The Role of Jelly Coats in Sperm-Egg Encounters, Fertilization Success, and Selection on Egg Size in Broadcast Spawners

Gregory S. Farley* **and Don R. Levitan†**

Department of Biological Science, Florida State University, Tallahassee, Florida 32306-1100

Submitted February 2, 2000; Accepted January 19, 2001

ABSTRACT: Sperm limitation may be an important selective force influencing gamete traits such as egg size. The relatively inexpensive extracellular structures surrounding many marine invertebrate eggs might serve to enhance collision rates without the added cost of increasing the egg cell. However, despite decades of research, the effects of extracellular structures on fertilization have not been conclusively documented. Here, using the sea urchin *Lytechinus variegatus*, we remove jelly coats from eggs, and we quantify sperm collisions to eggs with jelly coats, eggs without jelly coats, and inert plastic beads. We also quantify fertilization success in both egg treatment groups. We find that sperm-egg collision rates increase as a function of sperm concentration and target size and that sperm are not chemotactically attracted to eggs nor to jelly coats in this species. In fertilization assays, the presence of the jelly coat is correlated with a significant but smaller-than-expected improvement in fertilization success. A pair of optimality models predict that, despite the large difference in the energetic value of egg contents and jelly material, the presence of the jelly coat does not diminish selection for larger egg cell size when sperm are limiting.

Keywords: collisions, egg size, jelly coat size, fertilization, selection.

When sperm are limiting, selection can act on both male and female traits for enhanced fertilization success (Levitan 1993, 1996*a*, 1996*b*, 1998*a*, 2000*b*; Arnold 1994; Burd 1994). Broadcast spawning invertebrates are often sperm limited, and laboratory and field studies have documented that larger eggs, within and among species, are preferentially fertilized under sperm-limited conditions (Levitan 1993, 1996*a*, 1998*b*; Coma and Lasker 1997). The proposed mechanism generating this result is simply that larger egg targets are more likely to be struck by swimming sperm (Rothschild and Swann 1949, 1951; Vogel et al. 1982). This finding has generated the hypothesis that sperm availability can influence the evolutionary trade-off between egg size and number (Levitan 1993, 1996*a*, 1996*b*, 1998*a*, 1998*b*). However, the idea that fertilization kinetics might influence this trade-off has been challenged because of the notion that extracellular structures or chemoattractants may increase sperm-egg collisions yet may not represent as great a cost to fecundity as the trade-off associated with increased egg size (Podolsky and Strathmann 1996; Styan 1998). These contradictory ideas have not been resolved because data detailing the influence of extracellular materials on fertilization success are equivocal and because there have been no studies to date that have empirically quantified collision frequencies between sperm and eggs.

The eggs of many echinoderms are surrounded by jelly coats that can increase the diameter of the egg package by 100% or more (Harvey 1956). If increasing the overall target size of the egg package increases sperm-egg collision frequency, as predicted by theoretical models of fertilization kinetics (Rothschild and Swann 1949, 1951; Vogel et al. 1982), then jelly coats might be an efficient mechanism for enhancing fertilization (Rothschild and Swann 1951; Epel 1991; Podolsky and Strathmann 1996; Styan 1998). Jelly coats might also affect fertilization by attracting sperm chemically, eliminating the need for a physical structure to enhance target size. Sperm chemotaxis to egg extracts has been demonstrated in several taxa, including echinoderms, but numerous assays using echinoid egg extracts have failed to attract sperm (Miller 1985*a*, 1985*b*). The only demonstration of sperm chemotaxis in sea urchins involves the attraction of sperm to a component of the jelly coat in one species (*Arbacia punctulata*; Ward et al. 1985). No studies to date have demonstrated chemotaxis to whole eggs or determined whether chemotaxis results in increased levels of fertilization.

* E-mail: farley@bio.fsu.edu.

† E-mail: levitan@bio.fsu.edu.

Am. Nat. 2001. Vol. 157, pp. 626–636. 0003-0147/2001/15706-0004$03.00.

Despite half a century of laboratory investigations comparing fertilization in the presence and absence of the jelly coat (Tyler 1941; Rothschild and Swann 1951; Hagström 1956*a*, 1956*b*; Hagström and Markman 1957; Podolsky 1995), the evidence is ambiguous. Most studies suggest that removal of the jelly coat decreases the fraction of eggs fertilized at a given sperm concentration (Tyler 1941; Rothschild and Swann 1951; Hagström 1956*b*; Podolsky 1995). Hagström (1956*b*) also demonstrated the opposite result and suggested that decreases in fertilization success among jelly-free eggs were caused by the extremely acidic washes that previous investigators had used to remove jelly coats from eggs. In contrast, studies that quantified the rate, rather than the final percent, of eggs fertilized suggest that removal of the jelly coat causes an increase in fertilization, regardless of the method by which jelly coats were removed (Hagström 1956*a*; Hagström and Markman 1957). There are two problems with these studies: first, the possibility that eggs were damaged during jelly coat removal makes the impact of the jelly coat on fertilization difficult to interpret; and second, these fertilization assays do not provide direct evidence of the frequencies of sperm-egg collisions.

Here, by directly observing gametes of the sea urchin *Lytechinus variegatus*, we quantify the number of collisions between sperm and eggs. By comparing collision frequency among eggs with intact jelly coats, eggs gently stripped of jelly coats, and inert plastic beads, we explore the importance of target size to sperm-egg collision frequency, and we test for chemotactic attraction of sperm to egg jelly or to eggs. To explore the impact of jelly coat removal on the proportion of eggs fertilized, we remove jelly coats using only seawater and assay fertilization. Using a fertilization kinetics model developed by Styan (1998), we compare the predicted effect of removing jelly coats on fertilization with empirical measures of fertilization. Finally, to estimate how selection might influence both egg provisioning and the thickness of the jelly coat, we present a model that predicts the optimal egg and jelly coat size as a function of sperm limitation.

