

# The Role of Male Variation in Fertilization Success in Determining the Costs and Benefits of Polyandry in the Broadcast Spawning Urchin *Lytechinus variegatus*

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**Abstract.** Although the benefits to males mating with multiple females have been well documented, the benefits to females mating with multiple males (polyandry) are less studied, particularly the mechanism that might drive these potential benefits. Benefits of polyandry might stem from increasing the chance of mating with a high-quality or compatible male or stem from the ability of multiple males to fertilize more eggs than any single male. We examine the fertilization consequences of polyandry in the sea urchin *Lytechinus variegatus*. This species has variation in spine color, and we conducted matings between individual and pooled sperm from two males that matched or mismatched in color. The results indicate that (1) males with white spines achieved higher fertilization and were more likely to cause polyspermy than males with purple spines, and there was no effect of female spine color on fertilization; (2) when comparing the average success of individual matings with pooled-sperm matings, there was a net benefit to polyandry when purple-spine males were pooled, a net cost when white-spine males were pooled, and no difference when mismatched spine color males were pooled; and (3) the success under pooled-sperm trials, with any of the spine color combinations, never exceeded the success of the more successful male in the individual-male trials. Together these results suggest that the consequences of polyandry depend on the relation between sperm availability and the sensitivity of eggs to sperm limitation and polyspermy with respect to the specific set of available males. The potential fertilization consequences of a female spawning with multiple males might be associated primarily with increasing the amount of sperm available to fertilize her eggs and secondarily with increasing the chances

of mating with a higher-quality or more compatible male, as opposed to a diversity of males.

## Introduction

Although there is ample evidence that males can increase their fitness by mating with multiple females (polygyny; Bateman, 1948; Arnold, 1994), the evidence that females can benefit from mating with multiple males (polyandry; Arnqvist and Nilsson, 2000; Zeh and Zeh, 2001; Boulton and Shuker, 2013) is less resolved. The foundation of a sex bias in the costs and benefits of multiple mating originally stemmed from Bateman's principle, which states that because sperm outnumber eggs and because females invest more in offspring, female success is limited by resources to offspring production, and male success is limited by mating opportunities (Bateman, 1948). Counterexamples of this sex bias have been noted in species in which males provide parental care and females gain benefits from mating multiply (Arnqvist and Nilsson, 2000; Jones and Avise, 2001), and in cases where overcoming sperm limitation provides benefits for both males and females (Levitan, 1998), suggesting the utility of a resource investment and limitation approach for understanding mating patterns.

However, beyond these resource constraints, there are other potential reasons, based on the quality and diversity of gametes and offspring that are produced, for why individuals might benefit from multiple matings. When sperm are not limiting, females might still gain from multiple mates if the quality of sperm among males varies in the ability to fertilize eggs (Evans and Marshall, 2005; Purchase *et al.*, 2007; Evans and Sherman, 2013) or in the quality and diversity of offspring produced (Foerste *et al.*, 2003; Hosken *et al.*, 2003; Ivy and Sakaluk, 2005; Sprenger *et al.*, 2008).

The evidence for a benefit of polyandry based on patterns of fertilization generally comes from taxa that release both

Received 26 September 2017; Accepted 20 June 2018; Published online 27 September 2018.

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sperm and eggs into the environment for external fertilization. This spawning strategy provides an opportunity to address the fertilization component of the consequence of polyandry without the complications of parental care or the logistic difficulties of assessing fertilization in copulating species. The most straightforward benefit is that under sperm-limited conditions, additional males participating in a spawning event increase the local concentration of sperm and the likelihood of fertilization (Pennington, 1985; Levitan *et al.*, 1991, 1992; Levitan, 1993; Luttikhuisen *et al.*, 2011). This benefit can become a disadvantage when sperm are so numerous that eggs fail to develop (polyspermy, Rothschild, 1954; Styan, 1998; Franke *et al.*, 2002; Levitan *et al.*, 2007). This balance between the risk of sperm limitation and the risk of polyspermy produces an optimal range of sperm concentrations (Styan, 1998) or the number of spawning males (Levitan, 2004) that maximizes zygote production.

