fog-2, a Germ-Line-Specific Sex Determination Gene Required for Hermaphrodite Spermatogenesis in *Caenorhabditis elegans*

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ABSTRACT

This paper describes the isolation and characterization of 16 mutations in the germ-line sex determination gene *fog-2* (*fog* for feminization of the germ line). In the nematode *Caenorhabditis elegans* there are normally two sexes, self-fertilizing hermaphrodites (*XX*) and males (*XO*). Wild-type *XX* animals are hermaphrodite in the germ line (spermatogenesis followed by oogenesis), and female in the soma. *fog-2* loss-of-function mutations transform *XX* animals into females while *XO* animals are unaffected. Thus, wild-type *fog-2* is necessary for spermatogenesis in hermaphrodites but not males. The *fem* genes and *fog-1* are each essential for specification of spermatogenesis in both *XX* and *XO* animals. *fog-2* acts as a positive regulator of the *fem* genes and *fog-1*. The *tra-2* and *tra-3* genes act as negative regulators of the *fem* genes and *fog-1* to allow oogenesis. Two models are discussed for how *fog-2* might positively regulate the *fem* genes and *fog-1* to permit spermatogenesis; *fog-2* may act as a negative regulator of *tra-2* and *tra-3*, or *fog-2* may act positively on the *fem* genes and *fog-1* rendering them insensitive to the negative action of *tra-2* and *tra-3*.

In the nematode *Caenorhabditis elegans*, there are normally two sexes. The initial signal for sex determination is the ratio of the number of *X* chromosomes to sets of autosomes (MADL and HERMAN 1979). Diploid *XX* animals are self-fertilizing hermaphradites; dipoloid *XO* animals are males. Most tissues of these two sexes differ morphologically and/or biochemically. The term "hermaphrodite," when applied to *C. elegans*, describes a self-fertile animal with a female soma and a germ line that is first male, producing sperm, and then female, producing oocytes. Hermaphrodites of *C. elegans* reproduce either by self-fertilization or by cross-fertilization after mating with males.

The *C. elegans* hermaphrodite soma is essentially female. It is morphologically indistinguishable from the female soma of a closely related male/female nematode species *Caenorhabditis remanei* (SUDHAUS 1974), and is extremely similar to the female soma of *Panagrellus redivivus* (STERNBERG and HORVITZ 1981, 1982). The hermaphrodite soma is also indistinguishable from the soma of *C. elegans* females that arise as a consequence of mutations in sex determining loci (*e.g.*, *fem* mutations). In the hermaphrodite germ line, spermatogenesis occurs first, beginning during the last larval stage of development (L4) and ending soon after the molt into adulthood. From this brief period of spermatogenesis, about 320 sperm per hermaphrodite are produced from about 40 primary spermatocytes in each of two gonads. Then, each gonad switches to oogenesis and oocytes are produced continuously throughout adulthood. The sexual duality of the *XX* germ line in a female soma suggests that hermaphroditism in *C. elegans* is a property of the germ-line tissue. This is to be distinguished from hermaphroditism in the annelid *Lumbricus terrestris* (earthworm) in which each animal has a separate ovary and testis (sexual duality in both the germ line and soma) and reproduction occurs by mating (HICKMAN, HICKMAN and HICKMAN 1973).

In *C. elegans*, the *X/A* ratio is transduced by a set of genes that direct both sex determination and dosage compensation (VILLENEUVE and MEYER 1987; C. NUSSBAUM, L. MILLER, J. PLENEFISCH and B. MEYER, personal communication). These "transducer" genes, in turn, regulate both genes that direct dosage compensation plus genes that specify the sexual phenotype. Here, we focus on the later group of sex-determination genes; the transducer genes and dosage compensation genes are beyond the scope of this paper.

Seven sex-determining genes have been identified that specify sexual fate in all tissues of the animal—both somatic and germ line (HODGKIN and BRENNER 1977; HODGKIN 1980; DONIACH and HODGKIN 1984; HODGKIN 1986). In addition, one sex-determining gene has been identified that affects the sexual fate of a single tissue, the germ line (DONIACH 1986b; M. K. BARTON, personal communication). The germ-line and somatic mutant phenotypes of these sex-determining genes are summarized in Table 1. These genes must act downstream from the *X/A* ratio,

TABLE 1

Summary of sex determination genes used in this study

Genotype[a]	*XX* phenotype		*XO* phenotype	
	Germ Line	Soma	Germ Line	Soma
Wild type	Male then female (self-fertile)	Female	Male	Male
her-1(lf)[b]	Male then female (self-fertile)	Female	Male then female (self-fertile)	Female
fem-1(lf)[c,d]	Female	Female	Female	Female
fem-2(lf)[c,e]	Female	Female	Female	Female
fem-3(lf)[c,e,f]	Female	Female	Female	Female
fem-3(gf)[f]	Male	Female	Male	Male
tra-1(lf)[g]	Male then female	Male[h]	Male then female	Male[h]
tra-2(lf)[i]	Male	Incomplete male	Male	Male
tra-2(gf)[j]	Female	Female	Male	Male
tra-3(lf)[c,i]	Male then female	Incomplete male	Male	Male
fog-1[k]	Female	Female	Female	Male

[a] *lf*, loss-of-function; for these genes this is the probable null phenotype. *gf*, gain-of-function. Phenotypes are of homozygotes. For details of mutant phenotypes, consult references and text.
[b] HODGKIN (1980).
[c] Homozygous mutant derived from a homozygous mutant mother $m(-/-)$, $z(-/-)$.
[d] DONIACH and HODGKIN (1984).
[e] HODGKIN (1986).
[f] BARTON, SCHEDL and KIMBLE (1987).
[g] HODGKIN (1987); T. SCHEDL, unpublished observations. *tra-1(lf)* is included in this table for comparison with *tra-2(lf)* and *tra-3(lf)*. The interaction of *tra-1* and *fog-2* mutations will be discussed elsewhere.
[h] Gonad abnormal, HODGKIN (1987); T. SCHEDL, unpublished observations.
[i] HODGIN and BRENNER (1977); T. SCHEDL, unpublished observations.
[j] DONIACH (1986a), this paper.
[k] DONIACH (1986b); M. K. BARTON, person communication. It is unclear at this time whether *fog-1* alleles are *lf* or *gf*.

because mutations in them override this initial signal. Based on the results of a series of experiments in which the epistasis of mutations in these genes was analyzed, HODGKIN (1980, 1986) proposes that the sex-determining genes act in a cascade of negative regulation to control the state of *tra-1*, which in turn specifies somatic sexual phenotype.

The wild-type function of each of the sex-determining genes has been deduced from the phenotype of animals homozygous for a loss-of-function (*lf*) mutation in that gene. Thus, *fem-1(lf)*, *fem-2(lf)*, and *fem-3(lf)* *XX* and *XO* mutant animals are female instead of hermaphrodite and male, respectively (Table 1); therefore the wild-type *fem-1*, *fem-2*, and *fem-3* genes are required for male development in both the *XX* germ line and all *XO* tissues (NELSON, LEW and WARD 1978; DONIACH and HODGKIN 1984; KIMBLE, EDGAR, and HIRSH 1984; HODGKIN 1986). Similarly, *her-1(lf)* *XO* animals are hermaphrodite instead of male; therefore, the wild-type *her-1* gene is required for the development of male somatic tissues and for continuous spermatogenesis in *XO* animals (HODGKIN 1980; C. TRENT, P. SCHEDIN and W. WOOD, personal communication). Because *her-1(lf)* mutants are self-fertile (Table 1), wild-type *her-1* is not needed for hermaphrodite spermatogenesis. Finally, *tra-1(lf)*, *tra-2(lf)*, and *tra-3(lf)* *XX* animals are masculinized. The details of masculinization by mutations in each of the *tra* genes varies (Table 1). Both loss-of-function and gain-of-function mutant phenotypes of *tra-2* make this gene stand out as necessary for the switch from spermatogenesis to oogenesis in the hermaphrodite germ line. In contrast, the role of *tra-1* in the hermaphrodite germ line is unclear (HODGKIN 1987a; T. SCHEDL, unpublished observations). Therefore, the wild-type *tra-1*, *tra-2*, and *tra-3* genes are necessary for female development, but their roles in specification of that development differ (HODGKIN and BRENNER 1977).

The production of first sperm and then oocytes by the *C. elegans* hermaphrodite raises two major questions about the regulation of sex determination in the *XX* germ line: (1) how is male germ-line development initiated within a female soma? and, (2) how is the switch from male to female germ-line development affected? Since all uncommitted germ cells of the *XX* hermaphrodite are *XX*, whether a precursor of sperm or oocyte, the *X/A* ratio is not the primary signal for sexual choice in the hermaphrodite germ line. Instead, control of *tra-2* and *fem-3* activities appear to be important to germ-line sex determination in hermaphrodites. In particular, the phenotypes of gain-of-function (*gf*) mutations of *tra-2* and *fem-3* provide some insight into the genetic mechanisms of control over hermaphrodite germ-line development. Both *tra-2(gf)* and *fem-3(gf)* mutations affect the

sexual fate of the *XX* germ line, but have little or no effect on the *XO* germ line or the soma of either *XX* or *XO* animals. The *XX* germ line of *tra-2(gf)* mutants is feminized: germ cells that would normally differentiate as sperm become oocytes instead, and oogenesis continues throughout adulthood (DONIACH 1986a). Conversely, the *XX* germ line of *fem-3(gf)* mutants is masculinized: sperm are produced continuously, generating a vast excess of sperm, with no sign of oogenesis. Thus, germ cells that would normally differentiate as oocytes become sperm instead (BARTON, SCHEDL and KIMBLE 1987). Feminization of the *XX* germ line by *tra-2(gf)* mutations suggests that, in wild-type *XX* animals, *tra-2* activity might be modulated to permit spermatogenesis. DONIACH (1986a) suggests that *tra-2* is no longer sensitive to this modulation in *tra-2(gf)* mutants. Similarly, masculinization of the *XX* germ line by *fem-3(gf)* suggests that, in wild-type *XX* animals, *fem-3* activity is negatively regulated to permit the switch to oogenesis. BARTON, SCHEDL and KIMBLE (1987) suggest that *fem-3(gf)* mutants are no longer sensitive to this negative regulation.

