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The Contributions of Premating and Postmating Selection Episodes to Total Selection in Sex-Role-Reversed Gulf Pipefish

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abstract: Empirical studies of sexual selection often focus on events occurring either before or after mating but rarely both and consequently may fail to discern the relative magnitudes and interactions of premating and postmating episodes of selection. Here, we simultaneously quantify premating and postmating selection in the sex-role-reversed Gulf pipefish by using a microsatellite-based analysis of parentage in experimental populations. Female pipefish exhibited an opportunity for selection (I) of 1.64, which was higher than that of males (0.35). Decompositions of I and the selection differential on body size showed that over 95% of the selection on females arose from the premating phase. We also found evidence for a trade-off between selection phases, where multiply mating females had significantly lower offspring survivorship compared to singly mated females. In males, variance in relative fitness arose mainly from the number of eggs received per copulation and a small number of males who failed to mate. Overall, our study exemplifies a general approach for the decomposition of total selection into premating and postmating phases to understand the interplay among components of natural and sexual selection that conspire to shape sexually selected traits.

Keywords: sexual selection, opportunity for selection, parentage, cryptic mate choice.

Beginning with Charles Darwin’s first description of sexual selection (Darwin 1859, 1871), theoretical and empirical studies in this arena have focused largely on how events occurring before mating, such as female choice and male-male competition, affect reproductive success (Andersson 1994; Andersson and Simmons 2006; Jones and Ratterman 2009). Over the last several decades, research on mating competition has been linked with formal selection theory to produce a powerful set of inferential tools for the study of precopulatory sexual selection of the type Darwin originally described (Wade 1979; Wade and Arnold 1980; Arnold and Wade 1984a, 1984b; Hamon 2005; Shuster 2009). However, precopulatory mechanisms of mate choice are only part of the sexual selection story. A vast array of behavioral, morphological, and physiological traits can affect reproductive success during and after mating, and a major research enterprise has emerged and grown since the 1970s to focus attention on the fitness effects of these postmating processes (Parker 1970; Thornhill 1983; Birkhead and Pizzari 2002; Eberhard 2009). In recent years, sperm competition, cryptic female choice, and sexual conflict have taken center stage in the study of postmating processes. Any one of these mechanisms has the potential to reinforce or counteract the effects of precopulatory sexual selection. In addition, other forms of natural selection, such as fertility selection or offspring viability, have the potential to act in the same direction or in opposition to precopulatory sexual selection. Consequently, sexual selection is a complex process that does not stop at mating, as Darwin seemed to imply in his original treatise (Darwin 1871). To fully understand the total strength of selection acting on a sexually selected trait, we need to understand the mechanisms producing premating and postmating selection, including postmating natural and sexual selection, and the interactions between premating and postmating processes.

Typically, constraints imposed by experimental systems require premating and postmating events to be studied separately. However, the goal of elucidating potentially important trade-offs between premating and postmating phases of selection will require studies that measure the effects of both processes in the same individuals within a single experiment. In water striders (Gerris lacustris), premating and postmating mechanisms have been found to act antagonistically (Danielsson 2001), whereas in western rainbow fish (Melanotaenia australis), they act concor-
dantly (Young et al. 2010). Although these studies surveyed both episodes of sexual selection, they were unable to quantify the relative contributions of each episode due to empirical constraints, (e.g. limited sample sizes, an inability to assign total male fitness, or a failure to quantify selection coefficients). Thus, in many systems we are aware that both pre-mating and post-mating processes occur, and we may even know whether they reinforce or oppose one another, but we seldom know the relative magnitudes of the different phases of selection (Andersson and Simmons 2006; Hunt et al. 2009).

The theoretical and methodological framework necessary to divide total selection into its component parts exists (Arnold and Wade 1984a, 1984b), and its widespread application to empirical systems could contribute to a better understanding of the importance of pre-mating and post-mating natural and sexual selection in secondary sexual trait evolution. Three recent articles have adopted approaches related to those described by Arnold and Wade (1984a, 1984b) to decompose selection coefficients or variances in fitness into parts arising before and after mating. First, Pischedda and Rice (2012) conducted a study of Drosophila melanogaster in which they compared the variances in fitness arising from pre-copulatory and post-copulatory sexual selection. They found mating success and male fertilization success to be equivalent contributors to total male fitness. Second, Collet et al. (2012) focused on the covariance among selection episodes in Drosophila melanogaster and found no significant relationships between pre- and post-copulatory selection. They did, however, detect a positive relationship between sperm competitive success and offspring viability, suggesting that this component of natural selection acts in the same direction as post-copulatory sexual selection. Finally, Collet et al. (2012) found patterns of multiple paternity in seminatural groups of red junglefowl, Gallus gallus, to be correlated with the strength of sexual selection episodes. In replicate groups that exhibited low levels of multiple mating by females, male status, a target of pre-copulatory sexual selection, was strongly selected. However, in groups with high levels of multiple mating by females, there was little variation in the mating success of males, resulting in a stronger role for post-copulatory mechanisms and weaker overall sexual selection. This latter study demonstrates that the reproductive ecological setting can influence the relative importance of the various sources of selection.

