

DOES GENETIC DIVERSITY REDUCE SIBLING COMPETITION?

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An enduring hypothesis for the proximal benefits of sex is that recombination increases the genetic variation among offspring and that this genetic variation increases offspring performance. A corollary of this hypothesis is that mothers that mate multiply increase genetic variation within a clutch and gain benefits due to genetic diversity alone. Many studies have demonstrated that multiple mating can increase offspring performance, but most attribute this increase to sexual selection and the role of genetic diversity has received less attention. Here, we used a breeding design to generate populations of full-siblings, half-siblings, and unrelated individuals of the solitary ascidian *Ciona intestinalis*. Importantly, we preclude the potentially confounding influences of maternal effects and sexual selection. We found that individuals in populations with greater genetic diversity had greater performance (metamorphic success, postmetamorphic survival, and postmetamorphic size) than individuals in populations with lower genetic diversity. Furthermore, we show that by mating with multiple males and thereby increasing genetic variation within a single clutch of offspring, females gain indirect fitness benefits in the absence of mate-choice. Our results show that when siblings are likely to interact, genetic variation among individuals can decrease competition for resources and generate substantial fitness benefits within a single generation.

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Asexually reproducing organisms produce offspring that are almost genetically identical; in contrast, sexual organisms produce offspring that are genetically variable. Sexually reproducing parents produce genetically variable offspring because recombination of male and female genes produces novel gene combinations (Williams 1975; Maynard Smith 1978). If genetic variation among offspring results in differences in morphology, physiology, or behavior, then genetic variation may also generate variation in resource use among offspring within a single clutch (Bulmer 1980; Barton and Post 1986; Case and Taper 1986). The theoretical correlation between offspring genetic variation and offspring niche variation is one of the oldest proposed mechanisms to reconcile the costs of sexual reproduction relative to asexual reproduction (Williams 1975; Maynard Smith 1978).

The benefits of a genetically variable clutch could increase the fitness of sexually reproducing organisms within a single generation through two broad classes of mechanisms: frequency-dependent mechanisms and frequency-independent mechanisms (Young 1981). The most commonly cited frequency-dependent mechanism is the “resource partitioning hypothesis” (RPH). The RPH predicts that, because a clutch of sexually produced offspring is more genetically variable than a clutch of asexually produced offspring, sexually produced offspring will have greater variation in resource use (Maynard Smith 1978). Thus, sexually produced siblings are predicted to compete less and grow more, because their resource requirements overlap less than asexually produced siblings. According to the RPH, genetic variation among offspring can generate fitness benefits by reducing the

intensity of resource competition when siblings are likely to interact. A second, frequency-dependent mechanism is the “diversity-disease” or “monoculture” hypothesis: if the likelihood of disease or predation increases as the genetic similarity among individuals increases, then genetic variation among sexually produced siblings could also reduce the likelihood of disease and predation (Wolfe 1985; Zhu et al. 2000; Jokela et al. 2009). Alternatively, genetic diversity within a clutch can provide benefits via frequency-independent mechanisms (i.e., the lottery or bet-hedging hypothesis [Williams and Mitton 1973; Yasui 1998]). Unlike the RPH or diversity-disease hypothesis where the fitness returns of a genetically variable clutch are frequency-dependent, under the lottery hypothesis, the fitness returns of a genetically variable clutch are frequency-independent—only the fittest offspring will survive in a given environment. In the lottery hypothesis, because sexual recombination generates a greater range of genotypes, a sexually reproducing female is more likely to produce successful offspring in a greater range of environments (Williams and Mitton 1973; Yasui 1998).

Although the hypotheses described above were initially developed to understand the benefits of sexual reproduction, relative to its costs in comparison with asexual reproduction, they extend more generally to species where a females can mate with multiple males (Barton and Post 1986) or to situations in which the mating system or dispersal history results in aggregations of siblings or unrelated individuals (Tonsor 1989; Argyres and Schmitt 1992; Donohue 2003). For example, Barton and Post (1986) show analytically that, by producing a genetically variable clutch, females that mate with multiple males can have greater reproductive success than females that mate with only a single male. In contrast to recent hypotheses regarding role of sexual selection in generating the indirect genetic benefits of multiple mating (Arnqvist and Nilsson 2000; Jennions and Petrie 2000; Simmons 2005), Barton and Post (1986) predicted that benefits of multiple mating can occur in the absence of sexual selection. Specifically, it is the relative difference in genetic variation between clutches of half-siblings and clutches of full-siblings that can drive increases in offspring performance, and thereby increase the reproductive success of females that mate multiply (Schmitt and Antonovics 1986; Pakkasmaa and Aikio 2003; McLeod and Marshall 2009). Likewise, if sibling competition is strong, or if the environment is heterogeneous, there may be population-level benefits of genetic variation, as populations of unrelated individuals should have greater performance than populations of siblings (Griffiths and Armstrong 2001; Cheplick and Kane 2004; Mattila and Seeley 2007).