This paper describes loss-of-function mutations in a germ-line specific sex-determination gene, *fog-2* (*fog* for feminization of the germ line). *XX* animals homozygous for *fog-2* mutations are female, while *XO* animals are unaffected. Therefore, a homozygous *fog-2* strain can reproduce as a male/female strain, but not as a self-fertilizing hermaphroditic strain. This mutant phenotype indicates that *fog-2* is a regulator of hermaphrodite spermatogenesis. Analysis of the interaction of *fog-2* mutations with mutations in other sex determining genes provides a framework for placing *fog-2* within the regulatory hierarchy of sex determination as it applies to the *XX* germ line.

MATERIALS AND METHODS

General methods for culturing nematodes have been described by BRENNER (1974). Experiments were performed at 20° unless specified in the text. For all experiments worms were under continuous growth conditions and were not starved or recovering from the dauer state.

Nomenclature: For certain genes in the *C. elegans* sex determination pathway, some loss-of-function mutations exhibit dominance and some gain-of-function mutations are recessive. We therefore designate alleles as gain-of-function with the suffix *gf* and loss-of-function with the suffix *lf* instead of abbreviations for dominant and recessive. Numerically designated alleles without a suffix are assumed to be loss-of-function unless indicated to the contrary; this avoids confusion between "l" and "1" (see BARTON, SCHEDL and KIMBLE (1987) for a further description). For experiments where maternal and zygotic genotypes are important they are indicated by *m*( ) and *z*( ), respectively. For example, *m*(−/+), *z*(−/+) indicates a heterozygous mutant derived from a heterozygous mutant mother. All other nomenclature follows HORVITZ *et al.* (1979).

Strains: *C. elegans* var. Bristol isolate N2 is defined as wild type, and is the strain from which all other stocks are derived. Most of the mutations used in this study are listed in HODGKIN and RIDDLE (1988) and SWANSON, EDGLEY and RIDDLE (1984). The phenotypes of sex determination mutants are shown in Table 1 and are described and referenced explicitly in the text. The following mutations and chromosomal rearrangements were used [*daf* (abnormal dauer larva formation), *dpy* (dumpy), *emb* (embryonic lethal), *fem* (feminization of germ line and soma), *fog* (feminization of germ line), *her* (hermaphroditization), *lon* (long), *sup* (suppressor), *tra* (transformer), *unc* (uncoordinated)]:

Linkage group (LG) *I*: *fog-1(q187)*.
LG *II*: *dpy-10(e128)*, *tra-2(e1095, e1425, e1941gf, e2046gf, e2020gf)*, *unc-4(e120)*, *mnDf30*, *mnC1*.
LG *III*: *fem-2(e2105)*, *dpy-19(e1259)*, *unc-32(e189)*, *dpy-18(e364)*.
LG *IV*: *fem-1(e1991)*, *unc-24(e138)*, *fem-3(e1996, q20gf, q95gf, q96gf)*, *daf-15(m81)*, *dpy-20(e1282)*, *unc-30(e191)*, *tra-3(e1107)*.
LG *V*: *her-1(e1520, e1561)*, *him-5(e1490)*, *dpy-21(e428)*, *emb-4(hc60)*, *unc-51(e369, e1189)*.
LG *X*: *sup-7(st5)*, *lon-2(e678)*.

Isolation of *fog-2* alleles: Four methods were used to isolate *fog-2* alleles: 1. *Screen for germ-line feminizing mutants.* L_4 hermaphrodites (P_0), either N2 or *dpy-19* +/+ *unc-32* (markers used were for reasons irrelevant to this work), were mutagenized with 0.05 M ethyl methanesulfonate (EMS) for 4 hr (BRENNER 1974) and individual F_1 self-progeny were picked to agar-filled Petri plates. Using a dissecting microscope, the F_2 self-progeny were screened for the presence of females (spermless hermaphrodites) at 25°. Four *fog-2* alleles were isolated from 12,438 mutagenized haploid genomes; *q70* and *q71* were from N2 P_0 while *q154* and *q226* were from *dpy-19* +/+ *unc-32* P_0.

2. *Screen for mutations that fail to complement fog-2(q71). unc-51* hermaphrodites were mutagenized with EMS as described above and crossed with *fog-2(q71)* males, either at 15° or 25°. Non-Unc F_1 *XX* cross-progeny were picked away from *XO* males at the L4 stage (to ensure virginity) in groups of 25 to 50. Plates were screened for F_1 females among self-fertile sibs when all animals were adults. Females arising from the failure of the putative *fog-2* allele [*unc-51 fog-2(new)*] to complement *q71* in *trans* were crossed with N2 males and 8 to 12 F_2 L4 *XX* progeny were picked. For a new *fog-2* allele one expects the F_2 to be self-fertile and about half to segregate Unc-females (*unc-51* is tightly linked to *fog-2*, see Figure 1) while the remaining F_2 animals segregate non-Unc *(q71)* females. The possibility of recessive lethal events that were induced in *cis* to *unc-51* and that failed to complement *fog-2(q71)* was tested by searching for F_2 hermaphrodites that did not segregate either Unc or female animals; none of this class was found. New dominant Fem/Fog mutations, *e.g.*, *tra-2(gf)*, see below, caused up to 50% of the F_2 cross progeny to be female. Seven *fog-2* alleles were isolated from 23,407 mutagenized haploid genomes; *q86, q123, q124*, were obtained at 25°, while *q166, q167, q170*, and *q177* were obtained at 15°. *q86* was isolated in *cis* to *unc-51(e369)* while all others were in *cis* to *unc-51(e1189)*. To ensure independence of *fog-2* alleles only one mutant from a given cross was retained.

3. *Extragenic suppressors of fem-3(q20gf). fem-3(gf)* mutants are self-fertile at 15° and sterile (Mog) at 25°. Mog animals are sterile because they produce a vast excess of sperm and no oocytes and thus have a masculinization of the germline phenotype (Table 1; BARTON, SCHEDL and KIMBLE 1987). Mutations in *fog-2* suppress *fem-3(gf)*, so the *fog-2*; *fem-*

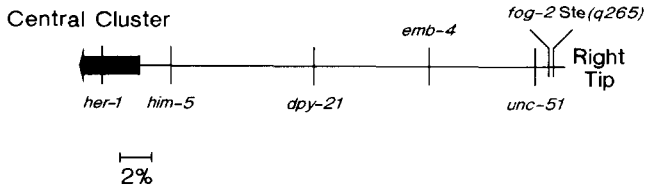


FIGURE 1.—Map of the right arm of chromosome *V*. The positions of *fog-2*, *emb-4*, and Ste(*q265*) (above the line) were determined by two- and three-factor crosses (Table 2; see MATERIALS AND METHODS). The positions of loci shown below the line are from SWANSON, EDGLEY and RIDDLE (1984) and HODGKIN and RIDDLE (1988).

3(gf) double mutant is self-fertile (see RESULTS). *fem-3(q20gf) dpy-20* L4 *XX* animals were mutagenized with EMS and allowed to produce self-progeny at 15°. Adult F_1 animals were picked, shifted to 25° and plates were screened for self-fertile F_2 animals. One *fog-2* allele, *q113*, was obtained by this procedure.

4. *Complementation suppression of fem-3(q95gf)/+; fog-2(q71)/+*. The strongest *fem-3(gf)* allele is *q95*, which is 100% Mog as a heterozygote at 25° (BARTON, SCHEDL and KIMBLE 1987). It was found that *fem-3(q95gf)/+; fog-2(q71)/+* is also Mog at 25°. However, when *fog-2(q71)* is homozygous, *fem-3(q95gf)/+* is no longer a dominant sterile (about 80–90% of animals are self-fertile). Thus a newly induced *fog-2* allele that fails to complement *fog-2(q71)* in *trans* will suppress the dominant sterility of *fem-3(q95gf)/+; fog-2(q71)/+*. *unc-51(e1189)* L4 *XX* animals were mutagenized with EMS and then crossed at 24° with *fem-3(q95gf) dpy-20*; *him-5 fog-2(q71)* males (from a 15° stock). Non-Unc F_1 animals were screened for self-fertile hermaphrodites (or eggs on the plate) among a sea of Mog animals and males. Any F_1 self-fertile animals were picked and new *fog-2* alleles were sought as F_2 Unc females. In a number of cases, the F_1 self-fertile animal had been mated by a sibling male (as judged by male progeny in the F_2), and as a result, more than one type of mutagenized *unc-51* chromosome was present. If the hermaphrodite had been mated, 12 non-Unc F_2 L4 *XX* animals were picked and F_3 Unc females were sought. As in the "screen for mutations that fail to complement *fog-2*," candidates were examined for sterile or lethal non-complementing alleles, but none was found. Four *fog-2* alleles (*q247*, *q249*, *q251*, and *q263*) were obtained from this procedure. In addition, a sterile mutation that does complement *fog-2*, and that does not suppress *fem-3(q95gf)/+*, Ste(*q265*), was fortuitously isolated in this mutagenesis. Ste(*q265*) is closely linked to *fog-2* (Figure 1), and thus useful in balancing *unc-51 fog-2* doubles. The phenotype of Ste(*q265*) *XX* animals is an arrest in gonadal development, lack of a vulva, and a reduced number of germ cells in which the only gametes to develop are sperm.

