CAENORHABDITIS ELEGANS FERTILIZATION-DEFECTIVE MUTANTS WITH ABNORMAL SPERM

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ABSTRACT

Seven new fertilization-defective mutants of *C. elegans* have been isolated and characterized; six are temperature sensitive, one is absolute and all are autosomal recessive. One mutation is in a previously described gene, while the other six define six new *fer* genes that appear to code for sperm-specific functions necessary for normal fertilization. In all *fer* mutants, both males and hermaphrodites accumulate sperm in near normal numbers. In hermaphrodites, mutant sperm contact the oocytes, but fail to fertilize them. Instead, the sperm are swept into the uterus by the passing oocytes and are expelled when oocytes are laid. Males of two *fer* mutants do not transfer sperm during copulation, but the other mutant males transfer sperm that fail to move to the spermatheca. Spermatozoa from *fer-1* and *fer-4* mutants are motility-defective *in vitro* as well as *in vivo*, and their pseudopods have an altered morphology. The period of development during which mutant hermaphrodites are temperature sensitive for fertility overlaps the time of sperm development. Some mutants are temperature sensitive throughout the entire period, and others are temperature sensitive during or just prior to spermiogenesis. In *fer-4*/+ and *fer-7*/+ males, the fertility of the mutation-bearing sperm is diminished, reducing the transmission ratio. This implies some post-meiotic expression of these genes.——This set of mutants provides a variety of functional and structural alterations in nematode sperm that should help identify and analyze gene products involved in sperm morphogenesis and motility.

SPERMATOZOA are highly differentiated cells, specialized to interact with eggs. During their differentiation, spermatozoa acquire both the ability to move to the site of fertilization and surface specializations that enable them to recognize an ovum and fuse with it. To study the genetic basis of this cellular differentiation, we have chosen to disrupt the development of sperm by means of mutations that cause sperm sterility (WARD and MIWA 1978). Genetic, anatomical and biochemical comparison of mutant with wild-type sperm may allow identification of gene products that are necessary for sperm differentiation, sperm motility and fertilization.

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The ease of cultivating *Caenorhabditis elegans* and isolating mutants, as well as the availability of many marker strains, makes this organism a convenient one for developmental genetic studies (BRENNER 1974; reviewed by EDGAR and WOOD 1977; RIDDLE 1978). Spermatogenesis and fertilization in *C. elegans* have several attractive features for genetic dissection of cellular differentiation and interactions. The worm is transparent, so the development of both sperm and oocytes and the process of fertilization can be directly observed (HIRSH, OPPENHEIM and KLASS 1976; WARD and CARREL 1979). The hermaphrodite is self-fertilizing, so that sterility can be recognized in a single animal without mating. Self-fertilization also ensures that most sterility mutations affect the gametes directly because few other cell types participate in fertilization. Once recognized in hermaphrodites, sterile sperm-defective mutants can be studied in males, which produce many more sperm.

The spermatozoa of *C. elegans*, like those of other nematodes, are nonflagellated, have no acrosome, lack a nuclear membrane and possess unusual membranous organelles. Their development has been described in detail (WOLF, HIRSH and MCINTOSH 1978). Their locomotion is mediated by a ruffling pseudopod and can be studied both *in vivo* and *in vitro* (WARD and CARREL 1979; NELSON 1979; NELSON and WARD 1980). The final steps of cellular maturation can also be studied *in vitro* (NELSON and WARD 1980).

The phenotypes of mutants in one fertilization-defective (*fer*) gene were described by WARD and MIWA (1978). In this paper, we describe the isolation of mutants in this and six additional *fer* genes and present genetic and phenotypic characterizations of these *fer* mutants.

MATERIALS AND METHODS

Strains: The nematode used in this study was *Caenorhabditis elegans* var. Bristol. The wild-type strain, N2, and the genetic markers were obtained originally from the collection of SYDNEY BRENNER. The following genetic markers were used: Linkage group I: *dpy-5(e61)*, *unc-13(e51)*, *unc-29(e1072)*; LGIII: *dpy-1(e1)*, *dpy-18(e364)*, *unc-69(e587)*, *lon-1(e185)*, *sup-5(e1464)*; LGIV: *dpy13(e184)*, *unc-30(e191*1)*; LGV: *dpy-11(e224)*, *dpy-11(e224)* +/+ *e1405*, *unc-39 (e257)*, *him-5(e1467* and *e1490)*, *unc-76(e911)*.

The map positions of these genes are shown in Figure 1. The following abbreviations are used throughout the paper for the description of phenotypes: Fer for fertilization-defective; Dpy for dumpy; Unc for uncoordinated; ts for temperature sensitive. The names of genes, mutations and strains follow the accepted nomenclature of *C. elegans* (HORVITZ *et al.* 1979). Strains previously designated HC are now called BA.

Strain BA17, *isx-1(hc17ts) IV*, has a temperature-sensitive mutation that abolishes spermatogenesis in hermaphrodites and causes sexual transformation of males (NELSON, LEW and WARD 1978). BA505 is *isx-1(hc17) IV*; *dpy-11(e224) V*. The sterile strains *A13* and *A34* were isolated and provided to us by R. S. EDGAR; they were crossed to wild type twice, and the sterile mutations were reisolated and assigned the corresponding *hc* numbers. The isolation of other sterile mutants is described in RESULTS.

Worm handling: Strains were grown on petri plates of NGM agar seeded with *E. coli* strain OP50, as described by BRENNER (1974). The phenotype of *fer* mutant worms was determined by cloning individual animals in 24-well Falcon microtiter plates or disposable beakers filled with 2 ml of NGM agar. The beakers ensure that no worms crawl from one well to another. When recovery of progeny was not necessary for further analysis, sterility phenotypes were scored by

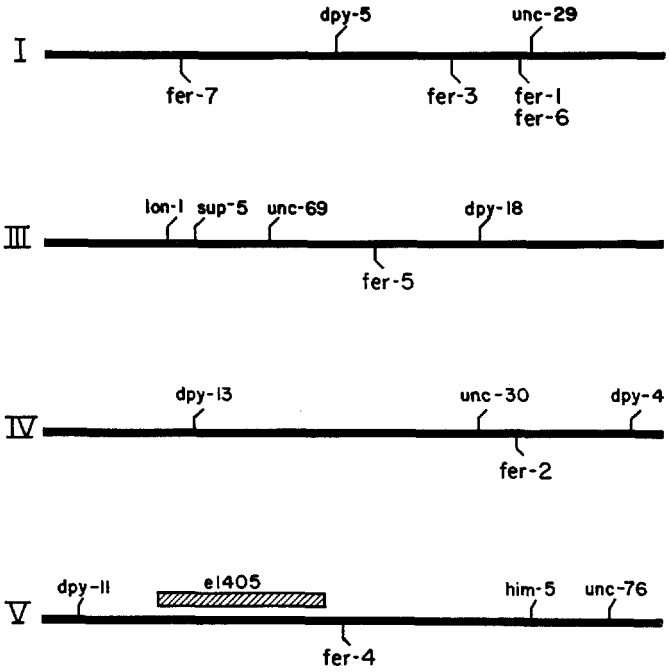

FIGURE 1.—An abbreviated genetic map showing the positions of *fer* genes relative to nearby markers on each chromosome.

placing young adult hermaphrodites on agar plates containing 1 to 2 mM Tramisole (American Cyanamid Company) for about 1 hr. Animals are immobilized by the drug within 3 min and 90% of the animals expel 2 or more eggs or oocytes from their uteri. By examination of the expelled contents, the worm can be scored as sterile or fertile; animals that cannot be scored can be transferred to regular growth microplates and scored later. Worms recover from the drug treatment within 6 hr, so that sterile worms can be rescued by mating with males, if desired. Unfertilized eggs were scored by flooding plates with 5 or 6 drops of 0.025% trypan blue or methylene blue, which stains unfertilized eggs but not eggs that have shells (WARD and CARREL 1979).