In addition to increasing sperm numbers, the likelihood of fertilization can depend on gamete traits. Sperm and eggs might vary in their ability to fertilize in general (gamete quality, *e.g.*, sperm swimming, egg target size, chemotactic ability; reviewed in Levitan, 1998), or they might vary in how they match with a specific mate (gamete compatibility). Variation in gamete recognition proteins has been shown to influence fertilization success because of genotype matching between males and females (Palumbi, 1999; Levitan and Ferrell, 2006; Levitan and Stapper, 2010) or because sperm protein variants have different affinities for the population of available eggs (Levitan and Ferrell, 2006; Levitan, 2012). Sperm variants demonstrated to have a lower affinity to eggs might be caused by their poor compatibility with any female's eggs or because they match with a rare matching egg receptor. A female can benefit from spawning with males releasing sperm with a low affinity to her eggs under conditions when polyspermy is a risk. Low-affinity males can also benefit under these polyspermic conditions if they are not outcompeted by higher-affinity males (Tomaiuolo and Levitan, 2010; Levitan, 2018). Together these gamete quality and affinity traits are thought to underlie the fertilization advantages of polyandry for females.

Releasing eggs in the presence of sperm from multiple males is thought to provide a diversity of sperm variants that can together fertilize more eggs than the sperm from a single male, as noted in fish (Garant *et al.*, 2001; Purchase *et al.*, 2007), sea urchins (Evans and Marshall, 2005), and polychaete worms (McLeod and Marshall, 2009). What remains uncertain is whether females benefit from mating with a diversity of males, because different males fertilize a different subset of eggs, or because it increases the likelihood of mating with a high-quality male. The notion that variation in compatibility increases the fraction of eggs fertilized from a single female could be caused by at least two mechanisms. First, if eggs from a single female are produced from homogenous diploid tissue, they could have identical compatibility traits. In this scenario, if different males have different levels of compatibility to this specific female,

then mating multiply might increase the chances of finding a male with a high match. Second, the eggs released by a single female could vary in their compatibility traits, because of haploid or epigenetic expression in the genes that affect the attraction, activation, or fusion of sperm to eggs (Tomaiuolo and Levitan, 2010). In this scenario, a single female produces a range of egg phenotypes that vary in the compatibility that might best be matched by a diversity of males. In the former case, polyandry might lead to higher reproductive success than the average success of a single male, but it would never exceed the success of the most compatible male. In the latter case, polyandry could lead to higher reproductive success than even the most compatible male, because multiple males can together fertilize more eggs than any one male. Although several studies have examined the effects of polyandry on fertilization success (*e.g.*, Evans and Marshall, 2005), these alternative mechanisms and their consequences have not been resolved.

Here we examine the consequences of polyandry in the sea urchin *Lytechinus variegatus*. This species has genetically determined intrapopulation polymorphism in spine color, varying from white to purple to green (Wise, 2011). Evidence suggests that these spine colors can predict patterns of fertilization in this species. There appear to be two compatibility groups: white-spine sea urchins have higher fertilization when crossed with each other, as do the combined green- and purple-spine sea urchins (Moscoso, 2017). This phenomenon of color-associated fertilization has also been noted in another species of sea urchin, *Paracentrotus gaimardi* (Lopes and Ventura, 2012), and is associated with variation in the sperm Bindin protein that is known to influence compatibility (Calderon *et al.*, 2010). We conducted crosses within and between the two most common colors in our location (St. Joseph Bay, FL): sea urchins with white spines and those with purple spines. Crosses were conducted with the sperm from single males or with the sperm pooled from two males that either matched or mismatched in color. We considered the consequence of polyandry compared to the average success of both males and compared to the success of the best male, in order to explore under what conditions polyandry might offer benefits.

