Developmental Genetics of Chromosome *I* Spermatogenesis-Defective Mutants in the Nematode *Caenorhabditis elegans*

Steven W. L'Hernault,[1] Diane C. Shakes[2] and Samuel Ward[3]

Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210

Manuscript received December 30, 1987
Revised copy accepted July 1, 1988

ABSTRACT

Mutations affecting *Caenorhabditis elegans* spermatogenesis can be used to dissect the processes of meiosis and spermatozoan morphological maturation. We have obtained 23 new chromosome *I* mutations that affect spermatogenesis (*spe* mutations). These mutations, together with six previously described mutations, identify 11 complementation groups, of which six are defined by multiple alleles. These *spe* mutations are all recessive and cause normally self-fertile hermaphrodites to produce unfertilized oocytes that can be fertilized by wild-type male sperm. Five chromosome I mutation/deficiency heterozygotes have similar phenotypes to the homozygote showing that the probable null phenotype of these genes is defective sperm. Spermatogenesis is disrupted at different steps by mutations in these genes. The maturation of 1° spermatocytes is disrupted by mutations in *spe-4* and *spe-5*. Spermatids from *spe-8* and *spe-12* mutants develop into normal spermatozoa in males, but not in hermaphrodites. *fer-6* spermatids are abnormal, and *fer-1* spermatids look normal but subsequently become abnormal spermatozoa. Mutations in five genes (*fer-7*, *spe-9*, *spe-11*, *spe-13* and *spe-15*) allow formation of normal looking motile spermatozoa that appear to be defective in either sperm-spermathecal or sperm-oocyte interactions.

SPERMATOGENESIS is a complex process during which undifferentiated germ cells develop into motile spermatozoa. Dramatic cytological changes occur as spermatocytes undergo the two meiotic divisions that precede formation of the haploid gamete. Mutations that affect spermatogenesis exist in a number of organisms, including man (*e.g.*, AFZELIUS 1985), but genetic analysis of this process is limited to a few organisms such as mice (reviewed by HANDEL 1987), *Drosophila* (reviewed by HACKSTEIN 1987 and LIFSCHYTZ 1987) and the nematode *Caenorhabditis elegans* (HIRSH and VANDERSLICE 1976; WARD and MIWA 1978; ARGON and WARD 1980; EDGAR 1982). The hermaphroditic mode of reproduction in *C. elegans* makes it especially well-suited for genetic analysis of spermatogenesis. Screening for defective sperm in normally self-fertile hermaphrodites avoids selection of mutants in male mating behavior, but permits identification of animals that will produce progeny when mated to wild-type males.

Sperm are produced by both the *C. elegans* male, which has one sex chromosome (*XO*), and the hermaphrodite, which has two sex chromosomes (*XX*) (NIGON 1949). Spermatogenesis begins in the male during the L4 (last) larval stage and continues throughout the life of the animal (KLASS, WOLF and HIRSH 1976). The hermaphrodite gonad produces sperm only during the L4 stage, and these sperm are stored until they fertilize one of the oocytes produced by the adult gonad (HIRSH, OPPENHEIM and KLASS 1976). Sperm introduced into a hermaphrodite during copulation with a male will outcompete hermaphrodite-produced sperm and outcross progeny will result (WARD and CARREL 1979). Fertilization with either male or hermaphrodite sperm occurs inside the hermaphrodite, and embryonated eggs are laid 8–10 hr after fertilization (HIRSH, OPPENHEIM and KLASS 1976).

Many mutations that eliminate spermatogenesis or cause production of defective sperm will cause adult hermaphrodites to lay oocytes rather than embryonated eggs. Hermaphrodites can be scored for this phenotype several hours after they have been picked simply by inspecting plates for the presence of oocytes under the dissecting microscope. Such self-sterile mutant hermaphrodites are *spe* (*spe*rmatogenesis-defective) if their oocytes can be fertilized by mutant sperm inseminated by a heterozygous wild-type male (SIGURDSON, SPANIER and HERMAN 1984). To date, more than 60 *spe* mutations have been recovered by various laboratories (*e.g.*, HIRSH and VANDERSLICE 1976; WARD and MIWA 1978; ARGON and WARD 1980; EDGAR 1982; BURKE 1983; SIGURDSON, SPANIER and HERMAN 1984; this paper and unpublished observa-

[1] To whom correspondence should be addressed. Present address: Department of Biology, Emory University, 1555 Pierce Drive, Atlanta, Georgia 30322.
[2] Present address: Department of Molecular and Cellular Biology, Biosciences West Building, University of Arizona, Tucson, Arizona 85721.
[3] Present address: Section of Genetics and Development, 220 Bradfield Hall, Cornell University, Ithaca, New York 14853.

tions). A subset of previously studied *spe* mutants that made haploid sperm in normal numbers were called *fer* (for *fer*tilization-defective) to distinguish them from premeiotic mutants in several previous studies (*e.g.*, WARD and MIWA 1978; ARGON and WARD 1980). This distinction is no longer being made, and the designation *spe* is now used for all new mutations that interfere with spermatogenesis whether they act pre- or postmeiotically (mutations previously named *fer* will retain their names).

Feminization (*fem*; NELSON, LEW and WARD 1978; DONIACH and HODGKIN 1984; KIMBLE, EDGAR and HIRSH 1984; HODGKIN 1986) or feminization of the germline (*fog*; SCHEDL and KIMBLE 1988; M. K. BARTON and J. KIMBLE, unpublished observations) mutations can also cause hermaphrodites to stop laying embryonated eggs and start laying oocytes but, unlike *spe* mutants, *fem* and *fog* mutant hermaphrodites do not produce sperm. The germline of *fem* and *fog XO* animals (which in wild type makes only sperm) makes either sperm and oocytes or only oocytes, and the soma of *XO fem* animals (which in wild type is always male) can be hermaphrodite. Consequently, these non-*spe* hermaphrodites that are recovered as oocyte-laying animals during *spe* mutant hunts can be easily identified.

We would like to identify all genes that confer a *spe* phenotype, and approximately 36 genes on all six *C. elegans* chromosomes that confer this phenotype have, thus far, been discovered (HIRSH and VANDERSLICE 1976; WARD and MIWA 1978; ARGON and WARD 1980; EDGAR 1982; BURKE 1983; SIGURDSON, SPANIER and HERMAN 1984; our unpublished observations). This paper describes our genetic analysis of spermatogenesis genes on chromosome *I*; we confined our efforts to a single chromosome in order to obtain multiple alleles of *spe* genes and to allow the use of genetic strategies for recovery and maintenance of nonconditional *spe* mutations. We have found 11 chromosome *I spe* genes, six with more than one allele, and both temperature sensitive (ts) and nonconditional mutations have been recovered.

MATERIALS AND METHODS

Strains, culture conditions and genetic nomenclature: *C. elegans* var. Bristol (strain N2) was the wild-type strain used in all experiments (BRENNER 1974). The following genes and mutations were used in this study: *dpy-5(e61) I*, *unc-29(e1072) I*, *unc-11(e47) I*, *unc-13(e51, e450, e1091) I*, *unc-15(e73) I*, *dpy-14(e188) I*, *bli-4(e937) I*, *rol-1(e91) II*, *unc-32(e189) III* (BRENNER 1974); *him-5(e1490) V* (HODGKIN, HORVITZ and BRENNER 1979); *lin-11(n566) I*, *lin-17(n671) I* (FERGUSON and HORVITZ 1985); *tra-1(e1099) III* (HODGKIN and BRENNER 1977); *eDp6 III* (HODGKIN 1980); *sup-7(st5) X* (WATERSTON 1980). The chromosome *I* deficiencies *sDf4* (ROSE 1980), *sDf5*, *sDf6* (ROSE and BAILLIE 1980), *hDf6* (K. S. MCKIM, A. M. HOWELL and A. M. ROSE, unpublished results) and *nDf23*, *nDf24* and *nDf25* (FERGUSON and HORVITZ 1985) and the free duplication *sDp2* (ROSE, BAILLIE, and CURRAN 1984; HOWELL *et al.* 1987) were also employed. Culturing, handling and genetic manipulations of *C. elegans* were performed as described (BRENNER 1974), and standard nomenclature is used (HORVITZ *et al.* 1979). The entire broods of *cis* (++/ab) heterozygotes, reared at 20°, were analyzed during two factor recombination mapping (ROSE and BAILLIE 1979), and symmetric 95% confidence limits were tabulated (MAINLAND, HERRERA and SUTCLIFFE 1956). Nonconditional chromosome I *spe* mutations were balanced either by the free duplication *sDp2* or to complementing chromosome *I* deficiencies. Experiments were performed at 16°, 20° or 25°, as noted.

Screen for new chromosome *I* sperm-defective steriles: Ethyl methanesulfonate (EMS) was used as a mutagen (BRENNER 1974) for generating all the *spe* mutations used in this study. We have used four methods to identify picked mutant hermaphrodites that lay oocytes but produce progeny if mated to wild-type males. The first two methods are essentially equivalent to the M and S sets of BRENNER (1974). Mutant hermaphrodites that laid oocytes were mated to wild-type males and, if outcross progeny resulted, were considered *spe* candidates. These candidates were outcrossed once more and assigned to a chromosome by crosses to two triply marked strains that have a marker on each linkage group (TRENT, TSUNG and HORVITZ 1983). Our triply marked strain for chromosomes *I–III* uses the original markers for chromosome *I* (*dpy-5*) and *III* (*unc-32*) but replaces *bli-2 II* with *rol-1 II*; *bli-2* is very sick at 25° and cannot be used to score ts *spe* mutants. Only *spe* mutations located on chromosome *I* were examined further during the course of this study.

The other two methods used to screen for *spe* mutants involved mutagenesis of morphologically marked strains to permit recovery of mutations linked to easily scored markers. Young hermaphrodites that carried *dpy-5*; *rol-1*; *unc-32* were mutagenized in the first of these methods, outcrossed to wild-type males and 2-12 F_1 progeny were picked from each P_0 to individual plates. These F_1 progeny were allowed to lay eggs for one day at 20° after which they were removed, and the plate of eggs was shifted to 25° so potential ts *spe* mutants would not be missed. When an F_1 is *spe-/+*, about ¼ of its F_2 progeny are *spe/spe*; young non-Spe hermaphrodites lay only embryonated eggs while Spe hermaphrodites lay large numbers of oocytes. Plates containing large numbers of oocytes were identified 2 days after shifting to 25°, and six hermaphrodites homozygous for each one of the three morphological mutations were picked off each of these candidate plates. The plates containing these picked hermaphrodites were inspected one day later and, if oocytes were present, the *spe* mutation was recovered by mating these Spe hermaphrodites to wild-type males. If Spe hermaphrodites were predominantly of one morphological type, this suggested linkage of the *spe* mutation to that marked chromosome. We also recovered *spe* mutations out of mutagenized *dpy-5* hermaphrodites by essentially the same methods. Only mutations derived from different mutagenized P_0 hermaphrodites were kept in order to ensure that mutations were of independent origin. All mutations were outcrossed at least twice to wild type, and only those that exhibited linkage to *dpy-5* were examined in this study.

Complementation: Initially, all mutations were tested for complementation to one another until allelic series and map positions were established. Construction of *spe dpy-5 cis* double mutants made complementation tests of nonconditional *spe* mutations easier to perform because *dpy trans* heterozygotes (*spe-a dpy-5/spe-b dpy-5*) could be easily iden-

tified. Later in the analysis, two factor mapping, duplication mapping and phenotypic data were obtained for new *spe* mutations, and these suggested appropriate complementation tests. Mutations that did not fall into previously identified genes were three factor mapped and complementation tested to all *spe* genes within the same map interval.

Suppression studies: All nonconditional chromosome *I spe* mutations were tested for *sup-7(st5) X* suppressibility. Males that were *unc-13(e450) /+ I; sup-7(st5) X* were mated to *spe dpy-5(e61)* hermaphrodites. The F_2 broods were scored for the presence of self-fertile *dpy-5* hermaphrodites (with correction for crossing over between *dpy-5* and the *spe* mutation). If necessary, tentative *spe dpy-5 I; sup-7 X* candidates were picked and outcrossed to wild type to check for segregation of the oocyte-laying phenotype in the F_2. No chromosome *I spe* mutations were suppressible.