For measurement of total brood sizes, parents were transferred, singly or in small groups, to fresh growth plates every 12 to 24 hr. Egg-laying periods of hermaphrodites at 25° and 16° are roughly 3 and 7 days, respectively.

Temperature-shift experiments were essentially by the method of HIRSH and VANDERSLICE (1976). The synchrony of the worms on each plate was rechecked periodically, and animals that were too old or too young were removed.

Microscopy: Live nematodes were observed on a thin slab of agar according to the method of SULSTON and HORVITZ (1977). Nuclei were visualized by Feulgen staining as described in WARD and MIWA (1978) or Hoechst 33258 staining according to ALBERTSON, SULSTON and WHITE (1978). Slides were viewed with a Zeiss Universal microscope fitted with Nomarski differential interference contrast optics and with epifluorescence and were photographed as described by WARD and CARREL (1979). Sperm for microscopic observations were dissected in sperm medium (NELSON and WARD 1980). Cells for scanning electron microscopy were fixed in 1.25% glutaraldehyde and 1% formaldehyde in sperm medium for at least 1 hr, and post-fixed with 1% OsO_4 for 15 min. They were then dehydrated in graded alcohols, transferred to acetone, to CO_2 and critical-point dried in a Polaron apparatus. Samples were sputter-coated with gold-paladium in a Polaron E5100 sputterer and observed in a JEOL JSM-35 scanning electron microscope equipped with an LaB_6 filament that was operated at 8 to 15 kV.

Genetic methods: EMS mutagenesis conditions were as described by BRENNER (1974). ICR-170 mutagenesis was performed by growing worms in petri plates in the presence of 0.1 mg/ml of the drug for a generation (D. RIDDLE, personal communication).

Complementation tests were done by mating permissively grown males of one *fer* strain to restrictively grown hermaphrodites of another at 25°. After the presence of approximately 50% males among the progeny was ascertained, immature F_1 hermaphrodites were grown individually at 25° and their fertility scored.

All the *fer* genes were assigned to linkage groups by crossing *fer* males to canonical *dpy* or *unc* markers on each linkage group (see abbreviated genetic map) and Dpy or Unc F_2 individuals were cloned and tested for sterility at 25°. Between 20 and 50 clones were scored for each cross. Scarcity of sterile Dpy or Unc animals indicated linkage. Mapping was done as described in the text.

RESULTS

Isolation of mutants: We chose to isolate temperature-sensitive (ts) sterile mutants initially because they are easily maintained as homozygous strains. BA1 (= HC1) was obtained as an adventitious mutant in a nonchemotactic strain (WARD and MIWA 1978). Some data on its phenotype are included here for completeness. BA13, BA23, BA24 and BA34 were isolated using the method of HIRSH and VANDERSLICE (1976): F_2 progeny of EMS-mutagenized wild-type hermaphrodites were cloned, and replica plates were tested for animals that were sterile and had laid many unfertilized eggs at 25°, but not at 16°. BA2, BA3, BA4 and BA6 were isolated by another protocol: F_1 (rather than F_2) progeny of mutagenized parents were cloned at the restrictive temperature (25°). Worms that segregated the desired recessive mutations were identified by the presence of large numbers of unfertilized eggs laid by the F_2 progeny in each clone that were homozygous for a mutation. The mutations were then recovered at the permissive temperature (16°) by segregation from the heterozygous siblings.

This second protocol is essentially that used by BRENNER (1974) to isolate his S set of mutants with visible phenotypes. It has two advantages: it minimizes the effort required for replica-plating mutant candidates and it allows for the isolation of absolute, as well as temperature-sensitive, mutants. In one such mutant hunt, six homozygous mutant strains that bred true were isolated from 1192 F_1 hermaphrodites. Two of them were ova-defective by the criterion described below and are not included in the present study. Several absolute sterile candidates were found in the same mutant hunt, but these were not pursued further. Sterile mutations that elude detection by this protocol are nonleaky dominant mutations, or mutations that show a maternal effect.

An absolute sterile mutant was isolated following ICR-170 mutagenesis by screening F_2 progeny plates for the presence of unfertilized eggs. As described below, this mutant is an allele of a complementation group previously defined by ts mutations.

Sterility phenotype of hermaphrodites: The wild-type *C. elegans* hermaphrodite (strain N2) produces about 285 zygotes at 16° and additional unfertilized eggs after it has used up its sperm (WARD and CARREL 1979). This pattern of

TABLE 1

Quantitation of sterility phenotypes

Gene	Strain	Growth temperature	Progeny	Unfertilized oocytes
Wild type	N2	25°	250	14
		16°	285	45
fer-1	BA1	25°	<1	205
		16°	302	17
	BA13	25°	3	160
		16°	149	7
	BA24	25°	15	282
		16°	280	50
	BA8	20°	<1	160
fer-2	BA2	25°	5	195
		16°	200	—
fer-3	BA3	25°	2	164
		16°	180	48
fer-4	BA4	25°	8	180
		16°	164	100
fer-5	BA23	25°	5	99
		16°	238	64
fer-6	BA6	25°	3	159
		16°	145	10
fer-7	BA34	25°	16	72
		16°	110	9

Numbers given are per hermaphrodite and are average counts of at least three groups of 5 to 20 animals each.

egg laying is similar at 25°, except that the total brood size is reduced (Table 1; also see HIRSH and VANDERSLICE 1976).

By contrast, hermaphrodites of nine ts strains described here produce only a few progeny, if any, at the restrictive temperature of 25°, but lay 72 to 282 unfertilized eggs (Table 1). At the permssive temperature, all ts mutants produce 100 to 300 progeny and less than 65 unfertilized eggs. These unfertilized eggs are laid late in the life cycle, like those of wild-type hermaphrodites. The total number of oocytes produced at 25° is generally smaller than the number of oocytes made by each strain at 16°, as in the wild type. Three strains, BA2, BA3 and BA4, produce three to six inviable embryos per hermaphrodite. The development of these embryos does not arrest at a particular stage.

Most *fer* mutations described here are leaky to some extent. Only one ts mutation, *hc1* is nonleaky, as is the absolute mutation *hc8*. In the others, between 0.5% and 8% of their oocytes are fertilized at the restrictive temperature. During mutant hunts, several other mutants that showed 10% leakage or more were not picked for further analysis.

There are some exceptions to the above general description of *fer* mutants: BA4 is exceptional in that it is somewhat sterile even at 16°; 38% of its oocytes are not fertilized at the permissive temperature. BA23 is exceptional because BA23 hermaphrodites produce many fewer oocytes at 25° than at 16°. BA34

produces fewer eggs than wild type at both temperatures. BA8 is completely sterile at all temperatures.

The *fer* strains have no visible phenotypes except for a slight sluggishness at 25° in some. They develop and grow at the same rate as wild type, initiating egg laying at 45 hr post-hatching, as do N2 hermaphrodites.

Hermaphrodite sterility is due to sperm defects: Table 2, column 3, shows that sterility of mutant hermaphrodites is not due to the absence of sperm. At the start of their egg-laying period, 45 hr post-hatching at 25°, the spermathecae of these hermaphrodites contain over 170 sperm. Since both sperm and eggs are produced in all strains, but fertile eggs are rare, they are designated *fer*, for fertilization-defective (WARD and MIWA 1978).

In order to distinguish sperm-defective from ova-defective mutations, *fer* hermaphrodites were mated to wild-type males. Hermaphrodites of all *fer* strains produced at least 100 progeny (some more than 200) after mating. Half of the progeny were males, confirming that the oocytes were fertilized by outcross sperm. This shows that oocytes of all these mutants are capable of being fertilized. Therefore, sperm of *fer* mutants must be defective. Precise quantification of oocyte fertility is difficult because mating efficiency varies, but the data are consistent with *fer* oocytes having normal fertility.

