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Evolutionary and experimental change in egg volume, heterochrony of larval body and juvenile rudiment, and evolutionary reversibility in pluteus form

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SUMMARY Heterochronic developmental plasticity of the juvenile rudiment and larval body of sea urchin larvae occurs in response to supply of food. Evolutionary increase in egg size can also be associated with earlier development of the juvenile rudiment. We examined effects of egg volume of feeding larvae on this heterochrony and other changes in larval form. (1) Evolutionary and experimental enlargements of egg volume did not accelerate formation of the rudiment relative to the larval body. Development of the larval body and juvenile rudiment was compared for the echinoids *Strongylocentrotus purpuratus* (with an egg of 78–82 μm) and *Strongylocentrotus droebachiensis* (with an egg of 150–160 μm diameter). Development of both larval body and rudiment were accelerated in *S. droebachiensis* relative to *S. purpuratus* but with greater acceleration of the larval body, so that the rudiment of *S. droebachiensis* was initiated at a later larval stage even though at an earlier age. Also, experimentally doubling the egg volume of *S. purpuratus* did not accelerate development of the juvenile rudiment relative to the larval body. (2) Both species exhibited similar plasticity in timing of rudiment development in response to food supplies. (3) Doubling egg volume of *S. purpuratus* produced a larval form more similar to that of *S. droebachiensis*. This result mirrors previous experiments in which larvae from half embryos of *S. droebachiensis* were more similar to larvae of *S. purpuratus*. Many of the effects of egg volume on larval form are similar against either species' genetic background and are thus evolutionarily reversible effects on larval form.

INTRODUCTION

Evolutionary divergence in egg size affects larval form (Sinervo and McEdward 1988; McAlister 2007; Reitzel and Heyland 2007) as well as size, growth, and survival of larvae (Hart 1995; Allen 2008; Marshall and Keough 2008). We here examine another potential effect of egg volume: timing of development of larval relative to juvenile structures (Bertram and Strathmann 1998).

Evolution of an abrupt metamorphic transformation often involves a partitioning of tissues into ephemeral larval structures that will be resorbed at metamorphosis and sequestered rudiments of postlarval tissues. The rudiments develop without compromises with larval function and enable rapid metamorphosis. Extreme partitioning has evolved numerous times, as in larvae of nemerteans, oweniid annelids, echinoderms, and bryozoans (Wilson 1932; Reed 1991; Hadfield 2000; Strathmann et al. 2008). Sea urchins with feeding larvae exemplify the partitioning of larval and postlarval structures (Arenas-Mena et al. 2000). Experimental studies have demonstrated the decoupling of development of their larval and postlarval structures (Boidron-Metairon 1988; Strathmann et al. 1992; Chino et al. 1994; Heyland and Hodin 2004). Larval bodies and juvenile rudiments are plastic in both timing of development and ultimate size. When larval food is abundant, development of the juvenile rudiment is accelerated, the larval arms and ciliary band that capture food remain shorter, and the juvenile formed at metamorphosis is larger. When larval food is scarce, larval arms and ciliary band grow longer, whereas development of the juvenile rudiment is delayed (Strathmann et al. 1992; Hart and Strathmann 1994; Bertram and Strathmann 1998). In unusual cases, the larval body can develop fully to the eight-armed stage, whereas the rudiment is absent (Davidson et al. 1995).

The decoupling of larval and juvenile developmental modules appears to confer functional advantages (Hart and Strathmann 1994). Developing a larger larval body increases the rates at which scarce food particles can be cleared from suspension. Allocating growth to a feeding machine while delaying development of postlarval structures may minimize the risky larval period when food is scarce. When food is abundant, a smaller larval body can gather sufficient food for rapid growth, and an early start on postlarval development may minimize the risky larval period when food is abundant.

Functional advantages of this plasticity for feeding larvae may have contributed to the evolution of this decoupling of larval and juvenile modules, but the decoupling may facilitate other evolutionary changes (Strathmann et al. 1992; Bertram and Strathmann 1998; Strathmann 2000; Hodin et al. 2001; West-Eberhard 2003). In the evolution of nonfeeding larvae, which develop from larger eggs, the larval arms and ciliary band are reduced or lost, whereas development of the juvenile rudiment is accelerated (Raff 1987; Emlet 1995). Earlier development of an enlarged left coelom is also evident in a facultatively feeding pluteus that can feed but does not require particulate food for development through metamorphosis (Snoke Smith et al. 2007).

Shifts in timing of rudiment development are mediated at least in part by thyroxin, either acquired exogenously from planktonic food or synthesized by the larva (Chino et al. 1994; Saito et al. 1998; Hodin et al. 2001; Heyland et al. 2004). Is accelerated development of the juvenile rudiment also a proximate effect of an increase in egg volume? For free-spawners in general and for sea urchins in particular, egg volume is correlated with maternal investment of organic material per offspring (Jaeckle 1995; Pernet and Jaeckle 2004; McEdward and Miner 2006). Are evolutionary increases in organic content of eggs associated with earlier development of the juvenile rudiment?

In a comparison of larvae within the species *Strongylocentrotus droebachiensis* (Bertram and Strathmann 1998), the nutritionally stressed mothers produced smaller eggs with slower development of both larval body and juvenile rudiment, but there was no shift in relative timing of development. However, the range of egg sizes was small (Bertram and Strathmann 1998). Meidel et al. (1999) similarly found little effect of maternal nutrition on larval size and form in *S. droebachiensis*. We therefore compared species with a larger difference in egg diameters: 78–82 μm for *Strongylocentrotus purpuratus* and 150–160 μm for *S. droebachiensis* (Strathmann 1987; Bertram and Strathmann 1998; McAlister 2007).

We also compared larval development of the two species at two concentrations of food to see whether the difference in egg volume was associated with a difference in plasticity in timing of rudiment development. Larvae of *S. purpuratus* and *Strongylocentrotus franciscanus* both exhibited plasticity in development of larval structures in response to food (Miner 2005; McAlister 2007), despite a large difference between these species in egg volume, although *S. purpuratus*, with the smaller egg, exhibited less plasticity in larval arm length (McAlister 2007). These studies did not examine effect of food supply on heterochrony in development of the juvenile rudiment.

To distinguish effects of egg volume from other effects of genetic divergence, we examined effects of an acute change in egg volume on timing of development of the juvenile rudiment. We doubled egg volumes of *S. purpuratus* to see whether the effect of an acute change in egg volume on timing of development of the juvenile rudiment resembled or differed from an evolutionary divergence in egg size.

