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Relatedness affects the density, distribution and phenotype of colonisers in four sessile marine invertebrates

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Genetic diversity has emerged as an important source of variation in the ecological properties of populations, but there are few studies of genetic diversity effects on colonisation processes. This relative scarcity of studies is surprising given the influence of colonisation on species coexistence, invasion, and population persistence. Here, we manipulated relatedness in experimental populations of colonising larvae in four sessile marine invertebrates. We then examined the influence of coloniser relatedness on the number, spatial arrangement and phenotype of colonisers following permanent settlement. Overall, relatedness influenced colonisation in all four species, but the effects of relatedness on colonisation differed among species. The variable responses of species to manipulations of relatedness likely reflect differences in intensity of inter- and intra-specific competition among adults, as well as the differential consequences of larval behaviours for each species. Relatedness appears to play an underappreciated role in the colonisation process, and we recommend that future studies of genetic diversity effects consider not only adult stages – the focus of most work to date – but also the importance of genetic diversity in early life history stages.

Intraspecific genetic diversity affects ecosystem function in a variety of ways (Hughes et al. 2008). Within a single generation, genetic diversity can affect the productivity and resilience of populations, as well as the trajectory of entire communities, but its effects can differ among environments, populations and species (Vellend 2006, Hughes et al. 2008). In some cases, individuals in more genetically diverse populations enjoy greater survival, growth and fecundity than individuals in less genetically diverse populations (Gamfeldt et al. 2005, Crawford and Whitney 2010, Koh et al. 2011), whereas in other cases, low diversity populations outperform high diversity populations (Tonsor 1989, Donohue 2003). Importantly, the weight of evidence suggests that ignoring genetic diversity can lead to inaccurate predictions of the forces structuring populations and communities (Hughes et al. 2008).

Genetic diversity effects can manifest throughout an organism's life, yet most studies focus on effects during the adult stage alone. Nevertheless, in both plants and animals there is evidence that genetic diversity can affect colonisation processes (Willson et al. 1987, Gamfeldt et al. 2005, Cote et al. 2007, Crawford and Whitney 2010). Colonisation is often the fundamental link between the dispersive life-history stages and the resident adult life-history stages. Thus, we might expect selection to favour colonisation strategies that increase the probability of settling in genetic contexts that enhance adult performance. For instance, Crawford and Whitney (2010) found that high diversity, polyclonal *Arabidopsis thaliana* populations had greater fitness than low diversity, monoclonal populations, and correspondingly, more seeds emerged in polyclonal seed banks than in monoclonal seed banks. In contrast, Donohue (2003) showed that limited dispersal likely favoured sibling cooperation in *Cakile edentula*. Interestingly, Koh et al. (2011) found that the processes underlying the benefits of genetic diversity differed between the benthic and dispersive phase of the bacterium *Serratia marcescens*, suggesting that the effects of genetic diversity may not be consistent among life-history stages. These studies show that more studies of genetic diversity on nonadult stages are needed, as these effects can enhance, obscure or modify effects in later life-history stages.

Broadly, the effects of genetic diversity on populations can be attributed to additive or nonadditive processes. Nonadditive effects manifest when the outcomes of interactions among unrelated individuals differ from those among related individuals. Genotype-dependent interactions can manifest by avoidance of particular genotypes (Grosberg and Quinn 1986, Pfennig et al. 1993), intraspecific niche partitioning (Robinson and Wilson 1996, Sotka 2003), or facilitation among genotypes

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(Karvonen et al. 2012). Hence, when nonadditive processes underlie the effects of diversity, the properties of mixed-genotype populations cannot be predicted based on the properties of the constituent single-genotype populations (Hughes et al. 2008). Conversely, in additive effects, the properties of mixed-genotype populations are determined by the additive contribution of the genotypes represented in the population. For example, Wiernasz et al. (2008) showed that populations of western harvester ants *Pogonomyrmex occidentalis* with higher genetic diversity commenced foraging earlier than populations with lower genetic diversity. Populations with higher genetic diversity therefore, foraged for longer periods and likely acquired more resources than populations with lower genetic diversity. However, the ants with the earliest onset times descended from a nonrandom subset of patrilines. Thus, the onset of foraging in populations with differing levels of genetic diversity could be predicted based on the greater likelihood of sampling patrilines with early onset times in populations with greater genetic diversity. Our understanding of genetic diversity effects rests on correctly identifying the processes that underlie these effects, because although the outcomes of additive and nonadditive processes may be similar, their ecological and evolutionary consequences differ considerably.