Fate of hermaphrodite sperm: When oocytes pass through the spermatheca in a wild type, they carry some sperm with them into the uterus. All these sperm crawl back into the spermatheca and fertilize subsequent oocytes (WARD and CARREL 1979). In contrast, the nonfunctional sperm of *fer* hermaphrodites are progressively lost from the spermathecae of mutant hermaphrodites (Table 2, columns 4 through 7). By the time 40 to 70 oocytes have passed through the

TABLE 2

Hermaphrodite sperm

Mutant		Sperm in spermatheca t=0	Sperm in spermatheca t=18 hrs	Oocytes	Progeny	Sperm swept / egg
fer-1	*hc1**	211 ± 13	60 ± 4**	75	0	2 ± 0.2
fer-1	*hc24*	296 ± 27	144 ± 11	38	16	2.5 ± 0.5
fer-2	*hc2*	252 ± 33	105 ± 8	48	4	2.8 ± 0.7
fer-3	*hc3*	267 ± 26	67 ± 9	36	<1	5.5 ± 0.8
fer-4	*hc4*	295 ± 11	100 ± 32	54	10	2.9 ± 0.5
fer-5	*hc23*	281 ± 28	—	—	—	—
fer-6	*hc6*	216 ± 26	—	—	—	—
fer-7	*hc34*	171 ± 8	10 ± 6	~50	~10	2.5 ± 0.2

* From WARD and MIWA 1978; **t = 22 hr.

Synchronized groups of 20 worms were grown at 25°. Half of each group was fixed, Feulgen-stained and their sperm counted at the onset of egg laying ($t = 0$). The other half was allowed to lay eggs for 18 hr. They were then fixed, stained and their sperm counted. The numbers presented are the mean per hermaphrodite and the standard error of the mean. The counts at $t = 0$ include some primary spermatocytes that each contribute 4 to the sperm count. The oocytes column shows total number that passed through the spermathecae in 18 hr. The progeny column shows the number of fertile eggs. The sperm swept per egg column shows the number of sperm lost from the spermatheca (excluding those that fertilized an egg) for each egg or oocyte that has passed. By a t test, only *fer-3* differs significantly from the others.

spermathecae of sterile hermaphrodites, most of the sperm have been swept out. When live animals are observed with the compound microscope, the oocytes can be observed carrying sperm with them into the uterus, as in wild-type animals. These sperm fail to migrate back through the spermathecal valve. Instead, they are carried down the uterus by the oocytes and are subsequently expelled through the vulva when eggs are laid. Sperm are lost at a similar rate per oocyte for all the mutants except *fer-3*, which loses sperm faster. The loss of mutant sperm contrasts sharply with the efficient utilization of sperm in wild-type hermaphrodites (WARD and CARREL 1979) and in permissively grown *fer* animals, in which nearly every sperm fertilizes an oocyte.

Genetics: The mutations described here were assigned to genes by complementation tests of all pair-wise combinations. Seven complementation groups are defined by the present set of mutants: six of them contain just one isolate each and one complementation group has multiple isolates (*hc1*, *hc8*, *hc13* and *hc24* fail to complement each other). The mutations were assigned gene names, as shown in Table 1, column 1.

Two cases of ambiguous complementation were encountered: heterozygous F_1 animals from BA34 × BA2 and BA34 × BA23 crosses repeatedly gave approximately equal numbers of oocytes and embryos. However, the three mutations are linked to different chromosomes and were assigned to different genes on this basis. The ambiguity may arise because *hc34* is so leaky.

Several *fer* mutations were isolated by D. HIRSH and co-workers in a search for spermatogenesis mutants (HIRSH and VANDERSLICE 1976; L. EDGAR, personal communication). Four of these were tested for complementation to the mutants described here; *b232* fails to complement either *hc1* or *hc24* and therefore is a fifth allele of *fer-1*. Three mutations on linkage group IV, *b48*, *b82* and *b126*, complement *hc2*, and are therefore in different genes.

Mapping: The mutants were assigned to linkage groups as described in MATERIALS AND METHODS. This is shown in Table 3, column 2. Four genes are located on LGI, one on LGIII and LGV and two on LGIV. Most of these genes were mapped by three -factor crosses of *fer* males to canonical *dpy unc* double-mutant strains. F_2 single recombinants were cloned and tested at 25° for segregation of sterile F_3 animals. The results of these crosses are listed in Table 3, and the map positions of the mutants are shown in Figure 1. Three alleles of *fer-1 I* (*hc1*, *hc13* and *hc24*) map to the same locus, near *unc-29*; *fer-2 IV* is located between *unc-30* and *dpy-4*; *fer-4V* is not covered by the deletion *e1405*, as all the progeny from a cross between *hc4* males and + *e1405/e224* + hermaphrodites were fertile at 25°; and *fer-6 I* is tentatively assigned a position indistinguishable from that of *fer-1*. Only one recombinant was obtained from a three-factor cross (Table 3), and it could have been a double recombinant. *fer-6* complements all *fer-1* alleles, and its sperm have different anatomical alterations from those of *fer-1* (S. WARD and Y. ARGON, unpublished). *fer-7 I* was mapped by a two-factor cross to *unc-29* and was found to lie 1.5 cM to the left of *dpy-5*. This value was obtained by normalizing the number of recombinants to a parallel two-factor cross of *dpy-5* and *unc-29*.

TABLE 3

Three-factor crosses used to locate fer *mutations*

Mutation	Linkage group	Mapped to the markers	Number of each recombinant genotype obtained			
			D F + / D + U	D + + / D + U	+ F U / D + U	+ + U / D + U
fer-1(hc1)	I	*dpy-5 unc-29*	9	2	1	11
(hc13)		*dpy-5 unc-29*	24	0	1	12
fer-3(hc3)	I	*dpy-5 unc-29*	8	7	4	6
fer-6(hc6)	I	*dpy-5 unc-29*	20	1	0	14
fer-7(hc34)	I	*dpy-5 unc-29*	0	13	12	0
fer-5(hc23)	III	*unc-69 dpy-18*	5	4	5	6
fer-2(hc2)	IV	*unc-30 dpy-4*	6	1	2	2
fer-4(hc4)	V	*dpy-11 unc-76*	9	7	8	8

The indicated mutants were assigned to the linkage groups shown in column 2 and were three-factor crossed to Dpy-Unc double markers. (For the actual gene arrangement, see Figure 2.) The numbers represent dumpy (D) or uncoordinated (U) single recombinants whose *fer* genotype (F) was verified.

Temperature sensitivity: Temperature shifts were used to determine the period during development that an animal has to spend at the permissive temperature in order to be fertile. The end of the temperature-sensitive period (TSP) is the latest time in development at which an animal can be shifted up to the restrictive temperature without being completely fertile. The beginning of the TSP is the latest time at which an animal can be shifted from the restrictive to the permissive temperature without decreasing the number of its progeny. The TSP's of six strains were determined by shifting synchronized populations of hermaphrodites. Two shift experiments, representing the two classes found, are shown in Figure 2.

The up and down shifts of *fer-4*, *fer-5*, *fer-1(hc1)*, *fer-1(hc24)* and *fer-7* are symmetric and intersect below their midpoint, at about 15 to 20% fertility. The shift curves of *fer-2* and *fer-3* represent the other class: their up-shifts are steep, yet their down-shifts are more gradual, making it difficult to determine accurately the start of their TSP's.

The TSP's of these six mutants are summarized in Figure 3. Clearly, there are two groups: *fer-1(hc1)*, *fer-1(hc24)*, *fer-5* and *fer-7* have short TSP's (5 to 10 hr) late in their development; *fer-2*, *fer-3* and *fer-4* have long (approximately 24 hr) TSP's. All six TSP's overlap spermatogenesis, and most of them end just after the completion of sperm development. This is consistent wtih their sperm-defective phenotype.