## Materials and Methods

*Lytechinus variegatus* (Lamarck, 1816) specimens were collected from St. Joseph Bay (29°45'45.1''N, 85°23'44.7''W), off the northwest coastline of Florida, during the months of September, October, and November 2016. Sea urchins were brought to the laboratory and maintained in aquaria filled with filtered seawater. For each experimental day (block), spawning was induced by injecting the urchins with 1 mL of 0.5 mol L<sup>-1</sup> KCl. Sperm was collected in clean, dry vials kept on ice. Eggs were collected in clean, seawater-filled vials. Individuals were randomly selected (with respect to color) and injected with KCl until we had a complete block of two white-spine males and females and of two purple-spine males and females (eight sea

urchins total). Sperm was kept dry on ice until dilution and immediate use. Sperm was diluted to a final experimental concentration *via* three 10-fold dilutions. All crosses were carried out in glass scintillation vials that contained 1 mL of the stock egg suspension, 1 mL of 100-fold diluted sperm, and 8 mL of seawater. Egg concentration was estimated by 3 replicate counts of the stock egg suspension, and then the stocks were readjusted by adding or subtracting seawater, to set egg concentration at 500 eggs per experimental vial. Sperm in this species tend to slow and have reduced ability to fertilize after around 30 minutes (Levitan, 2000). To minimize sperm aging, all crosses were conducted within 3 minutes of diluting (activating) sperm with seawater. Fertilization success was estimated at least 1 hour following the cross, by counting at least 100 eggs by using a compound microscope. Diluted sperm samples were taken from every male and were preserved in formalin in order to determine sperm concentration, by using eight replicate counts from a hemocytometer.

In the first set of crosses, all males and females were independently crossed in single-male trials (16 crosses; Fig. 1). In the second set of crosses, sperm from two males was pooled by combining equal volumes of each male's stock sperm suspension (polyandry trials) and was independently crossed with each female. Four sperm pools were created: two white-spine

males, two independent purple- and white-spine males, and two purple-spine males. These 4 vials of pooled sperm were crossed with the 4 females, for a total of 16 crosses (Fig. 1). Because the same stock sperm suspension was used in both the single and polyandry trials, the average sperm concentration of the two males crossed with a particular female, individually or pooled, had the same sperm concentration. A total of 17 blocks (experimental days) were conducted of this experiment.

#### Statistical analysis

Percent fertilization data were arcsine transformed and analyzed with analyses of covariance (ANCOVAs) in SAS (SAS Institute, Cary, NC). Unreported analyses conducted with logit-transformed data revealed the same pattern and magnitude of significance for all analyses.

To test for patterns of compatibility independent of spine color in the single-male trials, an ANCOVA, with the response variable being the arcsine-transformed proportion of eggs fertilized, examined the main effects of male identity, female identity, and their interaction with the covariates of sperm concentration and the polynomial of sperm concentration, to account for the non-linearity caused by polyspermy, and the interactions between these covariates and the main effects.

To test for patterns of fertilization associated with spine color, ANCOVAs, with the response variable being the arcsine-transformed proportion of eggs fertilized, were independently examined for the single-male and polyandrous-male trials that tested main effects of male and female spine color and the interaction of male and female spine color, blocked by day, with the covariates of sperm concentration, the polynomial of sperm concentration, and the interactions between these covariates and the main effects.

To examine the consequences of polyandrous matings, ANCOVAs, with the response variable being the fertilization difference between the polyandrous and individual males (either average or the more successful male of the two), were examined with the main effects of male spine color, female spine color, and their interaction, blocked by day, with the covariates of sperm concentration, the difference in the fertilization success of the two males in the individual trial, and the interaction of these covariates with spine color.