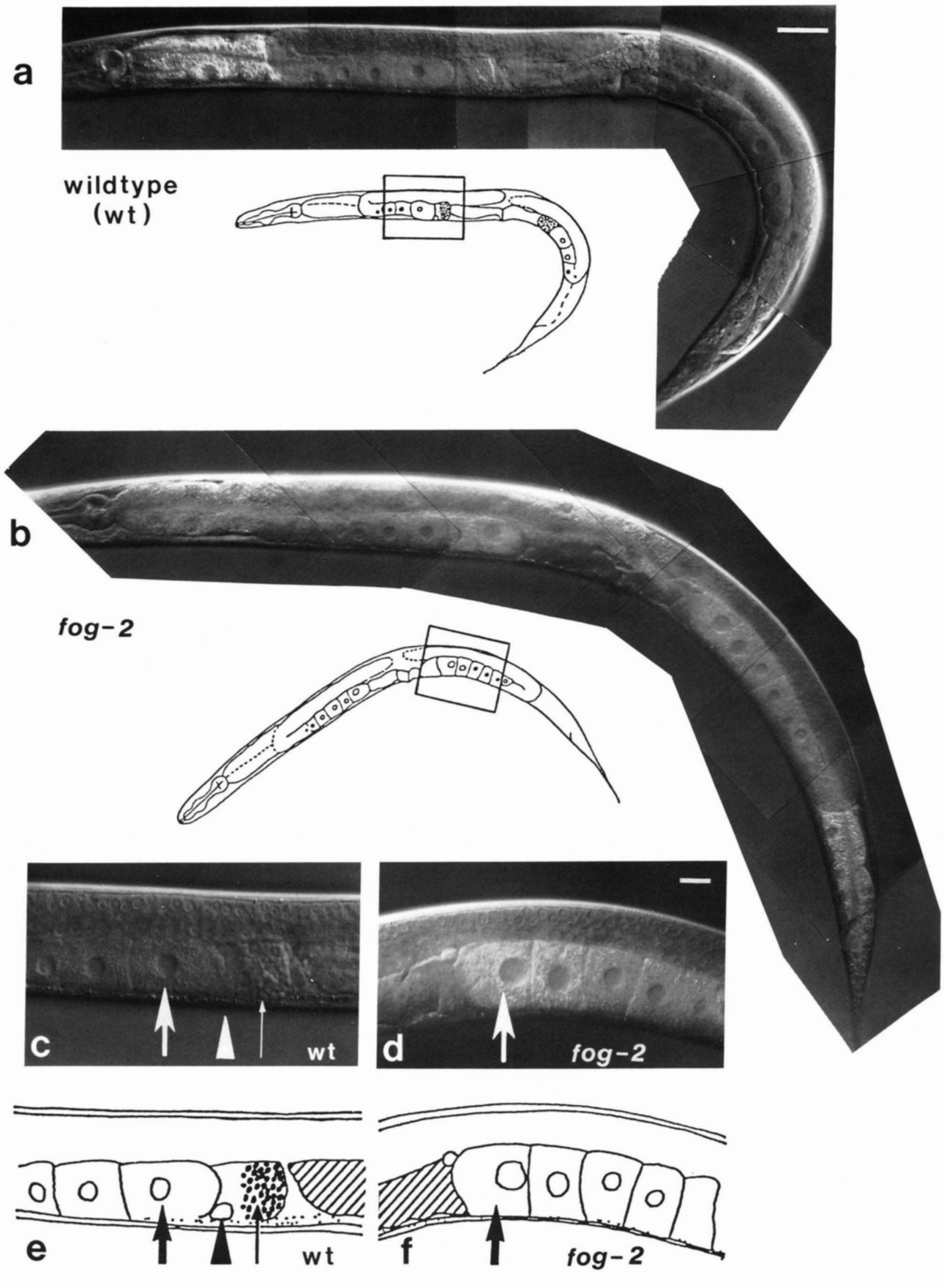
All putative *fog-2* alleles were out-crossed at least four times to N2. Where applicable, *fem-3(q20gf* or *q95gf) dpy-20* were removed during out-crossing based on loss of the tightly linked *dpy-20* marker and the absence of the Mog phenotype of *fem-3(gf)/+* at 25°. All alleles were then tested (or retested) for failure to complement *fog-2(q71)*. Mapping showed that all alleles are tightly linked to *unc-51* on the right arm of chromosome *V* (see below). The *fog-2* alleles isolated linked to *unc-51* were maintained as heterozygotes balanced by *emb-4(hc60)* or by Ste(*q265*). For construction of male/female strains (see below), the linked *unc-51* was removed by two factor crosses.

Analysis of *fog-2* mutants: Females can be distinguished from hermaphrodites either using a dissecting microscope or by Nomarski differential interference microscopy. With a dissecting microscope, adult females are non-eggbearing, and as such, the ventrally located uterus is empty and appears as a clear patch. Further, unfertilized oocytes accumulate in females giving the proximal arm of the gonads a striped appearance. To identify females unequivocally, animals were examined by Nomarski optics with a Zeiss Planapo 63X lens at ×630 magnification. By Nomarski, females in L_4 lethargus or as young adults lack sperm and primary spermatocytes in both the gonad and the spermatheca. The gametes that develop most proximally are oocytes (see RESULTS and Figure 2 for further description).

To determine the penetrance of the Fog phenotype, *XX* L4 animals were picked to individual plates and examined by the dissecting microscope as adults [about 24 hr (20 or 25°) or about 36 hr (15°)] for a female morphology and the absence of eggs/larvae. Selected animals were further examined by Nomarski. In some situations (such as strain constructions), L4 *XX* animals were transferred in groups of 20 to 50 and scored as above.

Males (*XO*) were examined by the dissecting microscope for the presence of a male tail, the absence of a vulva and for mating behavior. The following sexually dimorphic structures were examined by Nomarski optics for morphology and size to learn if there was any feminization of males: the germ line, the male gonad consisting of a single reflexed arm and vas deferens (KLASS, WOLF and HIRSH 1976; KIMBLE and HIRSH 1979), and the bursal fan, sensory rays (9 pairs), and copulatory spicules of the male tail (SULSTON, ALBERTSON and THOMSON 1980). Further, the type and position of gametes and germ cells within the gonad (HIRSH, OPPENHEIM and KLASS 1976) and the presence of yolk in the pseudocoelom (refractile droplets, KIMBLE and SHARROCK 1983; DONIACH 1986a) were scored. For each of the *fog-2* alleles, more than 40 *XO* animals

FIGURE 2.—Wild-type and *fog-2(q71) XX* germ-line phenotypes. (a and b), Composite photomicrographs using Nomarski optics. Focal plane was adjusted to show the two gonad arms. Scale bar = 40 μm. Line drawings are shown below. (c and d), Higher magnification photomicrographs of boxed gonad region from (a) and (b), respectively. Scale bar = 10 μm. Line drawings of gamete type and position in gonad are shown below. (a and c), Wild-type young adult hermaphrodite. (b and d), *fog-2* young adult female. (c), Gametes in the proximal part of the anterior gonad arm of a hermaphrodite. The first, most proximal, germ cells have differentiated as sperm. Subsequent germ cells have differentiated as oocytes. (d), Gametes in the proximal part of the posterior gonad arm of a *fog-2* female. The first, most proximal, germ cells have a transformed sexual fate and have differentiated as oocytes instead of sperm. There is no evidence of sperm or spermatogenesis. Note that in both the hermaphrodite (c) and the female (d) the spermatheca (striped in drawings) is empty. Thick arrows—oocytes. Oocytes are very large cells, with a large smooth nucleus, and have a granular cytoplasm. Immature oocytes are smaller, have a large nucleolus, and also have a granular cytoplasm. Thin arrow—sperm. Sperm are small, with a tiny elevated refractile nucleus. Arrow head—primary spermatocyte. Note that the pattern of gametogenesis in the anterior and posterior gonads are equivalent in the hermaphrodite, and similarly, they are equivalent in the *fog-2* female. For further morphological details see HIRSH, OPPENHIEM and KLASS (1976) and KIMBLE and WARD (1988).


a
wildtype
(wt)
b
fog-2
c
wt
d
fog-2
e
wt
f
fog-2

were examined. Because feminization (the appearance of yolk and oocytes) can be observed only in older adult males for certain mutations (DONIACH 1986a; and see below), *fog-2* alleles *q71*, *q86*, *q123*, and *q247* were scored over time; about 20 males for each were examined at about 24, 48, 72, 96, and 120 hr. post L4 at 20°.

For all of the *fog-2* alleles (except *q177*), *XO* animals were shown to be functional males by construction and propagation of male/female strains. Adult *fog-2* females were crossed with *fog-2*/+ males from which ten single male cross progeny (either *fog-2* or *fog-2*/+) were mated with a single adult *fog-2* female. Male/female strains were established from matings in which both parents were homozygous *fog-2*. In general, the *fog-2* male/female strains were the source of animals for strain constructions, scoring phenotypes and were continuously propagated by mating a single male and a single female. A sex ratio of one was observed for these male/female strains [*e.g.*, *fog-2(q226)* mating produced 107 females and 108 males], in contrast to what is observed for some other male/female nematode species (TRIANTAPHYLLOU 1973).

The mating efficiency of *fog-2* males was found to be equivalent to wild-type males. This was determined by a mating competition assay. Twenty-four young adult males, 12 for *fog-2(q71)* and 12 for N2, were placed with four *fog-2* females (2 hr at 20°), and then the males were removed. The genotype of the cross progeny was determined by picking *XX* L4 animals over 2 days and scoring their phenotype. Female progeny were generated by mating with a *fog-2* male; hermaphrodite progeny by an N2 male. From two such mating competitions, 186 females and 171 self-fertile hermaphrodites were found.

All *fog-2* mutants were tested for heat sensitivity (alleles isolated at 23–25°) or cold sensitivity (alleles isolated at 15°). Male/female strains or *unc-51 fog-2(q177)* self-fertile animals were placed at the appropriate temperature for at least two generations. Then, the proportion of L4 *XX* animals that developed as female or self-fertile adults was determined. None of the *fog-2* alleles was temperature sensitive.

The recessive nature of *fog-2* alleles was shown by crossing *fog-2* males with *unc-51* animals; non-Unc *XX* cross progeny [*m*(+/+), *z*(−/+)] were scored for self-fertility. The possibility of maternal effects was tested in strong *fog-2* mutants. A maternal rescue effect was tested by determining the proportion of *unc-51 fog-2* [*m*(−/+), *z*(−/−)] females segregating from an *unc-51 fog2*/+ + mother. All Unc self-fertile animals found were recombinants. A maternal absence effect was tested by determining the proportion of *fog-2*/+ [*m*(−/−), *z*(−/+)] females segregating from a cross of *fog-2* females by N2 males. No maternal effects were found (see RESULTS).

