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FERTILIZATION SELECTION ON EGG AND JELLY-COAT SIZE IN THE SAND DOLLAR *DENDRASTER EXCENTRICUS*

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Abstract.—Organisms with external fertilization are often sperm limited, and in echinoids, larger eggs have a higher probability of fertilization than smaller eggs. This difference is thought to be a result of the more frequent sperm-egg collisions experienced by larger targets. Here we report how two components of egg target size, the egg cell and jelly coat, contributed to fertilization success in a selection experiment. We used a cross-sectional analysis of correlated characters to estimate the selection gradients on egg and jelly-coat size in five replicate male pairs of the sand dollar *Dendraster excentricus*. Results indicated that eggs with larger cells and jelly coats were preferentially fertilized under sperm limitation in the laboratory. The selection gradients were an average of 922% steeper for egg than for jelly-coat size. The standardized selection gradients for egg and jelly-coat size were similar. Our results suggest that fertilization selection can act on both egg-cell and jelly-coat size but that an increase in egg-cell volume is much more likely to increase fertilization success than an equal change in jelly-coat volume. The strengths of the selection gradients were inversely related to the correlation of egg traits across replicate egg clutches. This result suggests the importance of replication in studies of selection of correlated characters.

Key words.—Correlated characters, echinoids, egg size, fertilization, jelly coat, selection gradient.

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The theory of sexual selection holds that, when sperm and males compete, female offspring production should not be limited by sperm availability and females should not be selected for increased mating success (see, e.g., Bateman 1948; Arnold 1994). However, in plants (Burd 1994) and broadcast-spawning marine invertebrates (Levitan 1995) pollen and sperm can be limiting, and females in these taxa may also be under selection for increased fertilization success (Burd 1994; Levitan 1996a, 1998a,b). This notion has been illustrated in sea urchins, where egg traits appear to be under such selection (Levitan 1993, 1996a,b, 1998b). Laboratory and field studies have documented that the percentage of eggs fertilized is correlated with egg size within (Levitan 1996a) and among (Levitan 1993, 1998b) closely related sea-urchin species and that egg traits may be adapted to particular spawning conditions in ways that optimize fertilization success (Levitan 1993, 1996a,b, 1998b; Thomas 1994a,b; Podolsky 1995; Podolsky and Strathmann 1996; Styan 1998).

What has remained unclear is what attribute of the egg or which component of egg size is actually responsible for increases in fertilization efficiency and what role the jelly coat plays in this interaction (Lillie 1915; Tyler 1941; Hagstrom 1956; Hagstrom and Markman 1957; Levitan 1995; Podolsky 1995; Podolsky and Strathmann 1996; Farley and Levitan 2001). The two factors that determine the fertilization rate of eggs are the rate at which sperm and eggs collide (collision rate) and the rate at which these collisions result in fertilization (fertility rate). The concentration of gametes, the movement of sperm, and the size of the egg target determine the collision rate (Vogel et al. 1982). The fertility rate is a more complicated function of the interaction of sperm and eggs and is a function of how likely a spermatozoon is to penetrate extracellular layers and fuse with the egg cell surface. The jelly coat may play a role in both rates, because it increases the overall target size of the egg and provides a barrier that must be penetrated by the sperm before fertilization is possible.

The sand dollar *Dendraster excentricus* (an irregular echinoid) has a thick jelly coat that increases the egg target diameter approximately twofold and the cross-sectional area fourfold (Podolsky 1995). We conducted a selection experiment by exposing eggs to conditions of sperm limitation and examining how egg-cell and jelly-coat size contribute to the likelihood of fertilization. The results suggest that eggs with large cell size and thick jelly coats are preferentially fertilized under these conditions but that fertilization selection acts to increase the ratio of egg-cell to jelly-coat material.

MATERIALS AND METHODS

We collected *D. excentricus* from Brady's beach, located in Barkley Sound, British Columbia, Canada (48°50′N, 125°09′W), on 8 July 1998. Laboratory experiments were conducted in July 1998 at the Bamfield Marine Station, Bamfield, British Columbia.

