

Impacts of Adelphophagic Development on Variation in Offspring Size, Duration of Development, and Temperature-Mediated Plasticity

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Abstract. Adelphophagic development, where embryos consume sibling embryos or nurse eggs, is particularly common in marine caenogastropods and some families of polychaetes. When exogenous nutrition is provided before hatching, egg size and hatching size can be uncoupled, but advantages and constraints of adelphophagic development compared to development from large eggs are unknown. Here we examine temperature-mediated plasticity in offspring size, brooding duration, and fecundity in the adelphophagic marine gastropod *Crepidula* cf. *onyx*. We use these data combined with previously published data on two planktotrophic *Crepidula* and two *Crepidula* species that develop from large eggs to test hypotheses about the consequences of adelphophagic development and patterns of variation in offspring size. In *Crepidula* cf. *onyx*, egg size shows no significant effect of temperature. Hatching size is significantly larger at 28 °C than at 23 °C but proceeds from fewer eggs per capsule at 28 °C. Hatching size is therefore decoupled from both egg size and the number of eggs per capsule. Although development is faster at the higher temperature, broods are produced roughly every 26–27 days at both temperatures. Increased rate of development has been cited as a potential advantage of adelphophagic development in muricids, but the adelphophagic *C.* cf. *onyx* did not develop more quickly than *C. atrasolea* or *C. ustulatulina*, species that produce similarly sized hatchlings from large eggs. Comparisons across calyptraeid species support the role of adelphophagy in increasing variance in offspring size. This increased variability is primarily expressed within broods or among broods from the same female, not among females.

Introduction

Marine invertebrates have evolved a number of ways for small eggs packaged by mothers into gelatinous egg masses or capsules to develop into large hatchlings. For example, in volutid gastropods, few small eggs are deposited in a large capsule filled with intracapsular fluid. Consumption of this fluid can support the growth of a 65- μ m egg into a 12-mm hatchling (Penchaszadeh *et al.*, 1999; Penchaszadeh and Miloslavich, 2001). Significant embryonic growth after consumption of intracapsular fluid has also been reported for *Urosalpinx cinerea* (Rivest, 1986). More commonly, extra-embryonic yolk is used to provide embryonic nutrition in addition to yolk provided by the egg itself. Among molluscs that develop in gelatinous egg masses, such extra-embryonic yolk can take the form of unusually large polar bodies (*e.g.*, some aeolid nudibranchs, Goddard, 1991), or smears of extracapsular yolk within the egg mass jelly in saccoglossans (Allen *et al.*, 2009; Krug, 2009).

By far the most common type of extra-embryonic nutrition in caenogastropods and polychaetes are nutritional eggs or embryos (Thorson, 1946, 1950; Fioroni, 1982). This includes oophagic development in which nurse eggs fail to develop, and adelphophagic development in which nurse embryos initiate development but are consumed by their siblings before development is complete. Very little is known about the developmental mechanisms of nurse egg or embryo specification, and in most cases it is not known if the nurse embryos would be able to develop if they were not consumed by their siblings. The nurse eggs of *Crepidipatella dilatata*, which never initiate cleavage, arrest development prior to the completion of meiosis and appear to be unfertilized (Gallardo and Garrido, 1987). The nurse eggs of the polychaete *Boccardia proboscidia* are activated and produce a fertilization envelope, but the yolk subsequently

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becomes compartmentalized in a process resembling apoptosis (Smith and Gibson, 1999). Since many species with adelphophagic development are polygamous and store sperm, the genetic relationships between the successful embryos and their nutritive siblings could also foster extreme sib-competition (Kamel *et al.*, 2010), which has been investigated only in the gastropod *Solenosteira macrospira*.

The ecological consequences of oophagic or adelphophagic development compared to development from large yolky eggs are also poorly understood and based on few studies (Spight, 1975; Rivest, 1983; Kamel *et al.*, 2010). Development from large eggs and development from small eggs with embryos that consume nurse eggs or albumin can both result in large hatchlings (Spight, 1975; Rivest, 1983; Collin, 2003a). Large hatchlings are thought to be advantageous as they have higher growth and survival (Spight, 1976; Rivest, 1983; Moran, 1999; Moran and Emler, 2001; Sman *et al.*, 2009; but see Jacobs and Sherrard, 2010), as well as larger feeding structures at settlement (Kosman and Pernet, 2011) and better competitive ability (Marshall *et al.*, 2006).

A potential advantage of development with nurse eggs over development from large eggs is that egg size and offspring size can be uncoupled. The evolution of adelphophagy could be favored if structural or energetic reasons limit maximum egg size (Strathmann, 1995). This idea would be supported if hatchlings produced with extra-embryonic nutrition were much larger than related species that develop without exogenous nutrition. This does occasionally occur. For example, the calyptraeids *Crepidula philippiana* and *Maoricrypta monoxyla* each produce a single embryo and many nurse eggs per capsule, and these embryos hatch at twice the size typical of direct-developers from large eggs (Gallardo, 1996; Collin, 2003a). However, calyptraeids with nurse eggs more typically produce hatchlings of similar sizes to those produced from large eggs (Collin, 2003a). Adelphophagic development could also uncouple more subtle changes in egg size, resulting from maternal physiological status (such as age, size, nutritional status) from hatching size.

