Sperm Competition in the Absence of Fertilization in *Caenorhabditis elegans*

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Manuscript received October 13, 1998
Accepted for publication January 28, 1999

ABSTRACT

Hermaphrodite self-fertilization is the primary mode of reproduction in the nematode *Caenorhabditis elegans*. However, when a hermaphrodite is crossed with a male, nearly all of the oocytes are fertilized by male-derived sperm. This sperm precedence during reproduction is due to the competitive superiority of male-derived sperm and results in a functional suppression of hermaphrodite self-fertility. In this study, mutant males that inseminate fertilization-defective sperm were used to reveal that sperm competition within a hermaphrodite does not require successful fertilization. However, sperm competition does require normal sperm motility. Additionally, sperm competition is not an absolute process because oocytes not fertilized by male-derived sperm can sometimes be fertilized by hermaphrodite-derived sperm. These results indicate that outcrossed progeny result from a wild-type cross because male-derived sperm are competitively superior and hermaphrodite-derived sperm become unavailable to oocytes. The sperm competition assays described in this study will be useful in further classifying the large number of currently identified mutations that alter sperm function and development in *C. elegans*.

ALTHOUGH the nematode *Caenorhabditis elegans* usually produces progeny by self-fertilization, genetic analyses are feasible because males will successfully sire outcross progeny after crossing. This preferential production of outcross progeny results from the competitive superiority of male-derived sperm in fertilizing oocytes (Ward and Carrel 1979; LaMunyon and Ward 1995, 1998). The ameboid sperm derived from male worms are larger and can crawl more quickly than hermaphrodite-derived sperm (LaMunyon and Ward 1998). Therefore, large male-derived sperm can displace smaller sperm from their position in the reproductive tract and take precedence during fertilization (Ward and Carrel 1979; LaMunyon and Ward 1998). In typical crosses, hermaphrodite self-fertility is functionally suppressed.

The spermatheca is the normal site of sperm storage and fertilization in the hermaphrodite reproductive tract (Ward and Carrel 1979; Schedl 1997; McCarter *et al.* 1999). The hermaphrodite produces spermatids during the L4 larval stage and then produces only oocytes as an adult (Kimble and Ward 1988). Hermaphrodite-derived spermatids in the ovotestes enter the spermatheca at the onset of ovulation and undergo spermiogenesis to become bipolar motile spermatozoa (Ward and Carrel 1979; Ward *et al.* 1983; Shakes and Ward 1989). Male worms start producing spermatids during the L4 larval stage and production continues throughout adult life (Wolf *et al.* 1978). Male-derived spermatids undergo spermiogenesis after ejaculation and this conversion is dependent on an unknown substance(s) in seminal fluid (Ward and Miwa 1978; Shakes and Ward 1989; LaMunyon and Ward 1994). These spermatozoa must migrate from the site of ejaculation just under the vulva through the uterus to the spermatheca. Fertilization occurs in the spermatheca when a single spermatozoon enters a receptive oocyte, apparently by engulfment (Ward and Carrel 1979; McCarter *et al.* 1999). Sperm penetration can occur anywhere on the surface of the oocyte (Goldstein and Hird 1996). Following fertilization, the egg completes meiosis, secretes a shell, passes through the uterus, and is laid before hatching.

In this study, we used mutants that are spermatogenesis defective (*spe*) or fertilization defective (*fer*) to examine the relationship between sperm competition and fertilization. All *spe* and *fer* mutant hermaphrodites display a self-sterile phenotype. Furthermore, such *spe* or *fer* mutations render mutant males unable to sire cross progeny despite normal copulatory behavior. Most *spe* and *fer* mutants cause defects during spermatogenesis so that spermatozoa either do not form or have cytologically obvious defects (reviewed in L'Hernault 1997). A few *spe* and *fer* mutants form spermatozoa that are ultrastructurally indistinguishable from wild type and appear to function normally in all assayed processes except for sperm-egg interactions (L'Hernault 1997; Singson *et al.* 1998). Here we show that crossing such mutant males to hermaphrodites causes sperm competition to occur in the absence of fertilization. Therefore, sperm competition and fertilization are mechanistically independent and sperm precedence is not due to an enhanced ability of male-derived sperm to penetrate

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oocytes. The effectiveness of sperm competition is apparently dependent on normal sperm motility and the number of spermatozoa transferred from the male to the hermaphrodite during crosses. This dependence on sperm number was shown in an assay that allows the detection of hermaphrodite self-fertilization in the presence of relatively few male-derived spermatozoa. This assay also indicates that sperm precedence is not always complete. We conclude that the predominantly outcross progeny produced by a hermaphrodite following a normal cross are due to the competitive superiority of male-derived spermatozoa and that hermaphrodite-derived spermatozoa lose access to receptive oocytes. Finally, the sperm competition assays developed for this study will aid in distinguishing between *spe* and *fer* mutants that affect fertilization and those that affect other aspects of sperm function and development.