Phenotypic analysis: Brood and/or oocyte counts were performed on individual hermaphrodites that were picked and transferred either every day (if at 25°) or every second day (if at 16°) until they stopped laying or died. Temperature sensitive periods were determined as previously described (HIRSH and VANDERSLICE 1976). Male sperm development and morphology were examined *in vitro* after dissection in sperm medium (SM): 50 mM HEPES titrated to pH 7.0 or 7.8 with NaOH, 50 mM NaCl, 25 mM KCl, 5 mM $CaCl_2$, 1 mM $MgSO_4$, plus 10 mg/ml polyvinylpyrrolidone, PVP 40 (NELSON 1979; NELSON and WARD 1980; NELSON, ROBERTS and WARD 1982). SM allows primary spermatocytes to develop into haploid spermatids. Some spermatids were treated *in vitro* with the sperm activator pronase so that both their differentiation and the motility of resulting spermatozoa could be examined (NELSON and WARD 1980; WARD, HOGAN and NELSON 1983). Sperm development and motility in hermaphrodites were studied by examining the spermathecae of live worms dissected in SM medium. Nuclear events were studied by either fixing worms in Carnoy's solution and staining the DNA with DAPI (4,6-diamidino-2-phenylindol) or Hoechst 33258, or dissecting worms in SM containing lipid-soluble Hoechst 33342. Light and electron microscopic techniques were essentially as previously described (WARD, ARGON and NELSON 1981).

Many of our phenotypic observations were performed before our *spe* mutations were properly balanced (see above). This was possible because linkage of a *spe* mutation to *dpy-5* permits identification of probable *spe dpy-5* mutants in mixed populations. Phenotypes that were initially determined by examining *spe dpy-5* animals have all been confirmed by subsequent analysis of *spe* non-*dpy-5* animals.

RESULTS

Isolation of mutations and genetic analysis: We have identified 23 new, independently derived chromosome *I* mutations that result in abnormal spermatogenesis, in addition to the six chromosome *I* mutations previously described (ARGON and WARD 1980). We used four different strategies to recover these *spe* mutations (see MATERIALS AND METHODS), and all mutations proved to be recessive. The most efficient strategy was isolation of mutations in *cis* to *dpy-5* since this established linkage to chromosome *I* and allowed convenient identification of sterile hermaphrodites during outcrossing and complementation testing. *spe* genes that are distant from *dpy-5*, such as *spe-13*, (about 21 map units; Table 1) still exhibit linkage; this suggests that at least 42 of the 48 known map units on chromosome *I* have been sampled for *spe* mutations in our study. Our collection includes both temperature-sensitive and nonconditional mutations. Many nonconditional *spe* mutations were found to lie under the free duplication *sDp2* (ROSE, BAILLIE and CURREN 1984; HOWELL *et al.* 1987), which could be used to balance the sterile mutant allele, usually on a *dpy-5* marked chromosome. These duplication-bearing strains are stable, and they permit both strain maintenance and genetic manipulation. Nonconditional steriles that do not lie under *sDp2* are maintained as heterozygotes to a complementing chromosome *I* deficiency. Genes are positioned on the chromosome *I* map (Figure 1) by two and three factor, duplication and deficiency mapping and the results appear in Tables 1, 2 and 3.

Description of wild-type spermatogenesis: Wild-type spermatogenesis in *C. elegans* has been described in detail previously (reviewed by WARD 1986). These results are summarized in diagrammatic form in Figure 2 and explained here in order to make our descriptions of mutant phenotypes understandable. Spermatogenesis occurs similarly in both the hermaphrodite and male. Primary spermatocytes develop from spermatogonia as a syncytium with a central core of cytoplasm, the rachis, connecting peripheral nuclei. Primary spermatocytes (which are 4*N*) separate from the rachis after entering meiosis, go through one division to give rise to secondary spermatocytes (which are 2*N*) and a second division to give rise to four haploid (1*N*) spermatids. Cellular components not needed by the mature sperm, such as ribosomes, are partitioned into the residual body before the spermatids separate. The hermaphrodite gonad produces sperm only during the L4 (last) larval stage and then switches to produce only oocytes in the adult. Spermatids made during the L4 stage are activated, become crawling spermatozoa (Figure 2b) and are stored in a chamber called the spermatheca where they fertilize incoming oocytes.

Male spermatogenesis, as in the hermaphrodite, also begins during the L4 stage but, unlike the hermaphrodite, continues throughout the adult life of the worm. Conversion of male-produced spermatids to amoeboid spermatozoa (termed either sperm activation or spermiogenesis, as it is in hermaphrodites) occurs during copulation with hermaphrodites. These male-derived spermatozoa crawl through the hermaphrodite uterus to reach the spermatheca, where they fertilize many oocytes because they displace hermaphrodite-produced spermatozoa (WARD and CARREL 1979). Male spermatids can also be converted into spermatozoa *in vitro* by treatment with a protease, weak bases such as triethanolamine or the ionophore monensin (NELSON and WARD 1980; WARD, HOGAN

TABLE 1

Two-factor mapping in *cis*[a]

Gene	Genotype of heterozygote	Parental types	Recombinant types	Percent recombination (100 *P*)[b]
spe-4	*spe-4 dpy-5/++*	222 wt[c] 78 Dpy Spe	4 Spe 1 Dpy	0.63–3.8
spe-8	*spe-8 lin-17/++*	223 wt 65 Lin Spe	13 Spe 13 Lin	5.7–13
spe-9[d]	*spe-9 dpy-5/++*	234 Dpy Spe	36 Dpy	5.4–8.7
spe-11	*spe-11 dpy-5/++*	1352 wt 460 Dpy Spe	1 Spe 0 Dpy	0.06
spe-12	*spe-12 dpy-5/++*	493 wt 138 Dpy Spe	14 Spe 13 Dpy	2.8–6.1
spe-13	*spe-13 dpy-5/++*	639 wt 144 Dpy Spe	92 Spe 90 Dpy	18–24
spe-15	*spe-15 dpy-5/++*	512 wt 111 Dpy Spe	60 Spe 56 Dpy	14–21
fer-6	*fer-6 dpy-5/++*	665 wt 183 Dpy Spe	32 Spe 44 Dpy	6.8–11

[a] Two-factor crosses were performed as described by BRENNER (1974) for markers in *cis*.
[b] The 95% confidence values limits on two-factor data are listed, except for *spe-11*.
[c] wt = wild type.
[d] Only *dpy* animals were scored.

and NELSON 1983). Resulting spermatozoa, whether produced *in vivo* or *in vitro*, use their single pseudopod to crawl across the substrate. Sperm motility is necessary for *in vivo* fertility because spermatozoa can be swept out of the spermatheca and into the uterus by passing oocytes. These spermatozoa must crawl out of the uterus and back into the spermatheca or they will eventually be pushed out of the hermaphrodite reproductive tract (WARD and CARREL 1979). Unlike most animal sperm, *C. elegans* sperm do not have a flagellum or an acrosome.

Phenotypes of mutations affecting meiosis: *spe-4(hc78, hc81)*—Spermatogenesis arrests in *spe-4* males during the formation of haploid spermatids from mature primary spermatocytes. The dissected testis of wild-type virgin adult males reveals spermatocytes developing in syncytium along the rachis, a few free spermatocytes (not joined to the rachis) that are completing the nuclear and cytokinesis events of meiosis and hundreds of accumulated spermatids (like Figure 3a). In contrast, *spe-4* males produce a few cells that appear to be in the process of completing meiosis but fail to produce any spermatids (Figure 3b); *spe-4/sDf6* males exhibit the same phenotype (data not shown). Some of these free cells will form the typical cloverleaf structures of syncytial spermatid/residual body clusters (Figures 2a and 3c), but most look like primary spermatocytes (Figure 3c). There "spermatocytes" differ from wild type because they have completed the nuclear events of meiosis and have four haploid nuclei in syncytium (Figure 3d). The total number of haploid nuclei visualized in a DAPI stained *spe-4* male is similar to the number observed in a wild-type male (data not shown).

The *spe-4* adult hermaphrodites, unlike males, do not retain the developmentally arrested spermatocytes that they form. Some apparently haploid sperm nuclei appear in L4 *spe-4* larvae during the normal time of spermatogenesis (data not shown), but they must be lost or resorbed because no sperm or haploid nuclei are observed after DAPI staining of the adult reproductive tract of hermaphrodites (Figure 4a). *spe-4* oocytes pass through the spermatheca, resume meiosis and eventually become polyploid. These polyploid oocytes resemble the oocytes in old wild-type hermaphrodites that have exhausted their sperm supply (WARD and CARREL 1979) or the oocytes of later-acting *spe* mutants such as *fer-1* (WARD and MIWA 1978). *spe-4* bearing worms lay fewer of these oocytes than wild type or many of the later acting mutants (Table 4).

spe-5(hc93, hc110)—Spermatogenesis in *spe-5* males, as in *spe-4* males, arrests during development of spermatids from spermatocytes. *spe-5* males never accumulate large numbers of spermatids, but they do produce some free sperm cells that consist of a mixture of primary and secondary spermatocytes as well as a few spermatids (Figure 5, a and b). Many of these free sperm cells contain haploid nuclei; the number of haploid nuclei generally correlates with the cytological stage of arrest (compare Figure 5, b and c). Although *spe-5* males produce a large number of haploid nuclei, diploid nuclei and disorganized nuclear divisions are also observed in DAPI stained

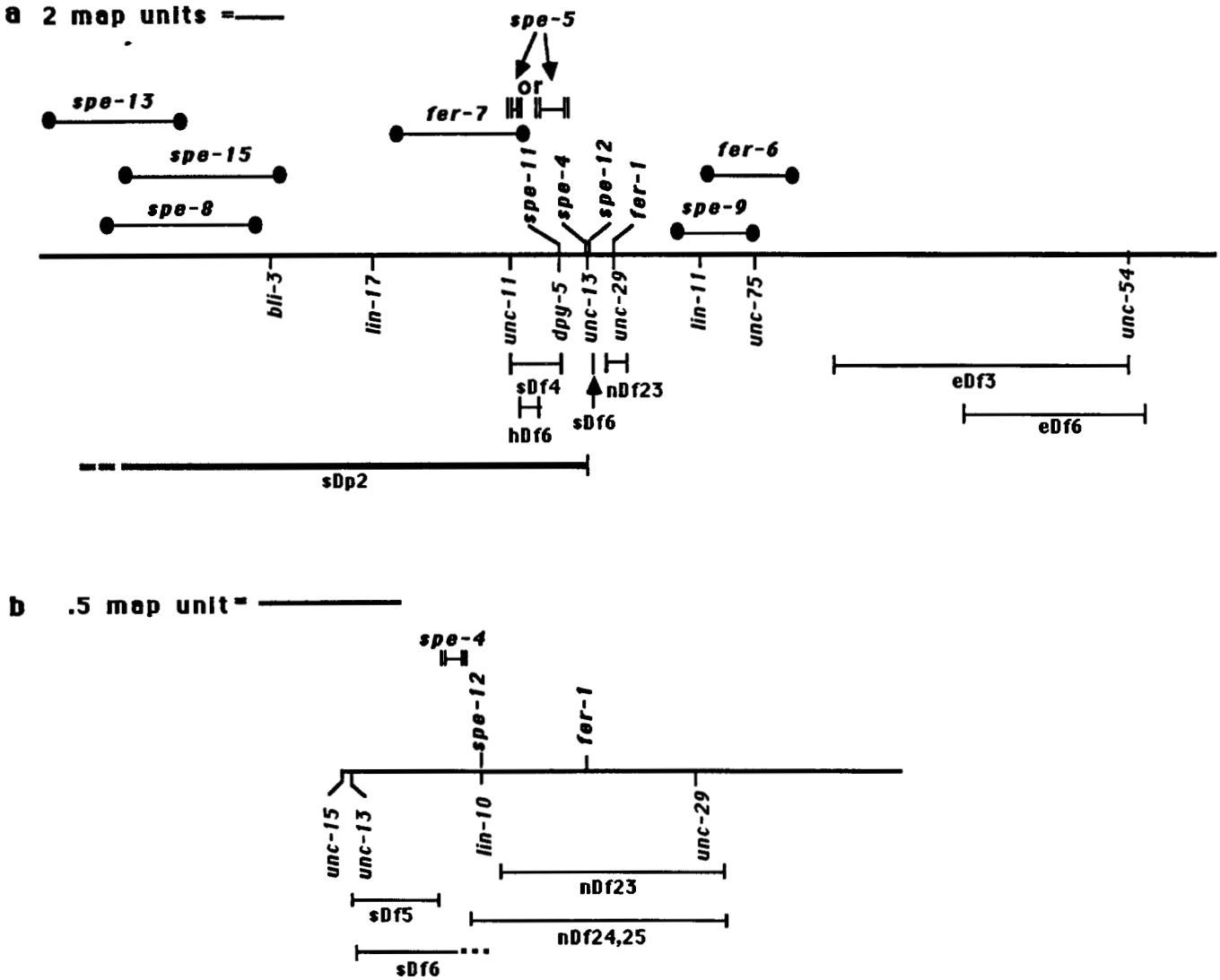

FIGURE 1.—a, A partial genetic map of *C. elegans* chromosome *I*. Genes that cause spermatogenesis defects (*spe*) are drawn above the line that represents chromosome *I*, and morphological and other markers are drawn below the line (the positions of these markers are from EDGLEY and RIDDLE (1988). Ninety-five percent confidence intervals of *spe* gene two factor mapping data are indicated by closed circles (see MATERIALS AND METHODS and Table 2). The extent of deficiencies (*Df*'s) and duplications (*Dp*'s) are indicated below the lines. b, Expanded genetic map of *C. elegans* showing the region of chromosome *I* extending from *unc-15* to *unc-29*.