Material and Methods

Models of Fertilization Kinetics

Perhaps the most widely used model of fertilization kinetics was developed by Vogel et al. (1982; their "Don Ottavio" model, hereafter "VCCW"). The model was derived from principles of molecular kinetics, so it assumes Brownian motion of eggs and sperm. It also assumes permanent adhesion of a sperm to the first egg with which it collides.

In the VCCW model, the proportion of eggs fertilized in a sample (Φ) is predicted as a function of the initial sperm concentration (S_0; sperm/μL), the initial concentration of virgin eggs (E_0; eggs/μL), the time of sperm-egg exposure (t; s), a rate constant describing collisions (β_0; mm^3/s), and a rate constant describing fertilization (β; mm^3/s):

$$\Phi = 1 - e^{(-\beta S_0/\beta_0 E_0)(1-e^{-\beta_0 E_0 t})}. \quad (1)$$

The rate constant describing sperm-egg collisions (β_0) is estimated by multiplying sperm swimming speed (ν; mm/s) and the area of the egg cross section (σ; mm^2):

$$\beta_0 = \nu\sigma, \quad (2)$$

where $\sigma = \pi(\text{egg radius})^2$.

Using empirical estimates for the proportion of eggs fertilized in a sample (Φ), the initial sperm concentration (S_0), the initial egg concentration (E_0), the time of sperm-egg exposure (t), and the rate constant describing collisions (β_0), the model can be solved to estimate the value of the fertilization rate constant (β) with nonlinear regression (Marquardt method; SAS 1996). Once these parameters have been estimated, the formula can be solved for the concentration of sperm needed to fertilize 50% of eggs. This provides an intuitive measure of gamete performance. The ratio β/β_0 is another useful measure that provides an indication of fertilization efficiency. This ratio of the estimated fertilization constant to the collision constant has three possible interpretations: the proportion of colliding sperm that initiate fertilization, the fraction of the egg surface available for fertilization (Vogel et al. 1982), or the proportion of sperm that is viable.

Styan (1998) modified the VCCW model to account for zygote loss due to polyspermy. Polyspermy is a developmental failure caused by multiple sperm penetrating the egg and has been noted in nature (Brawley 1992) and in the laboratory (Styan and Butler 2000) when eggs are exposed to high sperm concentrations. Styan's (1998) model subtracts away polyspermic zygotes from all zygotes to predict the proportion of monospermic zygotes (φ_{mono}) as follows:

$$\varphi_{\text{mono}} = 1 - e^{-x} - (1 - e^{-x} - xe^{-x})(1 - e^{-b}), \quad (3)$$

with

$$x = \frac{\beta S_0}{\beta_0 E_0}\left(1 - e^{-\beta_0 E_0 t}\right), \quad (4)$$

and

$$b = \frac{\beta S_0}{\beta_0 E_0}\left(1 - e^{-\beta_0 E_0 t_b}\right). \quad (5)$$

The new variable t_b is the time (s) necessary to establish a block to polyspermy.

Empirical data confirm that variation in gamete traits can influence fertilization. As predicted by these kinetics models, increases in sperm velocity (Levitan 2000*b*), gamete longevity (Pennington 1985; Havenhand 1991; Oliver and Babcock 1992; Benzie and Dixon 1994), sperm-egg contact time (Levitan et al. 1991), and egg size (Levitan 1993, 1996*a*, 1996*b*, 1998*a*) all result in increased fertilization success under sperm-limited conditions.

Gamete Collection and Manipulation

We collected sea urchins (*Lytechinus variegatus*) from subtidal populations in the northeastern Gulf of Mexico. Urchins were kept in running seawater at the Florida State University Marine Laboratory and fed turtlegrass (*Thalassia testudinum*).

We dissected randomly selected individual urchins and excised the gonads (sensu Vogel et al. 1982). Male gonads were punctured, placed in scintillation vials, and stored on ice. Female gonads were punctured, placed in petri dishes, covered with approximately 50 mL of artificial seawater, and kept at room temperature. We separated eggs from the gonads by gently shaking punctured gonads. To avoid contaminating eggs with sperm, we rinsed all dissection tools in fresh water between dissections. Fresh water is lethal to sea urchin sperm (Rothschild and Swann 1951).

Sperm

We diluted dry sperm (collected as it was expelled from the excised gonads) into 10-fold serial dilutions in scintillation vials. Because sperm at lower concentrations rapidly lose mobility and fertilizing ability (the "respiratory dilution effect" of Chia and Bickell 1983; Levitan et al. 1991; Levitan 1993, 2000*b*), we diluted sperm immediately before use. For observations of sperm-egg collisions, we used three serial dilutions of sperm; sperm concentrations in these suspensions ranged between 10 and 1,000 sperm/μL. For each male urchin used, we fixed 1 mL of diluted sperm in formalin and counted those sperm on a hemacytometer to obtain accurate estimates of sperm concentration.

Eggs

We randomly subdivided the eggs from each female to generate two treatment groups: eggs with intact jelly coats and stripped eggs (from which the jelly coat was removed and rinsed away). Intact eggs were left sitting in artificial seawater in petri dishes.