In benthic marine communities, competition for resources is intense, interactions typically occur over relatively small scales, and most often with immediate neighbours (Sebens 1986, Buss 1990). Importantly, the number of colonisers entering a population plays a key role in determining the productivity and stability of populations, as well as species coexistence and invasion (Gaines and Roughgarden 1987, Underwood and Keough 2001). Furthermore, studies suggest that larval relatedness can affect the spatial arrangement of settlers (Keough 1984, Grosberg and Quinn 1986) and the supply of recruits into a population (Gamfeldt et al. 2005, McLeod and Marshall 2009). Recent studies have also shown that genetic diversity can enhance productivity in adult populations of two marine invertebrates from different phyla (Aguirre and Marshall 2012a, b). Therefore, there are good reasons to expect that genetic diversity will play a role in colonisation in this group. Here, we examined the effects of relatedness on colonisation by generating populations of sibling larvae and populations of larvae that had no parents in common in four sessile marine invertebrates. We will use the term 'unrelated' to describe populations of larvae with no parents in common, but we acknowledge that there may be some level of relatedness (e.g. common grandparents) among individuals in our unrelated treatments. For both sibling and unrelated treatments, we measured the timing of settlement and spatial arrangement of settlers. The duration of the larval period is particularly important for the four species considered here; all have nonfeeding larvae and many studies show that prolonging the larval period can result in the depletion of energy reserves available for post-metamorphic growth (Pechenik et al. 1998, Burgess and Marshall 2011a). Thus, if relatedness affects the time that larvae take to settle, relatedness will indirectly affect the phenotype (quality) of recruits.

Of the four species considered here, we have previously shown that decreased relatedness increases population productivity in *Ciona intestinalis* and *Bugula neritina* (Aguirre and Marshall 2012a, b), and therefore we expected that settlement also would be greater and denser in unrelated populations than sibling populations of these species. Although the effects of relatedness in *Botrylloides violaceous* remain untested to the best of our knowledge, sibling larvae of the ecologically similar species *Botryllus scholsseri* settled in closer proximity than unrelated larvae, possibly because the greater likelihood of fusion among siblings reduces intraspecific competition (Grosberg and Quinn 1986). Consequently, we predicted greater and denser settlement of *B. violaceous* in sibling populations. *Ascidia ceratodes* is a dominant late-successional species, and spatial variation in the settlement of *A. ceratodes* plays a role in determining the settlement of hetrospecifics, as well as species coexistence (Claar et al. 2011, Edwards and Stachowicz 2011). Unfortunately, the effects of relatedness on the settlement of *A. ceratodes* or a closely related species are unknown, and so we had no prior expectation for the effects of relatedness on the settlement of this species. However, given the influence of *A. ceratodes* on the benthic invertebrate community at the study location, we included this species in our experiment to examine the possibility that relatedness can generate spatial variation in the settlement of a dominant competitor.

Methods

Study site and species

The species considered in our study are common in the sessile invertebrate community of the Spud Point Marina, Bodega Harbour, California, USA (38°19′41.60″N, 123°3′23.11″W). The benthic invertebrate community in Bodega Harbour is dominated by suspension feeding organisms (ascidians, bryozoans, sponges, mussels and anemones) with a free-swimming larval stage. The larvae of these species are often nonfeeding, and typically disperse for hours to days before larvae attach to unoccupied substrates (biotic or abiotic) and metamorphose. Settlement is defined here as attachment to the substrate and metamorphosis. Unoccupied space in these communities is generated by senescence or disturbance (natural or anthropogenic), and the colonisation of space occurs primarily through larval settlement. Because most of the adults in the community are sessile, competition for space and suspended food particles is intense (Byrnes and Stachowicz 2009, Edwards and Stachowicz 2010), and the choice of settlement site can have persistent effects on adult performance (Grosberg 1981, Claar et al. 2011).

Here, we examine the effects of relatedness on the larval behaviour of four species that span a representative range of growth forms and competitive strategies in benthic marine invertebrate communities: the arborescent bryozoan; *Bugula neritina*, the colonial ascidian; *Botrylloides violaceous*, and the solitary ascidians; *Ascidia ceratodes* and *Ciona intestinalis*. For simplicity, we will refer to these

Table 1. Summary of species biology and experimental details.

	Ascidia ceratodes	*Botrylloides violaceous*	*Bugula neritina*	*Ciona intestinalis*
Growth form	upright solitary	encrusting colonial	arborescent colonial	upright solitary
Fertilisation	external	internal	internal	external
Spawning method	strip (gametes)	light shock + strip (larvae)	light shock	strip (gametes)
Native?	native	nonnative	nonnative	nonnative
Parents known	both	mother	mother	both
Larvae bowl^{-1}	10	3	10	10
Families in sibling treatments	1	1	1	1
Families in unrelated treatments	10	3	10	10
Time intervals	early: <7 h late: <21 h	early: <2 h late: <4 h	early: <2 h late: <4 h	early: <7 h late: <21 h
Bowls treatment^{-1} block^{-1}	20 (10 time interval^{-1})	15 (total)	15 (total)	20 (10 time interval^{-1})

Notes: The experiment was replicated in two blocks (block 1: 10–13 July, block 2: 8–11 August) with all species represented in each block. Within each block, the experiments for each species were done on different days, but the order in which species were done was randomised between blocks.

species by their genus names. A brief summary of important biological characteristics and details of the experiment can be found in Table 1.

