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EVOLUTION OF EGG TARGET SIZE: AN ANALYSIS OF SELECTION ON CORRELATED CHARACTERS

ROBERT D. PODOLSKY
Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599-3280
E-mail: podolsky@unc.edu

Abstract.—In broadcast-spawning marine organisms, chronic sperm limitation should select for traits that improve chances of sperm-egg contact. One mechanism may involve increasing the size of the physical or chemical target for sperm. However, models of fertilization kinetics predict that increasing egg size can reduce net zygote production due to an associated decline in fecundity. An alternate method for increasing physical target size is through addition of energetically inexpensive external structures, such as the jelly coats typical of eggs in species from several phyla. In selection experiments on eggs of the echinoid *Dendraster excentricus*, in which sperm was used as the agent of selection, eggs with larger overall targets were favored in fertilization. Actual shifts in target size following selection matched quantitative predictions of a model that assumed fertilization was proportional to target size. Jelly volume and ovum volume, two characters that contribute to target size, were correlated both within and among females. A cross-sectional analysis of selection partitioned the independent effects of these characters on fertilization success and showed that they experience similar direct selection pressures. Coupled with data on relative organic costs of the two materials, these results suggest that, under conditions where fertilization is limited by egg target size, selection should favor investment in low-cost accessory structures and may have a relatively weak effect on the evolution of ovum size.

Key words.—Egg size, fertilization, jelly coat, organic cost, phenotypic correlation, selection, target size.

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In many marine invertebrates, fertilization takes place in open water following the release of sperm and eggs. Under conditions where reproductive success is chronically limited by sperm availability, adults and gametes should be under selection for mechanisms to increase sperm-egg contact. One such mechanism could involve changes in the physical size of the egg, because enhancing the size of the ''target'' for sperm could increase the probability of sperm-egg collision (Rothschild and Swann 1951; Vogel et al. 1982; Levitan 1993). Models of egg size evolution have traditionally focused on postzygotic consequences of egg size for larval or juvenile growth or survival (Vance 1973; Christiansen and Fenchel 1979; Emlet et al. 1987). In contrast, one implication of the target size hypothesis is that prezygotic benefits to fertilization instead could drive the evolution of egg size and, in turn, anisogamy in broadcast-spawning species (Levitan 1996).

As an explanation for the evolution of egg size, one potential limitation of the target size hypothesis is that, given a fixed allocation to reproduction, producing larger eggs can lead to a reduction in egg number (Vance 1973; Smith and Fretwell 1974). Scaling arguments predict that larger eggs of constant organic density will produce fewer zygotes, because the increase in rate of surface area-dependent collision is less than the decrease in volume-dependent fecundity (Podolsky and Strathmann 1996). As a result, an increase in egg target size would increase zygote production only if achieved at low organic cost. Such a mechanism could involve internal egg hydration (Robertson 1996) or the addition of low-cost external structures (Podolsky 1995).

However, even if target size were increased at low cost, associated changes in egg chemistry or structure could hinder reproduction in other ways. For example, increased hydration could compromise physiological processes underlying development or limit the capacity to store eggs before release (Robertson 1996). Comparisons among invertebrate species show that organic density generally does not decline as a function of egg size to the degree necessary to offset the fecundity cost (Jaeckle 1995; Podolsky and Strathmann 1996). Similarly, a comparison of several dozen fish species found no evidence of decreasing egg organic density as a function of volume and levels of hydration were not correlated with the probability of successful fertilization as related to ecological conditions (Robertson 1996). Although the exact costs are uncertain, these observations do not support the hypothesis that evolution has adjusted target size through changes in egg hydration.

An alternative, less costly means of increasing target size is to enclose eggs within a larger accessory structure such as a jelly coat, hull, or layer of follicle cells (Rothschild and Swann 1951; Epel 1991; Podolsky and Strathmann 1996). Such structures can increase the target size of eggs several-fold (Strathmann 1987). Although these structures could avoid interference with physiology or reduce limitations on storage in the female (Podolsky and Strathmann 1996), they could have other disadvantages for the process of fertilization. For example, large enclosing structures could act as physical barriers to sperm or reduce the egg surface available for sperm-egg fusion (Hagström 1956a; Buckland-Nicks 1993). The question therefore remains whether accessory structures can be not only cost efficient but also an effective means for enhancing the frequency of collision and fertilization through changes in target size.

Here I test the importance of target size in fertilization success, and I address the relative effectiveness of increased investment in ova versus extracellular coats. I exposed free-spawned eggs to selection on fertilization success, using sperm as the agent of selection. In contrast to analyses where complex characters may be under multiple selection pressures (Reznick and Travis 1996), the relative simplicity of target size as a character and fertilization as a performance measure

