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Variation in paternity in the field cricket *Teleogryllus oceanicus*: no detectable influence of sperm numbers or sperm length

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Recent attention has focused on the role that sperm competition may play in the evolution of sperm morphology. Theoretical analyses predict increased sperm size, decreased sperm size, and no change in sperm size in response to sperm competition, depending on the assumptions made concerning the life history and function of sperm. However, although there is good evidence that sperm morphology varies widely within and between species, the adaptive significance of this variation has not been examined. Here we document significant intraspecific variation in sperm length in the field cricket, *Teleogryllus oceanicus*. Sperm length did not influence the rate of migration of sperm from the spermatophore to the female's spermatheca. We performed sperm competition trials in which we varied the numbers of sperm transferred by each of two males that differed in the length of sperm they produced. Neither sperm length nor the number of sperm transferred influenced paternity. The same results were obtained using two different methods for assigning paternity. The distribution of paternity across a female's mates was highly variable, with frequently one, or more in the case of females mated to four males, principal sire. There were no mating order effects on paternity. These data show that sperm do not mix randomly in the female's spermatheca. We discuss several alternative explanations for the patterns of paternity observed. *Key words:* crickets, paternity, sperm length, sperm competition success, sperm numbers, *Teleogryllus oceanicus*. *[Behav Ecol 14:539–545 (2003)]*

Sperm competition, the competition between sperm from two or more males for the fertilization of a given set of ova (Parker, 1970, 1998), is now widely recognized as a pervasive force generating selection on male reproductive behavior, morphology and physiology (Birkhead and Møller, 1992, 1998; Simmons, 2001b; Smith, 1984). Recent theoretical analyses have begun to explore how sperm competition might influence the evolution of sperm morphology (Ball and Parker, 1996, 1998; Kura and Nakashima, 2000; Parker, 1993; Parker and Begon, 1993).

Parker and his colleagues' game theory approach has generated a number of predictions specific to the fertilization processes and to assumptions concerning the relationship between sperm morphology and parameters such as sperm swimming speed and longevity. For external fertilizers, Ball and Parker (1997) envisaged a continuous process during which fertilization occurs rapidly on spawning, and sperm maximize swimming speed at the expense of longevity to ensure high rates of collision with fertilizable eggs. These models predict that sperm length should increase with sperm competition intensity (number of males in competition) if sperm longevity decreases with sperm length. If sperm longevity increased with sperm length, however, sperm length should decrease with increasing sperm competition intensity (Ball and Parker, 1997). Stockley et al. (1997) found a trade-off between sperm length and longevity across species of fish, predicting a positive relationship between sperm competition intensity and sperm length. However, contrary to prediction, they found that sperm length decreased with sperm competition intensity. Although Balshine et al. (2001) found a positive relationship between their measure of sperm competition and sperm length across species of African cichlids, they had no information regarding the relationship between sperm length and longevity, so that it is unclear in which direction sperm length is predicted to change in this group.

For internal fertilizers, Parker's (1993) theoretical analysis predicted that sperm size should remain small irrespective of sperm competition risk (the probability that females mate with more than one male), unless some special circumstance is advocated. For example, sperm competition could favor increased sperm size if (1) ejaculate size can only increase by increased sperm size, (2) the competitive benefits of sperm size increase with the number of sperm in competition, (3) sperm size effects survival and sperm competition risk increases with female remating interval, or (4) sperm size increases competitive ability at the expense of survivorship, but sperm competition risk decreases with female remating interval. Despite the need for special circumstance, positive relationships between sperm length and sperm competition risk have been found across butterflies (Gage, 1994), birds (Briskie et al., 1997), and some mammals (Gomendio and Roldan, 1991) but not others (Hosken, 1997).

Thus, the general significance of sperm competition for the evolution of sperm morphology remains unclear. Theoretical studies predict positive, negative and no effects of sperm competition on sperm size, depending on the underlying assumptions made. These assumptions have rarely been evaluated in empirical studies that report associations between sperm competition and sperm length. This means that reported associations between sperm competition and sperm length may not be taken as evidence for a direct causal relationship. Indeed, in birds the influence of sperm competition on sperm length seems to be indirect via its

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influence on the length of sperm storage structures in the female reproductive tract (Briskie et al., 1997). To draw firm conclusions, we need more information on how sperm morphology influences sperm life-history and function.