In species where females can control the moment of hatching, adelphophagy may also allow the adjustment of hatching size and stage on the basis of cues experienced by the mother during brooding. For example, the worm *Boccardia proboscidea* broods capsules that contain nurse eggs, small embryos that hatch as planktotrophic larvae, and large embryos that hatch as lecithotrophs (Gibson, 1997). If the mother delays hatching, most of the small embryos are eaten by the larger embryos. If she accelerates hatching, both small and large embryos survive to hatching (Kamel *et al.*, 2010; Oyarzun and Strathmann, 2011).

Nurse egg development has also been proposed to act as a diversifying bet-hedging strategy. By increasing the variance in hatching size compared to species with large eggs,

females may benefit, depending on the conditions of an uncertain environment, from the high survival of the large hatchlings or the increased number of smaller offspring from a single brood (*e.g.*, Marshall *et al.*, 2008). In *Searlesia dira* (now *Lirabuccinum dirum*) and several *Nucella* species, high variation in hatching size is produced when each female makes a set frequency of nurse eggs and viable embryos but allocates them randomly to each capsule (Spight, 1976; Rivest, 1983). Because empty capsules are seldom observed, this mechanism of nurse egg allocation is unlikely to apply to species that place very few embryos in each capsule (Rivest, 1983) like the vermetid *Petalocochus montereyensis* (Hadfield, 1989). However, hatchlings in these species vary in size due to different allocation of nurse eggs to each capsule (Hadfield, 1989).

Adelphophagic development could increase variation in hatching size in four ways: (1) competition among embryos within capsules could increase variation among capsule-mates; (2) different allocation of nurse eggs among capsules could increase variation among capsules; (3) different proportion of nurse eggs per brood could increase temporal variation in offspring size within females; and (4) different proportion of nurse eggs per brood among females could increase variation among females relative to species with large eggs. Different times to hatching could also affect the among- and within-female variation in hatchling size if normally developing embryos turn on each other after nurse eggs are consumed. Patterns of variation in hatching size have been linked to mode of development for invertebrates in general by Marshall and Keough (2008), who suggest that direct-developers have higher variation in offspring size than do planktotrophs and that the relative contribution of within- and among-female variation should change with mode of development (Marshall *et al.*, 2008). They did not compare how variation from adelphophagic species contributed to the high values obtained for all direct-developers.

It has also been suggested that development mediated by nurse eggs may proceed more rapidly than development from large eggs (Spight, 1975). This idea stems from a review of muricid development times, combined with the observation that early development occurs faster in small eggs. However, in some nurse-egg feeders, development is temporarily delayed during a special stage during which the nurse eggs are consumed. In others, nurse-egg consumption continues through development with no noticeable reduction in development rate (Stöckmann-Bosbach, 1987). As development rates are very sensitive to temperature, strong tests of this hypothesis require observations made at known temperatures.

Here we raised the adelphophagic embryos of *Crepidula cf. onyx*. This species was referred to as "*Crepidula aff. onyx* Panama" in Collin (2003b) and corresponds to vouchers at the Field Museum FMNH299420 and GenBank numbers AF546020, AF545954, and AF545877. The develop-

ment of the embryos was compared with that of two *Crepidula* species with large eggs and direct development and with that of two species with small eggs and planktotrophic development to address the following questions: (1) Are the effects of temperature on hatching size and egg size uncoupled in *C. cf. onyx*? (2) Does *C. cf. onyx* have greater variation in hatching size than direct-developing species without nurse eggs? (3) Does *C. cf. onyx* have faster development than direct-developing *Crepidula* without nurse eggs? We also take advantage of previous work on *Crepidula* species with different development to compare patterns in variation in hatching size with the patterns from a review of the literature (Marshall and Keough, 2008; Marshall *et al.*, 2008).

Materials and Methods

Experimental methods

Specimens of *Crepidula cf. onyx* were collected from the intertidal of the Pacific coast of Panama from Chumical (8.874°N, 79.644°W) and Playa Venado (8.886°N, 79.596°W) near the town of Veracruz just outside Panama City. Small juveniles were collected, assigned to pairs, and placed in transparent 350-ml cups following the protocols previously used to study egg size in *Crepidula* (Collin and Salazar, 2010; Collin, 2012). Thirty-five replicates were raised at each of two temperatures: 23 °C and 28 °C. These temperatures are typical of the average sea temperature experienced during upwelling (typical range of 18–24 °C) and non-upwelling (28–29 °C) in the Bay of Panama (Collin, 2012; Kerr *et al.*, 2012). They were fed 50×10^3 cell/ml *Isochrysis galbana* daily, and the water was changed three times a week.