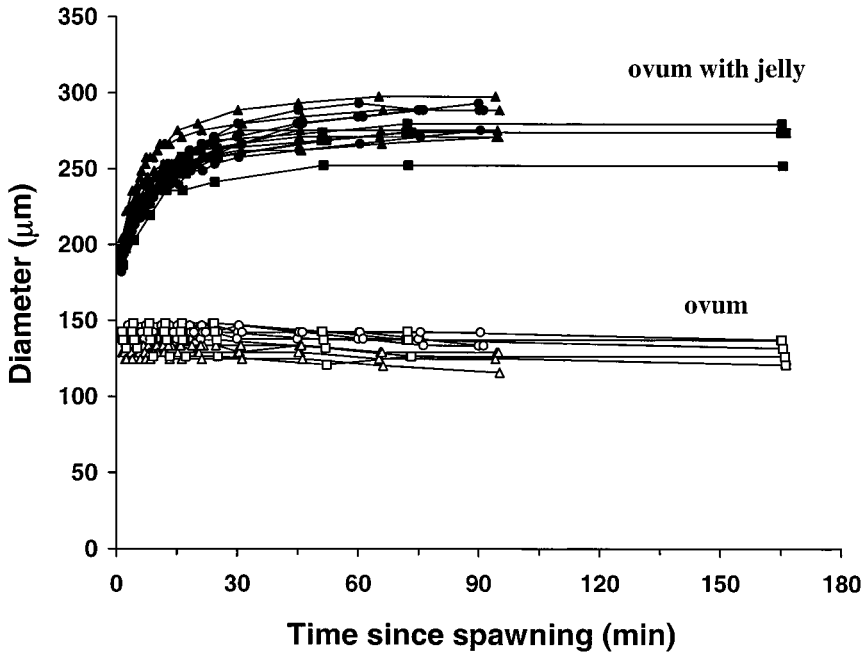

FIG. 1. Changes in the size of the ovum and jelly coat after contact with seawater. Five eggs for each of three females were measured at 200× magnification on a cooled microscope stage starting one minute after spawning and at regular intervals. Lines connect points for individual eggs, and the three different symbols represent different females. On average, jelly coats expanded to 80% of their maximum thickness within 15 min of contact with seawater and were relatively stable in size after about 60 min.

allows a relatively straightforward interpretation of fitness consequences. I used gametes of the sand dollar *Dendraster excentricus* for which two characters—ovum size and jelly coat size—contribute to overall target size. These characters are correlated but differ in important ways: jelly has less than 2% of the organic density of the ovum, but comprises about 93% of the total egg volume and increases cross-sectional area sixfold (Podolsky 1995). Thus, the jelly shell around the ovum of *D. excentricus* appears to be a highly cost-efficient means of substantially increasing physical size.

By fertilizing a large percentage of eggs and comparing the remaining unfertilized portion to an unfertilized control group, I measured the shift in target size following selection and compared the actual shifts to predictions of a model in which fertilization probability was proportional to target size. I then used an analysis of selection on correlated characters to evaluate the independent contributions of the two characters to fertilization success. These analyses are used to address the following questions: (1) Is fertilization under sperm-limited conditions biased toward larger overall target sizes? (2) How well does target size quantitatively predict the probability of fertilization? (3) How much does sinking speed, independent of size, contribute to fertilization probability? (4) What are the relative contributions of variation in ovum size and jelly coat size to selection by sperm on fertilization success? (5) What are the implications for egg size evolution of investment in the two materials?

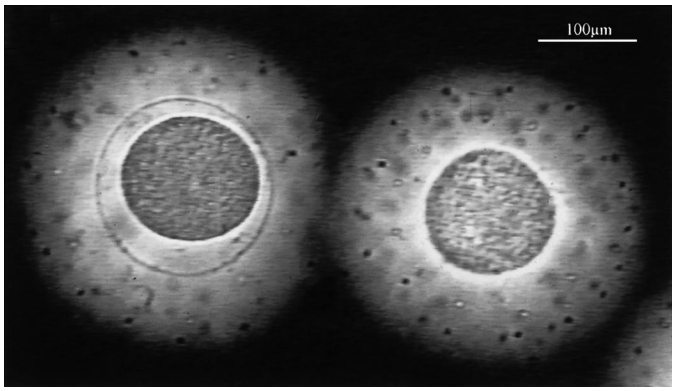

FIG. 2. Image from videotape showing fertilized and unfertilized eggs suspended in Sumi ink, which outlines the clear jelly coat and its embedded pigment cells. To avoid the apparent changes in ovum and jelly coat size associated with fertilization, only eggs that remained unfertilized were used in analyses.

METHODS

The egg jelly coat of the sand dollar *Dendraster excentricus* (Echinodermata: Echinoidea) forms a regular, spherical shell around the ovum and is persistent on eggs unless actively removed (Podolsky 1995). The coat is a polysaccharide-glycoprotein complex (Vasseur 1952; Bonnell et al. 1994) that is compressed around the ovum before spawning but rapidly expands on contact with seawater (Fig. 1; see also Bolton et al. 2000). For size measurements, eggs were viewed under 200× magnification in a suspension of Sumi ink, which is excluded by the clear jelly coat (Fig. 2; Schroeder 1980). Adults were collected from intertidal habitats near Friday Harbor, Washington, and spawned using KCl injection (Strathmann 1987). Eggs were kept cool and sperm were stored cool and undiluted before use.

Spawned eggs show natural variation in target size, defined

here as the maximum cross-sectional area including the ovum and extracellular layers. To examine whether fertilization results in ''selection'' on target size, I compared the sizes of eggs that remained unfertilized under conditions of high and negligible fertilization (Levitan 1996). I used only unfertilized eggs because lifting of the vitelline membrane and other changes associated with fertilization could cause apparent changes in the size of the ovum or jelly coat (see Fig. 2). Two sperm concentrations were used in each trial. ''Postselection'' cohorts were exposed to a sperm concentration that resulted in approximately 80% fertilization, which created strong selection pressure but maintained enough unfertilized eggs for measurement. ''Preselection'' cohorts were inseminated with an extremely dilute sperm suspension, such that eggs were exposed to sperm and seminal fluid, but with $<$ 1% fertilization. Pre- and postselection eggs are therefore analogous to cohorts ''before'' and ''after'' selection in a cross-sectional analysis (Lande and Arnold 1983). Members of the ''after'' cohort are the 20% of eggs that remain unfertilized, and changes in mean trait values represent the effects of selection by sperm.