We removed jelly coats from eggs by swirling the eggs in beakers. Flow induced shear forces must be quite large (on the order noted in crashing waves) to damage gametes to the point where subsequent fertilization is affected (Mead and Denny 1995; Thomas et al. 1999; M. Denny, personal communication). We placed eggs to be stripped into a 250-mL beaker, covered them with approximately 50 mL of fresh artificial seawater, and gently rotated the beaker by hand at three revolutions per second for 20 min to induce shear. We rinsed these eggs on a Nitex sieve with at least 100 mL of artificial seawater to wash away the components of the jelly coat and resuspended rinsed eggs in 30–40 mL of fresh artificial seawater by rinsing them from the Nitex mesh.

We suspended at least 30 eggs from each treatment group in artificial seawater and Sumi ink. Among intact eggs, we measured jelly coats; among stripped eggs, we confirmed that jelly coats had been removed. We also checked for contamination of eggs with sperm.

We then quantified collisions of sperm with eggs from each treatment group, using modified 1-mL syringes in order to manipulate single eggs at low flow rates. We cut the sliding plastic plunger of a 1-mL syringe to half its original length and reserved the end with the rubber stopper. We then affixed a finely threaded machine screw, threaded through a nut, to the plunger of each syringe and glued the nut to the top of the syringe barrel (the end opposite the needle). Slowly twisting the screw incrementally depressed the plunger, which in turn expelled extremely small amounts of liquid from the syringe tip. To the end of the syringe originally designed to hold a needle, we attached a clear micropipette tip, which permitted us to watch individual eggs progress toward the open end of the tip as we twisted the screw-top plunger.

These syringes allowed us to inject single eggs into 1-mL pools of sperm without changing the effective volume of the sperm pool or introducing a great deal of turbulence. We used a separate modified syringe for each egg treatment group and drew each egg into the appropriate syringe immediately before use to avoid storing eggs in the plastic pipette tip.

Sperm-Egg Collisions with and without Jelly Coats

We placed 1 mL of freshly diluted sperm in a deep-welled glass depression slide on the stage of a trinocular microscope fitted with a low-light-level video camera. The camera was connected to a video monitor and a VCR. Using a modified syringe, we gently injected a single egg into the pool of sperm. At 312.5-fold magnification, the depth of field enabled us to clearly see swimming sperm throughout the sample. Identifying sperm that collided with the jelly coat was relatively straightforward; freely swimming sperm suffered a sudden, marked decrease in velocity. Likewise, sperm colliding with the equator of the egg were easily

seen. Once sperm swam over or under the egg (into the circular projection of the egg on the videotape), we were unable to see them because the egg itself was too dark, but we saw many sperm that passed briefly over or under the egg and then continued swimming. We assumed that sperm that swam over or under the egg and did not reemerge had collided with the egg surface. Our criteria for determining sperm-egg collisions were supported by observations of sperm with transparent column-chromatography beads (see "Collisions of Sperm with Beads"), which allowed us to see colliding sperm clearly.

We videotaped all events, starting with the introduction of the egg and ending when the fertilization membrane had expanded to its full size. In those observations in which fertilization did not occur, we terminated the videotape after 20 min. *Lytechinus variegatus* sperm become less active and lose viability after 20 min (Levitan 2000*b*), as is the case with many other species (Vogel et al. 1982; Levitan et al. 1991; Benzie and Dixon 1994). From each of 15 male-female urchin pairs, we generated six independent data points: we injected individual eggs of both types (with and without jelly coats) into sperm at three different concentrations, using freshly diluted sperm for each egg.

Collisions of Sperm with Beads

To examine the possibility of chemical attraction of sperm to eggs in the treatment groups, we quantified the number of sperm collisions with nonbiological targets. We injected Sephadex (Pharmacia Biotech) column-chromatography beads 80–110 mm in diameter into pools of sperm in the same manner used to inject eggs and videotaped subsequent sperm-bead interactions. Because beads were more transparent than eggs, we were able to observe individual sperm colliding with the surfaces of beads. Using sperm from 15 male urchins, we exposed 35 total beads to sperm at various concentrations. We videotaped these trials for 5 min.

Fertilization Assays

Observations of collisions between sperm and single eggs provide a less powerful estimate of the proportion of eggs fertilized than assays of fertilization using several thousand eggs. Therefore, we performed fertilization assays across a gradient of sperm concentration represented by seven serial dilutions. For assays, we used 17 male-female urchin pairs that were not used in videotaped observations of sperm-egg interactions.

We first generated both stripped and intact egg treatment groups following the same methods described above and adjusted egg concentration to approximately 200 eggs/mL in both treatment groups. We exposed both types of eggs to seven serial dilutions of sperm. Ten seconds after adding sperm to each vial, we added 5 mL of 0.55 M KCl and swirled vials to ensure thorough mixing. Potassium chloride immobilizes sperm without interrupting the development of fertilized eggs (Schuel 1984), and it does not induce fertilization in unfertilized eggs.

We placed vials in running seawater in a wet table for 2 h following the introduction of KCl. After this incubation time had passed, we counted at least 200 eggs from each vial and scored each egg as fertilized or unfertilized. The 2-h wait allowed embryos to develop to the two- or four-cell stage, thus making fertilized/developed eggs distinct from unfertilized eggs.

Results

Direct Observations of Sperm-Egg Collisions

Eggs with intact jelly coats averaged 193.6 ± 0.056 μm (mean ± SD) in total target diameter; eggs stripped of jelly coats had an average target diameter of 99.4 ± 0.006 μm. Differences in diameter were significant (*t*-test for unequal sample variances, $P < .0001$).

