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Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegatus*

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The theoretical prediction that fast sperm should be more effective at fertilizing eggs has never been documented empirically. Interspecific comparisons suggest an inverse relationship between sperm velocity and sperm longevity, but this trade-off has never been demonstrated within a species. Here I investigate how sperm velocity and sperm longevity influence the patterns of fertilization in the sea urchin *Lytechinus variegatus*. In the laboratory, I examined 11 male–female pairs of sea urchins for variation in sperm velocity and sperm longevity, and determined the correlations of these traits with the percentage of eggs fertilized with serially diluted sperm. Males with faster sperm had higher rates of fertilization than males with slower sperm. Within individual males, as sperm aged they slowed down and showed a reduced percentage activity and lower rates of fertilization. Across males, the average velocity of freshly spawned sperm was inversely related to sperm longevity. These results establish the possibility that sperm traits are adapted for varying conditions along a continuum from sperm limitation to sperm competition.

Keywords: sperm; fertilization; life-history strategies; echinoids

1. INTRODUCTION

A fundamental characteristic of sperm is their ability to swim, yet, to the author's knowledge, no study to date has documented how sperm velocity influences fertilization rates in free-spawning organisms. Free-spawning invertebrates are characterized by 'primitive' sperm which are simple in structure and thought to be adapted for swimming in search of eggs in the sea (Franzen 1987). Theory predicts that fast sperm should collide with eggs more frequently than slow sperm (Rothschild & Swann 1951; Vogel *et al*. 1982) and variation in sperm velocity within (Levitan *et al*. 1991; Levitan 1993) and between (Gray 1955; Levitan 1993) species has been documented, but no tests have determined whether this variation in sperm velocity influences their fertilization rates.

Another characteristic of sperm is their relatively short life span. In many species, sperm lose their viability within seconds to minutes after dilution in seawater (Levitan 1995). In contrast, eggs are typically viable for several hours after spawning (see, for example, Pennington 1985).

Swimming fast and swimming for a long period both require energy, so a trade-off between the two seems likely. The optimal combination of speed and endurance may depend on the chances of fertilization. It has been suggested that fast sperm are advantageous under conditions of sperm competition and that long-lived sperm are advantageous under conditions of sperm limitation (Levitan 1993, 1998*b*). Evidence for this trade-off has been implied by correlations across closely related species (Levitan 1993), but a negative correlation between velocity and longevity has never been demonstrated within a species. Intraspecific comparisons provide the most direct evidence of a trade-off, because they are free from other trait differences which can confound comparisons across species (Roff 1992).

Here I use laboratory experiments to show that, in the sea urchin *Lytechinus variegatus*, both sperm velocity and sperm longevity influence their fertilization rates and that these traits are negatively correlated. This result suggests that different combinations of sperm velocity and sperm longevity may reflect selection on sperm traits in order to optimize fertilization success under varying conditions of sperm limitation and sperm competition.

2. METHODS

(a) *Urchin and gamete collection*

Lytechinus variegatus is a common, shallow-water sea urchin found in the grass flats of the Caribbean and the Gulf of Mexico. Experiments were conducted in the summer and autumn of 1996 with sea urchins collected from the Gulf of Mexico off Turkey Point of the Florida panhandle (29°50′ N, 84°30′ W). On each experimental day, one male sea urchin and one female sea urchin were used as replicates. Gametes were obtained by injection of each sea urchin with 1 ml of 0.55 M KCl. Sperm were collected with a pipette as they were extruded from the gonopore of the male sea urchin and were placed in a Petri dish on ice. Eggs were collected by inversion of the spawning female urchin on a Petri dish containing filtered seawater at ambient temperature (*ca*. 20 °C).

(b) *Laboratory experiment*

After spawning, the egg concentration was adjusted to approximately five eggs per microlitre in a stock egg suspension. Sperm were then diluted to one of a variety of concentrations (1/25–1/1500 dilution or 1.25×10^3–3.78×10^5 sperm μl^{-1}) as a stock sperm suspension. Because 'dry' (undiluted) sperm can retain viability for many hours whereas diluted sperm age rapidly (Levitan *et al*. 1991; Levitan 1993), only one sperm-dilution trial was performed at a time. Each sperm dilution was tested for its fertilization rate and sperm velocity at a variety of ages. Although both the eggs and sperm were ageing in this

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experiment, the egg viability in echinoids, as in most invertebrates, is several hours longer than the dilute sperm viability and is less likely to cause differences in the fertilization rates (reviewed by Levitan 1995).