MATERIALS AND METHODS

Worm strains and culture: *C. elegans* culture and manipulation was essentially as described by Brenner (1974). Strain N2 was considered wild type. Crosses of three or four males to single hermaphrodites were conducted on petri plates containing agar seeded with *Escherichia coli* strain OP50. Worms were raised and crossed at 16° or 25°. The permissive temperature is 16° and the nonpermissive temperature for all temperature-sensitive (ts) mutations used in this study is 25°. The paternity of the progeny produced by all crosses was determined with the aid of the recessive morphological marker *dpy-5(e61)*. Worms homozygous for *dpy-5* are shorter and fatter than wild-type worms. In many experiments, the mutation *him-5(e1490)* was used because it produces males at high frequency with no adverse effects on sperm (Nelson and Ward 1980; L'Hernault and Roberts 1995). Genetic markers and balancer chromosomes are listed according to linkage groups as follows: LGI—*dpy-5(e61)*, *spe-8(hc53)*, *spe-9(eb19)*, *spe-9(hc52ts)*, *spe-9(hc88ts)*, *spe-13(hc137ts)*, *fer-1(hc13ts)*, *fer-14(hc14ts)*, *sDp2*; LGIII—*daf-4(e1364)*; LGV—*him-5(e1490)*. Descriptions of all mutations can be found in L'Hernault (1997) or Hodgkin (1997).

Analysis of sperm competition and fertility: An experiment to test the ability of *him-5* males to produce outcross progeny and suppress hermaphrodite fertility was conducted at 16°. Twelve L4 larval stage *dpy-5* hermaphrodites were individually picked to plates with a small spot of *E. coli* that had been seeded the previous day. After being allowed to produce self-progeny for 1 day, the hermaphrodites were transferred to fresh plates and each was crossed to four *him-5* males. Each cross was transferred to a fresh plate for each of the next 4 days. Total progeny counts and the paternity of the progeny produced each day were determined. Dpy worms were scored as self-progeny and wild-type worms were scored as outcross progeny. Paternity was scored after 2 days when the Dpy phenotype could be scored unambiguously. The percentage of self-progeny *vs.* outcross progeny produced for each individual cross on each day was determined. These values were then averaged for all 12 crosses to determine the percentage total progeny from each day.

Experiments designed to test the ability of various mutants to participate in sperm competition were conducted at 25°. L4 larval stage *dpy-5* hermaphrodites were crossed with three or four male worms that were homozygous for *him-5(e1490)* and the mutations *spe-9(eb19)*, *spe-13(hc137ts)*, *fer-14(hc14ts)*, or *fer-1(hc13ts)* for ~24 hr. Adult worms were then removed from the plates, and progeny were scored after 2 days. Because *spe-9(eb19); him-5(e1490)* worms are nonconditionally sterile, this stock is maintained with a transgene containing wild-type copies of *spe-9* in the YAC Y47H9 linked to *rol-6(su1006)* (Singson *et al.* 1998). This stock segregates ~85% non-Roller males that are sterile and ~15% non-Roller mosaic males that are fertile. To decrease the chance of inadvertently including a mosaic worm that had a wild-type copy of *spe-9* in the germline, only three males were used for crosses with *spe-9(eb19)* males. The appearance of transgenic F_1 Rol progeny indicated that a male was non-Spe-9 and these crosses were excluded from subsequent analysis. Unmated controls and controls crossed to *him-5* males were included in every experiment and examined in parallel to control for variables such as food quality, the steady-state temperature of incubators, the general health of worm stocks, and other factors. While overall fertility rates were variable, they were consistent within each experiment relative to matched controls.

