( I_s \) for a negative binomial can be shown to be \( (X_{ms} + 1)/X_{ms} \). Thus, as mean mating success increases, the maximum value of \( I_s \) possible under a negative binomial distribution of mating success converges toward a value of one. This maximum is partially, but not completely alleviated, by our use of a Gaussian distribution. Eventually, \( I_s \) asymptotes in our model as well. As the variance in mating success increases, more and more individuals accumulate in the zero class and further increases in the variance have smaller and smaller effects on \( I_s \).

These limitations associated with the choice of mating success distributions are probably not a major source of concern in the present analysis, as constraints associated with the energetic cost of producing eggs likely prevent female pipefish from having an extremely large number of mates (Berglund et al. 1989; Berglund and Rosenqvist 1990). If the maximum number of mates for females is relatively modest, then the distribution of female mating success is probably reasonably well approximated by a negative binomial or our Gaussian distribution. However, some taxa are characterized by mating success distributions that are quite different from those considered in the present study. In species such as elephant seals, for instance, in which many males have extremely low mating success while a few males have extremely high mating success (Le Boeuf and Reiter 1988; Fabiani et al. 2004), the approach we have described here would probably produce poor estimates of the mating success distribution. However, in these cases the method could be adapted by using different underlying distributions of mating success, including nonparametric ones if necessary.

In terms of syngnathid mating systems, our results are novel because they provide the first reliable estimates of mating system measures based on Bateman’s principles for both sexes from a natural population of pipefish. Our results are consistent with previous studies showing that \( S. floridae \) is polygynandrous, with males and females mating with an average of 1.8 and 1.7 mates per male pregnancy, respectively. These values are well within the range of average values of male mating success observed across five populations of dusky pipefish studied along the Gulf and Atlantic Coasts of North America (Mobley and Jones 2009). Mobley and Jones (2009) found that dusky pipefish male mating success ranged from 1.06 to 2.52 mates per pregnancy and that average female mating success was quite variable depending on the sex ratio. Interestingly, Mobley and Jones (2009) also included a sample from the same site we used for the present study and found a mean male mating success of 1.06. Their sample was collected in late August of 2005, so it was from a year earlier, but about a month later in the breeding season, compared to the sample analyzed in the present study. The vast majority of the males from the 2005 sample had received eggs from only a single female, whereas we found in the present study that most males had mated with two or three females. These differences suggest that dusky pipefish mating systems show temporal variation, either within breeding seasons, between breeding seasons, or both. Further work will be necessary to clarify exactly how and over what time scales these mating patterns vary.
Our estimates of measures based on Bateman’s principles in *S. floridiae* are similar in some ways to those derived from captive breeding populations of *S. typhle*. For instance, we found that female Bateman gradients were significantly steeper than zero and that females exhibited large opportunities for sexual selection and selection. These results are in line with those reported from experimental studies of *S. typhle* (Jones et al. 2000, 2005), and they suggest that dusky pipefish females have the potential to experience very strong sexual selection. Our best estimates of $I$, and $I$ (Table 1) are more similar to the values observed in populations of *S. typhle* with an excess of females compared to populations with an even sex ratio or an excess of males. This observation lends additional support to the idea that natural populations of polygynandrous pipefish are typically characterized by female-biased operational sex ratios (Vincent et al. 1994). Our results for males are also similar to those derived from captive populations of *S. typhle* (Jones et al. 2000, 2005). For instance, male dusky pipefish, like *S. typhle*, exhibit a significantly positive Bateman gradient and relatively low values of $I$, and $I$. The exception to this pattern occurs under a male-biased sex ratio in *S. typhle*, where the values of $I$, and $I$ increase to 0.9 and 1.2, respectively (Jones et al. 2005).

The major difference between our study and the experimental studies of *S. typhle* is that our dusky pipefish female Bateman gradient is not significantly greater than that of males. The steep Bateman gradients of female pipefish are usually interpreted as evidence of strong competition for mates among females, and indeed this interpretation is well supported by field and laboratory studies. Female pipefish in the Genus *Syngnathus* tend to have much higher potential reproductive rates than males and most females collected during the breeding season are capable of mating immediately upon capture, suggesting that females are more limited by access to empty brood pouches than by their intrinsic ability to produce eggs (Berglund et al. 1989; Vincent et al. 1994; Scobell et al. 2009). For males, however, the interpretation of a steep Bateman gradient is less transparent. One possibility is that the steep Bateman gradient provides evidence that male reproductive success is limited in part by access to mates, and this interpretation is supported to some degree by the observation that females of *S. typhle* exhibit preferences for larger males (Berglund et al. 1986). Another equally plausible interpretation, however, is that larger males, possessing larger brood pouches, require more matings to fill their pouches compared to smaller males. This situation would result in a positive correlation between mating success and reproductive success without the need to invoke competition for access to mates. These observations underscore the need to consider $I$, $I$, and $\beta_{SS}$ in concert in the characterization of mating systems.

