

MALE-BY-FEMALE INTERACTIONS INFLUENCE FERTILIZATION SUCCESS AND MEDIATE THE BENEFITS OF POLYANDRY IN THE SEA URCHIN *HELIOCIDARIS ERYTHROGRAMMA*

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Abstract.—Numerous studies have reported that females benefit from mating with multiple males (polyandry) by minimizing the probability of fertilization by genetically incompatible sperm. Few, however, have directly attributed variation in female reproductive success to the fertilizing capacity of sperm. In this study we report on two experiments that investigated the benefits of polyandry and the interacting effects of males and females at fertilization in the free-spawning Australian sea urchin *Heliocidaris erythrogramma*. In the first experiment we used a paired (split clutch) experimental design and compared fertilization rates within female egg clutches under polyandry (eggs exposed to the sperm from two males simultaneously) and monandry (eggs from the same female exposed to sperm from each of the same two males separately). Our analysis revealed a significant fertilization benefit of polyandry and strong interacting effects of males and females at fertilization. Further analysis of these data strongly suggested that the higher rates of fertilization in the polyandry treatment were due to an overrepresentation of fertilizations due to the most compatible male. To further explore the interacting effects of males and females at fertilization we performed a second factorial experiment in which four males were crossed with two females (in all eight combinations). In addition to confirming that fertilization success is influenced by male \times female interactions, this latter experiment revealed that both sexes contributed significant variance to the observed patterns of fertilization. Taken together, these findings highlight the importance of male \times female interactions at fertilization and suggest that polyandry will enable females to reduce the cost of fertilization by incompatible gametes.

Key words.—Cryptic female choice, external fertilization, fecundity, genetic compatibility, multiple mating, sperm competition.

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One of the most enduring questions in evolutionary biology is why females typically mate with several males when the sperm from a single male are often sufficient to fertilize their entire complement of eggs. This question is especially pertinent for species in which there are no obvious direct benefits (e.g., nutrient donations, paternal care, territorial defense, etc.) to mating multiply. In such mating systems females are thought to obtain genetic (or indirect) benefits from polyandry (Yasui 1997; Jennions and Petrie 2000). A variety of such benefits have been proposed, although these can largely be categorized as either intrinsic, where paternal genes have an additive effect on a female's fitness via the enhanced quality of her offspring (Yasui 1997), or specific, where a female's fitness depends on the interaction between parental genotypes (Zeh and Zeh 1996, 1997, 2003; Tregenza and Wedell 2000). The former category proposes that postmating sexual selection, in the form of sperm competition (Parker 1998) or cryptic female choice (Eberhard 1996), increases the probability of fertilization by genetically superior males, who in turn pass on viability (Watson 1998; Hosken et al. 2003) or "attractiveness" genes (Curtis 1991; Keller and Reeve 1995) to their offspring. Such benefits are contingent solely on the genetic quality of sires, and postmating sexual selection will favor *intrinsically* high-quality males during sperm competition or cryptic female choice (e.g. Hosken et al. 2003). In contrast, specific benefits depend on the interacting effects of male and female genotypes (either at fertilization or subsequently during embryo or offspring development), and will generate (nondirectional) sexual selection for compatible male \times female crosses.

Increasingly, studies reporting genetic benefits of polyandry indicate that females engage in polyandry to ensure against the risk of fertilization by genetically incompatible sperm (reviews by Zeh and Zeh 1997, 2003; Tregenza and Wedell 2000). Much of this evidence is based on the findings that when females are restricted to mating repeatedly with a single male they suffer relatively high rates of hatching failure (Olsson et al. 1996; Zeh 1997; Tregenza and Wedell 1998, 2002; Newcomer et al. 1999) and/or greater variance in the quality, quantity, or hatching success of their offspring (Zeh 1997; Tregenza and Wedell 1998; Fedorka and Mousseau 2002). The conclusion that such benefits are due to the selection of compatible partners, rather than intrinsically high-quality ones, is drawn from the observation that the effects of male genotype on female reproductive success are often inconsistent among females. Few animal studies, however, have reported male \times female interactions on fertilization success directly, which will inevitably arise if postmating sexual selection mediates the merging of compatible gametes (but for examples of how male-female interactions influence fertilization in flowering plants see reviews by Barrett 2003; Bernasconi et al. 2004). Animal studies that have considered male-female interactions have tended to focus on internally fertilizing species in which the direct assessment of fertilization is problematic. For example, several insect studies have reported strong interacting effects of male and female genotypes on variance in sperm precedence, which is estimated through paternity assignment following female multiple mating (e.g., Lewis and Austad 1990; Wilson et al. 1997;