Sterility of fer *males:* The *fer* mutations were isolated by hermaphrodite sterility. The fertility of *fer* males has been assessed because males would be the preferred source of these cells for biochemical analysis and because *fer* mutations might have a differential expression in the two sexes, as has been the case with mutations affecting sexual development (HODGKIN and BRENNER 1977; NELSON, LEW and WARD 1978).

The fertility of *fer* males was assessed by mating them to *isx-1*; *dpy-11* her-

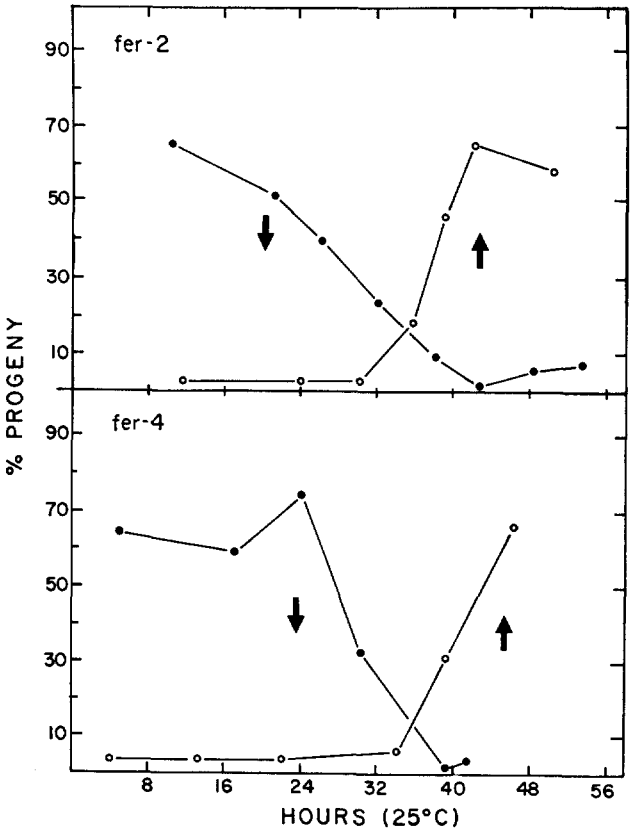

FIGURE 2.—Results of shifting growth temperature between 16° and 25° are shown for *fer-2*(hc2) and *fer-4*(hc4). Each point is the percentage of eggs that were fertilized and produced progeny after shifting 12 or more hermaphrodites down (solid circles ↓) or up (open circles ↑). Times are normalized to 25°.

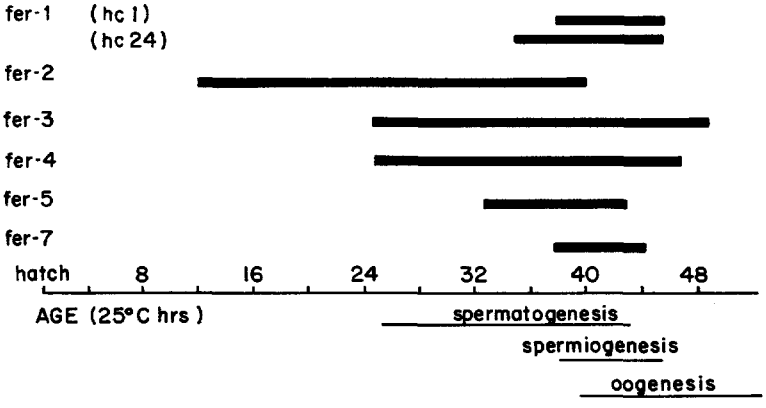

FIGURE 3.—The developmental periods during which the mutants are temperature sensitive as in Figure 2. All times are normalized to the growth rate at 25°. The timing of spermtogenesis, spermiogenesis and oogenesis are also indicated. Egg laying begins at 45 hr.

maphrodites. These dumpy animals are sterile when grown at 25°, due to absence of spermatogenesis (NELSON, LEW and WARD 1978). The *dpy* mutation was included to increase the efficiency of male sperm transfer (WARD and CAREL 1979). Wild-type males can sire up to 90 progeny from these "females." When permissively grown *fer* males were mated to these hermaphrodites, most sired 40 to 70 progeny (Table 4). *fer-2* and *fer-6* males are less fertile than other *fer* males, and their male stocks are difficult to maintain, suggesting sterility at 16°. *fer* males grown restrictively and tested with *fer* "females" sired two progeny each at most. The *fer* males, therefore, are *ts*-sterile and, like the hermaphrodites, this phenotype is slightly leaky.

The sterility of males could result from defective sperm or from inability to transfer sperm during mating. Sperm transfer was measured directly by staining mated hermaphrodites and recording the distribution of sperm. As shown in Table 5, *fer-1*, *fer-3* and *fer-4* males transfer sperm during copulation (as do *fer-2* males, for which data are not shown). *fer-5* and *fer-6* males were never observed to transfer sperm.

When sperm were transferred, over 95% were *in utero*, mostly near the vulva. However, occasionally a hermaphrodite mated by *fer-3*, *fer-4* or *fer-1* males contained a few sperm in or near the spermathecae, showing that some male sperm of these strains can reach the site of normal fertilization. This observation is consistent with the leakiness of the sterility phenotype of these males (Table 4).

The failure of mutant hermaphrodite sperm to return to the spermathecae after being pushed into the uterus by passing oocytes and the failure of most

TABLE 4

Male fertility

Males used in cross	Growth temperature (°C)	Oocytes	Defective embryos	Progeny	Total eggs
—	—	23	0	0	23
+/+	25	27	0	48	75
fer-1(hc24)	25	87	2	2	91
	16	45	7	40	92
fer-2(hc2)	25	6	0	0	6
	16	—	—	16	>16
fer-3(hc3)	25	2	0	1	3
	16	47	7	69	123
fer-4(hc4)	25	33	2	2	37
	16	55	4	53	112
fer-5(hc23)	25	25	1	0	26
fer-6(hc6)	25	17	0	0	17
	16	35	0	13	48

To quantify fertility of Fer males, 12 to 16 males grown at the designated temperature were mated to four to six sterile *isx-1;dpy-11* hermaphrodites at 25° for three to five hr (see text). The mated hermaphrodites were transferred to fresh growth plates each day, and their total broods were recorded. Each mating was repeated several times. Since the efficiency of matings varies, the numbers given are per hermaphrodite from representative matings (excluding those that gave no outcross progeny or oocytes) and are not averages.

TABLE 5

Male sperm transfer

Males used in cross	Total number stained	Number with sperm	Number of sperm transferred	
			Median	Range
fer-1(hc1)	35	14	28	7–230
fer-3(hc3)	32	13	19	8–275
fer-4(hc4)	19	13	18	5– 75
fer-5(hc23)	24	0	0	0
fer-6(hc6)	24	0	0	0

The indicated males were mated to *isx-1;dpy-11* hermaphrodites, as described in the legend for Table 4. After mating, the hermaphrodites were fixed and stained. The number of sperm in each hermaphrodite was determined and is presented as median and range due to the variability in such matings.

male sperm to reach the spermathecae suggest motility defects of these sperm, as proposed for *fer-1* sperm by WARD and MIWA (1978). This prediction was confirmed by studying male sperm motility *in vitro*. In sperm medium, spermatozoa attach to substrate by their pseudopods and can translocate (NELSON 1979). The shape of wild-type spermatozoa *in vitro* and their mode of movement are similar to those observed *in vivo*. Microscopical observations of spermatozoa from *fer* males showed that under similar conditions most are unable to translocate and have various motility defects (ARGON 1979).