## Results

A total of 16 complete blocks and 1 partial block were conducted. In the first test, we examined whether male and female identity and their interaction, independent of spine color, influenced fertilization success. This test revealed a significant effect of both male and female identity but not their interaction. The covariates of sperm concentration, the polynomial of sperm concentration, and the interaction between male identity and the polynomial of sperm concentration were all found to

Individual	Sperm			
Eggs	W1	W2	P1	P2
W1	0.4	0.5		
W2				
P1				
P2			0.3	0.4

Pooled	Sperm			
Eggs	W1W2	W1P1	W2P2	P1P2
W1	0.42			
W2				
P1				
P2				0.37

**Figure 1.** Experimental design of crosses of *Lytechinus variegatus*. Each experimental block used two white-spine (W1, W2) and two purple-spine (P1, P2) males and females (eight total). In the individual trials, each of the possible 16 crosses was conducted using 1 male and 1 female. In the pooled treatment, 16 crosses were conducted pooling 2 white-spine males (W1W2), 2 independent pools of purple- and white-spine males (W1P1, W2P2), and 2 purple-spine males (P1P2) crossed independently with all 4 females. Hypothetical data are presented in select cells. In these hypothetical examples, female success in crosses of white-spine males is lower under polyandry (0.42) compared to the average success of the two independent trials (0.45); while in crosses with purple-spine males, polyandry (0.37) has higher success than the average of the two independent trials (0.35). In all these hypothetical examples, polyandry never has higher success than the most successful individual male but always does better than the least successful male.

**Table 1**

*Analysis of covariance testing fertilization success (arcsine transformed) as a function of male and female identity and their interaction with the covariates of sperm concentration and the polynomial of sperm concentration (polysperm)*

Source	df	Type III SS	MS	F	P > F
Female ID	23	1.626	0.071	2.48	0.0015
Male ID	6	1.497	0.249	8.76	<0.0001
Sperm	1	0.338	0.338	11.85	0.0009
Polysperm	1	0.439	0.439	15.42	0.0002
Female × male	86	1.626	0.019	0.66	0.9686
Male × polysperm	14	2.730	0.195	6.85	<0.0001
Error	80	2.279	0.208		
Corrected total	267	42.211			

SS, sum of squares; MS, mean square.

be significant (Table 1). The next set of analyses examined success as a function of spine color for crosses involving sperm from single males (individual trials) and pooled sperm from each of the two males (polyandrous trials). Both sets of trials revealed a significant effect of sperm concentration, the polynomial of sperm concentration, and the interaction between male spine color and sperm concentration (Table 2). There was no evidence of an effect of female spine color or the interaction of male and female spine color, either as a main effect or through their interactions with the covariates. In the individual trials, white-spine males had higher fertilization at lower sperm concentrations compared to purple-spine males. Polyspermy was evident at the highest sperm concentrations (Fig. 2A). In the polyandrous trials, pooled sperm from white-spine males had higher success at low sperm concentrations and exhibited more polyspermy at high sperm concentrations, compared to pooled sperm from purple-spine males or a mixture of sperm from white- and purple-spine males (Fig. 2B).

To examine the consequences of polyandry compared to the average success of individual males, the ANCOVA revealed a significant interaction of male spine color and sperm concentration and a positive relationship between the magnitude of the difference in fertilization of the two individual males and the benefits to polyandry (Table 3). There was no evidence of an effect of female spine color or an interaction between male and female spine color. The least square means indicated that, overall, females benefited by mating with multiple purple-spine males compared to individual purple-spine males, females had no benefits by mating with pooled sperm from a purple- and white-spine males, and females had reduced success when mating with pooled sperm from white-spine males compared to individual white-spine males (Fig. 3). Males of all spine colors had a similar response to polyandry at low sperm concentrations, but as sperm concentration increased, purple-spine males demonstrated an increasing benefit to polyandry while white-spine males demonstrated an increasing cost (Fig. 3).

The parallel test comparing polyandry with the most successful male in the trial yielded the same patterns of significance (Table 3), but the least square means revealed that no combination of male spine color resulted in a benefit to polyandry (Fig. 3A); females always benefited by mating with the more successful male as opposed to the pooling of both males.