There is some evidence that variation in sperm motility can influence fertilization success (Birkhead et al., 1999; Levitan, 2000). Moreover, in sea urchin, there is a trade-off between sperm velocity and longevity (Levitan, 2000). Nevertheless, although there is now good evidence for widespread within species variation in sperm size (Morrow and Gage, 2001a; Simmons et al., 1999; Ward, 1998;), unfortunately, our knowledge of the significance of this variation for traits such as sperm motility and longevity is extremely limited. A trade-off between sperm length and longevity has been shown across species of fish (Stockley et al., 1997). Gomendio and Roldan (1991) reported a positive association between sperm length and swimming speed across just five species of mammal. Although detailed analyses of flagella movements do suggest that the longer the sperm flagellum, the greater the propulsive forces generated (Katz and Drobnis, 1990), whether this relationship represents a general phenomenon across mammals awaits the collection of data from an increased number of species and an appropriate comparative analysis. The same conclusion was reached by Bressac et al. (1991) in their study of the two sperm morphs of the *Drosophila obscura* species group; long sperm have higher beat frequencies and wave propagation velocities than do short sperm. However, in this case long and short sperm have different functions (Snook and Karr, 1998; Snook et al., 1994), and behavioral differences between sperm types may be related to their functions rather than to their lengths. Finally, male fishfly, *Parachauliodes japonica*, produce bundles of sperm, and bundle size is positively associated with swimming speed (Hayashi, 1998). The swimming speed of sperm bundles may be adaptive because sperm are transferred from an externally attached spermatophore that the female often removes and eats before insemination is completed (Hayashi, 1996). Faster sperm swimming speed, and thus greater sperm transfer, would offer obvious benefits to males producing larger sperm bundles (Hayashi, 1998).

Our knowledge of the significance of variation in sperm size for fertilization success is even more limited. Studies of bulb mites (Radwan, 1996) and nematodes (LaMunyon and Ward, 1998, 1999) have found positive associations between sperm size and competitive fertilization success. However, both of these taxa are unusual in that they have amoeboid-like sperm that move by pseudopodial action. Only one study has examined the importance of sperm length for sperm competition success in a species with more typical flagellate sperm. Morrow and Gage (2001b) used lines of field crickets, *Gryllus bimaculatus*, artificially selected for long or short sperm in sperm competition trials, finding that sperm length had no influence on a male's competitive fertilization success.

Thus, additional studies on the adaptive significance of variation in sperm morphology are greatly needed. Here we examine the influence of sperm length on the fertilization success of the Australian cricket, *Teleogryllus oceanicus*. Typical for crickets, sperm are transferred from an externally attached spermatophore. This allows us to manipulate the number of sperm transferred. First, we examined the influence of sperm length on the rate of transfer of sperm from the spermatophore to the sperm storage organ of the female. Second, we examined the paternity of males with short or long sperm when competing in a two male situation in which the relative numbers of sperm in the sperm storage organ of the female are also manipulated. Finally, by using allozyme markers, we extend our competitive situation to one in which females mate with four males.

METHODS

Animals used in this study were derived from a population collected from Cairns, Northern Queensland, Australia. Approximately 35 field mated females were allowed to oviposit, and the resultant offspring were used to establish a population of approximately 2000–3000 individuals. For these experiments, fourth- to sixth-generation crickets were used. Males and females were isolated from stock culture as penultimate instar nymphs and housed in single sexed cultures until used in experiments.

