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Temperature-mediated plasticity and genetic differentiation in egg size and hatching size among populations of *Crepidula* (Gastropoda: Calyptraeidae)

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Offspring size is a key characteristic in life histories, reflecting maternal investment per offspring and, in marine invertebrates, being linked to mode of development. Few studies have focused explicitly on intraspecific variation and plasticity in developmental characteristics such as egg size and hatching size in marine invertebrates. We measured over 1000 eggs and hatchlings of the marine gastropods *Crepidula atrasolea* and *Crepidula ustulatulina* from two sites in Florida. A common-garden experiment showed that egg size and hatching size were larger at 23 °C than at 28 °C in both species. In *C. ustulatulina*, the species with significant genetic population structure in cytochrome oxidase I (COI), there was a significant effect of population: Eggs and hatchlings from the Atlantic population were smaller than those from the Gulf. The two populations also differed significantly in hatchling shape. Population effects were not significant in *C. atrasolea*, the species with little genetic population structure in COI, and were apparent through their marginal interaction with temperature. In both species, 60–65% of the variation in egg size and hatching size was a result of variation among females and, in both species, the population from the Atlantic coast showed greater temperature-mediated plasticity than the population from the Gulf. These results demonstrate that genetic differentiation among populations, plastic responses to variation in environmental temperature, and differences between females all contribute significantly to intraspecific variation in egg size and hatching size. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **99**, 489–499.

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INTRODUCTION

Documenting patterns of intraspecific variation is vital to understanding the evolutionary dynamics of any trait (Bernardo, 1996). Despite this, studies of intraspecific variation are uncommon in certain fields. Several reviews have called specifically for more detailed studies of intraspecific variation in offspring size or propagule size (Bernardo, 1996; Marshall & Keough, 2008). Bernardo (1996) argues that the commonly studied patterns in interspecific variation do not provide adequate information to understand the evolutionary dynamics of offspring size, and that studies of within and among population variation are vital to make progress in this area. He specifically cites the traditional focus on the evolution of optimal offspring size, instead of on the evolution of reaction norms in offspring size or other aspects of plasticity, as an impediment to understanding evolutionary patterns in offspring size. Studies of intraspecific variation in propagule size in marine invertebrates are particularly rare (Marshall & Keough, 2008; Moran & McAlister, 2009) and plasticities are seldom examined.

PROPAGULE SIZE IN MARINE INVERTEBRATES

Egg size and hatching size have been the focus of many studies of life histories of marine invertebrates, yet there are few explicit studies of their intraspecific variation (Hadfield & Strathmann, 1996; Jones, Todd

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& Lambert, 1996; Marshall & Keough, 2008). Interspecific differences in these characteristics are often extreme and are associated with different modes of development: planktonic versus benthic, feeding versus nonfeeding, and direct verses indirect. Interspecific variation in egg size and/or hatching size can also affect fertilization rate and success (Levitan, 2006), development time (Kohn & Perron, 1994; Emlet, 1995), predation rates by zooplankton (Allen, 2008), hatching size (Kohn & Perron, 1994), settlement size (George, Cellario & Fenaux, 1990; Hart, 1995; Allen, Zakas & Podolsky, 2006), and juvenile growth rate and survival (Rivest, 1983; Moran & Emlet, 2001; Marshall & Keough, 2003). Intraspecific variation in offspring size generally has similar but smaller effects than intraspecific variation on many stages of the life cycle.

The existing literature suggests that there is widespread intraspecific variation in egg size and hatching size (Hadfield & Strathmann, 1996; Marshall & Keough, 2008), although there is no clear picture of how this variation is partitioned within and among broods, females, and populations. Similarly, few studies have examined how plastic responses to environmental factors such as temperature, or maternal effects such as responses to maternal size or nutrition effect offspring size in marine invertebrates.

The studies that do examine intraspecific variation in offspring size have found relationships between egg size or hatching size, and maternal size (Ito, 1997; Chaparro *et al.*, 1999; Marshall, Styan & Keough, 2000), temperature (Skadsheim, 1989; Honkoop & Van Der Meer, 1998), and latitude or tidal height (Hadfield, 1989; Lardies & Castilla, 2001). In addition, egg sizes have been found to vary among populations (Barnes & Barnes, 1965; Jones *et al.*, 1996), and can show patterns consistent with seasonal or lunar periodicity (Lessios, 1987).