Worms with a mutation in the *spe-8* gene provide a sensitive assay for copulation and seminal fluid transfer. *spe-8(hc53)* hermaphrodites are sterile due to a defect in spermiogenesis (L'Hernault *et al.* 1988; Shakes and Ward 1989). Spermatids in these mutant hermaphrodites do not become motile and are quickly lost from the reproductive tract (L'Hernault *et al.* 1988). However, these *spe-8* hermaphrodite-derived spermatids will become spermatozoa when exposed to male seminal fluid transferred to the hermaphrodite during copulation. Such *spe-8* hermaphrodite-derived spermatozoa can successfully fertilize oocytes (Shakes and Ward 1989). Single *spe-8(hc53) dpy-5(e61)* L4 hermaphrodites were crossed to three or four male worms raised at 16° or 25°. Each cross was transferred to a fresh plate daily for a minimum of 3 days. The paternity of progeny was determined by scoring for the Dpy phenotype. *spe-9*, *spe-13*, *fer-14*, and *fer-1* males can transfer functional sperm activator(s) as determined by the production of *spe-8* self-progeny or as previously noted for *fer-1* (LaMunyon and Ward 1994, 1995). In many crosses, neither self-progeny nor outcross progeny were produced, indicating that successful sperm and/or seminal fluid transfer had not occurred; such crosses were not included in subsequent analyses. There are several possible explanations for such "failed" crosses. First, as noted by others (Hodgkin 1983), N2 and mutants derived from N2 (all the male mutants employed in this study were derived from N2) do not copulate or transfer sperm with 100% efficiency. Another likely reason for unsuccessful crosses is the absence of competent *spe-8* spermatids within the hermaphrodite at the time seminal fluid transfer occurred during copulation. The spermatids produced by *spe-8* hermaphrodites are pushed out of the spermatheca (where fertilization occurs) after ovulation commences, so they cannot maintain their position within the reproductive tract. Consequently, the successful transfer of seminal fluid during mating must occur prior to or shortly after ovulation begins for a *spe-8* hermaphrodite to be able to produce self-progeny. Alternatively, some failed mates could be due to the complete suppression of *spe-8* hermaphrodite self-fertility by mutant sperm competition. The increased proportion of failed mates seen for the *spe-9(eb19)* experiment relative to other experiments is likely due to the decreased number of males in each mate (three vs. four in all other experiments). For this experiment, mates where transgenic worms were present in the brood were not included in subsequent analyses (Table 2).

Sperm transfer experiments: Sperm transfer and tracking experiments will be described in detail elsewhere (K. L. Hill and S. W. L'Hernault, unpublished results). It was discovered that male-derived sperm labeled by incubating worms in the fluorescent vital dye SYTO 17 (Molecular Probes, Eugene, OR) could be detected in the reproductive tract of unlabeled hermaphrodites after mating and these sperm are competent to produce outcross progeny. Although it is difficult to visualize individual SYTO 17-labeled *C. elegans* sperm, the larger sperm produced by *Caenorhabditis remanei* can be individually distinguished after labeling and sperm transfer (K. L. Hill and S. W. L'Hernault, unpublished results). Additionally, if the dye were to label a nonsperm component of the male ejaculate, this component would have to be transported to and preferentially accumulate in the spermatheca. *daf-4(e1364)* hermaphrodites were used as sperm recipients because they have decreased gut granule autofluorescence relative to wild-type worms under rhodamine illumination (K. L. Hill and S. W. L'Hernault, unpublished results). Similar experiments utilizing the dye Nile blue A were previously reported by Ward and Carrel (1979).

RESULTS

Sperm competition in *C. elegans*: After males are added to virgin hermaphrodites, a rapid change from 100% self-progeny to almost 100% outcross progeny can be observed (Figure 1A). In this experiment, the change from production of self-progeny to outcross progeny took ~2 days with intermediate numbers of self-progeny *vs.* outcross progeny seen after 1 day. Of offspring produced, 76% were a result of outcrossing and of the total progeny, only 24% were a result of selfing in this experiment (Figure 1B). As male-derived sperm became depleted, self-progeny were observed with increasing frequency (Figure 1A). Hermaphrodite self-fertility is therefore functionally suppressed as a result of male mating as noted previously (Ward and Carrel 1979).

Participation of sperm in competition is independent of the capacity to fertilize oocytes: We investigated the ability of mutant spermatozoa derived from males to participate in sperm competition with hermaphrodite-derived spermatozoa (Table 1). We examined the number and paternity of progeny produced from crosses after 1 day for the dramatic change in hermaphrodite self-fertility that is illustrated in Figure 1. Young, virgin *dpy-5* hermaphrodites produced an average of 25–44 self-progeny in 1 day depending on the conditions of the particular experiment (see materials and methods). When crossed to *him-5* males, *dpy-5* hermaphrodites produce about half as many self-progeny over this time period although overall fertility is similar to unmated hermaphrodites because of the production of outcross progeny. Crossing *dpy-5* hermaphrodites to *him-5* males carrying the *spe-9*, *spe-13*, or *fer-14* mutations that are defective in fertilization (L'Hernault *et al.* 1988; L'Hernault 1997; Singson *et al.* 1998) also causes suppression of hermaphrodite self-fertility. However, unlike a cross to *him-5* control males, a cross to these *spe* or *fer* mutant males produced no outcross progeny. In contrast to these results, the crossing of male *fer-1* mutants to hermaphrodites does not cause a statistically significant decrease in self-fertility. This result is presumably due to the fact that sperm produced by *fer-1* mutant males do not show normal motility (Ward and Miwa 1978; Argon and Ward 1980; Achanzar and Ward 1997).

