Genetic Compatibility Underlies Benefits of Mate Choice in an External Fertilizer

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abstract: Mate choice is a common feature of sexually reproducing species. In sessile or sedentary external fertilizers, however, direct interactions between reproductive partners are minimal, and instead mate recognition and choice must occur at the level of gametes. It is common for some sperm and egg combinations to have higher fertilization success than others, but it remains unclear whether differences in fertilization reflect gamete-level mate choice (GMC) for paternal quality or parental compatibility. Here, we examine the mechanisms underlying GMC in an externally fertilizing ascidian. A manipulative mate-choice assay confirmed that offspring viability was greater in clutches where we allowed GMC than in clutches where we precluded GMC. A complementary quantitative genetic experiment then revealed that paternal quality effects were generally weaker than parental compatibility effects, particularly for the trait combination underlying the benefits of GMC. Overall, our data suggest that gametes that are more compatible at fertilization produce more viable offspring than gametes that are less compatible at fertilization. Therefore, although the regalia we typically associate with sexual selection are absent in external fertilizers, mechanisms that allow females to bias fertilization in favor of some males over others produce significant fitness benefits in organisms reproducing via the ancestral strategy.

Keywords: mate choice, sexual selection, external fertilization, *Ciona*, marine invertebrate.

Introduction

Predicting a male's contribution to offspring performance is crucial to maximizing the reproductive benefits of female mate choice. Accordingly, males of many species have evolved a wide diversity of acoustic, visual, chemical, and behavioral traits that signal their quality as potential mates (Andersson 1994). These traits can advertise the direct benefits females gain from mating with a potential male (e.g., protection, material resources, or parental care) but may also indicate indirect benefits obtained by investing in the genetic quality of offspring (Kirkpatrick 1996; Simmons 2005). In many systems, males provide no direct benefits; hence, the benefits of female mate choice may depend entirely on a male's genetic contribution to offspring performance (Kirkpatrick and Ryan 1991).

Traditionally, indirect benefits of female mate choice were presumed to follow classical patterns of identity by descent (Fisher 1930; Zahavi 1975; Kirkpatrick and Ryan 1991). For instance, a male capable of acquiring resources, surviving to maturity, and becoming successful in male-male competition is likely to carry genetic advantages that will be inherited by their offspring (good-genes hypothesis). In addition, the sons of attractive males are likely to become successful males because they inherit the genes that made their fathers successful (sexy-sons hypothesis). Both the good-genes and sexy-sons hypotheses predict that females discriminate among males on the basis of heritable genetic variation in paternal quality (Kokko et al. 2003, 2006).

A second class of hypotheses for the indirect benefits of female mate choice focuses on the genetic compatibility of a male (Zeh and Zeh 1996; Neff and Pitcher 2005; Ivy 2007). According to the genetic compatibility hypothesis, it is the genetic interaction between males and females that determines differences in offspring performance. Benefits of mate choice driven by parental compatibility include both inbreeding and outbreeding avoidance (Tregenza and Wedell 2000) as well as more complex genomic interactions (Zeh and Zeh 1996). For example, male and female siblings may have the highest breeding values in a population, but offspring resulting from matings between siblings are likely to have reduced fitness as a result of inbreeding depression. Importantly, in this context, no one male is superior across all females; instead, some male-female combinations are more compatible than others. These prevailing theories for indirect genetic benefits of mate choice can therefore be viewed as debating the relative impacts differences in paternal quality and parental compatibility have on variation in offspring performance (Tregenza and Wedell 2000; Neff and Pitcher 2005; Simmons 2005).

When females mate with multiple males, bet-hedging and ecological genetic diversity effects can also increase female

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reproductive success and thereby enhance, modify, or obscure the benefits of mate choice driven by sexual selection (Barton and Post 1986; McLeod and Marshall 2009; Aguirre and Marshall 2012*a*; Garcia-Gonzalez et al. 2015). When bet-hedging benefits occur, multiple mating enhances female reproductive success by avoiding mating exclusively with low-quality males or by buffering females from demographic and environmental stochasticity (Garcia-Gonzalez et al. 2015). Ecological genetic diversity effects, on the other hand, are an emergent property of the spatial distribution of genetic diversity (Hughes et al. 2008; Crawford and Whitney 2010; Aguirre and Marshall 2012*b*; Aguirre et al. 2013). Ecological genetic diversity effects arise when greater genetic diversity enhances resource partitioning or disease resistance in multiply sired clutches, compared with singly sired clutches (Hughes et al. 2008). Although not possible in all study systems, experimental designs and analytical methods to partition the effects of selection from bet-hedging and ecological genetic diversity effects exist (Tilman et al. 1997; Loreau and Hector 2001; Johnson et al. 2006; McLeod and Marshall 2009; Garcia-Gonzalez et al. 2015) and allow the role of sexual selection in driving the benefits of mate choice to be examined with greater precision.

External fertilization is the ancestral reproductive strategy for all animals, and it remains the predominant reproductive strategy in many marine animal groups (Wray 1995). In these species, individuals generally lack the overt morphological and behavioral signatures of sexual selection common in internal fertilizers. Nonetheless, fertilization success can differ substantially among individuals of the same population (Palumbi 1999; Levitan 2012), and in many cases it is interactions between sperm and eggs that determine which individuals reproduce most successfully (Palumbi 1999; Levitan and Ferrell 2006; Levitan 2012; Evans and Sherman 2013). Importantly, because fertilization and embryogenesis are external and mate choice occurs at the level of gametes, many of the biological and logistical difficulties associated with studies examining the drivers of mate choice can be overcome when using external fertilizers as a model experimental system (McLeod and Marshall 2009; Garcia-Gonzalez et al. 2015).