Clark et al. 1999; Nilsson et al. 2003). However, as paternity is usually estimated from offspring rather than from embryo genotypes (but see Carter et al. 2000), such studies cannot distinguish differential zygote or embryo mortality from differential sperm use at fertilization (see discussions by Gilchrist and Partridge 1997; Olsson et al. 1999; Byrne and Roberts 2000; Simmons 2001; Mack et al. 2002; Birkhead et al. 2004).

It is relatively simple to measure fertilization success directly in external fertilizers, in contrast to internally fertilizing animals. In these organisms, well-established artificial fertilization techniques have the added advantage of controlling several variables (e.g., the timing and order of mating, sperm density, and sperm-to-egg ratios), which may otherwise influence fertilization. Sea urchins provide excellent model systems for studying gamete interactions and the process of fertilization (Vacquier and Moy 1977; Zigler et al. 2003). In these species, as in other marine free spawners, specific genotype interactions between males and females can have important consequences for fertilization success (Vacquier 1998). For example, in sea urchins the sperm protein, bindin, mediates sperm-to-egg attachment and therefore strongly influences fertilization success, both among closely related species (Metz et al. 1994; Levitan 2002; McCartney and Lessios 2002) and within individual species (Palumbi 1999). Importantly, the alleles that code for these proteins often exhibit substantial intraspecific variation and thus have the potential to mediate gamete recognition at the individual level (Metz and Palumbi 1996; Biermann 1998; Palumbi 1999). Although the mechanisms maintaining this variation are unknown, eggs can show strong affinities for specific sperm types on the basis of the males' bindin genotype, leading to positive assortative fertilizations between eggs and sperm with similar genotypes (Palumbi 1999).

In this study we report the results from two experiments designed to examine the direct fertilization consequences of polyandry and the interacting effects of males and females at fertilization in the Australian sea urchin *Heliocidaris erythrogramma*. Because of the advantages of assortative pairings between particular male and female genotypes in sea urchins, we predicted that polyandry would confer fertilization advantages on females. To test this prediction we performed two artificial fertilization experiments. In the first, we manipulated female mating status and examined the consequences for fertilization success. In this initial experiment we used a split clutch design in which sperm concentrations were identical within replicate blocks but varied slightly among blocks. Hence, we were able to compare fertilization rates under both treatments and at different sperm concentrations (see Materials and Methods). Within clutches we replicated each fertilization event to ensure that fertilization success by individual males (or male pairs in the polyandry treatment) was repeatable. We predicted that the fertilization benefits of polyandry would depend on the interacting effects of male and female genotypes at fertilization: when two competing males differ in their capacity to fertilize a particular female's eggs, polyandry will confer advantages to the female because it will provide scope for assortative combinations of compatible gametes. By contrast, where the ejaculates of two males exhibit similar fertilizing capabilities with a particular female, we predicted there would be no net fertilization ben-

efit of polyandry. In the second experiment we used a factorial design to test for male \times female interaction effects on fertilization success. This latter experiment allowed us to determine whether sperm from individual males exhibited similar fertilizing capacities among different females.

MATERIALS AND METHODS

Study Species

Heliocidaris erythrogramma is a common sea urchin that is endemic to Australia and inhabits coastal waters (maximum depth 35 m) in the southern part of the continent. Eggs and sperm are freely shed into the water column where they combine and develop into free-swimming larvae that later metamorphose into benthic juveniles. During the spawning season (which lasts approximately three months during the summer) this species typically occurs in high-density, mixed-sex aggregations (Laegdsgaard et al. 1991).

For the fertilization trials we used a sperm concentration (approximately 7.0×10^5 sperm per ml^{-1} ; see below) that resulted in 13–96% of the eggs being fertilized across treatments. These values fall within the natural range for *H. erythrogramma* and other sea urchin species in similar habitats (Styan 1997; Franke et al. 2002). Indeed, sperm concentrations, sperm-egg contact times, and consequently fertilization rates in the field exhibit considerable variation in free-spawning marine invertebrates and can result in both sperm limitation and polyspermy in the same population (Franke et al. 2002; Marshall 2002). At the study population (Bare Island, Sydney), the density of urchins ranges from 1–45 individuals per m^2 (J. P. Evans and D. J. Marshall, unpubl. data), suggesting that sperm concentrations (and the likelihood of polyandry) will be highly variable under natural conditions.

