Geographical variation in offspring size effects across generations

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Offspring size is thought to strongly affect offspring fitness and many studies have shown strong offspring size/fitness relationships in marine and terrestrial organisms. This relationship is strongly mitigated by local environmental conditions and the optimal offspring size that mothers should produce will vary among different environments. It is assumed that offspring size will consistently affect the same traits among populations but this assumption has not been tested. Here I use a common garden experiment to examine the effects of offspring size on subsequent performance for the marine bryozoan *Bugula neritina* using larvae from two very different populations. The local conditions at one population (Williamstown) favour early reproduction whereas the other population (Pt. Wilson) favours early growth. Despite being placed in the same habitat, the effects of parental larval size were extremely variable and crossed generations. For larvae from Williamstown, parental larval size positively affected initial colony growth and larval size in the next generation. For larvae from the other population, parental larval size positively affected colony fecundity and negatively affected larval size in the next generation. Traditionally, exogenous factors have been viewed as the sole source of variation in offspring size/fitness relationship but these results show that endogenous factors (maternal source population) can also cause variation in this crucial relationship. It appears offspring size effects can be highly variable among populations and organisms can adapt to local conditions without changing the size of their offspring.

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The study of offspring size has long fascinated evolutionary biologists because of the immense variation offspring sizes observed among and within species (Lack 1947). One of the first attempts at understanding the evolution of offspring size was the graphical model of Smith and Fretwell (1974). This model (and others since) assume that the resources a female has available for reproduction are limited and therefore females can either produce many small offspring or fewer, larger offspring (Vance 1973, Levitan 1993, Hendry et al. 2001). A central assumption of this model is that offspring size positively affects offspring fitness. Consequently, for a given set of environmental conditions a single optimal offspring size should be selected which balances the costs of larger, fitter young and more numerous but less fit young. Accordingly, there are a number of studies that have shown that larger offspring have greater fitness than smaller offspring (Bagenal 1969, Einum and Fleming 2000, Moran and Emlet 2001, Marshall and Keough 2003a, Marshall et al. 2003) but the relationship between offspring size and fitness is not universal.

Different environments can select for different optimal offspring sizes (Stearns 1992). The effects of environmental variation on optimal offspring size have been explored in both empirical and theoretical studies (McGinley et al. 1987, Mousseau and Fox 1998, Moran and Emlet 2001). These studies have shown that the

effects of offspring size are strongly mitigated by the local environment. For example, Einum and Fleming (1999) found that under the relatively benign conditions of the laboratory, offspring size had little effect on subsequent performance, but under harsher field conditions there were stronger effects. Given the strong interaction between the environment and offspring size, it is reasonable to expect that among environments, offspring size will vary. Indeed, many studies have shown wide variation in offspring size among populations (Williams 1994, Ramirez-Llodra 2002, Marshall and Keough 2003a). These differences in offspring size are generally viewed as local adaptations for a particular population (Reznick and Yang 1993, George 1994, Moran 1999, Hendrickx et al. 2003). However, in some organisms, changing offspring size may not be adaptive or evolutionary accessible (Bernado 1996). For example, for organisms with complex life histories, increasing offspring size may increase performance in one life history stage but decrease performance in another (Marshall et al. 2002). Alternatively, physiological or anatomical constraints may prevent mothers from producing offspring of optimal size (Bernado 1996, Ramirez-Llodra 2002). Therefore, some organisms may be unable to change the size of their offspring in order to adapt to local conditions. Nevertheless, there are other mechanisms of local adaptation of offspring that do not require the changing of offspring size although they have received far less attention. For example, among different populations, offspring size may affect different traits. Given that many other life history traits can exhibit local adaptation (Warner 1997, Brown et al. 2001), it seems reasonable to expect that offspring size effects may differ among different populations but this has not been tested in the field. More generally, most studies of offspring size effects are limited to a single population (Moran and Emlet 2001, Marshall and Keough 2003b); how the effects of offspring size vary among populations has not been examined in detail. This is remarkable given the wide variation in offspring sizes observed among populations.