Intraspecific variation in Atlantic and Gulf *Crepidula*

We used a common garden experiment to compare Atlantic and Gulf populations of two species of *Crepidula* (Gastropoda: Calyptraeidae), to document temperature-mediated plasticity in egg size and hatching size, and to examine differences among populations. In one species, the populations are reciprocally monophyletic at the cytochrome oxidase I (COI) locus. In the other species, the two populations share haplotypes. Therefore, this experiment also tests the hypothesis that the species with more population structure in COI also shows more differences between populations in life history traits. If divergence in COI reflects levels of divergence across the entire genome, as is often assumed, then divergence in COI should be associated with divergences in other traits, such as offspring size.

Development and reproduction are well studied in calyptraeid gastropods and phylogenetic studies have demonstrated rapid evolution of mode of development and concomitant changes in egg size (Collin, 2004; Collin *et al.*, 2007). *Crepidula atrasolea* Collin 2000 and *Crepidula ustulatulina* Collin 2002 (the 'southern species' of *C. convexa*; Collin, 2001) co-occur along the coast of Florida and both brood, have large eggs, and previously reported variation in egg size. *Crepidula atrasolea* has direct development with embryos that hatch as crawling juveniles. Egg diameter is reported as 335 μm (Collin, 2003) and hatching size as 900 μm (*Crepidula* cf. *plana*; Hoagland, 1986) or 1002 μm (Collin, 2003). Development of *C. ustulatulina* includes lecithotrophic larvae that swim for minutes to hours prior to settling. Egg diameter is reported as 300–340 μm and hatchling shell lengths are reported as 744 μm (Collin, 2003), 840 μm (*Crepidula* cf. *convexa*; Hoagland, 1986) or 630–700 μm (*Crepidula* cf. *convexa*; Hoagland, 1984).

Phylogeographic analysis has shown significant differences in population structure in mitochondrial (mt)COI between the two species (Collin, 2001). *Crepidula atrasolea* shows little genetic structure along the coast of Florida. Populations from Mote Marine Laboratory, near Sarasota on the Gulf coast and Fort Pierce on the Atlantic coast of Florida share COI haplotypes that differ by one or two base pairs and are not reciprocally monophyletic. By contrast *C. ustulatulina* has significant population structure and the reciprocally monophyletic COI haplotypes from Mote and Fort Pierce differ by 2% (Collin, 2001). Animals from both populations interbreed successfully in the laboratory (R. Collin, unpubl. data), supporting the conspecific identity of the different populations.

In the present study, we used animals from these two populations raised at two temperatures to determine: (1) whether egg size and hatching size show temperature-mediated plasticity; (2) whether the species with distinct COI haplotypes among populations showed greater population-level differences in egg size and hatching size than the species that shares COI haplotypes among populations; and (3) whether population × temperature effects are also greater in the species with distinct COI haplotypes among populations than the species that shares COI haplotypes.

MATERIAL AND METHODS

Brooding females and small juveniles of both species were collected from less than 1 m depth at low tide near the breakwater at the Harbor Branch

Oceanographic Institute in Fort Pierce on the Atlantic coast of Florida, and in the seagrass meadow in Sarasota Bay north of the Mote Marine Laboratory on the Gulf coast of Florida. Pairs of hatchlings from the same population were transferred to 350-mL plastic cups and raised for approximately 1 year in incubators at either 23 °C or 28 °C (within the range of temperatures experienced during the reproductive season). The larger animal in each pair became female after 3–4 months, and the smaller animal developed as male; thus, eggs were obtained from only one animal per pair. Water was changed every other day and 3.86×10^6 cells of *Isochrysis galbana* culture was added to the cups daily. At large (reproductive) sizes, this was probably a limiting ration because the water was cleared hours before the next scheduled feeding.

Females were checked twice each day for new eggs, which could be seen through the transparent plastic of the cups without disturbing the females. Uncleaved eggs were collected, placed on a slide with a no. 1 cover slip, supported by large 'clay feet' to ensure that pressure from the cover slip did not squash or alter the egg size or shape in any way, and photographed. We aimed to photograph twenty round, uncleaved eggs from each female, but could not always find 20 when the females were small or cleavage had begun. Hatchlings were collected within 12 h of hatching naturally from the egg capsule, and preserved in 70% ethanol. The shells were stained blue, oriented flat and photographed. Prior to photographing, the eggs or hatchlings from each female, a stage micrometer was photographed with the same magnification. Only a single brood of eggs and a single brood of hatchlings were measured from each female.

Using the software IMAGEJ (Abramoff, Magelhaes & Ram, 2004), the micrometer photograph was used to calibrate the measurements, and the Shape Descriptor plug-in was used to measure the area, diameter or major and minor axes, aspect ratio (major axis/minor axis) and roundness ($4 \times \text{area}/\pi \times \text{diameter}^2$) of each egg or hatchling. Eggs with a roundness of less than 94 were eliminated from the analysis because eggs elongate prior to cleavage and changes in shape can alter size estimates.