Increases in sperm concentration were correlated with increasing rates of sperm-egg collision in both intact (fig. 1, *solid line*) and stripped eggs (fig. 1, *dashed line*). We applied a fourth-root transformation to the data to reduce heteroscedasticity (Chang and Winnell 1981; Downing 1981; France 1987) and performed least squares regres-

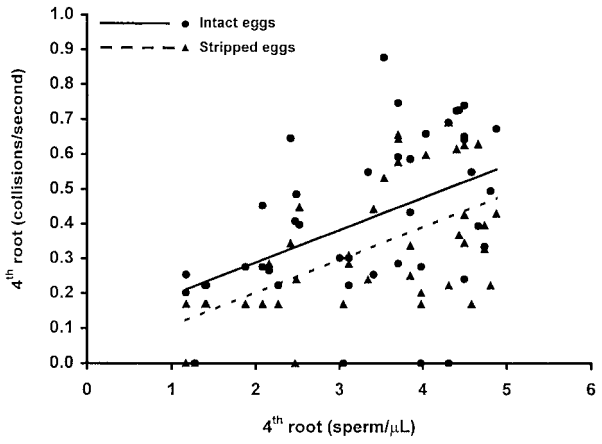

Figure 1: Comparison of sperm-egg collision rates among eggs with intact jelly coats (*filled circles*) and eggs without jelly coats (*filled triangles/dashed line*). Lines are linear regressions through fourth-root transformed data (*solid line*, eggs with jelly coats: $y = 0.093x + 0.10$, $r^2 = 0.213$, $P < .005$; *dashed line*, eggs without jelly coats: $y = 0.096x + 0.01$, $r^2 = 0.332$, $P < .0001$). Eggs with intact jelly coats accrue collisions at a significantly faster rate (ANCOVA, $P < .05$).

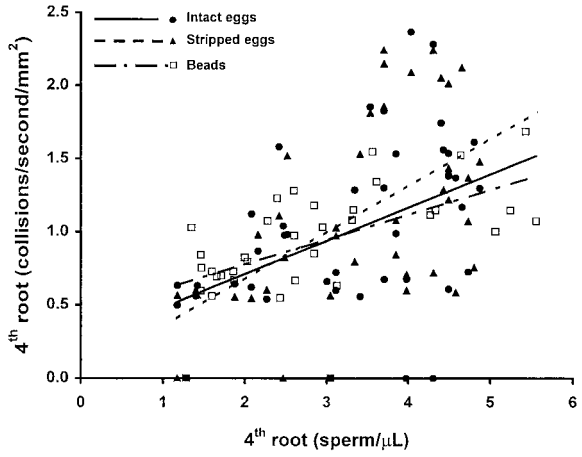

Figure 2: Collision rates standardized by target area for three target types: eggs with intact jelly coats (*filled circles/solid line*; $y = 0.23x + 0.25$, $r^2 = 0.199$, $P < .005$), eggs without jelly coats (*filled triangles/dashed line*; $y = 0.32x + 0.03$, $r^2 = 0.337$, $P < .0001$), and plastic beads (*open squares/dot-dashed line*; $y = 0.17x + 0.43$, $r^2 = 0.345$, $P < .0005$). The three lines are not significantly different (ANCOVA, $P > .6$), so area-adjusted collision rates with sperm are similar for egg and bead targets. We cannot detect chemotactic attraction of sperm to eggs with or without jelly coats.

sions of collision rate as a function of sperm concentration. Regressions were significant and positive in slope for both egg treatment groups. ($P < .005$ for intact eggs, and $P < .0001$ for stripped eggs.) Eggs with jelly coats accrue more collisions with sperm than eggs without jelly coats across the entire range of sperm concentration tested (fig. 1; ANCOVA, $P < .05$). We then did a second ANCOVA, adjusting collision rate by the circular cross sections of the targets. This ANCOVA was not significant ($P > .5$), which indicates that the significant difference in the collision rates (fig. 1) can be explained by target size differences. If chemotaxis is acting, it is neither enhanced nor diminished by the presence of the jelly coat.

To examine whether chemotaxis can be detected at all, we compared collision rates among intact eggs, stripped eggs, and egg-sized plastic beads. As with eggs, the rate of sperm-bead collision increased linearly with sperm concentration ($P < .005$). We again standardized collision rates by target sizes and compared the collision rate per target area as a function of sperm concentration for the three treatment groups (fig. 2). Adjusted by target size, these three treatment groups were not significantly different (ANCOVA, $P > .6$). We cannot detect chemotactic attraction of *Lytechinus variegatus* sperm to eggs in our samples.

We examined the probability of fertilization as a function of the number of collisions using logistic regressions. Eggs with jelly coats appear to need more collisions with sperm to become fertilized than eggs without jelly coats (fig. 3), but this trend is not significant.

Fertilization Assays

Across a range of sperm concentrations, fertilization success was higher among eggs with jelly coats than among eggs without jelly coats (fig. 4, *top panel*). To test for differences in fertilization success between egg treatment groups, we transformed the proportion of eggs fertilized (ln [proportion fertilized + 1]) and executed an analysis of variance (table 1). Eggs with jelly coats were fertilized more often than eggs without jelly coats at similar sperm concentrations ($P = .039$).