We injected sand dollars with 0.55 M KCl to induce spawning. The eggs were collected in ambient-temperature (12°C) filtered seawater. The sperm was kept in its dry, concentrated form and placed over ice.

The egg concentration of the stock suspension was adjusted to range from 3000 to 5000 eggs per milliliter. Each of seven scintillation vials was filled with 8.0 mL filtered seawater and 1.0 mL of the egg suspension. One of the seven was used as the control vial and was not fertilized.

A separate series of scintillation vials was used for serially diluting sperm. One scintillation vial was filled with 9.9 mL of filtered seawater and 0.1 mL of the collected sperm. The sperm suspension was then run through a 10-fold serial dilution into five additional vials, each containing 9.0 mL filtered seawater. To each egg vial, 1.0 mL of one of the sperm suspension was added, such that the six egg vials contained serially diluted sperm.

Two hours after the addition of sperm, we determined which of the vials produced the fertilization rate closest to

50%. The vial selected always ranged between 45% and 55% of eggs fertilized. Unfertilized eggs in that vial (50% fertilized) and the control vial (0% fertilized) were measured. Fertilized eggs were not measured because they change shape and size through development (Levitan 1996a).

We mixed eggs from both treatments with Sumi ink to visualize the jelly coat (Schroeder 1980). Egg-cell and egg-cell-plus-jelly-coat diameters were measured in 250 eggs per treatment and replicate at 100× under a compound microscope with an ocular micrometer. The egg-cell volume and total volume were calculated from these diameter measurements according to the formula for the volume of a sphere. Jelly-coat volume was calculated by subtraction of the egg-cell volume from the total volume. To ensure that eggs from the control vials and experimental vials were measured as nearly simultaneously as possible, samples of 25–50 eggs from the two sets were measured alternately until all 500 eggs were measured. Each replicate used a different male and female; five independent replicates of the experiment were conducted.

Analysis of Selection on Correlated Characters

We analyzed data by first conducting paired *t*-tests of treatment and control egg-cell and jelly-coat sizes of unfertilized eggs. We then calculated the selection differentials and gradients (sensu Lande and Arnold 1983) for these correlated characters. Because we could not follow the fates of individual eggs and thus determine the original sizes of the eggs after fertilization (Levitan 1996a), we compared the two treatments in a cross-sectional analysis of selection on correlated characters (Lande and Arnold 1983). The cross-sectional method is used when two populations are examined, one before and the other after a round of selection, and is useful when paired measurements cannot be made on each individual (in this case eggs). The method can be used if the following assumptions can be made: (1) no genetic evolution of the characters has taken place between the two populations; (2) the relevant environmental conditions are similar; (3) no differential immigration or emigration by different phenotypes has taken place; and (4) there have been no ontogenetic changes in the characters examined (Lande and Arnold 1983). Because this is a laboratory study of randomly assorted eggs from the same clutch (in each replicate), and because only unfertilized eggs are examined at the same time after spawning, this study meets all the necessary criteria. The statistical confidence of this analysis comes from replicating pairs of experimental and control egg clutches.

We calculated the mean volumes of egg and jelly coat in the control treatment ($\bar{x}_c$) and in the unfertilized eggs from the sperm treatment ($\bar{x}_{uf}$). We then estimated the mean size of the traits in the fertilized eggs in the sperm treatment ($\bar{x}_f$). Because half the eggs were fertilized in the sperm treatments, the mean trait sizes in the sperm treatment before sperm were added ($\bar{x}_b$) was calculated as

$$\bar{x}_b = (\bar{x}_f + \bar{x}_{uf})/2,$$

where $\bar{x}_f$ and $\bar{x}_{uf}$ are the mean trait sizes in the eggs that are fertilized and unfertilized. Because all eggs are random samples from the same female, $\bar{x}_b = \bar{x}_c$, and this equation can be solved for $\bar{x}_f$.

The selection differentials (s) for egg and jelly coat volume can be calculated as the change in mean size after selection ($\bar{x}_f - \bar{x}_b$). The selection gradient (β) is calculated as

$$\beta = P^{-1}S$$

where P is the variance-covariance matrix of the characters before selection and S is the vector of the selection differentials (Lande and Arnold 1983).