Experiment 1. Fertilization Success under Polyandry and Monandry

Our first experiment had two aims. First, we compared the fertilization success of eggs exposed to the sperm from a single male (monandry) with that of eggs exposed to two males (polyandry). To test our prediction that polyandry will result in enhanced fertilization success we compared fertilization rates under both treatments within individual egg clutches. Our second aim was to test whether an interaction between male and female identity explained a significant amount of variation in fertilization success. To address both of these aims we used a factorial design in which the fertilization success of egg batches from two females (nominally f_1 and f_2) was assessed under polyandry (average fertilization success of eggs exposed to the sperm of males m_A and m_B simultaneously) and monandry (average fertilization success of eggs exposed to m_A and m_B separately). Hence, the 2 male \times 2 female crosses constituted a block (i.e., for monandrous fertilization crosses: $m_A \times f_1$; $m_A \times f_2$; $m_B \times f_1$; and $m_B \times f_2$; and for polyandrous crosses: $m_A + m_B \times f_1$ and $m_A + m_B \times f_2$), which was repeated 10 times, yielding fertilization success estimates for $N = 20$ females under monandry and polyandry. This enabled us to compare fertilization success following monandrous and polyandrous matings within individual females. Within each block the concentration of

sperm used for each of the fertilizations was identical. Among blocks, however, sperm concentrations varied slightly (see Artificial Fertilizations), allowing us to compare fertilization rates under monandry and polyandry under nominally low and high sperm concentrations (see below). Furthermore, we were able to estimate the variance components of fertilization success attributable to males, females and their interaction (see Statistical Analyses below).

Artificial fertilizations.—Reproductively mature *H. erythrogramma* were collected from Bare Island, Sydney (151°23' E, 33°99' S) in January 2004. Once in the laboratory, the urchins were induced to spawn with an intracoelomic injection of 5 ml of 0.5M KCl. Each urchin was maintained in a separate container during the spawning to prevent cross-contamination. Once the urchins had spawned, we collected the eggs and sperm in separate containers until required for the artificial fertilizations (these were always performed within 20 minutes of gamete collection). Sperm concentrations were estimated using a modified Fuch-Rosenthal hemocytometer (three replicate counts per male). Although sperm concentrations varied among blocks (mean = 6.9×10^5 sperm per ml⁻¹; range 3.5–8.9 × 10⁵), we ensured that within blocks (i.e., for the crosses: $m_A \times f_1$; $m_A \times f_2$; $m_B \times f_1$; $m_B \times f_2$; $m_A + m_B \times f_1$ and $m_A + m_B \times f_2$) they were identical.

For each block, we split the eggs from female f_1 into seven separate vials at a concentration of 50 eggs per ml⁻¹. This process was repeated for female f_2 . For the monandry treatment, two replicate vials of eggs from the female were exposed to the sperm from male m_A . A further two vials of eggs from the female were then exposed to sperm from male m_B using the same sperm concentrations. For the polyandry treatment, eggs in two vials from the female were exposed to a 1:1 mixture of sperm from both m_A and m_B simultaneously (adjusted to the same concentration as in the monandry treatment). We ensured that sperm concentrations and sperm-to-egg ratios were constant across the monandrous and polyandrous treatments within each block. Fertilization success was measured 2 h after exposure of eggs to sperm by examining approximately 50 eggs from each vial; eggs were classed as fertilized if regular cell division had occurred (see Marshall et al. 2000). For each of the two females, the remaining (seventh) vial of eggs was kept free from sperm as a control for any errant fertilizations arising from contamination by sperm (these did not exceed 1% in any replicate).