Here I use a common garden experiment to examine how offspring size affects subsequent performance for offspring of parents from two very different populations for the marine bryozoan *Bugula neritina* L. In a previous study, it was found that offspring size affected a number of different offspring traits that were strongly related to offspring fitness (Marshall et al. 2003). Here, I test how larval size affects the subsequent growth and reproduction of the adult colonies that came from larvae originating from two populations with very different life history characteristics. Because the effects of offspring size can cross generations in this species (Marshall et al. 2003), I also examined how parental larval size and origin affects the size (and thus fitness) of the next generation.

Methods

Study species and populations

Adult *Bugula neritina* colonies are sessile, grow by asexual budding, and, when reproductive, they brood their larvae in obvious brood structures (ovicells) and can easily be induced to release their larvae. Larvae spend only a short time in the plankton, existing on internal energy reserves. As *Bugula neritina* grows, the colony bifurcates at regular intervals, and by counting the number of bifurcations on a line from colony base to tip, the number of zooids in each colony can be estimated. Colony fecundity can be estimated by counting the number of ovicells on an entire colony (Marshall et al. 2003).

The two source populations of *Bugula neritina* differed greatly in their population dynamics and their life history characteristics. In one population, Williamstown, colonies typically die off at the beginning of winter because of freshwater inputs from a local river. At another population, Pt. Wilson, colonies can persist throughout the year. Colonies from Williamstown typically begin reproduction at a much smaller size than colonies from Pt. Wilson (pers. obs; results here).

Breakwater Pier, Williamstown is located ∼6 km west of Melbourne, extends approximately 300 m from the shore and is sheltered from the prevailing weather by a rocky breakwater on its southern side. Point Wilson Explosives Reserve Pier is located ∼50 km km southwest of Melbourne and extends approximately 3 km from the shore without a breakwater. The common garden in which the experiment was conducted was St. Kilda Yacht Club Pier. This pier is located 6 km southeast of Melbourne, extends approximately 400 m from the shore and is protected by a rocky breakwater on its western side. Public access is restricted at all three sites.

Relationship between size and reproduction in wild colonies

Casual observations strongly suggested that Williamstown colonies became reproductively mature at a far smaller size than Pt. Wilson colonies. I wanted to quantify this observation and collected colonies of a range of sizes from Pt. Wilson and Williamstown on December 10, 2002 and December 2, 2002 respectively. I estimated the size of the colonies by counting the number of bifurcations along a single branch as in Marshall et al. (2003) under a 10 × magnification on a dissecting microscope. To estimate the fecundity of each colony, I counted the number of ovicells present on each colony.

Common garden experiment: collection and settlement of larvae

Reproductively mature *Bugula neritina* colonies of approximately the same size were collected from both Pt. Wilson and Breakwater Pier using S.C.U.B.A. on 27 November 2000. The colonies were kept in the dark overnight in lightproof aquaria with re-circulating seawater. The following day the colonies were exposed to bright, fluorescent light, after which colonies began releasing larvae immediately. The larvae from colonies of the same origin were pooled. I then haphazardly selected larvae from each pool, measured them as in Marshall et al. (2003) and allowed them to settle on their own individually marked settlement plates (25×50 mm) as described in Marshall et al. (2003). I measured and settled 23 larvae from colonies originating from Pt. Wilson and 23 larvae from colonies originating from Breakwater Pier. The size of larvae from both sites was very similar in range and distribution. Twenty four hours after settlement, I transferred the colonies to St. Kilda Marina. I affixed the small settlement plates to a single, large (400×600 mm) Perspex backing plate using stainless steel bolts. The position of the settlement plates was randomized. The backing plate was then suspended by nylon rope face down, had a small weight attached to prevent movement and was suspended at a depth of ~ 2.5 m below the mean low tide level.