We fitted our empirical fertilization data to Styan's (1998) model using SAS (Marquardt nonlinear regression; SAS 1996). We calculated β_0, the rate constant for sperm-egg collision, as the product of sperm swimming velocity and the circular "target size" of the egg in each group (Vogel et al. 1982). The target size of eggs with jelly coats was the area of cross section of the egg and jelly coat; target size of eggs without jelly coats included the area of the egg cell only. Iterating Styan's model allowed us to estimate β, the fertilization rate constant, and t_b, the time to establish a block to polyspermy, for each egg treatment group (table 2); it also generated 95% confidence intervals (CIs) about mean fertilization for each egg treatment group (fig. 4, *bottom panel*, *solid* and *dashed lines*). We calculated F_{50}, the sperm concentration necessary to fertilize 50% of the eggs in each treatment group. Among

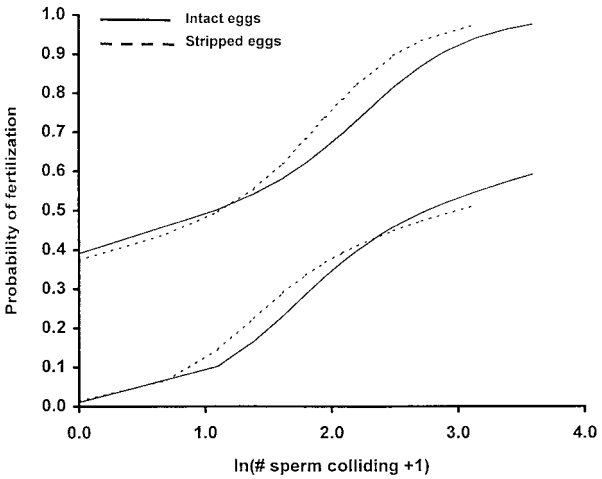

Figure 3: Logistic regressions of the probability of fertilization as a function of the number of collisions with sperm. Pairs of lines indicate 95% CIs about the regression curves. Eggs with jelly coats (*solid lines*) appear to need more collisions with sperm to become fertilized than eggs without jelly coats (*dashed lines*), but this trend is not significant because the CIs overlap.

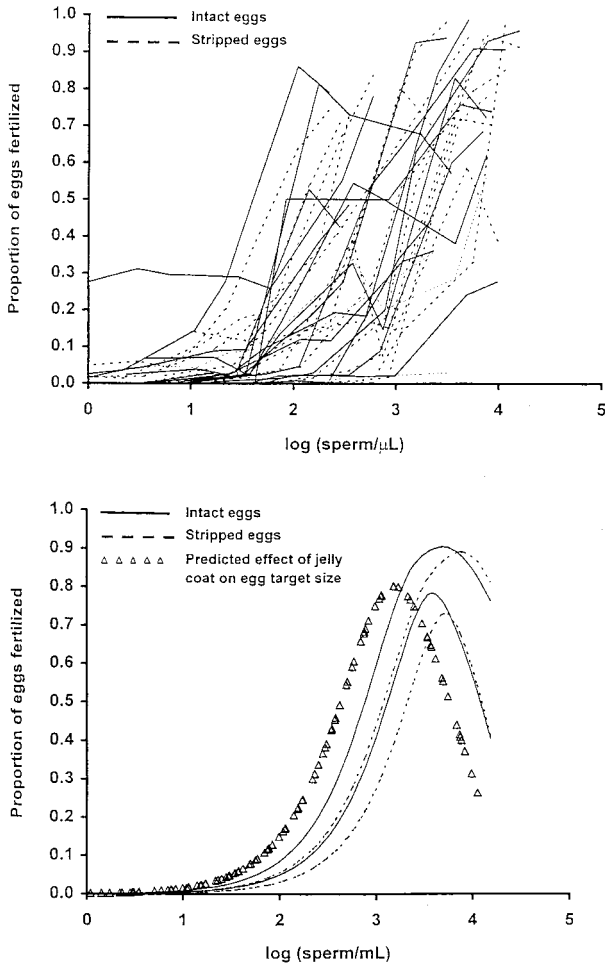

Figure 4: Assays of fertilization success. *Top panel*, raw estimates of fertilization success for eggs with intact jelly coats (*solid lines*) and eggs without jelly coats (*dashed lines*); $N = 17$ in both groups. Curves are generally sigmoid and not bell shaped, which indicates that polyspermy probably did not affect fertilization success in our trials. *Bottom panel*, upper and lower 95% confidence limits about mean fertilization for eggs with intact jelly coats (*solid lines*) and eggs without jelly coats (*dashed lines*). Confidence intervals were generated using Styan's (1998) fertilization-kinetics model, which incorporates egg concentration and sperm-egg contact time; hence, CIs are narrower than fertilization data in the *top panel* might suggest. Eggs with jelly coats (*solid lines*) are fertilized more often in assays than eggs without jelly coats (*dashed lines*), although only marginally so (ANOVA, $P = .0648$). Predictions of fertilization success among eggs with jelly coats (*open triangles*), based on the increase in target size and assuming equal collision efficiency in the two groups, are much greater than observed fertilization in that group.

eggs with jelly coats, F_{50} was 1,010.98 sperm/μL; among eggs without jelly coats, F_{50} was 1,774.56 sperm/μL. Eggs without jelly coats require nearly double the sperm to achieve 50% fertilization.