Although theoretical predictions of the benefits of increasing population-level genetic diversity are intuitively appealing, empirical evidence for such benefits remains equivocal. In some studies, groups of full-siblings have been shown to outperform groups of half-siblings and unrelated individuals (Willson et al.

1987; Tonsor 1989; Donohue 2003), whereas in others, the benefits of developing in groups of siblings or unrelated individuals are context-dependent (Andalo et al. 2001; Pakkasmaa and Aikio 2003). Furthermore, studies that test the benefits of genetic variation among offspring, and that also preclude the confounding effects of sperm competition, differences in maternal investment among offspring sired by different males, and differences in parental identity are surprisingly rare. In studies where females are allowed to mate with multiple males, sperm competition can favor biases in paternity toward males that produce high-quality offspring (Fisher et al. 2006), or males that produce low-quality offspring (Bilde et al. 2009). In such a case, any effects of genetic variation can be obscured by, or mistaken for, the effects postmating sexual selection on offspring performance. Furthermore, in internally fertilizing organisms, females can alter the provisioning given to embryos sired by different males, thereby confounding differences in genetic variation with differences in maternal provisioning (Marshall and Ellstrand 1986; Cunningham and Russell 2000). Thus, it remains unclear as to whether there are benefits of genetic diversity in organisms that mate multiply that are independent of the effects of sexual selection.

Externally fertilizing species are an ideal model system in which to explore the consequences of genetic diversity because the mating system allows us to generate groups of individuals with different levels of relatedness while removing the influence of sperm competition, maternal effects, and parental genetic identity. Here, using the solitary ascidian *Ciona intestinalis*, we manipulated the genetic diversity of populations by generating full-sibling monocultures, half-sibling polycultures, and unrelated polycultures. We measured metamorphic success in the laboratory, and postmetamorphic performance (both survival and size) in the field to determine the influence of genetic diversity on overall performance. In our experiment, we excluded the influence of sperm competition and sexual selection by fertilizing eggs in single-pair matings, and then mixing larvae of known parentage to generate larval populations of varying relatedness. In externally fertilizing species, maternal provisioning of offspring occurs exclusively before fertilization, so in our experiments we can also exclude biases in maternal investment among the larvae assigned to different treatments. Lastly by using a diallel breeding design to generate our experimental populations (McLeod and Marshall 2009), we can eliminate genetic identity effects—all offspring within a block are descendants of the same set of males and females, and differ only in terms of their relatedness to the individuals in their population. [Correction made here after initial online publication.] Our results suggest a positive role of genetic variation among individuals on performance and provide a strong evidence for the benefits of genetic diversity in sexually reproducing animals. Furthermore, we show that these benefits are likely driven by greater resource partitioning in populations with greater genetic diversity.

Methods

STUDY SITE AND SPECIES

All experiments and collections were conducted at the Lincoln Cove Marina, Port Lincoln, South Australia (34°44'28.76''S, 135°52'02.88''E), between July and August 2008. The field site is a sheltered marina connected to a large bay via a canal with regular tidal exchange. Ambient water temperature during the experiment was ~14°C. *Ciona intestinalis* is an invasive, filter-feeding, solitary ascidian, that has colonized human modified environments (e.g., pontoons, boat hulls, aquaculture facilities) throughout the world (Lambert and Lambert 2003; Ramsay et al. 2008; Zhan et al. 2010). Like many marine invertebrates, *C. intestinalis* has a biphasic life-history in which a swimming, nonfeeding larva metamorphoses into a sessile, feeding adult. Competition for space and food among sessile marine invertebrates is intense (Buss and Jackson 1981; Buss 1990) and in *C. intestinalis* competition is density dependent (Marshall and Keough 2003). Adult *C. intestinalis* are dominant competitors in marine invertebrate communities, often found in dense (>5000 individuals m⁻²) monocultures (Havenhand and Svane 1991; Lambert and Lambert 2003; Blum et al. 2007). Furthermore, there is evidence that *C. intestinalis* prefers food particles of a certain size (Lesser et al. 1992) and feeding can deplete phytoplankton in the surrounding water column (Petersen and Riisgard 1992; Riisgard et al. 1996; Byrnes and Stachowicz 2009).