Two predominant processes govern mate recognition and mate choice in marine external fertilizers: sperm chemotaxis and gamete recognition proteins (Evans and Sherman 2013). In conditions where sperm are limiting, chemoattractants released from unfertilized eggs generate a chemical gradient that guides motile sperm toward eggs, thereby increasing the effective target sizes of eggs (Jantzen et al. 2001; Riffell et al. 2004; Evans et al. 2012). Moreover, in species with overlapping distributions and synchronized spawning events, there is evidence that sperm can distinguish egg-derived chemoattractants from different species and that sperm deliberately swim toward conspecific eggs (Riffell et al. 2004). Importantly, recent studies also indicate that sperm can discriminate among conspecific eggs on the basis of these chemical cues (Evans et al. 2012; Oliver and Evans 2014). Gamete recognition proteins then operate at the point of contact between gametes. Proteins present on the sperm acrosome and the egg vitelline coat function as a lock-and-key mechanism that, through a series of biochemical reactions, allows the successful passage of the sperm through the vitelline coat and, subsequently, fusion with the egg membrane (Vacquier 1998; Palumbi 1999). The gamete recognition proteins of many external fertilizers are structurally diverse and highly polymorphic and evolve rapidly in response to environmental conditions (Levitan and Ferrell 2006; Levitan 2012).

Despite substantial progress in determining the biochemical processes driving variability in fertilization success in many externally fertilizing marine invertebrates, few studies have determined whether differences in fertilization success reflect mate choice for paternal quality or parental compatibility (but see Oliver and Evans 2014). Here, we examine the mechanisms that underlie the benefits of mate choice in an externally fertilizing ascidian, *Ciona robusta* (formerly known as *Ciona intestinalis*; see Brunetti et al. 2015 and Pennati et al. 2015). We investigated whether offspring in clutches where we allowed gamete-level mate choice (GMC) had, on average, greater viability than offspring in clutches where we experimentally precluded GMC, and we tested whether the benefits of GMC were driven by selection or by ecological genetic diversity effects. By employing a quantitative genetic breeding design, we were further able to partition the contributions of paternal quality and parental compatibility to variation in offspring viability. Finally, we examined differences in the amount of variation in paternal quality and parental compatibility aligned in the direction of the trait combination underlying the benefits of GMC.

Methods

General Laboratory and Field Methods

The field site and general laboratory and field methods are the same as those presented in Aguirre and Marshall (2012*a*) and Aguirre et al. (2014); therefore, here we present only the most relevant details. Data are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.c175g (Aguirre et al. 2016). Although *Ciona robusta* is hermaphroditic, adults collected from the field site were randomly assigned as either males or females and mated in accordance with the experimental design presented below. For all life-cycle stages, individuals developed in 35-mm petri dishes. More importantly, the density of offspring in each dish was standardized at the beginning of each life-cycle stage; therefore, differences in offspring viability be-

tween life-cycle stages accumulate across the life cycle. We used standard strip-spawning and fertilization protocols to extract gametes, standardize gamete concentrations, and combine gamete suspensions in fertilization dishes (Aguirre and Marshall 2012*a*; Aguirre et al. 2014). In particular, we used a mean sperm concentration of 2×10^6 sperm mL^{-1} and a mean egg concentration of 3×10^3 eggs mL^{-1}. To assay fertilization success, 2 hours after the initial exposure to sperm we subsampled a clutch of 70–120 eggs and counted the number of fertilized eggs (those that had begun regular cell division) and unfertilized eggs (no or abnormal cell division). At this time, fertilized eggs were between the 2- and 8-cell stages. We then transferred 70–100 embryos to hatching dishes with a pipette. Excess eggs and embryos were discarded. On the basis of pilot experiments and prior experience with this species at this study site (Marshall and Bolton 2007), we believe that the sperm and egg concentrations used in this study were not in a region that causes severe polyspermy. In our experience, *C. robusta* eggs that are polyspermic either fail to show any cell division or show abnormal cell division from the first or second cleavage. Abnormally dividing embryos were rare, compared with eggs showing no cell division, and both were classified as unviable.

Thirty minutes after the first larvae began to hatch (20–23 hours after fertilization at ambient seawater temperature of ~14°C) we haphazardly sampled 45 apparently healthy, swimming larvae and transferred them to settlement dishes with a pipette. Settlement dishes were roughened with sandpaper and conditioned in laboratory aquaria with flow-through seawater for 2 days to facilitate settlement. The embryos remaining in hatching dishes were allowed an additional 3 hours to hatch. Pilot experiments indicated that embryos that failed to hatch within 3 hours of the first hatchlings in their dish were unviable (see Aguirre et al. 2014). Therefore, to estimate embryo viability, we counted only the number of larvae that hatched within 3 hours of the first hatchings in each dish, and the remaining embryos were discarded.

Larvae were allowed 24 hours in constant darkness, at ambient seawater temperature, to attach and metamorphose. The larvae of *C. robusta* are positively phototactic, and a common artifact associated with maintaining larvae in small volumes of seawater in ambient light is that larvae will swim toward the surface, attach to the surface tension of the water, and, in some cases, metamorphose. Maintaining larvae in constant darkness promotes settlement on the dish rather than at the air-water interface, and in our experiments we did not observe any metamorphosed juveniles that were not attached to the dish. Pilot experiments indicated that larvae that delay metamorphosis more than 24 hours are unviable as juveniles (see Aguirre et al. 2014); thus, larval viability was measured as the number of larvae that attached and began metamorphosis within 24 hours of hatching. Any swimming larvae that remained in the dishes were discarded, and the dishes were placed in flow-through aquaria to allow juveniles to acclimate to natural seawater conditions before being transplanted to the field.