In summary, this study shows that care must be taken in estimating mating system measures based on Bateman’s principles, or any other metric that relies on variances in mating or reproductive success, when some offspring are missing from the sample subjected to parentage analysis. Even when a relatively small percentage of offspring or clutches is missing, estimates of the variances in mating and reproductive success, as well as the regression of reproductive success on mating success, can be severely biased. We also suggest a possible solution to this problem, based on maximum likelihood and approximate Bayesian computation, and apply our new approach to parentage data from a natural population of pipefish. Our results show that both males and females exhibit Bateman gradients that are significantly positive, but the total potential for precopulatory sexual selection in females is at least three times higher than in males in this species, confirming that dusky pipefish are sex-role reversed. Our approach should facilitate future comparative studies of mating systems, hopefully contributing to a better understanding of the causes and consequences of mating system evolution within and among populations and species.

**ACKNOWLEDGMENTS**

We thank N. Ratterman, C. Small, and C. Engelmen for help collecting samples. Dr. R. Fuller and three anonymous referees provided valuable suggestions for improvement to our manuscript. This work was supported by a grant from the National Science Foundation to AGJ.

**LITERATURE CITED**


Associate Editor: R. Fuller

Appendix. Estimation of Mating System Variables in Open Populations

**ESTIMATION OF THE VARIANCE IN MATING SUCCESS FROM A SAMPLE**

Here, we develop an approach to produce a maximum-likelihood estimate of the true variance in mating success when only a sample of clutches is available for parentage analysis. We describe the approach for pipefish, in which males are pregnant and can
be collected with their full complement of progeny and females are the sex for which mating and reproductive success must be inferred from the clutches present in the males. Thus, female pipefish suffer from the bias problem described in this article and males do not. For species in which females carry the offspring, the same logic obviously applies but the issues involving males and females will be reversed relative to pipefish. From an empirical standpoint, the approach we outline here requires the following types of data: (1) an estimate of the average number of mates per male (the sex collected with the groups of offspring), a value that is easily calculated from the parentage analysis, (2) the apparent mating and reproductive success of each female that deposited eggs in the males, inferred from the parentage analysis (many of these values will be underestimates unless all males have been collected), (3) an estimate of the sex ratio, and (4) an estimate of the local breeding population size.

To accomplish our goal, we need an expression for the probability of the observed data given a hypothesized distribution of mating success. Actual studies of parentage in nature typically sample without replacement, so the hypergeometric probability formula is appropriate for our purposes. If we define a clutch as a batch of offspring from the same mother and father, then each clutch will be arranged in a single male’s brood pouch, possibly with other clutches from different mothers. Each pregnant male will contain one or more clutches, and some clutches will escape sampling if some males are not assayed. We define \( M_j \) as the proportion of females that mothered \( j \) clutches in the total population. However, some of these mothers will have fewer clutches represented in our sample than they mothered in the population at large. If we define \( S_i \) as the probability that a female will have an apparent mating success of \( i \) in our sample, then we can calculate \( S_i \) directly by using the following equation:

\[
S_i = \sum_j f(i; N, j, n)M_j.\tag{1}
\]

The term \( f(i; N, j, n) \) is given by the hypergeometric probability formula as follows:

\[
f(i; N, j, n) = \frac{j! (N-j)! (n-i)! (N-i)!}{n! (j-i)!}. \tag{2}
\]

where \( N \) is the total number of clutches in the population and \( n \) is the number of clutches in the sample. Thus, for a given hypothesized distribution of mating success in the total population, described by the values of \( M_j \) over all \( j \), we can calculate a distribution of apparent mating success in a sample of \( n \) clutches, and this distribution is given by \( S_i \) over all \( i > 0 \). These \( S_i \) values are not directly estimable from the empirical data, but their calculation is a necessary step to obtain the probability of the data given a hypothesized mating success distribution (see eq. 3 below). In addition, the value of \( S_0 \) is not used in our procedure, and our approach could be used even if no physical females were collected, provided a large proportion of the males were available for assay, the sex ratio was known, and an estimate of the population size was available.