The combination of experimental and comparative methods (allometric engineering) has provided a powerful means of testing hypotheses on consequences of evolutionary change in egg size and the associated evolutionary divergence in other traits. Experimental reduction of volume of eggs of *S. droebachiensis* produced larvae resembling those of a related species of sea urchin, *S. purpuratus*, which has smaller eggs (Sinervo and McEdward 1988). We performed the complement to Sinervo and McEdward's (1988) experiment, enlarging the small eggs of *S. purpuratus* by a factor of 2. Fusion of sea urchin eggs can produce enlarged larvae (Tyler 1935; Bennett and Mazia 1981a, b). To the extent that acute changes in egg volume mimic the evolutionary changes in larval development, evolutionary change in egg size is sufficient to account for evolutionary divergence in larval development, without other genetic changes. Therefore, in addition to measuring larval and rudiment sizes, we noted whether experimentally doubling the egg volume of *S. purpuratus* would produce a larva more similar in form to that of *S. droebachiensis*. With an approximately 7-fold difference in egg volume for these species, a doubling of egg size for *S. purpuratus* does not produce an egg as large as that of *S. droebachiensis*, but egg doubling is sufficient to indicate associated changes in larval form and development, as did the half and quarter egg volumes of *S. droebachiensis* that were produced by Sinervo and McEdward (1988). It has remained uncertain whether the effects of egg cytoplasm volume on larval form would occur with either species' genetic background. Genetic differences between species could result in different effects of egg volume on morphogenesis. To the extent that experimental changes in egg volume produce the same effects on larval development in both species, the evolutionary divergence in larval form could be reversed simply by an evolutionary change in egg volume.

METHODS

Sources of parents

We collected ripe adult purple sea urchins (*S. purpuratus*) in Juan de Fuca Strait at Slip Point on the Olympic Penninsula on March 19, 1995. We collected green sea urchins (*S. droebachiensis*) from Deadman Bay on San Juan Island on March 25, 1995. Both sites are

on the coast of the NE Pacific Ocean in the state of Washington. Animals were held at the Friday Harbor Laboratories in continuous-flow aquaria near ambient sea temperatures (8–10°C) and fed bull kelp (*Nereocystis luetkeana*) at weekly intervals until use.

Interspecies comparison

Larval rearing was slightly modified from the methods of Bertram and Strathmann (1998). On April 19, 1995 we created two families of *S. droebachiensis* and two families of *S. pupuratus* by combining the sperm of one male with eggs from one female for each family. At 2 days after fertilization, we transferred 150 late-stage gastrulae from each family to a glass jar containing 1500 ml of filtered seawater. Larvae were first fed at 2 days postfertilization. Each family was reared at each of two levels of *Rhodomonas* sp. as food (high and low food rations of 5000 and 200 cells/ml) for a total of eight jars. Incubating culture jars in seatables supplied by the laboratory's seawater system maintained temperatures, 10.6 ± 0.8°C (mean $\pm$ SD), near those of ambient seawater at that season. Water for larval cultures was filtered through a 0.45-μm membrane filter and continuously stirred with motor driven paddles at 10 strokes per minute (Strathmann 1987).

At 2- to 4-day intervals, we removed five larvae from each jar and preserved them in 4% formalin buffered with $CaCO_3$. We videotaped the larvae and from taped images measured midline body length, postoral rod length, stomach length, invagination depth, and rudiment diameter (Fig. 1). Video images were measured with free software, NIH Image.

The measurements of size are correlated with performance of functions. Growth in length of the postoral arm rod is correlated with length of other arms, with length of the ciliary band, and thereby with maximum rate at which planktonic food can be cleared from suspension (Strathmann et al. 1992; Hart and Strathmann 1994). Midline body length is a measure of larval size less sensitive to changes in body form in response to food. Size of the stomach (as stomach length) may indicate digestive capacity, a nutrient store, or both. An invagination of ectoderm on the left side of the larva is the first sign of rudiment formation, and the depth of this invagination was measured before the ectoderm contacted the underlying coelom. After contact of the ectodermal invagination and coelom, rudiment diameter was measured as the diameter of the contact between ectoderm and mesoderm, and later as the diameter of the rudiment formed by both tissue layers.

Statistical comparisons were based on the jar means. Each jar provided one datum per date. Thus the mean for a measurement on a sample of plutei removed from a jar on a given day was a datum, and statistics were on day-jar means, as in Bertram and Strathmann (1998), rather than with individual plutei nested within jars. Values of n were number of day-jar means. With each jar representing one family in a given treatment, tests of differences between families were not possible. Instead, variation among families was included in the error term: variation among jars within treatments. A t-test at a particular age tested for differences in initial size. Tests for differences in growth rates or rates of development in form were conducted in two steps. First we estimated the slope for the relationship between the measured dimension and time within each jar. Second, we tested for the differences in slopes with a pooled variance t-test.

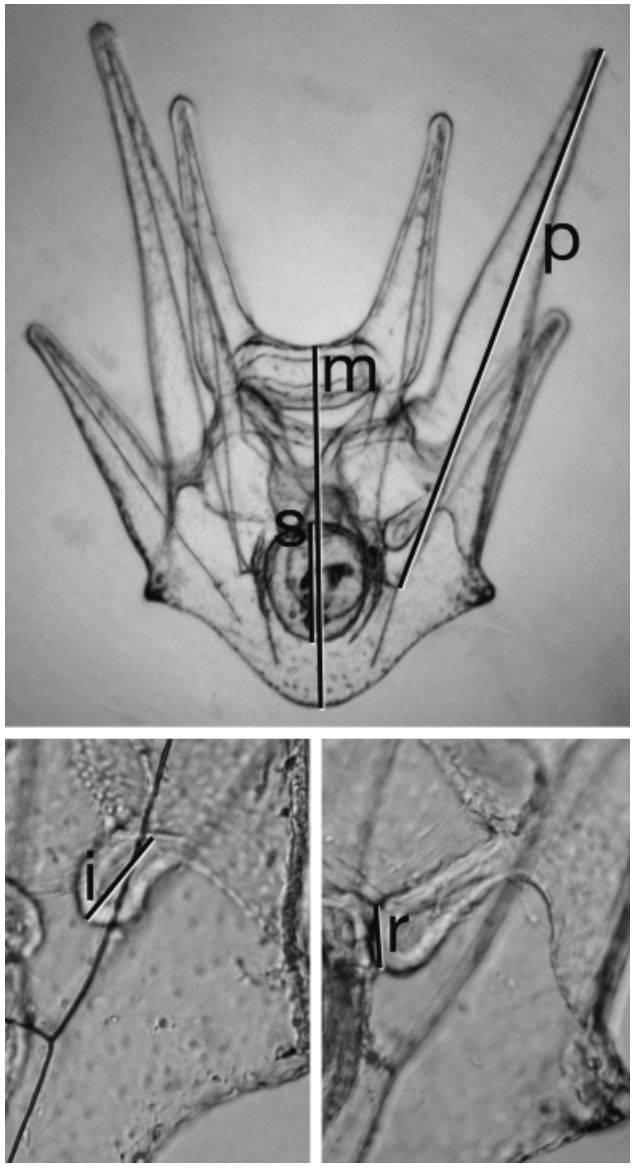

Fig. 1. Measurements of larval structures: *m*, midline body length; *p*, postoral rod length; *s*, stomach length; *i*, depth of invagination at early stage of rudiment formation; *r*, diameter of rudiment (initially the diameter of the contact between the ectodermal invagination and the hydrocoel).