The Gulf pipefish (Syngnathus scovelli) has been characterized sufficiently from both pre-copulatory and post-copulatory perspectives to provide a priori predictions regarding the relationships among different phases of selection. The Gulf pipefish is a sexually dimorphic species found along the Gulf of Mexico coastline and the Atlantic coast of Florida (Dawson 1985). As in all members of the family Syngnathidae (pipefishes, seahorses, and seadragons), this species exhibits unilateral male parental care. Male S. scovelli have a brood pouch on their ventral surface, which allows the males to receive eggs from the female and to carry developing offspring over the course of an approximately 2-week male pregnancy. Mature females tend to be larger than males and possess permanent iris-descent bars on their keeled abdomens that males lack (Jones and Avise 2001). The Gulf pipefish is sex role reversed in that females compete to gain access to mating opportunities with males. Molecular studies have shown that Gulf pipefish females mate with multiple males during the time frame of a male pregnancy and that males are rarely impregnated by more than one female per pregnancy (Jones and Avise 1997; Jones et al. 2001).

Studies of sexual selection in Gulf pipefish show that larger females have an advantage over smaller females in both pre-copulatory and post-copulatory phases. A micro-satellite-based study of parentage shows that field-collected males mate with the largest, most ornamented females (Jones et al. 2001), and laboratory no-choice studies indicate that mating occurs more quickly when males are given large females as potential mates as opposed to small females (Paczolt and Jones 2010). Precopulatory sexual selection on female Gulf pipefish is among the strongest ever documented for females of any species (Jones et al. 2001).

Postmating phenomena are somewhat more complex and also less well understood than pre-mating processes in Gulf pipefish. Events occurring after mating in Gulf pipefish are best understood from work on Gulf pipefish and the congeneric broad-nosed pipefish, Syngnathus typhle. In all studied species in the genus Syngnathus, males have a brief period of receptivity, after which their pouch seals irreversibly until the end of the pregnancy. Thus, even if they have an incompletely filled pouch, pregnant males cannot mate after pouch closure until they give birth. In S. typhle, males usually mate with multiple females per pregnancy and receive more eggs from their first mates, compared to later mates (Partridge et al. 2009). Males also give birth to a number of offspring substantially smaller than the number of eggs they initially receive (Ahnesjö 1992; Partridge et al. 2009), and embryos with larger mothers tend to experience higher survivorship in the brood pouch compared to eggs from smaller females (Ahnesjö 1996; Partridge et al. 2009; Mobley et al. 2011). Thus, the data thus far indicate that some form of postmating natural or sexual selection is acting in S. typhle (Sagebaken et al. 2011; Kvarnemo et al. 2011).
Gulf pipefish differ from broad-nosed pipefish in that male Gulf pipefish normally receive eggs from only one female per pregnancy, a feature that paves the way to investigate postmating selection in a slightly simpler system than that offered by *S. typhle* (Jones and Avise 1997; Jones et al. 2001). Paczolt and Jones (2010) conducted an experiment spanning two pregnancies for each focal male, in which they mated each male with two small females, two large females or a combination of large and small females. Their results showed that males preferred to mate with larger females over smaller females and that the eggs of larger females were more likely to produce viable offspring at the end of the pregnancy. They further documented a trade-off between broods, in which a male whose previous brood originated from a large female experienced a reduction in survivorship of about 12%–14% in his subsequent brood. Thus, Gulf pipefish, like broad-nosed pipefish, display enough variance in fitness among male broods that postmating selection could be important for the evolution of secondary sexual traits in females. Importantly, the strength of postmating selection in principle could be estimated and compared to premating sexual selection by partitioning the variance in relative fitness into premating and postmating components. An examination of the total magnitude of fitness variance arising from postmating processes should permit a diagnosis of the relative importance of premating and postmating processes as well as an assessment of whether these mechanisms reinforce or oppose one another.