Assessment of sperm number and sperm length

Spermatophores were removed from the subgenital pouch of sexually mature males and placed onto a clean dry microscope slide that had been marked on its reverse side with a 4×6 grid, each grid square measuring 5×5 mm. The spermatophore was ruptured in 10 μl of distilled water to liberate the sperm mass. Ampulla fragments were removed, and the sperm mass was gently mixed into the water. Additional 10-μl aliquotes of water were added until the sperm mixture was homogeneously spread across the entire grid. Slides were left to dry. By using a compound microscope, the number of sperm contained within a single $\times 20$ amplification field of view (approximately 1 mm^2) were counted from 16 haphazardly selected grid squares. Only sperm heads were counted. The numbers of sperm counted across the 16 grid squares of a slide were highly repeatable ($F_{161,2430} = 7.354$, $p < .001$; repeatability estimate, 0.864). Because each grid square was 25 mm^2, the total number of sperm contained in the spermatophore was estimated as the average of the 16 individual 1-mm^2 counts multiplied by 600 (25 mm^2 per square and 24 squares per grid). From a total of 61 spermatophores, six were devoid of sperm and not included in the analysis.

The process of sperm migration from the spermatophore to the female's spermatheca was examined by experimental removal of spermatophores after copulations by previously unmated females. A single male and female was placed into a small plastic mating box ($7 \times 7 \times 5$ cm) and observed under red light. After copulation, spermatophores were experimentally removed after a randomly assigned preset attachment time of either 5, 10, 15, 20, 30, or 60 min. Females were dissected and the spermathecae removed. Sperm counts were made after the protocol described above. From 105 dissections, five spermathecae were devoid of sperm and not included in the analysis.

We examined variation in the length of sperm contained within spermatophores produced by individual males. Spermatophores were removed from the male's genital pouch and placed onto a clean dry microscope slide. Spermatophores were ruptured in 20 μl of distilled water, and fragments of broken ampulla were removed. The sperm mass was mixed into the water, smeared across the slide, and allowed to dry. Slides were observed under $\times 10$ magnification. For each male, the lengths of 10 individual sperm were measured by using the measurement explorer function of the Optimas Image Analysis software package. Sperm were chosen haphazardly, subject to the condition that they showed no signs of physical damage.

Sperm competition trials

We used two methods for assigning paternity. First we used a morphological marker, white eye (*we*), that arose in our laboratory culture two generations after being brought in from the field. White-eyed individuals were isolated from

stock culture and bred true for two generations. We performed preliminary trials to explore the genetic basis of the *we* mutation. Crosses between white-eyed and wild-type, black-eyed (*be*) males and females produced black-eyed offspring. Crosses between these hybrid individuals produced white-eyed and black-eyed offspring in simple mendelian ratios: For nine crosses, the proportion of white-eyed offspring ranged from 0.18–0.30 with a mean ± SE of 0.24 ± 0.01; ratio of *we*:*be* deviated significantly from the expected 0.25 for only one of the nine crosses, with $p = .01$, which is expected by chance alone given the nine replicate crosses performed and a Bonferroni critical $p = .006$ when the test wide $\alpha = 0.05$. These data indicate that the phenotypic expression of the marker was homozygous recessive. Importantly, the lack of deviation from mendelian ratios in the hatched offspring indicates that there is no differential fertilization associated with sperm carrying the mutation per se, and no differences in embryo viability, making the mutation a suitable neutral marker for paternity studies.

Our second technique used allozyme variation at three polymorphic loci (two alleles at the manose-6-phosphate isomerase locus, two alleles at the phosphoglucose isomerase locus, and four alleles at the β-esterase locus) to determine paternity. Alleles were scored by starch-gel electrophoresis using tris-EDTA-borate buffer. Genotypic frequencies of offspring of known parentage did not deviate from that expected based on mendelian inheritance. The genotypes of animals to be used as parents were predetermined using muscle from one hind femur. The use of allozyme variation at these three loci allowed us to examine the outcome of sperm competition when females mated with more than two males, and to confirm the patterns observed using the *we* mutation.