After they had spent 24 hours in laboratory aquaria, we haphazardly selected 10 juveniles in each dish and circled them with a graphite pencil. The pencil marks allowed us to distinguish focal individuals from naturally settled nonfocal individuals throughout the experimental period. Excess juveniles were removed with forceps. The dishes were then attached to 500 × 500-mm PVC panels and transferred to the field site. Juvenile viability was measured as the number of circled juveniles remaining in each dish after 2 weeks in the field. Dishes were examined for nonfocal individuals on days 1, 2, and 7, and any nonfocal individuals were removed with forceps. Unlike other sessile marine invertebrates, where secondary settlement is common (e.g., mussels), juvenile *C. robusta* that detach from hard substrates are unlikely to reattach; hence, we assumed that only attached juveniles were viable.

Experimental Overview

To determine whether the benefits of GMC reflect choice for paternal quality or parental compatibility in *C. robusta*, we used two complementary experiments. Our overall conclusions use the data collected in both experiments to determine whether the benefits of GMC reflect choice for paternal quality or parental compatibility; hence, we assumed that genetic quality and genetic compatibility effects operated similarly in our two experiments. The two experiments were conducted consecutively, using parents from the same source population, such that embryos, larvae, and juveniles from both experiments were in the laboratory or field at the same time for some of their development. In experiment 1, we used a manipulative mate-choice assay to examine whether allowing the potential for GMC results in greater offspring viability. Experiment 1 had three objectives: first, to confirm that GMC enhances female reproductive success by increasing offspring viability; second, to determine the life-cycle stages contributing most strongly to the indirect benefits of GMC; and third, to uncover the process by which GMC confers indirect fitness benefits. Specifically, does selection for better-performing offspring or ecological genetic diversity effects underlie higher offspring viability in treatments where we allowed GMC? In experiment 2, we used a quantitative genetic breeding design to partition paternal quality and parental compatibility components of phenotypic variation in offspring viability. Experiment 2 had three objectives: first, to quantify the magnitude and orientation of paternal quality and parental compatibility effects on viability across the life cycle; second, to determine how much of the variation in paternal quality and parental compatibility lies along the axis describing the greatest differences in mean

multivariate viability between mate-choice treatments in experiment 1; and third, to examine differences in the integration of fertilization success (sensu Hansen and Houle 2008) with respect to embryo, larval, and juvenile viability for both paternal quality effects and parental compatibility effects. Overall, our approach allowed us to directly compare the amount of variation in paternal quality and parental compatibility aligned in the trait combination underlying the benefits of GMC.

A Digression on Scale

Our data in experiments 1 and 2 and the inference from our analyses are centered on variation in the probability of viability and therefore are measurements made on an absolute scale (Houle et al. 2011). However, we analyzed the data in experiments 1 and 2 by using a generalized linear mixed model (glmm), where the resulting parameter estimates are on the scale of the link function—for our binary viability scores we used a logit link function. Technically, there are no permissible transformations that maintain the meaning of measurement for data measured on an absolute scale (Hansen and Houle 2008; Houle et al. 2011). Accordingly, to make biological inferences about the variance in the probability of viability, we back-transformed our estimates of the variance components from the logit scale to the probability scale, using the delta method. The delta method uses the projection $\mathbf{D}^{T}\Sigma\mathbf{D}$ to give a first-order approximation for the variance of a transformed parameter. In our case, Σ is the logit-scale estimate of the covariance matrix (e.g., the sire covariance matrix in experiment 2), and $\mathbf{D}$ is a diagonal matrix. The entries on the diagonal of $\mathbf{D}$ are the first derivatives of the logit function with respect to the logit-scale estimates of the sample means (e.g., the mean viability for each life-cycle stage in experiment 2). All the parameter estimates for the analyses of experiments 1 and 2 are back-transformed from the logit scale to the probability scale with the delta method.

Experiment 1: Manipulative Mate-Choice Assay

Using a split-clutch, split-ejaculate experimental design, we exposed replicate clutches of eggs from each female to the sperm of three males in isolation (no-choice treatment) and the sperm of the same three males mixed in equal proportions (choice treatment; McLeod and Marshall 2009). Each block ($n = 10$) consisted of three females and three males crossed in all possible combinations to produce nine full-sibling families in each block. These full-sibling families were the "no-choice" treatments (three dishes for each female). In addition, for each female in each block, there was a "choice" treatment (one dish for each female), in which we allowed eggs access to the sperm of the same three males represented in the no-choice treatment. Therefore, for each female we measured fertilization success, as well as embryo, larval, and juvenile viability, in a scenario where we constrained GMC and a scenario where we allowed GMC.

Experiment 1, Objective 1: Are There Indirect Benefits of GMC? To test the hypothesis that GMC has indirect benefits for reproductive fitness, we fitted a multivariate glmm analog of a multivariate ANOVA, using the lme4 package in R, version 3.0.2. The response vector consisted of the binary viability scores (1 = viable and 0 = unviable) for each individual in each life-cycle stage. Our model included life-cycle stage as a fixed factor considered at four levels: fertilization, embryo, larval, and juvenile. Treatment was a fixed factor considered at two levels: no-choice and choice. Block and female nested within block were random factors, and dish nested within female and block was an observation-level random factor (Elston et al. 2001). To ensure a balanced design at the female level (there were three no-choice dishes but only one choice dish for each female), for the no-choice treatment we used the mean proportional viability of the three no-choice dishes for each life-cycle stage as the response value.