Each replicate was analyzed separately, and paired *t*-tests were conducted to determine whether the selection gradients were different from zero and different from one another. The selection gradients were also examined as a function of the degree of correlation between the traits in each replicate egg clutch.

In our analysis, we choose to use volume as our variable rather than diameter, cross-sectional area, or a conversion to caloric content. The rationale was that volume is a scale-independent measure of size, whereas a change in diameter or cross-sectional area in a small egg is hard to compare in the same unit change in a large egg. A conversion to caloric value was not used because volume and caloric value are poorly correlated within echinoderm species (McEdward and Morgan 2001), rendering an analysis of correlated characters invalid. Considering caloric investment would be appropriate in an analysis that included both fertilization and development because energy content may influence postzygotic growth and survival. Such an analysis of egg and jelly, using an optimality approach, has been done (Farley and Levitan 2001), but for an investigation of fertilization selection, it is target size rather than energy content that influences sperm-egg collisions.

Results

Mean egg-cell volume ranged from 0.00112 to 0.00135 mm^3 and differed significantly across the five females (ANOVA $P < 0.0001$). Mean jelly-coat volume ranged from 0.0131 to 0.0223 mm^3 and also differed significantly across the five females (ANOVA $P < 0.0001$).

Egg-cell and jelly-coat volumes of the unfertilized eggs from the sperm treatment were smaller than those from the control treatment (Fig. 1). These differences were significant and apparent in all five replicates (Table 1). Eggs of all sizes were capable of fertilization, and at the highest sperm concentration, 95–100% of eggs were fertilized. Only under sperm limitation were larger eggs preferentially fertilized and therefore removed from the pool of eggs that were measured.

The Analysis of Selection on Correlated Characters

The within-female correlations between egg and jelly-coat volumes ranged between 0.19 and 0.50 (Table 2). The among-female correlation between mean egg size and mean jelly-coat volume was 0.41 for the five females. Larger eggs were weakly associated with larger jelly coats.

The mean selection differentials were 0.000161 (SE = 0.0000132) for egg cells and 0.00519 (SE = 0.002193) for jelly coats. A paired *t*-test indicated a marginally nonsignificant difference between these selection differentials ($P = 0.08$).