Our goals in this study were to investigate the relative roles of premating and postmating selection in artificial breeding aggregations of Gulf pipefish. In particular, we used a microsatellite-based parentage analysis to quantify the opportunity for selection, which provides a measure of the maximum strength of selection that can operate on any trait, and to partition this measure into components arising from number of mates, number of eggs transferred per mating, and offspring survivorship within the male’s brood pouch. We also directly quantified selection differentials on male and female body size, a trait known to be a target of sexual selection in female pipefish, and we partitioned these selection differentials into premating and postmating components.

**Material and Methods**

Sexually mature female and pregnant male Gulf pipefish were collected from shallow seagrass beds in the Gulf of Mexico near Aransas Pass, Texas, using a seine net between July and October, 2010, and transported to Texas A&M University in College Station, Texas. Once in the laboratory, fish received freshwater baths to remove any external parasites, were acclimated to a salinity of 26 ppt, and were separated by sex and group housed in a flow-through system until the males gave birth. Before entering the experiment, fish were anesthetized with clove oil and marked with three visible implant fluorescent elastomer tags (Northwest Marine Technology) for identification using the protocol of Woods and Martin-Smith (2004). We used two colors, yellow and blue, and each fish received one of eight marking patterns, each of which consisted of three bands with a combination of at least one yellow and one blue mark to prevent mating preference based on marking colors. No mortalities occurred during the marking process, and marks did not affect mating success as evidenced by no difference in mating success between the eight different color combinations across replicates ($F = 0.911$, $df = 7$, $P = .506$). Photographs were taken after marking the fish on the first, eighth, and fifteenth days of the experiment to document any changes in fish size or health. The sexes were separated for 3 days to recover from the marking procedure.

Seven independent replicates were run over the course of the experiment, involving a total of 112 pipefish. Each replicate contained eight nonpregnant males and eight sexually mature females together in a 100-L tank for 14 days. Tanks were equipped with biological filters, and 10% water changes were performed daily. During each replicate, males were checked daily for pregnancies. On day 8 of a male’s pregnancy, the male was euthanized using MS222 and the eggs were dissected from the pouch. At this stage, reduced eggs that failed to develop can easily be distinguished from successfully developing embryos (Paczolt and Jones 2010). The numbers of viable embryos and reduced eggs were recorded and used to calculate embryo survivorship as the number of eggs that developed successfully divided by the total number of eggs initially transferred by the female (data available from the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.bk03n; Rose et al. 2013). To assign maternity to each brood, four embryos from the anterior end of the pouch and four from the posterior end of the pouch were preserved in ethanol for DNA extraction. We extracted DNA from the embryos by using 150 $\mu$L of a Chelex and proteinase K solution (25 mL millipure water, 199 $\mu$L of proteinase K [20 mg/mL], and 1.25 g Chelex) per embryo. The embryos were incubated in the solution at 56°C for 1 h followed by 100°C for 8 min. All females and nonpregnant males were euthanized 18 days after the replicate began. After each adult fish was euthanized, the dorsal fin was removed and preserved in ethanol for DNA extraction using the Gentra Genomic DNA Purification Kit (Qiagen).

Maternity was assigned using three hypervariable dinucleotide microsatellites (*micro25.10, micro25.22, and micro22.3*) previously developed by for *Syngnathus scovelli* (Jones and Avise 1997; Partridge et al. 2009). Microsatellite
polymerase chain reaction (PCR) conditions followed previous studies (Jones and Avise 1997; Jones et al. 1999), and PCR products were analyzed on an Applied Biosystems 3730xl DNA analyzer at the Cornell University Life Sciences Core Laboratories Center, Ithaca, New York. Microsatellite alleles were sized with Peak Scanner software. Gulf pipefish have unambiguous paternity; our goal was to use the microsatellite data to match each embryo to its unknown mother. Maternal alleles were determined by subtracting the known paternal allele from each offspring’s genotype. Each brood contained at most two maternal alleles per locus, indicating that each pregnant male had received eggs from only a single female and facilitating maternal genotype reconstruction. Each reconstructed maternal genotype matched only one of the eight females in the corresponding experimental replicate, allowing us to exclude all but one female as the mother of each brood. We assigned parentage in all broods except for those of two males whose premature deaths resulted in underdeveloped progeny from which we were unable to amplify microsatellite loci.