In *C. intestinalis*, fertilization and embryo development are external—both sperm and eggs are broadcast into the surrounding environment. Eggs are negatively buoyant and are sometimes entrained within a mucous matrix released during spawning (Svane and Havenhand 1993). Sperm are capable of penetrating this mucous matrix and fertilizing eggs, but dispersal can be limited by the entrainment of eggs and larvae in the mucous matrix (Svane and Havenhand 1993; Petersen and Svane 1995). These features of *Ciona* biology suggest that, at scales relevant to this study, interactions among full-siblings (eggs entrained in the mucous matrix fertilized by a single male), half-siblings (eggs entrained in the matrix fertilized by multiple males), and unrelated (a cohort of unrelated, dispersive larvae) individuals occur in the wild. Below, we first describe our overall experimental design for generating our treatments of interest, and we then provide specific information on the execution of the experiment.

EXPERIMENTAL DESIGN

We used a diallel breeding design to generate three relatedness treatments at the larval stage (Fig. 1); full-sibling monocultures (larvae with the same parents), half-sibling polycultures (mixtures of larvae from three half-sibling families), and unrelated polycultures (mixtures of larvae from three unrelated families). [Correction made here after initial online publication.] Each block

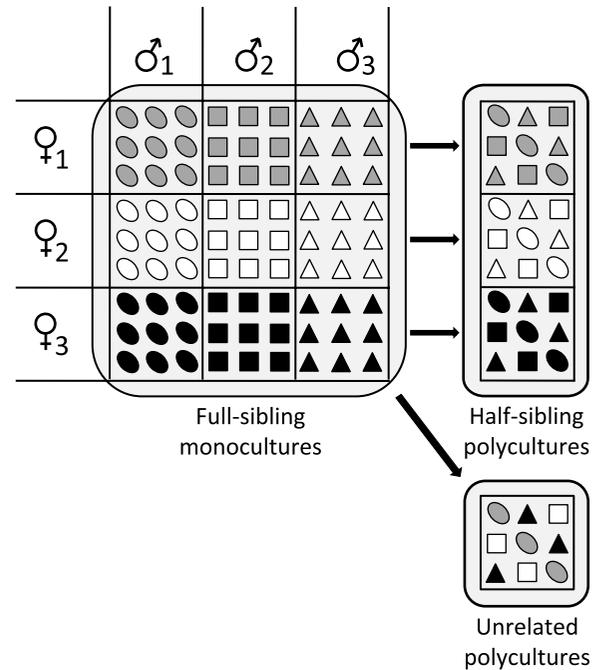


Figure 1. Schematic of methods used to generate full-sibling monocultures, half-sibling polycultures, and unrelated polycultures. Symbols indicate paternal half-sibling families. Colors indicate maternal half-sibling families.

consisted of three males crossed with three females in all possible combinations to produce nine full-sibling families (Fig. 1). Different males and females were used in each block. To create full-sibling monocultures, we placed 45 larvae from each full-sibling family in a separate settlement dish (a total of nine dishes per block: one for each full-sibling family). In each block, there were three maternal half-sibling families (Fig. 1). To create half-sibling polycultures, we placed 15 larvae from each maternal half-sibling family (a total of 45 half-sibling larvae) into a separate settlement dish (a total of three dishes per block: one for each maternal half-sibling family). Although in our experimental design it is possible to generate three unique, unrelated polycultures for each block, due to logistical constraints we created only one unrelated polyculture per block. To create unrelated polycultures we placed 15 larvae from three unrelated families (a total of 45 larvae) in one settlement dish (Fig. 1). Importantly, at the level of comparison, there were no systematic differences in genetic identity between treatments.