To create these treatments, I added eggs to a series of finely graded sperm concentrations between 10^1 and 10^5 sperm ml^{-1}, allowed eggs to sink through each sperm suspension, filtered off sperm and resuspended eggs in filtered seawater so that additional sperm-egg contact was negligible. I then sampled from the numerous containers to find the desired levels of fertilization for the pre- and postselection cohorts and used only these containers for analysis. Eggs were considered fertilized if they showed a fertilization envelope and cleavage; under the nonsaturating conditions used in experiments, uncleaved eggs with a fertilization envelope (i.e., polyspermic eggs) are rare. I repeated the experiment for five different male-female pairs. Low and high sperm concentrations ranged across trials from 10^1 to 10^2 ml^{-1} and $10^{4.25}$ to 10^5 ml^{-1} for the pre- and postselection treatments, respectively.

To avoid potential biases in estimates of selection on target size, fertilization conditions were controlled in several important ways. (1) Jelly coats were allowed to expand to full size before insemination (Fig. 1). (2) Eggs sank through the sperm cloud for the duration of the contact period, so that full target area was available to sperm throughout. (3) Sperm that had contacted eggs during this period were allowed to complete fertilization. In many fertilization studies, sperm activity is stopped shortly after insemination by adding KCl, hypo-osmotic sea water, or sodium-laurel sulfate (Hagström and Hagström 1954; Presley and Baker 1970; Schuel 1984). By eliminating sperm that have contacted the egg but not yet penetrated the jelly coat, such methods could create an artifactual bias against eggs with large jelly coats (Farley and Levitan 2001). The method used here also avoided any potential ionic effect on jelly coat size. (4) After fertilization, washing, and resettlement, eggs were immediately videotaped in large batches, alternating between subsamples from the two treatments, and later digitized for measurement. This short interval minimized the potential contribution to treatment differences of any residual change in coat size. (5) The potential fertilizability of all eggs from a given female was verified by inseminating subsamples at a sperm concentration that was known to result in complete fertilization (10^6 ml^{-1}).

To measure eggs I loaded samples into multiwell plates and used an inverted microscope to capture multiple images on videotape. I later measured the diameter of individual eggs with and without the jelly coat to the nearest 0.1 μm, recording major and minor axes, and calculated areas and volumes based on average diameters. Within each trial, I measured equal numbers of eggs for the pre- and postselection cohorts, ranging across trials from 236 to 450 per group (total = 3210 eggs digitized and measured). I used unpaired *t*-tests to compare the distributions of total target area for unfertilized eggs pre- and postselection.

To test the assumption that fertilization probability of a given egg is predictable from its size, I compared observed shifts in size frequencies to those expected under a model where fertilization was a linear function of target area. This situation avoids the need for an explicit kinetics model (Styan and Butler 2000) because the fertilization rate is already known, and the simple assumption that fertilization is proportional to target size can be tested directly. For each trial, the model divided the unfertilized (preselection) cohort into 20 equal-sized classes and then fertilized 80% of those eggs, distributed in proportion to the median target sizes for each class. Thus, the expected proportion of fertilized eggs in each size class was the product of the class target area and the original proportion in each class. To generate the predicted distribution of unfertilized eggs, the model then subtracted the fertilized proportion from the original, and normalized across classes to a cumulative frequency of one. To test the assumption, I compared for each trial the observed postselection distribution of target sizes to the predicted distribution using a Kolmogorov-Smirnov one-sample test (Siegel 1956).

Because sperm-egg collision may depend not only on target size but also on sperm-egg contact time, as determined by egg sinking speed (Podolsky 1995), I examined the relationship between fertilization success and sinking speed independent of size. For each trial, I measured differences between cohorts in sinking speed of unfertilized eggs using analysis of covariance (ANCOVA), with target size as the covariate. Sinking speed (u_e) was estimated for individual eggs based on their volumes of jelly and ovum material as well as densities for the two materials (1.023 and 1.054 g ml^{-1}, respectively; Podolsky 1995) and for sea water (1.0225 at 13°C; Smith 1974), using Stokes equation for low Reynolds number flow,

$$u_e = \left(\frac{2gR_e^2(\rho_e - \rho_{sw})}{9\mu_{sw}} \right) \quad (1)$$

where g is gravitational acceleration (9800 mm s^{-2}), R_e is egg radius, ρ_e is egg density (mg μl^{-1}), and ρ_{sw} and μ_{sw} are the density and viscosity (mg mm^{-1} s^{-1}) of seawater at 13°C (Dorsey 1968). Because regression data did not meet assumptions of linearity, values were rank-transformed before analysis (Conover 1982). Differences in intercept were not tested where the assumption of equal slopes was rejected.