1. Morphological alterations of fer *spermatozoa:* We have begun morphological characterization of mutant sperm by electron microscopy. Detailed descriptions of sperm from *fer* mutants will be presented elsewhere. Our observations so far show that most *fer* mutations cause characteristic morphological alterations of spermatozoa. Examples of two such alterations are shown in Figure 4. *fer-1* spermatozoa from permissively grown males (Figure 4a) are indistinguishable from wild-type spermatozoa. On the other hand, spermatozoa from sterile *fer-1* males form only a very short pseudopod that can wiggle but cannot cause sperm translocation *in vitro*. Typical *fer-1* mutant male spermatozoa are shown in Figure 4b. WARD and MIWA (1978) failed to detect these differences between *fer-1* and wild-type sperm because they inadvertently compared spermatids and not spermatozoa, and the media they used for sperm handling were not optimal.

fer-4 male spermatozoa exhibit another defect: they extend pseudopods of normal length but of irregular shapes. These pseudopods have smaller projections and less of them than those in the wild type (Figure 4c). The pseudopods of *fer-4* spermatozoa also fail to exhibit any ruffling movement and the cells do not translocate *in vitro*.

Sperm rescue: Sperm dysfunction could arise because of sperm-autonomous defects or because of defects in seminal fluid. To determine whether wild-type sperm and seminal fluid can rescue some *fer* sperm, a mixture of normal and defective sperm was introduced into the uteri of sterile hermaphrodites by comating with both sterile and wild-type males. In order to distinguish the out-

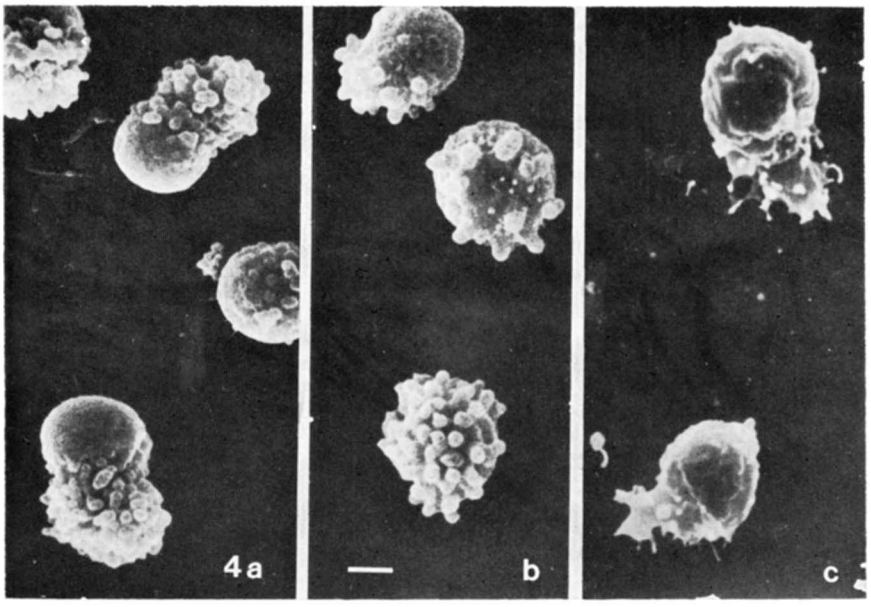

FIGURE 4.—Scanning electron micrographs of mutant sperm. (a) Spermatozoa from *fer-1 (hc1); him-5(e1490)* males grown at the permissive temperature of 16°. These are indistinguishable from wild type grown at 16° or 25°. (b) Spermatozoa from *fer-1(hc1); him-5(e1490)* males grown at the restrictive temperature of 25°. (c) Spermatozoa from *fer-4(hc4)* males grown at 25°. Bar = 1 μm.

cross progeny, the *fer* males were heterozygous for visible markers, and the hermaphrodites were homozygous for these markers. The cross is diagrammed in Figure 5 and the results given in Table 6. Of the progeny of doubly mated $\frac{fer\text{-}1\ unc29}{fer\text{-}1\ unc29}$ animals, only a few were from mutant males, no more than the number of outcross progeny obtained by singly mating the hermaphrodites with $\frac{fer\text{-}1\ unc29}{fer\text{-}1\ +}$ males. A similar result was obtained with doubly mated $\frac{fer\text{-}3\ dpy\text{-}5}{fer\text{-}3\ dpy\text{-}5}$ animals. Only those hermaphrodites producing at least one marked male were included in Table 6 to ensure that the mated animals scored were indeed mated by both genotypes. Because there was no significant improvement in their fertility by co-mating with wild type, we conclude that wild-type sperm and seminal fluid do not improve the fertility of *fer-1* and *fer-3* male sperm.

Transmission ratios: Temperature shifts of *fer* mutants show that TSP's occur during the formation of sperm, and microscopic analysis shows that the functions of *fer* genes are needed to make morphologically normal spermatozoa. To find out whether any of the *fer* genes are expressed post-meiotically, we determined the transmission ratio of several *fer* alleles from *fer*/+ heterozygotes, as has been done in mice, Drosophila and mosquitoes (reviewed by BEATTY 1975). The results are presented in Table 7. In some experiments, the *fer-*

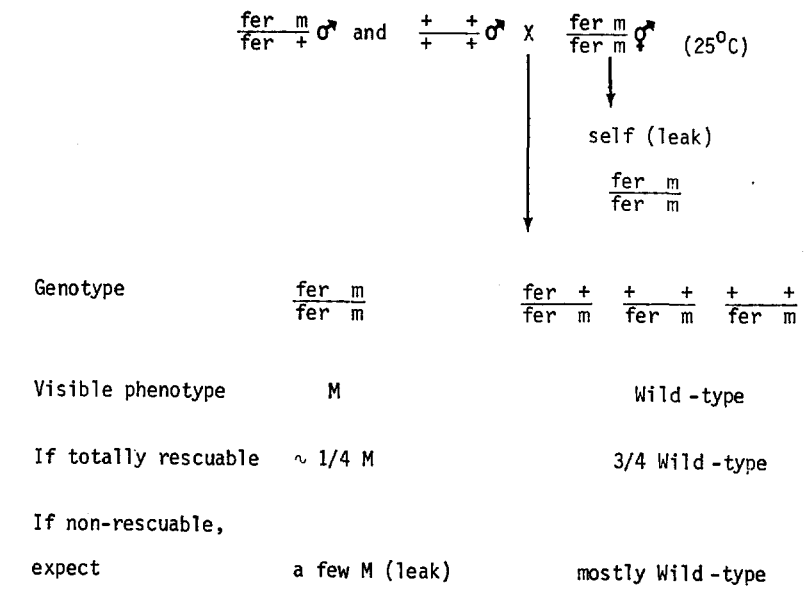

FIGURE 5.—The scheme used in co-mating experiments. *fer* indicates either *hc1* or *hc3*; *m* indicates the linked marker used, *e1072* or *e61* (see text).

TABLE 6

Fer sperm rescue

Genotypes of males	Number of mated hermaphrodites	Wild-type progeny	Marked progeny
hc3e61 / *hc3* +	15	5.7 ± 2.1	5.8 ± 2.2
hc3e61 / *hc3* +; + + / + +	7	71.1 ± 48.5	4.0 ± 2.8
hc1e1072 / *hc1* +	10	3.1 ± 1.2	2.0 ± 1.8
hc1e1072 / *hc1* +; + + / + +	4	115.5 ± 69.6	7.5 ± 6.4

Males and hermaphrodites of the genotypes shown in Figure 5 were grown at the restrictive temperature. Five hermaphrodites were mated simultaneously by five Fer males and five wild-type males at 25° for 12 hr. Control matings were by 10 Fer males. The markers used were *dpy-5(e61)* linked to *fer-3(hc3)*, and *unc-29(e1072)* linked to *fer-1(hc1)*. After matings, individual hermaphrodites were transferred to growth plates, and all their progeny were scored. The results shown are average (± SD) progeny counts from only those animals that gave rise to outcross progeny of both genotypes. By a nonparametric *t* test, the probability that the differences in marked progeny arose by chance is greater than 0.1 for both *fer* mutants; thus, the differences are not significant.

bearing chromosomes were marked with closely linked *unc* or *dpy* mutations. Equal transmission of the two alleles was found for *fer-1(hc1)* (WARD and MIWA 1978), *fer-1(hc24)* and *fer-3(hc3)* (Table 7). Two other mutants, *fer-4(hc4)* and *fer-7(hc34)*, have reduced transmission from heterozygous males when grown at restrictive temperature. *fer-4* also shows a reduced transmission from heterozygotes grown at 16° consistent with its partial sterility at permissive temperature.