General Laboratory and Field Methods

Sessile adults were collected from floating pontoons at the field site approximately 1 m below the water surface. Adults were maintained in flow-through aquaria in the laboratory for 48 h before gametes were extracted. To extract gametes, first, we made

a slit along the length of the tunic to expose the sperm duct and the oviduct then, we randomly assigned individuals as males or females and made a small incision in either the sperm duct (males) or the oviduct (females) and extracted gametes with a syringe. For each female, a clutch of eggs (100–200 eggs) was not exposed to sperm and treated as a control: any cell division in this clutch indicated contamination by self-sperm and the entire block was discarded. Sperm was filtered through 25- μm mesh to prevent the accidental transfer of eggs. Eggs were allowed to sit in fresh seawater for 30 min prior to the addition of sperm. A constant sperm concentration (2.00×10^6 sperm.mL⁻¹) that resulted in high fertilization success (85–100%) was used within 15 min of sperm extraction. To fertilize eggs, a well-mixed 0.1-mL egg solution and 0.25-mL dilute sperm solution were mixed in a 35-mm Petri dish. Excess sperm were rinsed and eggs placed in fresh seawater 30 min after the initial exposure to sperm. After 2 h, a random subsample of 70–100 fertilized eggs was placed in a new Petri dish with fresh seawater to standardize the density of zygotes across all families.

Larvae hatched between 20 and 23 h postfertilization. We created settlement dishes by roughening Petri dishes with sandpaper and placing them in flow-through aquaria for 48 h before use. Thirty minutes after the first larvae began to hatch, 45 swimming larvae were selected haphazardly and transferred to settlement dishes filled with fresh seawater in accordance with the experimental design described above. Twenty-four hours after we placed larvae in the settlement dishes, we counted the number of larvae that were alive in each dish, and of those that were alive how many larvae had attached to the Petri dish. Individuals that were still alive but had not attached within the 24 h were discarded because pilot studies in southern Australia showed that larvae that delay metamorphosis for >24 h are unable to successfully complete metamorphosis (D. J. Marshall unpubl. data). We then placed the dishes with the attached larvae in flow-through aquaria for an additional 24 h to allow individuals to complete metamorphosis. Forty-eight hours after larvae were initially placed in settlement dishes we counted the number of attached larvae that had successfully completed metamorphosis. Then, we randomly selected 10 focal individuals in each dish to be transferred to field and removed all other individuals with forceps.

In the field, dishes containing our focal recruits were attached to large PVC panels (550 mm \times 550 mm \times 6 mm) suspended facedown 1 m below the floating pontoons to imitate the orientation of wild individuals at the field site. Two panels, separated vertically by 50 mm, were used in this experiment. After 7, 14, and 28 days in the field, we measured offspring survival by counting the number of focal recruits remaining in each dish. Because higher density can cause lower survival in *C. intestinalis* (Marshall and Keough 2003), we standardized the density of recruits when we transferred dishes to the field, and after each census (10

recruits per dish at day 0, 5 recruits per dish after day 7 and 3 recruits per dish after day 14), to minimize the effect that differences in recruit density among treatments (due to differences in metamorphic success or postmetamorphic survival) may have in our experiment. After 35 days in the field, to estimate postmetamorphic size, we photographed individuals under a compound microscope and measured recruit side area with Image Pro Express version 5.1 image-analysis software (Marshall and Keough 2003; Jacobs et al. 2008). Note that the last standardization of recruit density was done after 14 days, and so our differences in postmetamorphic size among treatments are not independent of differences in postmetamorphic density among treatments after 28 days in the field (see Results).

The performance metrics in our study were: metamorphic success—the probability of successfully completing metamorphosis after 48 h given that larvae were alive and had attached within 24 h posthatching; postmetamorphic survival—the joint probability of survival after 7, 14, and 28 days in the field; and postmetamorphic size—mean recruit side area after 35 days in the field.