Crepidula individuals are protandrous hermaphrodites with socially cued, environmentally determined sex allocation. Typically, when raised in pairs, the smallest individual in each cup remains male while the larger becomes female (Collin *et al.*, 2005). Females, which brood numerous transparent capsules between the neck and the substratum, were checked twice a day for eggs or hatchlings. The number of days to hatching and the number of days between natural hatching and the deposition of the subsequent brood were recorded where possible. Ten to twenty live uncleaved eggs were photographed and measured with Image J ver. 1.44o (Abramoff *et al.*, 2004) (following Collin and Salazar, 2010; Collin, 2012). Within 12 h of hatching naturally, juveniles were collected and preserved in ethanol. Subsequently they were photographed and the shells measured with Image J (Abramoff *et al.*, 2004). Because cleavage is synchronous within a capsule but not between capsules, egg size was measured for whatever uncleaved eggs were available and generally represents eggs from one to three capsules. Juveniles hatched simultaneously; therefore they rep-

resented a random sample of the entire brood and could not be assigned to individual capsules.

Effective diameter (*i.e.*, the average of the major and minor axis) of eggs with a roundness (*i.e.*, minor axis/major axis) of at least 94, and the hatchling shell length (*i.e.*, major axis of the dorsal view) were measured using the Shape Descriptor ver. 1u plug-in for Image J. We aimed to measure three broods of eggs and three broods of hatchlings from each female, but collecting uncleaved eggs proved difficult as females generally lay in the evening. Therefore, the number of egg measurements is smaller than the number of measurements for hatchlings.

After three broods of eggs and three broods of hatchlings had been collected, the total number of capsules and the number of eggs per capsule for a subsample of five capsules were counted for the next three broods. All measurements for each female were collected within 18 months of initiation of the treatment, although measurements for most females were completed in less than a year. Measuring uncleaved eggs requires the removal of the brood from the female before oviposition is complete; therefore, the total number of capsules could not be counted for these broods. Because some broods were collected after cleavage had begun, “brood number” in the statistical analyses does not reflect every brood produced; rather, it reflects the relative order of the broods included in that analysis.

Analyses

Effects of temperature, maternal identity, and individual brood on egg size and hatching size were examined with a restricted maximum likelihood (REML) estimate of a mixed model ANCOVA with maternal identity (“female”) as a random effect nested within the fixed effect of temperature treatment, with brood number as a random effect nested within female, and with female length and brood date as covariates. The unlikely possibility of preservation effects was eliminated, as the time that hatchlings were held in ethanol before being measured was never significant. Effects of temperature and maternal identity on capsule number, eggs per capsule, total eggs per brood, time to hatching, and inter-brood period for three broods from each female were also analyzed with a mixed model ANCOVA with female as a random effect nested within temperature and with maternal length as a covariate. Stepwise removal of nonsignificant factors subsequent to the analysis of the full model did not alter any of the results reported here. All statistical analyses were conducted using JMP 9 (SAS Institute, Cary, NC).

We compared variation in offspring size in *C. cf. onyx* with four other species of *Crepidula* that have been raised under the same laboratory conditions (Collin and Salazar, 2010; Collin, 2012). Two of these, *Crepidula incurva* and *Crepidula cf. marginalis*, co-occur with *C. cf. onyx* in the

Table 1Summary of the effects of temperature on reproduction of *Crepidula cf. onyx*

Temperature (°C)	Egg size in μm (mean, SE)	Hatching size in μm (mean, SE)	Capsule number (mean, SE)	Eggs/capsule (mean, SE)	Eggs/brood (mean, SE)
23	156.89 (0.97)	966.54 (10.08)	13.63 (0.59)	139 (3.98)	1906 (112.59)
28	158.62 (1.27)	1059.13 (10.94)	12.11 (0.67)	119 (4.43)	1443 (128.12)

intertidal of Panama. The other two, *Crepidula atrasolea* and *Crepidula ustulatulina*, occur in shallow-water habitats along both coasts of Florida and much of the Gulf of Mexico, where they experience similar ranges in water temperature. All five species grow to similar sizes in the laboratory (*C. incurva*, 11–18 mm; *C. cf. marginalis*, 10–23 mm; *C. cf. onyx*, 14–24 mm; *C. atrasolea*, 9–23 mm; *C. ustulatulina*, 7–15 mm). To determine if development with nurse eggs increases the variation in hatching size, we compared (1) the overall coefficient of variation (CV) for hatching size for each species (*i.e.*, the CV of every hatching we measured); (2) the average within-brood CV for each species (*i.e.*, the average of the CVs calculated for each individual brood); and (3) the relative variance in hatching size explained by variation among females, among broods within females, and within broods (*i.e.*, the percent variation explained by the random effects of female, brood nested within female, and residual variation in an REML ANOVA). The within-brood CVs were compared across species and temperatures using ANOVA. The overall CVs in hatching size in each species were compared using 95% confidence intervals generated with 1000 replicate bootstrap samples generated by a modified version of a script written for JMP (Ramón V. León, Univ. Tennessee).