I then examined the independent contributions of ovum and jelly coat size to fertilization success. Because ovum and jelly volumes contribute to target area and may be correlated with one another, I used an analysis of selection on correlated characters (Lande and Arnold 1983). The selection differential (s), a vector of differences in mean values for each

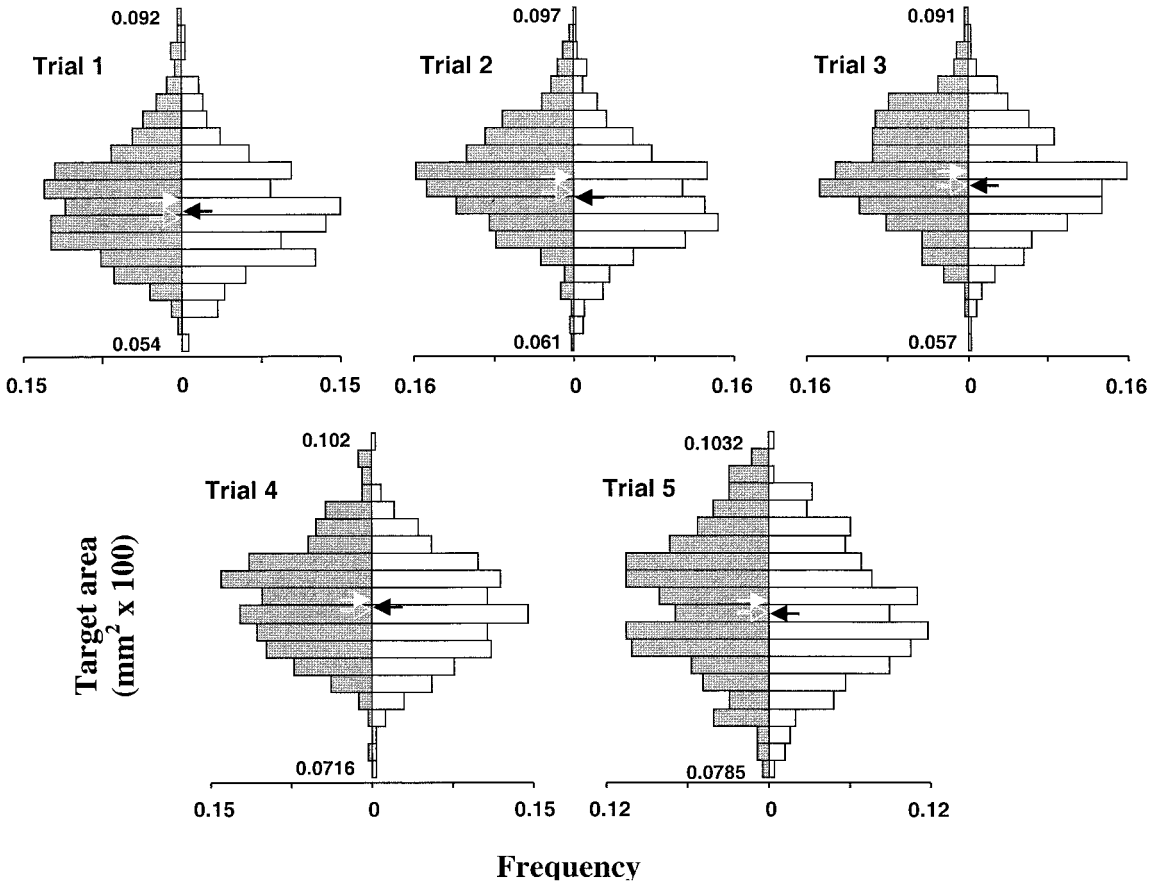

FIG. 3. Results of selection experiments. Frequency distributions for five trials showing the distribution of total egg target areas ($mm^2 \times 100$) for unfertilized eggs in the preselection (shaded bars) and postselection (open bars) cohorts. Numbers on the vertical axis show the midclass target areas for the smallest and largest classes. Arrows show the position of the mean target area for the preselection (white filled) and postselection (black filled) cohorts, as well as the model prediction (white outline; see text for explanation). Mean target areas in the two treatments differed significantly in each trial (see Table 1). Sample sizes as in Table 1.

trait before and after selection, includes both direct effects of selection and indirect effects through selection on correlated characters. Direct effects alone are described by the directional selection gradient (β), which is the vector of coefficients from a multiple regression of the traits on relative fitness. For a cross-sectional analysis, $\beta = P_b^{-1}s$, where P_b is the phenotypic variance-covariance matrix of the two traits before selection. Selection differentials (or gradients) were standardized to units of phenotypic standard deviations by dividing (or multiplying) by the standard deviation of the preselection cohort (Endler 1986).

I also analyzed the data for evidence of quadratic selection (''stabilizing'' selection in Lande and Arnold 1983). The quadratic selection gradient (γ) is a matrix of regression coefficients describing the direct effects of selection on the variance of characters (diagonal elements) and the direct effects on covariance between characters (off-diagonal elements). In a cross-sectional analysis, γ is calculated as: $\gamma = P_b^{-1}(P_a - P_b + ss^T)P_b^{-1}$ where *b* and *a* index the phenotypic variance-covariance matrix of traits before and after selection. The matrix ss^T, which is the outer product matrix of the selection differential vector *s*, makes estimates of quadratic selection independent of directional selection. Quadratic selection gradients were standardized to units of standard deviations by multiplying the *ij*th element by $\sigma_i\sigma_j$, where *i* and *j* index the standard deviations for ovum and jelly volume from the preselection cohort (Lande and Arnold 1983).