Standard length, the distance from the tip of the fish’s snout to the end of the caudal peduncle, was measured for each fish from the photographs taken throughout the experiment using ImageJ (National Institutes of Health, Bethesda, MD). We calculated the absolute and standardized selection differentials (s and s′, respectively) as the covariance between standard length and relative fitness (Lande and Arnold 1983). The standardized selection differential (s′) is in units of phenotypic standard deviations, while the absolute selection differential (s) is in centimeters. We decomposed the total selection differential into three successive, multiplicative episodes (Arnold and Wade 1984a), including premating selection (number of mates), followed by two episodes of postmating selection (number of eggs transferred per mating and proportion of surviving embryos). The last two episodes are averages within in-ternal genotype matched only one of the eight females in the corresponding experimental replicate, allowing us to exclude all but one female as the mother of each brood. We assigned parentage in all broods except for those of two males whose premature deaths resulted in underdeveloped progeny from which we were unable to amplify microsatellite loci.

Results

Characterization of the Gulf Pipefish Mating System

Two males died during pregnancy and two other males experienced complete brood loss (i.e., none of the progeny in their pouches survived to day 8). These other males were included in our calculation of total selection on males but not in the calculation of total selection on females, because we were unable to genotype the progeny for these failed broods. We conducted additional analyses with these broods added to either the most successful or least successful females and found that their omission had minor effects on our partitioning of selection. One female was removed from the analysis, because her only mate died shortly after mating. Thus, of the original 112 individuals, our data set contained 54 surviving males and 55 females across seven experimental replicates (Rose et al. 2013).

Including the two males that died, 51 males mated (91%) and only 5 males (9%) failed to mate. As expected, each mated male received eggs from only 1 female; within each male’s brood pouch, the four anterior end embryos and the four posterior end embryos invariably had the same mother. Males that mated did not differ significantly in standard length from unmated males (mated males: 8.7 cm, SE = 0.12; unmated males: 8.1 cm, SE = 0.38; Wilcoxon rank sum test: n = 54, P = .1563, with two males that died excluded), but with only five unmated males we had little power to detect a difference.

The parentage analysis indicated that 26 females (47%) did not mate, 15 (27%) mated with a single male and 14 (26%) mated multiply, having either two mates (18%) or three mates (7%). The mated females were significantly larger than the unmated females (mated: 9.9 cm, SE = 0.11; unmated: 9.6 cm, SE = 0.11; Student’s t-test: n = 55, P = .04). However, there was no significant difference between the size of females that mated singly versus multiply in body length (Student’s t-test: n = 29, P = .63; single: 9.96 cm, SE = 0.150; multiple: 9.86 cm, SE = 0.156) or standard body depth (Student’s t-test: n = 29, P = .97; single: 0.47 cm, SE = 0.119; multiple: 0.48 cm, SE = 0.123).

We found that 32 males received eggs from multiply mated females and 15 carried the broods of singly mated females, (excluding the two males that died and two males with complete reduction). There was no difference in latency to mate for males that mated with singly mating versus multiply mating female partners (singly: mean = 3.1 days, SE = 0.87; multiply: 3.7 days, SE = 0.59; Student’s t-test: n = 47, P = .58). However, smaller males took significantly longer to mate than larger males (n = 47, r = −0.38, P = .008). The males that had longer delays before mating also had smaller broods than the earlier mating males (n = 47, r = −0.31, P = .03), a pattern partly explained by the observation that larger males had larger broods (n = 47, r = 0.38, P = .007). Males that mated with singly mated females received significantly more eggs (Student’s t-test: n = 47, P = .0118) and also had a higher number of surviving offspring than males
mated to multiply mated females (Student’s t-test: \( n = 47, P = .0014 \)). On average, males that mated with singly mated females received 34 eggs (SE = 2.5) and had 33 surviving offspring (SE = 2.5), whereas the males that mated with multiply mated females received 26 eggs (SE = 1.7) and had an average of 23 surviving offspring (SE = 1.7; fig. 1). Males that mated with multiply mated females had a significantly higher number of reduced eggs with an average of three reduced eggs (SE = 0.5), resulting in 9% mortality of the brood, while males that mated with singly mated females lost an average of only 0.6 eggs (SE = 0.739) or 2% of the brood. Thus, males that received eggs from multiply mating females had significantly less successful broods compared to males that mated with singly mating females (Wilcoxon rank sum test: \( n = 47, P = .0241 \)).