For the estimation procedure, we will construct hypothesized mating success distributions \( (M_j) \) by assuming an approximately Gaussian distribution of mating success, as we did in the simulation procedure described in the main text. The total probability of the observed dataset can then be approximated by using multinomial probabilities, given the values of \( S_i \) calculated from equation (1). We define \( m \) as the total number of females with offspring in our sample and \( m_k \) as the number of females in our dataset with apparent mating success \( k \) in the parentage analysis. Thus, \( m_1 \) is the number of females inferred to have mated once in the parentage analysis, whether the physical specimen was collected or not, \( m_2 \) counts the number of females inferred to have mated twice, and so on. The values of \( m_k \) for our study can be discerned from Table S3 by counting the number of females with different values of mating success. Thus, \( m_1 = 19 \), \( m_2 = 6 \), \( m_3 = 5 \), and \( m_4 = 1 \) in our study. For any hypothesized mating success distribution, then, we can obtain the probability of observing our data as:

\[
\Pr(X_1 = m_1, X_2 = m_2, \ldots, X_k = m_k) = \frac{m!}{m_1!m_2!\cdots m_k!} (S_1)^{m_1} (S_2)^{m_2} \cdots (S_k)^{m_k}. \tag{3}
\]

To find the maximum likelihood solution of the standard deviation in mating success, we first use the observation that mean mating success of females can be calculated from the mean mating success of the assayed pregnant males and the sex ratio of the sample of adults. We then step through different values of the standard deviation in mating success and calculate the probability of the observed data at each step. We consider values of the standard deviation ranging from zero to three times the mean mating success (before truncation and rounding of the distribution), divided into 500 equal increments. The distribution of mating success that gives the highest probability according to equation (3) provides the maximum-likelihood estimate of the standard deviation in mating success. If the estimate is at the upper end of the range of standard deviations considered, then the range can be expanded to include even larger values.

Note that this estimation procedure requires data for only those females with nonzero observed mating success in the parentage data. The justification for this approach is that the number of females who actually failed to mate is unknowable from the parentage data without knowledge of the mean mating success of the females who did mate successfully, a value that cannot be
obtained directly from the parentage data (Table S1). Thus, the zero mating success class arises in our estimation procedure as a natural consequence of the variance in the distribution of mating success. That is, as more females are estimated to have high mating success, some females must have low (possibly including zero) mating success to achieve the correct mean mating success. This constraint was appreciated by Wade and Shuster (2004), but their solution implicitly assumes strict polygyny or polyandry, in which members of one sex have a maximum mating success of one, and is therefore not applicable to polygynandrous species such as the dusky pipefish.

In summary, the estimation of the mating system distribution for females in pipefish requires the following steps: (1) collect a sample of pregnant males and subject them to parentage analysis. (2) Calculate the mean mating success of males. (3) Estimate the sex ratio of breeding adults. (4) Use the mean mating success of males and the sex ratio to calculate the mean mating success of females. (5) Estimate \( N_f \), the local breeding population size (we accomplished this by using a genetic mark-recapture approach). (6) Step through hypothetical mating success distributions (described by \( M_j \)) and calculate the probability of the observed mating success distribution for females (given by \( m_k \) values). These hypothetical mating system distributions could in principle follow any desired distribution, including nonparametric ones, but a major constraint is that the mean female mating success must be reconciled with the mean male mating success and the sex ratio. (7) Finally, the hypothesized mating success distribution that maximizes the value of equation (3) provides the maximum likelihood estimate of the female mating success distribution. In our study, we bootstrapped across females to produce 90% and 95% confidence intervals for the variance in female mating success.

**ESTIMATION OF THE VARIANCE IN REPRODUCTIVE SUCCESS AND THE BATEMAN GRADIENT**

Once an estimate for the distribution of mating success is obtained, we jointly estimate the variance in reproductive success and the Bateman gradient. We adopt an approach similar to approximate Bayesian computation (Beaumont et al. 2002), in which we simulate populations under different parameter values, sample from the populations according to the sampling regime used in the actual study, and calculate summary statistics from the simulated samples. The simulated populations with sample summary statistics most similar to those calculated from the empirical dataset provide the maximum-likelihood estimates of the parameters of interest.