Tests for amber alleles of *fog-2*: The amber suppressor tRNA mutation *sup-7(st5)* (WATERSTON 1981; WILLS *et al.* 1983) was used to test whether any of the *fog-2* alleles were amber mutations. For *fog-2* alleles linked to the *unc-51* marker, Unc females were crossed with *dpy-18(e364)*/+; *sup-7*/0 males, and non-Unc cross progeny picked. In the next generation, Unc L4 animals were removed and scored as self-fertile or female. Since only one-fourth of the *unc-51 fog-2* animals would be homozygous for *sup-7*, greater than 60 Unc animals were scored for each allele tested. No suppression was observed in tests at either 20° or 25° for *fog-2*(q86, *q123*, *q124*, *q166*, *q167*, *q170*, *q247*, *q249*, *q263*).

For *fog-2* alleles that were not marked, *fog-2/unc-51(e369)* strains homozygous for *sup-7* were constructed. The phenotype of *unc-51(e369* or *e1189)* is paralysed, dumpy and egg laying defective (Egl). The *unc-51* allele *e369* is suppressible by *sup-7* and *unc-51(e369)*; *sup-7(st5)* homozygotes have a coily Unc, non-Dpy non-Egl phenotype at 20° and 25°. *fog-2* males were crossed to *sup-7*; *unc-51(e369)* hermaphrodites, non-coily cross progeny picked and allowed to self. F_2 animals were picked individually to obtain the stock *fog-2/unc-51(e369)*; *sup-7* identified by producing coily Unc, but no paralyzed Unc progeny. From this, greater than 50 non-Unc L4 animals were picked to search for suppressed *fog-2* animals that do not segregate Uncs. No suppression was observed at either 20° or 25° for *fog-2* (*q70*, *q71*, *q113*, and *q226*). The leaky alleles *q177*, *q154*, and *q251* were not tested.

Genetic mapping: *fog-2* was initially mapped to the right tip of LG *V* near *unc-51*. *fog-2* was positioned by three- and two-factor crosses (Table 2, A and B, respectively) in relation to *dpy-21* and *unc-51*. Two loci [Ste(*q265*) and *emb-4*] used for balancing marked *fog-2* mutants were also positioned by three-factor crosses. Figure 1 summarizes the map of the right arm of LG *V* and was derived from Table 2 and the current *C. elegans* map (HODGKIN and RIDDLE 1988).

***tra-2(gf)* mutants:** Two *tra-2(gf)* mutants (*q122gf* and *q179gf*) were isolated as dominant *XX* F2 females in the course of the *fog-2* complementation screen. They were characterized in a manner similar to that detailed above for *fog-2* mutants. These mutants are similar to the *tra-2(gf)* alleles described by DONIACH (1986a), *XX* animals are females while *XO* animals are essentially wild type males (see Table 1).

The *tra-2(q122gf)* allele shows a strong dominant *XX* feminizing phenotype: 100% of *q122gf*/*q122gf*, *q122gf*/+, and *q122gf*/*tra-2(lf)* [for either *tra-2(e1095* and *e1425)*] were female in both the germ line and the soma ($n > 200$ for each). Males (*XO*) were found to be functionally normal as judged by construction of male/female strains. When examined by Nomarski, *q122gf* homozygotes were morphologically normal except in two regards. First, 8% lacked germ cells in the adult gonad. Second, older males (72 hr or more post L4 at 20°) showed feminization in the germ line [59% ($n = 73$) showed evidence of oogenesis] and in the intestine (30% had yolk in the pseudocoelom). Three-factor mapping of *q122(gf)* with respect to *dpy-10 unc-4* on LG *II* was consistent with the position of *tra-2(lf)*: of 11 Dpy recombinants, one was *dpy-10 tra-2(q122gf)* and ten were *dpy-10* +, and of 11 Unc recombinants, nine were *tra-2(q122gf) unc-4* and two were + *unc-4*. *dpy-10* is about 0.1% to the left of *tra-2* while *unc-4* is 1.2% to the right (HODGKIN and RIDDLE 1988). Demonstration that the *q122(gf)* mutation is an allele of *tra-2* was obtained by isolating tightly linked revertants of the dominant *XX* germ line feminizing phenotype. These revertants have a *tra-2(lf)* phenotype and fail to complement *tra-2(lf)* (P. OKKEMA, personal communication).

The *tra-2(q179gf)* allele, while exhibiting a dominant gain-of-function phenotype in the germ line, also has recessive loss-of-function (hypomorphic) characteristics in the soma [see DONIACH (1986a) for a further discussion of this phenotype]. The germ-line feminization is incomplete: 73% of *q179gf*/*q179gf* animals were female ($n = 108$), 8% of *q179gf*/+ animals were female ($n = 66$), and *q179gf*/*tra-2(e1095)* animals were 100% self-fertile ($n = 66$). Somatic masculinization was observed in *q179gf XX* homozygotes. They had a truncated tail and some animals were Egl. The penetrance of the tail masculinization and the Egl phenotypes was increased in *q179gf*/*tra-2(e1095)* animals. *XO* males were morphologically and functionally normal. Mapping yielded four *dpy-10 tra-2(q179gf)* and nine *dpy-10* + recombinants and 13 *tra-2(q179gf) unc-4* and three +

TABLE 2

Map data for region of Chromosome *V* around *fog-2*

A. Three-factor crosses

Gene (allele)	Parental genotype	Recombinant phenotype	Recombinant genotype	Number
fog-2(q71)	*fog-2/dpy-21 unc-51*	Dpy non-Unc	dpy-21 + fog-2 / dpy-21 unc-51 +	77
		Unc non-Dpy	+ unc-51 + / dpy-21 unc-51 +	135
	dpy-21 fog-2/unc-51	Dpy non-Fog	dpy-21 unc-51 + / dpy-21 + fog-2	33
		Dpy non-Fog	dpy-21 + + / dpy-21 + fog-2	1
Ste(*q265*)	*unc-51 q265/fog-2*	Unc non-Ste	unc-51 fog-2 + / unc-51 + q265	9
		Unc non-Ste	unc-51 + + / unc-51 + q265	4
emb-4(hc60)[a]	*emb-4/dpy-21 unc-51*	Dpy non-Unc	dpy-21 emb-4 + / dpy-21 + unc-51	8
		Dpy non-Unc	dpy-21 + + / dpy-21 + unc-51	8
		Unc non-Dpy	+ emb-4 unc-51 / dpy-21 + unc-51	10
		Unc non-Dpy	+ + unc-51 / dpy-21 + unc-51	12

B. Two-factor crosses

Gene (allele)	Heterozygous parent	Segregants	Map distance[b]
fog-2(q71)	*dpy-21 fog-2/+ +*	647 wild type 192 Dpy Fog 52 Dpy 50 Fog	11.5%
	unc-51 fog-2/+ +	852 wild type 221 Unc Fog 2 Unc 5 Fog	0.65%
unc-51(e369)	*dpy-21 unc-51/+ +*	690 wild type 147 Dpy Unc 53 Dpy 48 Unc	11.4%

[a] The embryonic lethality of *emb-4(hc60)* (MIWA *et al.* 1980) was scored by cloning 12 L4 progeny from each of the initial recombinants and scoring for a brood of dead embryos at 25°.

[b] Map distance (or recombination frequency, p) was calculated from R (total number of recombinants/total number of progeny) according to the formula $R = p - p^2/2$ (BRENNER 1974). Two-factor crosses were performed at 20°.

unc-4 recombinants. A recessive roller mutation is tightly linked to *q179gf* and has not yet been separated.

Three other *tra-2(gf)* alleles have also been employed in this study (*q101gf*, *q103gf*, and *q244gf*; Z. ROSENQUIST, M. K. BARTON and P. OKKEMA, personal communication). Each of these alleles is strong; *tra-2(gf)* and *tra-2(gf)/+ XX* are 100% female ($n > 50$). Homozygous *XX* animals also have a normal female soma while *XO* males are unaffected.

Double heterozygote constructions: Double mutant heterozygotes were generated by crosses as detailed below and their phenotypes analysed by picking individual L4 *XX* cross progeny and examining them as adults about 24 hrs later (20°) for female morphology and the presence of eggs and larvae on plates. In some cases animals were further examined by Nomarski. The crosses to generate the desired maternal and zygotic genotypes were as follows. For *fog-1* *m*(−/−), *z*(−/+), *XX* females homozygous for *fog-1(q187)* (M. K. BARTON, personal communication) were crossed to

fog-2(q71) or control N2 males. For *fem-1* $m(-/-)$, $z(-/+)$, *XX* females homozygous for *fem-1(e1991) dpy-20*, an amber, putative null allele (Doniach and Hodgkin 1984), were crossed to *fog-2* or N2 males. For *fem-1* $m(+/+)$; $z(-/+)$, *fem-1(e1991) dpy-20*/+ + males were crossed to *dpy-20*; *fog-2* females or to control *unc-24 dpy-20* hermaphrodites and Dpy cross progeny scored. For *fem-2* $m(-/-)$, $z(-/+)$, *XX* females homozygous for the putative null allele (Hodgkin 1986) *fem-2(e2105)* were crossed with *fog-2* or N2 males. For *fem-3* $m(-/-)$, $z(-/+)$, *XX* females homozygous for the putative null allele (Hodgkin 1986) *fem-3(e1996) dpy-20* were crossed with *fog-2* or N2 males. *fem-3* $m(-/+)$; $z(-/+)$ were obtained by crossing Unc hermaphrodites *unc-24 fem-3(e1996)* + *dpy-20/unc-24* + *daf-15* + to homozygous *dpy-20*; *fog-2* or control *dpy-20*; *him-5* males and Dpy progeny scored. For *fem-3* $m(+/+)$, $z(-/+)$, + *fem-3(e1996) dpy-20*/+ + males were crossed to *unc-24* + *dpy-20*; *fog-2* females or control *unc-24* + *dpy-20* hermaphrodites. The statistical significance of the difference between proportions [females/(females + self-fertiles)] for given genotypes was determined by the z-test (Freund 1973) at $P < 0.05$.