Common garden experiment: data collection

Approximately one month after settlement, on December 28, I brought the colonies back into the laboratory and assessed mortality, growth and reproduction. Colonies were transported between the field and laboratory in lightproof, insulated aquaria containing 10 l of seawater. The travel time between the field and laboratory was approximately 30 min. After collection, I measured the number of bifurcations and ovicells present on each colony as described above. The colonies were then returned to the field on December 29.

I re-examined the colonies approximately three weeks later on January 19, 2001 but used slightly different methods to assess growth and fecundity. As the colonies had greatly increased in size, it became impractical to measure colony size by counting the number of bifurcations in each colony. Instead, I measured the longest length of the colony from the base of the colony to the tip to the nearest millimeter. To estimate colony reproduction, I kept the colonies in the dark overnight and exposed them to bright light the following day. Whilst the colonies were exposed to light, each colony was kept in its own individual container. The colonies were exposed to bright light for ~ 1 h and any larvae that were released by each colony during this time were collected using a pipette. I fixed the collected larvae with

a few drops of formalin, counted them and then measured a subsample ($n = 30$) of larvae using the technique described in Marshall et al. (2003).

Statistical analysis

Larval size explained little of the variation in colony survival so I used a χ^2 test to examine the effect of larval origin on colony survival four weeks after deployment in the field. There was no further mortality of the colonies after this time. To examine the effect of larval origin and size on colony growth, I used ANCOVA where larval size was a covariate and larval origin was a factor. To examine the effects of larval origin on colony fecundity (measured as the number of ovicells per colony) four weeks after deployment into the field, I used ANCOVA where larval origin was a factor and colony size was included as a covariate. Given that I found an effect of larval origin on colony growth, the size ranges of colonies with differing larval origins did not completely overlap. As such, I omitted the three largest Pt. Wilson from the ANCOVA analysis (Quinn and Keough 2002); this omission did not affect the outcome of the analysis.

I examined the effects larval size and origin on fecundity of colonies at seven weeks (measured as the number of larvae that were released by each colony) with ANCOVA where larval origin was a factor and larval size at seven weeks was a covariate. I also examined the effect of larval origin on the size of larvae produced by colonies after seven weeks in the field with ANCOVA where larval origin was a factor and larval size was a covariate. Finally, I tested whether newly collected, wild colonies from Williamstown and Pt. Wilson had different numbers of ovicells per unit colony size by using ANCOVA where origin was a factor and colony size (no. of bifurcations) was a covariate. For each ANCOVA, I first tested for homogeneity of slopes and in cases where slopes were heterogeneous, I report the test for the interaction between the factor and covariate.

Results

Wild colonies from Williamstown initiate reproduction at a much smaller size than colonies from Pt. Wilson (origin $\times$ colony size interaction: $F_{1,17} = 22.1$, $P < 0.001$; Fig. 1).