Data were analysed using the REML (restricted maximum likelihood) algorithm analysis of variance as implemented in JMP, version 5.1 (SAS Institute Inc.) with eggs produced by each female nested within population as a random effect. REML can provide more reliable estimates of variance for unbalanced models than standard analysis of variance (Quinn & Keough, 2002) and is recommended for analysing random effects. *P*-values for the random nested effect were calculated on the likelihood value of the model using a *Z*-test and Tukey's honestly significant difference (HSD) test were used to compare group means. Egg diameter was used since it is the most commonly used measure of egg size in the literature. Because there were significant differences between populations and temperatures in hatchling shapes, hatchling area was used as the measure of hatchling size for statistical analyses. Correlations between female size and egg size or hatching size were examined using analysis of covariance (ANCOVA) on average egg size for each brood, with population and temperature as direct effects and female size at the time the brood was laid as a covariate. Stepwise removal was used to eliminate nonsignificant interactions. These datasets were smaller than the total egg and hatchling datasets because female size was not measured for all of the animals when eggs and hatchlings were collected.

Variation in egg size is usually inferred to reflect variation in energy content and therefore maternal investment. However, egg size does not always reflect energy content because levels of hydration as well as allocation to different yolk components can vary among eggs and among mothers (McEdward & Carson, 1987; McEdward & Coulter, 1987; Moran & McAlister, 2009). It is not easy to verify the relationship between size and energy content of individual eggs because it is difficult to obtain accurate measures of energy content for such tiny samples. If egg size does reflect energy content, then egg size should be correlated with hatching size. Unfortunately, removing eggs from brooding *Crepidula* results in the abandonment of the entire brood and the eggs do not develop normally away from their mother. Therefore, we examined the correlation between egg size and hatching size from different broods from the same mother using ordinary least squares regression on brood means.

RESULTS

Egg size

The overall average egg diameter was 339 μm for *C. atrasolea* and 304 μm for *C. ustulatulina* (Table 1). For both species, smaller sample sizes were obtained in the high temperature treatment (Table 1). This was primarily because eggs cleaved quickly at high temperatures, making it difficult to collect eggs when they were still round. In addition, adult snails in the high temperature treatment did not appear to produce broods as frequently as those at the lower temperature.

For *C. atrasolea*, there was a significant effect of temperature and female on egg size but no interaction between temperature and population (Fig. 1, Tables 1, 2) and no direct effect of population. Egg size was smaller (overall average 333 μm) at 28 °C than in the 23 °C treatment (overall average 341 μm). Eggs

Table 1. Summary of egg size and hatching size in *Crepidula atrasolea* and *C. ustulatulina*

	Fort Pierce (23 °C)	Fort Pierce (28 °C)	Mote (23 °C)	Mote (28 °C)	Total	Individual females mean (range)
Crepidula atrasolea						
Egg diameter						
Average (μm)	344.4	330.1	337.7	337.1	338.8	299–394
SD (μm)	23.2	19.6	16.4	17.2	20.6	9.2 (3.3–55.7)
Number of eggs	556	240	645	214	1655	18 (10–23)
Number of females	30	14	33	12	89	89
Hatching length						
Average (μm)	1074	1005	1025	987	1026	850–1227
SD (μm)	93.2	93.4	97.1	103.7	102.0	57.2 (28.0–141.6)
Number of eggs	511	322	663	404	1880	17.7 (6–20)
Number of females	27	20	35	25	107	107
Crepidula ustulatulina						
Egg diameter						
Average (μm)	306.3	281.5	307.5	301.5	303.5	248–354
SD (μm)	25.4	22.0	17.5	17.7	22.0	10.0 (3.8–61.0)
Number of eggs	376	133	568	224	1301	17.6 (9–20)
Number of females	22	8	30	13	73	73
Hatching length						
Average (μm)	932.1	873.4	930.7	905.8	916	740.0–1160.5
SD (μm)	89.3	95.6	88.3	101.2	95.5	53.7 (19.7–133.9)
Number of eggs	578	287	645	513	2023	18.9 (9–20)
Number of females	30	16	34	28	108	108

Table 2. Restricted maximum likelihood analysis of varianceof egg size of *Crepidula atrasolea* and *Crepdiula ustulatulina*

Crepidula atrasolea				
Fixed effects	d.f.	SS	*F*-ratio	*P*-value
Temperature	1	613.47	4.49	**0.03**
Population	1	0.09	0.0007	0.98
Temperature × population	1	412.17	3.02	0.08
Random effect	d.f.	Variance ratio	Z-ratio	*P*-value
Female [population]	88	2.00	6.20	**< 0.0001**
Crepidula ustulatulina				
Fixed effects	d.f.	SS	*F*-ratio	*P*-value
Temperature	1	1691.06	10.36	**0.001**
Population	1	865.53	5.30	**0.02**
Temperature × population	1	605.89	3.71	**0.05**
Random effect	d.f.	Variance ratio	*Z*-ratio	*P*-value
Female (population) random	70	2.00	5.50	**< 0.0001**

Significant *P*-values in bold.

from 28 °C and 23 °C from Fort Pierce differ from each other but not from the eggs from Mote (Tukey's HSD test; Fig. 1). Most of the variation in egg size (65%) was a result of the significant effects of individual females.