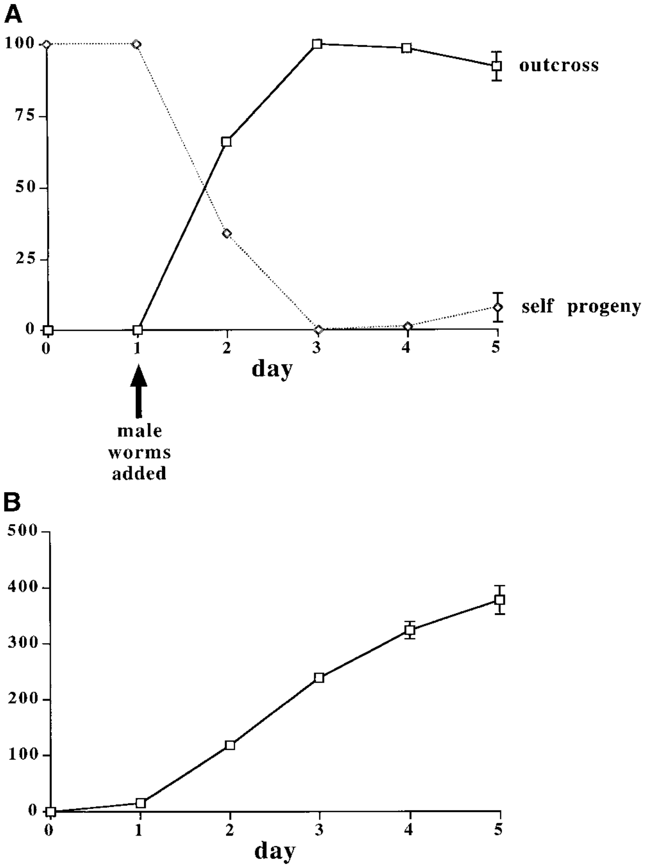

Figure 1.—Sperm competition leads to the production of predominantly outcross progeny. (A) Squares represent the average percentage of total progeny produced each day that were a result of outcrossing. Diamonds represent the average percentage of total progeny produced each day that were a result of selfing. (B) Squares represent the average total number of progeny produced during each day of the experiment. The error bars large enough to be visible on this graph represent the standard deviations of the mean values.

During the course of our mating experiments, we observed an occasional hermaphrodite that produced no progeny and laid only unfertilized oocytes. This suggests that fertile hermaphrodites could be sterilized by being crossed to fertilization-defective *spe-9*, *spe-13*, or *fer-14* mutant males. This outcome was observed in ~6% of crosses when all worms were raised at 25°. This effect was not seen in crosses with wild-type or *fer-1* mutant males. The results of these experiments indicate that sperm competition can occur in the absence of fertilization.

Self-fertilization in the presence of male sperm: *spe-8* mutant hermaphrodites provide a very sensitive assay for crosses because self-fertility is induced by seminal fluid transferred during copulation (see materials and methods). One or more male seminal fluid components apparently activate spermatids produced by a

TABLE 1

Analysis of sperm competition

Male genotype	Self-progeny[a]	Outcross progeny[a]	Total progeny[a]	n	Ability to compete
spe-9					Yes
Not crossed	42 ± 4	0	42 ± 4	17	
him-5(e1490)	24 ± 2***	23 ± 4	47 ± 4	15	
spe-9(eb19); him-5(e1490)	25 ± 3**	0	25 ± 3	14	
spe-13					Yes
Not crossed	27 ± 3	0	25 ± 3	15	
him-5(e1490)	13 ± 2***	5 ± 1	18 ± 2	16	
spe-13(hc137ts); him-5(e1490)	13 ± 3***	0	13 ± 3	17	
fer-14					Yes
Not crossed	28 ± 4	0	26 ± 4	20	
him-5(e1490)	14 ± 3**	7 ± 3	21 ± 4	18	
fer-14(hc14ts); him-5(e1490)	13 ± 2***	0	13 ± 2	21	
fer-1					No
Not crossed	44 ± 4	0	44 ± 4	35	
him-5(e1490)	27 ± 3**	14 ± 3	41 ± 4	35	
fer-1(hc13ts); him-5(e1490)	44 ± 4*	0	44 ± 4	34	

After 1 day, adult worms were removed from plates and progeny were scored after 2 days. All male worms were crossed to *dpy-5(e61)* hermaphrodites. *P, not significant; ** $P \leq 0.01$; *** $P \leq 0.001$. Data compared statistically to unmated controls with Student's t-test.