All of the mortality of colonies that originated from both sites occurred in the first four weeks after deployment into the field and no further mortality occurred for the remainder of the experimental period until the experiment was stopped on January 20, 2001. Williamstown colonies had lower survivorship than Pt. Wilson colonies (Williamstown: 52%, Pt. Wilson: 82%; Pearson $\chi^2 = 4.847$, df $= 1$, $P < 0.05$).
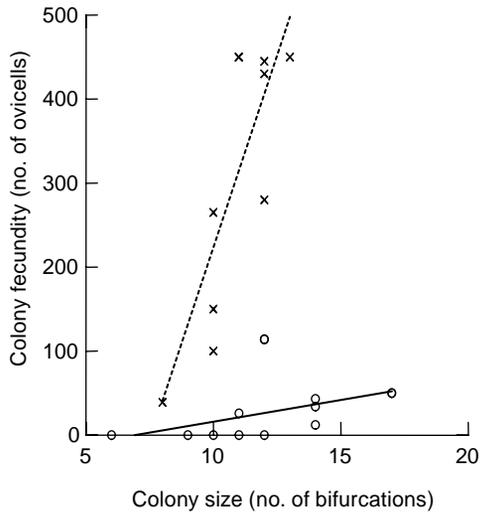
Fig. 1. Relationship between colony size (measured as no. bifurcations) and colony fecundity (measured as no. bifurcations) for *Bugula neritina* at two populations, Williamstown (colonies represented by crosses, dashed line) and Pt. Wilson (colonies represented by circles, solid line), Victoria, Australia.
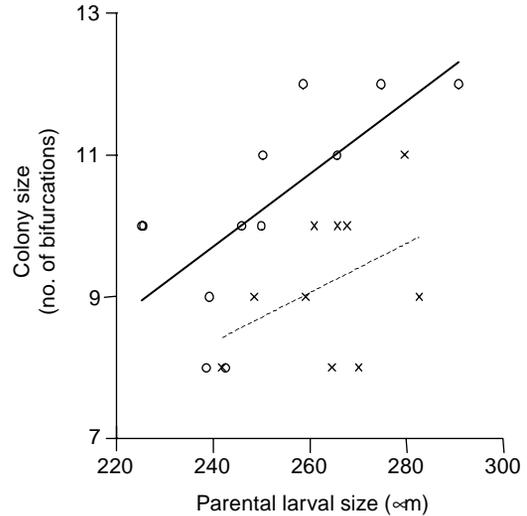

Fig. 2. Effect of larval size and origin on subsequent growth of *Bugula neritina* colonies after four weeks in the field at St. Kilda Marina, Victoria, Australia. Each point represents a single colony where crosses represent Williamstown-origin colonies and circles represent Pt. Wilson-origin colonies.

Growth in the first four weeks after settlement was dependent on the size of larvae and the origin of those larvae. Colonies that originated from larger larvae grew more than colonies that originated from smaller larvae. Overall, those colonies that came from larvae from Pt. Wilson grew larger overall than Williamstown colonies (Table 1, Fig. 2). The origin of larvae also affected the relationship between colony size (measured as the number of bifurcations per colony) and colony fecundity (measured as the number of ovicells on each colony) as shown by a significant interaction between colony size and larval origin using ANCOVA (larval origin × colony size: $F_{1,15} = 5.384$, $P = 0.035$). Williamstown colonies tended to have more ovicells per colony for their size than Pt. Wilson colonies.

Seven weeks after settlement, I could no longer detect an effect of original larval size on colony size but larval origin still had a strong effect with Pt. Wilson colonies being larger than Williamstown colonies (ANCOVA: larval size: $F_{1,16} = 3.34$, $P = 0.086$; larval origin: $F_{1,16} = 5.76$, $P = 0.029$). There was a strong interaction between the effect of larval size and origin on the number of larvae released by colonies seven weeks after settlement (ANCOVA: larval origin × larval size: $F_{1,10} = 7.146$,

$P = 0.023$). For colonies from Pt. Wilson, colonies from larger larvae had higher fecundity than colonies from smaller larvae. In contrast, there appeared to be no relationship between colony fecundity and initial larval size for colonies from Williamstown (Fig. 3)

The relationship between parental larval size and the size of larvae produced by those colonies was strongly affected by the origin of the larvae from which those colonies came (ANCOVA: larval origin × larval size:
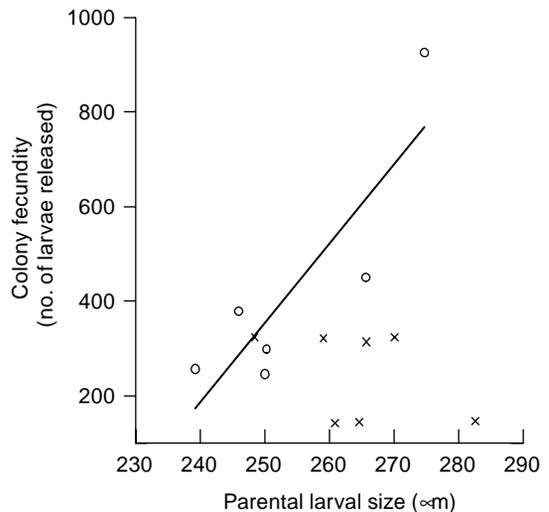

Fig. 3. Effect of parental larval size and origin on the fecundity of *Bugula neritina* colonies (measured as no. larvae released per colony) after 7 weeks in the field at St. Kilda Marina, Victoria, Australia. Each point represents the number of larvae released by Williamstown-origin colonies (crosses) and Pt. Wilson-origin colonies (circles).