To judge the significance of all coefficients I used permutation tests, which resample observed values without replacement and can give more reliable estimates than bootstrap methods that resample with replacement (Efron and Tibshirani 1993). For each trial and each coefficient, I calculated a test-statistic by randomly reallocating all log-transformed ovum-jelly volume pairs to ''before'' and ''after'' cohorts, repeating this procedure 1000 times to generate a distribution for the statistic. *P*-values were assigned based on the proportion of values that were more extreme than the observed coefficient. Tests of coefficients were one-tailed for directional selection and two-tailed for quadratic selection.

RESULTS

Eggs with larger overall targets had a selective advantage in fertilization. In all trials, the target size distribution of eggs that remained unfertilized shifted to lower values after 80% of eggs were fertilized (Fig. 3). Although shifts in mean

TABLE 1. Mean target area ($mm^2 \times 100$) of unfertilized eggs before and after selection and unpaired *t*-tests comparing the means of these distributions. On the right, mean target area ($mm^2 \times 100$) after selection as predicted by the model, which assumed that 80% fertilization selected eggs in proportion to their target sizes. Also shown is the proportion of the predicted shift shown by the actual shift in mean target size ((before-after)/(before-predicted)), and *P*-values for Kolmogorov-Smirnov one-sample tests comparing the observed (preselection) and predicted distributions (ns, $P > 0.05$). Sample sizes are the number of eggs measured for each cohort.

Trial	*n*	Mean target area (1 SD)		*t*	*P*-value, *t*-test	Mean target area predicted by model	Proportion of predicted shift	*P*-value K-S test
		Before	After					
1	300	7.06 (0.15)	6.93 (0.91)	−2.62	<0.005	6.82	0.54	ns
2	450	7.98 (0.60)	7.76 (0.72)	−5.97	<0.001	7.82	1.33	$P < 0.05$
3	373	7.53 (0.62)	7.39 (0.23)	−3.62	<0.002	7.36	0.82	ns
4	236	8.77 (0.49)	8.64 (0.39)	−2.84	<0.005	8.64	1.03	ns
5	246	9.12 (0.62)	9.01 (1.08)	−2.28	<0.02	8.99	0.78	ns

target area were small—on average, about 0.27 SD units—these shifts were statistically significant (Table 1). Because in all trials still higher sperm concentrations resulted in 100% fertilization (data not shown), this result cannot be attributed to the presence of small, unfertile eggs.

Actual shifts in target size were consistent with the prediction that fertilization probability is directly proportional to overall target size. On average, the actual shift in mean target size was 0.90 (±0.13 SE; $n = 5$) times the model prediction, a result not significantly different from 1, although with large variation (Table 1). In four of five trials, Kolmogorov-Smirnov one-sample tests showed no significant difference between the predicted and observed effects of selection. Therefore, overall target size was a good quantitative predictor of relative fertilization success.

Holding target size constant, eggs that sank more slowly through the sperm cloud were more likely to be fertilized. In trials 2–4, ANCOVA found that unfertilized eggs in the postselection cohort had significantly faster sinking speeds than those in the preselection cohort, indicating that fertilized eggs removed from the population on average had slower sinking speeds (Fig. 4). In the other two trials, intercepts were not compared because the assumption of equal slopes was violated (i.e., regression lines crossed).

Within females, the correlation between ovum size and jelly coat size was positive and significant in four trials and marginal in the fifth (Table 2). This phenotypic correlation indicates the potential for indirect selection on one character through correlation with the other. In addition, measurements for five females in this study and six additional females re-