General methods

Mature adults were carefully collected by hand from the floating docks at Spud Point Marina, Bodega Harbour. Adults were then transported to the laboratory in insulated aquaria. In the laboratory, adults were held in dark, aerated aquaria for 24–48 h before they were used in experiments. For all species, adults were collected at least 20 m apart. We believe the 20 m distance between the adults we sampled reduced the likelihood of sampling closely related individuals for three reasons. First, although to the best of our knowledge direct estimates of the larval dispersal for the species considered in our study are unavailable; studies of ecologically similar sessile marine invertebrates suggest that if suitable settlement substrates are available, larvae disperse only small distances (<10 m: Olson 1985, Olson and McPherson 1987, Davis and Butler 1989, Bingham and Young 1991, Stoner 1992). Second, for *Bugula* and *Ciona* at least, indirect estimates of larval dispersal also indicate that realised dispersal distances may be considerably smaller than potential dispersal distances (Petersen and Svane 1995, Burgess and Marshall 2011b). Last, molecular studies of the *Botrylliodes* population in Bodega Harbour suggest that at the sampling scale considered here it was unlikely that we sampled fragments of the same clone (Bock et al. 2011).

To collect *Bugula* larvae, each adult (>30 adults in each block) was transferred to its own beaker filled with filtered seawater. Beakers were then placed under a bright light until adults began releasing larvae (Table 1). For *Botrylloides*, each adult colony (>30 adults in each block) was transferred to its own beaker filled with filtered seawater, placed under a bright light, and then torn into small ($\sim$1–2 cm^2) pieces to release larvae (Table 1: Marshall et al. 2006). To collect the larvae of *Ciona* and *Ascidia*, we used standard strip-spawning techniques to extract gametes (Table 1: Aguirre and Marshall 2012a, Lambert and Epel 1979). We then crossed ten males with ten females in all possible combinations to create one-hundred full-sibling families in each block. Larvae hatched between 15–17 and 17–20 h post-fertilisation for *Ascidia* and *Ciona*, respectively.

Experimental design

We created experimental larval populations with two levels of relatedness – siblings and unrelated – in *Ascidia*, *Botrylloides*, *Bugula* and *Ciona*. The experiment was replicated in two blocks with all species represented in each block. Sibling treatments for species with internal fertilisation consisted of ten larvae (*Bugula*) or three larvae (*Botrylloides*) from the same mother; unrelated treatments consisted of the same total number of larvae, but each larva came from a different mother (Table 1). Sibling treatments for externally fertilising species (*Ascidia* and *Ciona*) consisted of ten larvae from the same full sibling family, and unrelated treatments consisted of ten larvae, each from a different full-sibling family (Table 1). Sibling and unrelated treatments for each species have the same total number of larvae, and the same number of replicates per treatment (Table 1). However, there were differences among species in the total number of larvae, and the number of replicates per treatment (Table 1).

For each species, we counted the number of larvae that had settled at two time intervals. The duration of the time periods varied among species (Table 1) as preliminary observations indicated that few *Ascidia* or *Ciona* larvae settle within the first 3–4 h of hatching, whereas the larvae of *Botrylloides* and *Bugula* begin to settle within minutes of release. For all species, preliminary observations indicated that few larvae settle after the termination of the late time interval. We quantified the spatial arrangement of settlers after the late time interval using the methods described in Clark and Evans (1954).

Because only maternal identity was known for species with internal fertilisation (*Botrylloides* and *Bugula*), we assumed that larvae in sibling treatments were half or full siblings (Table 1). In unrelated treatments, larvae had different mothers and we assumed that larvae also had different fathers. For species with external fertilisation (*Ascidia* and *Ciona*), paternal and maternal identity was known, and consequently, individuals in sibling treatments were

full-siblings, and larvae in unrelated treatments had no common parents (Table 1). Importantly, even if some of the adults used to generate sibling and unrelated treatments were siblings themselves, the relative difference in relatedness among our treatments would decrease, and thereby reduce our ability to detect treatment effects. Hence, we believe ours is a conservative test of the effects of relatedness on colonisation.

Experimental procedures

Swimming larvae were transferred to cylindrical glass bowls (90 mm diameter) that were filled with 200 ml of filtered seawater in accordance with the experimental design presented above. We then floated a roughened polycarbonate disk (90 mm diameter × 0.75 mm thickness) on the water surface, fixing it in place with adhesive tape. We then transferred the bowls containing larvae to a dark, temperature controlled room (~15°C) until larvae settled.

We could not confidently distinguish swimming from attached larvae of *Ascidia* and *Ciona* by eye, and we could not sample all the disks fast enough by using a microscope. Therefore, to ensure that the same families and combinations of families were represented in sibling and unrelated treatments for both early and late time intervals, we created two replicate bowls for each sibling and unrelated treatment – one replicate bowl was assigned to the early time interval and the second bowl was assigned to the late time interval (Table 1). After each time interval, we transferred the corresponding disks to new bowls filled with filtered seawater, and allowed the attached larvae 24 h to complete metamorphosis. We assume that mortality, if any, was unbiased with respect to our treatments during this period. Swimming larvae were discarded and the original bowls were inspected for attached larvae with a microscope. Individuals that had successfully completed metamorphosis (apoptosis of the tail) after 24 h were considered settled and their positions were marked on the disk.