whole mounts of *spe-5* males (data not shown). This variable arrest of spermatogenesis has been observed for *spe-5(hc93)*, *spe-5 (hc110)*, *spe-5(hc93/hc110)* and *spe-5(hc93/sDf4)*.

spe-5 adult hermaphrodites, unlike males, do not retain the developmentally arrested spermatocytes that they form. Some apparently haploid sperm nuclei appear in L4 larvae during the normal time of spermatogenesis, but they must be resorbed or lost because few, if any, sperm or haploid nuclei are observed after DAPI staining of the reproductive tract of young adult hermaphrodites (data not shown, but similar to *spe-4* in Figure 4a). *spe-5(hc93)* hermaphrodites sometimes produce one or two progeny indicating that fertile spermatozoa must develop occasionally (Table 4). Most oocytes produced by *spe-5* hermaphrodites do not get fertilized and become polyploid (similar to *spe-4* hermaphrodites; Figure 4a). *spe-5* hermaphrodites lay fewer of these oocytes than wild type or many of the later acting spermatogenesis defective mutants (Table 4).

Postmeiotically acting *spe* mutations: *spe-8(hc40, hc50, hc53, hc79, hc85, hc108, hc134*ts) and *spe-12(hc76)*—Mutations in *spe-8* and *spe-12* cause indistinguishable phenotypes so our data on these two genes will be discussed together. The *spe-8* gene is presently defined by seven nonconditional alleles. *spe-8* mutant hermaphrodites are completely self-sterile, with the exception of *hc134*ts hermaphrodites, which produce a few progeny at 16° (Table 4 and data not shown). There is one allele of *spe-12*, and mutant *spe-12* hermaphrodites rarely produce any young (Table 4); this is also true for *spe-12/nDf24* hermaphrodites (data not shown).

TABLE 2

Three-factor crosses[a]

Gene	Genotype of heterozygote	Phenotype of selected hermaphrodite	Genotype of selected hermaphrodite (with respect to unselected marker)
spe-5	*spe-5/dpy-5 unc-11*	Dpy non-Unc	3/20 *spe-5/+*
		Unc non-Dpy	5/21 *spe-5/+*
spe-8	*spe-8/lin-17 dpy-5*	Dpy non-Lin	12/12 *spe-8/+*
		Lin non-Dpy	0/12 *spe-8/+*
spe-9	*spe-9/unc-75 dpy-5*	Dpy non-Unc	57/57 *spe-9/+*
		Unc non-Dpy	0/44 *spe-9/+*
spe-11	*spe-11/unc-13 dpy-5*	Dpy non-Unc	0/22 *spe-11/+*
		Unc non-Dpy	24/24 *spe-11/+*
spe-12	*spe-12/unc-29 dpy-5*	Dpy non-Unc	10/11 *spe-12/+*
		Unc non-Dpy	1/11 *spe-12/+*
spe-13	*spe-13/dpy-5 unc-11*	Dpy non-Unc	18/18 *spe-13/+*
		Unc non-Dpy	0/12 *spe-13/+*
spe-15	*spe-15/dpy-5 lin-17*	Dpy non-Lin	21/21 *spe-15/+*
		Lin non-Dpy	0/16 *spe-15/+*
fer-6	*fer-6/dpy-5 unc-75*	Dpy non-Unc	18/18 *fer-6/+*
		Unc non-Dpy	0/18 *fer-6/+*

[a] Three factors crosses were performed as described by BRENNER (1974). F_1 heterozygotes of genotype *spe/ab* will segregate A non-B and B non-A F_2 recombinants, which were picked and scored for expression of a Spe phenotype in 1/4 of the F_3 self-progeny.

TABLE 3

Deficiency and duplication mapping of *spe* genes[a]

Gene	*sDp2*	*hDf6*	*sDf4*	*sDf5*	*sDf6*	*nDf25*	*nDf24*	*nDf23*	*eDf3*	*eDf6*
spe-4	+			+	0	+	+			
spe-5	0	+	0							
spe-8	0									
spe-9	+								+	+
spe-11	0	+	0							
spe-12	+			+	+	0	0	+		
spe-15	0									
fer-1	+				+	0	0	0		
fer-6				+				+		+

[a] + indicates complementation, 0 indicates noncomplementation; no symbol indicates the test was not performed.

spe-8 or *spe-12* adult hermaphrodites contain spermatids that fail to differentiate into spermatozoa. These nonmotile spermatids are rapidly swept out of the spermatheca (Table 5) into the uterus by passing oocytes (Figure 4b); a similar phenotype has been described previously for *fer* mutants, which have spermatids that form abnormal spermatozoa (ARGON and WARD 1980). Unmated males bearing *spe-12* (either *spe-12/spe-12* or *spe-12/nDf24*; data not shown) or any of the four *spe-8* alleles, *hc40*, *hc50*, *hc79* or *hc108*, accumulate normal looking spermatids in normal numbers. Surprisingly, mating induces maturation of *spe-8* and *spe-12* male spermatids into cytologically normal spermatozoa. These male-derived spermatozoa are fertile in hermaphrodites of the same or different genotype and normal outcross progeny are produced. Thus, *spe-8* or *spe-12* males are normally fertile while unmated *spe-8* or *spe-12* hermaphrodites are self-sterile. Combining *spe-8* males with *spe-8* hermaphrodites or *spe-12* males with *spe-12* hermaphrodites creates a stable male/"female" gonochoristic strain.

C. elegans males differ from hermaphrodites because they have only one *X* chromosome instead of two (NIGON 1949). In order to test whether the differing fertility of *spe-8* and *spe-12* males and hermaphrodites was a result of *X* chromosome dosage, we have used the transformer gene *tra-1* to construct strains that have fertile (albeit, at reduced levels relative to wild type) phenotypic males with a *XX* genotype (HODGKIN and BRENNER 1977). The fertility of these males was assessed by the mating scheme outlined in Figure 6, and the results indicate that *spe-8* and *spe-12* phenotypic males produce functional spermatozoa, even if they have the *XX* genotype of a hermaphrodite.

spe-9 (*hc52*ts, *hc88*ts)—Unmated *spe-9* males, grown at the restrictive temperature (25°), accumulate large numbers of normal-appearing spermatids that activate *in vitro* following pronase treatment to give cytologically normal, crawling spermatozoa (data not shown). *spe-9*(*hc88*ts) males are apparently infertile at restrictive temperatures; eight matings of four *spe-9*(*hc88*ts) males and one *dpy-5* hermaphrodite failed to yield any outcross progeny. *spe-9* hermaphrodites (either *spe-9* allele), raised under restrictive conditions, contain spermatozoa that are motile in the spermatheca and crawl *in vitro* (data not shown), but are slowly swept out of the spermatheca by passing oocytes (Table 5). *spe-9*(*hc88*ts) hermaphrodites are completely self-sterile while *spe-9*(*hc52*ts) hermaphrodites can still produce a few young under restrictive conditions (Table 4).

The time period during development when *spe-9*(*hc88*ts) is temperature sensitive for fertility (the TSP) has been determined by growing mutant hermaphrodites at 16° (the permissive temperature) or 25° (the restrictive temperature) and shifting worms of various ages to the other temperature. The total number of progeny produced by animals that were shifted from one temperature to the other was then determined and is summarized in Figure 7. The TSP for the sterile phenotype is between 30 and 45 hr of postembryonic development; this is during the L4 larval period, when spermatogenesis occurs in the wild-type hermaphrodite (HIRSH, OPPENHEIM and KLASS 1976).

spe-11(*hc77*ts, *hc90*)—Hermaphrodites bearing the temperature sensitive allele of *spe-11*, (*hc77*), produce a few progeny under restrictive growth conditions while mutant hermaphrodites bearing the other allele (*hc90*) are nonconditional and completely self-sterile

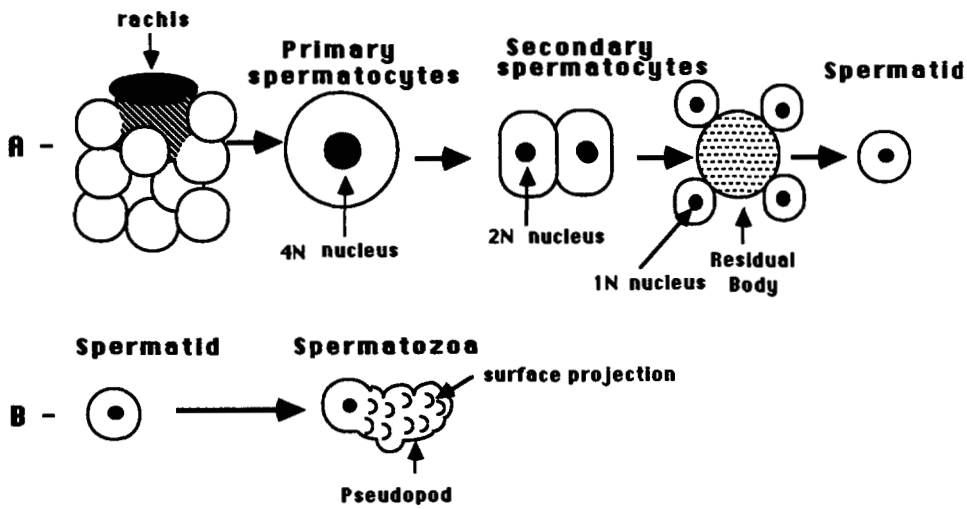

FIGURE 2.—Summary of *C. elegans* spermatogenesis. a, Stages of meiosis. Primary spermatocytes develop while in syncytium with the rachis. Mature primary spermatocytes bud off the rachis, undergo a first meiotic division and resulting secondary spermatocytes can separate or stay in syncytium; only the latter is illustrated. Four haploid spermatids subsequently develop from these syncytial secondary spermatocytes during the second meiotic division. Much of the cytoplasm present in the secondary spermatocytes ends up in the residual body during the cytoplasmic volume reduction that accompanies spermatid differentiation. b, Postmeiotic stages. The sessile spermatid, lacking any obvious cellular polarity, is converted into an actively crawling spermatozoan with a polarity that is indicated by its single projection-studded pseudopod.

(Table 4). The *trans* heterozygote *hc77/hc90* is also leaky, producing small broods of approximately the same size as *hc77* homozygotes (Table 4). Brood sizes of *hc77/sDf4*, however, are lower than *hc77* homozygotes (Table 4). The *hc90* hermaphrodites never produce any progeny, and the phenotype of *hc90/sDf4* animals is the same as *hc90* homozygotes.

The few progeny produced by *spe-11*(*hc77*ts) hermaphrodites at 25° are produced early in the egg-laying period. Preliminary temperature shift experiments indicate that *spe-11*(*hc77*ts) hermaphrodites can produce more progeny if they are shifted to permissive conditions as young adults after spermatogenesis has been completed. This demonstrates that mature *spe-11* spermatozoa can reverse their sterile phenotype after shift-down to the permissive temperature, and suggests that the *spe-11* gene product is itself temperature reversible, since mature *C. elegans* sperm do not synthesize proteins (they lack ribosomes: reviewed by WARD 1986).