We divided the fitted estimate of β by our calculated estimate of β_0 to generate an estimate of β/β_0 for each egg treatment group (table 2). Among eggs without jelly coats, β/β_0 was 0.0284, which is 2.4 times greater than our estimate of β/β_0 for eggs with jelly coats ($\beta/\beta_0 = 0.0119$). We then used the fitted estimates for β and t_b and the calculated estimates of β_0 and β/β_0 (table 2) in Styan's (1998) model to predict fertilization among hypothetical egg cells as large as *L. variegatus* eggs with jelly coats. We set $\beta/\beta_0 = 0.00589$ to reflect the average target size of intact eggs in our treatments and set $\beta/\beta_0 = 0.0284$ and $t_b = 0.709$ s to reflect the values estimated for eggs without jelly coats. This prediction assumes that the target size of eggs changed but that the characteristics of the egg did not. The prediction of fertilization success in such hypothetically enlarged eggs lies above the 95% CI for eggs with jelly coats (fig. 4, *bottom panel*, *open triangles*), which indicates that while jelly coats did improve fertilization success, they did not provide as large a benefit to fertilization as predicted by doubling the size of egg cells.

Optimization Model of Egg and Jelly Coat Size

Our data indicate that the presence of a jelly coat around an egg enhances sperm-egg collision frequency by increasing egg target size. Because the energetic value of jelly material is far less than egg yolk material (Podolsky 1995; Bolton et al. 2000), it has been suggested that jelly coats could weaken selection for increased egg cell size under sperm-limited conditions (Podolsky 1995; Podolsky and Strathmann 1996). To examine the effects of egg jelly on optimal egg size, we modified a model developed to predict optimal egg size in echinoids (Levitan 2000*a*) to include the effects of a jelly coat.

Because the size of planktotrophic echinoids at metamorphosis is independent of egg size (Emlet et al. 1987; Levitan 2000*a*), we used the number of metamorphs as a proxy for fitness, given a constant allocation to reproduction (Vance 1973; Strathmann 1985; Levitan 1996*b*, 2000*a*). The optimal combination of egg size and jelly coat size results

Table 1: ANOVA testing differences in percent fertilization between stripped-egg and intact-egg treatment groups in fertilization assays

	df	SS	*F*	*P*
Sperm dilution	6	120.895	116.461	<.0001
Egg treatment group	1	.745	4.305	.039
Dilution × treatment group	6	.480	.462	.836
Male-female pair number (unreplicated block)	16	10.601	…	…
Error	208	35.986	…	…

Note: Model: ln (proportion of eggs fertilized + 1) = unreplicated block + sperm dilution + egg type + sperm dilution × egg type.

Table 2: Estimated values for rate constants for fertilization (β) and collision (β_0), ratio of rate constants (β/β_0), and time to establish a block to polyspermy (t_b) for eggs in each treatment group

	β (mm^3/s)	β_0 (mm^3/s)	β/β_0	t_b (s)
Intact eggs	.0007	.00589	.0119	.489
Stripped eggs	.00004	.00155	.0284	.709

Note: Estimates of β and t_b were obtained by iterating the Styan (1998) fertilization-kinetics model in SAS (1996), estimates of β_0 were derived by multiplying egg cross section and sperm swimming velocity, and estimates of β/β_0 were obtained by dividing estimates of β by estimates of β_0.

in the maximum number of metamorphs for a given set of parameter values. From Levitan (2000*a*), the number of metamorphs at time t (N_t) is a function of the number of eggs (N_e), the proportion of eggs fertilized (F), the development time in the plankton (T), and the instantaneous mortality rate (M):

$$N_t = FN_e e^{-MT}. \tag{6}$$

Development time (T) is negatively and proportionately related to absolute egg size (S_a):

$$T = \left(\frac{S_{fp}}{S_a - 1}\right) + T_{fp}, \tag{7}$$

where S_{fp} (0.010306 mm^3) and T_{fp} (14 d) are the egg size and development time of larvae that do not need to feed in the plankton in order to complete metamorphosis (facultative planktotrophs; Levitan 2000*a*). This relationship conforms to empirical data on egg size and development time from both extant echinoid species with planktotrophic development and independent contrasts of phylogenetic data (Levitan 2000*a*).

The number of eggs (N_e) is determined by the total energetic allocation to egg and jelly material (C_t) divided by the investment per individual egg (C_e) and jelly coat (C_{jc}):

$$N_e = \frac{C_t}{C_e + C_{jc}}. \tag{8}$$

The energetic cost is assumed to be 207.5 $\mu g/\mu L$ for egg material and 2.9 $\mu g/\mu L$ for jelly coat material, based on values calculated for the echinoid *Dendraster excentricus* (Podolsky 1995).

The proportion of eggs fertilized in a sample (Φ) was predicted using the fertilization kinetics models of VCCW (see our eq. [1]) and Styan (see our eq. [3]). In our model, we adjusted the rate constant for sperm-egg collision (β_0) so that the egg cross-sectional area (σ) is a function of the total size of the egg plus jelly coat. We set the collision efficiency (β/β_0) at 0.25. This value is set to reflect our empirical observation that, on average, four sperm collided with an egg before it was fertilized. Varying the value of β/β_0 has no effect on the patterns noted below other than to shift the positions of the predicted curves along the sperm concentration axis. We assume that the fertilization efficiency of eggs with and without jelly coats is identical.

We varied sperm concentration in the model from 0.015 to 25,000 sperm/μL in 37 increments increasing by 50%. At each sperm concentration, we varied egg volume from 0.0001 to 0.01 mm^3 in increments of 0.0001 mm^3, and at each egg volume, we varied jelly coat volume from 0.001 to 1.0 mm^3 in increments of 0.0001 mm^3. The optimal solution at each sperm concentration was the combination of egg and jelly coat volumes that resulted in the maximum number of metamorphs. The model assumed constant mortality rate (0.15/d), egg concentration (0.01 eggs/μL), and sperm-egg contact time (10 s). Variation in these parameters results in shifts of optimal egg size but does not alter the qualitative findings (Levitan 2000*a*). When we used Styan's (1998) fertilization kinetics model, we kept all parameters identical to those we used in the VCCW model and set t_b (the time required for blocks to polyspermy to become effective) to 2 s.