In contrast, because the larvae of *Bugula* and *Botrylloides* are large and conspicuous we could confidently distinguish swimming and attached larvae by eye, so, in these species, we used repeated measurements on the same bowls during early and late time intervals (Table 1). Larval positions were marked on a template during each time interval, and larvae that remained in the same location between time intervals were considered to have settled during the early interval. As with *Ascidia* and *Ciona*, disks were transferred to a new bowl that was filled with filtered seawater after the late time interval, and attached larvae were allowed 24 h to complete metamorphosis (formation of the ancestrula and formation of ampullae for *Bugula* and *Botrylloides*, respectively). Swimming larvae were discarded, and the original bowl was checked for attached larvae with a microscope. The position of all settlers was marked on the disk.

The proportion of larvae that settled during each time interval was calculated by counting the number of settlers on each disk and dividing by the total number of larvae in each dish (Table 1). Nearest neighbour distances between settlers after the late time period were calculated by measuring Euclidian distances between settlers. For *Ascidia*, *Bugula* and *Ciona*, there was a clear preference for downward-facing surfaces (100% of the settlers were attached to the disk), and so two-dimensional distances between settlers could be calculated. In contrast, for *Botrylloides*, ~60% of larvae settled on either the sides or base of the glass bowl, and therefore, we calculated three-dimensional distances between settlers using Pythagoras's theorem. Encountering three-dimensional structures of this scale is commonplace for larvae of these species in nature (e.g. mussel beds, aggregations of solitary ascidians, crab pots). The proportion of *Botrylloides* larvae settling on the sides or base of the glass bowl did not differ significantly among treatments (Aguirre unpubl.).

Statistical analysis

We analysed the effects of relatedness on settlement success and spatial arrangement of settlers with a mixed-model ANOVA. Fixed factors were species (four levels representing the four species) and relatedness (siblings and unrelated), whereas block was a random factor. Time was a fixed repeated factor in settlement success analyses. We followed standard stepwise model reduction protocols and removed nonsignificant interactions between fixed and random factors from the models. In settlement success analyses, the response variable was the proportion of larvae that settled in each dish at each time period (arcsine square-root transformed). In spatial arrangement at settlement analyses, the response variable was the index of aggregation value for each late time interval disk. Index of aggregation values range between $R = 0$ and $R = 2.1491$, which correspond to perfectly aggregated (individuals occupy the same spatial unit) and perfectly overdispersed (individuals are equidistant), respectively. To examine the deviations from a random spatial arrangement for each species-by-relatedness combination, we used a one sample t-test comparing the index of aggregation values to a test value of $R = 1$. Importantly, index of aggregation values account for differences in settler densities, and so values are comparable among treatments and species.

To determine if additive or nonadditive effects drove the differences in settlement between sibling and unrelated treatments we used Monte Carlo resampling (Johnson et al. 2006) to generate artificial unrelated populations for each species in each block. If the mean settlement of the observed unrelated populations fell within the 95% confidence intervals of the artificially generated unrelated populations, we interpreted this as support for additive effects. In contrast, if the mean settlement of the observed unrelated populations fell outside the 95% confidence intervals of the artificially generated unrelated populations we interpreted this as support for nonadditive effects.

Results

Proportion settled

Each species reacted differently to experimental manipulations of larval relatedness (species × relatedness: $F_{3,176} = 3.855$, $p = 0.011$), but the effects of relatedness for each

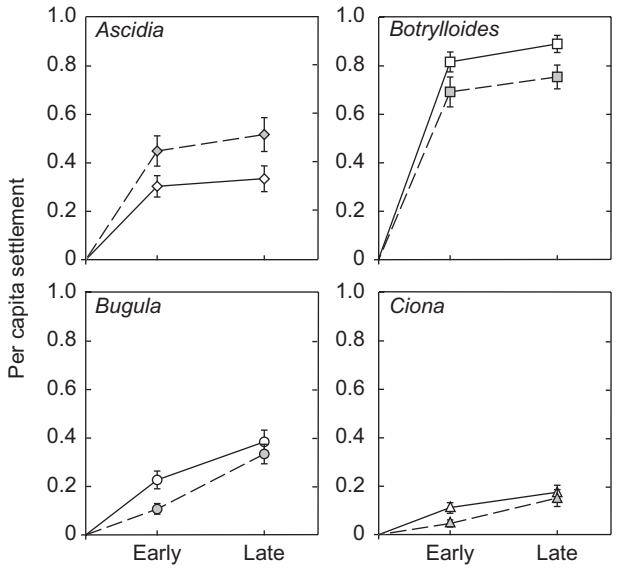

Figure 1. Proportional settlement (mean ± SE) of *Ascidia*, *Botrylloides*, *Bugula* and *Ciona* in sibling (open symbols and solid line) and unrelated (closed symbols and dashed line) larval populations for early and late time intervals (see methods for duration of time interval for each species).