The variation in offspring size in *Crepidula* can be placed

in the context of data from other invertebrates by comparing the overall CV of offspring size with data in Marshall and Keough (2008). The prediction that the relationship between among- and within-female variation depends on mode of development can also be assessed for *Crepidula* by calculating the CV of offspring size for various partitions of the data (Marshall *et al.*, 2008). These analyses were conducted for all the available data and for a reduced dataset that included only the first brood for each female, for comparison with other published studies.

Results

Effects of temperature on the reproduction of C. cf. onyx

The effects of temperature on reproduction of *Crepidula cf. onyx* were different from the effects demonstrated for non-adelphophagic *Crepidula*. Unlike other species of *Crepidula* in which egg size and hatching size are smaller at 28 °C than at 23 °C, *C. cf. onyx* did not show a significant effect of temperature on egg size (Tables 1, 2), and hatching size showed a significant increase with increased temperature (Tables 1, 2). The number of eggs per capsule and the total number of eggs per brood decreased significantly with increasing temperature (Tables 1, 3), while the number of

Table 2ANOVA table of the effects of temperature on egg size and hatching size in *Crepidula cf. onyx*

Effects	df	<i>F</i>	<i>P</i>	Total variation (%)
Egg Size				
Temperature	1	1.13	0.30	
Female [temperature] Random				46.66
Brood # [temperature, female] Random				29.25
Date	1	0.02	0.88	
Maternal length	1	0.01	0.93	
Hatching Size				
Temperature	1	36.42	<0.001	
Female [temperature] Random				4.49
Brood # [temperature, female] Random				25.65
Date	1	2.70	0.10	
Maternal length	1	0.32	0.57	
Days in tube	1	3.06	0.08*	

* Does not become significant with stepwise removal of nonsignificant factors. Statistically significant *P* values are highlighted in bold.

Table 3

ANOVA table of the effects of temperature on capsules per brood and eggs per capsule in *Crepidula cf. onyx*

Effects	df	F	P	Total variation (%)
Capsules per brood				
Temperature	1	3.81	0.06*	
Female [temperature] Random				26.47
Maternal length	1	0.73	0.40	
Eggs per capsule				
Temperature	1	3.29	0.08#	
Female [temperature] Random				61.71
Maternal length	1	0.46	0.50	
Total Eggs per brood				
Temperature	1	6.72	0.014	
Female [temperature] Random				38.14
Maternal length	1	0.09	0.77	

* Not significant with stepwise removal of maternal length.

Significant with stepwise removal of maternal length ($P = 0.02$).

Statistically significant P values are highlighted in bold.

capsules did not differ between the temperature treatments (Tables 1, 3).

Are C. cf. onyx hatchlings more variable in size than other direct-developers?

Crepidula cf. onyx does show greater within-brood coefficient of variation in hatching size and a greater overall CV in hatching size than the four non-adelphophagic species for which data are available: direct-developing *Crepidula atrasolea* and *C. ustulatulina* and planktrophic *C. incurva* and *C. cf. marginalis*. Analysis of variance of the coefficient of variance of each brood showed a significant effect of species ($df = 4$; $F = 488.93$; $P < 0.0001$) but no significant effect of temperature and no significant interaction between species and temperature ($df = 1$; $F = 2.42$; $P = 0.12$; $df = 4$; $F = 1.89$; $P = 0.11$ respectively) (Fig. 1A). The *post hoc* Tukey HSD test showed that *Crepidula cf. onyx* broods had significantly higher CVs in hatching size at both temperatures than the other species. The CVs in hatching size are lowest for *C. cf. marginalis* and *C. incurva* and intermediate for *C. atrasolea* and *C. ustulatulina* (Fig. 1A). The higher CV per brood in *C. cf. onyx* supports the idea that adelphophagic development increases the variation among hatchlings from the same brood.

The CVs in hatching size calculated across all hatchlings for each species show a similar pattern (Fig. 1B). Bootstrap-generated 95% confidence intervals of the CVs in hatching size show that *C. cf. onyx* has the highest overall CV at 28 °C and the second highest CV at 23 °C. The two direct-developers from large eggs are intermediate, and the planktotrophs have the lowest CV across all measured eggs.