We used a model-selection approach to determine whether offspring in the mate-choice treatment had greater mean multivariate viability than offspring in the no-choice treatment. Specifically, we compared the fit of a model including the main effects of life-cycle stage and treatment, as well as the life-cycle stage × treatment interaction, with a model including only the main effects of life-cycle stage and treatment. Support for a fixed life-cycle stage × treatment interaction (confirmed with a χ^2 nested log-likelihood ratio test) indicated that allowing GMC resulted in differences in mean multivariate viability between the choice and no-choice treatments but that the effects of the mate-choice treatment differed among life-cycle stages. Similarly, if a reduced model (i.e., considering the main effects of life-cycle stage and treatment but not their interaction) indicated support for an effect of the mate-choice treatment, we would conclude that allowing the opportunity for GMC resulted in differences in mean multivariate viability and that differences between the choice and no-choice treatments were in the same direction and of the same magnitude for all life-cycle stages. Although our model-selection approach was not identical to a traditional multivariate ANOVA, it was asymptotically equivalent (Wright 1998). In addition, although our interpretation focused on the effects of mate choice on multivariate offspring viability, in figure 1 (*right*) we display the univariate results.

Experiment 1, Objective 2: Which Life-Cycle Stages Contribute Most Strongly to the Benefits of GMC? To determine the life-cycle stages contributing most strongly to dif-

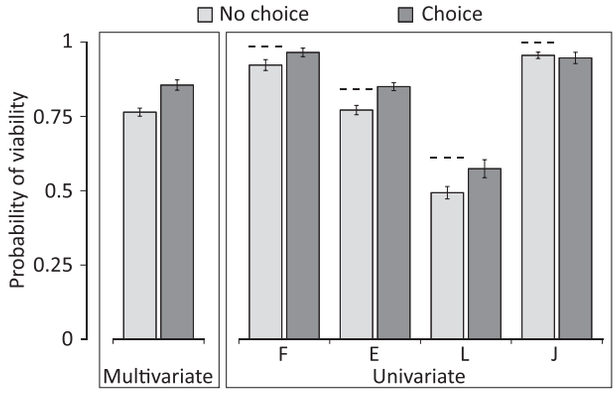

Figure 1: Mean (±SE) offspring viability in the experimental treatment where we precluded mate choice (no choice) and the treatment where we allowed mate choice (choice). Values for multivariate offspring viability in choice and no-choice treatments are the corresponding mean (±SE) canonical variate (CV) scores for $\boldsymbol{a}_{max}$ on the probability scale. For univariate offspring viability, the letters F, E, L, and J denote the fertilization, embryo, larval, and juvenile life-cycle stages, respectively. The dashed line above the no-choice treatment for the univariate data indicates the mean (±SE) viability of the best constituent no-choice dish for each female. We do not show the corresponding CV score for multivariate transgressive overyielding because the canonical variate is specific to each analysis.

ferences in mean multivariate viability between the choice and no-choice treatments, we fitted an additional glmm to estimate the hypothesis (**H**) and error (**E**) sums of squares and cross-products (SSCP) matrices. The **H** matrix is the life-cycle stage × treatment SSCP matrix, and the most appropriate **E** for our experimental design was the life-cycle stage × block SSCP matrix (Quinn and Keough 2002). The product of these two matrices gives the total SSCP matrix **A** (i.e., $\mathbf{A} = \mathbf{HE}^{-1}$). The eigenvalues of **A** are the foundation for the familiar multivariate ANOVA test statistics (e.g., Hotelling-Lawley trace, Pillai's trace, and Roy's greatest root), and the eigenvectors of **A** describe the directions of greatest mean multivariate difference between treatments (as in linear discriminant analysis). For our purposes here, we were interested in the eigenvectors of **A**, as we establish support for multivariate effects of GMC on offspring viability by using the model-selection procedure described above. For our two-level mate-choice treatment, **A** will have only one nonzero eigenvector (i.e., a single value is required to describe the difference between two observations), $\boldsymbol{a}_{max}$, which describes the axis underlying the multivariate differences in mean offspring viability between the choice and no-choice treatments. Accordingly, larger values for the loadings of a life-cycle stage in $\boldsymbol{a}_{max}$ indicate stronger contributions to the benefits of GMC.

Experiment 1, Objective 3: Do Genetic Diversity Effects in Multiply Sired Clutches Underlie Indirect Benefits of GMC? The statistical model to test for ecological genetic diversity effects (known as transgressive-overyielding analyses) is the same as the model used to test for mean differences in offspring viability described in "Experiment 1, Objective 1." The only difference is the response values for the no-choice treatment. Rather than comparing the mean proportional viability for each female, in this objective we compared the viability of the best constituent no-choice dish to the viability of the choice dish for each female. In transgressive-overyielding analyses, support for a fixed life-cycle stage × treatment interaction driven by greater mean viability in the choice treatment would suggest that at least some of the benefits of GMC were driven by ecological genetic diversity effects. In other words, the benefits of GMC cannot be attributed solely to sexual selection for paternal quality or parental compatibility.