For *C. ustulatulina*, there were significant effects of temperature, population, and female on egg diameter (Fig. 1, Tables 1, 2), and a marginally significant interaction between temperature and population (Table 2). Egg size was larger (overall average 307 μm) at 23 °C than it was at 28 °C (overall average 294 μm). Again, the majority of variation (62%) in egg size was a result of the effect of female.

There was no clear effect of maternal size on egg size in either species. For *C. ustulatulina*, there was

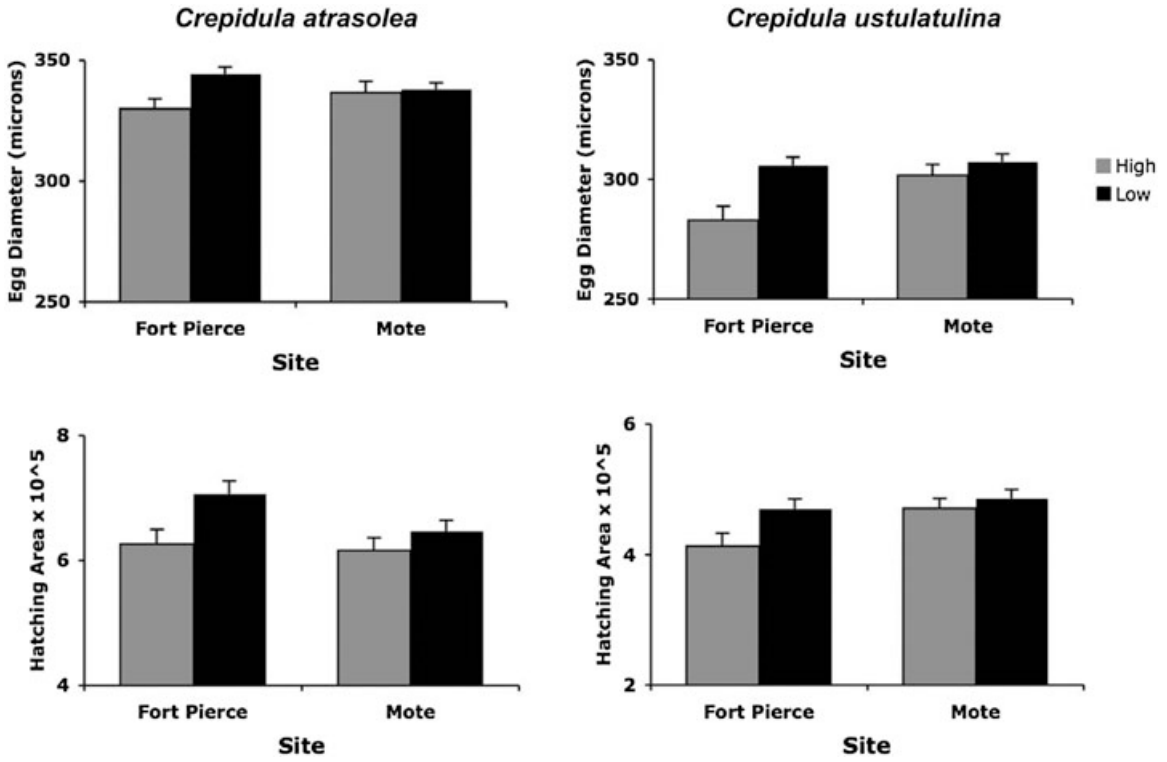

Figure 1. Effects of rearing temperature and population of origin on egg size and hatching size in *Crepidula atrasolea* and *Crepidula ustulatulina*. Restricted maximum likelihood analysis of variance showed a significant effect of temperature in all four cases and a significant effect of population for egg size and hatching size in *C. ustulatulina*. Error bars indicate the standard errors.

no significant effect of maternal size on egg size (ANCOVA: $F = 0.02$; $N = 38$; $P > 0.9$) in the total dataset, nor when the dataset was partitioned by population or temperature. There was no significant effect of maternal shell length on egg diameter in *C. atrasolea* when eggs from both temperature treatments were analysed together (ANCOVA: $F = 0.25$; $N = 77$; $P > 0.6$;). There was also no significant effect of temperature for this dataset, which is smaller than our total dataset, although there was a significant interaction between the effects of temperature and maternal shell length ($P < 0.003$; $N = 77$). At 23 °C, egg size decreases with maternal size ($r^2 = 0.12$; $N = 59$, $P = 0.007$) but, at 28 °C, egg size increases with maternal size ($r^2 = 0.24$; $N = 18$; $P = 0.04$).