Sperm are swept out of the spermatheca very slowly by passing oocytes in *spe-11*(*hc90*) or restrictively raised *spe-11*(*hc77*ts) hermaphrodites (Figure 4c and Table 5), and motile spermatozoa are observed in the spermatheca of live hermaphrodites (data not shown). *spe-11* oocytes sometimes undergo a few cell divisions (either allele; Figure 4c). This oocyte phenotype appears to be due to the presence of *spe-11* sperm because we have occasionally observed hermaphrodites that lack sperm in one of the gonadal arms (this phenomenon also occasionally occurs in wild-type hermaphrodites; unpublished observations), and the oocytes formed by the spermless arm remain as single cells, but become polyploid like most other *spe* mutants (data not shown). Restrictively grown *spe-11*(*hc77*ts), but not *spe-11*(*hc90*), hermaphrodites also produce a number of round (rather than the normal oblong) thinly shelled eggs, in addition to many unshelled oocytes and a few apparently normal embryos (Table 4). These thinly shelled eggs, unlike the normal embryos, do not hatch.

Unmated *spe-11* males accumulate spermatids that are cytologically wild type and can be induced by pronase treatment to differentiate *in vitro* into spermatozoa that propel themselves across the substrate normally (data not shown). The fertility of *spe-11*(*hc77*ts) *him-5* males was assessed by mating them in excess to *dpy-5* hermaphrodites. A few of the total young present after three days of mating were outcross phenotypic wild types (13/472 or about 3%) indicating that *spe-11*(*hc77*ts) males, like *spe-11*(*hc77*ts) hermaphrodites, are only slightly fertile under restrictive conditions.

spe-13(*hc137*ts)—*spe-13* males grown at 25° produce normal numbers of spermatids that activate *in vitro* after pronase treatment to form normal-appearing spermatozoa. Electron microscopic analysis of unmated *spe-13* males reveals no obvious cytological defects in spermatocytes or spermatids (data not shown). *spe-13* males grown at 25° are apparently infertile, even though their spermatozoa appear cytologically normal, since they fail to sire progeny in mating experiments (80 *spe-13 him-5* males tested; 20 attempted crosses of four males to one *dpy-5* hermaphrodite at 25°).

spe-13 hermaphrodites are completely self-sterile at 25° and weakly self-fertile at 16° (Table 4). *spe-13* hermaphrodites grown at 25° produce spermatozoa that can crawl in the spermatheca but are rapidly swept out into the uterus by passing oocytes and are eventually lost (Table 4). These oocytes resume meiosis after passing through the spermatheca and

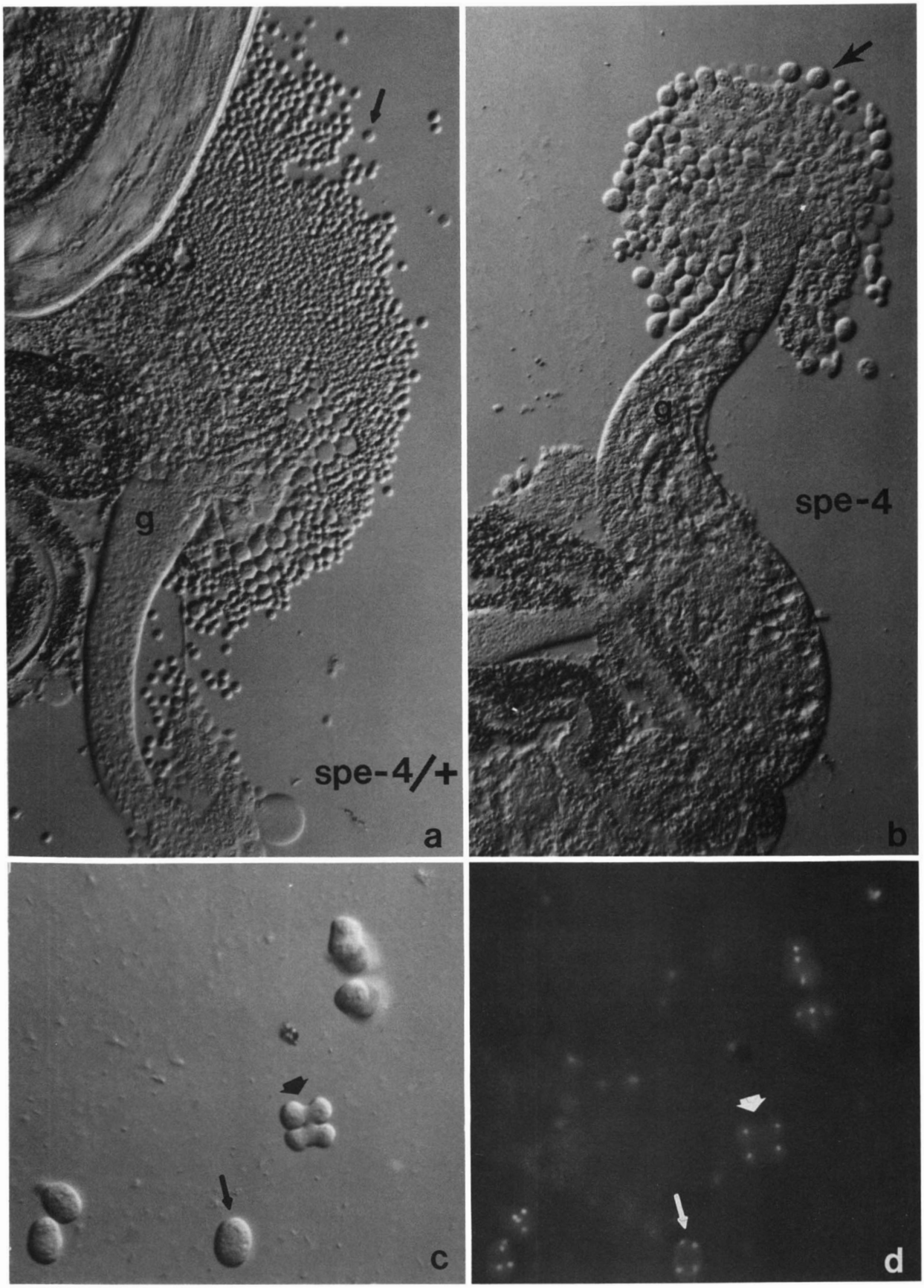

FIGURE 3.—a and b, Low magnification micrographs of dissected male gonads of *spe-4(hc78)/+* (a) and *spe-4(hc78)* (b) ×310. *spe-4/+* is indistinguishable from wild type, accumulating hundreds of spermatids (representative spermatid at arrow). *spe-4* males do not accumulate spermatids but arrest spermatogenesis at what appears to be primary spermatocytes (representative spermatocyte at the arrow), most of which remain attached to the syncytial gonad (g). c and d, Nomarski and fluorescence micrographs of DAPI stained *spe-4(hc81)* spermatocytes, ×750. arrow, a cell that looks like a spermatocyte by Nomarski optics (c) actually has four haploid nuclei when viewed by fluorescence microscopy (d). arrowhead, a cell that has attempted to complete meiosis but the haploid nuclei do not separate into spermatids, and the residual body is much smaller than wild type.

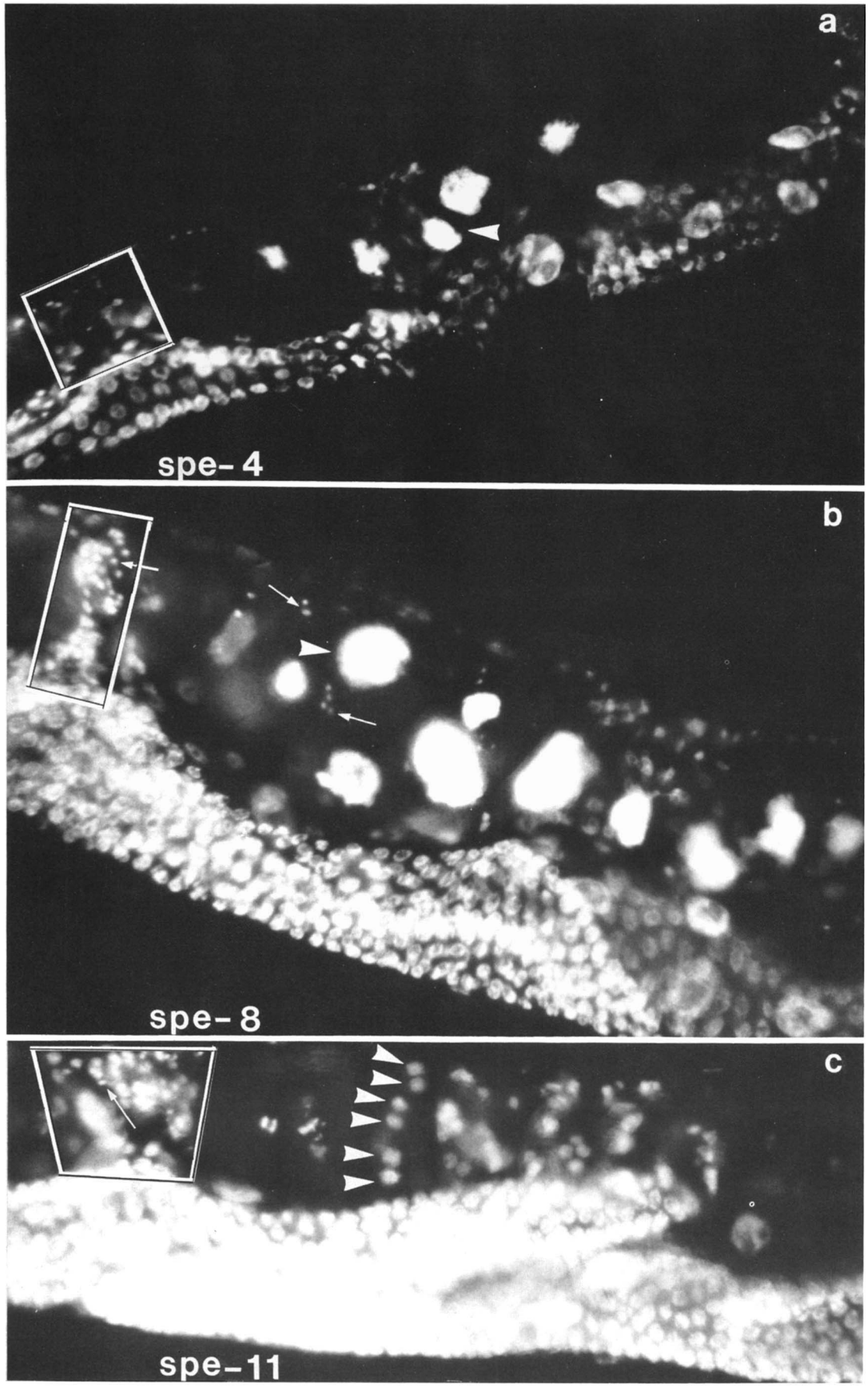

FIGURE 4.—Fluorescent photographs of DAPI stained hermaphrodites. The spermatheca appears in the boxed regions, sperm nuclei are indicated by small arrows and oocyte nuclei by large arrowheads. Analyses of nonconditional *spe* mutants were carried out in a *dpy-5* background, which appeared to have no effect on the Spe phenotype (see MATERIALS AND METHODS). a, *spe-4(hc81) dpy-5(e61)* hermaphrodites do not accumulate mature sperm in their spermatheca, yet oocytes are produced that become polyploid (arrowhead), ×500. b, *spe-8(hc50) dpy-5(e61)* hermaphrodites accumulate many sperm in their spermatheca but these are swept into the uterus (arrows to the right of the box) by passage of the oocytes that then become polyploid in the uterus (arrowhead), ×800. c, *spe-11(hc90) dpy-5(e61)* hermaphrodites accumulate many sperm in their spermatheca (arrow in box), and these sperm are not swept into the uterus by passing oocytes. A few oocytes undergo an orderly series of nuclear divisions (arrowhead) but they do not form normal embryos, ×800.