Experiment 2: Quantitative Genetic Breeding Design

We used a North Carolina II breeding design, whereby we crossed three sires with three dams in all combinations to produce nine full-sibling families in each block (19 blocks in total). For each sire × dam combination there were two replicate dishes. Overall, the experiment consisted of 57 sires and 57 dams and a total of 171 full-sib families. The components of interest in this study are the sire covariance matrix, **S**, and the sire × dam interaction covariance matrix, **I**. The **S** matrix summarizes how intrinsic differences among sires affect variation in offspring viability and therefore the magnitude and orientation of paternal quality effects. Alternatively, the **I** matrix summarizes how differences among sire × dam combinations affect variation in offspring viability and therefore the magnitude and orientation of parental compatibility effects.

Although the estimation procedures for **S** and **I** are identical to those used in estimating the additive and nonadditive genetic covariance matrices, respectively, we have chosen to refer to these components of variation as simply paternal quality and parental compatibility effects. For instance, a sire's contribution to fertilization success involves the interaction between the sire's genes and the environment before contact with the egg and therefore an environmental effect that occurs before the recombination of a sire's genes with the dam's genetic contribution. Accordingly, the sire's contribution to fertilization success includes a genetic component (e.g., the sire's gamete recognition protein genotype) but also an environmental component that manifests itself in the absence of the dam's genetic contribution to phenotypic variation. Importantly, in the genetic analysis of a nested full-sib–half-sib experimental design (Lynch and Walsh 1998), this prefertilization environmental component is confounded with the additive genetic compo-

nent of fertilization success (Marshall 2015). We therefore use the terms sire variation (**S**) and sire × dam variation (**I**) to represent paternal quality and parental compatibility effects, respectively, and our estimates of **S** and **I** are not multiplied by 4, as is typical in the genetic analysis of a nested full-sib–half-sib experimental design (Lynch and Walsh 1998).

Experiment 2, Objective 1: How Much Variation Is There among Sires and among Sire × Dam Combinations, and How Is This Variation Distributed? To quantify the variance in offspring viability attributable to paternal quality (**S**) and to parental compatibility (**I**) we fitted a multivariate glmm, using the MCMCglmm package in R, version 3.0.2 (Hadfield 2010). Our genetic analysis closely followed that presented in Aguirre et al. (2014), and additional details can be found there. The multivariate glmm considered sire, dam, sire × dam, and dish as random factors as well as life-cycle stage and block as fixed factors. The response vector consisted of the binary viability scores (1 = viable and 0 = unviable) for each individual in each life-cycle stage. As discussed in Aguirre et al. (2014), the effects of viability selection on estimates of quantitative genetic parameters (Hadfield 2008; Steinsland et al. 2014) are explicitly considered in a multivariate genetic analysis where the viability scores for preceding life-cycle stages are included in the analysis (Steinsland et al. 2014).

Priors for the location effects were normally distributed and diffuse around a mean of 0 and a variance of $\pi^2/3$. For the variance components, priors conformed to a scaled, noncentral *F* distribution with the location parameter equal to 0 and the scale parameter equal to $\text{diag}[\boldsymbol{p}(1-\boldsymbol{p})]$, where $\boldsymbol{p}$ is the vector of mean proportional viabilities at the dish level for each life-cycle stage. For binary responses where there is only one trial per observation (individuals are either viable or unviable), it is not possible to estimate the residual covariation (Hadfield 2014). Consequently, we constrained the prior for the residual term to fit an identity matrix for the residual covariance (Hadfield 2014). The Markov chain Monte Carlo (MCMC) chain had a length of 1,050,000 iterations, a burn-in period of 50,000 iterations, and a sampling interval of 1,000 iterations, resulting in 1,000 MCMC samples of the posterior distributions of the parameters. We then used posterior prediction to confirm that our model was an adequate fit for our data. Specifically, we confirmed that $\boldsymbol{p}$ was within the 95% highest posterior density (HPD) interval of the posterior predictive distribution for the corresponding life-cycle stage.

*Experiment 2, Objective 2: Of the Variation Captured by **S** and **I**, How Much Is Oriented in the Direction of $\boldsymbol{a}_{\max}$?* We used projection to quantify the variance in paternal quality (**S**) and the variance in parental compatibility (**I**) aligned in the direction of the greatest benefits of GMC ($\boldsymbol{a}_{\max}$). For **S**, the projection is $\boldsymbol{S}_{\boldsymbol{a}_{\max}} = |\boldsymbol{a}_{\max}|^{\mathrm{T}}\mathbf{S}|\boldsymbol{a}_{\max}|$, and similarly for **I**, $\boldsymbol{I}_{\boldsymbol{a}_{\max}} = |\boldsymbol{a}_{\max}|^{\mathrm{T}}\mathbf{I}|\boldsymbol{a}_{\max}|$. Given that $\boldsymbol{S}_{\boldsymbol{a}_{\max}}$ and $\boldsymbol{I}_{\boldsymbol{a}_{\max}}$ quantify the variance in the direction of the benefits of GMC, the greater the value of $\boldsymbol{S}_{\boldsymbol{a}_{\max}}$ or $\boldsymbol{I}_{\boldsymbol{a}_{\max}}$, the greater the gains in relative fitness from choosing the correct mate and the greater the potential losses from choosing poorly. Hence, a significant difference in magnitude between $\boldsymbol{S}_{\boldsymbol{a}_{\max}}$ and $\boldsymbol{I}_{\boldsymbol{a}_{\max}}$ would indicate support for GMC in favor of paternal quality or parental compatibility. We examined significant differences in the magnitude of $\boldsymbol{S}_{\boldsymbol{a}_{\max}}$ and $\boldsymbol{I}_{\boldsymbol{a}_{\max}}$ by applying the projection above to each MCMC sample of the posterior distributions of **S** and **I**. Then we determined a significant difference between $\boldsymbol{S}_{\boldsymbol{a}_{\max}}$ and $\boldsymbol{I}_{\boldsymbol{a}_{\max}}$ by examining the overlap between their 95% highest posterior density (HPD) intervals. Specifically, nonoverlapping 95% HPD intervals would indicate that differences between the magnitudes of $\boldsymbol{S}_{\boldsymbol{a}_{\max}}$ and $\boldsymbol{I}_{\boldsymbol{a}_{\max}}$ are significant.