In our first experiment, we examined the relative influence of sperm numbers and sperm length on the outcome of sperm competition using the white-eyed marker in a two-male mating trial. White-eyed females were placed into a mating box and observed under red light. A male was introduced, and the pair was allowed to mate. The male was left to guard the female in order to prevent her from removing her spermatophore. After 50 min, the spermatophore of the first male was removed with forceps, and the male was replaced with a second male. After the pair had copulated, the second male's spermatophore was removed with forceps following a randomly assigned, preset attachment time of either 5, 10, 15, 30, 40, or 50 min. Before experimental matings, all males were assessed for sperm length following the protocol described above. We selected pairs of males to mate with each female from the extremes of the sperm length distribution, thus maximizing the differences between males. We found that on average white-eyed males had shorter sperm than did black-eyed males (see results), so that sperm length treatment coincided with the marker used to assign paternity. Nevertheless, we know from our preliminary experiments that the white-eye mutation per se has no influence on fertilization capacity (see above). The mating order was thus either first-male short sperm (*we*) second-male long sperm (*be*) or vice versa. After the female had mated with both males, she was provided with a pad of moist cotton wool in which to oviposit and left for 7 days. Egg pads were incubated for 2 weeks at 25°C, or until no further hatchlings appeared. All offspring were scored for eye color on hatching, and the proportion of offspring sired by the second male to mate, P_2, was calculated. The total number of offspring per family ranged from 14–286 with an average of 123 ± 9.5. Females that produced less than 10 offspring were not included in the analysis.

In our second experiment, we used males differing in genotype at the β-est locus to specifically examine the mechanism of sperm competition. In this experiment, the combined spermatophore attachment time for both first and second males was 60 min. However, spermatophores were experimentally removed so that the ratio of first male to second male attachment times were 50:10, 40:20, 30:30, 20:40, and 10:50. After both matings had been completed, females were provided with a pad of moist cotton wool in which to oviposit and left for 7 days. Pads were incubated, and hatchlings were transferred to single family cages and reared for 2–3 weeks. This period of rearing was required to allow nymphs to reach a size suitable for genotyping. The genotypes of 52 randomly selected offspring from each pairing were scored.

In our final experiment, we used variation at the β-est, *mpi*, and *pgi* loci to assign paternity to four males that had mated to a single female. The experiment consisted of mating six females, each to a different set of four males. Before mating, all males and females were screened for allozyme genotypes using muscle from one hind femur. Genotypes were chosen so that offspring could be assigned unambiguously to each of the female's four mates. Over 200 potential parents were screened to provide appropriate genotypes for the experiment. Males to be used in experiments were also assessed for sperm length following the protocol described above. Females were provided with each male separately. Spermatophores and males were removed 60 min after copulation. No female removed her spermatophore before the 60 min period. All females completed their four copulations within a period of 5 h. They were then provided with a pad of moist cotton wool to oviposit and left for 7 days. Egg pads were incubated, and hatchlings were transferred to single family boxes and reared for 2–3 weeks before genotyping. A total of 100 randomly selected offspring were genotyped for each female.

RESULTS

Sperm numbers and sperm length

There was no significant difference in the numbers of sperm contained within spermatophores produced by white-eyed or black-eyed males (mean number of sperm per spermatophore: *we*, 51,501 ± 5576; *be*, 42,825 ± 4632; $t = 1.13$; $df = 52$; $p = .26$). The rates of sperm migration from spermatophore to spermatheca did not differ between male phenotypes (Figure 1). The number of sperm in the spermatheca increased with spermatophore attachment time (ANOVA on log-transformed sperm counts: $F_{1,47} = 76.67$, $p < .001$) and was not influenced by male phenotype ($F_{1,47} = 1.65$, $p = .205$) and there was no interaction ($F_{1,47} = 0.78$, $p = .380$). Thus, our manipulation of spermatophore attachment time provided predictable variation in the relative numbers of sperm transferred by competing males.