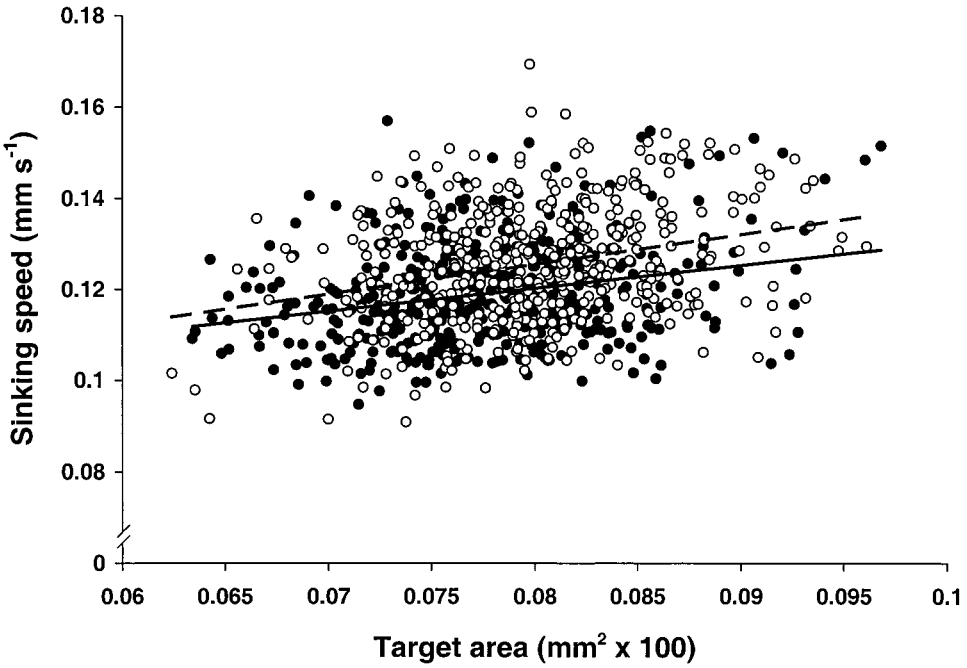

FIG. 4. The relationship between calculated sinking speed and target area of unfertilized eggs before (filled circles, solid regression line) and after (open circles, dashed line) selection (trial 2 only). In trials 2–4, unfertilized eggs in the post-selection cohort had significantly faster sinking speeds than those in the pre-selection cohort (trial 2: $F_{1,897} = 50.2$, $P < 0.0001$; trial 3: $F_{1,743} = 9.04$, $P < 0.0027$; trial 3: $F_{1,469} = 4.42$, $P < 0.036$), indicating that slower-sinking eggs had been fertilized, on average, at a higher rate. In trials 1 and 5, the assumption of equal slopes was violated and intercepts were not compared. The covariate, target area, was significant in all trials. To meet assumptions of linearity, values were rank-transformed before analysis.

TABLE 2. Mean ovum and jelly volumes (μl × 100) before and after selection. Values were log-transformed before the selection analysis. Coefficients (*r* and *P*-values are for the correlation between ovum and jelly volumes using the pre-selection cohorts. Sample sizes as in Table 1.

	Mean volume (1 SD)					
	Before		After			
Trial	Ovum	Jelly	Ovum	Jelly	*r*	*P*
1	0.124 (0.024)	1.292 (0.182)	0.120 (0.019)	1.257 (0.181)	0.159	<0.050
2	0.114 (0.016)	1.587 (0.171)	0.105 (0.014)	1.525 (0.177)	0.256	<0.001
3	0.097 (0.012)	1.461 (0.166)	0.094 (0.013)	1.420 (0.164)	0.170	<0.001
4	0.113 (0.013)	1.839 (0.163)	0.111 (0.011)	1.800 (0.159)	0.093	<0.080
5	0.115 (0.015)	1.959 (0.164)	0.112 (0.016)	1.927 (0.154)	0.285	<0.001

ported previously (Podolsky 1995) showed a positive relationship among females between mean ovum and jelly coat volumes (Fig. 5).

In all trials, both ovum and jelly volume experienced significant directional selection, as shown by the selection differential *s* (Table 3). This measure can include effects of both direct and indirect selection on each character. Jelly coat volume was identified as a direct target of selection in all five trials and ovum volume in three trials, as indicated by the selection gradient β (Table 3). Thus, in two trials ovum volume may have changed, in part, due to its correlation with jelly volume. In both of these trials selection was in the expected direction, thus it is possible that ovum size would have been identified as a direct target in a larger sample. On average, the standardized selection coefficients for jelly volume (mean ± SE = −0.209 ± 0.018) and ovum volume (−0.221 ± 0.063) were not statistically different, indicating that the two materials had similar effects on enhancing fertilization by increasing target size.

In contrast to direct selection, the analysis of quadratic selection showed no consistent pattern. Ovum volume showed weak quadratic selection that was negative in two trials and positive in two, whereas total volume and jelly volume showed quadratic selection in one or two trials (Table 3). These variable results do not indicate a consistent pattern of quadratic selection independent of directional selection.

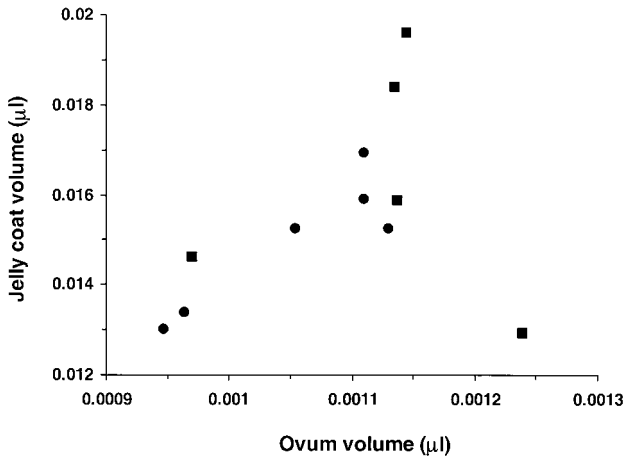

FIG. 5. Relationship between mean ovum volume and mean jelly coat volume for eleven females (Spearman's rank correlation; r_s = 0.43, $P < 0.09$, $n = 11$; excluding one outlier: $r_s = 0.91$, $P < 0.0001$, $n = 10$). Squares are means from preselection eggs for the five females used in this study. Circles are means from a different study (Podolsky 1995) in which ten eggs were measured for each of six females. Jelly coats had expanded fully before measurement.

DISCUSSION

Fertilization was biased toward eggs with larger target areas. Although the potential importance of target size in fertilization has been cited previously (Rothschild and Swann 1951; Epel 1991; Levitan 1993; Podolsky and Strathmann 1996), this analysis is the first to show a quantitative correspondence between a predicted effect of target size and the outcome of selection by sperm. Furthermore, the analysis partitioned this effect between two structural components of target size—ovum and jelly—and showed that on a per-volume basis they contributed about equally to variation in fertilization success. Although ovum and jelly volume are the only characters that contribute to physical target size, this result of equivalent contributions to fertilization success was not a necessary outcome. For example, it was possible that larger jelly coats would enhance sperm-egg collision (Farley and Levitan 1998) but somehow interfere with sperm-egg fusion (as suggested by Hagström 1956a; Hagström and Markman 1957). Because this study measured fertilization, and not just collision rate, it provides an aggregate measure of the relative effectiveness of jelly and ovum as materials for enhancing fertilization.