One millilitre of the first stock sperm suspension was placed into each of four to six pairs of scintillation vials containing 8 ml of filtered seawater. One vial in the pair was used to assay the fertilization rate and the other for microscopic examination of the sperm swimming velocity. Each of the four to six pairs was used for a different sperm age. Immediately after the addition of the sperm, the 'time 0' vials were treated. One millilitre of the egg suspension was added to the fertilization rate vial, bringing the final volume to 10 ml. Ten seconds later, additional fertilization was stopped by the addition of 10 ml of 0.55 M KCl (Schuel 1984). I stopped fertilization after only 10 s to eliminate the effects of sperm ageing within each sperm age treatment. After 1 h, at least 100 eggs were inspected with a compound microscope for the presence of a fertilization membrane or further development.

Simultaneously to the addition of the eggs to the fertilization rate vial, a sample of a few drops was taken from the sperm velocity vial and placed on a glass slide. A glass cover-slip with a clay support on each corner was placed over the sperm specimen and pressed down until the top of the specimen touched the cover-slip. The slide was then placed on the stage of a compound microscope and videotaped at $\times 400$ magnification for 2 min (Levitan *et al.* 1991; Levitan 1993). The plane of focus was set midway between the glass slide and cover-slip to minimize the influence of the glass walls on sperm movement (walls greater than several body lengths away have a negligible influence on swimming cells) (Winet 1973). The field of view was shifted at 15 s intervals to minimize the risk of sampling a spermatozoon more than once.

At each of several sperm ages ranging from 5 to 120 min, an aliquot of eggs was placed into one of the next pair of vials. When time permitted, another dilution was made and another age series begun. The sperm dilutions and ages at egg addition varied between replicates to cover a range of overlapping values.

I estimated the egg concentration by counting the number of eggs in replicate 0.1 ml aliquots of the stock egg suspension. The sperm concentration was estimated for each stock sperm suspension by replicate counts using a haemocytometer. Eleven replicates of the entire experiment were conducted and each replicate used an independent male and female urchin.

(i) *Sperm activity*

I traced sperm movement onto acetate sheets by marking the sperm's position in each video frame while it moved through the field of view. Only sperm that swam parallel to the glass slide and remained in the plane of focus were scored. Sperm that were not moving or were swimming through the plane of focus were ignored (Levitan *et al.* 1991; Levitan 1993). Distance was calibrated from the image of a stage micrometer videotaped at the same magnification before the addition of sperm. I measured sperm distances with MTV computer software by scanning each acetate sheet image and tracing the sperm paths with a computer mouse. The sperm velocity was estimated from 25 sperm per treatment as the distance moved divided by the number of frames in which the sperm was scored (at 30 frames s^{-1}).

The sperm videotapes were also examined for the percentage of active sperm at each dilution and age. The first 100 sperm encountered on the videotape were scored as either moving or non-moving.

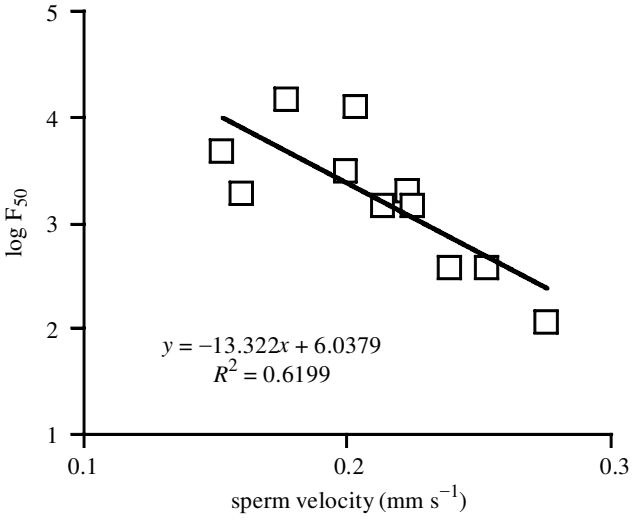

Figure 1. Influence of sperm velocity on fertilization in freshly diluted sperm. The F_{50} is the number of sperm per microlitre needed to fertilize 50% of a female's eggs; a low value indicates high-performance gametes.