The selection gradient is a measure of the proportional in-

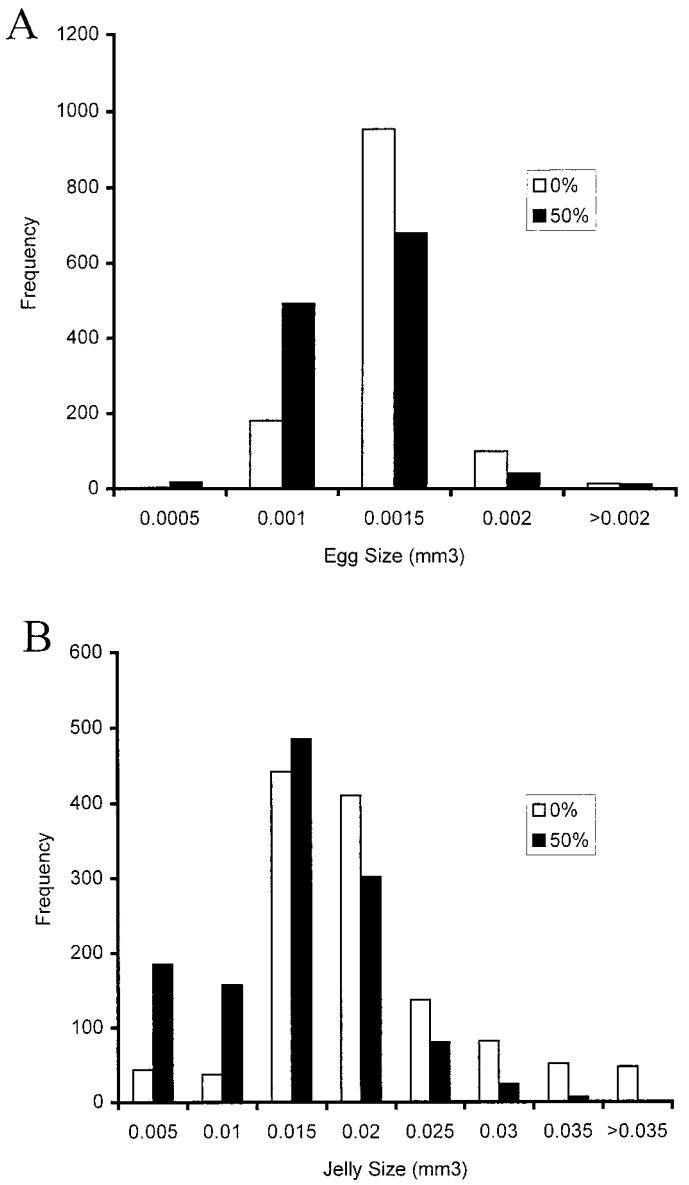

FIG. 1. Frequency distribution of unfertilized eggs in control (0% fertilized) and treatment (50% fertilized) populations. Each histogram is of the sum of the five replicate male-female pairs. (A) Distribution of egg-cell sizes. (B) Distribution of jelly-coat sizes.

TABLE 2. Variance-covariance matrix of egg and jelly coat size in the control treatments for the five females. Diagonal elements are the variances in each trait, elements below the diagonal are covariances.

Female		Egg	Jelly
1	Egg	1.21×10^{-7}	
	Jelly	1.18×10^{-6}	4.60×10^{-5}
2	Egg	8.05×10^{-8}	
	Jelly	1.34×10^{-6}	1.74×10^{-4}
3	Egg	3.39×10^{-8}	
	Jelly	9.22×10^{-8}	5.85×10^{-6}
4	Egg	4.69×10^{-8}	
	Jelly	1.94×10^{-7}	7.26×10^{-6}
5	Egg	3.03×10^{-8}	
	Jelly	7.06×10^{-8}	4.35×10^{-6}

crease in the measure of fitness (fertilization success) with an increase in one unit (1 μL) of volume. The selection gradients varied as a function of the degree of correlation between egg-cell and jelly-coat sizes across egg clutches; as the correlation increased, the absolute value of the selection gradient decreased (Fig. 2A). However, the percent difference in the selection gradients did not vary consistently with the degree of correlation (Table 3). The selection gradients for egg-cell size were between 390% and 1774% (average 922%) greater than the selection gradient for jelly-coat size. Paired *t*-tests indicate that the selection gradients on egg size were significantly greater than the gradients on jelly-coat size ($P < 0.05$). The selection gradients for egg-cell size were significantly different from zero ($P < 0.05$). The jelly-coat selection gradients were not significantly different from zero ($P = 0.10$).

We calculated standardized selection gradients by multiplying the selection gradient by the before-selection (control) standard deviation. The result provides an estimate of the proportion increase in fitness with one standard deviation difference in the trait (Fig. 2B). Because the variances in the selection gradients were much larger for jelly volumes (average 4.76×10^{-5}) than for egg volumes (6.26×10^{-8}), the standardized selection gradients were more similar and not significantly different from each other ($P = 0.11$). Both egg ($P < 0.01$) and jelly-coat ($P < 0.05$) standardized selection gradients were significantly different from zero.

DISCUSSION

Larger eggs and eggs with thicker jelly coats were preferentially fertilized under sperm limitation. The selection gradients indicate that, for a given change in volume, increases in egg-cell size have the most influence on fertilization suc-

TABLE 1. Mean sizes of egg traits in control (0% fertilized) and of fertilized egg traits in experimental (50% fertilized) treatments and the selection differentials (S) in each replicate. Asterisks indicate significant differences in students *t*-test between experimental and control populations in each trait.