HATCHLING SIZE

The average hatchling length was 1026 μm for *C. atrasolea* and 916 μm for *C. ustulatulina* (Table 1). For *C. atrasolea*, there was a significant effect of temperature and female on hatchling area, and no significant effect of population or temperature × population (Fig. 1, Tables 1, 3). The average hatchling length was smaller (995 μm) at 28 °C than at 23 °C (1046 μm). Again, most of the variation in hatching size (65%) was a result of the significant effect of female.

For *C. ustulatulina*, there was a significant effect of temperature, population, and female on hatchling area (Fig. 1, Tables 1, 3), and no significant temperature × population effect. Hatchling length was larger (931 μm) at 23 °C than it was at 28 °C (894 μm). Hatchlings from Fort Pierce were smaller than those from Mote at both temperatures. Again, the majority of variation (65%) in hatching size was as result of the effect of female.

The effect of female size explained very little of the variation in hatchling size. Hatchling area was not significantly correlated with maternal size for *C. atrasolea* for the total dataset (ANCOVA: $F = 0.55$; $N = 107$; $P > 0.7$), nor when the dataset was partitioned by temperature or population. Hatchling size showed no effect of maternal size in *C. ustulatulina* for the entire dataset (ANCOVA: $F = 2.62$; $N = 101$; $P > 0.1$). However, when the dataset was partitioned by population, the Mote population showed a small but significant increase in hatchling size with maternal shell length ($N = 58$; $r^2 = 0.10$; $P < 0.05$). The Fort Pierce population did not show any significant relationship between the two variables.

Table 3. Restricted maximum likelihood analysis of variance of hatching size of *Crepidula atralsolea* and *Crepidula ustulatulina*

Crepidula atrasolea				
Fixed effects	d.f.	SS	*F*-ratio	*P*-value
Temperature	1	4.14×10^{10}	7.45	**0.006**
Population	1	1.62×10^{10}	2.92	0.09
Temperature × population	1	7.88×10^{9}	1.42	0.23*
Random effect	d.f.	Variance ratio	Z-ratio	*P*-value
Female (population) random	104	1.81	6.70	**< 0.0001**
Crepidula ustulatulina				
Fixed effects	d.f.	SS	F-ratio	*P*-value
Temperature	1	1.67×10^{10}	4.97	**0.03**
Population	1	1.72×10^{10}	5.13	**0.03**
Temperature × population	1	5.61×10^{9}	1.67	0.20
Random effect	d.f.	Variance ratio	*Z*-ratio	*P*-value
Female (population) random	105	1.92	6.81	**< 0.0001**

*Removal of the nonsignificant interaction effect does not alter the result. Significant *P*-values in bold.

There is some consistency in relative offspring size from brood to brood, suggesting a maternal component. Hatchling size in one brood was positively correlated with egg diameter in a different brood from the same female, although this explained only a small part of the variance in hatchling size (*C. atrasolea*: $r^2 = 0.11$; $N = 39$; $P = 0.04$; *C. ustulatulina* $r^2 = 0.11$; $N = 56$; $P = 0.01$; Fig. 2).

Hatchling shape

Analysis of hatchling shape was based on aspect ratio, the ratio of major to minor axes. For *C. atrasolea*, there was a significant effect of temperature and female, but not of population (Table 4) on aspect ratio. Hatchlings at 23 °C were longer and thinner than those in the high temperature treatment (Fig. 3). A substantial amount of the variation in shape (40%) was a result of differences among females.

For *C. ustulatulina*, temperature, population, and female were significant effects on hatchling shape (Table 3). Similar to *C. atrasolea*, hatchlings at low temperature were longer and thinner than the ones in the high temperature treatment (Fig. 3). In addition, the two populations differed in hatching shape: hatchlings from Fort Pierce are significantly longer and thinner than those from Mote. Thirty-five percent of the variation was a result of differences among females.