We found significant between male variation in the length of sperm contained in a single ejaculate. The variance between males was greater than the variance between 10 sperm produced by the same male ($F_{200,1809} = 28.65$, $p < .001$; repeatability estimate, 0.965). Thus, males varied predictably in the length of sperm they produced. We also assessed the repeatability of mean sperm-length differences across three successive ejaculates of a subset of 12 *be* males. The variance between males was significantly greater than the variance between ejaculates of the same male ($F_{11,\ 24} = 2.90$, $p = .014$; repeatability estimate, 0.656). Some of the variation in sperm length between males was associated with differences in eye color; on average *we* males had shorter sperm than did *be* males (*we*, 1.098 ± 0.003 mm; *be*, = 1.137 ± 0.002 mm; $t = 9.68$, $df = 206$, $p < .001$). Thus, *we* males transferred the same number of shorter sperm than did *be* males, allowing us to

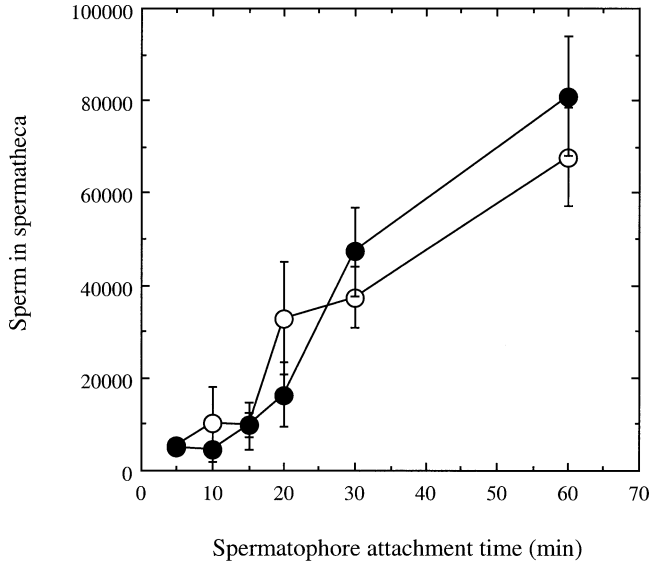

Figure 1
The mean (±SE) number of sperm recovered from a female's spermatheca after varying durations of spermatophore attachment for both wild-type black-eyed (filled symbols) males and white-eyed (open symbols) males.

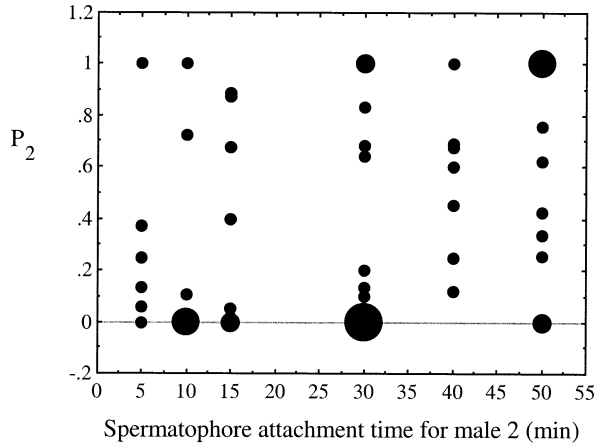

Figure 2
The proportion of offspring sired by the second male to mate (P_2) in relation to the spermatophore attachment duration of the second male, when the first male's spermatophore had been attached for 50 min (increasing size of symbols represents increasing numbers of overlapping data).

assess the relative importance of sperm numbers and sperm length in determining paternity.

Sperm competition trials

Morphological marker

Our experimental design was such that we had two groups of females, those mated first to a male with short sperm and a 50-min spermatophore attachment, followed by a male with long sperm and spermatophore attachments that varied from 5–50 min. Conversely, females were mated first to a male with long sperm and a 50-min spermatophore attachment, and second to a male with short sperm and spermatophore attachments that varied from 5–50 min. If sperm numbers influence fertilization success, we would expect to see an increase in P_2 with increasing spermatophore attachment time. If longer sperm confer a fertilization advantage on males, we would expect to see an interaction between spermatophore attachment time and sperm length of the second male; P_2 should rise more rapidly with spermatophore attachment time when second males had longer sperm than did the female's first mate, compared with second males that had shorter sperm than did the female's first mate. Our prescreening ensured that pairs of competing males differed in sperm length (paired $t = 6.56$, df $= 47$, $p < .001$). However, neither spermatophore attachment duration of the second male (≈sperm number) nor his sperm length influenced paternity (Table 1, Figure 2). Importantly, there was no significant interaction.