TABLE 4

Quantitation of sterile phenotype[a]

Gene	Allele	Growth temperature	Progeny (SE)	Shelled eggs[b]	Oocytes (SE)	*n*
Wild type (Bristol)		16°	350 ± 54	>1	179 ± 25	15
		25°	183 ± 40	>1	122 ± 22	15
spe-4	*hc78*	16°	0	0	36 ± 4	15
	hc81	16°	0	0	77 ± 11	17
spe-5	*hc93*	16°	<1	<1	44 ± 5	30
	hc110	16°	0	0	43 ± 2	18
spe-8	*hc50*	16°	<1	<1	346 ± 28	17
	hc53	16°	0	0	241 ± 27	15
	hc79	16°	<1	<1	346 ± 22	17
	hc134	16°	11 ± 2	<1	187 ± 30	26
	hc134	25°	<1	<1	365 ± 28	14
spe-12	*hc76*	16°	<1	0	349 ± 25	15
spe-9	*hc52*	16°	330 ± 74	~1	214 ± 9	15
	hc52	25°	<1	<1	222 ± 25	15
	hc88	16°	380 ± 43	<1	174 ± 13	15
	hc88	25°	0	0	168 ± 16	16
spe-13	*hc137*	16°	12 ± 9	~2	735 ± 30	17
	hc137	25°	0	0	312 ± 17	17
spe-15	*hc75*	16°	9 ± 4	<1	368 ± 16	18
	hc75	25°	<1	<1	119 ± 7	19
spe-11	*hc77*	16°	305 ± 56	<1	159 ± 20	15
	hc77	25°	8 ± 5	7 ± 6	114 ± 19	15
	hc77/hc90	25°	7 ± 6	ND[c]	ND	18
	hc77/sDf4	25°	>1	ND	ND	11
	hc90/sDf4	20°	0	ND	ND	15
	hc90	16°	0	0	378 ± 26	17

[a] Oocyte and/or progeny counts were performed on 15–30 individual hermaphrodites until they either died or had stopped laying.
[b] These shelled eggs do not subsequently hatch.
[c] ND = not determined.

eventually become polyploid (data not shown but similar to *spe-8* in Figure 4b). Surprisingly, while the self-sterility phenotype of *spe-13* is slightly temperature sensitive (Table 4), the sweep of hermaphrodite spermatozoa is dramatically temperature sensitive (Table 6). *spe-13* hermaphrodites raised at 16° retain most of their sperm, even after 196 oocytes have been laid. In contrast, *spe-13* hermaphrodites raised at 25° have swept out all of their sperm after 197 oocytes have been laid (Table 6). Interestingly, while 25° grown *spe-13* worms lay about as many oocytes as the total number of oocytes produced by unmated wild-type hermaphrodites, 16° grown *spe-13* hermaphrodites, which contain sperm that are infertile but are not swept out by passing oocytes, produce 1.4 times as many oocytes as unmated wild-type hermaphrodites (note that most oocytes get fertilized and give rise to progeny in the case of wild type; Table 4).

spe-15(*hc75*ts)—*spe-15* hermaphrodites are slightly self-fertile at 16° (Table 4), and their young are produced throughout the life cycle, unlike most of the other *spe* mutants whose progeny (if any) are produced during the first day of adulthood. *spe-15* hermaphrodites are almost completely self-sterile and lay many oocytes at 25° (Table 4). *spe-15* males produce large numbers of spermatids that activate *in vitro* after pronase treatment to form spermatozoa that are cytologically indistinguishable from wild type (data not shown).

fer-1(*hc1*ts, *hc13*ts, *hc24*ts, *hc82*ts, *hc91*ts, *hc47*, *hc80*, *hc136*, *b232*ts)*fer-1* has been studied in detail previously (WARD and MIWA 1978; ARGON and WARD 1980; WARD, ARGON and NELSON 1981), and only new information will be discussed. The nine alleles all show the same sperm phenotype: infertile spermatozoa with stubby motile pseudopods. The alleles do differ from one another enough to indicate they must represent at least five distinctive mutations. The alleles *hc47*, *hc80* and *hc136* are nonconditional, exhibiting complete sterility at both 16° and 25°. The remaining alleles are temperature sensitive and are fertile at 16° in all cases. The restrictive temperature is 20° for *hc82*, *hc91* and *b232* while it is 25° for *hc1*, *hc13* and *hc24*. As previously noted (WARD and MIWA 1978; ARGON and WARD 1980), *hc24* produces significantly more progeny at 25° than either *hc1* or *hc13*.

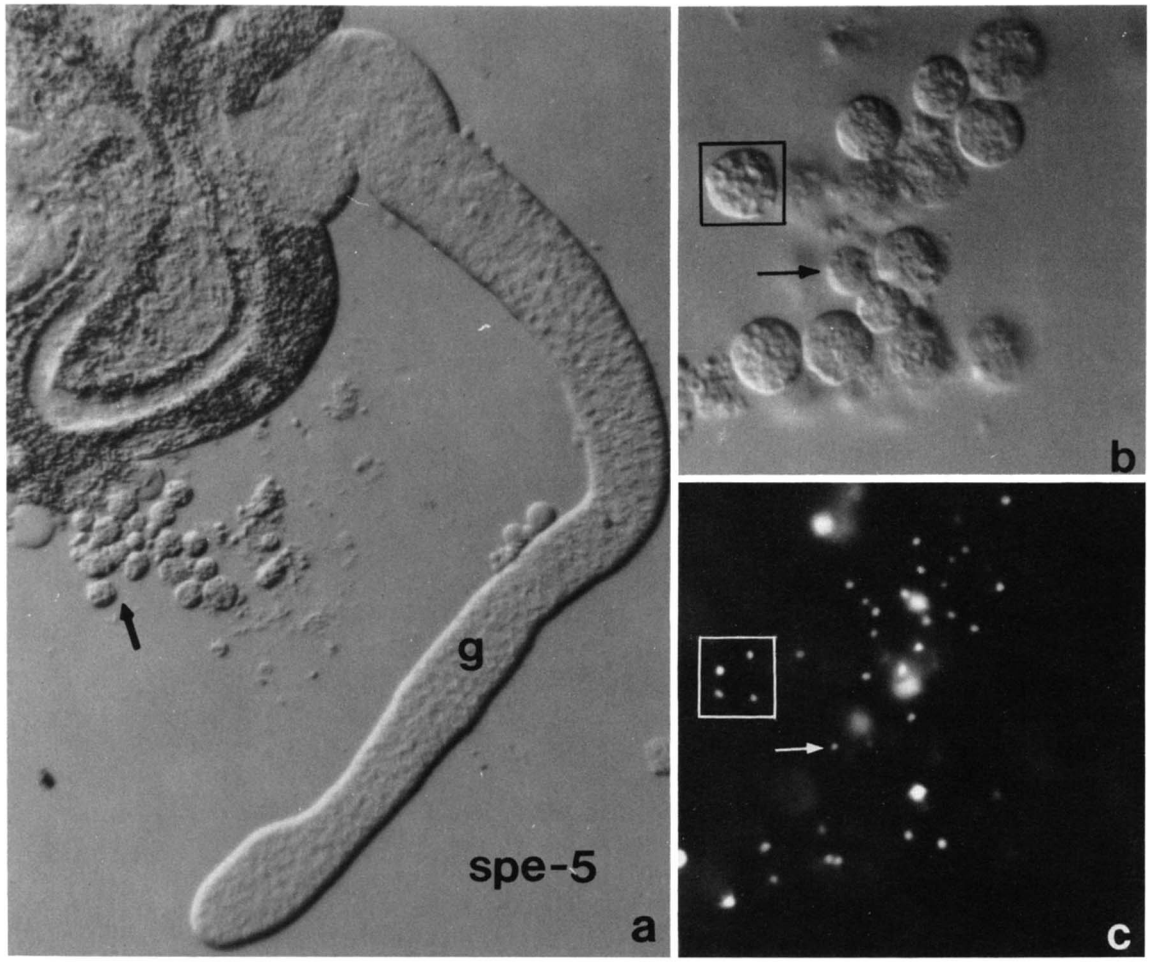

FIGURE 5.—a, Low magnification Nomarski micrograph of a *spe-5(hc93)* dissected gonad (g). The arrow indicates a field of spermatocytes and spermatids, ×290. b and c, Nomarski (b) and fluorescence (c) micrographs of Hoechst 33342 stained *spe-5(hc93)* sperm. Cells that appear by Nomarski optics (b) to be primary spermatocytes (boxed) actually have four haploid nuclei (c). The cells that look like spermatids (arrow) also have normal looking haploid nuclei, ×740.

TABLE 5

Hermaphrodite sperm[a]

Gene	Allele	Sperm/ Hermaphrodite		Initial sweep/oocyte		Later sweep/oocyte	
		N	$\bar{X}$ SE *t*=0	*N*	$\bar{X}$ SE *t*=0	*N*	$\bar{X}$ SE *t*=12 or 18
dpy-5	*e61*	8	225 ± 14	9	0.1 ± 0.03		
spe-11	*hc90 e61*	11	116 ± 9	15	0.1 ± 0.02		
spe-9	*hc52*ts	9	121 ± 16	9	1.0 ± 0.1		
	*hc88*ts	5	167 ± 28	5	0.8 ± 0.3		
spe-8	*hc50 e61*	9	183 ± 16	4	3.7 ± 1	9	3.6 ± 0.4
spe-12	*hc76*	8	246 ± 22	7	11.0 ± 2.3	8	6.2 ± 0.5
spe-13	*hc137*ts	13	282 ± 16	13	9.1 ± 1.6		

[a] Synchronized groups of worms were grown at 20° or 25° for temperature sensitive (ts) mutations. Worms were fixed and stained with Hoechst 33258 or DAPI at the onset of egg laying ($t = 0$) and, in two cases, after a number of hours of egg laying ($t = 12$ or 18). The counts at $t = 0$ include some primary spermatocytes that each contribute four to the sperm count. The sweep/oocyte shows the number of sperm lost from the spermatheca (excluding those that fertilized an egg) for each egg or oocyte that has passed. At $t = 0$, all oocytes and/or eggs are still in the uterus while a number of oocytes and/or eggs have been laid by $t = 12$ or 18 hr. and these expel sperm from the uterus.

The *trans* heterozygote *hc13/hc24* is more penetrant than *hc24/hc24* (virtually all *hc24/hc24* produce in excess of eight young/hermaphrodite/generation, see ARGON and WARD 1980; 2/16 *hc13/hc24* produced one young each and the other 14 were completely sterile). Electron microscopic examination of *fer-1(hc1)/nDf23* spermatozoa isolated from males raised under restrictive growth conditions (data not shown)