DATA ANALYSIS

The effects of relatedness on metamorphic success, postmetamorphic survival, and postmetamorphic size were analyzed using a mixed-model ANCOVA. The response variable in each analysis was the mean value for each performance metric for each relatedness treatment for each block. We treated relatedness as a fixed, continuous factor and block as a random, categorical factor. There were eight blocks where full-sibling monocultures, half-sibling polycultures, and unrelated polycultures were represented. Block was an experimental convenience and removed from the analyses if $P > 0.15$ (Quinn and Keough 2002). To explicitly test for differences in performance between full-sibling monocultures and half-sibling polycultures we used a mixed-model ANOVA. The response variable in each analysis was the mean value for each performance metric for each relatedness treatment for each block. We treated relatedness as a fixed, categorical factor with two levels (full-sibling monocultures and half-sibling polycultures) and block as a random, categorical factor. To test for correlations in family rank performance between the three performance measures (metamorphic success, postmetamorphic survival, and postmetamorphic size) we used Spearman's rho. We calculated bootstrapped confidence intervals for the correlations that were considered significant if the confidence interval for the correlation did not overlap zero.

The greater probability of sampling high (or low) performing individuals in treatments with greater diversity is the foundation of the lottery hypothesis for the benefits of sexual reproduction (Williams and Mitton 1973), the bet-hedging hypothesis for the benefits of multiple mating (Yasui 1998), sampling effects in

experiments investigating the relationship between biodiversity and ecosystem function (Tilman et al. 1997; Loreau and Hector 2001), and more recently, the benefits of kin-recognition in plants (Masclaux et al. 2010). The benefits of diversity when driven by sampling effects are a consequence of: first, the increased probability of sampling high performing individuals in treatments that include a greater range of genotypes or species, second, positive selection for high performing individuals, and last, the overrepresentation of high performing individuals in treatments with greater diversity. These frequency-independent benefits of diversity differ from frequency-dependent benefits of diversity, because in frequency-dependent effects, performance in treatments with higher diversity is an emergent property of diversity itself. That is, the performance of a high diversity population cannot be predicted based on the performances of the constituent species in isolation, rather it is the diversity among individuals that allows resources to be partitioned or greater protection from disease and predation. A simple way to differentiate between frequency-dependent and frequency-independent processes is to test for transgressive overyielding. Transgressive overyielding analyses (Tilman et al. 1997; Cardinale et al. 2007; Stachowicz et al. 2007) determine whether the increase in performance in populations with greater diversity was due to the overrepresentation of high performing individuals (i.e., frequency-independent processes) or an emergent property of diversity (i.e., frequency-dependent processes). The null hypothesis in transgressive overyielding analyses is that the performance of high diversity treatments is driven by the overrepresentation of individuals from high performing families. Therefore, a significant treatment effect in transgressive overyielding analyses indicates resource-partitioning or disease-diversity effects. Failure to detect significant transgressive overyielding favors the null and so suggests lottery or bet-hedging effects drive the benefits of increasing genetic diversity.

To test for transgressive overyielding, we compared the performance of the best performing full-sibling monoculture to the performance of the unrelated polyculture in each block using a one-tailed *t*-test (Cardinale et al. 2007; Stachowicz et al. 2007). We tested for transgressive overyielding for each performance metric (metamorphic success, postmetamorphic survival, and postmetamorphic size) independently for the two extremes of our relatedness treatments (i.e., full-sibling monocultures vs. unrelated polycultures), then we examined transgressive overyielding for overall performance for full-sibling monocultures versus half-sibling polycultures and for full-sibling monocultures versus unrelated polycultures. Overall performance was a composite measure: for each dish we calculated the joint probability of pre- and postmetamorphic survival, and then multiplied survival by mean postmetamorphic size. Response variables in transgressive overyielding analyses for overall performance were the same as for analyses where each metric was considered independently

(the performance of the best performing full-sibling monoculture compared with the mean performance of half-sibling or unrelated polycultures). Full-sibling monocultures and unrelated polycultures were represented in eight blocks, thus there were eight replicates of each relatedness treatment for the overyielding test for full-sibling monocultures and unrelated polycultures. There were an additional two blocks where only full-sibling monocultures and half-sibling polycultures were represented. Therefore, to make use of all the available data, there were ten blocks for the overyielding test for full-sibling monocultures and half-sibling polycultures. For overall performance, we used resampling to assess the significance of the test statistic. Generating composite measures of performance can be inappropriate in some cases (Shaw et al. 2008), but for a simple experimental designs like the one presented here, resampling methods can produce reliable tests of hypotheses (Shaw et al. 2008).