Table 1
ANCOVA examining the influence of spermatophore attachment time (≈sperm number) and relative sperm length (shorter or longer) on the arcsine transformed proportion of offspring sired by the second male to mate with a doubly mated female

Source	Mean squares	df	F	p
Sperm number	0.8033	1	2.589	.115
Sperm length	0.0949	1	0.306	.583
Number × length	0.0019	1	0.006	.937
Error	0.3103	46		

Allozyme markers

In our previous experiment, females varied in the total numbers of sperm received from their two mates, as well as the relative numbers from each male. In our two male experiment using allozyme markers, we held constant the total spermatophore attachment time, and thus total number of sperm received by the female, but varied only the relative proportions of sperm in order to more accurately assess the basic mechanism of sperm competition. Because sperm appear to be transferred at a constant rate (Figure 1), a model of random sperm mixing would predict a positive relationship between the ratio of first-male sperm to second-male sperm and P_2, with a slope of approximately 1.0 (Parker et al., 1990). However, consistent with our previous experiment using morphological markers, we found no significant influence of spermatophore attachment time for the second male on his paternity ($F_{1,20} = 1.29$, $p = .270$) (Figure 3). These data suggest that sperm are not randomly mixed in the sperm stores or used in proportion to their representation.

In our final experiment, we assessed the paternity of four males competing for fertilizations within each of six females. Across females, all four males obtained, on average, an equal proportion of offspring (Friedman's $\chi^2 = 2.28$, df $= 3$, $p = .52$), indicating that there were no significant mating order effects. However, within individual females paternity deviated from the 25% for each male expected by a model of random sperm mixing (Table 2). We note that the data in Table 2 might suggest, on average, a higher paternity for the first male to mate. However, a previous experiment, in which the paternity of single focal males were assessed when females mated four times ($N = 32$), similarly found no order effects and no tendency for first males to gain priority (Simmons, 2001a).

There was no significant effects of sperm length on paternity. The proportion of offspring sired by males 1–4 were not independent, so to avoid pseudo replication, we calculated separate Spearman rank correlations between sperm length and paternity for each female. There were no

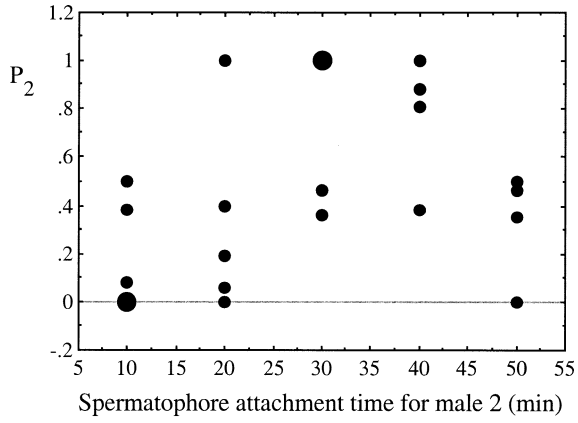

Figure 3
The proportion of offspring sired by the second male to mate (P_2) in relation to the spermatophore attachment duration of the second male. The first male's spermatophore had been attached for 60 min minus the second male's attachment duration, so that the total numbers of sperm received by the female from both males remained constant across all matings (increasing size of symbols represents increasing numbers of overlapping data).

consistent patterns in paternity relative to the sperm lengths of competing males (Figure 4). Spearman rank correlations between sperm length and paternity ranged from 1.0 to −0.95, with a mean that did not differ from zero, 0.125 ± 0.312, $N = 5$ (for one female, the sperm length of one male could not be assessed, and for another female, none of the males could be assessed).