Ovum and jelly volumes were positively correlated, such that selection on one character could lead to indirect selection on the other (Lande and Arnold 1983). Jelly volume was consistently a direct target of selection, although evidence in two trials was not strong enough to conclude that ovum volume was selected directly. On average, however, the shift in both characters due to direct selection was around 20% of a standard deviation unit. Because jelly coats make up 93% of the absolute volume of an egg and show standard deviations 10 times greater than for ova, absolute shifts in jelly coat volume were considerably larger.

For a given target size, eggs that sank more slowly through the sperm cloud had an additional fertilization benefit. This observation supports the assumption that increased contact time will improve chances for sperm-egg collision (Vogel et al. 1982) and is consistent with experiments and models showing that jelly coat removal contributes to a decline in fertilization rate by altering egg suspension time (Podolsky 1995). The addition of a jelly coat brings the egg close to neutral buoyancy and can double the suspension time, reflecting an important potential benefit of jelly coats and other accessory structures under natural conditions.

TABLE 3. Results of the selection analysis. Standardized directional selection differentials (s') and gradients (β') for mean ovum and jelly volume, and standardized quadratic selection gradients (η') for variance of characters and covariance between characters. Values were standardized to units of standard deviations calculated from the preselection cohort (see Table 1). Negative values for directional coefficients represent declines in mean volume after selection, whereas negative values for quadratic coefficients represent declines in variance or covariance. Significance of coefficients was determined with a permutation test (see text); tests for directional coefficients are one-tailed, and for quadratic coefficients are two-tailed. Sample sizes as in Table 1.

	s' (means)		β' (means)		η' (variances, covariance)		
Trial	Ovum	Jelly	Ovum	Jelly	Ovum	Jelly	Ovum-Jelly
1	−0.146*	−0.203**	−0.117	−0.185*	−0.298*	0.115	−0.036
2	−0.518**	−0.378**	−0.451**	−0.263**	0.012	0.239*	0.094
3	−0.293*	−0.253*	−0.257*	−0.209*	0.386*	0.157	−0.165*
4	−0.192*	−0.249**	−0.170*	−0.233**	−0.295*	0.080	0.017
5	−0.156*	−0.189*	−0.110	−0.157*	0.397*	0.056	−0.261*
Mean	−0.261	−0.254	−0.221	−0.209	0.040	0.130	−0.070
SE	0.069	0.033	0.063	0.018	0.154	0.032	0.064

* $P < 0.05$, ** $P < 0.01$.

Research Approach

A long history of research includes speculation about the role of egg accessory coats as physical devices for sperm-collection (Rothschild and Swann 1951; Epel 1991; Buckland-Nicks 1993; Podolsky 1995; Levitan 1996). One experimental approach to this question has involved the removal of these structures to assess their overall importance to fertilization (Lillie 1914; Loeb 1914; Tyler 1941; Rothschild and Swann 1951; Hagström 1956a, b; Vacquier et al. 1979; Podolsky 1995). This approach has raised methodological questions, given that procedures used to remove jelly coats vary among species and could be harmful to eggs (Loeb 1914; Hagström 1959; Vacquier et al. 1979). Furthermore, because the jelly coat can play several roles in fertilization, this procedure can confound various physical and chemical effects of coat removal on fertilization.

To address these concerns, previously I used two independent removal methods, coupled with a model of fertilization kinetics, to compare overall effects of jelly coat removal to those expected from an equivalent change in target size (Podolsky 1995). I found consistently between methods that about half of the overall effect of removal would be predicted by the size change alone, which suggests that under some conditions physical and chemical attributes of jelly coats can play equally important roles in fertilization success.

The method used in the present study avoids entirely these potential problems of methodology and interpretation by examining natural variation in ovum and jelly volume and by allowing sperm to carry out selection. The results showed not only that larger target size is an advantage in fertilization, but that fertilization success does not depend strongly on which material alters target size. Therefore, the main conclusion of these experiments is that jelly may be equally effective as a material for increasing target size. This result is important to current debate in life-history theory because the means to effectively increase target size at relatively low cost can substantially weaken selection on ovum size to increase fertilization success (Podolsky 1995).

Using a model the scaled fertilization probability in proportion to target size, I found that the average shift in target size following selection was 0.9 times the expected shift, although with large variation (Table 1). A similar study (Levitan 1996) examined changes in target size (ovum cross-sectional area only) of sea urchin eggs after 50% of eggs were fertilized. When I applied the same target size model to data shown in that study, the actual shift in ovum cross-sectional area was 2.6 (for *Strongylocentrotus franciscanus*) to 4.9 (for *S. purpuratus*) times the predicted shift. What accounts for this difference between observed and predicted effects of target size is not clear. The unidentified effect may be related to jelly coat size or another variable correlated with ovum size, such as the density of sperm receptors or other aspects of egg fertilizability. Such effects could similarly explain interspecific differences in size-dependent fertilization rates (Styan and Butler 2000).

