nature

INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

Volume 382 No. 6587 11 July 1996 $10.00

Sperm limitation at sea

Vestigial **control of wing formation**

Star-forming filaments in Orion

Oxygen and evolution

Effects of gamete traits on fertilization in the sea and the evolution of sexual dimorphism

Don R. Levitan

Department of Biological Science, Florida State University, Tallahassee, Florida 32306-2043, USA

THE evolution of egg and sperm[1,2], and more derived forms of sexual dimorphism, is thought to be driven by sperm competition and postzygotic survival; males are limited by fertilizations, females by resources[3]. Evidence of sperm competition comes from internal fertilizers, or cases where sperm are deposited on eggs[4], but in free-spawners, the ancestral mating strategy (refs 1,5 but see ref. 6), females are often sperm limited[7,8]. Laboratory experiments on sea urchins demonstrate that intraspecific differences in gamete attributes, such as egg size, can influence rates of fertilization. Field experiments in which gametes are released and recaptured demonstrate that the influence of gamete traits on fertilization is not overwhelmed by sea conditions, and that variation in gamete traits can have important fitness consequences. These results suggest a new mechanism for the evolution of anisogamy and sexual dimorphism, in which sperm limitation is important, and natural selection for enhanced fertilization acts on females as well as males.

Given that sperm limitation is ubiquitous among free-spawners[7], it is possible that variation in egg traits influences female fertilization success[9]. Laboratory experiments on the sea urchin *Strongylocentrotus franciscanus* indicate that male–female pairs differ 25-fold in the amount of sperm needed to fertilize 50% of eggs (the f_{50} value). Although there is seasonal variation in gamete quality in echinoids[10], most of the variation in f_{50} can be explained by the combined effects of mean egg size and the quality of sperm (Table 1).

In a second experiment, the size of an egg influenced its probability of fertilization, even within a single clutch. When sperm were limiting, larger eggs were fertilized preferentially (Fig. 1).

Enhanced fertilization may be a result of more frequent sperm collisions with larger eggs[9], or arise from other aspects of egg quality, such as age, number, or distribution of sperm-receptor sites, that may covary with egg size. The correlation of larger egg sizes with higher fertilization rates has now been documented among *Strongylocentrotus* species[9], within species, and within individual females.

Because turbulent water movement can reduce the probability of fertilization to almost zero, seconds after gamete release[11], the influence of gamete traits on fertilization may be swamped by environmental conditions, and so the fitness consequences of variation in gamete traits might be minimal. Paired laboratory and field experiments performed in Barkely Sound, British Columbia, Canada, tested whether individual differences in fertilization could be detected under field conditions. Laboratory tests determined the f_{50} of gametes from a single male and female. Additional samples from the same male and female spawn were released into the ocean (3–8 m depth) under natural conditions. Sperm were released first, with eggs being released into the sperm plume after a predetermined sperm dispersal time. After two minutes, the free-drifting eggs were recaptured and inspected for evidence of fertilization (Fig. 2*a*). Daily estimates of turbulent mixing were calculated from video images of the change in area (height and width) of the sperm cloud. Current velocity of the water at the height of gamete release ranged from 0 to 85 cm s^{-1} during the experiments (sampled at 0.5-s intervals). As the sperm dispersal time increased, average efficiency of fertilization decreased (Fig. 2*a*). However, dispersal time accounted for only

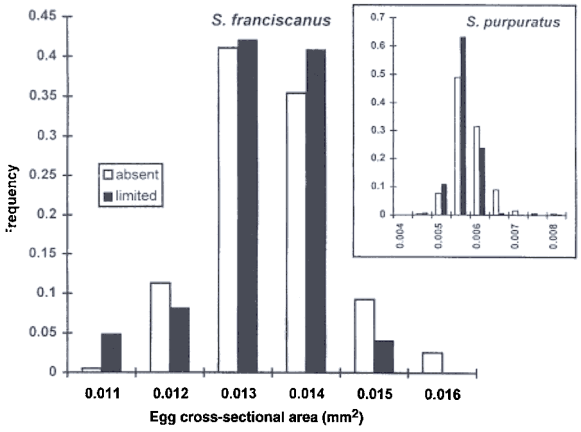


FIG. 1 Size distribution of unfertilized eggs within a single female, when sperm were absent or limiting. In a subset of the laboratory experiments detailed in Table 1, unfertilized eggs were measured that were randomly selected from experimental vials that had approximately 50% of eggs fertilized, and from vials, from the same female, that had no eggs fertilized ($N = 3$ females). When approximately 50% of eggs were fertilized (limiting), the distribution shifted towards smaller eggs than when none were fertilized (absent) ($G = 18.9$, $P < 0.001$). This result indicates that, when sperm are present but not concentrated enough to fertilize all eggs (limiting), larger eggs are preferentially fertilized. At higher sperm concentrations, all eggs, regardless of size, were fertilized (not shown), indicating that smaller eggs can be fertilized if sperm are abundant. Inset, a second species, *S. purpuratus*, demonstrated a similar relation of larger eggs being preferentially fertilized under conditions of sperm limitation ($G = 65.6$, $P < 0.001$, $N = 3$).