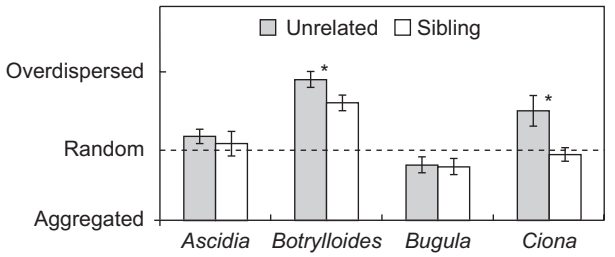

Figure 2. Spatial arrangement of *Ascidia*, *Botrylloides*, *Bugula* and *Ciona* settlers in sibling and unrelated larval populations for the late time interval (mean ± SE). Index of aggregation values vary between 0 and 2.1491, which correspond to aggregated and overdispersed arrangements of settlers, respectively. Asterisks indicate significant differences among relatedness treatments for each species. Dashed line denotes a random arrangement of settlers.

species were consistent across time intervals (time × species × relatedness: $F_{3,176} = 0.695$, $p = 0.556$). When we explored the settlement patterns for each species independently (Fig. 1), we found that for both *Botrylloides* and *Ciona*, larvae settled in greater numbers in sibling rather than unrelated treatments, though the effect was marginally non-significant for *Botrylloides* (relatedness: $F_{1,38} = 5.816$, $p = 0.021$ and $F_{1,51} = 3.919$, $p = 0.053$ for *Ciona* and *Botrylloides*, respectively). The pattern was reversed for *Ascidia* – more larvae settled in unrelated treatments than sibling treatments (relatedness: $F_{1,32} = 6.928$, $p = 0.013$). Last, for *Bugula* a greater number of larvae settled in sibling treatments than unrelated treatments for the early time interval but the difference between treatments was small for the late time interval (time × relatedness: $F_{1,57} = 3.657$, $p = 0.061$). Patterns of settlement among species also varied between blocks (species × block: $F_{3,176} = 3.991$, $p = 0.009$) and times (time × species: $F_{3,176} = 4.721$, $p = 0.003$), but in neither case was there an interaction with relatedness.

All species showed additive responses to manipulations of relatedness – the mean settlement of the observed unrelated populations fell well within the 95% confidence intervals of the artificially generated unrelated populations. Thus, settlement differences between sibling and unrelated populations appeared driven by positive selection for families with higher (i.e. *Ascidia*) or lower (i.e. *Botrylloides*, *Bugula* and *Ciona*) settlement in unrelated treatments.

Spatial arrangement

In general, the effects of relatedness on the spatial arrangement of settlers were consistent with patterns of settlement – species with greater settlement in sibling populations also tended to settle in closer proximity ($F_{1,45} = 4.286$, $p = 0.040$ and $F_{1,16} = 8.377$, $p = 0.010$ for *Botrylloides* and *Ciona*, respectively; Fig. 2). Conversely, when settlement was greater in unrelated larval populations (*Ascidia*) or differences in settlement among relatedness treatments were variable among time intervals (*Bugula*), there was no significant difference in the spatial arrangement of settlers between sibling and unrelated treatments ($F_{1,28} = 0.727$, $p = 0.400$ and $F_{1,43} = 0.023$, $p = 0.88$, for *Ascidia* and *Bugula* respectively; Fig. 2). Moreover, both sibling and unrelated settlers of *Botrylloides* were overdispersed ($t_{25} = 6.043$, $p < 0.001$, $t_{21} = 8.902$, $p < 0.001$, respectively; Fig. 2), but siblings appeared less overdispersed than unrelated settlers. There was no effect of relatedness on the spatial arrangement of *Bugula* settlers, however settlement was slightly aggregated for both sibling and unrelated larvae ($t_{23} = -2.013$, $p = 0.056$, $t_{21} = -1.813$, $p = 0.084$, respectively; Fig. 2). Although unrelated settlers of *Ciona* were overdispersed ($t_6 = 2.562$, $p = 0.043$; Fig. 2), siblings settled randomly ($t_{11} = -0.624$, $p = 0.545$; Fig. 2). Last, although *Ascidia* settled in greater numbers in unrelated populations, the spatial arrangement of settlers in sibling and unrelated populations appeared random ($t_{14} = 0.532$, $p = 0.603$, $t_{16} = 1.927$, $p = 0.072$, respectively; Fig. 2).

Discussion

We found strong effects of relatedness on colonisation in all four species. For the two traits measured (settlement success and spatial arrangement at settlement) in the four species we investigated, relatedness had significant effects in six of the eight instances. Furthermore, in addition to direct effects of relatedness on the number and arrangement of settlers, our results suggest there may be indirect effects mediated by differences in settler phenotype. Overall, we predict that because relatedness affected colonisation, the genetic diversity, abundance, arrangement and phenotype of colonisers are all likely to covary spatially. Furthermore, the species-specific effects of relatedness on colonisation may result in interspecific differences in spatial covariation among these factors.

Differences in the number and spatial arrangement of colonisers, as well as the interactions between these two

factors, can confound our interpretations of the relative importance of genetic diversity effects in adult populations (Donohue 2003, Agashe and Bolnick 2010). For example, Agashe and Bolnick (2010) showed that the effects of genetic diversity in flour beetles *Tribolium castaneum*, were context-dependent: genetic diversity increased population productivity at high conspecific densities, but there were no effects of genetic diversity at low conspecific densities. Given that genetic diversity affects the number of colonisers in plants (Willson et al. 1987, Crawford and Whitney 2010), vertebrates (Cote et al. 2007), and invertebrates (Gamfeldt et al. 2005, Agashe and Bolnick 2010), experiments integrating the effects of genetic diversity on colonisation and adult performance are needed to further our understanding of biodiversity effects.