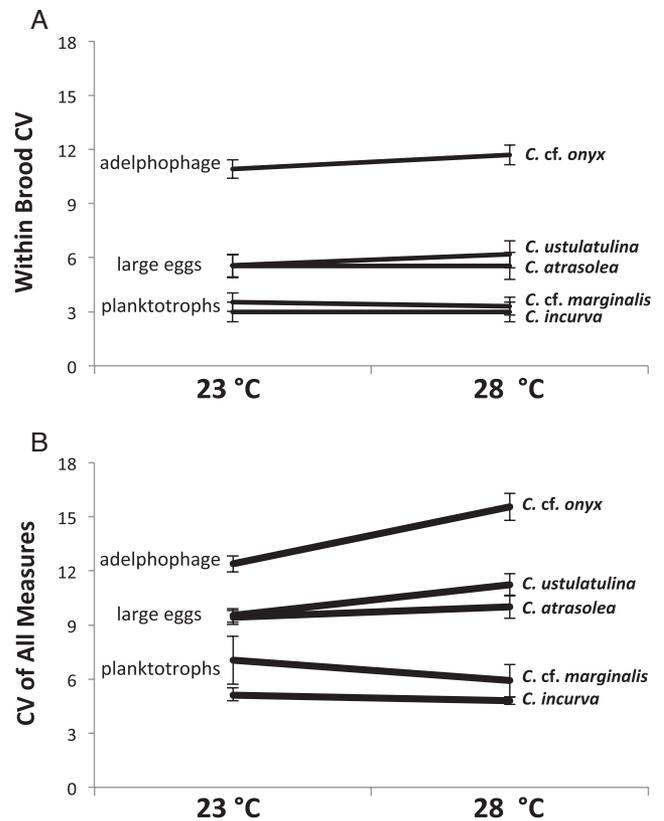


Figure 1. Relationship between species' coefficient of variation (CV) in hatching size and temperature for 5 species of *Crepidula*. (A) CV calculated within each individual brood; error bars represent standard errors. (B) CV calculated across all measurements for each species; error bars are the standard errors calculated from 1000 bootstrap replicates. *Crepidula atrasolea* and *C. ustulatulina* have development from large eggs, *C. cf. marginalis* and *C. incurva* are planktotrophs, and *C. cf. onyx* is adelphophagic.

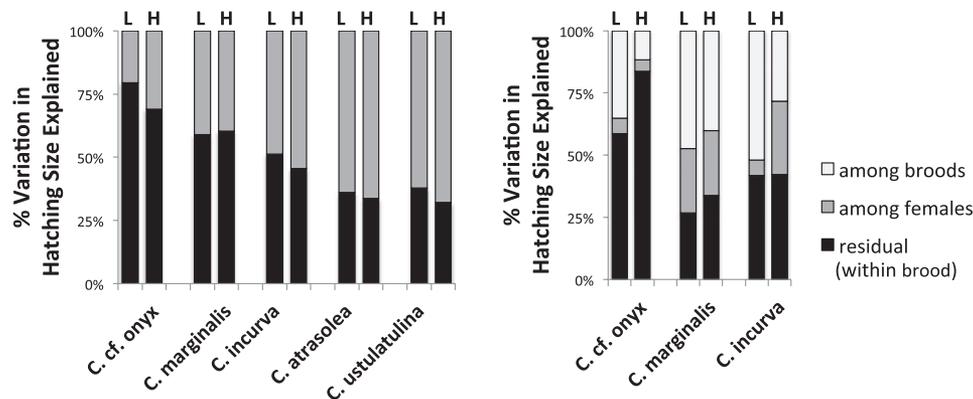


Figure 2. Proportion of variation in hatching size explained in a restricted maximum likelihood nested ANOVA. The graph on the left shows the results when only a single brood was analyzed for each female, with female as a random effect. The graph on the right shows the results when 3 broods were analyzed for each female, with brood as a random effect nested within the random effect of female. Data were not available for *Crepidula atrasolea* and *C. ustulatulina*. Data from each species and temperature were analyzed separately. Temperature is indicated above each bar: L = 23 °C and H = 28 °C.

Within- versus among-female variation

When only a single brood is measured per female, more variation in hatching size is attributable to differences among females for the species with large eggs compared to the other species (Fig. 2A). The smallest proportion of variation in hatching size is attributable to differences among females for *C. cf. onyx* (Fig. 2A; REML ANOVA with the effect of female as a random effect). In this case, the residual variance reflects variation within the single brood measured for each female. When multiple broods were measured per female, the results are quite different. The residual (within-brood) variation is still smaller in the planktotrophs than in *C. cf. onyx*, but the estimated percent variation explained by the among-female component was considerably reduced (Fig. 2B). This shows that a large proportion of what appear to be differences among females when only a single brood is examined can be attributed to variation among broods from the same female.

Comparisons of the within- and among-female CVs showed a similar pattern (Table 4). When only one brood per female is measured, in all species but *C. cf. onyx* the within-female CV is smaller than the among-female CV. However, when multiple broods for each female are included, all three species with multiple broods per female show a larger CV within females than among females.

Is time to hatching faster in *C. cf. onyx*?

Temperature had a significant effect on time to hatching ($df = 1$; $F = 201.67$; $P < 0.0001$) and the interbrood period ($df = 1$; $F = 8.94$; $P = 0.004$) in *C. cf. onyx*. The covariate of maternal length did not contribute significantly to variation in either duration ($df = 1$; $P > 0.10$). Maternal identity contributed to 22% of the variation in days to hatching, but

less than 7% of the variation in interbrood period. As expected, time to hatch was longer (19.05 d) at 23 °C than at 28 °C (14.61 d), but the interbrood period was shorter at 23 °C (7.87 d) than at 28 °C (12.85 d). These opposite relationships with temperature result in a similar overall brood frequency at the two temperatures, with broods being produced every 26.92 d at 23 °C and every 27.46 d at 28 °C.