Statistical Analyses of Experiment 1

We compared fertilization success under monandry and polyandry at two sperm concentration ranges. We first allotted the blocks into a nominally high and low sperm concentration group. The median sperm concentration used in experiment 1 was 7.3×10^5 ; we assigned blocks into a low or high sperm concentration group according to whether they fell below or above this median value. We then analyzed the effects of the polyandry treatment, sperm concentration, and their interaction using a partly nested analysis of variance in which sperm concentration and monandry/polyandry treatment were fixed factors (two levels in each) and female was a blocking factor. We analyzed raw data, rather than arcsine square-root-transformed percentage fertilization data, as they

TABLE 1. Fertilization assays for the male (m) × female (f) crosses (experiment 2). The number before parentheses denotes the number of fertilizations performed for each cross.

	Male 1	Male 2	Male 3	Male 4
Female 1	2($m_1 \times f_1$)	2($m_2 \times f_1$)	2($m_3 \times f_1$)	2($m_4 \times f_1$)
Female 2	2($m_1 \times f_2$)	2($m_2 \times f_2$)	2($m_3 \times f_2$)	2($m_4 \times f_2$)

were not badly skewed and transformation did not improve the distribution.

Our design for experiment 1 involved 10 blocks of 2 males × 2 females. For each block, the percentage of eggs fertilized across the four monandrous crosses ($m_A \times f_1$; $m_A \times f_2$; $m_B \times f_1$; $m_B \times f_2$) was used to estimate the variance in fertilization success attributable to males, females, and their interaction (see Lynch and Walsh 1998, pp. 599–602).

Experiment 2. Male-by-Female Interaction Effects on Fertilization Success

Following experiment 1, which revealed a significant male × female interaction on fertilization success (see Results), we performed a second experiment that enabled us to further explore male × female interactions at fertilization across a wider range of male genotypes. We used a crossed factorial design involving four males and two females (i.e., $N = 8$ male × female crosses; Table 1). In this experiment, the eggs of each female were exposed to the sperm from each male (using a fixed concentration 7.0×10^5 sperm per ml⁻¹). We performed repeated fertilizations for each male-female pair to ensure that the fertilization success for specific male-female combinations was repeatable. The fertilization protocol and the assessment of fertilization success followed the methods described above. This simple design allowed us to investigate the interacting effects of males and females on fertilization success both graphically (see Fig. 3) and analytically, using a two-way analysis of variance (ANOVA) with the identity of males and females entered as random factors.

RESULTS

Experiment 1

Our analysis of the fertilization data from experiment 1 revealed significant differences in fertilization rates between treatments. Across both sperm concentrations, the mean fertilization success of eggs exposed to the sperm of two males simultaneously was significantly greater than the mean success for eggs exposed to sperm from each of the same two males separately (Table 2; Fig. 1). Consistent with this finding, our subsequent analysis revealed that the magnitude of the fertilization benefit in the polyandry treatment was strongly positively correlated with the difference in fertilization rates between males A and B in the monandrous treatment ($r = 0.66$, $N = 20$, $P = 0.001$; Fig. 2). This latter result indicates that females will benefit more from polyandry when ejaculates from different males vary in their capacity to fertilize eggs.

If the fertilization benefit of polyandry were due to an overrepresentation of fertilizations due to the most compatible male, the fertilization rates for the most successful males

TABLE 2. Summary of results (experiment 1) from a partly nested analysis of variance showing the effect of sperm concentration (low <730,000 sperm per ml⁻¹; high >730,000 sperm per ml⁻¹) and treatment (polyandry and monandry) on fertilization success in *Heliocidaris erythrogramma*. MS, mean square; df, degrees of freedom. *P*-values <0.05 are bold.

Source	df	MS	<i>F</i>	<i>P</i>
Between subjects				
Sperm concentration	1	0.813	5.6	0.03
Female (sperm concentration)	18	0.005		
Within subjects				
Treatment	1	0.169	33.8	<0.001
Treatment × sperm concentration	1	<0.001	0.005	0.943
Treatment × female (sperm concentration)	18	0.005	0.363	0.991
Error	80	0.012		

in the single-male treatment should be similar to the fertilization rates in the polyandrous treatment. For instance, for a given block, if the $m_A \times f_1$ cross resulted in higher fertilization success than the $m_B \times f_1$ cross, the fertilization rate arising from $m_A \times f_1$ should be similar to the fertilization rate when both males compete in the polyandrous cross (i.e., $m_A + m_B \times f_1$). In accordance with this prediction we found that within pairs of males, fertilization rates for the most successful males under monandry were not significantly different from the fertilization rates when sperm from both males were used in the polyandrous trials (paired *t*-test: $t = 0.258$, $df = 19$, $P = 0.799$).