DISCUSSION

Consistent with previous studies (Morrow and Gage, 2001a; Simmons et al., 1999; Ward and Hauschteck-Jungen, 1993), we found significant and highly repeatable differences between males in sperm length. The rate of transfer of sperm from the spermatophore to the female's spermatheca was not influenced by the length of sperm produced by a male; white-eyed males had significantly shorter sperm than did black-eyed males, yet their sperm accumulated in the female's spermatheca at the same rate. Neither did we find an impact of sperm length on a male's fertilization success. By using the morphological marker to assign paternity, short-spermed white-eyed males had the same competitive fertilization success as that of long-spermed black-eyed males. One of Parker's (1993) special circumstances under which an increase in sperm length could be favored by sperm competition was if the competitive benefits of sperm size increased with the number of sperm in competition. However, we found no significant interaction between differences in sperm length of competing males and the total numbers of sperm competing for fertilizations. If *T. oceanicus* conformed to Parker's special circumstance, we would have expected longer-spermed males to have a competitive advantage when both males had transferred a complete complement of sperm compared with when one male had transferred only a fraction of his sperm complement. Moreover, by using allozyme markers to assign paternity to four black-eyed males in competition, we similarly failed to find an influence of relative sperm lengths on fertilization success, even though four times the amount of sperm were in competition for eggs.

Morrow and Gage (2001b) also failed to find an effect of sperm length on fertilization success by using lines of crickets artificially selected to have long or short sperm. Otronen et al. (1997) found that male dung flies, *Scatophaga stercoraria*, producing longer sperm were more likely to have sperm stored in the female's spermathecae than were males producing shorter sperm. However, they did not examine the effects, if any, of differential storage on fertilization success. Subsequent microevolutionary experiments (Hosken and Ward, 2001; Hosken et al., 2001) suggest that sperm length may not be important in sperm competition for this species, because experimental removal of sperm competition via enforced monogamy resulted in decreased sperm production but had no influence on the length of sperm. Similar experiments with *Drosophila melanogaster* have also failed to find an evolutionary response in sperm length to the experimental removal of sperm competition (Pitnick et al., 2001). Finally, Simmons and Kotiaho (2002) found very little additive genetic variance in sperm length of dung beetles, *Onthophagus taurus*, consistent with patterns of variation seen in traits subject to stabilizing rather than directional selection (Houle, 1992; Pomiankowski and Møller, 1995). Together,

Table 2
Number of offspring sired by each male when females mated multiply

	Offspring sired				
Female	First male	Second male	Third male	Fourth male	χ^2
1	8	52	18	22	43.0*
2	81	8	8	3	167.9*
3	13	41	10	36	29.8*
4	59	2	33	6	84.4*
5	66	0	0	34	120.5*
6	54	0	34	12	68.6*
Mean	47	17	17	19	

One hundred offspring were selected at random for allozyme screening.
* $p < .01$.

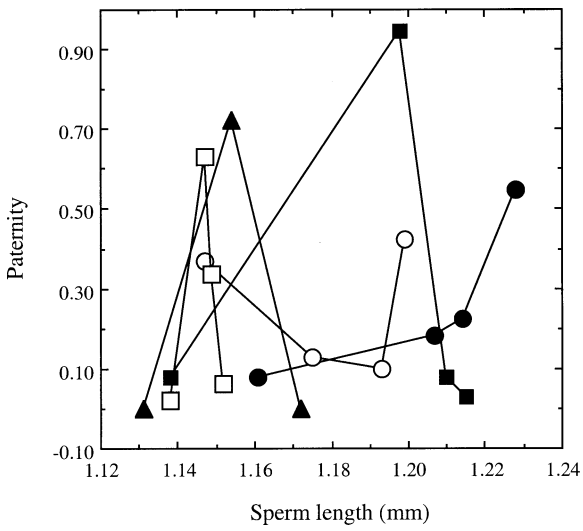

Figure 4
The proportion of offspring sired by each of four males mated to a single female in relation to the length of sperm produced by those males. The different symbols represent the five different females for which data were available. Note that for one female (triangles) the length of sperm produced by one of the competitors was not available and thus his paternity is not shown.

these results suggest that sperm length in these internally fertilizing species may not be important for competitive fertilization success. Nevertheless, there is increasing evidence to suggest that sperm length may coevolve with physical characteristics of the female's sperm storage organs. Studies of birds (Briskie et al., 1997), featherwing beetles (Dybas and Dybas, 1981), *Drosophila* (Pitnick et al., 1999), stalk-eyed flies (Presgraves et al., 1999), and moths (Morrow and Gage, 2000) have all revealed covariation between sperm length and the dimensions of female reproductive tracts. In birds, the positive association seen between sperm competition risk and sperm length arises because sperm competition is associated with an increase in the length of sperm storage tubules, and sperm length appears to track changes in the length of sperm storage tubules (Briskie et al., 1997). The adaptive significance of covariation between sperm and female reproductive tract morphology remains an intriguing problem.