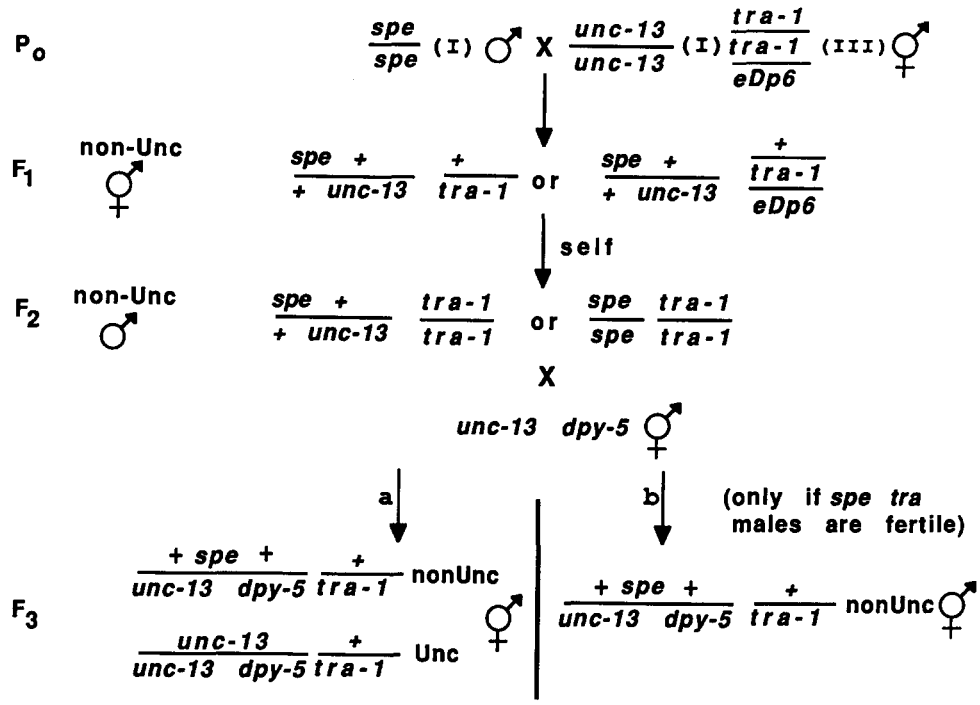

FIGURE 6.—Scheme by which *spe-8* and *spe-12 XX* males were created and tested for fertility. Phenotypic *unc-13* hermaphrodites bearing a recessive allele (*e1099*) of the transformer mutation *tra-1* (balanced by the free duplication *eDp6*) were crossed to *spe/spe* (either *spe-8*(*hc50*) or *spe-12*(*hc76*) males. Resulting F_1 non-Unc hermaphrodites were picked and allowed to segregate F_2 broods. All F_2 males will be *tra-1* homozygotes, except for rare cases of *X* chromosome nondisjunction, and about one-third of the F_2 non-Unc males will be *spe* homozygotes. One hundred twenty F_2 non-Unc males of unknown genotype were individually mated to several *unc-13 dpy-5* tester hermaphrodites and scored 4 days later for the presence of outcross progeny. Overall mating efficiency of tra-1 *XX* males was 30% for the *spe-8* crosses and 22% for *spe-12* crosses. The genotype of most of the F_2 non-Unc males (about two thirds) is *spe* +/+ *unc-13*, so most outcross F_3 broods contain Unc hermaphrodites (arrow at "a," between F_2 and F_3); mating efficiency of *tra-1* males with this genotype was 24% for either *spe* gene. Some of the outcross F_3 broods should be composed exclusively of phenotypic wild-type hermaphrodites if *spe tra* F_2 males were fertile (arrow at "b," between F_2 and F_3). A fraction of the successful outcrosses (17/36 for *spe-8* and 7/26 for *spe-12*) lacked both males and *unc-13* hermaphrodites in their F_3 broods, which indicated that these *tra-1* males were most likely homozygous *spe*. However, F_2 *spe unc-13*(+)/++; *tra-1* males can arise because of F_1 recombination, and these would be false positives, if they successfully mated. The frequency of these false positives is a function of both the map distance between the *spe* gene and *unc-13* (see Figure 1) and the mating efficiency of these males. If the mating efficiency of *spe unc-13*(+)/++; *tra-1* males is assumed to be about 24%, then, of the above-mentioned crosses attributed to *spe-8* or *spe-12 tra-1* males, no more than 6/17 *spe-8* or 1/7 *spe-12* crosses are likely to be false positives due to F_1 recombination.

reveals a spermatozoan phenotype that is indistinguishable from the *fer-1*(*hc1*)/*fer-1*(*hc1*) homozygote described previously (WARD, ARGON and NELSON 1981).

fer-3(*hc3*ts)—*fer-3*(*hc3*ts) had previously been assigned to chromosome *I* (ARGON and WARD 1980). Linkage of this gene was reinvestigated and it was found to be unlinked to *dpy-5* and was linked to *rol-1* and *unc-4*. This gene has not been pursued further in this study since it is clearly on chromosome *II*.

fer-6(*hc6*ts)—One temperature sensitive allele of *fer-6* has been previously described as a male-sterile mutant with abnormal spermatids (ARGON and WARD 1980; WARD, ARGON and NELSON 1981). We have found that, in addition to male-sterility, the *fer-6* strains characterized previously (ARGON and WARD 1980; WARD, ARGON and NELSON 1981) are temperature sensitive for uncoordinated movement, and have a growth rate that is retarded by ~1 day at 25° with respect to wild type (data not shown). We have found that the *hc6* mutation fails to complement *fer-5*(*hc23*ts), a gene previously described as being on chromosome *III* (ARGON and WARD 1980). Linkage of *hc23* was reinvestigated and it was found to be linked to *dpy-5*. Furthermore, *hc23* fails to complement *hc6* in all aspects of the phenotype described above. Creation of a *fer-6*(*hc6*ts) *dpy-5 cis* double resulted in loss of the temperature sensitive effects on worm movement and growth rate. Two factor mapping of *fer-6*(*hc6*ts) *dpy-5* produced non-Dpy isolates that were non-Unc and grew at wild-type rates, suggesting that these original aspects of the *fer-6* phenotype were conferred by mutation(s) that were separable from the mutation causing the *spe* phenotype. These data suggest that *fer-5*(*hc23*ts) is a reisolate of *fer-6*(*hc6*ts) since it is unlikely that two ts mutations of independent origin would also have the same secondary mutations; the *fer-5* gene no longer exists.

fer-7(*hc34*ts)—We have confirmed the previously published data on *fer-7* (ARGON and WARD 1980) and

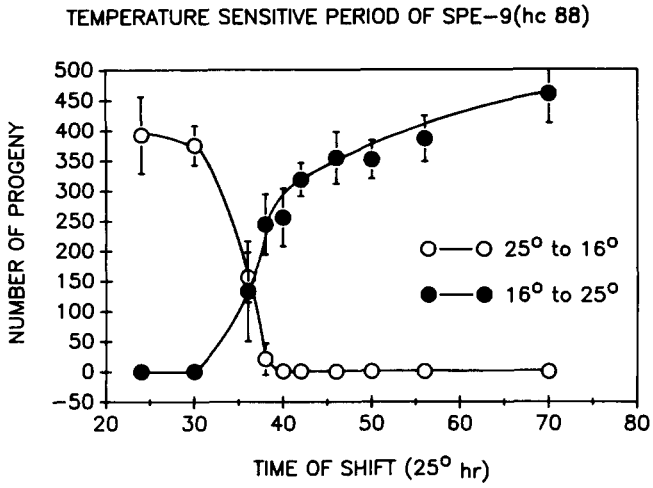

FIGURE 7.—Results of shifting *spe-9*(*hc88*ts) worms between 16° and 25°. Each point is the mean number of progeny produced per worm after shifting 10 hermaphrodites down (open circles) or up (solid circles). The error bars are the standard deviations of the mean values. Times are normalized to the 25° growth rate which is about twice the 16° growth rate.

TABLE 6

***spe-13* hermaphrodite sperm at different temperatures[a]**

Raised at 16°			Raised at 25°		
Oocytes and eggs laid	Sperm present	*n*	Oocytes and eggs laid	Sperm present	*n*
0	320 ± 8	9	0	310 ± 16	8
51	359 ± 24	5	54	74 ± 13	3
132	206 ± 17	5	110	77 ± 7	10
196	211 ± 11	5	197	0	5

[a] Temperature dependence of sperm sweep rate in *spe-13* hermaphrodites. Hermaphrodites were raised at 16° or 25°, and synchronized L4 larvae were placed as groups of 15 worms/plate. The worms were transferred daily to fresh plates, a few were fixed and stained with DAPI and the average number of oocytes and eggs laid per worm, up to that point, was calculated. Sperm are quickly swept out of the hermaphrodite gonad by passing oocytes at 25°, but most sperm are retained in the spermatheca of worms raised at 16°. The sperm count (± the standard error of the mean) for which no oocytes or eggs have been laid was determined in young hermaphrodites that had oocytes in their uterus. At 25°, the worms laid about 200 oocytes during the next 48 hr, whereas at 16°, the worms took 120 hr to lay about 200 oocytes.

have shown that *fer-7* complements at least one allele of all the chromosome *I spe* genes that map in the same region.

Other mutations: We have also recovered five mutations that appeared to be *spe* because unmated hermaphrodites lay oocytes but produce progeny following successful mating to wild-type males. However, these mutations were not studied in detail because they either had gonadal and somatic abnormalities (*hc45*), erratic expression of the sterile phenotype (*hc45*, *hc84*, *hc109*, *hc111*), or result in a switch in germline specific sex determination (*hc94*) and were, therefore, not sperm specific. These mutations complement each other and all other chromosome *I spe* and *fer* genes. *hc94* has been determined to be an allele of *fog-1* (*f*eminization *o*f the *g*ermline; M. K. BARTON and J. KIMBLE, unpublished data), and heterozygous *hc94*/+ males contain oocytes. The other four mutants all contain spermatids that become apparently normal spermatozoa after pronase treatment.

DISCUSSION

We have identified and described 23 new *C. elegans* mutations on chromosome *I* that affect spermatogenesis. These mutations could be identified because they cause normally self-fertile hermaphrodites to lay oocytes. We have chosen to confine our efforts to chromosome *I* because several well-studied spermatogenesis-defective (*spe*) genes were already mapped to this chromosome (ARGON and WARD 1980), balancers were available for much of this chromosome (*e.g.*, HOWELL *et al.* 1987), and we wanted to recover multiple alleles. We have recovered 16/23 of our new chromosome *I spe* mutations in *cis* to the morphological marker *dpy-5* to facilitate balancing and other genetic manipulations of these sterile strains. The remaining seven chromosome *I spe* mutations were not recovered on a morphologically marked chromosome *I*, but were subsequently shown to exhibit linkage to *dpy-5*. A few chromosome *I spe* genes might not be identified because they show only weak linkage to *dpy-5* due to the effects of recombination. Such *spe* genes, if they exist, will most likely be on the distal right arm of chromosome *I*; it is about 27 map units from *dpy-5* to the right end of the chromosome *I* genetic map. Two *spe-8* alleles (18 map units to the left of *dpy-5*) and one *spe-9* allele (eight map units to the right of *dpy-5*) were actually recovered as linked *dpy-5* doubles. The *spe-13* gene is unambiguously linked to *dpy-5* and, since this gene is 21 map units to the left of *dpy-5*, it seems probable that our screening procedures have sampled at least 85% (42 map units) of chromosome *I*.

The new chromosome *I spe* mutations, together with six previously isolated mutations (ARGON and WARD 1980), define 11 complementation groups; six of these complementation groups are defined by multiple alleles. Twenty-two of the 23 new chromosome *I spe* mutations were recovered from 3838 picked mutant F_1's for an overall EMS induced forward mutation rate of $\sim 6 \times 10^{-3}$ chromosome *I spe* mutations/gamete. BRENNER (1974) calculated the average EMS induced forward mutation rate for *C. elegans* to be $\sim 5 \times 10^{-4}$ mutations/gene/gamete. This figure suggests there should be about 12 *spe* genes in the 42 map unit interval of chromosome *I* we have sampled. Two of these genes, *spe-8* and *fer-1*, have mutation rates that are somewhat higher than the other *spe* genes (1.6×10^{-3} and 1.3×10^{-3} mutations/gene/gamete, respectively) suggesting that they are favored targets for

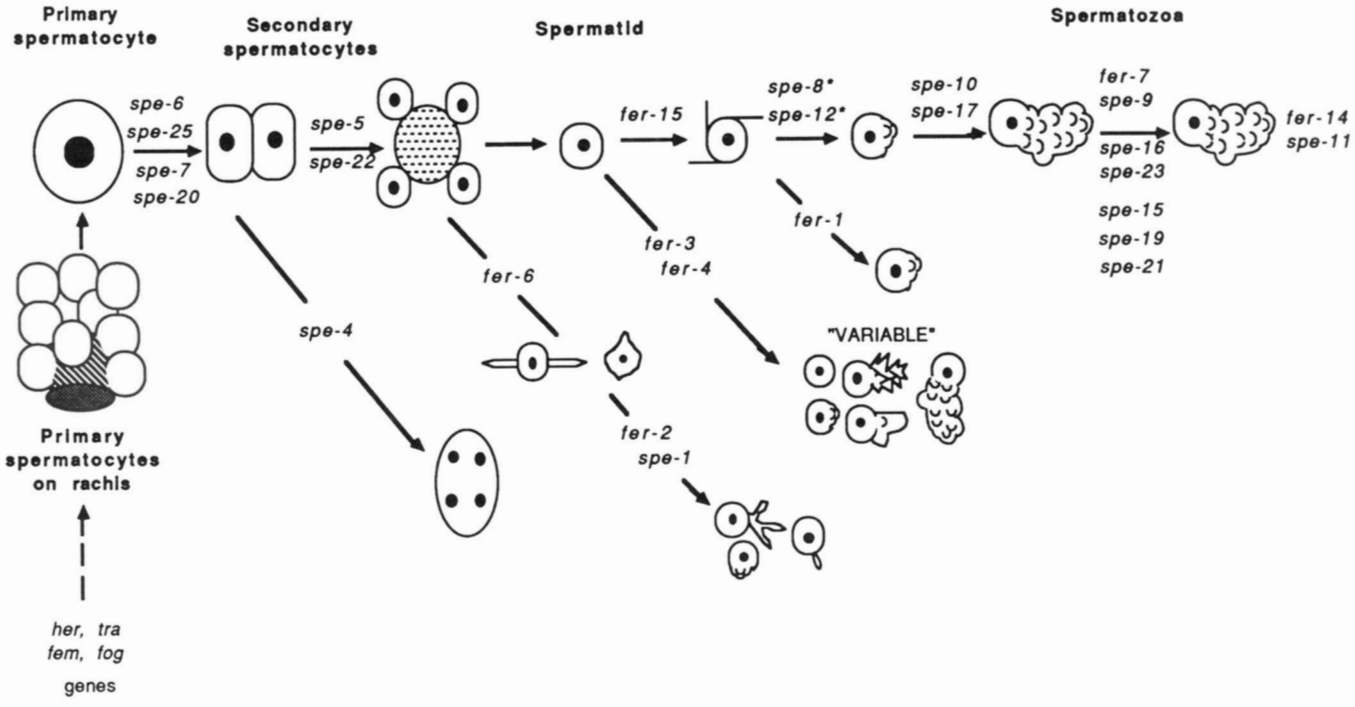

FIGURE 8.—Stages of normal spermatogenesis are shown diagrammatically as an ordered pathway of morphogenesis. Genes are placed on the pathway at the stage that is altered by mutations in a gene. Sperm phenotypes of *spe-8* and *spe-12* are different in the hermaphrodite (*) and the male, which has spermatozoa that are cytologically indistinguishable from wild type. Below the pathway are shown abnormal intermediates that accumulate when the gene is mutated. The last step on the pathway represents mutant spermatozoa that are cytologically normal and can enter oocytes.