DISCUSSION

The data obtained in the present study on egg size and hatching size in these two species of *Crepidula* show that temperature-mediated plasticity, genetic differentiation among populations, and differences between females, all contribute to significant variation in offspring size and shape. In both species, the majority of variation in egg size and hatching size was a result of differences among females. Similarly, in both species, egg size and hatching size decreased significantly with increasing temperature, and this pattern was more pronounced in the Atlantic population than the Gulf population. The species differed in the effect of population on offspring size: In *C. ustulatulina*, the species that shows populations differentiation in COI, there was a strongly significant difference in egg size and hatching shape between populations and a significant difference in hatchling size. In *C. atrasolea*, the species lacking differentiation in COI, there was no evidence of differences among populations in egg size, hatching size, or hatching shape. Finally, although there was a significant temperature–population interaction only for egg size, the animals from Fort Pierce showed a pattern of greater response to temperature than those from Mote for all three variables. The possible causes and consequences of these different results are discussed below.

Are the effects of temperature adaptive?

Egg size and hatching size both show a phenotypic response to temperature, with larger offspring being produced at the lower temperature in both species. Such decreases in egg size and hatching size with increasing temperature are common not just in marine invertebrates (Honkoop & Van Der Meer, 1998), but also in insects (Ernsting & Isaaks, 2000; Fischer, Brakefield & Zwaan, 2003) and marine fishes (Bengtson, Barkman & Berry, 1987; Kokita, 2003). Many studies interpret such temperature-mediated plasticity as an adaptive response linked to differential juvenile growth or survival at different temperatures, and seek to quantify reproductive output under

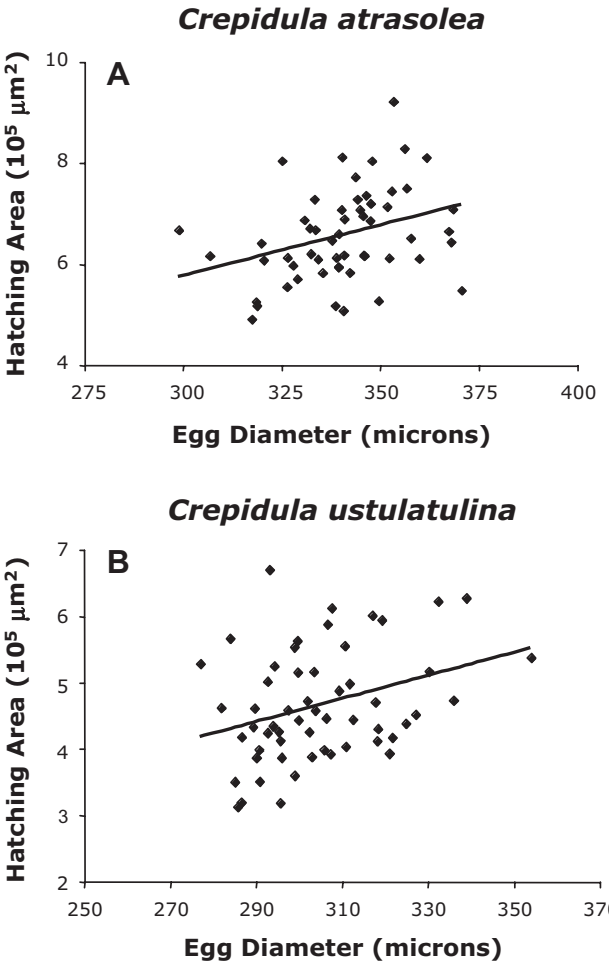

Figure 2. Scatter plots of average egg size for a single brood for each female versus average hatching size for a single brood for the same female. A, *Crepidula atrasolea* (trend line is the ordinary least squares (OLS) regression; $r^2 = 0.11$; $N = 39$; $P = 0.037$). B, *Crepidula ustulatulina* (trend line is OLS; $r^2 = 0.11$; $N = 56$; $P = 0.013$).