Multiply mated females transferred an average of 59 eggs (SE = 3.0) to their mates collectively and had 53 successful offspring (SE = 3.2) with two or three mates, a figure significantly higher than that for singly mated females, who averaged 34 eggs (SE = 2.9) transferred and 33 successful offspring (SE = 3.0; fig. 2). However, multiply mated females transferred fewer eggs per mate (25.9, SE = 2.4) than singly mated females (34.1, SE = 2.3), a statistically significant difference (Student’s t-test: \( n = 29, P = .009 \)). With respect to postmating selection, multiply mated females had a significantly higher number of eggs that failed to develop compared to singly mated females (multiply mated: mean 6.43 reduced eggs, SE = 0.68; singly mated: 0.6 reduced eggs, SE = 0.659; Wilcoxon rank sum test: \( n = 29, P < .0001 \)). As a result, singly mated females had a higher percentage of their eggs survive compared to multiply mated females (98% vs. 89% brood survivorship, respectively; fig. 2).

To determine the effects of mating order on number of eggs transferred, survivorship of consecutive broods, and size of the mates we analyzed the first two broods of multiply mated females. We included the first and second matings for females that had their first two matings occur on the same day because we were not able to determine the order of the matings. We found that both the number of eggs transferred and egg survivorship were not significantly different between a female’s first and second matings when compared using paired t-tests (number of eggs: \( n = 9, P = .383, SE = 3.13 \); egg survivorship: \( n = 9, P = .210, SE = 0.079 \)). The first mating had an average of 23 eggs with 89% survivorship, while the second mating averaged 26 eggs with 78% survivorship. We also found no difference in the size of the male mate between the first (8.5 cm) and second (8.6 cm) matings, suggesting that there was no order effect on male size (paired t-test: \( n = 9, P = .157, SE = 0.322 \)). The sample sizes for these comparisons are small so subtle differences between first and second matings for females would be difficult to detect in this study.

**Partitioning the Opportunity for Selection**

The total opportunity for selection \( (I) \), which incorporates all three episodes of selection, was much higher in females (1.641) than in males (0.345), and this difference was significant, as evidenced by nonoverlapping 95% confidence intervals (table 1). We performed the decomposition of \( I \) separately for each replicate, and table 1 shows means and confidence intervals across replicates. In females, the variance in fitness due to differences among individuals in mating success \( (I_s) \), also known as the opportunity for sexual selection, \( I_s \), was by far the most important contributor to the total opportunity for selection (table 1). Variance in mating success was responsible for 96.4% of the variance in fitness (table 1). The two phases of postmating selection that we measured, namely number of eggs transferred per mating and proportion of embryos surviving during the pregnancy, contributed only 4.0% and 0.6%, respectively, to the total opportunity for selection in females. Thus, in Gulf pipefish females, precopulatory sexual selection made a larger contribution to the total variance in relative fitness than did postmating mechanisms.

The decomposition of the total opportunity for selection in males showed two major sources of variance in fitness. The first was determined by whether or not a male mated, and this source of variance made up 43.2% of the op-
Our results for the selection differential (s) on female standard length was generated by the first episode of selection, mating success. This premating phase of sexual selection contributed to a positive covariance between female size and fitness and was 155.1% the magnitude of the total selection differential (table 2). Even though the selection differential was non-significant at \( \alpha = 0.05 \) in this case, our observation that mated females were significantly larger than unmated females (see above) suggests that this pattern is real. Both postmating phases of selection opposed premating selection, so the total selection differential was smaller than the selection differential caused by mating success alone (table 2). The selection differential arising from embryo survivorship in females was significantly negative (table 2), bolstering the notion that premating selection opposed postmating selection in female Gulf pipefish in our experiment. This result was consistent with the observation that females with greater mating success, which tended to be larger than females with lower mating success, experienced lower per capita offspring survivorship (fig. 1).

In males, the decomposition of the selection differentials showed that larger males experienced greater reproductive success (table 2). This result was significant for the standardized selection (s) differential on male standard length and nearly significant for the absolute selection differential (s, table 2). However, the source of selection on males is different from that on females. In males, there was no evidence for a relationship between body size and embryo survivorship. Rather, most of the selection differential arose from the first two episodes of selection, only the second of which was statistically significant (table 2).

**Discussion**

Our most interesting results concerned the operation of selection in females, the strongly sexually selected sex in the sex-role-reversed Gulf pipefish. Our data showed that almost all of the selection in females was attributable to premating rather than postmating selection in our experimental populations. We found this result to be surprising, because several studies have shown that postmating selection can occur in pipefish (Partridge et al. 2009; Silva et al. 2009; Paczolt and Jones 2010; Mobley et al. 2011). However, until this study, no experiments in pipefish (and few in any species) had quantified both pre- and postmating selection episodes in the same individuals as part of a single experiment. Our comprehensive approach re-
Differences among males in number of eggs received, however, could be either sexual selection, if females chose to allocate eggs strategically (Silva et al. 2009), or natural selection, if males with larger brood pouches simply accommodated larger numbers of eggs (Berglund et al. 1986b). Regardless, the total opportunity for selection on males (0.345) was about a fifth of the magnitude of the opportunity for selection on females (1.641), suggesting that sexual selection is more important in female than in male Gulf pipefish.