Table 1. Analysis of Co-variance on effect of larval size and larval origin on the size of *Bugula neritina* colonies after four weeks in the field. Model is reduced after testing for the homogeneity of slopes.

Source	df	Mean square	F-ratio	P
Larval size	1	12.21	11.807	0.003
Origin	1	13.11	12.677	0.002
Error	19	1.03		

$F_{1,12} = 6.832$, $P = 0.023$). The size of larvae produced by Williamstown colonies increased with colony size whilst for Pt. Wilson colonies, larval size decreased with colony size (Fig. 4).

Discussion

Effects on growth and reproduction

Offspring size strongly affected offspring fitness in this study but these effects were extremely variable. Despite being transplanted into the same habitat, the effects of larval size on the subsequent growth and reproduction of adult *Bugula neritina* colonies depended strongly on the population from which larvae came (summarized in Table 2). This variation appears to be due to either genetic effects or very early maternal effects (Keough 1989). For larvae from Williamstown (where colonies typically die off at the end of the year), increased larval size resulted in an increase in colony growth and a large increase in colony fecundity. For larvae from Pt. Wilson (where colonies can persist year-round), colony growth rates during their first for weeks were higher overall than those from Williamstown. For larvae from Pt. Wilson, increased larval size again resulted in increased colony growth but in contrast to those from Williamstown, larval size had little effect on colony fecundity. These results are in accordance with my observations of wild colonies from both sites where reproduction is initiated at a far smaller size for Williamstown colonies than for Pt. Wilson colonies. It appears that larval resources are utilized differently, according to their maternal source

Table 2. Summary of the effects of larval size and larval origin on the subsequent growth and reproduction of *Bugula neritina* colonies at St. Kilda Marina, Victoria, Australia. √ indicate the detection of the effect and × indicate that no effect could be detected.

Response variable	Interaction	Larval size	Origin
Colony size after 4 weeks	×	√	√
Fecundity after 4 weeks	√		
Colony size after 7 weeks	×	×	√
Fecundity after 7 weeks	√		
Offspring size after 7 weeks	√		

population and there is a strong trade off between growth and reproduction in this colonial species. Furthermore, it appears that the allocation of larval resources is relatively fixed despite the larvae being transplanted to a new habitat. Interestingly, colonies that came from Williamstown larvae had higher mortality than Pt. Wilson larvae. It may be that the increased initial allocation of colonies to reproduction increases their chances of suffering mortality but this remains to be tested.

The variation in the effects of offspring size on adult traits may be adaptive. At Pt. Wilson, colonies that come from larger larvae may "use" their advantage to attain a larger size as an adult. For many colonial organisms, increased size results in higher fitness because larger colonies are more resistant to predation, partial mortality and disturbance (Sebens 1987, Davis 1988). In contrast, colonies from Williamstown are likely to be under intense pressure to reproduce as soon as possible because mortality at the end of summer is very high due to freshwater pulses (D. Marshall, per. obs.). This mortality is unlikely to be colony size specific and consequently, larger larvae from Williamstown may "use" their size advantage to reproduce sooner and have higher initial fecundity.