Measures on plutei were not repeated; sampling was without replacement.

Experimental doubling of egg volume

On July 13, 1995, we induced spawning of a single female of *S. purpuratus* by injection with 0.53 M KCl. Eggs were fused with the chemical procedure of Bennett and Mazia (1981a, b), with some modifications. We removed egg jelly by sieving and washing in acidic (pH 5) seawater. Eggs were added to approximately 5 ml of normal seawater (pH about 8) in a cellulose nitrate tube (Beckman

Centrifuge Tube) until they formed a thin layer on the bottom. Most of the seawater was then removed and the eggs incubated in calcium-free seawater containing 0.2% cysteine (cysteine hydrochloride) and 1.2 mg/ml pronase at room temperature (near 20°C). We gently swirled the test tube for 5–10 min. Fusion was induced in hypotonic artificial seawater containing 25 mM Ca. The denuded eggs were suspended in this artificial seawater for 5 min after which the solution was removed. We diluted the eggs 10-fold with a solution of approximately 0.015 mg/ml poly(Arg) (Sigma, St. Louis, MO, USA) of molecular weight 120,000 in the artificial seawater for 70 min, then removed most of the solution and chilled the eggs to ambient seawater temperature. We washed the eggs twice with seawater containing 1.5 mg/ml arginase (Sigma), with 5 min between each rinse, then washed the eggs twice with normal seawater. After 75 min, we transferred the eggs to a chamber constructed from a cellulose nitrate tube and designed to facilitate removal of individual eggs with a Pasteur pipette.

We inspected the products of fusion with a dissecting microscope, selected successfully fused pairs (products of fusion of two eggs) by size, and transferred them to glass culture dishes. Unfused eggs that had been subjected to the same treatment were selected as controls. Diameters of eggs in an unbiased sample from the fused and control treatments were measured to the nearest 2 μm with an ocular micrometer. We fertilized the fused and control eggs 6.5 h after the addition of the solution containing pronase by adding seawater containing egg jelly (from the remaining eggs) and fresh motile sperm from a single male.

At 6 days after fertilization we transferred individual larvae to six-well tissue culture plates with a single larva per well, each in 10 ml of 0.45 μm filtered seawater. Each culture plate had three control and three experimental larvae. The larvae were in a chamber with a light cycle of 16-h light and 8-h dark and temperature of 11.9 ± 0.25°C (mean ± SD), range 11.5–13.0°C. Larvae were fed 10,000 cells of *Rhodomonas* sp./ml and transferred into new filtered water and new food every 3 days. Lack of stirring and less frequent water changes prompted the choice of a higher algal concentration.

Effects of experimental enlargement of eggs of *S. purpuratus* were analyzed with larvae with the normal number of parts. Not all of the embryos produced by fusion of eggs regulate properly to form normal larvae. Larvae with extra parts were eliminated from the analysis, though of interest because they demonstrated that larval body plans unknown in nature were viable. As examples, neither a bifurcating gut nor extra arms prevented development to advanced larval stages with juvenile rudiments (Fig. 2).

We videotaped individual larvae under the 4 × and 10 × objective lenses of a compound microscope to obtain images for measurements for the ontogentic series. During measurements, each larva was protected from crushing by plasticene feet (modeling clay) that supported the cover glass, and the glass slide was chilled in a cooling chamber, which circulated ambient seawater. Following videorecording, the larvae were returned to their individual rearing containers with newly filtered water and new food. Video images were measured as in the interspecies comparison. Because plutei were measured and then replaced for further development, we used repeated measure analyses for ontogenetic series.

For later rearing to metamorphosis, some larvae were continued in the incubator and others were in containers immersed in a seatable aquarium for temperature control. For measurements of test diameter at metamorphosis we induced metamorphosis of larvae with tube feet protruding from the juvenile rudiment. Metamorphosis was induced by placing the individual larvae in containers that had been soaked for 2–7 days in the seawater aquaria to produce a biofilm. Induction was continued over a range of larval ages until 28 metamorphosed juveniles had been obtained. The experiment was terminated 71 days after fertilization.

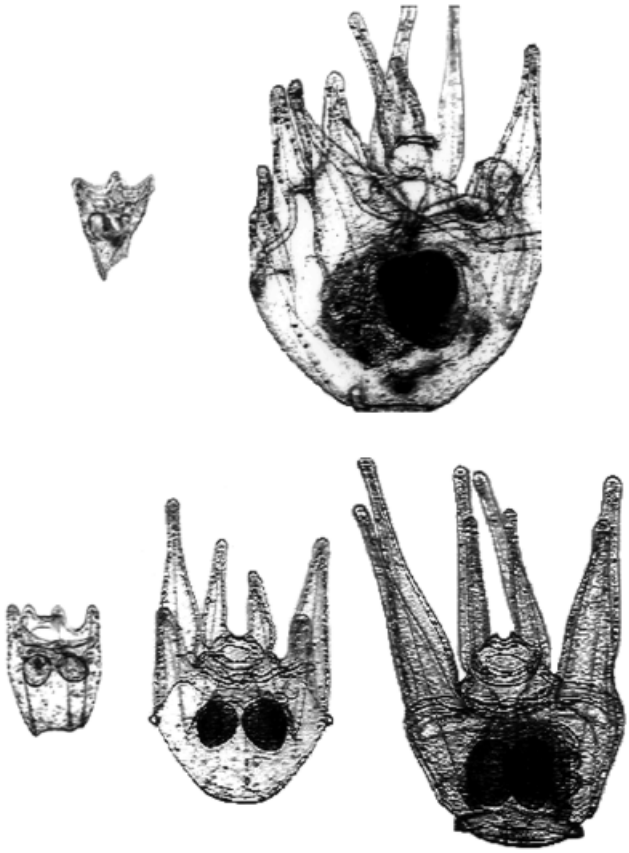

Fig. 2. Ontogenetic series of two abnormal larvae of *Strongylocentrotus purpuratus* from fused eggs, one with extra arms (top), the other with a bifurcating gut (bottom), both developing to late stages with an echinus rudiment.