Characterization of the Gulf Pipefish Mating System

The results of the parentage analysis in our study parallel the findings of previous studies describing the mating system of Gulf pipefish. The vast majority of the males in the experiment mated and each of them received eggs from a single female, a result which mirrors those from microsatellite-based studies of natural populations of Gulf pipefish (Jones and Avise 2001; Jones et al. 2001). Our results also show that males, the choosier sex, tended to mate with larger females, which is the same pattern found in

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Table 1: The partitioning of the opportunity for selection in Gulf pipefish by selection episode

<table>
<thead>
<tr>
<th>Source of variance in fitness</th>
<th>Symbol</th>
<th>Male value (95% CI)</th>
<th>Male (%)</th>
<th>Female value (95% CI)</th>
<th>Female (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premating selection (mating success, ( w ))</td>
<td>( I_1 )</td>
<td>0.149 (-0.037, 0.335)</td>
<td>43.2</td>
<td>1.582 (0.878, 2.287)</td>
<td>96.4</td>
</tr>
<tr>
<td>Postmating selection arising from number of eggs transferred (eggs per mate, ( w ))</td>
<td>( I_2 )</td>
<td>0.098 (0.049, 0.147)</td>
<td>28.4</td>
<td>0.065 (0.022, 0.108)</td>
<td>4.0</td>
</tr>
<tr>
<td>Covariance between ( w_1 ) and ( w_2 ):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unweighted</td>
<td>COI(1, 2)</td>
<td>0.105 (-0.013, 0.224)</td>
<td>30.5</td>
<td>0.519 (0.383, 0.655)</td>
<td>31.6</td>
</tr>
<tr>
<td>Weighted by number of mates</td>
<td>COI(1, 2</td>
<td>0)</td>
<td>0.000 (0.000, 0.000)</td>
<td>0</td>
<td>-0.006 (-0.157, 0.028)</td>
</tr>
<tr>
<td>Change in covariance between number of eggs (( w_{1,2} )) and eggs per mate (( w )) caused by precopulatory sexual selection</td>
<td>COI(12, 2</td>
<td>1) - COI(12, 2)</td>
<td>-0.097 (-0.210, 0.017)</td>
<td>-28.0</td>
<td>-0.528 (-0.651, -0.404)</td>
</tr>
<tr>
<td>Variance in number of eggs (( w_{1,2} ))</td>
<td>Subtotal: ( I_{12} )</td>
<td>0.256 (0.072, 0.439)</td>
<td>74.1</td>
<td>1.574 (0.806, 2.341)</td>
<td>95.9</td>
</tr>
<tr>
<td>Covariance between number of eggs (( w_{1,2} )) and embryo success (( w_s )) caused by first two episodes of selection</td>
<td>( I_3 )</td>
<td>0.060 (0.004, 0.117)</td>
<td>17.5</td>
<td>0.009 (0.002, 0.016)</td>
<td>0.6</td>
</tr>
<tr>
<td>Change in covariance between total fitness (( w_{1,2,3} )) and embryo success (( w_s )) caused by first two episodes of selection</td>
<td>COI(123, 3)</td>
<td>0.122 (0.000, 0.244)</td>
<td>35.3</td>
<td>0.540 (0.406, 0.674)</td>
<td>32.9</td>
</tr>
<tr>
<td>Total opportunity for sexual selection (( w_{1,2,3} ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( I )</td>
<td></td>
<td>0.345 (0.154, 0.537)</td>
<td>100</td>
<td>1.641 (0.747, 2.535)</td>
<td>100</td>
</tr>
</tbody>
</table>

Note: The most important values are shown in boldface type, and the covariance terms are shown for the sake of completeness. Further details regarding the methods of partitioning and the interpretation of the covariance terms can be found in Arnold and Wade (1984a, 1984b). Note that the covariance terms largely cancel one another out and make at most a small contribution to the overall opportunity for selection. We calculated the partitioning for each tank separately and report the means across tanks in this table and 95% confidence intervals.
Syngnathus typhle (Berglund and Rosenqvist 1990) and another polyandrous pipefish, Nerophis ophidion (Berglund et al 1986a). This choosiness results in many females failing to mate, because females have the potential to produce many more eggs than males can fit into their brood pouches (Berglund et al. 1989; Scobell et al. 2009). In addition, females continuously mature eggs in *Syngnathus* pipefish, so mature females are almost always immediately capable of mating upon collection from the wild (Begovac and Wallace 1987). Thus, the failure of some females to mate in our experiment is not likely a consequence of female infertility.