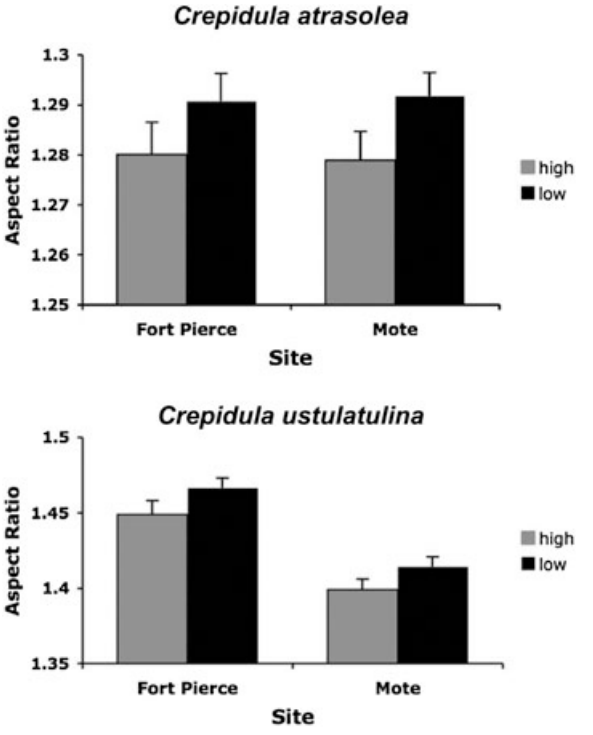

Figure 3. Effects of rearing temperature and population of origin on hatchling shape for *Crepidula atrasolea* and *Crepidula ustulatulina*. Restricted maximum likelihood analysis of variance showed a significant effect of temperature on both species as well as a significant effect of population on *C. ustulatulina*. Error bars indicate the standard errors.

different conditions (Fischer *et al.*, 2003). Other studies have interpreted this response as a physiological response to stress (George, 1996), either directly from elevated temperatures or via nutritional stress as a result of a higher metabolic rate at higher temperatures. Finally, Van Voorhies (1996) suggested that temperature effects are ubiquitous on cell size in ectotherms.

Stress appears unlikely to be operating in the case of *Crepidula* offspring size. The temperatures employed in the present study were not physiologically stressful for adult animals and were typical of temperatures experienced in the field. Water temperatures at the collection sites for both populations showed similar annual ranges and temperatures in considerable excess of 28 °C. Water temperature measured at mid-day once a month for 5 years from the collecting site in Fort Pierce ranged from 13.8–37.7 °C, with an average of 25.5 °C. Four months of the year have average temperatures higher than 28 °C (S. Reed, pers. comm.). Ten years of monthly data from the seagrass meadow near Mote Marine Laboratory are similar and ranged from 12.8–37.2 °C, with an average of 24.4 °C. The 75% quartile was 28.91 °C (K. Dixon, pers. comm.).

Stress could also have resulted from food limitation; however, it is unclear in which direction nutritional stress would be expected to act. Nutritional stress could have the direct consequence of reduced egg size as a result of reduced resources available to the female (Fox, 1993; Bertram & Strathmann, 1998). However, it could also induce an adaptive maternal response: Because juveniles of both species are unlikely to disperse far from the mother, mothers might be expected to produce larger propagules under nutritional stress (Qian, 1994) to increase their chances of survival. The first scenario suggests that if higher temperatures or larger sizes result in nutritional stress, egg size should decrease with tempera-

Table 4. Restricted maximum likelihood analysis of variance of hatchling shape as measured by aspect ratio

Crepidula atrasolea				
Fixed effects	d.f.	SS	*F*-ratio	*P*-value
Temperature	1	0.0048	4.3	**0.038**
Population	1	1.25×10^{-7}	0.0001	0.99
Temperature × population	1	3.7×10^{-5}	0.033	0.86
Random effect	d.f.	Variance ratio	*Z*-ratio	*P*-value
Female (population) random	104	0.66	6.40	**< 0.0001**
Crepidula ustulatulina				
Fixed effects	d.f.	SS	*F*-ratio	*P*-value
Temperature	1	0.011	4.90	**0.027**
Population	1	0.11	46.73	**< 0.0001**
Temperature × population	1	0.00004	0.019	0.89
Random effect	d.f.	Variance ratio	*Z*-ratio	*P*-value
Female (population) random	105	0.53	6.31	**< 0.0001**

Significant *P*-values in bold.

ture and with maternal size, consistent with our results indicating that egg size decreases with temperature. The second scenario suggests that egg size should increase with nutritional stress and therefore with temperature and with size. This is consistent with our result that egg size increased with maternal size, for *C. atrasolea* at 28 °C, and in *C. ustulatulina* from Mote. Direct tests of the effects of food limitation must be made to determine exactly how this factor contributes to variation in egg size in *Crepidula*.

It is difficult to address the two other possible explanations: adaptive trade-offs and physiological allometry. We did not collect data on the survival or fitness of offspring of different sizes at different temperatures, or the offspring size–clutch size trade-offs necessary to examine the effects of temperature in terms of optimal fitness and survival. We therefore could not directly evaluate the adaptive scenario.

However, it is unlikely that the almost ubiquitous pattern among invertebrates of larger eggs at lower temperatures could be a result of detailed fitness tradeoffs in such diverse organisms as butterflies, marine gastropods, and soil nematodes. Reduced cell size at higher temperatures has been demonstrated in fish blood cells, nematode cells, and various *Drosophilia* epidermal cells (Van Voorhies, 1996; Azevedo, French & Partridge, 2002). One possible explanation for this general effect of temperature is the fundamental scaling relationships between oxygen supply and consumption. This predicts that cells or eggs produced at high temperatures should be smaller than those produced at lower temperatures (Woods, 1999). Unfortunately, we cannot evaluate this hypothesis without additional data. It would be necessary to measure oxygen gradients within the eggs themselves and demonstrate that levels are low enough in the centre of the egg to effect development (Woods, 1999). To our knowledge, such data are not currently available for any marine invertebrate.