Results

METAMORPHIC SUCCESS, POSTMETAMORPHIC SURVIVAL AND POSTMETAMORPHIC SIZE

Populations with greater genetic diversity performed significantly better than populations with lower genetic diversity (Fig. 2). With regard to metamorphic success, unrelated and half-sibling polycultures are predicted to have 28% and 19% higher metamorphic success, respectively, than full sibling monocultures ($F_{1,22} = 9.296$, $P = 0.006$; Fig. 2A). Our results also show populations with greater genetic diversity tended to have higher postmetamorphic survival and greater postmetamorphic size ($F_{1,22} = 4.276$, $P = 0.051$ and $F_{1,13} = 11.565$, $P = 0.005$ respectively). Although variation among replicates within diversity treatments was considerable, our results predict that unrelated polycultures will have 28% greater metamorphic success (Fig. 2A), 70% greater postmetamorphic survival (Fig. 2B), and those surviving individuals will be on average 88% larger than individuals in full-sibling monocultures, after 35 days in the field (Fig. 2C). The death of all individuals between 14 and 35 days for some unrelated polyculture treatments resulted in only six replicates for unrelated polycultures in analyses for postmetamorphic size (Fig. 2C). Comparing full-sibling monocultures and half-sibling polycultures, we found half-sibling polycultures had significantly greater metamorphic success than full-sibling monocultures ($F_{1,14} = 7.923$, $P = 0.014$ and), but for postmetamorphic survival and size the difference between full-sibling monocultures and half-sibling polycultures was marginally nonsignificant ($F_{1,14} = 4.466$, $P = 0.053$ and $F_{1,7} = 4.583$, $P = 0.070$ respectively). There was no correlation in rank family performance (Spearman's rho) between metamorphic success and postmetamorphic survival or metamorphic success and postmetamorphic size (mean r_s ($\pm 95\%$ CI):

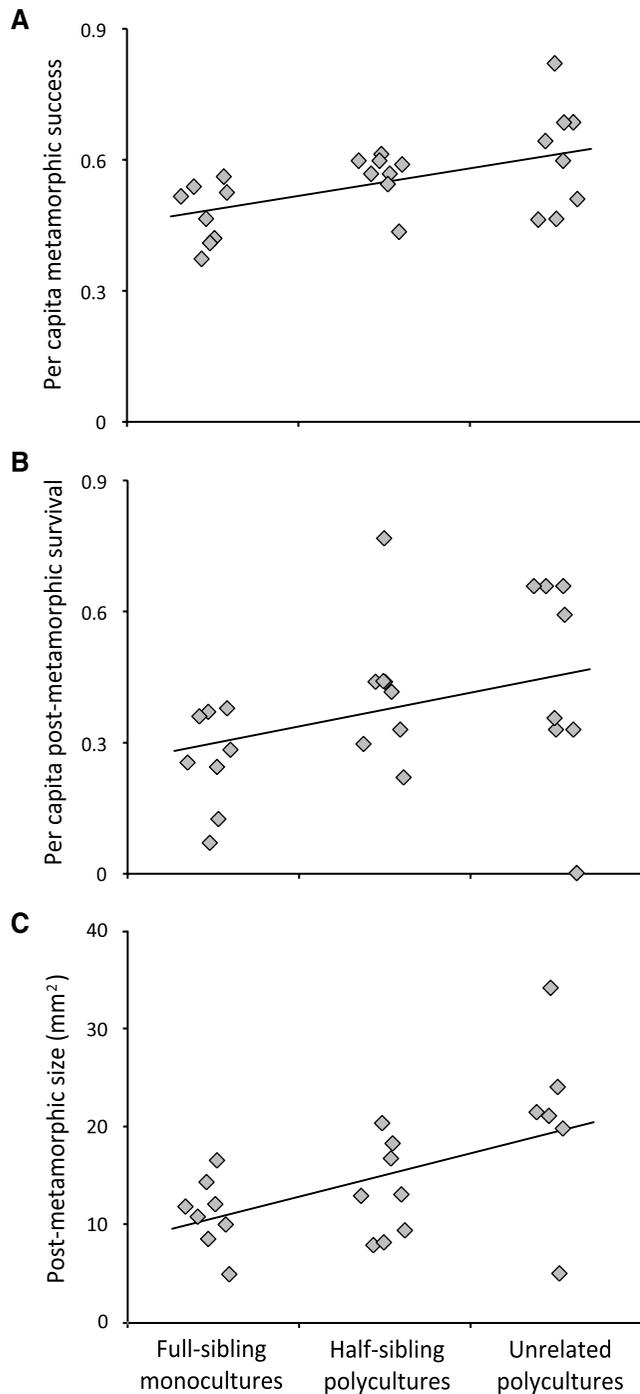


Figure 2. Populations with greater genetic diversity had greater metamorphic success (panel A), postmetamorphic survival (panel B), and postmetamorphic size (panel C). Symbols indicate treatment means for each block and are offset for display purposes.