***her-1(lf) fog-2* double mutant constructions:** *XO* animals homozygous for a putative null allele of *her-1(e1520)* (Hodgkin 1980) have a soma that is female and a germ line that first makes sperm and then oocytes; these *XO* animals are self-fertile (Table 1). *him-5(e1490)* was used to generate *XO* animals by nondisjunction of the *X* among progeny of homozygotes (Hodgkin, Horvitz and Brenner 1979). *dpy-21(e428)* was used to distinguish *XX* and *XO* animals independent of sexual phenotype; *XX* animals are Dpy while *XO* animals are non-Dpy (Hodgkin 1980, 1983, 1986). *dpy-21 fog-2(q71)* non-Dpy (*XO*) males were mated with *her-1 him-5 dpy-21* Dpy (*XX*) hermaphrodites. Dpy cross progeny + + *dpy-21 fog-2*/*her-1 him-5 dpy-21* + were cloned; they segregated F_2 Dpy females (*dpy-21 fog-2* homozygotes), Dpy hermaphrodites (*her-1 him-5 dpy-21* homozygotes), and Dpy parental hermaphrodites (+ + *dpy-21 fog-2*/*her-1 him-5 dpy-21* +). Twenty-one Dpy F_2 hermaphrodites were picked to individual plates to obtain animals in which a cross-over event had occurred between *him-5* and *fog-2* (distance of about 18%, see Figure 1). Three recominant F_2, *her-1 him-5 dpy-21 fog-2*/*her-1 him-5 dpy-21* +, were found and they segregated the following progeny types: *her-1 him-5 dpy-21* homozygotes that were self-fertile Dpy(*XX*) or non-Dpy(*XO*), parental *her-1 him-5 dpy-21 fog-2*/*her-1 him-5 dpy-21* + self-fertile Dpy(*XX*) or non-Dpy(*XO*), and *her-1 him-5 dpy-21 fog-2* homozygotes that were female Dpy(*XX*) or female non-Dpy(*XO*). The genotype of the non-Dpy (*XO*) females was confirmed by mating with *fog-2* males. For one such cross, 31 cross-progeny were males, and 10 were females. In addition, a number of dead embryos were observed. These data suggest that the non-Dpy females were *XO* and homozygous for *fog-2*.

***tra-2(lf)*; *fog-2* double mutant constructions:** *XX* animals homozygous for putative null alleles of *tra-2* (*e1095* and *e1425* amber) are transformed into incomplete males (Table 1, Hodgkin and Brenner 1977). Heterozygotes show a semi-dominant Egl phenotype (Trent, Tsung and Horvitz 1983; Doniach 1986a). The *tra-2*; *fog-2* doubles were constructed with either *e1095* or *e1425* and with one of three alleles of *fog-2* (*q71*, *q124*, *q247*). + *tra-2(e1425) unc-4*/*dpy-10* + *unc-4* hermaphrodites were crossed with either *fog-2(q71* or *q124)* males and non-Unc cross-progeny were individually picked. F_2 Unc L4 animals were transferred *en masse* and were scored by dissecting and Nomarski microscopy either at about 24 or 72 hr (20°) later. Since only one-fourth of the Unc animals will be homozygous for *fog-2*, more than 60 animals were examined at each time point for both *q71* and *q124*. About 1% of the Unc animals were recombinants (female soma and self-fertile) of the genotype *tra-2(e1425) unc-4*/+ *unc-4* based on progeny testing. The remaining (99%) Unc animals were incomplete males indistinguishable by Nomarski from *tra-2(e1425) unc-4* alone.

In the course of constructing *tra-2(e1095)*; *fog-2(q71* or *q247)* double mutants, it was found that *tra-2(lf)*/+ partially suppresses the Fog phenotype. *unc-51 fog-2(q71* or *q247)* females were crossed with *tra-2(e1095)*/*tra-2(q122gf)* males, *XX* L4 cross progeny removed *en masse* and self-fertile animals picked to individual plates [*tra-2(q122gf)*/+ (*XX*) animals are female]. In the F_2 far more Unc animals were self-fertile than expected by recombination (see Table 2 and Figure 1). Unc self-fertile animals were picked and segregated Unc Tra (29%), Unc females (54%), and Unc self-fertile (17%; n = 141 total animals for two broods of *tra-2(lf)*/+; *unc-51 fog-2(q247)* hermaphrodites). Crossing of Unc self-fertiles with N2 males and analysis of F_2 progeny confirmed that the original genotype was *tra-2(e1095)*/+; *unc-51 fog-2(q247)*. Analysis of >30 adult Unc incomplete males segregating from a *tra-2(e1095)*/+; *unc-51 fog-2* parent by Nomarski showed no difference from *tra-2(lf)* alone. In this case the *fog-2* genotype is $m(-/-)$, $z(-/-)$. Similar results were obtained when *fog-2(q71)* was employed (data not shown).

Suppression of *fog-2* was also observed with animals heterozygous for a deficiency (*mnDf30*, Sigurdson, Spanier and Herman 1984) of the region around *tra-2*. A strain *mnDf30 unc-4*/+ *unc-4*; *fog-2(q71)* was constructed. Analysis of two broods showed segregation of dead embryos (27%, presumably *mnDf30* homozygotes), Unc females (57%) and Unc self-fertile hermaphrodites many of which were Egl (16%, n = 181 total embryos and animals for two broods). The genotype of self-fertile animals was verified by crossing with *dpy-10*/+ males (*dpy-10* is deleted by *mnDf30*). From this cross, about one-fourth the F_1 cross progeny were Dpy and *fog-2* segregated among progeny of all individually picked F1s.

***tra-3*; *fog-2* double mutant constructions:** *XX* animals homozygous for a putative null allele of *tra-3(e1107* amber) when derived from a homozygous mutant mother [$m(-/-)$, $z(-/-)$] are transformed into incomplete males (Table 1, Hodgkin and Brenner 1977). This phenotype is somewhat temperature sensitive; at 25° no animals are self-fertile, while at 15° some are self-fertile (Hodgkin 1986). At 25°, most animals have a male shaped somatic gonad (see below), while at 15° most animals have an intersexual somatic gonad.

tra-3(e1107) hermaphrodites were purged (allowed to exhaust all their sperm), crossed with *unc-51 fog-2*/+ + (either *q71* or *q247*) males and L4 cross progeny were individually picked. Non-Unc F2 animals were then individually picked, one-sixth of which have the genotype *tra-3(e1107)*/*tra-3(e1107)*; *unc-51 fog-2*/+ +. Such animals segregate a brood of all *XX* incomplete males with Unc animals homozygous for *fog-2*. Unc L4 animals were removed *en masse* and scored by Nomarski about 24 hr later (all at 25°). The same protocol employing *unc-51*/+ males provided a control population for comparison. The phenotype of *tra-3(e1107)* $m(-/-)$, $z(-/-)$; *unc-51 fog-2* was essentially identical to *tra-3* $m(-/-)$, $z(-/-)$; *unc-51* +. For both, *tra-3* with either *fog-2* or *fog-2*(+), all animals had a partially masculinized tail and all had yolk in the pseudocoelom. With *fog-2(q71)*, 17% had an abnormal/intersexual somatic gonad, 40% had a male somatic gonad with a male germ line and 41% had a male somatic gonad with a germ line

of first sperm and then oocytes (n = 59). Similar results were obtained with *fog-2(q247)*. With *fog-2(+)*, 21% had abnormal/intersexual somatic gonad, 42% had a male somatic gonad with a male germ line and 37% had a male somatic gonad and a germ line of first sperm and oocytes (n = 51). Note that an additional approximately 30% of Unc Tra animals for both *fog-2* and *fog-2(+)* were dead at the time of scoring due to the inability to defecate as a result of a defective anus. Essentially identical germ-line and somatic phenotypes were also observed when comparing a marked *tra-3*, *unc-30 tra-3* $m(-/-)$, $z(-/-)$ with or without *fog-2(q71)* [data not shown].

***fem-3(gf); fog-2* double mutant construction:** Gain-of-function *fem-3* mutants are self-fertile at 15° and Mog at 25°. The markers *unc-24* and *dpy-20* map about 1% to the left and right of *fem-3* respectively. Thus Unc Dpy animals segregating from *unc-24 fem-3(gf) dpy-20/+ + +* will be (>99%) homozygous for *fem-3(gf)*. Homozygous *fog-2* males were mated with *unc-24 fem-3(gf) dpy-20* hermaphrodites (raised at 15°) and *XX* F_1 cross progeny were picked and shifted to 25° as adults. The F_2 includes unsuppressed Mog Unc Dpy animals and suppressed self-fertile Unc Dpy animals. Self-fertile *unc-24 fem-3(gf) dpy-20; fog-2* stocks were established, and verified to be homozygous for both *fem-3(gf)* and *fog-2* by crossing Unc Dpy animals with N2 males (15°), picking and shifting 12 F_1 *XX* adults to 25°. All 12 F_1 hermaphrodites segregated Unc Dpy Mogs and non-Unc non-Dpy females. Animals heterozygous for *fog-2(q154* or *q71)* and homozygous *fem-3(q20gf)* were obtained by crossing *unc-24 fem-3(q20gf) dpy-20; fog-2(q154* or *q71)* hermaphrodites with *fem-3(q20gf) dpy-20; him-5* males (at 15° or 25°) and scoring *XX* Dpy cross-progeny.

Scoring the interaction of *fem-3(gf)* with *fog-2* was determined as follows. L4 Unc Dpy *XX* animals were picked to individual plates and examined as adults either about 24 hrs later (25°) or 48 hr later (15°) for self-fertile hermaphrodite, female, and Mog phenotypes. The Unc Dpy animals were from homozygous *fem-3(gf); fog-2* stocks. All worms that did not have eggs or larvae on the plate were scored by Nomarski. Animals found with cleaving or fertilized eggs in the uterus were scored as self-fertile hermaphrodites. Females were scored as defined above. Animals were scored as Mog if they did not have any self-progeny by 24 (25°) or 48 (15°) hr after the L4 stage and if their lack of progeny was due to a vast overproduction of sperm. Such animals include those that never make oocytes, and those that make oocytes days later than normal after producing a great excess of sperm. This procedure was also used to score the interaction between *tra-2(gf)* and *fem-3(gf)* and between *tra-2(gf)* and *fog-2* in a *fem-3(gf)* background (see below).