The model predicts that both optimal egg volume (fig. 5) and jelly volume (fig. 6) decrease with increases in sperm availability. Using the VCCW model, the response of egg volume to changes in sperm availability is of the same degree noted in previous models that did not include the effects of the jelly coat (fig. 5, *top panel*). The sole difference generated by incorporating jelly coats into the model is a shift in optimal egg cell sizes at lower sperm concentrations; this is because egg targets enlarged by jelly coats have an increased probability of fertilization. With or without jelly coats, optimal egg size is predicted to increase by 200% as sperm become limiting. Jelly coats are favored under sperm-limited conditions but are predicted to vanish as sperm become abundant (fig. 6).

When the possibility of polyspermy is incorporated into the model, optimal egg cell size varies to an even greater extent (fig. 5, *bottom panel*). Smaller eggs are predicted as sperm concentration increases beyond the point of maximal fertilization success because larger eggs are more likely to become polyspermic. Using this model, we determined that egg cell size becomes vanishingly small at extremely high sperm concentrations. The differences in optimal jelly volume are slight between the models that incorporate or omit polyspermy (fig. 6).

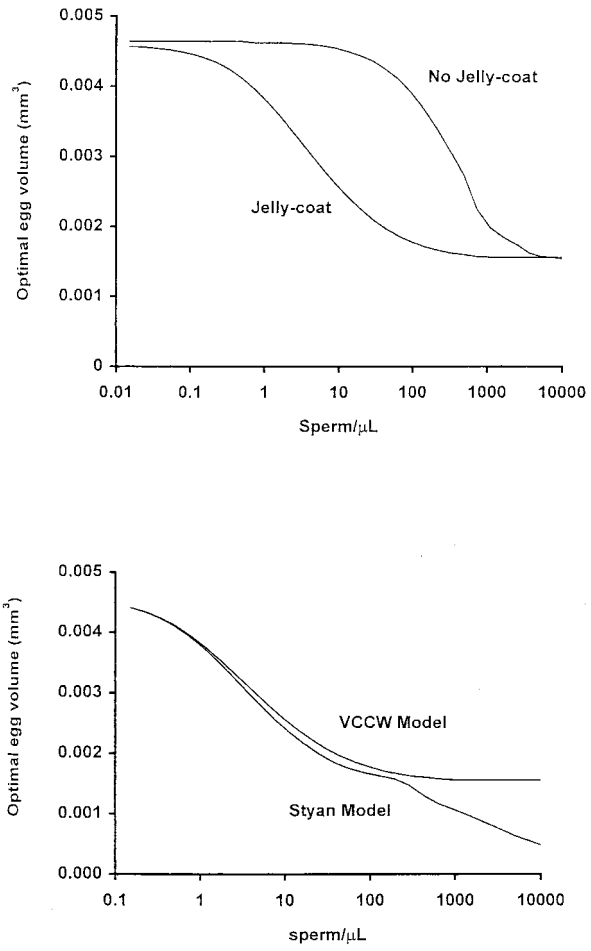

Figure 5: Optimal egg cell volume and jelly coat volume as predicted by our model. Optima are those combinations of egg and jelly coat volumes that produce the maximum number of metamorphic individuals at a given sperm concentration. Larger egg and jelly coat sizes are predicted under severe sperm limitation than when sperm are abundant. *Top panel*, optimal egg cell volume (mm^3) as a function of sperm availability. The presence of a jelly coat in the model shifts optimal egg volumes to lower sperm concentrations but does not change the prediction that different egg cell sizes should be selected at varying sperm concentrations. Larger egg cells are favored under sperm-limiting conditions in both models. Polyspermy is not considered in these models. *Bottom panel*, optimal egg cell volume (mm^3) as a function of sperm availability. The incorporation of polyspermy (Styan [1998] model, *lower curve*) into our model predicts even stronger selection on egg cell volume than the traditional model of fertilization kinetics (VCCW, *upper curve*) predicts. Styan's model predicts a reduction in egg size when sperm are abundant because large eggs are more likely to encounter superfluous fertilizing sperm.

Discussion

Egg target size is an important determinant of sperm-egg collision rates in *Lytechinus variegatus*, just as theoretical models of fertilization kinetics assume (Rothschild and Swann 1949; Vogel et al. 1982; Styan 1998). Intact targets were, on average, four times larger in area than stripped targets and accrued 2.2 times as many collisions with sperm across a range of sperm concentrations. When the rates of sperm-egg collision to intact eggs, stripped eggs, and plastic beads are standardized by target size, differences among treatment groups disappear. This result suggests that for *L. variegatus*, egg target size, not chemotactic attraction of sperm to egg jelly coats, drives sperm-egg collision frequency in still water.

In fertilization assays, the presence of the jelly coat is correlated with a significant increase in fertilization success. Eggs with jelly coats were fertilized more often than eggs without at similar sperm concentrations. However, using the observation that jelly coats, on average, quadruple the target size of eggs in this species and the assumption that eggs and jelly coats are equally efficient surfaces for fertilization, we predicted a target-size-based increase in fertilization success that was greater than the benefit we actually observed.