As shown in Table 7, we have examined several different markers to assess their effect on the transmission of *hc4*. The transmission ratio is unchanged when the mutant chromosome is marked by *unc76(e911)* and mated to *lon2(e678)*; *unc76(e911)* hermaphrodites. The transmission is improved significantly when the chromosome is marked with *dpy-11(e224)* and mated to *dpy-11* hermaphrodites. This could be because Dpy gonads are shorter. The distance sperm must migrate in the Dpy uterus is 152 ± 17 μm (n = 24) as opposed to 195 ± 26 μm ($n = 36$) in the Fer uterus. In heterozygous *hc4*/+ hermaphrodites the transmission of *hc4* by selfing is reduced from the expected 0.25 to 0.20 ($n = 868$), equivalent to a male ratio of 0.4.

Effect of fer *males on fertile hermaphrodites:* WARD and CARREL (1979) have shown that mating with wild-type males reduces the fertility of hermaphrodite sperm. This inhibition of self-fertilization was observed even when no outcross fertilization occurred. We therefore asked if mating with sterile males reduces the fertility of hermaphrodite sperm. Extensive mating of fertile *dpy-11* hermaphrodites with sterile *fer* males did not reduce hermaphrodite self-fertilization (Table 8), whereas control matings wtih wild-type males or with permissively grown *fer-1* or *fer-5* males did. We conclude that these sterile *fer* males do not sterilize fertile hermaphrodites. Unexpectedly, mating with permissibly grown *fer-3* or *fer-4* males did not inhibit self-fertilization (Table 8). This ob-

TABLE 7

Transmission ratios

Male genotype	Temperature (°C)	Progeny scored	$\frac{Fer^-}{Fer^- + Fer^+}$	P
hc24/+	25	322	0.53 ± 0.10	0.2
hc3/+	25	682	0.48 ± 0.05	0.2
hc4/+	25	674	0.34 ± 0.05	<0.001
hc4/+	16	440	0.39 ± 0.04	<0.001
hc34/+	25	822	0.40 ± 0.04	<0.001
hc34/+	16	605	0.47 ± 0.02	0.25
hc4 e911/+ +	25	237	0.34 ± 0.01	<0.001
hc4 e224/+ +	25	582	0.45 ± 0.02	<0.025

Heterozygous *fer* males were grown at the indicated temperature and mated to the corresponding homozygous *fer* hermaphrodites. In several experiments, the *fer* chromosome was marked with a *cis*-visible marker: *hc3* with *dpy-5(e61)*; *hc34* with *unc-29(e1072)* and *hc4* with *unc76(e911)* or *dpy-11(e224)*. In these experiments, progeny counts were corrected for the expected recombination frequency. *P* values are χ^2 probabilities that the indicated ratios are not significantly different from 0.5.

TABLE 8

Matings of fertile hermaphrodites with sterile males

Males used in mating	Growth temperature (°C)	Total progeny	Self-progeny	Outcrossed progeny
unmated	25	100	100	0
+/+	25	186	24	162
	16	168	20	148
fer-1(hc1)	25	98	94	4
	16	122	12	110
fer-2(hc2)	25	114	114	0
	16	—	—	—
fer-3(hc3)	25	102	101	1
	16	119	105	14
fer-4(hc4)	25	88	87	1
	16	108	90	18
fer-5(hc23)	25	95	95	0
	16	74	39	35

Males grown either restrictively or permissively were mated to *dpy-11(e224)V* hermaphrodites. Matings were at 25°, and all males and hermaphrodites were serially transferred to fresh mating plates. The average brood size was different in two experiments (84 and 179), presumably because of slight temperature differences. The brood size within each experiment was, however, consistent. Therefore, brood sizes are normalized to 100 and the average for the two experiments is shown.

servation may reflect differences between male strains in the ability to outcompete hermaphrodite sperm.

DISCUSSION

Several results show that the mutants described here are defective in fertilization due to abnormal sperm: (a) they are sterile, but lay unfertilized eggs; (b) they make sperm in near normal numbers and store them in the spermathecae; (c) their oocytes are fertilizable by wild-type but not mutant sperm. Light and electron microscopic observations have confirmed this conclusion directly by finding morphological alterations in the sperm of *fer-1* through *fer-6* mutant males (Figure 4; S. WARD and Y. ARGON, in preparation).

Although the *fer* mutations were isolated in hermaphrodites, males of all mutant strains are also sterile. This is not necessarily expected, because out-fertilization demands sperm motility to a different extent than does self-fertilization and because there are functional differences between male and hermaphrodite sperm (WARD and CARREL 1979). Male sterility can result from inability to transfer sperm during copulation, from nonfunctional spermatozoa, or both. Among *fer* mutants, *fer-1 fer-2*, *fer-3* and *fer-4* males transfer sperm to mated hermaphrodites, whereas *fer-5* and *fer-6* males do not, although they appear to exhibit normal copulatory behavior.

The fraction of *fer* males that are able to transfer sperm is high when compared to male-sterile Drosophila mutants (reviewed by ROMRELL 1975). Only two male-sterile strains of Drosophila transfer sperm. Among sperm-specific

mutable functions, a large subset should be those functions that affect sperm motility. In Drosophila mutants having mature sperm, the sperm are nonmotile [*e.g.*, 1(1)v451; ms(2)3R; esc; ms(2)1; ms(2)10R]. Most *fer* mutants in *C. elegans* also exhibit defective motility (results presented here and our published observations), although *C. elegans* spermatozoa are ameboid and not flagellated. In particular, the phenotypes of *fer-1* and *fer-4* closely resemble that of the *KL-1* sterile mutation in *D. melanogaster* (KIEFER 1969). In all three mutants, male spermatogenesis seems normal by light microscopy, spermatozoa are deposited in uteri of mated females, but fail to reach the spermathecae in *C. elegans* or the seminal receptacle in *D. melanogaster*. Consequently, fertilization does not take place. The sperm are then forced out of the uterus by the passage of the oocytes. Ultrastructural analysis of *KL-1* sperm suggests an inefficient motile apparatus, as is suggested by our observations of *fer-1* and *fer-4* sperm *in vivo* and *in vitro*.

Most male-sterile mutants in the mouse and Drosophila are unable to transfer sperm due to anatomical or behavioral defects. Many of them are sterile because of deficiencies of germ line cells (*e.g.*, steel, tremulous, W. spotting). Other mutants have gonadal abnormalities, such as the abnormal connection of the *vas deferens* to the seminal vesicle in *px* (post-axial hemimelia, SEARLE 1964). The mouse mutations share pleiotropic effects in pigmentation, anemia, embryonic development and behavior. Likewise, sterility in Drosophila is associated with defects in development of the eye, wing or abdomen (ROMRELL 1975). Most *fer* mutations, on the other hand, have very specific defects in spermatogenesis, and no developmental or behavioral pleiotropy has been detected with the exceptions of *fer-5* and *fer-6* males, which fail to transfer sperm. Presumably, this is due to the method of isolation of the *fer* mutations, selecting hermaphrodites that are otherwise normal. The sperm of both *fer-5* and *fer-6* mutants are morphologically defective (S. WARD and A. ARGON, in preparation); in addition, males are impotent. It is possible that the wild-type alleles of these genes specify functions that are necessary for sperm maturation in both sexes and, in addition, are necessary in the copulatory organs of males. An alternative interpretation is that both sperm maturation and sperm-transfer machinery are under the same hormonal or neuronal control and that this control is defective.