(c) *Estimating the fertilization rates*

I calculated the amount of sperm needed to fertilize 50% of the eggs (F_{50}) by fitting the fertilization data to Vogel *et al.*'s (1982) fertilization kinetics model. This model predicts the proportion of eggs fertilized given the sperm (S_0, in sperm per microlitre) and egg (E_0, in eggs per microlitre) concentrations, sperm–egg contact time (t, 10 s) and two rate constants, i.e. the fertilization rate constant (b, in $mm^3\,s^{-1}$) and the collision rate constant (b_0, in $mm^3\,s^{-1}$):

$$\phi\infty = 1 - \exp\left(-\frac{\beta S_0}{\beta_0 E_0}(1 - e^{-\beta_0 E_0 t}) \right). \quad (1)$$

The best fitted values of β and β_0 were estimated for each sperm age and sperm dilution trial within each replicate by the Marquart method of nonlinear regression with the SAS statistical program (see Levitan *et al.* (1991) and Levitan (1993, 1996*a*) for similar analyses). With these rate constants, equation (1) was solved for the F_{50} given an egg concentration of 0.5 eggs μl^{-1} and a sperm–egg contact time of 10 s for each trial.

3. RESULTS

The sperm concentration did not influence the sperm velocity over the range of concentrations tested ($p = 0.79$ and $R^2 = 0.0003$), so I pooled the measurements of sperm velocity across all concentrations in order to test for variation in the sperm velocity across males and ages. The average velocities of freshly diluted sperm ranged from 0.153 to 0.275 mm s^{-1} across all males. Linear regression revealed that males with slow sperm needed up to two orders of magnitude more sperm to fertilize 50% of a female's eggs than did males with faster sperm (figure 1).

As the sperm aged, they slowed down (figure 2*a*) and their ability to fertilize eggs decreased (figure 2*b*). A multiple regression revealed significant effects of both sperm velocity ($p < 0.0001$) and sperm age ($p < 0.0001$) on the amount of sperm needed to fertilize 50% of a female's eggs ($R^2 = 0.70$). After 1 h, two orders of magnitude more sperm were needed to fertilize 50% of a female's eggs and after 2 h fertilization was nil.