RESULTS

Interspecific comparisons of growth rates and developmental timing

At high food levels, the ephemeral larval body of *S. droebachiensis* had greater initial size than that of *S. purpuratus*. With measurements for the two species as mean ± SD and n as the number of day-jar means, at 4 days after fertilization the midline body lengths were 357 ± 13.8 and 225.3 ± 0.3 μm ($t = 13.6$, df = 2, $P = 0.005$) (Fig. 3). In addition, the growth rates were more rapid for larvae of *S. droebachiensis* than for

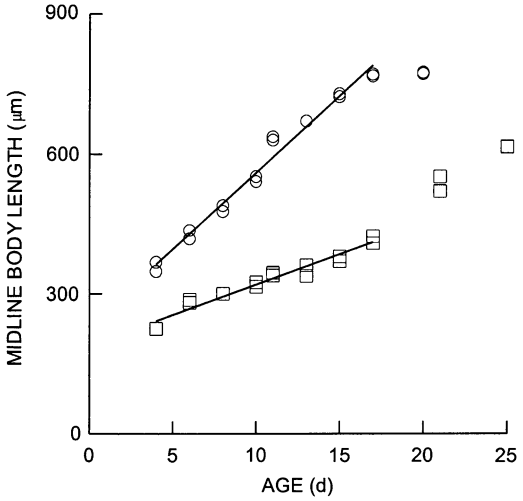

Fig. 3. Increase in midline body length of larvae of *Strongylocentrotus droebachiensis* (circles) and *Strongylocentrotus purpuratus* (squares), reared on the high food ration. Larval size increases more rapidly in *S. droebachiensis*, which has the larger egg.

S. purpuratus, with more rapid increases in midline body lengths and stomach lengths. The differences in slopes for size versus time were indicated by a *t*-test of the slopes from each jar ($n = 2$) for each species. For midline body lengths of larvae <20 days old, $t = 30.2$, $P = 0.001$, df = 2 (Fig. 3). For stomach lengths, $t = -41$, $P = 0.001$, df = 2.

The results were similar in the low food treatments, with larger initial larval body and faster growth for *S. droebachiensis* than for than *S. purpuratus*. Within species, at 4 days after fertilization, there was not yet a detectable effect of differences in concentration of food. Between species, at low food at age of 4 days, midline body lengths were 363.9 ± 26.9 for *S. droebachiensis* and 242.6 ± 1.9 μm for *S. purpuratus* ($t = 31.1$, df = 2, $P = 0.001$). At low food, we could not detect growth differences for *S. droebachiensis* and *S. purpuratus*. For larvae <20 days old, we could not detect differences in midline body length growth rates ($t = 2.7$, $P = 0.11$, df = 2), nor could we detect significant differences between growth rates of stomach lengths of *S. droebachiensis* and *S. purpuratus* at 6–19 days age, ($t = -1.1$, $P = 0.4$, df = 2).

At the high food ration, the development of the invagination for the juvenile rudiment was earlier for *S. droebachiensis* (first appearance at 6 days) than for *S. purpuratus* (first appearance at 13 days, Fig. 4A). The invagination also extended more rapidly for *S. droebachiensis* than for *S. purpuratus* (*t*-test on slopes, $t = 48.4$, $P = 0.0001$, df = 2). The echinus rudiment (as contact between ectodermal invagination and hydrocoel) also appeared earlier for *S. droebachiensis* (first appearance at 8 days) than for *S. purpuratus* (first appearance at 17 days, Fig. 4B). We could not detect interspecific differences in the rates of rudiment growth once rudiments appeared ($t = 3.7$, $P = 0.06$, df = 2).

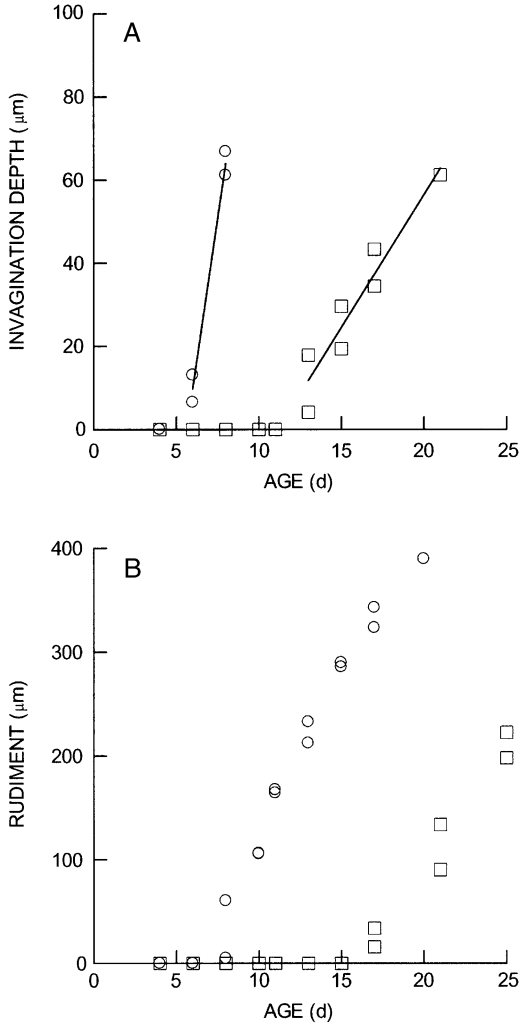

Fig. 4. Time of appearance and rate of growth of (A) invagination depth and (B) rudiment diameter for larvae of *Strongylocentrotus droebachiensis* (circles) and *Strongylocentrotus purpuratus* (squares), reared on the high food ration. Invagination and rudiment appear earlier and invagination increases more rapidly in *S. droebachiensis*, which has the larger egg.

The results were similar for larvae reared on low food rations. At low food, the invagination appeared earlier for *S. droebachiensis* (8 days) than for *S. purpuratus* (15 days). We could not detect differences in the rate of invagination extension between the species ($t = 1.9$, $P = 0.19$, df = 2). The echinus rudiment also appeared earlier for *S. droebachiensis* (10 days) than for *S. purpuratus* (21 days) and also without detectable interspecific differences in growth rates of rudiments once rudiments had appeared ($t = 0.02$, $P = 0.99$, df = 2).

Interspecific comparison of development of the rudiment relative to the larval body

We examined the influence of interspecific differences in maternal investment per offspring (indicated by egg size) on

the size or stage of the larval body (indicated by length of postoral rods) when juvenile structures (ectodermal invagination) are initiated. At both high and low food levels, the larvae of *S. droebachiensis* had longer postoral arm rods for a given stage of invagination than did those of *S. purpuratus* (Fig. 5, A and B). Larvae of *S. droebachiensis* did begin the invagination at an earlier age and, at high food, with subsequent faster growth of the juvenile rudiment (Fig. 4A), but although the larvae of *S. purpuratus* were older when the invagination began, their postoral rods were shorter than those of *S. droebachiensis* when the invagination of the juvenile rudiment began. At high food, the invagination first appeared when the postoral rods were approximately 400 μm in *S. droebachiensis* versus 300 μm in *S. purpuratus* (Fig. 5A). Similarly, at low food, the invagination first appeared when the postoral rods were approximately 450 μm in *S. droebachiensis* versus 350 μm in *S. purpuratus* (Fig. 5B). Because an immediate effect of greater maternal investment (i.e., a larger egg) was to produce a larger larval body, juvenile structures were accelerated relative to age but not relative to larval structures.