Comparisons of time to hatching for broods of *C. cf. onyx*, *C. atrasolea*, and *C. ustulatulina* (raised for Collin and Salazar, 2010) show a significant effect of species ($df = 2$; $F = 102.49$; $P < 0.0001$) and temperature ($df = 1$; $F = 1743.73$; $P < 0.0001$) and a significant interaction between the two ($df = 2$; $F = 120.90$; $P < 0.0001$) (Fig. 3). The *post hoc* Tukey HSD test showed that brood durations differed significantly for all combinations of species and temperatures, except for *C. cf. onyx* and *C. ustulatulina* at 23 °C (Fig. 3A). At 28 °C, *C. cf. onyx* had the longest brood duration of the three species, and at 23 °C, *C. cf. onyx* and *C. ustulatulina* had significantly shorter brood durations than *C. atrasolea* (19.14 and 18.65 d vs. 22.85 d, respectively). Comparison of the average time to hatching relative to average hatching size for the three species (Fig. 3B) shows that the duration of development in *C. cf. onyx* is not obviously fast for its hatching size, and therefore does not offer support for the hypothesis that adelphophagic development decreases development time.

Discussion

The detailed comparative data available for variation in egg size and hatching size for five species of *Crepidula* allow some of the hypotheses about the possible advantages of nurse-egg development to be tested. We found that, as is commonly assumed, adelphophagic development does pro-

Table 4

Variation in hatching size (measured as coefficient of variation, CV) in *Crepidula cf. onyx* compared to other *Crepidula* species

	One brood per female			All broods included		Total CV of all measurements (n)
	Average CV for each brood (number broods)	Average within female CV (number of females)	Among female CV of hatching size	Average within female CV of all offspring	Among female CV of the total mean hatching size for each female	
23°C						
<i>C. cf. onyx</i>	10.91 (96)	10.88 (31)	6.48	11.78	3.79	12.39 (1692)
<i>C. cf. marginalis</i> #	3.52 (96)	3.38 (33)	5.70	5.15	4.61	7.06 (1881)
<i>C. incurva</i> #	2.99 (85)	3.26 (29)	3.93	4.15	2.60	5.11 (1805)
<i>C. atrasolea</i> *	5.52 (63)	5.52 (63)	7.81	–	–	9.42 (1174)
<i>C. atrasolea</i> Mote	5.52 (36)	5.52 (36)	8.07	–	–	9.48 (663)
<i>C. atrasolea</i> ftp	5.54 (27)	5.54 (27)	6.87	–	–	8.67 (511)
<i>C. ustulatulina</i> *	5.56 (64)	5.56 (64)	7.75	–	–	9.53 (1223)
<i>C. ustulatulina</i> Mote	5.23 (34)	5.23 (34)	7.96	–	–	9.48 (645)
<i>C. ustulatulina</i> ftp	5.93 (30)	5.93 (30)	7.63	–	–	9.58 (578)
28°C						
<i>C. cf. onyx</i>	11.69 (89)	11.36 (30)	9.44	13.28	7.56	15.53 (1381)
<i>C. cf. marginalis</i> #	3.30 (103)	3.23 (36)	5.31	4.40	3.77	5.92 (1966)
<i>C. incurva</i> #	2.98 (86)	3.06 (29)	3.63	3.67	3.12	4.80 (1731)
<i>C. atrasolea</i> *	5.54 (45)	5.54 (45)	8.27	–	–	10.01 (726)
<i>C. atrasolea</i> Mote	5.67 (25)	5.67 (25)	9.27	–	–	10.50 (404)
<i>C. atrasolea</i> ftp	5.38 (20)	5.38 (20)	7.04	–	–	9.28 (322)
<i>C. ustulatulina</i> *	6.18 (44)	6.18 (44)	9.86	–	–	11.23 (800)
<i>C. ustulatulina</i> Mote	5.88 (28)	5.88 (28)	10.06	–	–	11.18 (513)
<i>C. ustulatulina</i> ftp	6.70 (16)	6.70 (16)	8.85	–	–	10.95 (287)

Shell length was measured as the major axis for juvenile shells and as Feret's length for larvae.

Boldface type indicates whether the within or among female variation is largest.

* From the data collected in Collin and Salazar (2010), where only a single brood was measured for each female. Data are reported when the two populations (Mote, Florida [Mote], and Fort Pierce, Florida [ftp]) were pooled and also shown separately, although values are generally consistent among populations.

Calculated from the experiment in Collin (2012).

duce more variable hatchlings than either direct development from large eggs or planktotrophic development from small eggs. Adelphophagic *Crepidula cf. onyx* showed significantly more variation among offspring from the same brood, and significantly more overall variation in offspring size than any of the other species. The CV in offspring size among females, however, was of a similar magnitude for all of the species. This suggests that increased variation in offspring size in *C. cf. onyx* is due to different consumption of nurse eggs within each capsule and/or different allocation of nurse eggs among capsules, but that the overall proportion of nurse eggs produced by each female is similar. A similar result was reported for *Searlesia dira*, where variation among capsules from the same female was much larger than among-female differences (Rivest, 1983).