By prolonging the duration of the larval period, the effects of relatedness on colonisation are likely to have lasting effects on adult populations. First, in marine invertebrates with nonfeeding larval stages, settlers with longer larval durations are more likely to settle in poorer quality habitats, and suffer reduced performance as adults than are settlers with shorter larval durations (Pechenik et al. 1998, Gribben et al. 2006). Second, larvae that settle quickly in the field are exposed to less predation than larvae of the same species that spend longer searching for a settlement site (Morgan 1995). Therefore, if siblings settle sooner than unrelated larvae, then differences in genetic diversity among larval populations are likely to persist post-settlement. However, if siblings delay settlement, then we expect groups of sibling larvae to disband (due to diffusion), and the level of genetic diversity among recruit populations to become more homogenous. Therefore, by affecting the pelagic larval duration, relatedness may affect not only the number and spatial arrangement of settlers, but also their phenotypes and genetic diversity at larger scales.

Importantly, different species responded differently to manipulations of relatedness. Our results for *Botrylloides* were consistent with our expectations. *Botrylloides violoceous* has a well described cell-cell recognition system that allows adults to discern the genetic identity of conspecific neighbours upon contact (Hirose et al. 1988). Individuals then fuse with genetically similar individuals, or reject fusion with genetically dissimilar individuals (Hirose et al. 1988). In space-limited communities fusion allows individuals to avoid overgrowth by conspecific neighbours and prevents the colonisation of neighbouring spaces by stronger conspecific or heterospecific competitors (Grosberg and Hart 2000). Hence, positive selection for greater settlement of *Botrylloides* in sibling populations provides a likely explanation for our results for *Botrylloides*.

Because we previously found that unrelated populations are more productive than sibling populations in *Ciona* and *Bugula* (Aguirre and Marshall 2012a, b) we expected greater settlement in unrelated populations of these species; however, this was not the case, and for *Ciona*, the opposite pattern was observed. This discrepancy could have resulted from differences in the source populations (Hughes and Stachowicz 2009), the concentration of larvae (Agashe and Bolnick 2010), the proportional representation of each family in unrelated treatments, or the material and orientation of the settlement surface. Indeed, the proportional settlement of *Ciona* in the experiment presented here was low, indicating that artefacts of the experimental design may have influenced the overall *Ciona* settlement, and potentially, the effects of relatedness on larval behaviour in this species. The composition of the surrounding community also may have played a role. For instance, *Ciona* is the dominant competitor for space in Port Lincoln, Australia where we conducted our previous experiment (Aguirre unpubl.); however, the presence of a stronger competitor, *Ascidia*, in Bodega Harbour could have resulted in different selection pressures on the behaviour of *Ciona* larvae and thus differences in the effects of relatedness on colonisation. Experiments examining the effects of relatedness on post-settlement performance of these species at Bodega Harbour are required to better understand the mechanisms that underlie the processes driving variability in larval behaviour.

Behavioural variability in marine larvae is widespread, and may reflect differences in the strength of selection on larval traits that determine patterns of settlement (Raimondi and Keough 1990). In our experiments, there were two dispersal strategies that would reduce the probability of developing near genetically unfavourable neighbours: delaying settlement or increasing overdispersion. Both behaviours increase the separation between interacting individuals just at different scales, and it is possible that variation in the costs of dispersal influence among population differences in larval behaviour. For example, *Bugula* settled sooner in sibling populations during our experiments in northern California, but once settled, there was little difference in the spatial arrangement of sibling and unrelated settlers. Conversely, in southern California, Keough (1984) found that sibling and unrelated larvae settled in similar numbers, but siblings settled in closer proximity than unrelated larvae. Thus, lower costs of delaying settlement for *Bugula* in the cooler water temperatures of northern Californian waters compared with southern California (Burgess and Marshall 2011c), may select for different dispersal strategies to enhance the likelihood of developing in favourable habitats, and thereby underlie variability in larval behaviour.

The presence of *Ascidia* is known to affect the settlement of heterospecifics (Claar et al. 2011), our results suggest that the settlement of *Ascidia* can also be affected by conspecifics: *Ascidia* settlement was greater in unrelated populations than sibling populations. Consequently, our results suggest that relatedness may play an important role in generating spatial variation in settlement of dominant competitors (Edwards and Stachowicz 2011). Importantly, spatial variation in the abundance of a dominant competitor has been shown to mediate coexistence in locations with different species compositions (*Ciona* at Avery Point, Connecticut; Edwards and Stachowicz 2011, and *Botryllus* at Eel Pond, Massachusetts; Grosberg 1981), and therefore, the community consequences of genetic diversity effects on colonisation may be profound. Moreover, because differences in settler density covary with differences in relatedness, our predictions of the outcomes of competitive interactions based on differences in density alone may be inaccurate, as the effects of density may differ depending on the genetic diversity of the population.