Although the postoral arm length of *S. droebachiensis* was longer than in *S. purpuratus* when the invagination for the rudiment began, the larval stages and body forms converged as the larvae grew larger. The convergence occurred on both the high food ration (Fig. 5A) and at the low food ration (Fig. 5B). The slopes for invagination depth versus postoral rod length converged at high food (*t*-test for difference of slopes, $t = 10.1$, $P = 0.01$, $df = 2$) but not significantly at low food ($t = 1.3$, $P = 0.3$, $df = 2$).

Interspecific comparisons of developmental plasticity in response to concentration of food

Larvae of both species responded to differences in the concentration of food with developmental plasticity that was similar in both pattern and magnitude of response (Fig. 6, A–D). The invagination developed earlier in relation to growth of larval arms for *S. droebachiensis* reared at high food than at low food. For larvae with length of postoral arm rods between 380 and 410 μm, the mean depth of invagination for larvae at high food concentration was 9.7 μm ($n = 2$ day-jar means), whereas larvae with this arm length at low food had not yet developed an invagination ($n = 1$ day-jar means) (Fig. 6A). The forms of the larvae, as measured by postoral rod length and invagination depth, diverged at the two concentrations of food as the larvae grew ($t = 9.1$, $P = 0.012$, $df = 2$). Similarly, the rudiment (contact between invagination and hydrocoel) developed at an earlier stage for *S. droebachiensis* reared at high food than at low food concentration. For larvae with lengths of postoral arm rods < 600 μm, mean diameter of the rudiment at high food concentration was 10.9 μm ($n = 6$ day-jar means), whereas the rudiment had not yet formed for larvae that had grown to this arm length at low food ($n = 7$ day-jar means) (Fig. 6B).

For *S. purpuratus*, the invagination also developed earlier for larvae reared at high food. For larvae with lengths of postoral arm rods between 260 and 350 μm, the mean invagination depth at high food concentrations was 13.7 μm ($n = 3$ day-jar means), whereas the larvae that had grown to this arm length at the low food concentration had not yet developed the invagination ($n = 3$ day-jar means) (Fig. 6C). The forms of the larvae, as measured by postoral rod length and invagination depth, appeared to diverge at the two concentrations of food as the larvae grew but the difference was marginally nonsignificant ($t = 3.8$, $P = 0.06$, $df = 2$). Similarly, the rudiment developed earlier for *S. purpuratus* reared at high food than at low food concentration. For larvae with lengths of postoral arm rods between 390 and 450 μm, the mean diameter of the rudiment at high food concentration was 24.7 μm ($n = 2$ day-jar means), whereas the rudiment had not yet formed for larvae that had grown to this arm length at low food ($n = 2$ day-jar means) (Fig. 6D).

For both species, the development of postlarval structures is similarly decoupled from development of ephemeral larval structures. Timing of the invagination and the rudiment shifts in response to food at early stages of larval development. The similar response of both species to differences in food concentration also demonstrated that the differences in egg size had little if any effect on sensitivity to concentration of food (Fig. 6, A and C; Fig. 6, B and D).

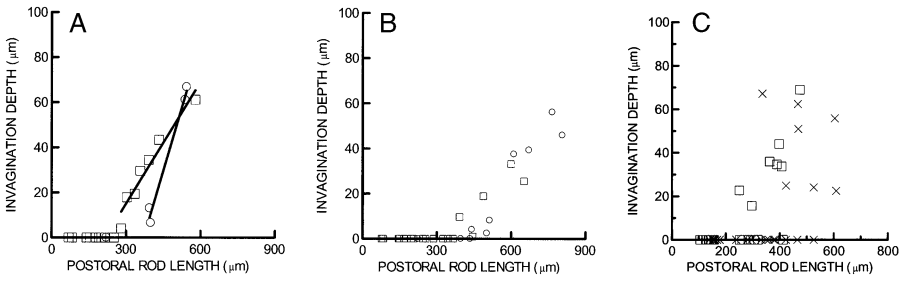

Fig. 5. The invagination begins at greater postoral rod length for *Strongylocentrotus droebachiensis* (circles) than for *Strongylocentrotus purpuratus* (squares) at both high (A, large symbols) and low (B, small symbols) food rations. The species converge in relative development of larval body and rudiment as they develop. (C) Similar plot for *S. purpuratus* from single egg (squares) and egg doubled in volume by fusion (×).

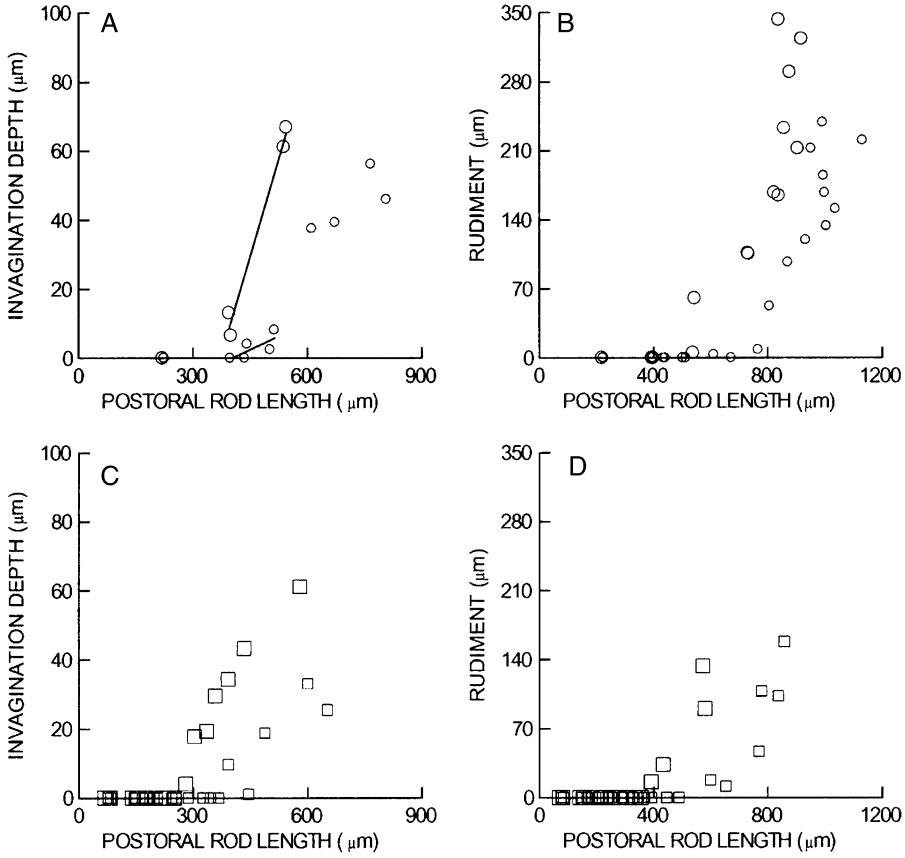

Fig. 6. Larval developmental plasticity in response to high food ration (large symbols) and low food (small symbols) for *Strongylocentrotus droebachiensis* (circles) and *Strongylocentrotus purpuratus* (squares). For comparison of the magnitude and pattern of the responses to food level, the plots have the same scales for both species.