An unmarked *fem-3(q20gf); fog-2(q71)* stock was constructed by crossing *dpy-20; fog-2(q71)* males with *fem-3(q20gf); unc-51* hermaphrodites (15°) and obtaining a non-Dpy, non-Unc F_2 *XX* suppressed self-fertile animal (25°) that fails to segregate Dpys or Uncs in the next generation. The stock was verified by crossing with N2 males as described above.

***tra-2(gf); fem-3(gf)* double mutant construction:** By a strategy analogous to the *fog-2; fem-3(gf)* constructions described above, *tra-2(gf)* males were mated with *unc-24 fem-3(gf) dpy-20* hermaphrodites and F2 Unc Dpy self-fertile animals (at 25°) were picked to obtain a suppressed self-fertile stock. As above, the stock was verified to be homozygous *unc-24 fem-3(gf) dpy-20; tra-2(gf)* by crossing with N2 males and analyzing the progeny segregating from 12 F_1 animals (at 25°). Scoring of the interaction between *tra-2(gf)* and *fem-3(gf)* was also analogous to that described above for *fog-2; fem-3(gf)*.

***tra-2(gf); fog-2* double mutant constructions:** Male/female strains homozygous for both *tra-2(gf)* and *fog-2* were constructed. *tra-2(q122gf)* homozygous males were crossed to *dpy-10 unc-4; fog-2* homozygous females. F_1 males and females from the cross were mated to each other *en masse* followed by 32 matings of non-Unc non-Dpy single F_2 males with single F_2 females. Two single matings were identified in which both parents were *tra-2(gf)/dpy-10 unc-4; fog-2* homozygous based on all (>30) Dpy Uncs being females. This was followed by three generations of single male/female matings to obtain lines that fail to segregate Dpy Uncs. A male/female line was verified to be homozygous *tra-2(q122gf); fog-2(q71)* by: (1) crossing males with *dpy-10 unc-4* hermaphrodites and obtaining only non-Unc non-Dpy cross progeny; (2) intercrossing the F_1 males and females from (1) and showing that about one-fourth the Dpy Unc animals were female (because the original line is homozygous for *fog-2*); and (3) crossing males with *unc-51*, intercrossing the F_1 males and females to show that about two-thirds of the Uncs were females (because the original line is homozygous *tra-2(gf)*). Males and females of *tra-2(q122gf); fog-2(q71)* were examined by Nomarski and found to be indistinguishable from *tra-2(q122gf)* alone (see above). Similarly, a *tra-2(q179gf); fog-2(q71)* male/female strain was constructed and verified to be homozygous for both *q179gf* and *q71*. It has the recessive *XX* truncated tail and roller phenotypes expected of *q179gf* homozygotes (see above).

Triple mutants: To construct *fog-2(lf); tra-2(gf); fem-3(gf)* triple mutants, homozygous *XX unc-24 fem-3(gf) dpy-20; tra-2(gf)* hermaphrodites were crossed with *fog-2(q71)* males and F_1 L4 cross progeny picked (all at 25°). Unc Dpy F_2 animals were picked and scored for Mog, self-fertile, and Fog phenotypes as described above for the *fem-3(gf); fog-2* double. All Unc Dpy animals are homozygous for *fem-3(gf)*, while one-sixteenth are homozygous for both *fog-2* and *tra-2(gf)*. In some cases, Unc Dpy triple mutant females were crossed with N2 males. Males cross progeny were examined for feminized phenotypes. Additionally, the Unc Dpy triple mutant females were shown to be homozygous for *fem-3(gf)* by picking 12 F_1 progeny and showing that all segregate Unc Dpy Mogs at 25°.

A homozygous strain *tra-2(e1941gf); unc-24 fem-3(q20gf) dpy-20; fog-2(q71)* was obtained by repeated rounds of picking self-fertile Unc Dpy animals and testing for the presence of *e1941gf* and *q71*. The genotype was verified by crossing with N2 males. All the male cross progeny tested (20) were shown to carry *e1941gf, q20gf* and *q71* by mating single males with *dpy-10 unc-4* or *unc-51* hermaphrodites and examining the phenotype of F2 animals at 25°. F_2 *dpy-10 unc-4* females were homozygous for *q71, unc-51* females were homozygous or heterozygous for *e1941gf* and *unc-24 dpy-20* Mogs were homozygous for *q20gf*.

RESULTS

Isolation and characterization of *fog-2* alleles: Sixteen recessive *fog-2* alleles have been isolated by four different protocols (see MATERIALS AND METHODS for details). The phenotype of homozygous *fog-2* mutants is the same for all alleles: *XX* animals are transformed from self-fertile hermaphrodites to females while *XO* males are unaffected (Figure 2; Table 3). In wild-type young adult hermaphrodites, sperm and pri-

TABLE 3

Phenotype of *fog-2* alleles[a]

XX			*XO*	
fog-2 allele	% Female	% Self-fertile (hermaphrodite)	Morphology	Mating[b]
+	0	00	Male	+
q177	73	27 (n = 117)	Male	ND[c]
q154	93	7 (n = 309)	Male	+
q251	98	2 (n = 442)	Male	+
q124	99.6	0.4 (n = 420)	Male	+
q70, q71, q86 *q113, q123, q166* *q167, q170, q226* *q247, q249, q263*	100%	0 (n > 250 to n > 1000)	Male	+

[a] See MATERIALS AND METHODS for scoring of phenotypes.
[b] Male/female strains.
[c] ND = not determined.

mary spermatocytes are the first and most proximal gametes to differentiate within the gonad, followed by maturing oocytes (Figure 2, a and c). In *fog-2 XX* young adults, the first and most proximal gametes to differentiate are oocytes (Figure 2, b and d). There is no evidence of sperm, spermatogenesis, or germ cell death in *fog-2* females. Further, oogenesis begins in *fog-2* mutants at about the time spermatogenesis begins in wild type. In late L4, signs of oogenesis are observed in the proximal arm of each gonad and germ cells have the morphology of immature oocytes and not that of primary spermatocytes. This *fog-2* phenotype is distinct from "females" generated by *spe* (spermatogenesis defective) and *fer* (fertilization defective) mutants that produce defective sperm and/or primary spermatocytes in the normal position and at the normal time (WARD and MIWA 1978; KIMBLE and WARD 1988). The soma of *XX fog-2* females is indistinguishable from that of wild-type hermaphrodites.

Unlike *XX* animals, *XO* animals homozygous for any of the *fog-2* alleles are unaffected in either germ line or soma. Detailed examination of the morphology of *XO* males by Nomarski (Table 3, see MATERIALS AND METHODS) showed no evidence of feminization for any of the alleles. Animals homozygous for four of the strong alleles (*q71*, *q86*, *q123*, and *q247*) were examined over time, with no evidence of feminization, even in old adults. For 15 alleles, males were shown to be fertile based on ability to mate and thus propagate biparentally as homozygous *fog-2* male/female strains. In a mating competition experiment, *fog-2(q71)* males were indistinguishable from N2 males in ability to sire cross progeny (see MATERIALS AND METHODS).

No maternal effects have been observed for *fog-2*—neither a maternal absence effect [$m(-/-)$, $z(-/+)$] nor a maternal rescue effect [$m(-/+)$, $z(-/-)$] (Table 4, second and third rows). Thus, zygotic *fog-2* activity is necessary and sufficient for hermaphrodite spermatogenesis. This is in contrast to the maternal effects observed for the *fem* genes. A maternal absence effect is observed for *fem-3(lf)* such that *fem-3*/+ $m(-/-)$, $z(-/+)$ *XX* and *XO* animals are partially feminized (see HODGKIN 1986; BARTON, SCHEDL and KIMBLE 1987 for details; Table 4). Thus, the absence of maternally contributed *fem-3*(+) impairs male germline and male somatic development in heterozygous progeny. A maternal rescue effect is observed for *fem-1* and *fem-2*, *XX* and *XO*, and for *fem-3 XO* animals such that *fem*/*fem* $m(-/+)$, $z(-/-)$ animals are only incompletely feminized (DONIACH and HODGKIN 1984; HODGKIN 1986). Here, maternally contributed *fem*(+) partially rescues homozygous *fem*(−) progeny such that some male development occurs in both the germ line and soma.

The recessive nature of *fog-2* alleles was demonstrated by crossing *fog-2* males with *unc-51* hermaphrodites and showing that all heterozygous *XX* cross progeny were self-fertile (Table 4, *top row*). Further, the brood size, which is limited by the number of functional sperm made (WARD and CARREL 1979), of *fog-2(q71)*/+ is not significantly different from that of wild type (306 ± 36 *vs* 328 ± 45, respectively, n = 10 in both cases; Student t-test, >95% confidence level). The penetrance of the *XX fog-2* phenotype has been ranked for the 16 alleles and is shown in Table 3. There are two classes of *fog-2* alleles. Four alleles are leaky (*q177*, *q154*, *q251*, and *q124*) and 12 are strong with complete penetrance of the Fog pheno-

type (*q70*, *q71*, *q86*, *q113*, *q123*, *q166*, *q167*, *q170*, *q226*, *q247*, *q249*, *q263*). None of the alleles is temperature sensitive, although all alleles were isolated at one of the standard growth extremes (15°, 4 alleles and 25°, 12 alleles) for *C. elegans*.

The *fog-2* alleles that have been isolated are likely to be loss-of-function. Eleven of the *fog-2* mutants were isolated at a frequency of 3.2×10^{-4} and 3×10^{-4} per haploid genome after EMS mutagenesis (by the "screen for feminizing mutations" and the "screen for mutations that fail to complement *fog-2*" respectively, see MATERIALS AND METHODS). This is within the bounds (10^{-3} to 10^{-4}) for the frequency of EMS induced loss-of-function mutations observed for other *C. elegans* genes (BRENNER 1974; GREENWALD and HORVITZ 1980; HODGKIN 1986). Additionally, all *fog-2* mutants are recessive. The four leaky alleles probably represent partial loss-of-function (hypomorphic) mutants that retain some *fog-2*(+) activity. The 12 strong alleles are indistinguishable and completely penetrant; they are likely to be complete loss-of-function or null alleles. The argument for nullity is weakened somewhat by the fact that none of the *fog-2* alleles is amber (see MATERIALS AND METHODS) and no deficiencies in the region have been isolated. However, since eleven *fog-2* mutants were obtained by either complementation screens or complementation suppression, alleles that have a sterile or lethal phenotype should have been recovered. Since no sterile or lethal alleles of *fog-2* have been isolated, the fully penetrant Fog phenotype of the 12 strong alleles probably represents the effect of the complete absence of the *fog-2*(+) activity. The strong allele *q71* was used for all double mutant constructions and is designated as the canonical putative *fog-2* null allele. In a number of cases, other strong alleles (*q86*, *q123*, or *q247*) were also employed and equivalent results were obtained (see below and see MATERIALS AND METHODS).