This difference in fertilization success may indicate that egg jelly coats are less efficient users of sperm than egg cells. However, it is possible that the fertilization assays underestimated fertilization success in the intact treatments because sperm trapped in the jelly coat may have been immobilized by KCl before they had a chance to fertilize the egg. This bias assumes that the time spent in the jelly is significant compared to the 10 s allowed for fertilization. There is a second line of evidence suggesting that jelly-free eggs are more efficient at turning collisions into fertilizations from the direct observations of sperm and eggs. Logistic regressions of probability of fertilization as a function of the number of sperm collisions (fig. 3) suggest that stripped eggs may need fewer collisions to

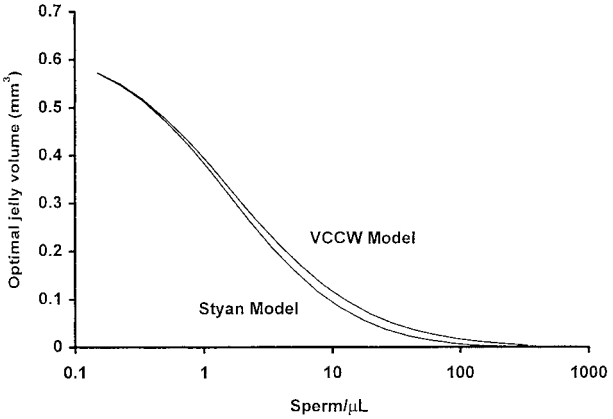

Figure 6: Optimal jelly coat volume (mm^3) as a function of sperm availability. Jelly coat volume is predicted to become vanishingly small at high sperm concentrations, both when polyspermy is important (Styan [1998] model, *lower curve*) or when it is ignored (VCCW model, *upper curve*).

achieve fertilization than eggs with intact jelly coats. However, this trend is not significant. These two results are consistent but not convincing evidence of differences in the fertilization efficiency of eggs with and without jelly coats.

Regardless of the efficiency of the jelly coat, our evidence from both direct observations of sperm-egg collisions and fertilization assays indicated that jelly coats increase collision frequency and the probability of fertilization. Because the jelly coat material has much less caloric value than egg material (Podolsky 1995; Bolton et al. 2000), it has been suggested that selection might favor minimizing egg size and maximizing jelly coat size in order to maximize parental reproductive success (Podolsky 1995; Podolsky and Strathmann 1996). However, investments in egg and jelly coat material do not provide equal returns. Larger egg cells not only provide an increased target size but also provide increased caloric resources for the embryo that can reduce time to metamorphosis (Vance 1973; Strathmann 1985; Levitan 1993, 1996*b*, 2000*a*). In contrast, jelly coats provide no nutrition to the embryo.

Our models of optimal egg and jelly coat size incorporated these differential costs and benefits and predicted that, as sperm become limiting, selection should favor both greater egg and greater jelly coat volumes. Optimal egg cell volume increased by 200% as sperm became limiting in the models, both when jelly coats were incorporated into the model and when they were omitted. Egg cell volume varied even more strongly when we modified the model to account for zygote loss due to polyspermy. These results support previous arguments (Levitan 1993, 1996*a*, 1996*b*, 1998*a*, 2000*a*) that egg cell size might respond to selection for increased fertilization success and weakens the hypothesis (Epel 1991; Podolsky 1995; Podolsky and Strathmann 1996; Styan 1998) that jelly coats can reduce or eliminate selection on egg cell size by serving as relatively inexpensive mechanisms for increasing egg target size.

Our studies were conducted in the laboratory. Fertilization in nature is influenced by water movement. Empirical studies of the influence of gamete traits on fertilization demonstrate egg size effects over a large range of flow conditions (Levitan 1996*a*, 1998*a*). Models of fertilization in flow maintain the assumption that egg target size and turbulent mixing (rather than sperm swimming ability) determine collision frequency (Denny 1988; Denny and Shibata 1989). What is unclear is the persistence of jelly coats under conditions of high shear forces in turbulent water. If jelly coats quickly dissipate following spawning, then overall levels of fertilization may be reduced, but this will not influence the strength of selection on egg cell size.

Enhancing the rate of sperm-egg collision does not appear to be the sole function of jelly coats. Rather, egg jelly probably performs a number of functions before and during fertilization. Eggs with jelly coats survive shear forces better than eggs without (Thomas et al. 1999; Bolton et al. 2000), which may be particularly important as eggs pass through the gonoduct during spawning (Thomas and Bolton 1999). Egg jelly has been implicated in increasing sperm respiration rates (Ohtake 1976; Kopf et al. 1979), increasing sperm motility (Foltz and Lennarz 1993), and promoting the expulsion of proteases (Foltz and Lennarz 1993) and bindin (Foltz and Lennarz 1993; Vacquier et. al. 1995) from the acrosome. Egg jelly may also promote species-specific recognition of eggs and sperm and may serve as a barrier to polyspermy as well (Bohus Jensen 1953; Hagström 1956*c*, 1959*a*, 1959*b*, 1964).

Although a variety of factors may have contributed to the origin of jelly coats, the thickness of jelly coats may be under selection for enhanced fertilization. Our evidence suggests that, in *L. variegatus*, jelly coats enhance collision rates and fertilization success because of enhanced target size and not through chemical attraction. However, the presence of jelly coats does not appear to diminish selection favoring larger egg cell size under sperm-limited conditions.

Acknowledgments

We thank A. Johnson, C. Styan, A. Winn, and two anonymous reviewers for making comments on various versions of the manuscript. D. Tinsley assisted with urchin collection, and the Florida State University Marine Laboratory donated laboratory space to the project. This work was supported by a National Science Foundation grant to D.R.L.

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Associate Editor: Mark A. McPeek