[a] SE.

spe-8 hermaphrodite so that they become spermatozoa that are competent to fertilize oocytes (L'Hernault *et al.* 1988; Shakes and Ward 1989; LaMunyon and Ward 1995; L'Hernault 1997). The results of crossing various mutant males to *spe-8* hermaphrodites are shown in Table 2. Virgin *spe-8* hermaphrodites are self-sterile. Crossing *spe-8* hermaphrodites to *him-5* males or permissively raised ts mutants that are fully fertile at 16° (*e.g.*, *spe-9*, *fer-1*) results in the production of almost all outcross progeny, as would be expected as a result of sperm competition. However, when these worms are crossed to sterile males, the production of self-progeny sharply increases. Intermediate or mixed outcross/self-broods result from crosses of hermaphrodites to mutant males that are leaky or not fully fertile at permissive temperatures (*e.g.*, *fer-14*, *spe-13*). The production of self-progeny by *spe-8* mutant hermaphrodites indicates that the seminal fluid of all mutant males used in this study contains functional sperm activator(s). Some crosses produced no progeny and were not included in subsequent analyses. The results from these experiments indicate that sperm competition is not absolute and, under the right conditions, some selfing can occur in the presence of male sperm.

Variable amounts of sperm are transferred during mating: The variable amounts of sperm competition seen in the experiments discussed above might depend on the number of male-derived sperm present in the hermaphrodite reproductive tract. Although we observed wild-type mating behavior associated with all of our mutant strains (Liu and Sternberg 1995) over the period of our experiments, it was impossible to determine how many successful copulation events took place. Therefore, we examined the amount of sperm that could be transferred from *him-5* males to hermaphrodites. Male-derived sperm can be labeled by staining male worms with the fluorescent dye SYTO 17 (K. Hill and S. W. L'Hernault, unpublished results). The amount of sperm transferred via mating can be observed within unlabeled recipient hermaphrodites. Transferred sperm are observed to accumulate in the uterus and/or spermatheca of the recipient hermaphrodite (Figure 2). Two extreme examples of the amount of sperm transferred in similar experiments are shown in Figure 2. Most crosses showed intermediate levels of sperm transfer while some crosses led to no sperm transfer (also see Singson *et al.* 1998). Previous studies in which mating was observed either on plates or under the compound microscope found that both the number of copulation events and the amount of sperm released per ejaculation can vary (Ward and Carrel 1979). Additionally, apparent copulation events frequently resulted in no sperm transfer.

DISCUSSION

Sperm competition can occur in the absence of fertilization: Sperm competition is a process in which sperm from one individual compete with the sperm of another individual to fertilize eggs (Birkhead 1996). Competition can also occur between the sperm derived from one animal when individual sperm differ from one an-

TABLE 2

Mutant males crossed to *spe-8* hermaphrodites

Male genotype	Temperature	Self-progeny[a]	Outcross progeny[a]	*n*	Failed crosses[b] (%)
Not crossed	16°	<1	NA[c]	28	NA
	25°	<1	NA	51	NA
him-5(e1490)	16°	1 ± 1	104 ± 7[d]	20	0
	25°	<1	128 ± 11[d]	20	0
spe-9(eb19); him-5(e1490)	16°	54 ± 6	<1	16	60
	25°	40 ± 8	0	10	66
spe-9(hc52ts); him-5(e1490)	16°	2 ± 1	119 ± 12[d]	19	5
	25°	8 ± 2	11 ± 3	11	56
spe-13(hc137ts); him-5(e1490)	16°	17 ± 3	20 ± 4	27	0
	25°	12 ± 2	1	28	20
fer-14(hc14ts); him-5(e1490)	16°	11 ± 3	4 ± 1	19	5
	25°	24 ± 8	<1	16	20
fer-1(hc13ts); him-5(e1490)	16°	<1	174 ± 11[d]	15	25
	25°	35 ± 6	0	16	20

[a] SE.

[b] Crosses were considered to have failed if worms produced no self-progeny or outcross progeny. Failed crosses were not included in brood size determination.

[c] NA, not applicable.