Effect on juvenile rudiment of experimentally doubling volume of *S. purpuratus* eggs

Control eggs of *S. purpuratus* ranged from 78.3 to 85.9 μm with a mean ± SD of 81.5 ± 1.9 μm ($n = 30$). Fused eggs ranged from 101 to 106.1 μm with a mean ± SD of 102.3 ± 1.9 μm ($n = 20$).

Doubling the egg volume of *S. purpuratus* did not accelerate development of the juvenile rudiment. The timing of the invagination was similar but slightly earlier for the control larvae ($\leq$ 13 days) in relation to the experimentally enlarged larvae ($\leq$ 18 d) (Fig. 7C). Thus doubling egg volume did not mimic the evolutionary divergence in timing of the invagination.

However, in the larvae from doubled egg volume, the postoral arms were larger than those of controls at initiation of the ectodermal invagination (Fig. 5C). In this respect the timing of the rudiment relative to the larval body with experimentally enlarged eggs of *S. purpuratus* was like that of the species with larger eggs.

Effect on body size of experimentally doubling volumes of *S. purpuratus* eggs

Experimental fusion of eggs of *S. purpuratus* produced development of larval bodies more similar in size to larvae of *S. droebachiensis*, a species with larger eggs (Fig. 7). The fused eggs produced larvae that were significantly larger than those from single ova controls at first measurement (5–6 days after fertilization) (Fig. 7). At 6 days, the midline body length for the control larvae was 292.3 ± 16.1 μm ($n = 8$) and for experimentally enlarged larvae was 351.8 ± 26.1 μm ($n = 9$), significantly different ($t = -5.5$, $P = 0.00006$, df = 15); the stomach length of the control was 83.3 ± 12.4 ($n = 8$) and of the fused was 98.9 ± 11.9 ($n = 9$), significantly different ($t = -3.3$, $P = 0.005$, df = 15).

The initial size advantage of the fused larvae appeared to diminish as development progressed, as indicated by simple univariate comparisons of size at age (Fig. 7). Statistical differences persisted until at least day 13 for stomach length and at least day 18 for midline body length. Analysis of variance of repeated measures of midline body length (at days 6, 13, 18, 22, 29, and 39) indicated a significant treatment effect on the midline body length ($n = 18$, $F = 11.2$, $P = 0.004$). That analysis, however, could not detect differences in the growth rates of control and experimentally enlarged larvae. The treatment by time interaction was not significant (Wilks' $\lambda = 0.85$, $F = 0.42$, df = 5,12, $P = 0.83$).

Larvae from single and fused eggs developed through metamorphosis. Mean age at metamorphosis was nearly the

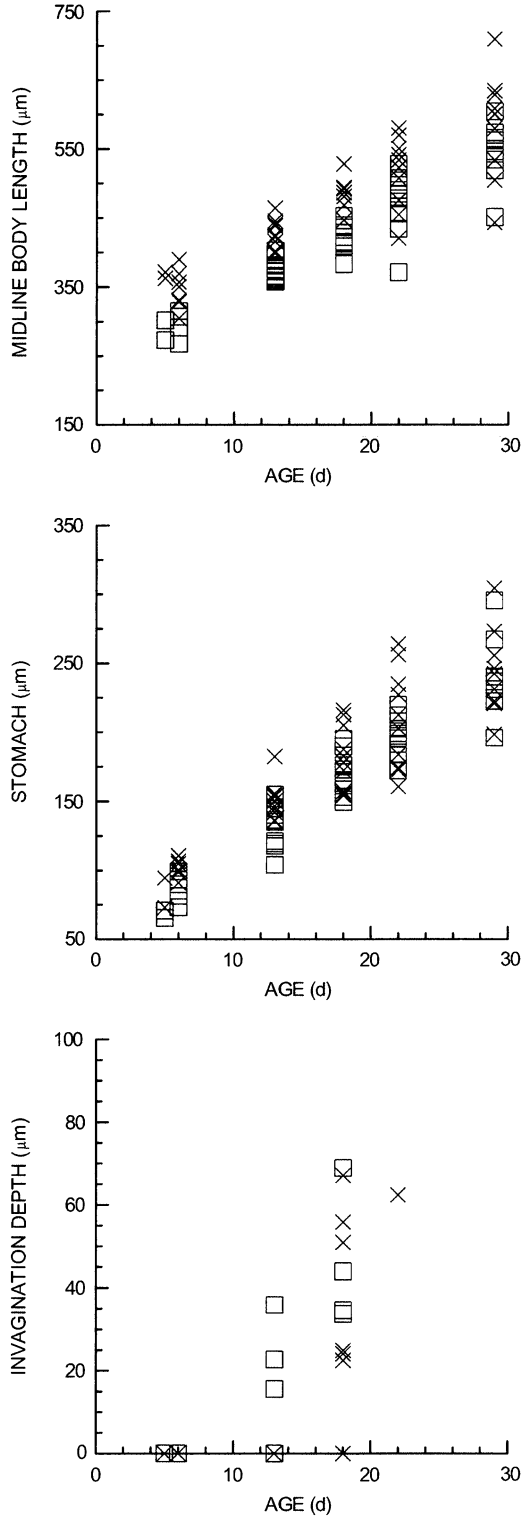

Fig. 7. Size at age for *Strongylocentrotus purpuratus* larvae from single eggs (squares) and those from eggs experimentally enlarged by fusion of two eggs (×). Dimensions and growth shown by midline body length, stomach, and invagination depth plotted against age.

Table 1. Mean test diameter (μm) and age (days) at metamorphosis for larvae from single eggs and eggs experimentally enlarged by fusion of two eggs

	Incubator		Seatable	
	Test	Age	Test	Age
Single	391 ± 50 (8)	53 ± 9 (8)	429 ± 40 (12)	51 ± 7 (12)
Double	412 ± 23 (4)	51 ± 5 (4)	445 ± 60 (4)	53 ± 5 (4)

Rearing was in an incubator and on a seatable aquarium. Measures are mean ± SD (*n*).

same for larvae from eggs of single and doubled volumes (Table 1). Mean test diameter at metamorphosis was greater for the juveniles from doubled eggs (Table 1), but a difference in size at metamorphosis could not be proven, either for those reared in the incubator ($P > 0.3$) or those on the seatable ($P > 0.5$), or for both groups combined ($P > 0.4$) (Table 1). Although we could not demonstrate an effect of egg doubling on size or age at metamorphosis, there was a marginally significant positive relationship between size and age at metamorphosis for the pooled data set ($y = 243 + 3.7x$; $F_{1,14} = 4.9$, $P = 0.04$). We could not detect differences in the test diameters of five surviving juveniles from enlarged eggs and three from single-egg controls at 30 days after metamorphosis.