The analysis of the variance components for fertilization success revealed a strong female effect, as well as a significant male × female interaction (Table 3). Female identity explained approximately 65% of the observed variance in fertilization success, male identity explained 13% and the interaction between males and females explained approxi-

mately 17%. The unexplained variation (i.e., within-female variability) was remarkably small (about 5%; Table 3).

Experiment 2

Consistent with the results obtained from the first experiment, our results from the 2 × 4 factorial experiment revealed a strong significant female effect on fertilization success, as well as a significant male × female interaction (Table 4; see Fig. 3). Fertilization success estimates for the repeated ($N = 2$) male × female crosses were highly repeatable (see repeated fertilization measures for each cross, Fig. 3), accounting for less than 6% of the variation in fertilization success (Table 4).

DISCUSSION

In accordance with our initial expectations we found that when eggs from female *H. erythrogramma* were assigned to the polyandry treatment they exhibited enhanced fertilization success in comparison to those assigned to the monandry treatment. We are confident that this difference in fertilization

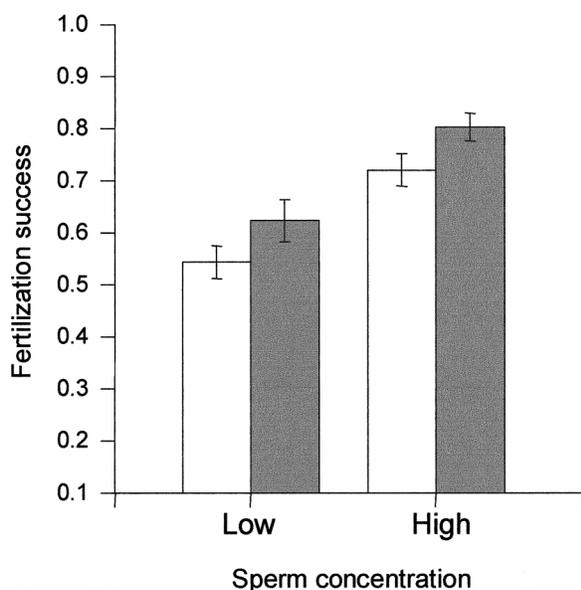


FIG. 1. The effect of polyandry and variable sperm concentrations on the fertilization success of *Heliocidaris erythrogramma*. Each bar represents the mean fertilization success (\pm SE) of females under monandry (open bars) and polyandry (shaded bars). The sperm concentrations were classed as low (<730,000 sperm per ml⁻¹) and high (>730,000 sperm per ml⁻¹).

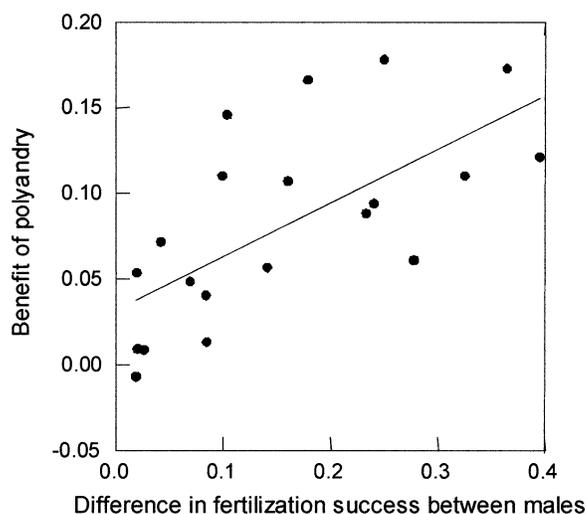


FIG. 2. The relationship between the net benefit of polyandry (net benefit = average fertilization success under polyandry – average fertilization success under monandry from both m_A and m_B) and difference in fertilization success between both males (m_A and m_B) in each replicate. Note that polyandry resulted in enhanced fertilization success in 19 of 20 cases.

TABLE 3. Summary of results from the factorial crosses in experiment 1 (crosses are for the monandry treatments: $m_A \times f_1$; $m_A \times f_2$; $m_B \times f_1$; and $m_B \times f_2$; see text for details). Variance components for each factor were calculated following Lynch and Walsh (1998). Additional test: male effect = female effect F : 3.33, $P = 0.036$. P -values <0.05 are bold.