21% of the variation in fertilization efficiency, so the remainder must be attributable to fluctuating environmental conditions or gamete quality.

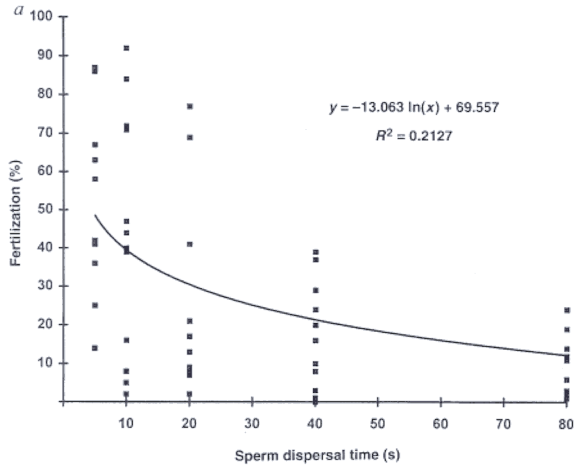
An overall field measure of gamete performance for a particular male–female pair on a particular day is the sperm dispersal time at which 50% of the eggs are fertilized (the t_{50} value). Laboratory gamete performance (f_{50}) explained 55% of the variation in field fertilization rates (t_{50}; Fig. 2*b*); gametes that performed well in the laboratory also performed well in the field. A multiple regression incorporating both the sperm diffusion data and the f_{50} data explained 61% of the daily variance in t_{50}.

These results provide at least two insights into the evolution of gamete traits. First, selection on gametes for enhanced fertilization success will be intense not only for males, but also for females. Second, the evolution of anisogamy and gender have been influenced by sperm limitation and selection for enhanced fertilization, rather than simply by sperm competition and postzygotic survivorship.

Theories of the evolution of anisogamy and optimal egg size invoke postzygotic factors as the selective agent on egg size[1,2,12,13]. This conclusion is reasonable if there is little variance in female fertilization success. Increasing evidence indicates that female fertilization success varies greatly[7], and my results indicate that, in free-spawning animals, egg size may be under intense selection for higher rates of fertilization.

Because egg size is an indication of maternal investment, selection on egg size is a function of maternal fitness. Assuming a constant total investment in reproduction, selection for producing a maximum number of eggs will result in vanishingly small eggs, but because lower egg size can decrease both fertilization and postzygotic survivorship[12,13], optimizing selection acts to produce an egg of intermediate size. My data support the hypothesis that sperm limitation can shift this optimal egg size dramatically[9,11] (Fig. 3).

All gender differences in morphology, physiology and behaviour stem from the evolutionary transition from isogamy to