## Discussion

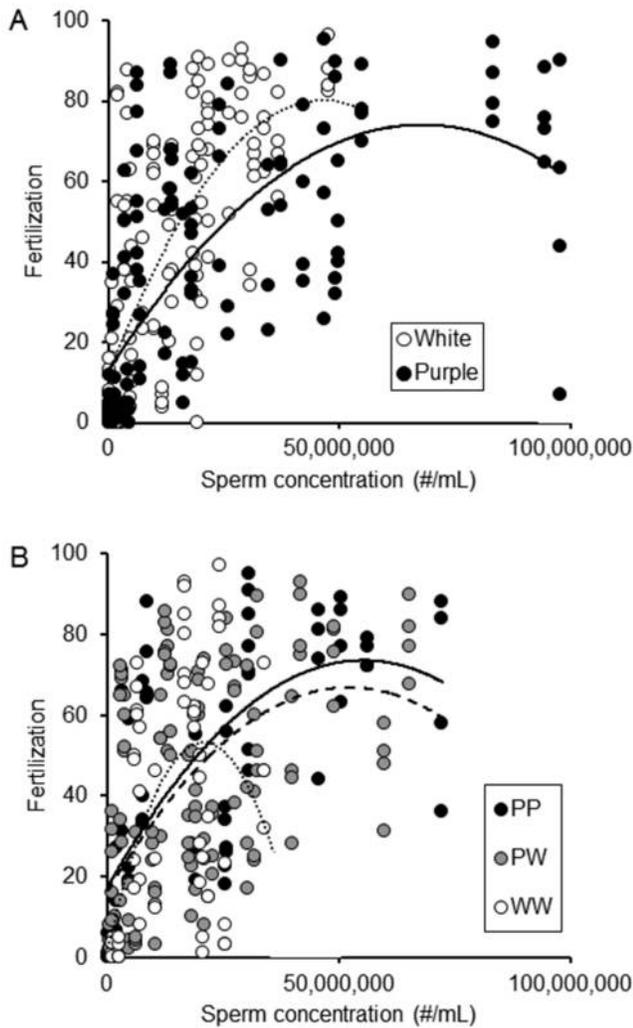
We detected a significant effect of male spine color on fertilization success in this sample of sea urchins collected from St. Joseph's Bay, Florida. We did not detect an effect of female spine color or the interaction between male and female spine color. More generally, we did not find any evidence of an individual male-by-female interaction on fertilization suc-

**Table 2**

*Analysis of covariance testing fertilization success in individual-male trials and polyandrous trials that combined the sperm from two males on arcsine-transformed proportions of fertilization success*

Source	df	Type III SS	MS	F	P > F
<b>Individual trials</b>					
Male	1	0.0049	0.0049	0.08	0.7712
Female	1	0.0438	0.0438	0.76	0.3831
Block	16	6.5897	0.4119	7.18	<0.0001
Sperm	1	3.5014	3.5014	61.06	<0.0001
Polysperm	1	0.9256	0.9256	16.14	<0.0001
Male × sperm	1	0.2576	0.2576	4.49	0.0351
Female × sperm	1	0.1382	0.1382	2.41	0.1219
Male × female	1	0.0018	0.0018	0.03	0.8599
Male × female × sperm	1	0.0027	0.0027	0.05	0.8273
Male × polysperm	1	0.1740	0.1740	3.03	0.0828
Female × polysperm	1	0.0673	0.0673	1.17	0.2798
Male × female × polysperm	1	0.0091	0.0091	0.16	0.6908
Error	240	13.7617	0.0573		
Corrected total	267	42.2109			
<b>Polyandrous trials</b>					
Male	2	0.3516	0.1758	3.51	0.0316
Female	1	0.0019	0.0019	0.04	0.8452
Block	16	9.8317	0.6145	12.26	<0.0001
Sperm	1	1.4750	1.4750	29.42	<0.0001
Polysperm	1	0.8807	0.8807	17.57	<0.0001
Male × sperm	2	0.4833	0.2416	4.82	0.0089
Female × sperm	1	0.0003	0.0003	0.01	0.9412
Male × female	2	0.0633	0.0317	0.63	0.5327
Male × female × sperm	2	0.0740	0.0370	0.74	0.479
Male × polysperm	2	0.5490	0.2745	5.48	0.0047
Female × polysperm	1	0.0006	0.0006	0.01	0.9105
Male × female × polysperm	2	0.0396	0.0198	0.39	0.6745
Error	234	11.7303	0.0501		
Corrected total	267	37.9604			