*Experiment 2, Objective 3: What Are the Relative Effects of Differences in Fertilization Success on Viability in Later Life-Cycle Stages for **S** and **I**?* Hansen and Houle (2008) call the projection above a measure of unconditional evolvability, which they then extend to formalize a measure for the integration of one trait with respect to remaining traits in the covariance matrix of interest. For example, the integration of fertilization success in the space of **S** is $i(\mathbf{S}_{\text{fert}}) = 1 - (\boldsymbol{b}^{\mathrm{T}}\mathbf{S}\boldsymbol{b}\boldsymbol{b}^{\mathrm{T}}\mathbf{S}^{-1}\boldsymbol{b})^{-1}$, where $\boldsymbol{b}$ is a vector with a coefficient of 1 for fertilization success and 0 for the remaining life-cycle stages. We investigated the integration of fertilization success in the space of **S** and **I** to explore differences in the degree to which biases in fertilization driven by GMC would result in correlated responses in viability during subsequent life-cycle stages. The integration metric is bounded between 0 and 1: a value of 0 indicates that no correlated responses are expected and a value of 1 that strong correlated responses are expected (Hansen and Houle 2008). We calculated the integration of fertilization success for each MCMC sample of **S** and **I**, then examined the overlap between the posterior distributions of $i(\mathbf{S}_{\text{fert}})$ and $i(\mathbf{I}_{\text{fert}})$ for evidence of a significant difference in the integration of fertilization success between paternal quality and parental compatibility effects.

Results

Experiment 1: Are There Indirect Benefits to Female Mate Choice?

In clutches where we exposed eggs to the sperm of three males simultaneously, the mean multivariate viability was greater than that in clutches where we exposed eggs to the sperm of the same three males in isolation ($\chi^2_3 = 22.558$, $P < .001$; fig. 1). In other words, offspring viability was higher

in clutches where we allowed GMC than in clutches where we experimentally precluded GMC. Moreover, transgressive-overyielding analyses showed that positive sexual selection was the most likely driver of the indirect benefits of GMC in our study. Specifically, we can reject ecological genetic-diversity effects because the mean multivariate viability in the choice treatment was significantly less than the mean multivariate viability in the best constituent no-choice treatment for each female (fig. 1; life-cycle stage × treatment: $\chi^2_3 = 27.524$, $P < .001$). In our experiments, we standardized the density of offspring at the beginning of each life-cycle stage; hence, although we found no significant difference between mate-choice treatments for juvenile viability, that does not imply that the benefits of GMC disappear during the juvenile stage. Overall, clutches where we allowed GMC produced 1.3 times as many viable juvenile offspring as clutches where we experimentally precluded GMC.

Examining the life-cycle stages underlying differences in multivariate viability between the choice and no-choice treatments (i.e., $\boldsymbol{a}_{\max}$) showed that differences in mean larval viability between the choice and no-choice treatments contributed most strongly to the indirect benefits of GMC, followed closely by differences in mean embryo viability (table 1; fig. 1). Mean differences in fertilization success between choice and no-choice treatments were of a smaller magnitude than those in embryo and larval viability (fig. 1). Nonetheless, the signs of the loadings for fertilization success, embryo viability, and larval viability were all positive, indicating that the choice treatment had greater mean viability than the no-choice treatment across all three life-cycle stages (table 1; fig. 1). Conversely, the negative sign on the loading for juvenile viability showed that mean juvenile viability was lower in the choice treatment than in the no-choice treatment (table 1; fig. 1). However, the magnitude of the loading for juvenile viability was small compared with that for the remaining life-cycle stages and thus contributed only weakly to differences among mate-choice treatments (table 1; fig. 1).

Experiment 2: How Much Variation Is There among Sires and among Sire × Dam Combinations, and How Is This Variation Distributed?

Offspring viability was more variable among sire × dam combinations than among sires, suggesting that parental compatibility had a greater effect on variation in offspring viability than did paternal quality (**I** and **S** in table 2, respectively). The greatest difference in variance between paternal quality and parental compatibility effects was for fertilization success, where the variance of parental compatibility effects was 44.7 times that of paternal quality effects (table 2). For the proportion of viable embryos and larvae, differences in variance were more modest but still considerable—the variance for parental compatibility effects was 2.7 and 1.7 times that for paternal quality effects for embryo and larval viability, respectively (table 2). Conversely, the variance of the proportion of viable juveniles for paternal quality effects was 2.0 times that for parental compatibility effects (table 2).

Given the large uncertainty surrounding the bivariate correlations between fertilization success and the remaining life-cycle stages (table 2), it is difficult to comment on the biological significance of the bivariate correlations. Nonetheless, for parental compatibility effects we did find a significant negative correlation between fertilization success and embryo viability (table 2). Although the exact cause of this negative correlation is unknown, we suspect that this may have occurred as a consequence of misclassifying polyspermic eggs as viable embryos. Given that polyspermy is most likely when gametes are highly compatible (Levitan 2004), this classification error would likely result in lower embryo viability in cases where sire × dam combinations have high fertilization success driven by high gamete compatibility.