0.032 (−0.227, 0.295); and 0.185 (−0.072, 0.424) respectively). However, there was a positive correlation in rank family performance between postmetamorphic survival and postmetamorphic size (mean r_s ($\pm 95\%$ CI): 0.337 [0.078, 0.569]).

TRANSGRESSIVE OVERYIELDING

We found no evidence for transgressive overyielding (i.e., frequency-dependent effects) for metamorphic success or post-metamorphic survival ($t_{14} = 0.753$, $P = 0.768$ and $t_{14} = -0.508$, $P = 0.310$), but we did find support for transgressive overyielding for postmetamorphic size ($t_{11} = -1.972$, $P = 0.040$) when comparing full-sibling monoculture and unrelated polyculture treatments. In addition to finding strong support for a mean differences in overall performance between full-sibling monocultures and unrelated polycultures, as well as between full-sibling monocultures and half-sibling polycultures ($P = 0.011$ and $P < 0.001$ respectively), we found significant effects in transgressive overyielding analyses for full-sibling monoculture versus unrelated polyculture and for full-sibling monoculture versus half-sibling polyculture treatments ($P = 0.036$ and $P = 0.052$ respectively), thereby indicating that frequency-dependent effects likely played a role in driving differences in overall performance among populations with different levels of genetic diversity.

Discussion

Genetic variation among offspring has been proposed as one of the major proximal benefits of sexual reproduction, outcrossing, and multiple mating (Maynard Smith 1978; Barton and Post 1986; McCall et al. 1989), yet empirical studies that clearly demonstrate an advantage of greater genetic diversity are rare. Here, we found that three fitness components (metamorphic success, post-metamorphic survival, and postmetamorphic size) all increased as relatedness among individuals decreased. This positive relationship between the performance of populations and the level of genetic diversity within a population (i.e., relatedness) was incremental: even small genetic differences among individuals within a population (i.e., differences in genetic diversity between groups of full-siblings and half-siblings [Barton and Post 1986]), were sufficient to generate differences in performance in sexually reproducing organisms within a single generation.

Our results suggest that, by mating with multiple-males and thereby increasing genetic variation among offspring within a single clutch, females may gain significant fitness benefits. Traditionally, the benefits of multiple mating are thought to occur because females that mate multiply increase the likelihood that they will mate with genetically superior or genetically more compatible males. The offspring sired by the best or most compatible males are then overrepresented in the offspring population because of selection on offspring genetic quality (Arnqvist and Nilsson 2000; Jennions and Petrie 2000; Simmons 2005). Hence, the benefits of multiple mating for females are often viewed as indirect and a product of sexual selection biasing offspring paternity in favor of the highest quality male. In our study, we constrained the

role of sexual selection by precluding female choice and sperm competition, yet we still saw increases in the performance of mixed clutches: overall performance of half-sibling polycultures was on average 110% greater than the best performing full-sibling monocultures. In other words, a female producing a single half-sibling clutch sired equally by three males of variable quality, would have more than double the reproductive success of a female whose eggs were sired by only the best male. Thus, the substantial enhancement in the performance of half-sibling populations that we found cannot be explained solely by selection on offspring genetic quality (i.e., frequency-independent effects), rather it seems that resource partitioning or diversity-disease effects (frequency-dependent effects) drive some of the benefits of mixed clutches (Barton and Post 1986).