The results presented here also support the idea that nurse-egg production uncouples egg size and hatching size. In this case the seemingly ubiquitous reduction in egg size and hatching size with increasing temperature, termed the size-temperature rule (Atkinson *et al.*, 2001; Moran and McAlister, 2009; Zuo *et al.*, 2012), was not detected. The

four other *Crepidula* species without adelphophagic development all conform to the size-temperature rule, with animals raised at the colder temperature producing larger eggs and larger hatchlings (Collin and Salazar, 2010; Collin, 2010, 2012). In contrast, egg size in *C. cf. onyx* showed no change with temperature, but there was a large significant increase in hatching size with increasing temperature. Our data are unable to resolve the cause of this effect. Since the total number of eggs per capsule decreases significantly with increasing temperature, this increase in hatching size cannot simply be an increase in the number of nurse eggs placed in each capsule. We did not count the number of nurse eggs *versus* normal embryos because the distinction does not become clear until the middle of development, and even then it is not always possible to distinguish embryos that will complete development from those that will be consumed (M. P. Lesoway, McGill University, unpubl. data). Therefore we cannot determine if the increased hatching size is due to a decreased number of normal embryos relative to specified nurse embryos in each capsule. This pattern could also occur if nurse eggs are not specified

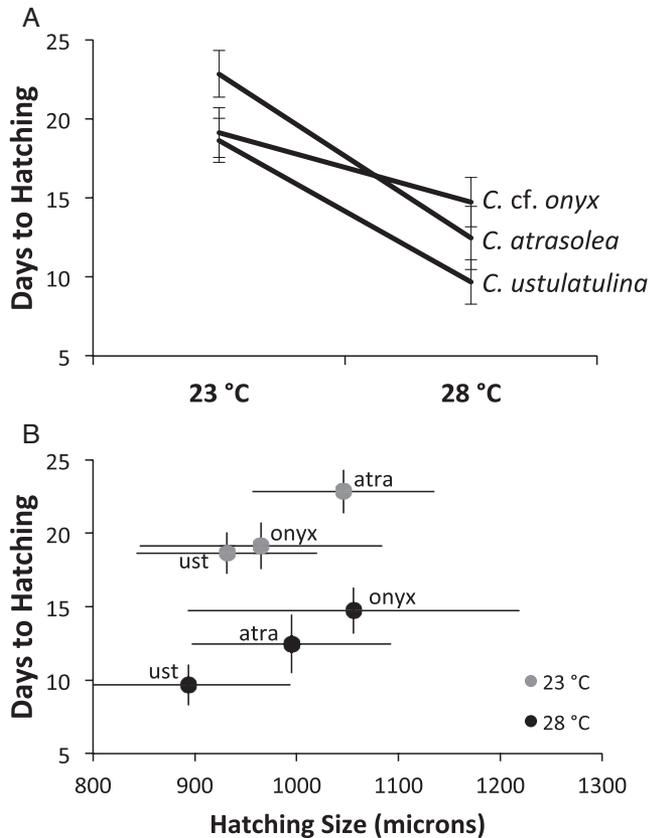


Figure 3. Development rates in three species of *Crepidula* with direct development. (A) Relationship between average days to hatching and temperature. (B) Relationship between hatching size, temperature, and days to hatching. *C. cf. onyx* does not have obviously faster development for a given hatching size than the two species with development from large eggs. Error bars show standard deviations.

before they are allocated to a capsule. In that case, the intensity of competition and siblicide among potentially developing embryos within a capsule could affect the number and size of the surviving hatchlings (Kamel *et al.*, 2010). It is also possible that temperature effects on developmental physiology result in the effect of temperature on hatching size, or that eggs contain more nutrients at high temperatures, or that development requires less energy (McEdward and Coulter, 1987; Moran and McAlister, 2009).

The idea that adelphophagic development can be completed more quickly than direct development from large eggs was proposed by Spight (1975), who found that muricids with nurse eggs took only 60% as long as those with large eggs to develop. This hypothesis is consistent with the idea that early development proceeds more rapidly in smaller than larger eggs. However, the relationship between egg size and early development rate has not been supported in comparative studies, which found that cleavage rate is associated with level of embryonic protection and time to swimming but not to egg size (Strathmann *et al.*, 2002;

Staver and Strathmann, 2002). The idea that adelphophagic development is faster is also not supported by our results. All three species examined seemed to fall on the same line representing the relationship between duration of development and hatching size. However, the relationship between temperature and duration of development differs for *C. cf. onyx*. The Q_{10} for duration of development is 3.36 for *C. atrasolea* and 3.71 for *C. ustulatulina*. *Crepidula cf. onyx*, in contrast, has a Q_{10} for duration of development of 1.69. These values are in the same range as those reported for muricids (Palmer, 1994), but the large difference between congeners is unexpected, and suggests that there may be some fundamental difference between adelphophagy and development from large eggs.