Female effect

The results obtained in the present study show that more than half of the variation in egg size and hatching size for each species of *Crepidula* was a result of differences among females. Such differences in egg size between females could result from genetic or environmental factors. Traits that are presumably highly linked to fitness and are therefore under selection are not expected to show high levels of genetic variation, although high heritibilities in egg size have been demonstrated for *Hydroides elegans* (Miles, Hadfield & Wayne 2007). Significant differences between females are frequently found in studies of marine invertebrate reproduction and most studies seek nongenetic factors to explain this variation. For example, in 13 species of sea urchins, significant differences were found between females whose eggs were measured on the same day (Lessios, 1987). Similarly, 20% of the variation in egg volume and 86% of the variation in egg energy content from mussels collected from several locations was a result of variation among females (Phillips, 2007). In these studies, as well as our own, it was not clear what differences among the females could contribute to such variation in egg size. Phillips (2007) ruled out effects of female body size and condition, and we found little indication of these factors having large effects in *Crepidula*. Among-female variation could be inflated by the artificial induction of spawning (Phillips, 2007), although this cannot be the case in *Crepidula*, which were allowed to spawn naturally. Similarly, the idea that small-scale differences in habitat could result in among-female variation in egg size would only apply

to field-collected animals, and not those raised their entire lives under laboratory conditions.

One factor that has been shown to affect egg size and quality in a number of studies is brood order. A reduction in egg size with successive broods has been found in a number of marine invertebrates (Gallager & Mann, 1986; Qian & Chia, 1992; Jones *et al.*, 1996; Ito, 1997). Because we only measured eggs from a single brood from each female, our experimental design does not allow us to test for an effect of brood order.

Population differentiation

We showed that genetic differentiation in mtCOI sequences between populations in *C. ustulatulina* is accompanied by differentiation in offspring size and shape. Such differentiation in life history characters is not evident in *C. atrasolea*, which also does not show differentiation in COI. This is the first time that differences in population genetic structure among congeners have been linked to among-population differences in developmental features.

Inter-population differentiation in developmental features has been reported for barnacles (Barnes & Barnes, 1965), polychaetes (Bridges & Heppell, 1996), and fishes (Johnston & Leggett, 2002; Kokita, 2003), although, in most cases, there is no information on the genetic structure of the populations. This makes it difficult to determine whether such differences are due to genetic differentiation or plastic responses to environmental differences between the sites. A single previous study, using *Adalaria proxima*, demonstrated that animals from populations that were differentiated with respect to allozymes (Todd *et al.* 1998) also differed in egg size, brood size, and hatching size (Jones *et al.*, 1996). If such genetic differences in offspring size and other reproductive features are common among genetically distinct populations, then the starting conditions required for evolutionary divergence in mode of development may be more common that previously appreciated.

Conclusions

Crepidula atrasolea and *C. ustulatulina* raised under controlled conditions produce eggs with a wide range of diameters. In *C. atrasolea*, the average egg from the brood with the largest eggs was twice the volume of the average egg from the brood with smallest eggs; in *C. ustulatulina*, there was a three-fold difference in volume between the broods with the largest and smallest egg sizes. Documentation of such significant variation lays the foundation for the in-depth study of variation in offspring size, which is necessary to develop a comprehensive theory of the evolution of offspring size (Bernardo, 1996). Bernardo advocates an approach that, instead of expecting a single optimal offspring size, embraces intraspecific variation and the complex interplay between optimal offspring size from both the parents' and offsprings' perspective, at the same time as taking into account pasticities and maternal effects. Certainly, our simple experiment yielded evidence for wide variation in offspring size and supported the idea that several factors play a roll in this variation.

From a macroevolutionary perspective, detailed studies of intraspecific variation are vital to bring a fuller understanding of the role of developmental variation in speciation. The link between genetic and developmental differentiation among populations is particularly important for understanding the diversification of marine invertebrates, where cryptic sister species with similar ecology often differ in development. Inter-population divergence in development could play a part in driving speciation in these groups, existing inter-population differences in development could be dragged along with speciation driven by other features, or differences in development among sibling species could evolve after speciation. There are few available data with which to assess these different scenarios. The results obtained in the present study, demonstrating genetic differentiation in offspring size among populations, lend support to the first two of these scenarios. However, more studies of intraspecific variation are necessary to assess the generality of this pattern and further distinguish between possible scenarios.

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