Effects on the subsequent generation

The interaction between offspring size and larval origin persisted through to affect the size and number of larvae produced in the subsequent generation. After 7 weeks in the field, larval size strongly affected the number of larvae that were released by Pt. Wilson colonies but there was no relationship between initial larval size and fecundity for Williamstown colonies. However, larval size increased with maternal larval size for colonies whose larvae originated from Williamstown but decreased with maternal larval size for colonies that originated from Pt. Wilson. This may represent a trade off between the number and size of offspring that a colony can produce. The differences in investment strategies between colonies from different populations may also be adaptive. Colonies that came from larger larvae from Williamstown produce larger larvae
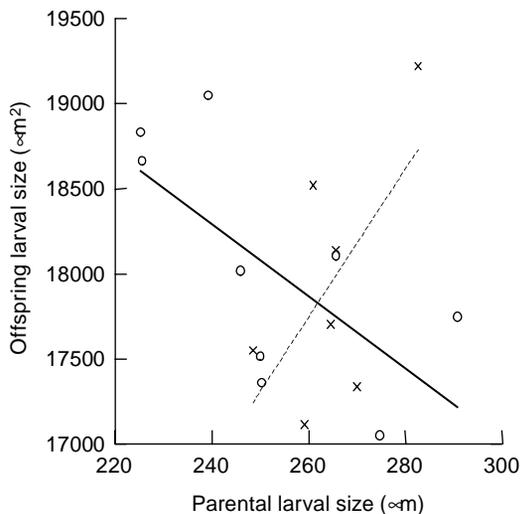
Fig. 4. Effect of parental larval size and origin on the size of larvae produced by *Bugula neritina* colonies after 7 weeks in the field at St. Kilda Marina, Victoria, Australia. Each point represents the mean size of 30 larvae for each colony for Williamstown-origin colonies (crosses) and Pt. Wilson-origin colonies (circles).

themselves. Larger larvae have a greater potential for dispersal than smaller larvae (Marshall and Keough 2003c). It may be that colonies that came from larger Williamstown larvae use their advantage to produce offspring that, although more expensive, are more likely to disperse further away. In contrast, colonies that came from larger larvae from Pt. Wilson produced smaller larvae themselves. Pt. Wilson is a relatively isolated population and it may be that producing smaller, less dispersive larvae (that are also less energetically expensive to produce) represents a strategy to increase the probability of retaining some offspring in the population. These possibilities remain highly speculative but others have suggested that mothers may influence the dispersal of their offspring in order to maximize their own fitness (McGinley et al. 1987).

Variable relationship between offspring size and fitness

Regardless of whether the observed variation in the effects of offspring size on the traits of offspring is adaptive or not, it remains clear that offspring size effects are not consistent even when offspring are placed into the same location. For *Bugula neritina* increasing the size of offspring in one generation will result in more, smaller offspring being produced in the next generation in one population (Pt. Wilson) or larger offspring being produced in another population (Williamstown). This variation in the effect of offspring size has important implications for our understanding of the evolution of offspring size. Most theoretical models assume that offspring size affects offspring traits consistently, although the strength of these effects may vary among different environments (Parker and Begon 1986, Hendry et al. 2001). My results demonstrate that offspring size can affect a range of different traits in different ways depending on the maternal source population. Variation in the relationship between offspring size and fitness is traditionally assumed to originate from external environmental factors (Mousseau and Fox 1998, Moran and Emlet 2001). It appears that variation in the relationship between offspring size and offspring fitness may also occur due to intrinsic factors and offspring size models should reflect this variation.

The effects of offspring size on offspring performance were remarkably persistent. Other studies on offspring size have suggested that maternal effects such as offspring size become less important as an offspring ages because the offspring's genotype begins to have an influence (Einum and Fleming 2000). This was the case for the effects of offspring size on colony growth but not reproduction – in *B. neritina* offspring size effects are pervasive and can cross generations. It appears that, with respect to offspring size, there are two ways in which

populations can adapt: by changing the size of offspring that are produced or by changing the traits which offspring size affects. Many studies have demonstrated that offspring size can affect a number of different traits simultaneously (Sinervo and Doughty 1996). It will be interesting to determine if the strength of the effect of offspring size on different traits varies among populations in other species.

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