Although correlations are a convenient summary of the association between two traits, it is the effect these associations have on the distribution of variation across the phenotypic space that determines the potential for evolutionary change. Comparing the magnitudes of $\boldsymbol{S}_{\boldsymbol{a}_{\max}}$ and $\boldsymbol{I}_{\boldsymbol{a}_{\max}}$ allowed us to directly compare the amount of variation in paternal quality and parental compatibility aligned in the direction of the multivariate trait combination describing the greatest benefits of GMC. Overall, our data suggest that the benefits of GMC result from biasing fertilization in favor of the most compatible male rather than the highest-quality male (fig. 2). The 95% HPD intervals of $\boldsymbol{S}_{\boldsymbol{a}_{\max}}$ and $\boldsymbol{I}_{\boldsymbol{a}_{\max}}$ did not overlap, indicating that there was significantly more variation in the direction of GMC for parental compatibility effects than for paternal quality effects (fig. 2). In addition, although the integration of fertilization success did not differ between paternal quality and parental compatibility effects ($i(\mathbf{S}_{\text{fert}})$ posterior mean [95% HPD]: 0.95 [0.43–0.99]; $i(\mathbf{I}_{\text{fert}})$ posterior mean [95% HPD]: 0.89 [0.13–0.99]), integration is a standardized metric (i.e., it is bounded within the 0–1 interval) and therefore does not capture the absolute differences in the numbers of viable offspring produced when GMC favors paternal quality or parental compatibility. Examining the variance of parental compatibility and paternal

Table 1: $\boldsymbol{a}_{\max}$ vector

Life-cycle stage	$\boldsymbol{a}_{\max}$
Fertilization	.220
Embryo	.490
Larval	.530
Juvenile	−.043

Table 2: **S** and **I** matrices

Life-cycle stage	Fertilization	Embryo	Larval	Juvenile
$\bar{p}$	.988	.752	.496	.753
S × 10^{-4}:				
Fertilization	**.126**	.135 (−.959, .999)	−.223 (−.999, .966)	.192 (−.956, .999)
Embryo	.235	**23.898**	−.788 (−.999, −.450)*	.472 (−.329, .999)
Larval	−.629	−30.633	**63.313**	−.704 (−.999, .114)**
Juvenile	.246	8.295	−20.142	**12.922**
I × 10^{-4}:				
Fertilization	**5.653**	−.244 (−.464, −.004)*	−.003 (−.243, .252)	.622 (−.356, .999)
Embryo	−4.667	**64.660**	.165 (−.113, .447)	−.388 (−.997, .136)
Larval	−.071	14.117	**113.203**	−.136 (−.992, .379)
Juvenile	3.799	−8.002	−3.723	**6.596**

Note: The posterior mean estimates of the variance for each life-cycle stage in **S** and **I** are displayed on the diagonal in boldface, the posterior mean estimates of the covariances are below the diagonal, and the posterior mean estimates of the correlations with corresponding 95% highest posterior density (HPD) intervals are above the diagonal. $\bar{p}$ denotes the posterior mean probability of viability for each life-cycle stage.

* Significant at 95% HPD.

** Significant at 90% HPD.

quality effects shows that there was, overall, 1.8 times as much variation in offspring viability among sire × dam combinations as among sires (comparing the sum of the diagonal elements of **S** and **I**; table 2). Consequently, we expect GMC to favor parental compatibility because there was greater opportunity for differences in mating success among individuals to emerge.

Discussion

We found evidence for benefits of gamete-level mate choice (GMC) in an externally fertilizing marine invertebrate. Eggs that were allowed to "choose" among the sperm of multiple males achieved higher fertilization success and higher postfertilization viability than eggs that had no choice. Moreover, these benefits of GMC were not driven by genetic diversity alone (McLeod and Marshall 2009); rather, they appeared to be driven by the biasing of fertilization in favor of genetically compatible males. These results suggest that compatibility at fertilization reflects postfertilization genomic compatibility. Whether these compatibility effects are indicative of a mechanism for avoiding inbreeding with related individuals (Tregenza and Wedell 2002) or a more sophisticated mate-choice mechanism (Zeh and Zeh 1996; Tregenza and Wedell 2000) remains unclear. Overall, our results show that despite limited opportunities for mate choice before fertilization, females reproducing via the ancestral reproductive strategy still influence mating interactions and bias paternity to maximize the performance of their offspring.

Our results help resolve a paradox regarding gamete interactions in external fertilizers. A consequence of broadcasting gametes into the surrounding environment is that sperm concentrations dilute rapidly and many eggs can remain unfertilized (Yund 2000). Given this strong selection on mating success imposed by sperm limitation, it is surprising that some gamete combinations are more compatible than others (Palumbi 1999; Levitan and Ferrell 2006; Levitan and Stapper 2010). If sperm limitation alone drove the evolution of gamete recognition, we would expect all individuals of a given species to be equally compatible, thereby ensuring successful fertilization whenever male and female gametes of the same species collide (Levitan 2004). Instead, variability in fertilization success is ubiquitous, implying that selection favors occasional fertilization failure be-