[d] Progeny counts were conducted for 3 days. All other progeny counts continued until the worms died or stopped producing progeny.

other by genotype (LaMunyon and Ward 1997). Sperm competition in *C. elegans* has been recognized previously and is somewhat unusual in this species because the principal mode of reproduction is by self-fertility within a hermaphrodite (Ward and Carrel 1979; LaMunyon and Ward 1995, 1997, 1998). However, this species also produces males and their sperm have precedence within the hermaphrodite after crossing (Ward and Carrel 1979) as illustrated in Figure 1. We have further defined the steps preceding fertilization in *C. elegans* by employing various mutants that are defective in sperm function. Specifically, we show that competition between male-derived sperm and hermaphrodite-derived sperm can occur when sperm are not competent to fertilize oocytes *in vivo*. Thus, sperm competition and fertilization are mechanistically independent. Sperm precedence must not be determined by a differential ability of male sperm to penetrate oocytes and activate blocks

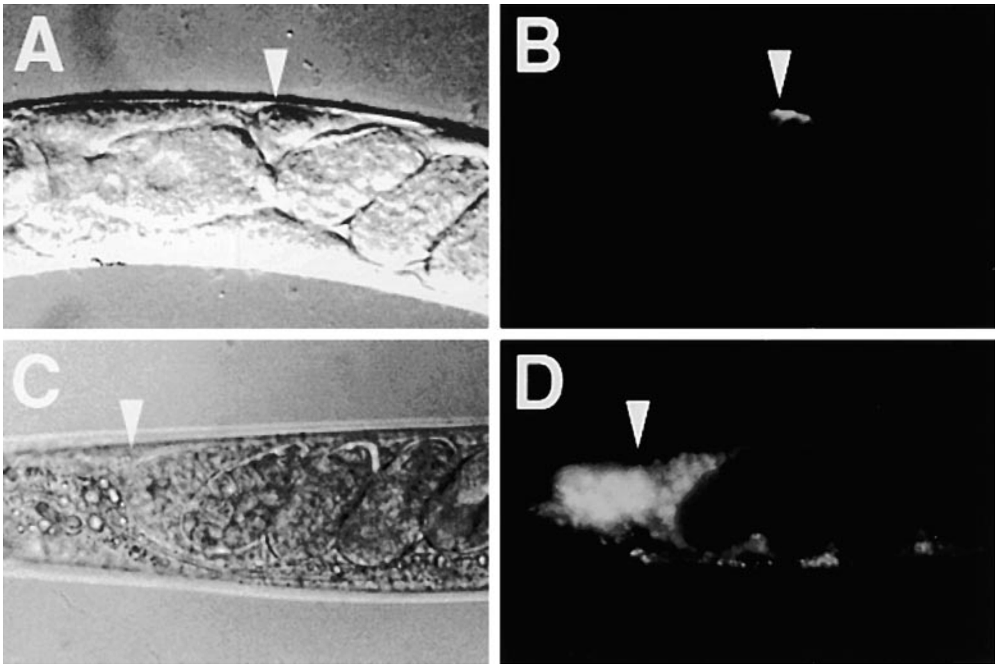

Figure 2.—Variable amounts of sperm can be transferred during crossing. (A and C) Nomarski DIC images and (B and D) corresponding fluorescent signal within the reproductive tract of crossed hermaphrodites. These unlabeled *daf-4* hermaphrodites were crossed to wild-type males that had been labeled with the fluorescent vital dye SYTO 17. Arrow heads point to the position of the spermatheca. (B and D) Accumulation of the fluorescent signal corresponds to the accumulation of sperm from males. (A and B) An example of a cross where very few sperm were transferred. (C and D) An example of a cross where a large amount of sperm were transferred. In this case, many sperm are seen in the uterus.

to polyspermy. Reduced self-fertility in hermaphrodites crossed to *him-5* males in which no outcross progeny were produced has previously been seen (Ward and Carrel 1979). We see this same effect in our control crosses. Our results are consistent with the proposed mode of sperm competition where larger sperm produced by males can move faster and displace the smaller hermaphrodite-derived sperm already present in the reproductive tract (LaMunyon and Ward 1998). The *spe-9*, *spe-13*, and *fer-14* genes appear to play a role in sperm-oocyte interactions and are required for fertilization (L'Hernault 1997; Singson *et al.* 1998). The *spe-9* gene encodes a sperm transmembrane protein that contains epidermal growth factor-like repeats and is a putative ligand for an as yet undetermined receptor on the oocyte (Singson *et al.* 1998). The *spe-13* and *fer-14* genes have not yet been cloned but have mutant phenotypes very similar to *spe-9* mutants (L'Hernault *et al.* 1988; L'Hernault 1997). In our experiments, male sperm derived from *spe-9*, *spe-13*, or *fer-14* mutants can displace the endogenous hermaphrodite-derived sperm and contact passing oocytes. However, no fertilization can result after these mutant sperm contact oocytes. Indeed, we have observed sperm from these mutants contacting passing oocytes without the fertilization that nearly always follows oocyte contact with wild-type spermatozoa (Ward and Carrel 1979; Singson *et al.* 1998; A. Singson and S. W. L'Hernault, unpublished results). By taking precedence over hermaphrodite-derived sperm, mutant male-derived sperm functionally reduce the self-fertility of the recipient hermaphrodite. We did not see this effect when we used *fer-1* mutant males in identical experiments. The *fer-1* gene encodes a transmembrane protein required for vesicle fusion during the transition of a spermatid into a spermatozoon (Achanzar and Ward 1997). Unlike *spe-9*, *spe-13*, and *fer-14* spermatozoa, *fer-1* mutant spermatozoa do not show normal motility (Ward and Miwa 1978; Argon and Ward 1980). Consistent with the hypothesis that sperm competition depends on sperm motility, the nonmotile sperm from *fer-1* mutant males do not cause a decrease in hermaphrodite self-fertility (Table 1).