One interesting limitation on reproduction in sex-role-reversed pipefishes is that females compete for access to mates but also bear a substantial cost of producing large eggs (Fitzpatrick et al. 1995). This energetic cost implies that successful females may experience constraints associated with the rate at which they are able to produce eggs (Berglund et al. 1989; Scobell et al. 2009; Braga Goncalves et al. 2011). Indeed, work on *Syngnathus scovelli*, *S. typhle*, and *N. ophidion* shows that females have a potential reproductive rate that is about two to three times higher than that of males (Berglund et al. 1989; Scobell et al. 2009). Thus, even the most successful females never achieve levels of mating success equal to successful males in the most polygynous species with conventional sex roles, in which some males can sire offspring with dozens of females during a breeding season (Le Boeuf 1974; Fabiani et al. 2004). Our results provide two lines of evidence that energetic constraints associated with egg production may play a role in female reproductive success in Gulf pipefish. First, the females that mated multiply transferred fewer eggs per mate on average when compared with the singly mated females (fig. 1) but had more total eggs transferred. Second, the eggs deposited by multiply mated females experienced lower average survivorship across all of their matings compared to the eggs deposited by singly mated females, suggesting lower overall survivorship rather than declining survivorship in multiply mated females (fig. 2).

That female mating success trades off with number of eggs transferred per mating and embryo survivorship could be explained by a number of possible processes. The simplest explanation might be that multiply mated females are running out of eggs and transferring some eggs before they are fully ripe, resulting in reduced embryo survivorship. This explanation is not consistent with our results, because we saw no difference in between a female’s first and second brood. Our sample size for this comparison was small (n = 9), however, as our study was not originally designed to explore this aspect of female reproductive success. Another possibility is that females exhibit a resource trade-off between investment in precopulatory displays and egg quality. Females may also strategically allocate their egg investment, in terms of number or quality, among males in a process analogous to strategic sperm allocation in species with strongly sexually selected males (Dewsbury 1982; Wedell et al. 2002). Recent work showing that female *S. typhle* females can alter the chemical composition of their eggs in response to male size supports this interpretation (Braga Goncalves et al. 2010). However, the reduced embryo survivorship we observed for multiply mating females could be partially explained by a male-mediated strategy, if males can sense their brood size and invest fewer resources in smaller broods. The patterns of brood reduction we observed in this experiment are probably not a consequence of genetic incompatibility (Zeh and Zeh 1996), as we would expect these incompatible mating events to be equally likely for all females regardless.

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**Table 2: Decomposition of selection differentials for males and females**

<table>
<thead>
<tr>
<th>Selection episode</th>
<th>Male s (95% CI)</th>
<th>%</th>
<th>Male s' (95% CI)</th>
<th>%</th>
<th>Female s (95% CI)</th>
<th>%</th>
<th>Female s' (95% CI)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premating selection</td>
<td>.034 (−.012, .079)</td>
<td>41.9</td>
<td>.087 (−.046, .220)</td>
<td>53.5</td>
<td>.140 (−.015, .295)</td>
<td>143.5</td>
<td>.209 (−.011, .429)</td>
<td>155.1</td>
</tr>
<tr>
<td>Postmating selection</td>
<td>.037 (−.001, .075)</td>
<td>46.0</td>
<td>.051 (.006, .096)</td>
<td>31.4</td>
<td>−.026 (−.093, .041)</td>
<td>26.9</td>
<td>−.044 (−.174, .086)</td>
<td>32.8</td>
</tr>
<tr>
<td>Postmating selection</td>
<td>.010 (−.044, .064)</td>
<td>12.1</td>
<td>.024 (.053, .102)</td>
<td>15.0</td>
<td>−.016 (−.029, −.004)</td>
<td>16.6</td>
<td>−.030 (−.056, −.005)</td>
<td>22.4</td>
</tr>
<tr>
<td>Total selection differential</td>
<td>.080</td>
<td>100</td>
<td>.162</td>
<td>100</td>
<td>.097</td>
<td>100</td>
<td>.135</td>
<td>100</td>
</tr>
</tbody>
</table>

Note: Here we show the contributions of mating success, number of eggs transferred per mate, and embryo survivorship to the total selection differential for standard length. We show both the absolute selection differential (s), in centimeters, and the standardized selection differential (s'), in units of phenotypic standard deviations, for each sex. Decompositions were performed separately for each experimental tank, and we show means across tanks and 95% confidence intervals (CI).
of whether they mated once or multiple times. Regardless of the underlying mechanism, and despite these apparent costs of multiple mating, females with multiple mates had significantly higher fitness than singly mated females, because each additional mate added much more fitness than the females lost due to the reduced number of eggs transferred per mate or decrease in egg survivorship.