The *fer* mutations described here are all autosomal. This is also true of all the spermatogenesis defective mutants that have been mapped so far (L. EDGAR, personal communication). Two additional *fer* mutants have recently been isolated in our laboratory and mapped to linkage groups II and X (T. ROBERTS, personal communication). Another sex-linked *fer* mutant, *let-9*, has been described by MENEELY and HERMAN (1979). Thus, *fer* mutants have been isolated on all six linkage groups of *C. elegans*. The mapping of *fer* genes does not reveal any special linkage relationships. They map among the clusters of genes that affect somatic tissues. Since these *fer* genes appear to code for germ line and not somatic functions, their map positions show that germ line genes are interdigitated on the chromosomes with genes whose function is needed in somatic tissues. BOVERI proposed that genes specific for the germ line might be on the ends of chromosomes in the parasitic nematode Ascaris because chromatin is lost from these

ends in somatic tissue, but retained in the germ line (see WILSON 1925). That the *fer* genes are not on the ends of chromosomes in *C. elegans* is consistent with the apparent absence of chromatin diminution in this species (SULSTON and BRENNER 1974; EMMONS, KLASS and HIRSH 1979).

In wild-type *C. elegans* hermaphrodites, copulation by males reduces hermaphrodite sperm fertility and stimulates oogenesis (WARD and CARREL 1979). The differing phenotypes of *fer* mutant males allow analysis of the mechanism of these two phenomena. Copulation and transfer of defective sperm by *fer-1*, *fer-3* and *fer-4* males is not sufficient to reduce hermaphrodite fertility (Table 8). Since the sperm themselves are defective in *fer-4* at least, it is unlikely that the inhibition of hermaphrodite sperm fertility is due to factors transferred in the seminal fluid. More likely explanations for this inhibition are displacement of hermaphrodite sperm from the walls of the spermatheca by male sperm or release of inhibitory factors from the male sperm. Oogenesis in spermless hermaphrodites can be stimulated by some mutant males, *fer-1*, but not by others, *fer-3* and *fer-4* (Table 4; also NELSON, LEW and WARD 1978). This confirms that copulation alone is not sufficient to stimulate oogenesis and suggests that some material transferred by the sperm is required, although the sperm need not reach the spermatheca. It is likely that normal oogenesis in unmated hermaphrodites is stimulated by the presence of hermaphrodite sperm because oogenesis is nearly normal in all the *fer* mutants that have sperm, but it is reduced in spermatogenic mutants with few sperm (NELSON, LEW and WARD 1978; L. EDGAR personal communication; J. MIWA and S. WARD, unpublished).

The TSP's of the *fer* mutants can be correlated with stages of sperm development, but the correlation is only approximate because spermatogenesis and spermiogenesis go on simultaneously. It is not known whether the TSP is the time of synthesis or the time of utilization of the mutant gene product (see HIRSH and VANDERSLICE 1976, for discussion of temperature-shift interpretation). The TSP's of *fer-3* and *fer-4* extend over the entire period of sperm development as if their defective gene product is synthesized or used throughout. The TSP of *fer-2* begins prior to the first meiotic events of spermatogenesis and ends prior to the completion of spermiogenesis. Two *fer-1* mutant strains and the *fer-7* mutant strain have short TSP's coinciding with spermiogenesis, as if their gene products are synthesized or used only during the final stages of sperm maturation. *fer-5* also has a short TSP, but it is slightly earlier, coinciding with the end of spermatogenesis.

Two experiments suggest that at least some of the *fer* genes are expressed in the sperm themselves during development. The altered transmission ratio of *fer-4* and *fer-7* implies post-meiotic gene expression that can occur only in the germ cells themselves (see below). The co-mating experiments show that the functions missing in *fer-1* and *fer-3* mutant sperm cannot be supplied by wild-type seminal fluid. It is still possible, however, that *fer-1* and *fer-3* sperm are defective because a function normally supplied by other cells is missing during their early development and cannot correct them later.

A fundamental question about sperm development is how much of the pheno-

type of spermatozoa depends on the diploid genotype of the organism and how much is under control of the haploid genome of the sperm. This question has evolutionary importance because haploid gene expression provides an opportunity for gametic selection and meiotic drive (ZIMMERING, SANDLER and NICOLETTI 1970; MULCAHY 1979). In plants, many cases of selective fertilization and gametic competition are known (HARTE 1975) because pollen cells undergo a variety of developmental and differentiative processes. In animals, however, diploid control is paramount in determining sperm phenotype, and haploid effects are rare. The *SD* locus in Drosophila (HARTL 1975), the *T/t* locus in mouse (BENNETT 1975) and M^D locus in *Aedes aegypti* (HICKEY and CRAIG 1966) are the only well-characterized genes that manifest distortion of segregation. The M^D locus in mosquitos increases the sex ratio in favor of males. In both the *SD* and *T/t* loci, the transmission ratio of alleles from heterozygous males is distorted in favor of the mutant alleles, whereas the segregation from heterozygous females is normal.

By a similar test of transmission ratio, the effects of *fer-1* and *fer-3* are under diploid control. However, mutations in *fer-4* and *fer-7* exhibit reduced transmission of the *fer*-bearing sperm from heterozygous males. Differential embryonic mortality cannot explain this result because no more than 1% of the eggs laid by homozygotes and heterozygotes in the course of these experiments failed to develop into adults. Differential production of gametes or reduced viability of sperm in the male gonad also cannot account for the transmission distortion, because both homozygous and heterozygous mutant worms produce normal numbers of sperm. In longitudinal EM sections of either homozygous or heterozygous *fer-4* male gonads, pycnotic or aberrant sperm are no more frequent (1 to 5%) than in wild type. Therefore, the altered transmission ratio must be due to reduced fertility of sperm bearing the mutant allele.

Since *fer-4* spermatozoa differ from wild-type sperm by light, scanning and transmission electron microscopy, we have tried to distinguish two populations of sperm derived from *fer4*/+ males, one resembling normal sperm and one resembling homozygous mutant sperm. All spermatozoa from such heterozygotes appear wild type; thus, the two haplotyes are not readily distinguishable morphologically.

In mice, the physiological state of the female affects the transmission ratio obtained from *t*/+ males. In delayed matings, there is less distortion of transmission of these alleles (BENNETT 1975). We cannot control the physiology of nematode hermaphrodites in a similar fashion, but the improvement of *hc4* transmission when mated to dumpy hermaphrodites could be related to the length of their uteri. If *hc4*-bearing sperm were slightly less motile than wild-type sperm, shortening the distance they have to travel would reduce the effect of the motility defect. The higher transmission of *hc4* from heterozygous hermaphrodites is consistent with this notion, but the hermaphrodite transmission ratio is still less than that of the *dpy* hermaphrodite, although hermaphrodite sperm have less distance to move. An alternative interpretation of the improved transmission ratio in matings with dumpy animals is the presence of a modifier

in the *fer-4 dpy-11* strain. The expression of segregation distortion in Drosophila and in Aedes is known to be affected by such modifiers (HARTL 1975).