Evolutionary Response to Selection

Although I found similar selection pressures on the two characters that contribute to target size, a response to selection will depend on at least three additional factors. First, evolution in either character will depend on their heritabilities and on genetic correlation between them (Falconer 1981). Broadcast spawners typically exhibit large phenotypic variation in ovum size and organic content, even within a single spawn (Lönning and Wennerberg 1963; Turner and Lawrence 1979; McEdward and Coulter 1987). In this study, ovum and jelly volumes showed significant variation and were phenotypically correlated both within and among females. Heritability and genetic correlation data are more scarce. For a polychaete with internal fertilization, Levin et al. (1991) estimated a high heritability ($h^2 = 0.75$) for ovum diameter, but I could find no estimates of heritability for size of ova or accessory structures in broadcast-spawning organisms. Such data are needed to evaluate the potential for evolutionary shifts in size due to selection on fertilization success.

A second factor is the relative costs of investment in the two materials. Measures of ash free dry mass, an estimate of organic content, showed that organic density of the ovum was more than 67 times greater than that of jelly (Podolsky 1995; see also Bolton et al. 2000). Given equal heritabilities, net selection (benefit relative to cost) for changes in jelly coat volume would be substantially greater than for changes in ovum volume. If so, then interspecific variation in sperm limitation would more likely be correlated with patterns of large interspecific variation in jelly volume than in ovum volume.

A third factor concerns what benefits other than target size are gained from investment in the two materials. As detailed in a large literature (reviewed by Emlet et al. 1987; Levitan 2000), increased investment in ova can have postzygotic benefits to larval or juvenile success that are unlikely with jelly. The magnitude of such benefits will depend on particular relationships that describe incremental gains to larval and juvenile feeding, growth and mortality (Hart 1995), and how these balance against disproportionate costs to fecundity (Podolsky and Strathmann 1996). Jelly, in contrast, has been implicated in a number of essential chemical roles in fertilization related to sperm activation, chemo-attraction, and the sperm acrosome reaction (SeGall and Lennarz 1981; Ward et al. 1985; Suzuki 1989; Vacquier and Moy 1997) as well as physical roles in buoyancy control, guidance of sperm, and protection (Szollosi 1964; Chia and Atwood 1982; Honegger 1983; Thomas et al. 1999). However, the quantitative relationship between jelly volume and these other processes has not been established. Given its extreme size, the jelly coat of *D. excentricus* is most likely related to physical functions, such as rates of sperm-egg collision or suspension times (as demonstrated here), or protection from shear forces (Thomas and Bolton 1999).

In fact, the potential for sperm attractants to increase chemical target size complicates interpretations of the ecological importance of physical target size. Rough estimates of distances over which sperm attractants can act range from about 50 to 500 μm from a source (Miller and King 1983; Maier and Muller 1986), which spans a size range that is similar to those of extracellular coats (Strathmann 1987). However, these estimates of chemical target size are taken from sperm in still water. Water motion and turbulence is likely to interfere more with the effects of chemical cues than of physical size, because a larger physical target will be important regardless of whether swimming, sinking or water motion is responsible for relative gamete movement. In any case, sperm attractants have not been found for eggs of *D. excentricus* nor for most echinoids (Miller 1985). Further complicating an ecological interpretation is the observation that some echinoderms release viscous strings of gametes that may behave in gamete encounter more as an aggregate mass than as individual eggs of a certain physical size (Thomas 1994).

Given the finding of consistent directional selection, what factors could limit physical target size? Organic costs of the two materials, cited above, offer part of the answer. Given the assumption that ovum and jelly contribute equivalently to sperm-egg encounter, as supported here, a model that incorporates fertilization, larval mortality, fecundity, and organic cost suggests that ovum size will vary by no more than a few percent under different degrees of sperm limitation (Podolsky 1995). With these assumptions, jelly coat size is instead expected to vary as a function of sperm limitation and to be optimized at an intermediate level that returns maximum benefit (zygote production) relative to cost (organic investment; Podolsky 1995).

It should be emphasized that results of this analysis do *not* imply that jelly coats evolved under selection for increased target size. Rather, *if* selection on fertilization success were important in the evolution of target size, then investment in low-cost extracellular structures would be evolutionarily favored as an equally effective but more efficient means of enhancing fertilization. Clearly this argument applies only to taxa that produce or can produce large extracellular structures.

Results in this paper lend to the hypothesis, not yet fully addressed, that jelly coats play a dual role in fertilization. As demonstrated here, under sperm limitation jelly coats can improve fertilization success by enhancing physical and chemical target size. On the other hand, work by Styan (1998) suggests that, at saturating sperm concentrations, large target size may reduce chances for successful development by increasing the risk of polyspermy. I hypothesize that jelly coats could also help to reduce polyspermy, especially relative to ova of equivalent size, by regulating the arrival times of sperm, or acrosomal processes, at the egg surface (for complementary views of their role in polyspermy, see Hagström 1956b; Vacquier et al. 1979; Lambert and Lambert 1981; Patricolo and Villa 1992). The most appropriate test of this hypothesis, and one that complements Styan's (1998) model, would be whether jelly coats increase the *variance* of sperm arrival times and thereby reduce the risk of coincident fertilizations. Such a versatile role would be especially relevant to the great variability of sperm concentrations encountered in nature (Levitan and Petersen 1995; Yund 2000) and the likelihood that selection on an ''optimal'' target size will therefore vary greatly across space and time.

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