Source	df	MS	F	P	Variance (%)
Males	10	0.056	2.36	0.096	12.97
Females	10	0.187	7.83	0.0015	65.38
Male \times female	10	0.024	7.65	<0.001	16.63
Error	40	0.003			5.01

success is a consequence of treatment effects (polyandry vs. monandry) rather than the influences of varying sperm-to-egg ratios, sperm concentrations, mating order, or differential maternal effects, because our artificial fertilization protocol enabled us to rigorously control these potentially confounding effects. Additionally, our results suggest that these fertilization benefits were mediated by the interaction between males and females at fertilization, a finding that was clearly illustrated by our second experiment from which we plotted fertilization success estimates following each specific male \times female cross (Fig. 3). Moreover, we found that the magnitude of the fertilization benefit in the polyandry treatment was positively correlated with the difference in fertilization rates between the two males in the monandrous treatment (see Fig. 2). Furthermore, the fertilization success of the most compatible male (under monandry) did not differ significantly from that under polyandry when that male's sperm were used together with those from the less compatible male. These results strongly suggest that the fertilization benefit of polyandry was due to an overrepresentation of fertilizations due to the most compatible male.

Benthic marine invertebrates, including sea urchins and other echinoderms, are known to form complex aggregations during the spawning season (Young et al. 1992; Hamel and Mercier 1995), which in turn are believed to facilitate spawning synchrony, increase local sperm concentrations and influence gamete encounter rates (Yund 2000). In the majority of species the mechanisms driving these aggregations remain largely unknown, although physical factors (moon phase, temperature, etc.) are commonly cited (Levitan 1995). However, in a number of marine invertebrate species (including echinoderms), females release sex pheromones that quickly stimulate such aggregations or otherwise influence male spawning readiness (Miller 1989; Hamel and Mercier 1996; Hardege and Bentley 1997). Furthermore, female echinoids typically modify their behavior during the spawning season; for example, by climbing structures that place them in optimal positions for expelling gametes (reviewed by Levitan 1998). Although such traits are often thought to arise as a consequence of natural selection pressure to minimize the risk of sperm limitation or egg predation, our results raise the intriguing possibility that these traits may additionally function to increase the likelihood that eggs will encounter the sperm from multiple males, thus facilitating fertilization by compatible gametes.

The findings presented here add to an increasing body of evidence that fertilization success is nonrandom with respect

TABLE 4. Summary of a two-way analysis of variance revealing the influence of males, females, and their interaction on fertilization success in the sea urchin *Heliocidaris erythrogramma* (experiment 2). Note that male identity percent variance was converted to zero because a negative variance component value was found (Lynch and Walsh 1998; Quinn and Keough 2002). P -values <0.05 are bold.

Source	df	MS	F	P	Variance (%)
Males	3	0.019	4.705	0.035	0
Females	1	0.114	28.558	0.001	57.24
Male \times female	3	0.031	7.685	0.010	37.24
Error	8	0.004			5.50

to male identity (Wilson et al. 1997; Clark et al. 1999; Palumbi 1999; Ward 2000), therefore supporting the idea that females exercise cryptic choice for compatible mating partners at the level of the gamete (Eberhard 1996; Birkhead 1998; Pitnick and Brown 2000). However, despite the strong interacting effects of males and females on fertilization success (experiment 2), we cannot rule out the possibility that, in addition to the specific benefits of polyandry highlighted by our study, females also gain intrinsic benefits from polyandry. Indeed, the results from the monandrous crosses in both experiments revealed weak male effects on fertilization (see Tables 3 and 4), although these should be interpreted cautiously given the strong interactions between males and females in both experiments. Moreover, sperm concentration, which may to some extent be controlled by males during natural spawnings, had a significant effect on fertilization success when we included this variable as a factor in our ANOVA (Table 2). Thus, in addition to aiding fertilization by compatible mating partners, polyandry may also favor males who produce more, intrinsically better, or highly competitive sperm.