The spatial arrangement of species in a community can affect coexistence in marine invertebrate communities (Idjadi and Karlson 2007, Hart and Marshall 2009). In communities with large competitive asymmetries, stronger competitors, such as *Botrylloides*, perform better when species are overdispersed, because encounters with weaker, heterospecific competitors are more likely than encounters with stronger, conspecific competitors. In contrast, weaker competitors, such as *Bugula*, perform better when species are aggregated because they are more likely to avoid stronger, heterospecific competitors and instead encounter weaker, conspecific competitors. Our results for the spatial arrangement of settlers in *Botrylloides* and *Bugula* generally reflect these two scenarios – settlement of *Botrylloides* was highly overdispersed, whereas settlement of *Bugula* was slightly aggregated. Furthermore, greater overdispersion in unrelated treatments for *Botrylloides* suggests that selection for overdispersion is stronger when neighbouring larvae are unrelated, presumably because fusion is less likely, and thereby competition with conspecifics is more severe. Interestingly, although *Ascidia* is a strong spatial competitor, the arrangement of *Ascidia* settlers appeared random, potentially reflecting the relatively weak effects of conspecific density on individual performance in *Ascidia* (Edwards and Stachowicz 2011).

In benthic marine communities, competition for resources is intense and species often differ substantially in their ability to acquire and maintain those resources. However, studies have shown that temporal and spatial variation in recruitment influences how species coexist in these systems (Chesson and Warner 1981, Edwards and Stachowicz 2010, 2011). In addition, evidence in other systems suggests that differences in genetic diversity among populations can affect the strength of competition among species (Vellend 2006). Our results suggest that the underlying drivers of both these mechanisms can be influenced by the effects of relatedness on colonisation success.

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References

Agashe, D. and Bolnick, D. I. 2010. Intraspecific genetic variation and competition interact to influence niche expansion. – Proc. R. Soc. B 277: 2915–2924.

Aguirre, J. D. and Marshall, D. J. 2012a. Does genetic diversity reduce sibling competition? – Evolution 66: 94–102.

Aguirre, J. D. and Marshall, D. J. 2012b. Genetic diversity increases population productivity in a sessile marine invertebrate. – Ecology 93: 1134–1142.

Bingham, B. L. and Young, C. M. 1991. Larval behavior of the ascidian *Ecteinascidia turbinata* Herdman; an in situ experimental study of the effects of swimming on dispersal. – J. Exp. Mar. Biol. Ecol. 145: 189–204.

Bock, D. G. et al. 2011. Looking at both sides of the invasion: patterns of colonization in the violet tunicate *Botrylloides violaceus*. – Mol. Ecol. 20: 503–516.

Burgess, S. C. and Marshall, D. J. 2011a. Are numbers enough? Colonizer phenotype and abundance interact to affect population dynamics. – J. Anim. Ecol. 80: 681–687.

Burgess, S. C. and Marshall, D. J. 2011b. Field estimates of planktonic larval duration in a marine invertebrate. – Mar. Ecol. Progr. Ser. 440: 151–161.

Burgess, S. C. and Marshall, D. J. 2011c. Temperature-induced maternal effects and environmental predictability. – J. Exp. Biol. 214: 2329–2336.

Buss, L. W. 1990. Competition within and between encrusting clonal invertebrates. – Trends Ecol. Evol. 5: 352–356.

Byrnes, J. and Stachowicz, J. J. 2009. Short and long term consequences of increases in exotic species richness on water filtration by marine invertebrates. – Ecol. Lett. 12: 830–841.

Chesson, P. L. and Warner, R. R. 1981. Environmental variability promotes coexistence in lottery competitive systems. – Am. Nat. 117: 923–943.

Claar, D. C. et al. 2011. Positive and negative effects of a dominant competitor on the settlement, growth, and survival of competing species in an epibenthic community. – J. Exp. Mar. Biol. Ecol. 399: 130–134.

Clark, P. J. and Evans, F. C. 1954. Distance to nearest neighbor as a measure of spatial relationships in populations. – Ecology 35: 445–453.

Cote, J. et al. 2007. Mother–offspring competition promotes colonization success. – Proc. Natl Acad. Sci. USA 104: 9703–9708.

Crawford, K. M. and Whitney, K. D. 2010. Population genetic diversity influences colonization success. – Mol. Ecol. 19: 1253–1263.

Davis, A. R. and Butler, A. J. 1989. Direct observations of larval dispersal in the colonial ascidian *Podoclavella moluccensis* Sluiter: evidence for closed populations. – J. Exp. Mar. Biol. Ecol. 127: 189–203.

Donohue, K. 2003. The influence of neighbor relatedness on multilevel selection in the Great Lakes sea rocket. – Am. Nat. 162: 77–92.

Edwards, K. F. and Stachowicz, J. J. 2010. Multivariate tradeoffs, succession, and phenological differentiation in a guild of colonial invertebrates. – Ecology 91: 3146–3152.

Edwards, K. F. and Stachowicz, J. J. 2011. Spatially stochastic settlement and the coexistence of benthic marine animals. – Ecology 92: 1094–1103.

Gaines, S. D. and Roughgarden, J. 1987. Fish in offshore kelp forest affect recruitment of intertidal barnacle populations. – Science 235: 479–481.

Gamfeldt, L. et al. 2005. Increasing intraspecific diversity enhances settling success in a marine invertebrate. – Ecology 86: 3219–3224.