The main effects were male spine color (Male) and female spine color (Female), blocked by experimental day, with the covariates of sperm concentration (Sperm), the polynomial of sperm concentration (Polysperm), and their interactions. Spine color in the individual trials was either white, purple, or all three combinations (WW, WP, PP) in polyandrous trials. SS, sum of squares; MS, mean square.



**Figure 2.** Fertilization success of *Lytechinus variegatus* females exposed to sperm from either (A) one male or (B) two males (polyandry). Males had either white or purple spines. (A) Lines are polynomial fitted to data for purple-spine males (solid symbols, solid line) or white-spine males (open symbols, dotted line) tested independently. (B) In the polyandrous trials, equal volumes of sperm from both males were mixed and used as the stock solution. Males in the polyandry trials had spines of either the same color (WW/PP) or a different color (WP).

cess and thus found no evidence of compatibility groups with this sample of sea urchins. We did find that males with white spines were more successful at fertilization and were more likely to cause polyspermy compared to purple-spine males. This finding could be caused by white-spine males having overall higher-quality sperm or because purple-spine males are more compatible with rare or locally absent female genotypes. We cannot generalize that this pattern of spine color-dependent male success holds across this species range, but, regardless, it provides a mechanism for examining how variation in male fertilization success influences the costs and benefits of polyandry. When the concentration of pooled sperm was identical to the average sperm concentration of the two males crossed independently,

there was a subtle benefit when the two males had the lower compatibility associated with purple spine color and a subtle cost when the two males had the higher compatibility associated with white spine color. The benefits to polyandry increased with the difference in the fertilization success of the two males, a result that mirrors that of an earlier study in another sea urchin species (Evans and Marshall, 2005). The finding that polyandry with white-spine males was costly to females but that polyandry with purple-spine males was beneficial to females was likely caused by the higher compatibility of white-spine male sea urchins to these eggs and their increased susceptibility to polyspermy. This notion is supported by the increasing cost of polyandry with increasing sperm concentration when females mated with white-spine males.

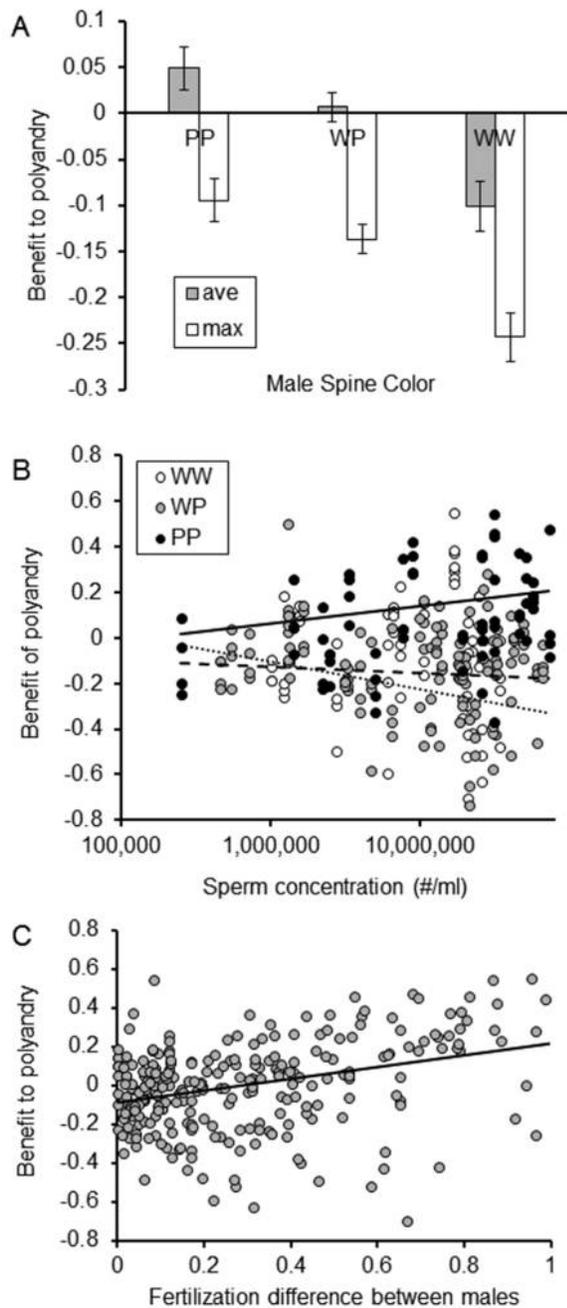
Females were always more successful when crossed with the more successful male in a pair, compared to when the two males were pooled; pooling sperm appears to dilute the sperm from the more successful male. This result is consistent with a