The effectiveness of sperm competition may depend on the amount of sperm transferred during mating: Sperm competition was shown to be incomplete in a sensitive *spe-8*-based assay for mating. There are several scenarios that could explain this observation. If very few sperm are transferred during a cross, a mixture of male and hermaphrodite-derived sperm would be present in the spermatheca (see below) and a combination of self-progeny and outcross progeny would be produced. In this same situation, if the male sperm are fertilization defective (for example, *spe-9* mutant sperm), only a slight, and probably undetectable, decrease in self-fertility would be expected. In our crosses to *spe-8* hermaphrodites, low sperm transfer probably accounts for most of the observed self-fertility. The *spe-8* hermaphrodite-derived sperm would be activated by the seminal fluid component(s) introduced during copulation but also would not be displaced by sperm competition. Consistent with this idea, fluorescent sperm transfer experiments indicate that the amount of sperm transferred in any given cross can vary (Figure 2). Indeed, it is well established that the quantity of inseminated sperm is important in determining the outcome of sperm competition in other systems (Birkhead 1996). Older hermaphrodites that were crossed only when they were young will eventually deplete their supply of male-derived sperm. In this situation, if any hermaphrodite sperm are still present when male sperm numbers decrease, self-progeny will again appear. This is consistent with our observations depicted in Figure 1A and previous data (Ward and Carrel 1979). If male sperm were fertilization defective, these sperm would not be depleted and would therefore lead to the continued suppression of hermaphrodite self-fertility. This appears to be the case in some of our experiments.

The maximum average number of self-progeny produced by *spe-8 dpy-5* hermaphrodites (54 ± 6, $n = 16$, 16°) is still below the average brood size for *dpy-5* worms without the *spe-8* mutation (162 ± 34, $n = 9$, 16°). Just as in failed mates, this low self-fertility could be due to sperm competition by fertilization-defective sperm transferred during the mating, hermaphrodite spermatid loss from the reproductive tract before the first copulation event, or other unknown factors. LaMunyon and Ward (1995) performed mating experiments that were similar to those described in this study yet they found higher *spe-8* self-fertilized broods after mating to *fer-1* males. This difference from our data could be due to the use of different morphological markers. LaMunyon and Ward estimated outcross progeny on the basis of the number of male offspring produced from unmarked *spe-8* hermaphrodites, while we looked for non-Dpy progeny from *spe-8 dpy-5* hermaphrodites. The *dpy-5* mutation reduces the fertility of hermaphrodites.

If sperm competition leads to the displacement of hermaphrodite sperm from the spermatheca, why are these sperm unable to fertilize oocytes in the uterus? There are several lines of evidence that fertilization in *C. elegans* can occur only in the spermatheca. The best evidence comes from the direct observations of Ward and Carrel (1979). The spermatheca is the only location where fertilization was observed. The first events that occur after fertilization, such as the increased Brownian and saltatory movement of granules in the oocyte cytoplasm and the initiation of eggshell formation, are seen when the egg is still in the spermatheca. If an oocyte passes through the spermatheca and into the uterus without being fertilized, it begins endoreplicating its genome. These polyploid oocytes are unlikely to be fertilized productively in the uterus. Sperm excluded from the spermatheca, as seen in Figure 2D and reported by LaMunyon and Ward (1998), would not