Previous studies suggested that the mechanism of sperm competition in *T. oceanicus* was one of sperm mixing; mating order effects were absent, and the pattern of mixed paternity persisted across both two male and four male matings (Simmons, 2001a). Data presented in the current study allow us to look more closely at the mechanism of sperm competition in this species. In contrast to other crickets (Parker et al., 1990; Sakaluk and Eggert, 1996; Simmons, 1987), it is clear that sperm do not mix randomly in storage because we failed to find a significant effect of the relative numbers of sperm transferred by two males on their share of fertilizations. This result was replicated using two methods for assigning paternity. In both cases, there was a positive trend for increased P_2 with increases in the numbers of sperm transferred by the second male, but these trends were weak and insignificant because values of P_2 were highly variable, with many males obtaining none or all of the fertilizations. It could be that some spermatophores failed to function or contain sperm, so that sperm from one or other male were not represented in the female's sperm store. However, if this were the case, we would expect to have found equivalent frequencies of failed sperm transfer in our sperm counts, which was not the case. In our dissections, we found 5% of cases in which sperm were not transferred from spermatophore to spermathecae, and 10% of spermatophores collected directly from males were devoid of sperm. In contrast 40% of P_2 values in Figure 2 were equal to zero or 1.0. In our four male mating trials, many males failed to gain significant paternity. Similar high frequencies of zero paternity were also characteristic of four male mating trials in Simmons' (2001a) study.

There are three possible explanations for the patterns of paternity observed in *T. oceanicus*. First, they may simply arise because of nonrandom mixing of sperm in the spermatheca. Harvey and Parker (2000) have shown that bimodal distributions of P_2 values can arise if sperm remain clumped within the female's sperm storage organs. Clumping of sperm from different males in the spermatheca could account for the weak tendency for P_2 to increase with sperm numbers, because longer spermatophore attachments should generated larger clumps of second-male sperm. With sperm clumping and the high variance in P_2 that this generates, very large sample sizes would be required to reveal an effect of sperm numbers on paternity. Conversely, the biological effect size of sperm number would be rather weak, as suggested by our data. Second, the patterns of paternity observed may arise because of intrinsic differences in competitive fertilization success between males that are independent of sperm numbers and sperm length. In domestic fowl (Birkhead et al., 1999) and Atlantic salmon (Vladic and Järvi, 2001), fertilization success is dependent on sperm mobility that varies between males. Finally, the patterns of paternity could represent cryptic female choice. Recent studies of crickets suggest that females mate multiply to avoid the costs of incompatibility, having higher hatching success when mating polyandrously (Simmons, 2001a; Tregenza and Wedell, 1998, 2002). The avoidance of incompatibility requires a mechanism by which females can selectively fertilize their eggs with sperm from compatible males, which could generate a principle sire(s) effect such as that seen in *T. oceanicus*. Female *T. oceanicus* do have a higher hatching success when mated to two different males, although elevated hatching success does not appear to be associated with paternity skewed to particular males (Simmons, 2001a).

In conclusion, sperm morphology, particularly in insects, exhibits rapid and divergent evolution (Simmons, 2001b; Sivinski, 1980, 1984). There is also widespread individual variation in sperm morphology within species (Morrow and Gage, 2001a). Nevertheless, little effort has been made to examine the adaptive significance of this variation. Although we failed to find an effect of sperm length on fertilization success in *T. oceanicus*, in general more studies of selection acting on sperm morphology are required before the growing theoretical base for the possible influence of sperm competition on sperm morphology can be evaluated.

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