The apparent null phenotype of *fog-2*, in which *XX* animals are females while *XO* animals are normal males, indicates that the *fog-2* product is necessary for specification of the male germ cell fate in an otherwise normal *XX* female soma. Also, *fog-2*(+) masculinizing activity is restricted to a single tissue, the germ line. The fact that *fog-2* mutants have no effect on *XO* males indicates that the *fog-2* product is not necessary *per se* for specification of the male germ cell fate. This contrasts with *fem-1*, *2*, *3*, and *fog-1* which are each necessary for spermatogenesis in both *XX* and *XO* animals.

Interaction of *fog-2* with other feminizing mutations: To test whether *fog-2* mutants could enhance germ-line feminization in *XX* animals with other feminizing mutations, double heterozygotes were constructed and analyzed. Homozygotes of *fog-1*, *fem-1*, *fem-2*, and *fem-3* feminize the germ line of both *XX* and *XO* animals while *fem-1*, *2*, and *3* also feminize the *XO* soma (Table 1). (Interactions with gain-of-function feminizing mutations in *tra-2* are discussed below; interactions with gain-of-function mutations in *tra-1* are beyond the scope of this paper.)

TABLE 4

Interaction of *fog-2* with *fog-1*, *fem-1(lf)*, *fem-2(lf)* or *fem-3(lf)* alleles in *XX* animals

Maternal (*m*) and zygotic (*z*) genotype of other feminizing loci[a]		Maternal (*m*) and zygotic (*z*) genotype of *fog-2*[b]	% Female
		m(+/+), *z*(−/+)	0 ($n > 250$)
		m(−/−), *z*(−/+)	0 ($n > 250$)
		m(−/+), *z*(−/−)	100 ($n > 250$)
fog-1	*m*(−/−), *z*(−/+)	*m*(+/+), *z*(+/+)	0 ($n = 87$)
	m(−/−), *z*(−/+)	*m*(+/+), *z*(−/+)	0 ($n = 151$)
fem-1	*m*(−/−), *z*(−/+)	*m*(+/+), *z*(+/+)	0 ($n = 128$)
	m(−/−), *z*(−/+)	*m*(+/+), *z*(−/+)	1 ($n = 148$)
	m(+/+), *z*(−/+)	*m*(+/+), *z*(+/+)	0 ($n = 253$)
	m(+/+), *z*(−/+)	*m*(−/−), *z*(−/+)	0.5 ($n = 206$)
fem-2	*m*(−/−), *z*(−/+)	*m*(+/+), *z*(+/+)	0 ($n = 144$)
	m(−/−), *z*(−/+)	*m*(+/+), *z*(−/+)	0 ($n = 102$)
fem-3	*m*(−/−), *z*(−/+)	*m*(+/+), *z*(+/+)	15 ($n = 78$)[c]
	m(−/−), *z*(−/+)	*m*(+/+), *z*(−/+)	40 ($n = 144$)[c,d]
	m(−/+), *z*(−/+)	*m*(+/+), *z*(+/+)	10 ($n = 112$)[e]
	m(−/+), *z*(−/+)	*m*(+/+), *z*(−/+)	30 ($n = 119$)[e,d]
	m(+/+), *z*(−/+)	*m*(+/+), *z*(+/+)	4 ($n = 92$)[f]
	m(+/+), *z*(−/+)	*m*(−/−), *z*(−/+)	23 ($n = 156$)[f]

[a] Alleles used are: *fog-1(q187)*, *fem-1(e1991)*, *fem-2(e2105)* and *fem-3(e1996)*.
[b] *fog-2(q71)*.
[c,d,e,f] Results indicated with the same letter are significantly different from each other [$P < 0.05$; Z-test (FREUND 1973)].

No *fog-1*/+, *fem-1*/+, or *fem-2*/+ heterozygotes are female (Table 4). In combination with *fog-2*/+, no females were observed for either *fog-1*/+ or *fem-2*/+ (Table 4). For *fem-1*/+; *fog-2*/+, a very low frequency of females was found, but it is unclear if the single events are significant. Of the three *fem* gene products, *fem-2* is thought to be required in the smallest amount for normal male development, while *fem-3* is required in the largest amount (HODGKIN 1986). The failure to observe a feminizing effect in the *fog-2*/+; *fem-1*/+ or *fem-2*/+ double heterozygotes may be a consequence of the small amount of *fem-1* and *2* products necessary for normal spermatogenesis.

Heterozygotes for *fem-3(lf)* show *XX* germ-line feminization that is dependent on the maternal genotype of *fem-3* (HODGKIN 1986; BARTON, SCHEDL and KIMBLE 1987). *fem-3*/+ animals were 4%, 10%, or 15% female depending on whether the mother was *fem-3*(+/+), *fem-3*/+, or *fem-3*/*fem-3*, respectively (Table 4). This is a consequence of both a haplo-

insufficiency for *fem-3* in *fem-3/+ XX* animals and a maternal absence effect in *fem-3/+* progeny from *fem-3* mutant mothers. An enhancement of the *XX* germ-line feminization phenotype occurred when *fem-3/+* was in combination with *fog-2/+*. For a given maternal genotype of *fem-3*, the percentage of females was significantly increased when animals were also *fog-2/+* (Table 4). Further, the difference in frequency of *fem-3/+; fog-2/+* females was significantly different when mothers were either *m(−/−)* or *m(−/+)* for *fem-3*. These results indicate that reduced amount of zygotic *fog-2* activity (as *fog-2/+*) in combination with a reduced amount of zygotic and/or maternal *fem-3* activity caused a significant decrease in the number of *XX* animals that were able to initiate spermatogenesis and become self-fertile. This is consistent with *fem-3* being the *fem* gene required in the largest amount for normal male development.

Interactions between *fog-2* and *her-1(lf)*: Loss-of-function alleles of *her-1* transform *XO* males into self-fertile hermaphrodites—the soma is female and the germ line produces sperm and then oocytes (HODGKIN 1980; Table 1). The *her-1(lf) fog-2* double mutant was constructed to ask if the spermatogenesis that occurs in a *her-1 XO* mutant hermaphrodite is dependent on *fog-2* activity.

Self-fertile strains were constructed that were heterozygous for *fog-2(q71)* and homozygous for the putative *her-1* null allele *e1520*, *her-1 him-5 dpy-21 fog-2/her-1 him-5 dpy-21* + (see MATERIALS AND METHODS). Such heterozygous strains segregated *her-1 him-5 dpy-21 fog-2* homozygotes that were either *XX* or *XO* as a consequence of the *him-5* mutation (HODGKIN, HORVITZ and BRENNER 1979). The *dpy-21* mutation permits *XX* (phenotypically Dpy) and *XO* (phenotypically non-Dpy) animals to be distinguished independently of sexual phenotype (HODGKIN 1980, 1983, 1986). The heterozygous strains segregated both Dpy(*XX*) females and non-Dpy(*XO*) females. Thus *her-1* transforms *XO* males into *XO* hermaphrodites and, in combination with *fog-2*, the *XO* hermaphrodites are transformed into females. This indicates that the spermatogenesis observed in a *her-1 XO* hermaphrodite is dependent on *fog-2* activity. Further, although *fog-2* mutants exhibit no phenotypic effect on *XO* animals that have a male soma, they do have an effect on *XO* animals that lack *her-1* activity and have a female soma and hermaphrodite germ line.

The additive effect of phenotypes in *XO* animals for the *her-1 fog-2* double mutant was also demonstrated with the temperature sensitive *her-1* allele, *e1561*. At the permissive temperature (15°) *her-1(e1561) XO* animals are male, while at the restrictive temperature (25°) they are self-fertile hermaphrodites (HODGKIN 1984). A *her-1(e1561) fog-2(q71)* male/female strain, propagating biparentally as *XX* females and *XO* males was obtained at 15°. When mated adults were shifted to 25° and progeny examined, both *XX* and *XO* females were obtained. A proportion of the females were shown to be *XO* by crosses using males marked with the *X*-linked gene *lon-2* and observing Lon male cross progeny.

Interactions between *fog-2* and *tra-2(lf)*: Loss-of-function mutations in *tra-2* transform *XX* animals into incomplete males. Homozygotes for putative *tra-2* null alleles (*e1095* or *e1425* amber) are similar to *XO* males, except that tail structures are incompletely masculinized and there is no mating behavior (HODGKIN and BRENNER 1977; Table 1). However, the germ line is identical to that of a wild-type male.

Examination of *tra-2(lf); fog-2* homozygous double mutants showed they were phenotypically indistinguishable from *tra-2(lf)* alone for both the germ line and soma. This was observed for all the different combinations of alleles tested: *tra-2(e1425)* with *fog-2(q71, q124* or *q247)* and *tra-2(e1095)* with *fog-2(q71 or q247)* (see MATERIALS AND METHODS for details). Self-fertile strains, *tra-2(e1095)/+; fog-2* (see below), were used to test the possibility that spermatogenesis in the *tra-2(lf); fog-2* double had been a consequence of rescue by *fog-2(+)* present in the mother. It was found that *tra-2(e1095); fog-2(q71* or *q247)* [*m(−/−), z(−/−)*] animals were also indistinguishable from *tra-2(lf)* alone. Therefore, *tra-2(lf)* is epistatic to *fog-2(lf)*. This indicates that the spermatogenesis observed in the *tra-2(lf) XX* mutants is not dependent on *fog-2* activity.