Effect on larval form of experimentally doubling volumes of eggs of *S. purpuratus*

It is striking that the form of the larvae from fused eggs was more similar to that of *S. droebachiensis* than that of *S. purpuratus* (Fig. 8). The *S. purpuratus* larvae from doubled eggs had relatively longer arms and a greater distance between the tips of the body rods at the posterior end of the larvae, both features of the early stage larvae of *S. droebachiensis*.

These differences did not last. Both postoral arm rods and midline body lengths were shorter in larvae from smaller eggs, but both measures increased along the same trajectory of relative body proportions. This convergence on the same trajectory in larval body proportions occurred both in the interspecies comparison (Fig. 9, A and B) and for larvae from single and experimentally doubled egg volumes (Fig. 9C) even though culture conditions differed in both size of containers and concentration of food.

DISCUSSION

A trait of special interest is the timing of development of the juvenile rudiment, as distinct from the larval body. In the

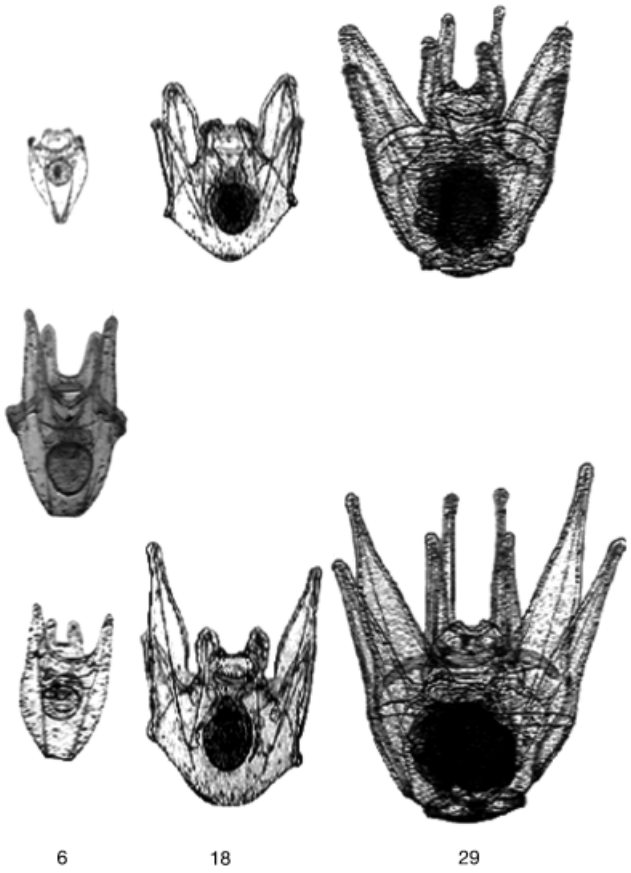

Fig. 8. Larvae of *Strongylocentrotus purpuratus* from single eggs (top row) and two fused eggs (bottom row) at 6, 18, and 29 days of age. Larva of *Strongylocentrotus droebachiensis* from a single egg (middle row) at 6 days of age.

divergence between species, the ephemeral larval body was accelerated relative to rudiment formation in *S. droebachiensis*, the species with the larger egg. The larger ova of *S. droebachiensis* results in larger sizes at age for both the ephemeral larval structures and the rudiments of postlarval structures, but the increase in size of larval arms is accelerated relative to development of the juvenile rudiment (Figs. 3–5). An alternative and perhaps expected outcome would have been reduced larval arms and accelerated juvenile development in larvae from larger eggs, given the trend toward these traits in animals with larger eggs and nonfeeding larvae (Strathmann et al. 1992), but this was not the case. Because development of the juvenile rudiment is accelerated relative to larval arms in *S. purpuratus*, in comparison to *S. droebachiensis*, material was allocated to rudiments of postlarval structures before the capacity for clearing food from suspension was as great as in *S. droebachiensis*. This difference between species was apparent at both high and low concentrations of food.

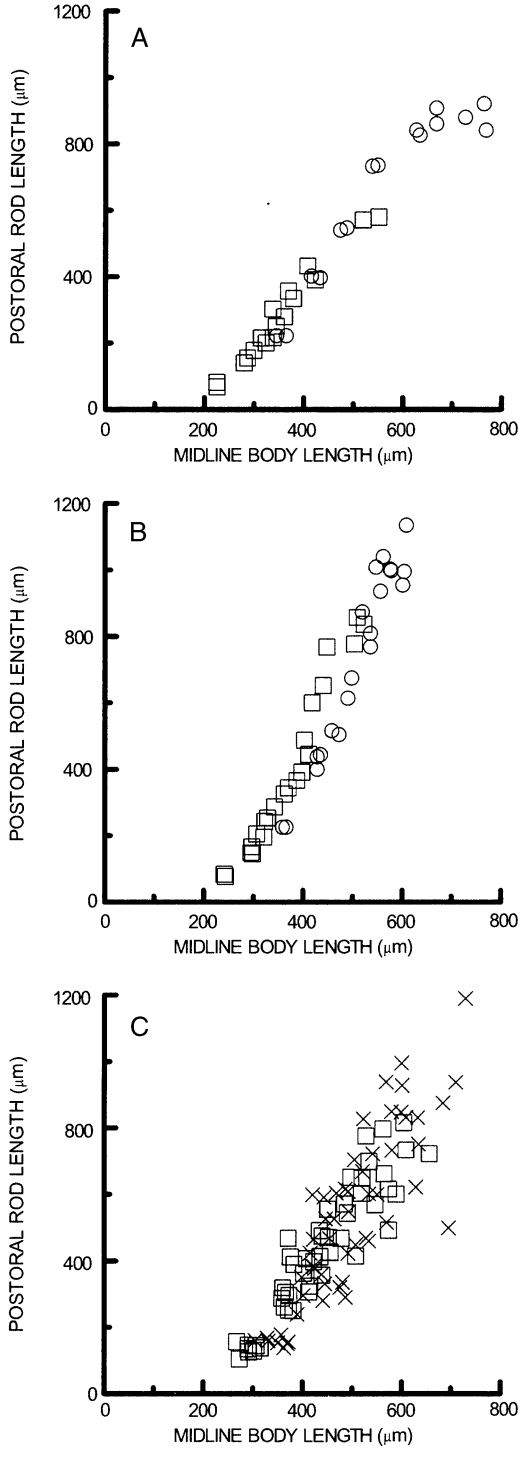

Fig. 9. Similar developmental change in larval body form, as measured by midline body length and postoral rod length, for *Strongylocentrotus droebachiensis* (circles) and *Strongylocentrotus purpuratus* (squares) at (A) high food and (B) low food and (C) for *S. purpuratus* from single eggs (squares) and fused eggs of double the volume (×). The larvae from small eggs come to resemble the larvae from large eggs.