The Relationship between Premating and Postmating Phases of Selection

Our results showed that the three episodes of selection in our experiment were not equally important in determining the total strength of selection. Our most interesting result is that postmating selection, arising from number of eggs transferred and embryo survivorship, made a small contribution to the total opportunity for selection in females. This result is especially surprising in light of recent studies that suggest that postmating selection is potentially important in Gulf pipefish and in the related broad-nosed pipefish, S. typhle. In Gulf pipefish, a previous laboratory experiment showed that males prefer to mate with larger females, males accept more eggs per mating from larger females, and eggs from larger females are more likely to result in viable offspring (Paczolt and Jones 2010). However, that experiment differed from this study in that it employed a no-choice mating design, in which some males were paired with small females for mating, which isolated the effects of postmating phenomena without the potentially obscuring effects of precopulatory sexual selection. Indeed, such an approach is frequently used in studies of postcopulatory sexual selection. In this experiment, we allowed both premating choice and postmating processes to occur in the same breeding aggregation. Consequently, males may have been able to mate with preferred females in almost every case, reducing the extent to which postmating mechanisms would become necessary in this study. This conclusion is bolstered by the observation that rates of embryo survivorship were much higher in this study than in the previous no-choice experiment of postmating selection (93% vs. 71%, respectively).

Our experimental design may have increased the likelihood of detecting a greater role of premating as opposed to postmating selection because of two important factors. First, we equalized the sex ratio and used only females that displayed their secondary sexual characters and were thus deemed ready to mate at the start of the experiment. As a consequence, males had a choice of many attractive females, and the fact that several females mated with three males suggests that the females in each tank collectively had far more eggs than the males had brooding space to accommodate. Second, our studies were conducted at a much higher breeding density than probably occurs in nature. As a result, each male had the opportunity to assess every female in the tank and mate with the best of the eight available females. We do not know how many females a male can sample in the field, but males likely incur greater costs from sampling additional mates in their natural habitats, which include predators and lower population density, than they did in our experiment. Overall, we conclude that postmating selection was not very important in the ecological setting simulated by our experimental tanks. Furthermore, because males receive eggs from only one female per pregnancy, postmating processes in Gulf pipefish do not include sperm or egg competition within a pregnancy. This aspect of the mating system also likely reduces the importance of postmating processes in Gulf pipefish compared to other species of syngnathids. Our study spanned only the length of a single male pregnancy, whereas male pipefish have repeated pregnancies during a prolonged mating season in nature. Trade-offs between male pregnancies could add another layer of complexity to postmating selection in Gulf pipefish. Thus, the extent to which postmating selection is generally important in Gulf pipefish remains an open issue, and a key question is how often males resort to mating with less attractive mates in natural populations due to demographic or ecological constraints.

This study and the few others of its kind (Droge-Young et al. 2012; Collet et al. 2012; Pischedda and Rice 2012) represent the next step in understanding the relative roles of precopulatory sexual selection and its interaction with postmating processes, including phenomena that fall into the realms of postcopulatory sexual selection, sexual conflict and natural selection. While no generalities have yet emerged due to the low number of studies to date, our results and those of Collet et al. (2012) lead us to predict that the relative importance of pre-versus postmating selection will be context dependent. We hope that researchers will adopt a standard approach to quantify sexual selection and measure total selection that facilitates easy comparison among studies. Arnold and Wade’s (1984a, 1984b) decomposition of the opportunity for selection and the selection differential provides an ideal framework. Detailed quantitative study of mating patterns and reproductive success promises to resolve the relative contributions of premating and postmating processes, as well as interactions between them, to total sexual selection.

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“At one time only (May, 1865) I found under a fallen trunk a prodigious number of workers of E. Mexicana. They were heaped and piled upon each other like the bees in a swarm. Attacking them with the end of a stick, I obliged them to disperse, but could find no entrance which they concealed, no eggs, no males nor females.” From “Notes on Mexican Ants” by Edward Norton (American Naturalist, 1868, 2:57-72).