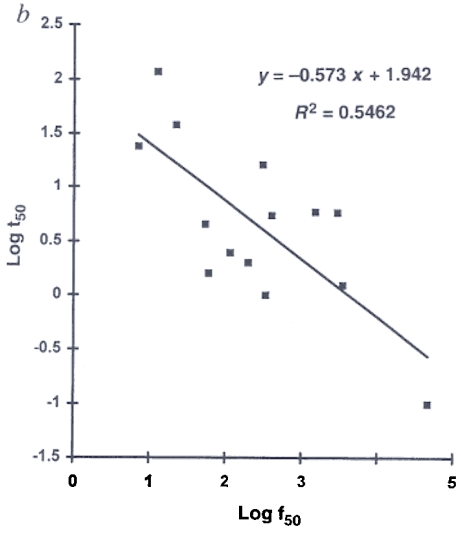


FIG. 2 *a*, Percentage of eggs fertilized as a function of sperm dispersal time. Dry sperm were diluted with filtered sea water mixed with fluorescein dye. Within 1 min of dilution, 5 ml of the sperm–dye solution was released upwards out of a syringe from a height of 0.5 m at a rate of 1 ml s^{-1} (1×10^7 sperm s^{-1}). The mean spawning rate of *S. franciscanus* injected with KCl was 1.12×10^7 sperm s^{-1}. After a predetermined sperm-dispersal time, $\sim 3 \times 10^7$ eggs mixed with dye were released into the centre of the sperm cloud. At 2 min after release, the free-drifting eggs were collected using a submersible pump, and water was sucked through a filter of mesh size 30 μm (ref. 17). The pump was run for 30 s within the gamete cloud and then rinsed out of the cloud for 60 s. Controls indicated that, after the 2-min sperm–egg interaction time, sperm were too diffuse to result in further fertilization, and all fertilization occurred *in situ* rather than in the filter chamber. Laboratory controls also indicated that the concentration of fluorescein used did not influence rates of fertilization. *b*, Time of sperm dispersal (t_{50}) in which 50% of eggs are fertilized in the field as a function of fertilizations in the laboratory f_{50} ($P = 0.00025$; regression remains significant at $P = 0.039$, with the bottom-right data point removed). A high t_{50} or a low f_{50} indicates high-performance gametes. Each data point represents a different day and environmental condition ($N = 14$). On a single day the spawn of one male and female were used for both laboratory and field experiments. Individual urchins were used only once.

TABLE 1 Effect of mean egg size and sperm quality on fertilization

Source of variation	d.f.	s.s.	m.s.	F	P
Egg size	1	5.215	5.215	16.17	0.0011
Male	7	5.255	0.751	2.33	0.0803
Residual	15	4.837	0.322		
Corrected total	24	15.307			

Parameter	Estimate	s.e.	P (parameter = 0)
Intercept	−27.216	7.262	0.0019
Egg size	−11.065	2.546	0.0006
$R^2 = 0.684$			

Analysis of covariance testing the effect of male quality (main effect) and log egg cross-sectional area (covariate) on the log amount of sperm needed to achieve 50% fertilization (f_{50}). Females (24) were paired with one of eight males (2–4 females per male). Each female was only used once. Dry concentrated sperm from a single male was run through eight 10-fold dilutions, and sperm solution (1 ml) was added to a vial with 9 ml filtered sea water containing ~5,000 eggs from a particular female. Percentage fertilization was assayed after 3 h. The f_{50} was calculated for each male–female pair by fitting the fertilization data from the eight serial dilutions to a fertilization-kinetics model[16] (Fig. 3). Abbreviations: d.f., degrees of freedom; s.s., sum of squares; m.s., mean-square; s.e., standard error.

anisogamy. Previous theories have invoked sperm competition and postzygotic fitness as the selective mechanisms driving the evolution of anisogamy[1,2]. Divergent selection on isogamous gametes favoured individuals that produced many small gametes (proto-sperm) able to outcompete other individuals for fertilizations when groups of individuals spawn simultaneously. At the other extreme, selection favoured individuals that produced large proto-ova that would optimize postzygotic success when fertilized by a proto-sperm.

Under the sperm-limitation hypothesis, divergent selection on isogamous gametes favoured individuals that produced many small gametes (proto-sperm), increasing the chance of finding another gamete in a diffuse medium, rather than the chance of outcompeting other sperm (see ref. 15 for a similar, but group-selectionist, argument). Individuals producing large gametes (proto-ova) were selected to increase the probability of fertilization and also postzygotic survivorship. Although both theories predict divergent gamete sizes, they differ in the mechanism driving anisogamy. In the extreme, sperm-limitation theory suggests that sperm competition is a derived condition, resulting from selection for internal fertilization as an escape from sperm limitation. Alternately, sperm limitation and sperm competition represent two ends of a continuum that may vary among taxa, habitats and spawning conditions[8]. Evidence supporting sperm limitation rather than sperm competition is likely to be found in female life-history characters, because both theories predict selection for many small sperm. Female characteristics that may have evolved to increase the probability of fertilization include adaptations in behaviour (aggregation and synchrony), morphology (egg size and structures on eggs, such as jelly coats or accessory cells, that may increase sperm collisions), and physiology (sperm chemoattractants)[7]. With the exception of egg size, these adaptations are independent of postzygotic survivorship, and would not have evolved under sperm-abundant (competitive) conditions. The sperm-limitation hypothesis does not obviate the importance of postzygotic factors, but allows for a more complete understanding of gamete evolution and the evolutionary trajectories of mating systems that later evolved from a free-spawning strategy. □