Larvae of *S. purpuratus* (egg diameter 78–82 μm) and *S. droebachiensis* (egg diameter 150–160 μm) exhibited similar plasticity in response to food. Both developed longer arms relative to size of rudiment at a low concentration of food. An alternative outcome, could have been less response in the species with the larger egg than in the species with the smaller egg, but the larger egg of *S. droebachiensis* did not decrease its sensitivity to food supply. Similarly, larvae of *S. franciscanus* (with an egg of 123–125 μm diameter) and of *S. purpuratus* both exhibited plasticity in response to food (Miner 2005; McAlister 2007). However, McAlister (2007) found less plasticity (in relative increase in arm length at low concentrations of food) in larvae of *S. franciscanus* when egg volume was experimentally reduced and also in larvae of *S. purpuratus*, from naturally smaller eggs. In McAlister's study, the lesser response of *S. purpuratus* was not entirely attributable to its lesser egg volume, indicating additional differences in plasticity between these species. Different foods for larvae and different measures of plasticity may have contributed to the seemingly different results in McAlister's study and our study. It remains to be seen whether lack of plasticity of arms relative to midline body length or a stomach dimension (McAlister 2008; Soars et al. 2009) extends to plasticity in relation to rudiment formation.

Species of clypeasteroid echinoids have provided ideal material for studies of the effect of egg size on development of feeding plutei. They develop through a similar pluteus stage but with a wide range of egg sizes and corresponding differences in dependence on particulate food for development through metamorphosis. An extreme, in *Clypeaster rosaceus* with a large egg, is development to metamorphosis with no requirement for particulate food (Emlet 1986; Herrera et al. 1996; Allen et al. 2006; Moran and Allen 2007; Zigler et al. 2008) and accelerated development of the left coelom (Snoke Smith et al. 2007). Comparative studies of clypeasteroids include the effect of egg size on plasticity in response to food. Among clypeasteroid echinoids, *Clypeaster subdepressus* (egg diameter 150 μm) and *Mellita tenuis* (egg diameter 99 μm) exhibited similar plasticity, but *Leodia sexiesperforata* (egg diameter 191 μm) did not change relative sizes of postoral arms and stomach in response to the concentration of food (Reitzel and Heyland 2007). The result for *L. sexiesperforata* is expected because the larvae are almost nonfeeding, requiring little food to develop to metamorphosis and metamorphosing without exogenous food when treated with thyroxine (Reitzel and Heyland 2007).

Our results with *S. purpuratus* and *S. droebachiensis* extend such comparisons by including development of the juvenile rudiment and also extend the range of egg sizes over which similar plasticity has been observed. Similar developmental responses to food of larvae from eggs greatly different in size is consistent with evidence that material supplied to planktotrophic larvae in the egg primarily forms larval structures rather than serving as a nutrient reserve (Moran and Allen 2007).

Experimentally doubling the volume of the egg did not accelerate the development of postlarval structures in absolute time. Size of rudiments at age was not increased by egg fusion. The effect of the experimental increase in egg size did not resemble the evolutionary divergence between species in this respect. The invagination for the rudiment did, however, develop when the larval arms were more advanced in the plutei from eggs experimentally doubled in volume, and in that respect the experimental change in egg volume resembled the evolutionary divergence between species.

The doubling may have been insufficient to obtain a detectable difference in age at rudiment formation (the 7-fold difference in egg volume between the species being greater than the 2-fold difference from egg fusion), or divergence in rudiment development of *S. purpuratus* and *S. droebachiensis* may have included factors other than divergence in the volume of ova. A third possibility is that the method of doubling egg volume by egg fusion delayed rudiment formation. The larger ova of *S. droebachiensis* were associated with both a larger initial larva and an earlier development of postlarval structures than in *S. purpuratus*, whereas an experimental increase in size of ova of *S. purpuratus* resulted in a larger initial larva but no detectable acceleration in development of the rudiment of postlarval structures.

Doubling egg volume of *S. purpuratus* did not change age at metamorphosis but may have increased size at metamorphosis. If the larger mean test diameter at metamorphosis observed for juveniles developing from doubled eggs is representative (despite the small sample size) (Table 1), then the result mirrors that with larvae from full and half eggs of *S. droebachiensis* (Hart 1995), in which larvae from half eggs developed to metamorphosis at the same mean age but with a smaller juvenile size.

When the volume of the small egg of *S. purpuratus* was experimentally doubled, the developing larvae were changed to resemble the larvae of *S. droebachiensis*, a sea urchin with larger eggs. The changes toward larvae of *S. droebachiensis* were in form (Figs. 5 and 8) as well as in increased initial larval size (Figs. 7 and 9). The similarity included changes in body proportions, such as relatively longer arms and greater distance between posterior ends of body rods. These changes in larval form were produced with no change in the *S. purpuratus* genotype. They were solely the result of a change in egg volume. The evolutionary divergence between these species in parental investment per offspring (as indicated by egg volume) produced the change in larval form.

This result mirrors that of Sinervo and McEdward (1988), who reduced egg volume of *S. droebachiensis* to produce a larva similar in size and form to the larva of *S. purpuratus*. In that experiment the change in larval form toward that of *S. purpuratus* occurred with the genotype of *S. droebachiensis*.

The effect of egg volume on larval form (as well as size and growth rate) therefore occurs with either species' genotype. The allometric engineering mimics interspecies differences in both directions. To the extent that experimental changes in egg volume have reproduced the interspecies divergence in form, the evolutionary divergence in larval form would be reversed by an evolutionary change in egg volume. The same conclusion applies to effects of egg volume on larval size and growth rate, but reversible evolution of these traits is less remarkable.

There are, of course, evolutionary divergences that are not reproduced by a change in egg volume. For one, Sinervo and McEdward (1988) noted that although *S. droebachiensis* from eggs of reduced volume resembled larvae of *S. purpuratus*, they had larger skeletons. In our study, experimentally enlarged larvae of *S. purpuratus* did not advance rudiment development as seen in *S. droebachiensis*.

Although a weakness of egg fusion is that regulation of fused embryos in forming plutei varies, making it necessary to discard some abnormal larvae, egg fusion has several advantages. It demonstrates that effects of reduction of egg size by blastomere separation are not artifacts of that method. Also, in comparisons between species, similar effects of enlargement of small eggs and reduction of large eggs demonstrates similar effects of egg volume with either genotype. There are other possibilities. Centrifugation of eggs can produce egg fragments enriched in different materials (Harvey 1956). It may be possible to produce large qualitative differences in egg composition by fusing lipid-enriched fragments of ova with intact ova.

Acknowledgments

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