**Table 3**

*Analysis of covariance testing the effect of polyandrous fertilization, characterized as the difference between success under polyandry minus the success of either the average fertilization of the two individual males (average success) or the more successful of the two males (maximum success)*

Source	df	Type III SS	MS	F	P > F
<b>Average success</b>					
Female color	1	0.0119	0.0119	0.36	0.5502
Male color	2	0.0953	0.0476	1.43	0.2412
Block	16	2.3911	0.1494	4.49	<0.0001
Sperm	1	0.0141	0.0141	0.42	0.516
Difference	1	0.8912	0.8912	26.77	<0.0001
Male color × sperm	2	0.2395	0.1197	3.6	0.0289
Female color × sperm	1	0.0034	0.0034	0.1	0.7507
Male color × difference	2	0.1608	0.0804	2.42	0.0915
Female color × difference	1	0.0552	0.0552	1.66	0.1991
Male color × female color	2	0.0117	0.0058	0.18	0.8392
Error	238	7.9238	0.0333		
Corrected total	267	13.5724			
<b>Maximum success</b>					
Female color	1	0.0158	0.0158	0.48	0.4911
Male color	2	0.0893	0.0446	1.34	0.2633
Block	16	2.3707	0.1482	4.45	<0.0001
Sperm	1	0.0167	0.0167	0.5	0.4793
Difference	1	0.5348	0.5348	16.08	<0.0001
Male color × sperm	2	0.2504	0.1252	3.76	0.0246
Female color × sperm	1	0.0025	0.0025	0.07	0.7848
Male color × difference	2	0.1622	0.0811	2.44	0.0896
Female color × difference	1	0.0581	0.0581	1.75	0.1875
Male color × female color	2	0.0156	0.0078	0.23	0.7909
Error	238	7.9173	0.0333		
Corrected total	267	12.5782			

Factors include male and female spine color, sperm concentration (Sperm), the difference in the fertilization success of the two males (Difference), and the interaction of these terms. Fertilization data were arcsine transformed. SS, sum of squares; MS, mean square.



**Figure 3.** Comparing fertilization success of *Lytechinus variegatus* under polyandry to the success of single males. (A) The benefit to polyandry, defined as the fertilization success (arcsine transformed) under polyandry minus the success in individual male trials using either the average success of the two males (ave) or the higher success of the two males (max). Data are the least square means (SE). (B) The benefit of polyandry compared to the average success of individual males as a function of sperm concentration for two white-spine males (WW, open symbols, dotted line), two purple-spine males (PP, solid symbols, solid line), and one white-spine male and one purple-spine male (WP, gray symbols, dashed line). (C) The benefit to polyandry compared to the average success of the two individual males as a function of the difference in the fertilization success of the two males in the individual trials.

study on polyandry in fish, in which the benefits of polyandry were largely a consequence of low fertilization in a few individual crosses with low-quality males (Purchase *et al.*, 2007). The study by Evans and Marshall (2005) found no difference in fertilization success between the most compatible male and the pooled-male treatments and concluded that the more compatible male outcompeted the less compatible male in pooled treatments; but it also found no evidence for a dilution effect of the more compatible male.