Although our study was not designed to investigate the

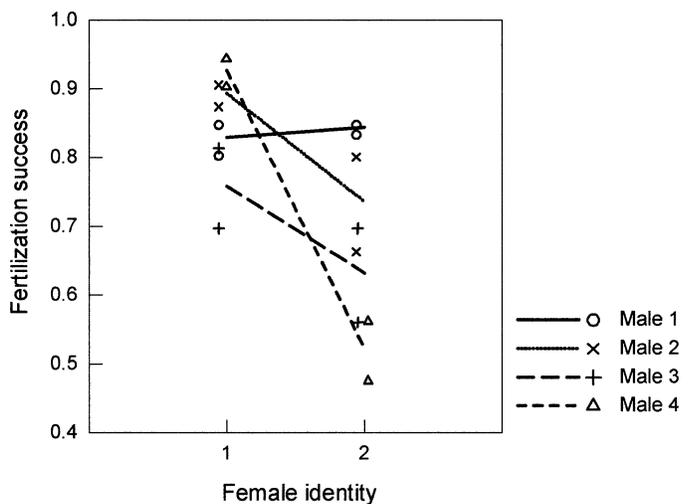


FIG. 3. The interacting effects of male and female identity on fertilization success. For each of the two females, replicate fertilizations were performed with sperm from each of the four males (denoted by the two male symbols for each female) to determine whether fertilization success estimates for specific female \times male crosses were repeatable (see text).

mechanisms underlying the interactions between eggs and sperm, previous studies have identified a number of molecules that influence male-female compatibility at fertilization (reviewed by Vacquier 1998). Among these, the sperm protein bindin forms a major component of the acrosomal vesicle and is known to strongly influence sperm-to-egg attachment and fertilization success in sea urchins (Vacquier and Moy 1977; Vacquier 1998). Bindin has evolved under positive selection in numerous sea urchin species that occur sympatrically with closely related congeners (Metz and Palumbi 1996; Biermann 1998), including *H. erythrogramma* (Zigler et al. 2003). In these species, positive selection for loci that code for bindin polymorphisms can facilitate species-specific gamete interactions (Metz et al. 1994) and may fuel reproductive isolation (e.g., Palumbi and Metz 1991; but see McCarty and Lessios 2004). However, differences among loci that code for bindin can also constitute important sources of intraspecific variation, with potentially far-reaching consequences for reproductive success at the individual level in some species (Palumbi 1999). In *H. erythrogramma*, however, where there is apparently limited intraspecific variation in alleles that code for bindin (Zigler et al. 2003), its function with respect to intraspecific gamete compatibility remains to be tested.

To the extent that cell surface molecules mediate sperm-egg compatibility at fertilization, the magnitude of the fertilization benefit derived from multiple mating should positively covary with the amount of variation for loci that code for these molecules. Hence, the reproductive benefits of polyandry, at least with respect to fertilization, should be greatest in populations in which strong positive selection increases polymorphism for alleles that code for gamete cell recognition molecules. Our results from the first experiment lend support for this idea by revealing that the degree to which rival ejaculates differ in their capacity to fertilize a clutch of eggs positively covaries with the reproductive benefits of polyandry (see Fig. 2).

In both of our experiments, the principal source of variation in fertilization success was female identity. In marine free spawners, egg size variation among and within individual egg clutches (as present in *H. erythrogramma*, Marshall et al. 2004) can influence fertilization kinetics strongly (Levitan 1993; Levitan 2000; Marshall et al. 2000). Specifically, larger eggs present larger targets for sperm and therefore a clutch of larger eggs requires a lower sperm concentration than a clutch of smaller eggs to achieve the same fertilization success (Marshall et al. 2000; 2002). Consequently, although we only chose females that were in spawning condition, intrinsic differences in the size distribution of eggs among females may have had a strong influence on fertilization success.

In conclusion, our findings highlight the influence of male \times female interactions on fertilization success in sea urchins, and therefore contribute to an emerging pattern that the reproductive benefits of polyandry often involve aspects of male-female compatibility (e.g., Bishop 1996; Olsson et al. 1996; Palumbi 1999; Mack et al. 2002). They also contribute to an increasing number of studies that have investigated sexual selection at the level of the gamete (see Birkhead 1998, 2000; Pitnick and Brown 2000). Studies spanning a broad range of taxonomic groups have highlighted the importance

of gamete recognition in mediating fertilization (reviews by Vacquier 1998; Swanson and Vacquier 2002). Despite this, however, we still lack a basic understanding of the mechanisms and functional significance of gamete recognition in mediating both intra- and interspecific fertilization.

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