For each iteration of the estimation procedure, we simulated mating and reproductive success for a population of breeding female pipefish. This procedure involved the following steps:

1. For a population of \( N_f \) females, where \( N_f \) is the estimated number of females in the local breeding population, each simulated female’s mating success was drawn at random from the mating success distribution estimated from the procedure described above (see section Estimation of the Variance in Mating Success from a Sample).
2. Each of the \( N_f \) females was assigned a value for reproductive success based on a hypothesized slope of the relationship between mating success and reproductive success and a hypothesized variance in reproductive success. In this estimation procedure, we stepped through a large number of hypothesized slopes and variances that bracketed the range of realistic values (see below). The mean reproductive success of females can be calculated from knowledge of the sex ratio and the mean reproductive success of pregnant males, so we constrained the values to adhere to this known mean. We can achieve any desired slope by realizing that the relationship between mating and reproductive success must pass through the bivariate mean and assuming a linear change (or no change in the case of a zero slope) in reproductive success as mating success departs from the mean. This approach gives an expected reproductive success for each value of mating success. We drew a random number from a Gaussian distribution with this mean and a specified variance in reproductive success to simulate each female’s reproductive success. At this point in the estimation procedure, we have a population of \( N_f \) females with simulated values of mating success and reproductive success, and this population adheres to a hypothesized value for the slope of reproductive success on mating success and a hypothesized variance in reproductive success.

3. We sampled clutches at random from the simulated population without replacement, as would occur in an actual study that sampled pregnant males from a natural population. In other words, each simulated female had a number of clutches in the population equal to her mating success and each clutch contained a proportion of her reproductive success (which we assumed was split equally between clutches). We sampled the same number of clutches that were sampled in the actual study (i.e., the number of males times the mean mating success of males). From the clutches sampled from the simulated population, we were able to calculate apparent values of mating success and reproductive success for each simulated female with clutches appearing in the sample. In short, the simulation procedure attempted to replicate as closely as possible the empirical steps used in the study to generate the actual dataset.

4. From the simulated sample of clutches, we calculated several summary statistics and compared them to the same summary statistics calculated from the empirical dataset.
The variables we used in this analysis were the slope of the least-squares regression of reproductive success on mating success, the overall observed variance in reproductive success among females in the sample, and the observed variance among females within each mating success category. We assessed the fit of the simulated data to the observed data by calculating a $\chi^2$-test statistic across these summary statistics, with the actual dataset providing the expected values and the simulated data providing the observed values. The values for the observed variance within each mating success category were weighted by the observed proportion of females in each category. For any expected value less than 1, we divide by one rather than the expected value to avoid instability in our estimates caused by low expected values. The value of the $\chi^2$-test statistic was recorded for the given combination of parameter values in the simulated population.

(5) Finally, we repeated steps (1) through (4) for a wide variety of hypothesized slopes and variances in reproductive success, and the simulated population with the lowest value of the $\chi^2$-test statistic provided the maximum-likelihood estimate for the slope of the Bateman gradient and the variance in reproductive success. For this analysis, we allowed the hypothetical standard deviation in reproductive success to range from zero to three times the mean. We also allowed the slope of the Bateman gradient to range from a positive value 20% steeper than a slope that would pass through the origin to a negative value of equal absolute magnitude. We divide the range of values for the standard deviation in reproductive success into 40 equal intervals, and the values for the slope of the Bateman gradient into 40 equal intervals, for a total of 1600 different combinations of values for the two parameters of interest. For each combination of parameters, we simulated 500 independent breeding populations, and averaged across these 500 replicates for the tests of goodness of fit.

To produce 90% and 95% confidence intervals for our estimates, we bootstrapped across females in the dataset (1000 replicates). For each bootstrap replicate, we resampled females with replacement and performed the entire estimation procedure, including the estimation of the mating success distribution, the estimation of the standard deviation in reproductive success, and the estimation of the Bateman gradient. Thus, any uncertainty in the estimation of the mating success distribution is included in our confidence intervals for the standard deviation in reproductive success and the Bateman gradient. All of the above procedures were implemented in a custom, Windows-based computer program written in C++. The source code and program are available by request from the corresponding author.

**Supporting Information**

Additional Supporting information may be found in the online version of this article at the publisher’s website:

- **Table S1.** A simulated dataset illustrating the issues that arise from incomplete sampling of parents.
- **Table S2.** Parentage data for the males used in this study.
- **Table S3.** Raw data for female mating and reproductive success inferred from the parentage analysis.