Variation in offspring size across modes of development

Marshall and Keough (2008) reviewed variation in offspring size across marine invertebrates and found an average CV of about 9%, with 14% for direct-developers and 3% for planktotrophs. They did not explicitly examine the role of adelphophagy, so direct-developers with nurse eggs were combined with those with large eggs. Analysis of only the gastropod data from their meta-analysis (after removal of the CV for *C. adunca* from Collin, 2000, which does not reflect normal variation in this species), shows an overall similarity with the entire dataset. Planktotrophic gastropods have the lowest CV (4.0%; $n = 15$), direct-developers and lecithotrophs with large eggs showed an intermediate CV (8.2%; $n = 5$), and direct-developers and lecithotrophs with nurse eggs had the highest CV (12.5%; $n = 9$). The data for calyptraeids (Collin, 2003a, data from table 1 for those including means and standard deviations) give a strikingly similar pattern: planktotrophs have the lowest CV (6.1%; $n = 17$), followed by direct-developers and lecithotrophs with large eggs (7.9%; $n = 8$); and then by direct-developers and lecithotrophs with nurse eggs with the highest CV (12.6%; $n = 7$). ANCOVA of these data show that planktotrophs had a significantly lower CV than adelphophages, but species with large eggs were not significantly different from the other two. The possible correlation between CV in hatching size and the mean size has been raised as a potential problem with the use of the coefficient of variation (Jacobs and Podolsky, 2010) but seems not to be a problem here as the ANCOVA failed to detect a relationship between CV and mean size or sample size.

Both the Collin (2003a) dataset and the data compiled by Marshall and Keough (2008) are idiosyncratic, with unbalanced sample sizes for each mode of development, measurements from different numbers of females and capsules per female, and different experimental conditions. Therefore these meta-analyses should be viewed as rough exploratory efforts at pattern detection. More detailed controlled studies are necessary to fully document these patterns and to

understand in more detail how offspring size varies with mode of development and whether this general relationship holds across a variety of different taxa.

The breakdown of CV in hatching size into within- and among-female components is also predicted to differ with mode of development (Marshall *et al.*, 2008). A model of female bet-hedging with offspring dispersal in unpredictable environments predicted that among-female variation should be greater than within-female variation in direct-developers, and within-female variation should be larger than among-female variation in planktotrophs (Marshall *et al.*, 2008). This is not borne out by our data on *Crepidula*. When a single brood is measured for each female, within-female CV is higher than among-female CV only for the adelphophagic species (Table 3). When multiple broods are examined, both the planktotrophs and *C. cf. onyx* had higher within-female than among-female CV in hatching size. Unfortunately, comparable data on multiple broods are not available for hatching size in the two species with large eggs.

Most published studies of invertebrate reproduction examine a single brood for each female. The data presented here show that including multiple broods per female can significantly alter the interpretation of patterns of variation: With multiple broods per female, the among-female variation is significantly reduced relative to the within-female variation. This could reduce the estimates of repeatability and heritability (Collin, 2010) and alter our ideas about the genetic underpinnings of offspring size. Logistical constraints limit our ability to obtain multiple clutches from some invertebrates. However, in many other iteroparous groups it should be possible to assess the different components of within-female variation, a necessary step before the results reported here can be generalized outside the calyptraeids.

Concluding thoughts

What are the advantages of oophagic and adelphophagic development? This kind of development has arisen numerous times, not just among gastropods but within various other groups of marine invertebrates as well as within insects and amphibians. Therefore, it is appealing to impute some kind of selective advantage, and to seek such potential advantages among the demonstrable consequences of nurse-egg development. At least in *Crepidula*, offspring produced by adelphophagic development are more variable in size than those produced from large eggs, leaving open the possibility that diversifying bet-hedging is one potential advantage. However, it is difficult to rigorously demonstrate that variation in any character is the result of selection for diversifying bet-hedging, and well-documented examples are rare (Simons, 2011). An alternate and novel hypothesis suggested by the results of the current study is that adel-

phophagic development allows animals to escape from the physiological constraints that underlie the temperature-size rule, thus allowing larger offspring to be produced at higher temperatures. This seems to be a consequence of adelphophagy in *C. cf. onyx*, but whether it is also true of other species with adelphophagic development remains to be demonstrated.

We should not, however, lose sight of the alternative: adelphophagy may simply be a means to an end and not itself selectively advantageous. Most obviously, adelphophagy is a way to produce large hatchlings. Both direct development with large eggs and direct development involving nurse eggs or nurse embryos could be the indirect result of selection for large offspring size, and not in themselves the object of selection. Like any set of alternate pathways to a single developmental outcome, the route taken may be the result of historical contingency rather than selection on characteristics of the developmental stages. The apparent diversity of development with extra-embryonic yolk compared to the diversity of development with large eggs could be because there are many distinct ways to disrupt early development and few distinct ways to generate large eggs. Since both kinds of development evolved numerous times from species with small eggs and planktotrophic development, the selective pressures underlying the origins of adelphophagy may be found by studying the patterns of genetic variation in egg size and number and how they relate to hatching size in planktotrophs, rather than in the consequences of adelphophagic development.

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