	Egg volume (mm^3)			Jelly volume (mm^3)		
	Control	Treatment	*S*	Control	Treatment	*S*
1	0.001085	0.001296	0.000211***	0.017303	0.022331	0.005028***
2	0.001093	0.001240	0.000147***	0.007047	0.020685	0.013638***
3	0.000982	0.001119	0.000137***	0.010774	0.013094	0.002320***
4	0.001201	0.001348	0.000147***	0.015532	0.017025	0.001493***
5	0.000953	0.001116	0.000163***	0.011073	0.014568	0.003495***

*** $P < 0.0001$.

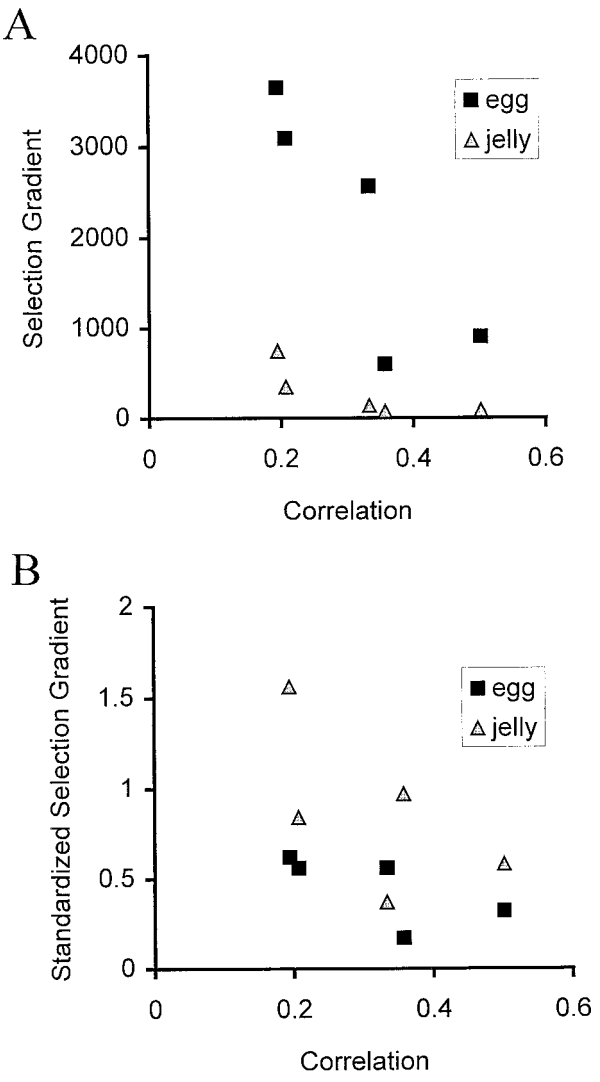

FIG. 2. Selection gradients for egg and jelly volume as a function of the correlation of the traits in the different male-female pairs. (A) Selection gradients. (B) Standardized selection gradients.

cess. The selection gradients were positive for both traits, but the gradient for egg-cell size was an average of 922% greater than that for jelly-coat size. An increase in the total target size of the egg may therefore be advantageous for fertilization success whether it results from increases in egg or jelly-coat size. The greater value of the egg-cell size-selection gradient suggests that, for any given total target size, fertilization success would increase as the ratio of egg-cell to jelly-coat volume increased. This result was also evident from an unreported analysis of selection coefficients of jelly-coat volume compared to those of total volume, which resulted in a significant positive selective gradient for total size (mean = 9703) and a significant negative selective gradient for jelly-coat size (mean = −9625) in these two highly correlated traits. An interpretation of these results is that, although jelly coats may increase the rate of collisions with sperm, penetrating and fertilizing eggs might be more difficult as the jelly coat thickens.

TABLE 3. Selection gradients, standardized selection gradients, and the correlation of egg and jelly volume.