These results suggest a fertilization benefit, when sperm concentration is held constant, for a female's eggs being exposed to sperm from a more successful or more compatible male, rather than several males releasing sperm with a diversity of fertilizing ability. Estimates of paternity under sperm competition in other sea urchin species indicate that males win in competition either because of higher compatibility, revealed by gamete recognition proteins (Palumbi, 1999; Levitan, 2012), or because of sperm swimming ability (Campbell *et al.*, 2016). A male's sperm competitive ability or, potentially, a female's egg choosiness might bias fertilization under polyandry toward one male (Levitan, 2018). The potential benefits of polyandry seem to lie in the increased likelihood of mating with a male better able to fertilize all of a particular female's eggs, rather than mating with males that are each more compatible with a subset of eggs.

These experiments, like others testing for the effects of polyandry on fertilization, attempt to hold sperm concentration constant or within a narrow range. This design eliminates the most important consequence of females mating with more than one male: the increase in local sperm concentration when multiple males release sperm into the water column. Laboratory tests indicate that increasing sperm concentration increases the chances of fertilization at lower levels and increases the likelihood of polyspermy at higher levels (Styan, 1998). Field tests indicate that increasing the number and proximity of spawning males increases female fertilization success until excess sperm results in polyspermy (Franke *et al.*, 2002; Levitan, 2004).

The current experiments test for how polyandry affects the quantity of zygotes produced but not for the quality of offspring produced. Male-by-female interactions on post-zygotic success have been noted (Evans *et al.*, 2007), and post-zygotic fitness benefits to polyandry have been observed (Foerste *et al.*, 2003; Hosken *et al.*, 2003; Ivy and Sakaluk, 2005; Sprenger *et al.*, 2008). What the current experiments indicate is that, under a set level of sperm availability, females would benefit by mating with the best available male rather than a diversity of males in a polyandrous group. The benefits of polyandry depend on the relative abilities of the available mates and whether the level of sperm availability is above or below the threshold at which polyspermy is a meaningful risk.

Although exposing eggs to a greater number of males might have increased the likelihood of finding evidence for intra-female variation in egg receptivity to specific males, we did slant

the experiment for finding this effect by pitting males with obvious differences in fertilizing ability. This is not to say that intrafemale variation in egg traits has no influence on the likelihood of a specific egg being fertilized. Various traits such as egg size (Levitan, 1993; Marshall *et al.*, 2002), extracellular layers (Farley and Levitan, 2001; Podolsky, 2001), and release of sperm chemoattractants (Riffell *et al.*, 2004) might all vary within a batch of eggs released by a single female and can shift the likelihood of fertilization. There is emerging evidence for variation in compatibility among individual males (Palumbi, 1999; Evans and Marshall, 2005 [but not Evans *et al.*, 2007 in the same species]; Marshall and Evans, 2005; Levitan and Ferrell, 2006; Levitan and Stapper, 2010), but evidence that subsets of eggs (or sperm) from an individual female (or male) exhibit differential patterns of compatibility has not been found and is not apparent in this study. This could be explained by diploid gonadal tissue producing a uniform set of gamete traits such as recognition proteins. Under such conditions an individual would always have higher compatibility with the best-matched mate than with a diversity of mates.

### Acknowledgments

We thank Jon Evans, Ellen Kosman, Kevin Olsen, Jose Moscoso, and Jen Kennedy for comments on this research. This work was funded by the National Science Foundation Division of Environmental Biology (1354272).

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