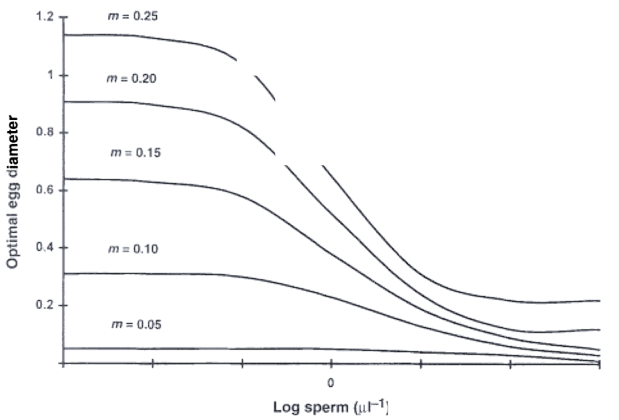


FIG. 3 The optimal egg size, which maximizes the number of successfully settling offspring in a clutch of eggs, varies as a function of ambient sperm concentration and the daily postzygotic larval mortality (m) (ref. 14). Under sperm limitation, optimal egg size increases and becomes more sensitive to changes in postzygotic mortality. The number of settling offspring is calculated as follows. Egg number is determined by 1,000/egg volume (mm^3), which is the number of eggs produced by 1 ml of egg material. Zygote number is the product of egg number, and the proportion of eggs fertilized (ϕ_∞) calculated from a fertilization-kinetics model[16] based on sperm (S_0; sperm per microlitre) and egg (E_0; 0.01 μl^{-1}) concentration, sperm–egg interaction time (τ; 10 min), the sperm–egg collision rate (β_0 ($mm^3 s^{-1}$); the product of egg cross-sectional area (mm^2) and sperm velocity (0.130 mm s^{-1}; ref. 9)), and the fertilization rate constant (β ($mm^3 s^{-1}$); 0.0000952; ref. 9): $\phi_\infty = 1 - \exp(-\beta S_0/\beta_0 E_0(1 - e^{-\beta_0 E_0 \tau}))$. The number of settling zygotes (N_s) is calculated from the number of zygotes produced (N_z) and larval mortality: $N_s = N_z e^{(-tm)}$ (modified from ref. 12), where the larval mortality is calculated by the length of the larval period (t (days) = 18.987 egg volume $(mm^3)^{-0.1156}$; ref. 14) and the daily mortality (m; range based on empirical range noted for the genus *Strongylocentrotus*; ref. 18). The plot represents the egg size with the peak (optimal) number of settling offspring for each combination of larval mortality and sperm concentration.

Received 3 October 1995; accepted 23 May 1996.

1. Parker, G. A. in *Sperm Competition and the Evolution of Animal Mating Systems* (ed. Smith, R. L.) 1–59 (Academic, New York, 1984).
2. Maynard Smith, J. & Szathmary, E. *The Major Transitions in Evolution* (W. H. Freeman, Oxford, 1995).
3. Bateman, A. J. *Heredity* **2,** 349–368 (1948).
4. Arnold, S. J. *Am. Nat.* **144,** S126–S149 (1994).
5. Wray, G. A. in *Ecology of Marine Invertebrate Larvae* (ed. McEdward, L.) 412–448 (CRC, Boca Raton, FL, 1995).
6. Rouse, R. & Fitzhugh, K. *Zool. Scripta* **23,** 271–312 (1994).
7. Levitan, D. R. in *Ecology of Marine Invertebrate Larvae* (ed. McEdward, L.) 123–156 (CRC, Boca Raton, FL, 1995).
8. Levitan, D. R. & Petersen, C. *Trends Ecol. Evol.* **10,** 228–231 (1995).
9. Levitan, D. R. *Am. Nat.* **141,** 517–536 (1993).
10. Benzie, J. A. H. & Dixie, P. *Biol. Bull.* **186,** 153–167 (1994).
11. Denny, M. W. & Shibata, M. F. *Am. Nat.* **134,** 859–889 (1989).
12. Vance, R. R. *Am. Nat.* **107,** 339–352 (1973).
13. Smith, C. C. & Fretwell, S. D. *Am. Nat.* **108,** 499–506 (1974).
14. Levitan, D. R. *Am. Nat.* **148,** 174–188 (1996).
15. Scudo, F. M. *Evolution* **21,** 285–291 (1967).
16. Vogel, H., Czihak, G., Chang, P. & Wolf, W. *Math. Biosci.* **58,** 189–216 (1982).
17. Mundy, C. N. *et al. Biol. Bull.* **186,** 168–171 (1994).
18. Rumrill, S. S. *Ophelia* **32,** 163–198 (1990).

ACKNOWLEDGEMENTS. I thank H. Brook, J. Dalby, D. Levitan and A. Netting for laboratory and field assistance, and T. McGovern, D. Simberloff and J. Travis for comments. This work was supported by Bamfield Marine Station and the NSF.

CORRESPONDENCE and requests for materials should be addressed to the author (e-mail: levitan@bio.fsu.edu).