If the reduced transmission ratio of *hc4* indeed reflects haploid gene expression, then the TSP of the mutant strain indicates that the *fer-4* gene is expressed both in the diploid and haploid states because its normal function is needed before the appearance of haploid nuclei in the gonad, as well as throughout the development of spermatozoa. Haploid gene effects necessitate transcription after the reduction division of meiosis. Evidence that some transcription occurs in secondary spermatocytes and in spermatids of various species has been reviewed by KIERZENBAUM and TRES (1978), although no reports on nematodes are included. In addition to transcriptional ability, there is a need to prevent the exchange of RNA and proteins between the spermatids, which are synctial in *C. elegans*, as well as in other species (WOLF, HIRSH and MCINTOSH 1978). One class of gene products that could be under a haploid control and that might not distribute evenly in the syncytium is membrane proteins. Some *t* alleles have been shown to control the expression of sperm surface anstigens, with two populations of spermatozoa in heterozygotes (YANAGISAWA *et al.* 1974). In men heterozygous for two histocompatibility loci, two antigenically distinct spermatozoa populations are found (FELLOWS and DAUSSET 1970). Another class of genetic products that might not distribute evenly is nuclear proteins. The *SD* distortion system is thought to involve the transition of somatic histones to sperm histones (KETTANEH and HARTL 1976). Attempts to identify mutant proteins in *fer-4* sperm, as well as in other *fer* mutant sperm, are in progress.

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LITERATURE CITED

ALBERTSON, D. G., J. E. SULSTON and J. G. WHITE, 1978 Cell cycling and DNA replication in a mutant blocked in cell division in the nematode *Caenorhabditis elegans*. Develop. Biol. **63**: 165–178.

ARGON, Y., 1979 Genetic and biochemical analysis of sperm-defective mutants of *Caenorhabditis* sperm. Ph.D. Thesis, Harvard University.

BEATTY, R. A., 1975 Genetics of animal spermatozoa. pp. 61–68. In: *Gamete Competition in Plants and Animals*. Edited by D. L. MULCAHY. North-Holland Publishing Co., Amsterdam.

BENNETT, D., 1975 The T-locus of the mouse. Cell **6**: 441–454.

BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77**: 71–94.

DAVIDSON, E. H., 1976 pp. 284–285, 356–358. In: *Gene Activity in Early Development*, Second edition, Academic Press, New York.

EDGAR, R. S. and W. B. WOOD, 1977 The nematode *Caenorhabditis elegans*: a new organism for intensive biological study. Science **198**: 1285–1286.

EMMONS, S. W., M. R. KLASS and D. HIRSH, 1979 Analysis of the constancy of DNA sequences during the development and evolution of the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. U.S. **76**: 1333–1337.

FELLOUS, M. and J. DAUSSET, 1970 Probable haploid expression of HL-A antigens on human spermatozoa. Nature **225**: 191–193.

HARTE, C., 1975 Competition in the haploid generation in *Oenothera*. pp. 31–42. In: *Gamete Competition in Plants and Animals*. Edited by D. L. MULCAHY. North-Holland Publishing Co., Amsterdam.

HARTL, D. L., 1975 Segregation distortion in natural and artificial populations of *Drosophila melanogaster*. pp. 83–92. In: *Gamete Competition in Plants and Animals*. Edited by D. L. MULCAHY. North-Holland Publishing Co., Amsterdam.

HICKEY, W. A. and G. B. CRAIG, 1966 Genetic disortion of sex ratio in a mosquito, *Aedes aegypti*. Genetics **53**: 1177–1196.

HIRSH, D., D. OPPENHEIM and M. R. KLASS, 1976 Development of the reproductive system of *Caenorhabditis elegans*. Develop. Biol. **49**: 200–219.

HIRSH, D. and R. VANDERSLICE, 1976 Temperature-sensitive developmental mutants of *Caenorhabditis elegans*. Develop. Biol. **49**: 220–235.

HODGKIN, J. A., 1974 Genetic and anatomical aspects of the *Caenorhabditis elegans* male. Ph.D. thesis, Cambridge University.

HODGKIN, J. and S. BRENNER, 1977 Mutations causing transformation of sexual phenotype in the nematode *Caenorhabditis elegans*. Genetics **86**: 275–287.

HORVITZ, H. R., S. BRENNER, J. HODGKIN and R. K. HERMAN, 1979 A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Molec. Gen. Genet. **175**: 129–133.

KETTANEH, N. P. and D. L. HARTL, 1976 Histone transition during spermiogenesis is absent in segregation distorter males of *Drosophila melanogaster*. Science **193**: 1020–1021.

KIEFER, B. I., 1969 Phenotypic effects of *Y* chromosome mutations in *Drosophila melanogaster*. I. Spermiogenesis and sterility in *KL-1* males. Genetics **61**: 157–166.

KIERSZENBAUM, A. L. and L. L. TRES, 1978 RNA transcription and chromatin structure during meiotic and post meiotic stages of spermatogenesis. Fed. Proc. **37**: 2512–2515.

KIMBLE, J. and D. HIRSH, 1979 The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. Develop. Biol. **70**: 396–417.

MENEELY, P. M. and R. K. HERMAN, 1979 Lethals, steriles and deficiencies in a region of the *X* chromosome of *Caenorhabditis elegans*. Genetics **92**: 99–115.

MULCAHY, D. L., 1979 The rise of angiosperms: a genecological factor. Science **206**: 20–23.

NELSON, G. A., 1979 The development and motility of nematode sperm and the genetic control of sex determination in *C. elegans*. Ph.D. Thesis, Harvard University.

NELSON, G. A., K. K. LEW and S. WARD, 1978 Intersex, a temperature-sensitive mutation of the nematode *Caenorhabditis elegans*. Develop. Biol. **66**: 386–409.

NELSON, G. A. and S. WARD, 1980 Vesicle fusion, pseudopod extension and ameboid motility are induced in nematode spermatids by the ionophore monensin. Cell **19**: 457–464.

RIDDLE, D. L., 1977 A genetic pathway for Dauer larva formation in the nematode *Caenorhabditis elegans*. Genetics **86**: s51–s52. ——, 1978 The genetics of development and behavior in *Caenorhabditis elegans*. J. Nematol. **10**: 1–16.

ROMRELL, L., 1975 pp. 735–745. Mutations influencing male fertility in *Drosophila melanogaster*. In: *Handbook of Genetics*, Edited by R. C. KING. Plenum, New York.

SEARLE, A. G., 1964 The genetics and morphology of two "luxoid" mutants in the house mouse. Genet. Res. **5**: 171–197.

SULSTON, J. E. and S. BRENNER, 1974 The DNA of *Caenorhabditis elegans*. Genetics **77**: 95–104.

SULSTON, J. E. and H. R. HORVITZ, 1977 Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. Develop. Biol. **56**: 110–156.

SUZUKI, D. T., T. KAUFMAN, D. FALK and the U.B.C. DROSOPHILA RESEARCH GROUP, 1976 Conditionally expressed mutations in *Drosophila melanogaster*. pp. 208–264. In: *The Genetics and Biology of Drosophila*, Vol. 1a. Edited by M. ASHBURNER and E. NOVITSKI. Academic Press, London, New York.

WARD, S. and J. S. CARREL, 1979 Fertilization and sperm competition in the nematode *Caenorhabditis elegans*. Develop. Biol. **73**: 304–321.

WARD, S. and J. MIWA, 1978 Characterization of temperature-sensitive, fertilization-defective mutants of the nematode *Caenorhabditis elegans*. Genetics **88**: 285–303.

WATERSTON, R. H. and S. BRENNER, 1978 A suppressor mutation in the nematode acting in specific alleles of many genes. Nature **275**: 715–719.

WILSON, E. B., 1925 *The Cell in Development and Heredity*. The MacMillan Company, New York.

WOLF, N., D. HIRSH and R. J. MCINTOSH, 1978 Spermatogenesis in males of the free-living nematode *Caenorhabditis elegans*. J. Ultrastruct. Res. **63**: 155–169.

YANAGISAWA, K., D. R. POLLARD, D. BENNETT, L. C. DUNN and E. A. BOYSE, 1974 Transmission ratio distortion at the T-locus: serological identification of two sperm populations in t-heterozygotes. Immunogenetics **1**: 91–105.

ZIMMERING, S. L. SANDLER and B. NICOLETTI, 1970 Mechanisms of meiotic drive. Ann. Rev. Genetics **4**: 409–436.

Corresponding editor: D. HARTL