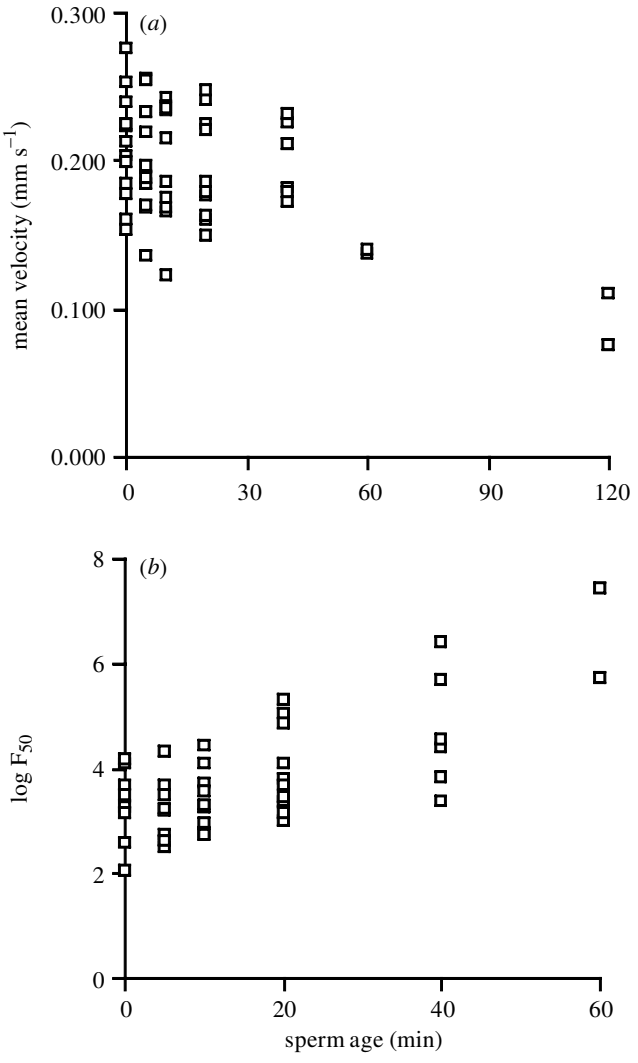

Figure 2. Sperm age influences sperm velocity and fertilization efficiency. (*a*) Sperm velocity decreases with sperm age. (*b*) Fertilization efficiency decreases with sperm age. No data plotted at 120 min because no fertilization took place at this sperm age.

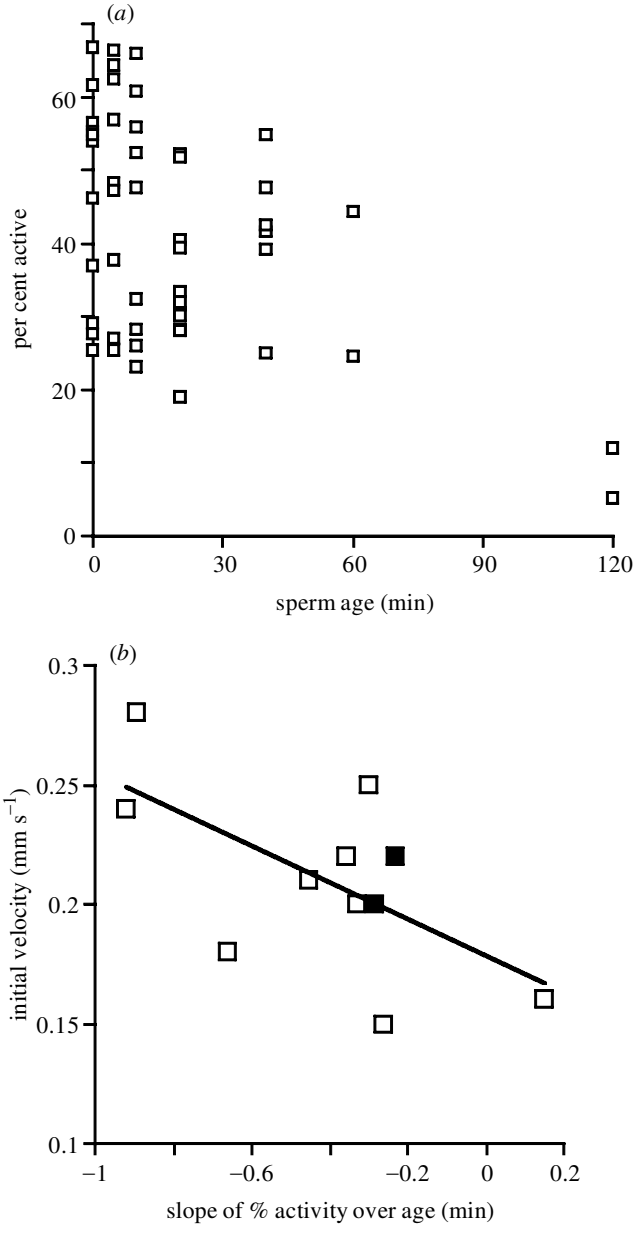

Figure 3. Sperm longevity and the trade-off of sperm velocity and longevity. (*a*) The percentage of active sperm decreases with sperm age ($R^2 = 0.24$ and $p < 0.0001$). (*b*) Trade-off of the slope of the relationship between sperm age and percentage activity calculated for each male and the average initial sperm velocity for each male ($R^2 = 0.38$ and $p < 0.05$). The two males observed for 120 min are highlighted with solid squares.