EMS mutagenesis. Poisson analysis of mutant allele frequencies by the method of MENEELY and HERMAN (1979), which reduces overemphasis on the more mutable genes such as *spe-8* and *fer-1*, indicates there are about 14 chromosome *I spe* genes. We conclude that there could be less than three unidentified chromosome *I spe* genes in the 42 map unit interval of chromosome *I* we have sampled.

Many, although not all, *spe* mutants have sperm that appear obviously different from wild type. The phenotypic effects of all of these *spe* mutations are recessive; since none are semidominant, they probably do not act in *cis* on sperm. A previously studied series of temperature sensitive *spe* mutations were all found to act late in spermatogenesis (ARGON and WARD 1980; WARD, ARGON and NELSON 1981). We have demonstrated that our screening procedures (described above) allow the recovery of mutations that permit analysis of most, if not all, of spermatogenesis. All cytologically identifiable stages in spermatogenesis now have at least one mutation (chromosome *I* or elsewhere, unpublished data) that either prevents or alters its transition to the next cellular stage. The phenotypes of the new chromosome *I* and previously described mutants are summarized in Figure 8.

Mutations in two chromosome *I* genes, *spe-5* and *spe-4*, act during meiosis to prevent formation of normal spermatids. Both *spe-5* and *spe-4* mutants produce mostly primary spermatocytes, but mutations in either gene occasionally allow partial progression of spermatocytes through the second meiotic division. *spe-4* spermatocytes never proceed further than the formation of incomplete spermatids connected to the residual body, whereas *spe-5* animals produce a few normal looking spermatids that must occasionally mature into functional spermatozoa since *spe-5(hc93)* hermaphrodites sometimes produce a few progeny. Most *spe-4* and *spe-5* spermatocytes, no matter at which cytological stage they arrest, contain haploid nuclei. This indicates that nuclear divisions of meiosis are not dependent on cytokinesis; similar results have been found in other experimental systems, such as yeast (reviewed by ESPOSITO and KLAPHOLZ 1981).

Mutations in the other nine chromosome *I spe* genes affect spermatogenesis postmeiotically. The phenotypes of three of these chromosome *I spe* genes (*fer-1*, *fer-6* and *fer-7*) have been discussed in detail elsewhere (WARD and MIWA 1978; ARGON and WARD 1980; WARD, ARGON and NELSON 1981). Mutations in any of the six recently identified chromosome *I spe* genes (*spe-8*, *spe-9*, *spe-11*, *spe-12*, *spe-13* and *spe-15*) permit formation of cytologically normal spermatids. Morphogenesis of spermatids into spermatozoa and/or spermatozoan function are altered by mutation in any of these six genes.

spe-8 and *spe-12* hermaphrodites accumulate sper-

matids that do not mature into spermatozoa. The spermatids that *spe-8* and *spe-12* males deposit in hermaphrodites during copulation become spermatozoa that crawl to the spermatheca and, like sperm from wild-type males (WARD and CARREL 1979), successfully fertilize the hermaphrodite's oocytes because they outcompete wild-type hermaphrodite spermatozoa. Thus, single mutations in either the *spe-8* or *spe-12* gene can convert the mode of *C. elegans* reproduction from hermaphroditic to male/"female," and *spe-8* and *spe-12* mutations have revealed a new class of *spe* mutants that are male-fertile. These differences between the hermaphrodite and male phenotype are not due to *X*-chromosome dosage because genotypic *XX spe-8* or *spe-12* worms can sire progeny when converted into males by *tra-1*. Therefore, the phenotypic sex and not the genotype of the worm determines if fertile spermatozoa can form. Recently, the response of hermaphrodite and male spermatids to various *in vitro* sperm activators has been examined. These results indicate that *spe-8* and *spe-12* directly affect spermatids rather than *in vivo* sperm activator(s) (D. C. SHAKES, S. W. L'HERNAULT and S. WARD, in preparation).

Four other new chromosome *I spe* mutants (*spe-9*, *spe-11*, *spe-13* and *spe-15*) all produce spermatids that activate *in vitro* to form crawling spermatozoa. Mating experiments, however, indicate that males carrying a mutation in three of these *spe* genes are sterile (*spe-15* has not been tested). Crawling spermatozoa are also observed in the spermatheca of unmated hermaphrodites carrying these mutations (*spe-15* has not been tested). Thus, in these mutants, as in most of our *spe* mutants, the hermaphrodite and male phenotype appears to be the same.

Mutants that produce cytologically normal spermatozoa are likely to have defects in spermatozoa-spermatheca interactions and/or spermatozoa-oocyte interactions. These two phenomena can be distinguished by examining sperm behavior in the hermaphrodite reproductive tract. The passage of oocytes through the reproductive tract usually, but not always, sweeps nonfunctional *spe* sperm out of the spermatheca (Tables 5 and 6) as has been shown previously (WARD and MIWA 1978; ARGON and WARD 1980). For example, *spe-8* and *spe-12* hermaphrodite spermatids are swept very rapidly (respectively, ~30 or ~100 times wild type; Table 5) by passing oocytes, presumably because they lack pseudopods and, therefore, can neither move nor interact with the spermatheca; both of these processes are known to be important in preventing the sweeping of wild-type spermatozoa (WARD and CARREL 1979). Although hermaphrodites with mutations in *spe-9* form normal looking spermatozoa, these sperm are also rapidly swept (~10 times wild type; Table 5). Therefore, this mutation probably is caused by defective spermatozoa-spermatheca interactions such that spermatozoa either get forced out of the spermatheca by passing oocytes or do not correctly recognize and/or position themselves within the spermatheca in the first place. The analysis of *spe-13* hermaphrodites proves to be more complicated; the sperm are rapidly swept in animals grown at 25° (~100 times wild type; Table 5) but are not swept at all in animals grown at 16° (Table 6), despite the fact that the animals are only slightly temperature sensitive for self-sterility. Perhaps a single protein encoded by the *spe-13* gene is required for sperm to interact with the spermatheca and oocytes. These two interactions might be differentially sensitive to the levels of this protein present in *spe-13*(*hc137*ts) mutant animals at 16° and 25°.

Spermatozoa made by *spe-11* hermaphrodites, unlike all other chromosome *I spe* mutations, are not swept out by passing oocytes, but they do interact with oocytes and often stimulate several nuclear divisions. Additionally, hermaphrodites carrying the hypomorphic allele *hc77*ts (see below), produce a number of round thinly shelled eggs (as opposed to wild-type oblong eggs). These thinly shelled "eggs" do not hatch, and it recently has been discovered that *spe-11* (*hc90*) hermaphrodite sperm enter and activate oocytes during self-fertilization but subsequently form an abnormal zygote (D. HILL and S. STROME, personal communication). Sperm from *spe-11* males cause these same abnormalities when they cross-fertilize wild-type oocytes, suggesting that defective *spe-11* sperm cause abnormal zygote formation (D. HILL and S. STROME, personal communication). These analyses of *spe-11* mutations have revealed a possible role for sperm-specific components in early embryogenesis. Our preliminary analysis of the *spe-11*(*hc77*ts) temperature sensitive period indicates that the defect affecting hermaphrodite self-sterility is temperature reversible during adulthood. These data suggest that mutant *spe-11* gene product can recover (at least) partial wild-type function if sperm are shifted from restrictive to permissive conditions because mature sperm lack ribosomes and, therefore, do not synthesize proteins (reviewed by WARD 1986).

Our genetic analysis of the *spe* mutations discussed in this paper indicates that their phenotypes probably result from single gene recessive mutations. Alleles of two genes, *fer-1* and *spe-8* arise at or slightly above the average forward (knockout) mutation frequency (see above), suggesting that some alleles should be null. All seven *spe-8* alleles cause hermaphrodites, but not males, to produce nonfunctional sperm, and only *hc134*ts shows any significant hermaphrodite self-fertility at 16°. The uniformity of these phenotypic traits in animals homozygous for any of the seven *spe-8* alleles strongly suggests that this is the null phenotype.

Likewise, all nine alleles of *fer-1* cause identical phenotypes in 25° grown mutant sperm at the light microscope level, despite the fact that this phenotype is only conditionally expressed in the six ts alleles; three ts alleles also cause the same ultrastructural defects (WARD, ARGON and NELSON 1981). *fer-1(hc1*ts) appears to be conditionally null since the ultrastructural phenotype of sperm from either *hc1/hc1* or *hc1/nDf23* males grown under restrictive conditions have the same Spe phenotype (*fer-1* fails to complement *nDf23*). In contrast, *fer-1(hc24*ts) appears to be conditionally hypomorphic since hermaphrodites become more penetrant for self-sterility when *hc24*ts is in *trans* to *fer-1(hc13*ts).

The *spe-4*, *spe-5* and *spe-11* genes are each defined by two alleles, and noncomplementing deficiencies exist for all three genes. Both alleles of *spe-4* and *spe-5* have the same phenotype, and *spe* heterozygotes in *trans* to noncomplementing deficiencies have the same cytological phenotype as the *spe* homozygote, suggesting that these nonconditional alleles eliminate gene activity. One allele (*hc77*ts) of *spe-11* is temperature sensitive and incompletely penetrant for sterility while the other allele (*hc90*) is nonconditional and completely sterile. When heterozygous in *trans* to a noncomplementing deficiency, the sterility of *spe-11(hc77*ts) is enhanced while *spe-11(hc90)* remains completely sterile. This indicates that *hc77*ts is hypomorphic for gene function, but *hc90* might eliminate gene activity. There are six other chromosome I *spe* genes (*fer-6*, *fer-7*, *spe-9*, *spe-12*, *spe-13*, and *spe-15*) for which the effects of existing mutations on gene function are less certain.