Gribben, P. E. et al. 2006. Less inhibited with age? Larval age modifies responses to natural settlement inhibitors. – Biofouling 22: 101–106.

Grosberg, R. K. 1981. Competitive ability infuences habitat choice in marine invertebrates. – Nature 290: 700–702.

Grosberg, R. K. and Quinn, J. F. 1986. The genetic-control and consequences of kin recognition by the larvae of a colonial marine invertebrate. – Nature 322: 456–459.

Grosberg, R. K. and Hart, M. W. 2000. Mate selection and the evolution of highly polymorphic self/nonself recognition genes. – Science 289: 2111–2114.

Hart, S. P. and Marshall, D. J. 2009. Spatial arrangement affects population dynamics and competition independent of community composition. – Ecology 90: 1485–1491.
Hirose, E. et al. 1988. A new type of the manifestation of colony specificity in the compound ascidian, *Botrylloides violaceus* Oka. – Biol. Bull. 175: 240–245.
Hughes, A. R. and Stachowicz, J. J. 2009. Ecological impacts of genotypic diversity in the clonal seagrass *Zostera marina*. – Ecology 90: 1412–1419.
Hughes, A. R. et al. 2008. Ecological consequences of genetic diversity. – Ecol. Lett. 11: 609–623.
Idjadi, J. A. and Karlson, R. H. 2007. Spatial arrangement of competitors influences coexistence of reef-building corals. – Ecology 88: 2449–2454.
Johnson, M. T. J. et al. 2006. Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. – Ecol. Lett. 9: 24–34.
Karvonen, A. et al. 2012. Synchronous attack is advantageous: mixed genotype infections lead to higher infection success in trematode parasites. – Proc. R. Soc. B 279: 171–176.
Keough, M. J. 1984. Kin-recognition and the spatial-distribution of larvae of the bryozoan *Bugula neritina* (L.). – Evolution 38: 142–147.
Koh, K. S. et al. 2011. Minimal increase in genetic diversity enhances predation resistance. – Mol. Ecol. 21: 1741–1753.
Lambert, C. C. and Epel, D. 1979. Calcium-mediated mitochondrial movement in ascidian sperm during fertilization. – Dev. Biol. 69: 296–304.
Marshall, D. J. et al. 2006. Offspring size effects mediate competitive interactions in a colonial marine invertebrate. – Ecology 87: 214–225.
McLeod, L. and Marshall, D. J. 2009. Do genetic diversity effects drive the benefits associated with multiple mating? A test in a marine invertebrate. – Plos One 4: e6347.
Morgan, S. G. 1995. The timing of larval release. – In: McEdward, L. R. (ed.), Ecology of marine invertebrate larvae. CRC Press, pp. 157–192.
Olson, R. R. 1985. The consequences of short-distance larval dispersal in a sessile marine invertebrate. – Ecology 66: 30–39.
Olson, R. R. and McPherson, R. 1987. Potential vs. realized larval dispersal: fish predation on larvae of the ascidian *Lissoclinum patella* (Gottschaldt). – J. Exp. Mar. Biol. Ecol. 110: 245–256.
Pechenik, J. A. et al. 1998. Metamorphosis is not a new beginning. – Bioscience 48: 901–910.
Petersen, J. K. and Svane, I. 1995. Larval dispersal in the ascidian *Ciona intestinalis* (L.). Evidence for a closed population. – J. Exp. Mar. Biol. Ecol. 186: 89–102.
Pfennig, D. W. et al. 1993. Kin recognition and cannibalism in spadefoot toad tadpoles. – Anim. Behav. 46: 87–94.
Raimondi, P. T. and Keough, M. J. 1990. Behavioural variability in marine larvae. – Austral Ecol. 15: 427–437.
Robinson, B. W. and Wilson, D. S. 1996. Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). – Evol. Ecol. 10: 631–652.
Sebens, K. P. 1986. Spatial relationships among encrusting marine organisims in the New England subtidal zone. – Ecol. Monogr. 56: 73–96.
Sotka, E. E. 2003. Genetic control of feeding preference in the herbivorous amphipod *Ampithoe longimana*. – Mar. Ecol. Progr. Ser. 256: 305–310.
Stoner, D. S. 1992. Vertical distribution of a colonial ascidian on a coral reef: the roles of larval dispersal and life-history variation. – Am. Nat. 139: 802–824.
Tonsor, S. J. 1989. Relatedness and intraspecific competition in *Plantago lanceolata*. – Am. Nat. 134: 897–906.
Underwood, A. J. and Keough, M. J. 2001. Supply-side ecology – the nature and consequences of variations in recruitment of intertidal organisms. – In: Bertness, M. D. et al. (eds), Marine community ecology. Sinauer, pp. 183–200.
Vellend, M. 2006. The consequences of genetic diversity in competitive communities. – Ecology 87: 304–311.
Wiernasz, D. C. et al. 2008. Mating for variety increases foraging activity in the harvester ant, *Pogonomyrmex occidentalis*. – Mol. Ecol. 17: 1137–1144.
Willson, M. F. et al. 1987. Sibling competition in plants: an experimental study. – Am. Nat. 129: 304–311.