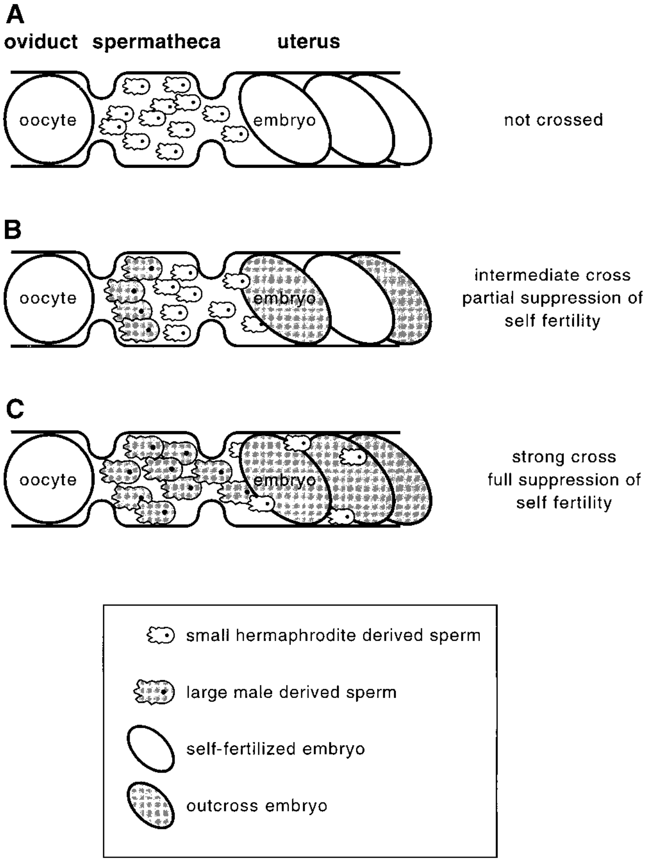

Figure 3.—A model for the mechanism of sperm competition and suppression of self-fertility in *C. elegans.* (A) Virgin hermaphrodites are fully self-fertile. (B) Hermaphrodites with a few male-derived sperm still have hermaphrodite-derived sperm present in the spermatheca. If an oocyte is not fertilized by a male-derived sperm it can still be fertilized by a hermaphrodite-derived sperm. (C) When hermaphrodites contain a large number of male-derived sperm, hermaphrodite-derived sperm are excluded from the spermatheca and full suppression of self-fertility is seen.

come in contact with oocytes that are receptive to fertilization. Finally, fertilization does not occur within the proximal oviduct, which is upstream of the spermatheca. In mutants where the spermatheca does not dilate to allow oocyte entry from the oviduct, fertilization is not observed (Clandinin *et al.* 1998).

A model consistent with our data and the proposed mechanism of sperm competition (Ward and Carrel 1979; LaMunyon and Ward 1998) is presented in Figure 3. In this model, unmated hermaphrodites display full self-fertility because no male sperm are present in the spermatheca (Figure 3A). If mating resulted only in the transfer of a few male-derived sperm, the situation depicted in Figure 3B would occur. The larger male sperm would take precedence in producing outcross progeny. When these sperm become depleted, selfing would resume. If the male-derived sperm were fertilization defective, hermaphrodite sperm not excluded from the spermatheca could fertilize passing oocytes. If a cross results in a large number of male-derived sperm being transferred to the hermaphrodite reproductive tract, the situation depicted in Figure 3C would occur. Hermaphrodite sperm would be completely excluded from the spermatheca and only outcrossing would occur. If the male-derived sperm were fertilization defective, an otherwise fully fertile hermaphrodite would be sterilized.

Analysis of sperm competition for classifying *spe* and *fer* mutants: To date, only 17 of the >60 *spe* and *fer* genes have been defined by multiple alleles (reviewed in L'Hernault 1997). The lack of multiple alleles for many of these genes suggests that many loci required for sperm function and development remain to be discovered (L'Hernault *et al.* 1988; L'Hernault 1997). Additionally, many of the known *spe* and *fer* mutations have not yet been characterized in great detail. Some of these mutations could cause defects in early aspects of sperm development, while others could alter sperm activation, motility, or interactions with oocytes during fertilization. Many of the steps that occur during fertilization in *C. elegans* after sperm contact oocytes are still poorly understood (Kemphues and Strome 1997; Singson *et al.* 1998). For instance, the region of the sperm cell that interacts with the oocyte is unknown. Additionally, the mechanisms that mediate sperm signals that coordinate oocyte maturation and ovulation as well as the nature of the block to polyspermy are also unknown (McCarter *et al.* 1997, 1999). The phenotypic assays described in this study will complement existing techniques for classifying these mutants. For example, *spe* or *fer* mutants where male-derived spermatozoa cannot translocate through the uterus and into the spermatheca would not be expected to display effective sperm competition. Understanding these mutants will help fully define the steps leading to fertilization in *C. elegans* and could complement studies of fertilization in other organisms.

We thank Andrea Dean, Grant MacGregor, members of the L'Hernault lab, and anonymous reviewers for critical comments on the manuscript. We also thank Andrea Ross for assistance with statistical analysis. K.L.H. was supported by a National Institutes of Health (NIH) predoctoral training grant (GM-08490-03). This work was supported by a National Science Foundation (NSF) Minority Postdoctoral Fellowship to A.S. and a NSF grant (IBN-9305058) and NIH grant (GM-40697) to S.W.L.

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Communicating editor: R. K. Herman