The sperm velocity was negatively correlated with sperm longevity. As the sperm aged, the percentage of active sperm decreased (figure 3*a*). The slope of this decrease in activity with age is an indication of that male's average sperm longevity. A male's initial average sperm velocity was negatively correlated with his average sperm longevity (figure 3*b*). Fast sperm have lower endurance than do slow sperm.

4. DISCUSSION

The present study provides two novel findings. The first is the evidence that sperm velocity influences the fertilization rate. The second is that sperm velocity and sperm longevity are inversely related within species. Both findings increase our basic understanding of the fertilization dynamics and life-history trade-offs involved in resource allocation within individual sperm.

The negative correlation between sperm velocity and the amount of sperm needed to fertilize eggs indicates that a 0.1 mm s^{-1} decrease in sperm velocity is correlated with an order of magnitude increase in the number of sperm needed to achieve 50% fertilization. Because males differ by more than 0.2 mm s^{-1} in their average velocity of freshly diluted sperm and the velocity difference between a male's fresh and aged sperm was also more than 0.2 mm s^{-1}, variation in sperm velocity within a species may play an important role in fertilization in nature.

However, a correlation between the sperm velocity and fertilization rate is not direct evidence that a higher sperm velocity results in higher rates of fertilization. Sperm velocity might, for example, be correlated with some other trait which results in higher rates of fertilization. Fast sperm might be overall 'better' sperm. For example, studies on sperm velocity and fertility in

humans have documented that males in infertile couples release fewer, slower and more abnormal sperm than do fertile sperm donors (Morales *et al*. 1988; Barratt *et al*. 1993). In the present case, however, fast sperm age more rapidly than slow sperm, suggesting that velocity and endurance trade off each other, and that fast sperm are not necessarily 'better' in all traits.

This second finding, that fast sperm have reduced endurance, has important implications for evolutionary trade-offs in gamete allocation. Under ideal conditions—still water, no competition for eggs and a perfect trade-off between longevity and velocity (such that each sperm travels the same total distance)—any combination of velocity and longevity will result in the same probability of colliding with an egg. In nature, however, these conditions are unlikely and different combinations of velocities and longevities may confer adaptive advantages under different conditions. For example, when sperm are competing for eggs and virgin eggs are quickly fertilized, selection may favour velocity over longevity. Conversely, when sperm are limiting and must drift via water currents to find eggs, selection may favour longevity over velocity (Levitan 1993, 1998*a*,*b*).

These expectations match the interspecific differences in sperm velocity and longevity among congeneric sea urchins. Among three species of *Strongylocentrotus* sea urchins, *Strongylocentrotus purpuratus* has the fastest but shortest-lived sperm (Levitan 1993). It lives at a high population density where female fertilization rates are near 100% (Levitan 1998*a*, 1999) and, presumably, sperm often compete for fertilizations (Levitan 1998*b*). *Strongylocentrotus droebachiensis* has the slowest but longest-lived sperm (Levitan 1993). It lives at the lowest population density (Levitan 1998*a*), where female fertilization success is generally less than 50% and can be near zero (Levitan 1998*a*, 1999) and is clearly sperm limited. *Strongylocentrotus franciscanus* has intermediate sperm traits (Levitan 1993), intermediate population density (Levitan 1998*a*) and intermediate levels of female fertilization (Levitan 1993, 1998*a*, 1999).

These patterns of sperm traits mirror the interspecific differences in egg traits. *Strongylocentrotus purpuratus*, the species with the highest levels of female fertilization, has small numerous eggs and requires the most sperm to fertilize 50% of a female's eggs, whereas *S. droebachiensis* has the largest and fewest eggs and requires the fewest sperm to fertilize 50% of a female's eggs. Again *S. franciscanus* has intermediate egg traits and an intermediate fertilization performance (Levitan 1993, 1998*a*).

Previous studies have indicated that egg traits in sea urchins represent different points along an adaptive continuum from sperm limitation to sperm competition (Levitan 1993, 1996*a*,*b*, 1998*a*,*b*; Styan 1998). The present study yields the first direct evidence that sperm traits also influence fertilization and trade off against each other. Different combinations of both sperm and egg traits might therefore be selected in order to optimize fertilization under varying environmental conditions.

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