	Gradient		Standardized gradient		
Pair	Egg	Jelly	Egg	Jelly	Correlation
1	904.0	86.1	0.32	0.58	0.5015
2	598.0	73.8	0.17	0.97	0.3572
3	3095.4	347.8	0.56	0.84	0.2072
4	2567.5	137.0	0.56	0.37	0.3332
5	3645.3	744.3	0.62	1.56	0.1946

In the majority of replicates, the jelly-coat-size-standardized selection gradient was larger than the egg-size-standardized selection gradient, but this trend was not significant. Overall, the standardized selection gradients were much more similar than were the selection gradients and suggest that eggs and jelly coats should respond at near equal rates to fertilization selection, given the levels of variation noted in these females.

Similar selection experiments have been conducted in the sea urchins *Strongylocentrotus franciscanus* and *S. purpuratus*, but only egg-cell size was examined (Levitan 1996a). As in the present study, both species demonstrated that larger eggs were preferentially fertilized under sperm limitation. Podolsky (1995) investigated egg-cell and jelly-coat sizes in two male-female pairs of *Dendraster excentricus*. In one pair, jelly-coat size had a greater selection gradient, and in the other egg-cell size had the greater value. Although Podolsky argued that the selective gradient was greater for jelly coats, the average values for the two male-female pairs were similar (egg cell = 4.31; jelly coat = 4.34). Podolsky's results are consistent with ours in that they suggest that jelly-coat and egg-cell sizes are under direct selection for fertilization success, but our study consistently demonstrated that selection on egg cells was severalfold larger than that on jelly coats.

Measurements of fertilization as a measure of fitness are not directly comparable to estimates of lifetime fitness, especially if trade-offs mean that variation in a trait increases one component of fitness while lowering another (Roff 1992). Egg size is such a trait, because increases in mean egg size are inversely related to the number of eggs produced when the total allocation of resources is constant (Vance 1973; Smith and Fretwell 1974). Models of optimal egg size that include this trade-off, independent of the influence of jelly coats, indicate that decreases in sperm availability can result in selection for increased egg size (Levitan 1993, 1996b, 2000). Models that estimate fitness, and that include fertilization effects of the jelly coat and the caloric value of egg-cell and jelly-coat material, indicate that increasing sperm limitation results in increases in both optimal egg size and optimal jelly-coat size. The magnitude of the effect of sperm availability on optimal egg-cell size is independent of the presence or absence of the jelly coat (Farley and Levitan 2001). These predictions hold in models that include and models that ignore the possibility of polyspermy at high sperm concentrations (Farley and Levitan 2001).

Our study and the above modeling do not consider the fitness consequences of the other potential benefits of jelly

coats. Jelly coats have been suggested to attract (Ward et al. 1985) and activate (Foltz and Lennarz 1993, Moy et al. 1996) sperm chemically, to aid in species recognition (Hagstrom 1956), and to protect the egg cell from physical damage (Thomas et al. 1999). For some of these functions the presence or absence of a jelly coat might be the only important variable, in others, like collision frequency, the size of the jelly coat might be under selection. The evolutionary origin of jelly coats might be independent of selection for increased collision frequency. The present study suggests that jelly-coat size might currently be under selection to increase fertilization success under sperm limitation.

The cross-sectional method used here has both disadvantages and advantages compared to a longitudinal analysis of correlated characters. A disadvantage is that regression techniques cannot be used to estimate the correlation coefficients, and there are no error estimates for the selection gradients in each population. Because cross-sectional methods are easier to replicate, however, multiple populations can be examined and error estimates can be made from the independent male-female pairs. In all five replicate egg clutches, the selection differentials and gradients had identical sign and rank order.

The degree to which traits are correlated is variable across populations. There is a striking relationship between the degree of correlation, within a trait comparison, and the selection gradients; increases in correlation were related to decreases in the selection gradients. As the correlation approaches one, the selection gradients should approach zero, because selection cannot distinguish between two perfectly correlated traits. If only one egg clutch had been examined, the conclusions would have been very sensitive to the degree of correlation in that particular clutch. Therefore, selection coefficients should be estimated from replicated experiments when practicable.

Under sperm limitation, selection favors larger eggs with larger jelly coats. Changes in egg-cell size result in much greater fertilization gains than do changes in jelly-coat size, but because jelly-coat size has a much higher variance, the two traits should respond to fertilization selection at similar rates.

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