The *spe* mutants studied in this paper were recovered because they cause hermaphrodites to lay oocytes. *C. elegans* is unusual among animals in that wild type hermaphrodites produce more eggs than the available sperm (reviewed by NIGON 1965). The gonad produces about 300 sperm during the hermaphrodite L4 larval stage, and they are stored in the spermatheca. Sperm production then ceases, and the gonad produces oocytes throughout adulthood. Wild-type oocytes are fertilized in the spermatheca with close to 100% efficiency (WARD and CARREL 1979), and each hermaphrodite lays about 350 fertilized eggs followed by 180 unfertilized oocytes at 16° (Table 4). However, wild type hermaphrodites can produce 1000–1500 progeny if they are repeatedly mated to males (HODGKIN 1983). Unmated *fem-1 XX* animals, which are true females because they never make any sperm (NELSON, LEW and WARD 1978; DONIACH and HODGKIN 1984), produce a few (~10) oocytes but can produce hundreds of progeny when mated (NELSON, LEW and WARD 1978). These data indicate that either the presence of sperm or a signal associated with spermatogenesis stimulates oogenesis. The number of oocytes produced by a *spe* hermaphrodite, however, seems to depend on when during spermatogenesis a *spe* mutation acts. Two chromosome *I spe* genes (*spe-4* and *spe-5*) block spermatogenesis during meiosis, and hermaphrodites mutant in either of these genes produce ~10–20% of the wild-type number of oocytes (Table 4). Mutants that arrest spermatogenesis post-meiotically generally produce about the same number of oocytes as wild type (see *spe-8* and *spe-12*, Table 4) as has been noted before by others (ARGON and WARD 1980). One striking exception to this rule is the post-meiotically acting temperature sensitive mutation *spe-13(hc137*ts) that has an unusual correlation between oocytes produced and sperm sweep rate. *spe-13* hermaphrodites rapidly sweep their sperm when raised at 25° and produce about the same number of oocytes as unmated wild-type hermaphrodites. In contrast, *spe-13* hermaphrodites raised at 16° do not sweep their sperm and produce 1.4 times more oocytes than unmated wild-type hermaphrodites. The longer persistence of sperm in the spermatheca at 16° appears to be responsible for stimulating excessive oocyte production in these mutant hermaphrodites, and is consistent with production of a direct signal by spermatozoa that stimulates oogenesis.

Spermatogenesis-specific mutations can be readily isolated in *C. elegans* because mutant screening is carried out in hermaphrodites. Delivery of sperm to the *in vivo* location where oocytes are fertilized is far less complicated in self-fertilizing hermaphrodites than in many dioecious organisms where male mating competence and successful copulation are required. The presence of defective sperm causes hermaphrodites to lay oocytes, rather than shelled eggs, and picked hermaphrodites that lay oocytes can be easily identified because self-fertilization in young adult wild-type hermaphrodites is quite efficient. Screens for oocyte-laying mutant hermaphrodites have, so far, permitted the identification of 36 *C. elegans spe* genes on all six chromosomes (this paper and our unpublished results). All of these *spe* mutations affect only sperm and do not have any apparent effects on worm growth, behavior or viability. Complementation screens have not been used to recover new alleles of any *spe* gene, and so, for many *spe* genes, we cannot be sure that available mutations reflect the null phenotype. However, 13 *spe* genes have been mapped to regions for which noncomplementing deficiencies exist (the five chromosome *I* genes described in this paper and our unpublished results). Twelve *spe*/deficiency *trans* heterozygotes do not have different phenotypes from the *spe* homozygote; the one exception is the *fer-4(hc4*ts)/deficiency heterozygote, which has gonadal abnormalities not observed in the *fer-4* homozygote (our unpublished observations). These re-

sults suggest that many *spe* genes probably exert their phenotypic effects only in sperm and do not affect somatic tissue.

Screening for spermatogenesis mutations in other experimental systems, such as *Drosophila* (reviewed by HACKSTEIN 1987 and LIFSCHYTZ 1987) or mice (reviewed by HANDEL 1987) through male-sterile screens leads to recovery of many mutations that affect cells in addition to or other than sperm. In fact, no genes that affect spermatogenesis exclusively have been uncovered in mice (reviewed by HANDEL 1987), and only a few such genes have been discovered in *Drosophila* (reviewed by HACKSTEIN 1987 and LIFSCHYTZ 1987). Commonly, either male mating behavior or secondary sexual characteristics are altered so that sperm cannot be deposited in the female. This phenotypic class, as discussed above, is eliminated when studying *C. elegans* because the hermaphroditic mode of reproduction does not require mating. Many male-sterile *Drosophila* mutations cause cytologically defective sperm, but these mutations subsequently proved to be alleles of genes that have a null-lethal phenotype. The *C. elegans spe* mutations that have been analyzed (a total of more than 30 genes on chromosome *I* and elsewhere) (WARD and MIWA 1978; ARGON and WARD 1980; WARD, ARGON and NELSON 1981; ROBERTS and WARD 1982a, b; our unpublished observations) are always associated with sperm defects, and the null phenotype of many of these genes probably does not affect tissues other than sperm (see previous paragraph). This does not mean that somatically expressed *C. elegans* genes are not also expressed in sperm but, rather, that our screening method has identified a class of genes whose activity seems limited to sperm. Mutations that act either pre or postmeiotically can be obtained, and in fact, mutant hermaphrodites that do not make any sperm but do make a few oocytes, such as *fem* (NELSON, LEW and WARD 1978; DONIACH and HODGKIN 1984) and *fog* (this paper) mutants can be identified by this screening method. This means that our screening method probably permits recovery of any *spe* mutation, no matter when it acts during spermatogenesis, and that many aspects of *C. elegans* spermatogenesis are accessible by genetic analysis.

We thank DANIEL BURKE, TOM ROBERTS and JACOB VARKEY for the original isolation of some of the alleles described here, and thank EILEEN HOGAN and MICHAEL SEPANSKI for excellent technical assistance. We thank JUDITH KIMBLE, ANN ROSE and SUSAN STROME and their laboratories for communicating unpublished results and thank the Rose laboratory for nematode strains. We thank ANDREW FIRE for suggesting the *spe tra* experiments and many helpful discussions. Some nematode strains used in this study were provided by the *Caenorhabditis* Genetics Center, which is supported by contract number NO1-AG-9-2113 between the National Institutes of Health and the Curator of the University of Missouri. This work has been supported by U.S. Public Health Service grant GM25243 to S.W., the Carnegie Institution of Washington, Public Health Service grant GM09684 to S.W.L. and a Carnegie Corporation Fellowship to D.C.S.

LITERATURE CITED

AFZELIUS, B. A., 1985 The immotile cilia syndrome: a microtubule-associated defect. CRC Crit. Rev. Biochem. **19:** 63–87.

ARGON, Y., and S. WARD, 1980 *Caenorhabditis elegans* fertilization-defective mutants with abnormal sperm. Genetics **96:** 413–433.

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

BURKE, D. J., 1983 A molecular genetic analysis of the major sperm proteins of the nematode *Caenorhabditis elegans*. Ph.D. thesis, Johns Hopkins University.

DONIACH, T., and J. HODGKIN, 1984 A sex-determining gene, *fem-1* required for both male and hermaphrodite development in *Caenorhabditis elegans*. Dev. Biol. **106:** 223–235.

EDGAR, L. G., 1982 Control of spermatogenesis in the nematode *Caenorhabditis elegans* Ph.D. thesis, University of Colorado.

EDGLEY, M. L., and D. L. RIDDLE, 1988 *Caenorhabditis elegans* genetic map. *Caenorhabditis elegans* Stock Center, University of Missouri, Columbia.

ESPOSITO, R. E., and S. KLAPHOLZ, 1981 Meiosis and ascospore development. pp. 211–287. In: *The Molecular Biology of the Yeast Saccharomyces, Life Cycle and Inheritance*, Edited by J. N. STRATHERN, E. W. JONES and J. R. BROACH. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

FERGUSON, E., and H. R. HORVITZ, 1985 Identification and characterization of 22 genes that affect the vulval cell lineages of the nematode *Caenorhabditis elegans*. Genetics **110:** 17–72.

HACKSTEIN, J. H. P., 1987 Spermatogenesis in *Drosophila*. pp. 63–116. In: *Spermatogenesis: Genetic Aspects. Results and Problems in Cell Differentiation 15*, Edited by W. HENNIG. Springer-Verlag, Berlin.

HANDEL, M. A., 1987 Genetic control of spermatogenesis in mice. pp. 1–62. In: *Spermatogenesis: Genetic Aspects. Results and Problems in Cell Differentiation 15*, Edited by W. Hennig. Springer-Verlag, Berlin.

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

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

HODGKIN, J., 1986 Sex determination in the nematode *C. elegans*: analysis of *tra-3* suppressors and characterization of *fem* genes. Genetics **114:** 15–32.

HODGKIN, J., 1980 More sex-determination mutants of *Caenorhabditis elegans*. Genetics **96:** 649–664.

HODGKIN, J., 1983 Male phenotypes and mating efficiency in *Caenorhabditis elegans*. Genetics **103:** 43–64.

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

HODGKIN, J., H. R. HORVITZ and S. BRENNER, 1979 Non-disjunction mutants of the nematode *Caenorhabditis elegans*. Genetics **91:** 67–94.

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

HOWELL, A. M., S. G. GILMOUR, R. A. MANCEBO and A. M. ROSE, 1987 Genetic analysis of a large autosomal region in *Caenorhabditis elegans* by use of a free duplication. Genet. Res. **49:** 207–213.

KIMBLE, J., L. EDGAR and D. HIRSH, 1984 Specification of male development in *Caenorhabditis elegans*: the *fem* genes. Dev. Biol. **105:** 234–239.

KLASS, M., N. WOLF and D. HIRSH, 1976 Development of the male reproductive system and sexual transformation in the nematode *Caenorhabditis elegans*. Dev. Biol. **52:** 1–18.

LIFSCHYTZ, E., 1987 The developmental program of spermiogenesis in *Drosophila*: a genetic analysis. Int. Rev. Cytol. **109:** 211–258.

MAINLAND, D. L., L. HERRERA and M. I. SUTCLIFFE, 1956 *Statistical Tables for use with Binomial Samples, Contingency Tests, Confidence Limits and Sample Size Estimates.* Department of Medical Statistics, New York University School of Medicine.

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.

NELSON, G. A., 1979 Nematode sperm motility. Carnegie Inst. Wash. Year Book **78:** 62–66.

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

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

NELSON, G. A., T. M. ROBERTS and S. WARD, 1982 *Caenorhabditis elegans* spermatozoan locomotion: amoeboid movement with almost no actin. J. Cell Biol. **92:** 121–131.

NIGON, V., 1949 Les modalités de la reproduction et le déterminisme du sexe chez quelques nématodes libres. Ann. Sci. Nat. (Zool.) **11:** 1–132.

ROBERTS, T. M., and S. WARD, 1982a Centripetal flow of pseudopodial components could propel the amoeboid movement of *Caenorhabditis elegans* spermatozoa. J. Cell Biol. **92:** 132–138.

ROBERTS, T. M., and S. WARD, 1982b Membrane flow during nematode spermatogenesis. J. Cell Biol. **92:** 113–120.

ROSE, A. M., 1980 Genetic studies of the gene coding for paramyosin in *Caenorhabditis elegans*: *unc-15* and the adjacent region. Ph.D. thesis, Simon Fraser University, Burnaby, B. C. Canada.

ROSE, A. M., and D. L. BAILLIE, 1979 The effect of temperature and parental age on recombination and nondisjunction in *Caenorhabditis elegans*. Genetics **92:** 409–418.

ROSE, A. M., and D. L. BAILLIE, 1980 Genetic organization of the region around *unc-15* (*I*), a gene affecting paramyosin in *Caenorhabditis elegans*. Genetics **96:** 639–648.

ROSE, A. M., D. L. BAILLIE and J. CURRAN, 1984 Meiotic pairing behavior of two free duplications of linkage group I in *Caenorhabditis elegans*. Mol. Gen. Genet. **195:** 52–56.

SCHEDL, T., and J. KIMBLE, 1988 *fog-2*, a germ-line specific sex determination gene required for hermaphrodite spermatogenesis in *Caenorhabditis elegans*. Genetics **119:** 43–61.

SIGURDSON, D. C., G. J. SPANIER and R. K. HERMAN, 1984 *Caenorhabditis elegans* deficiency mapping. Genetics **108:** 331–345.

TRENT, C., N. TSUNG and H. R. HORVITZ, 1983 Egg laying defective mutants of the nematode *Caenorhabditis elegans*. Genetics **104:** 619–647.

WARD, S., 1986 Asymmetric localization of gene products during the development of *Caenorhabditis elegans* spermatozoa. pp. 55–75. In: *Society for Developmental Biology 44th Annual Symposium*, Edited by J. G. GALL. Alan R. Liss, New York.

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

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

WARD, S., Y. ARGON and G. A. NELSON, 1981 Sperm morphogenesis in wild type and fertilization-defective mutants of *Caenorhabditis elegans*. J. Cell Biol. **91:** 26–44.

WARD, S., E. HOGAN and G. A. NELSON, 1983 The initiation of spermatogenesis in the nematode *Caenorhabditis elegans*. Dev. Biol. **98:** 70–79.

WATERSTON, R. H., 1980 A second informational suppressor, *sup-7 X*, in *Caenorhabditis elegans*. Genetics **97:** 307–325.

Communicating editor: R. K. HERMAN