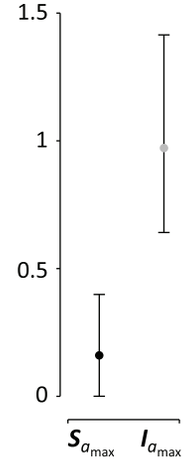

Figure 2: Posterior means and 95% highest posterior density (HPD) intervals for the projection of $\boldsymbol{a}_{max}$ on **S** and **I** ($\boldsymbol{S}_{a_{max}}$ and $\boldsymbol{I}_{a_{max}}$, respectively). Nonoverlapping 95% HPD intervals indicate a significant difference the contribution of paternal quality (black circle) and parental compatibility (gray circle) to the benefits of gamete-level mate choice. *Y*-axis values are in units of variance (×10^{-2}).

cause of the benefits of discriminating against the sperm of some males over that of others. Previously, it has been argued that the evolution of incompatibility reduces the risk of polyspermy (Levitan 2004), and similar arguments could be applied in our system (see "Results"). Nevertheless, our results suggest that the costs of losing some fertilizations to sperm limitation or polyspermy can be overcome by the benefits of biasing fertilization toward more compatible males because these pairings yield greater numbers of viable offspring. Specifically, we found that individuals that exercise GMC contribute, on average, 1.3 times as many juvenile offspring to the population as individuals where we precluded the opportunity for GMC. Moreover, our data suggest that if postfertilization differences in offspring viability between choosy and naïve females remain constant, choosy females could endure a 24% decline in mean fertilization success and still produce a number of juvenile offspring equivalent to that of females where mate choice is limited or absent.

Despite the finding that sire effects were strong and persistent, it is unlikely that the benefits of GMC we found were driven by paternal quality effects. The variance aligned in the direction of the benefits of GMC was 6.7 times as great for parental compatibility effects as for paternal quality effects. This result implies that differences between the proportions of the offspring population mothered by females choosing correctly and by females choosing poorly are considerably larger when sire-compatibility effects determine mating decisions. Hence, although eggs capable of biasing fertilizations in favor of the highest-quality male may produce greater numbers of viable offspring than eggs that are unable to choose, these benefits are only a fraction of the reproductive benefits that could be achieved by successfully biasing fertilization in favor of the most compatible males.

In a previous study on *Ciona robusta*, we showed that, even when we precluded the opportunity for GMC, benefits of mixed clutches emerged, and that some of those benefits were driven by selection (Aguirre and Marshall 2012*a*). In Aguirre and Marshall (2012*a*), instead of allowing sexual selection to bias mating success, we experimentally produced multiply sired clutches by manipulating the genetic diversity of the larval population, enforcing paternity from each male. Accordingly, in Aguirre and Marshall (2012*a*), differences in offspring viability between singly sired and multiply sired clutches were determined by natural selection alone. Despite these differences between our studies, both showed that the mechanism driving benefits of mixed clutches was selection for individuals from better-performing families. Interestingly, while these benefits prevailed long after our manipulations of clutch-level paternity, they waned sooner when sexual selection and natural selection were allowed to operate earlier in the life cycle.

The correlation between incompatibility at fertilization and reduced performance after fertilization could be driven by the evolution of inbreeding avoidance. Biparental inbreeding is thought to be a significant problem for sessile marine invertebrates with external fertilization, yet the fitness consequences of inbreeding are rarely tested, and the mechanisms by which these organisms avoid inbreeding are poorly resolved (Knowlton and Jackson 1993). Nevertheless, studies on *C. robusta* have shown that self-sperm (recall that *C. robusta* is a simultaneous hermaphrodite) have lower affinity with eggs than non-self-sperm (Harada et al. 2008; Yamaguchi et al. 2011; Saito et al. 2012). An extension of the self-incompatibility system to include less extreme forms of inbreeding would allow the genetics underlying gamete interactions to interact with the more complex genetics determining postfertilization genomic compatibility. Recent studies also suggest that mechanisms operating before gametes come into contact—sperm chemotaxis—depend heavily on the genetic identity of sperm and egg donors (Evans and Sherman 2013). For instance, Oliver and Evans (2014) showed that sperm of an externally fertilizing mussel preferentially swim toward the egg-derived chemoattractants of some females over those of others. Critically, they show that sperm preferences are biased toward the eggs of females likely to produce better-quality offspring. Determining support for the role of inbreeding avoidance in determining incompatibility at fertilization requires additional manipulative studies examining whether reproductive success differs predictably in response to the level of inbreeding (e.g., Tregenza and Wedell 2002).

External fertilization is the mating strategy from which all mating strategies evolved (Wray 1995). However, in the context of mate choice and sexual selection, external fertilization is arguably the mating strategy of which we know the least (Evans and Sherman 2013). In part, our lack of understanding stems from the historical perspective that it is difficult to envisage how such a primitive mating strategy, involving sexually monomorphic sexual partners, provides opportunities for sexual selection to operate (Darwin 1871; Arnold 1994; Levitan 2005). However, pioneering studies have succeeded in shifting our focus from interactions among sexual partners to interactions among the gametes themselves, thereby elucidating the influence that sexual selection has in shaping the evolutionary trajectories of populations in externally fertilizing species (Palumbi 1999; Levitan 2004; Levitan and Ferrell 2006). Our results contribute to this growing body of literature and show that females, via their eggs, enhance their own reproductive success by biasing fertilization in favor of the males likely to produce the greatest number of viable offspring. Moreover, we provide evidence that, although mate choice for paternal quality and for parental compatibility can operate simultaneously, differences in the relative magnitudes of these effects determine the opportunity for sexual selection and therefore the likely target of mate choice.

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