By increasing the genetic differences among individuals we may expect populations with greater genetic diversity to have greater variation in resource use and consequently lower competition for resources (Maynard Smith 1978). Lower competition for resources means that individuals in populations with greater genetic diversity have more resources to invest in defense, growth, and reproduction than individuals in populations with lower genetic diversity (Schmitt and Antonovics 1986; Griffiths and Armstrong 2001; Cheplick and Kane 2004). *Ciona intestinalis* feeds by filtering planktonic particles from the water column, and we suspect that reduced competition for food in populations with greater genetic diversity is a possible explanation for the results of our study (although we are uncertain how families partition the acquisition of food). Higher rates of infection or disease in populations with greater similarity could also drive differences in performance among populations in our study (Zhu et al. 2000; Altermatt and Ebert 2008; Jokela et al. 2009). However, we are unable to comment on the possibility or prevalence of infection or disease in wild populations of *C. intestinalis* or whether the infection increases with increasing genetic similarity among *C. intestinalis* neighbors. Further studies disentangling resource partitioning from diversity-disease effects on overall performance are required, but, for now, it appears that frequency-dependent mechanisms underlie the benefits of genetic diversity we found in our study.

We found the benefits of genetic diversity for metamorphic success and postmetamorphic survival were driven by frequency-independent mechanisms (i.e., lottery or bet-hedging effects), but benefits for postmetamorphic growth were frequency-dependent (i.e., resource partitioning or diversity-disease effects). These results demonstrate that frequency-dependent and frequency-independent benefits of increased genetic diversity can operate simultaneously, and in the same direction (Young 1981). Furthermore, although frequency-independent mechanisms were responsible for enhanced metamorphic success and postmetamorphic survival in unrelated polycultures, there was no correlation in

family performance between life-history stages. The nonsignificant correlation for pre- and postmetamorphic survival suggests that those families that were more likely to successfully metamorphose were not the same families that performed well postmetamorphosis.

Relatedness within our experimental populations strongly affected postmetamorphic growth. Individuals in unrelated polycultures were considerably larger than individuals in full-sibling monocultures and half-sibling polycultures. For most of our experiment we standardized the density of individuals across all populations after each census because, competition among *C. intestinalis* recruits can be strong and performance tends to be lower at higher densities (Marshall and Keough 2003). However, because standardizing the density of individuals only corrects differences in density after some arbitrary experimental period, we might still expect lower growth in unrelated populations due to greater postmetamorphic survival and therefore greater density. Instead, we found that treatments that resulted in the greatest survival also resulted in the greatest growth. This result suggests that despite higher densities, individuals in unrelated polycultures experience lower levels of realized competition than individuals in half-sibling monocultures and full-sibling polycultures. The predicted daily growth rate for individuals in unrelated and half-sibling polycultures was 88% and 20% greater, respectively, than that in full-sibling monocultures. Because *C. intestinalis* mature at a given size and not age (Yamaguchi 1975), an increase in postmetamorphic growth could decrease the time until reproductive maturity and therefore increase overall fecundity. Thus, differences in genetic diversity among populations could generate substantial differences in population growth rates.

In longitudinal studies, where manipulations of relatedness are done only once, effects in later development may become confounded by significant effects that occur earlier in development (Perez-Tome and Toro 1982). Given we found relatedness significantly affected metamorphic success, our results for postmetamorphic size and survival should be interpreted with this caveat in mind. Nevertheless, we believe that our estimates of the effects of relatedness postmetamorphosis are robust to differences in metamorphic success among treatments for three reasons. First, we standardized recruit density before deploying dishes to the field, thereby controlling for differences in recruit density caused by differences in metamorphic success among treatments (Lopez-Suarez et al. 1993). Second, if families differed substantially in metamorphic success, the proportional representation of each family after metamorphosis would not be equal in unrelated and half-sibling polycultures. Indeed, this is likely given we found frequency-independent effects drove increases in metamorphic success for populations with greater genetic diversity. However, because an under-representation of some families would increase the relative genetic similarity within populations, our results could

be viewed as conservative estimates of the potential effects of genetic diversity compared to a situation where family representation remains equal at metamorphosis. Third, if families with high metamorphic success also had greater postmetamorphic performance, differences in performance postmetamorphosis could be driven by the overrepresentation of high performing families. Because we found no correlation between metamorphic success and postmetamorphic survival or postmetamorphic size, we also consider such an effect unlikely.

Although long predicted by theory, the positive correlation between genetic variation among individuals in a population and performance has been difficult to prove empirically. Moreover, in studies where benefits of genetic diversity have been shown, the mechanisms that generate these benefits have remained largely unknown. Here, we provide evidence that both frequency-dependent and frequency-independent mechanisms can operate simultaneously and drive differences in performance among groups of individuals that vary in relatedness.

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