Animals heterozygous for *tra-2(lf)* and homozygous for *fog-2* are partially suppressed. When *tra-2(e1095)/+; fog-2* animals are examined, 26% (*q247*) or 28% (*q71*) were self-fertile (see MATERIALS AND METHODS). The same result was obtained if a deficiency for the region around *tra-2*, *mnDf30* (SIGURDSON, SPANIER and HERMAN 1984) was used; 33% of *mnDf30/+; fog-2(q71)* homozygotes were self-fertile. This suppression of the Fog phenotype suggests that *fog-2* mutants fail to undergo spermatogenesis as a consequence of *tra-2(+)* feminizing activity. When *tra-2(+)* activity is reduced in the *tra-2(lf)/+; fog-2* double mutant some spermatogenesis can occur in the absence of *fog-2* activity. Note that in contrast to *tra-2(lf); fog-2* homozygotes which have a masculinized soma (see above), in *tra-2(lf)/+; fog-2* mutants, spermatogenesis occurs in animals with an essentially female soma. The suppression of *fog-2* by *tra-2(lf)/+* shown here, and the enhancement of *fem-3(gf)* by *tra-2(lf)/+* described by BARTON, SCHEDL and KIMBLE (1987) reveal a haplo-insufficiency for *tra-2* in the germ line. A haplo-insufficiency for some somatic characteristics has also been demonstrated for *tra-2* (TRENT, TSUNG and HORVITZ 1983; DONIACH 1986a).

Interaction of *fog-2* with *tra-3*: An incomplete male phenotype is observed for *XX tra-3* homozygotes

(using the putative null allele *e1107*) when they are derived from a homozygous mutant mother (HODGKIN and BRENNER 1977; Table 1). *tra-3(e1107) m(−/−), z(−/−) XX* pseudomales have an incompletely masculinized tail, continue to synthesize yolk, and, in general (79%), have a male somatic gonad. The germ line is also incompletely masculinized; 37% of animals first make sperm and then oocytes (n = 51, at 25°). Essentially the same germ-line and somatic phenotypes were obtained when *tra-3(e1107) m(−/−), z(−/−)* was homozygous for either *fog-2(q71* or *q247)* (see MATERIALS AND METHODS). No additional feminization of either the soma or germ line was observed, (41%, n = 59 make sperm and then oocytes). Thus, *tra-3* is epistatic to *fog-2*.

Both *tra-2* and *tra-3* are epistatic to *fog-2*. In contrast, *fem-1*, *2*, and *3* are epistatic to *tra-2* and *tra-3* in both germ line and soma (DONIACH and HODGKIN 1984; HODGKIN 1986), and *fog-1* is epistatic to *tra-2* and *tra-3* in the germ line (DONIACH 1986b; M. K. BARTON, personal communication). These results suggest that the role of *fog-2* in promoting the male germ cell fate is fundamentally different from that of *fem-1*, *2*, *3*, and *fog-1*.

***fog-2* suppresses *fem-3(gf)*:** Gain-of-function mutations in *fem-3* result in complete masculinization of the germ line of *XX* animals while the female soma is unaffected (BARTON, SCHEDL and KIMBLE 1987). Thus germ cells that would have normally undergone oogenesis instead undergo spermatogenesis. The three *fem-3(gf)* alleles used in this study have the following ranking of mutant strength: *q20gf* < *q96gf* < *q95gf*. Note that the *fem-3(gf)* allele *q20gf* (and probably *q96gf* and *q95gf*) does not simply increase the amount of *fem-3* but rather causes unregulated or inappropriate activity (BARTON, SCHEDL and KIMBLE 1987).

When *fem-3(gf); fog-2 XX* double mutants were constructed, the Mog and Fog phenotypes were both suppressed resulting in self-fertility at 25° (Table 5). The penetrance of the suppression of *fem-3(q20gf)* by *fog-2* at restrictive temperature (25°) was complete (100% self-fertile) for the seven *fog-2* alleles tested. Four of the alleles, *fog-2(q154, q124, q226,* and *q71)*, were isolated independent of their suppression of *fem-3(gf)* while three, *fog-2(q113, q247,* and *q249)*, were isolated in selections for *fem-3(gf)* suppressors. At 15°, the permissive temperature for *fem-3(q20gf)*, the double mutants remain fully self-fertile. A similar result was obtained with doubles of *fem-3(q20gf)* and *fem-1(hc17ts)* or *fem-2(b245ts)* (BARTON, SCHEDL and KIMBLE 1987). Two alleles, *fog-2(q71* and *q154)*, were further tested for suppression in one copy and found to be weak dominant suppressors of *fem-3(q20gf)*. Interestingly, the leaky allele, *q154*, suppresses as well in one or two copies as the putative null allele *q71* (Table 5). The stronger *fem-3(gf)* allele, (*q96gf*) was also completely suppressed by *fog-2(q71)*. For the strongest *fem-3(gf)* allele, *q95*, *fog-2(q71)* did not suppress at 25°, but at 15° *q71* partially suppressed *q95*, significantly increasing its self-fertility (Table 5).

TABLE 5

Interaction of *fog-2* with *fem-3(gf)* in *XX* animals[a]

Allele		% Self-fertile[b]	
fem-3(gf)	*fog-2*	15°	25°
q20gf[c]	+	100 (n > 200)	0 (n > 200)
q20gf	*q154*/+	100 (n = 60)	7 (n = 213)
q20gf	*q154*	100 (n = 75)	100 (n = 103)
q20gf	*q124*	100 (n = 66)	100 (n = 72)
q20gf	*q226*	100 (n = 72)	100 (n = 96)
q20gf	*q113*	100 (n = 72)	100 (n = 88)
q20gf	*q247*	100 (n = 72)	100 (n = 72)
q20gf	*q249*	100 (n = 72)	100 (n = 72)
q20gf	*q71*/+	100 (n = 50)	5 (n = 210)
q20gf	*q71*	100 (n > 200)	100 (n > 200)
q96gf	+	100 (n > 200)	0 (n > 200)
q96gf	*q71*	100 (n = 98)	100 (n = 125)
q95gf	+	28 (n = 72)	0 (n > 200)
q95gf	*q71*	83 (n = 72)	0 (n > 200)

[a] From stocks homozygous for both *fem-3(gf)* and *fog-2*. See MATERIALS AND METHODS for details.
[b] The remaining animals that were not self-fertile were Mog.
[c] Data from BARTON, SCHEDL and KIMBLE (1987).

An unmarked *fem-3(q20gf); fog-2(q71)* strain was constructed and found to be essentially identical to wild type except for an increased brood size. Brood sizes for *fem-3(q20gf); fog-2(q71)* were 415(±24) at 15°, 487(±39) at 20°, and 299(±41) at 24° compared to N2 which were 317(±32) at 15°, 355(±37) at 20°, and 247(±55) at 24°. Males were functionally and morphologically normal. The normal male phenotype of *fem-3(gf); fog-2* contrasts to that of *fem-3(q20gf)* with *fem-1(hc17)* or *fem-2(b245)* in which *XO* animals are still mutant due to lack of suppression of the *XO* somatic feminizing effects of *fem-1* and *fem-2* alleles (BARTON, SCHEDL and KIMBLE 1987).

Thus, an essentially normal self-fertile strain is regenerated in the *fem-3(q20gf* or *q96gf); fog-2* doubles by mutual suppression of the Fog and Mog phenotypes. The continued spermatogenesis observed in adult *fem-3(gf)* mutants must depend on *fog-2* germ-line masculinizing activity. In the absence of *fog-2* activity, adult spermatogenesis stops and oogenesis ensues. However, spermatogenesis (in late L4 and as a young adult) and the switch to oogenesis occurs in the absence of *fog-2* activity in the *fem-3(q20gf)* and *q96gf)* mutants. Therefore, *fem-3(gf)* obviates the requirement for *fog-2* activity to initiate hermaphrodite spermatogenesis and switch to oogenesis.

***tra-2(gf)* mutants:** Most gain-of-function alleles of *tra-2* have a dominant feminizing effect on the *XX* germ line, have essentially no effect on the *XX* soma, and have essentially no effect on the germ line or

soma of *XO* males (Table 1; DONIACH 1986a; this paper). This phenotype contrasts with that of *tra-2(lf)* alleles—masculinization of both germ line and soma of *XX* animals with no effect on *XO* males (HODGKIN and BRENNER 1977). The gain-of-function phenotype of *tra-2(gf)* alleles in the germ line of *XX* animals may be the result of increased, unregulated, or inappropriate *tra-2* activity (DONIACH 1986a).

Two *tra-2(gf)* mutants were isolated in the "screen for mutations that fail to complement *fog-2*" (see MATERIALS AND METHODS). The phenotype of *tra-2(q122gf)* is strong: *XX* animals are female in both the germ line and the soma as homozygotes, as heterozygotes, and in *trans* to a putative *tra-2* null allele. *q122gf XO* animals are generally unaffected, although old males show some oogenesis and yolk synthesis. The phenotype of *tra-2(q179gf)* is weaker: *XX* animals show semidominant germ-line feminization and recessive somatic masculinization (truncated tail and Egl). The somatic masculinization increases in *trans* to a putative *tra-2* null allele (see MATERIALS AND METHODS). *tra-2(q179gf) XO* males are unaffected. Weak *tra-2(gf)* alleles like *q179gf*, which are gain-of-function in the germ line but partial loss-of-function in the soma, are discussed in more detail by DONIACH 1986a).

No maternal absence effect, such as that of *fem-3(lf)* (HODGKIN 1986; BARTON, SCHEDL and KIMBLE 1987), was observed for *tra-2(gf)* alleles. Neither heterozygous *tra-2(gf)/+ XO* males derived from homozygous *tra-2(gf)* mothers nor *+/+ XX* hermaphrodites derived from *tra-2(gf)/+* mothers were feminized (data not shown; DONIACH 1986a).

Interaction of *tra-2(gf)* with *fem-3(gf)*: Examination of *tra-2(gf)*; *fem-3(gf) XX* homozygous double mutants reveals mutual suppression of germline feminizing and masculinizing phenotypes resulting in self-fertility at both 15° and 25° (Table 6; BARTON, SCHEDL and KIMBLE 1987). When *fem-3(q20gf)* was used, the degree of suppression depended on the *tra-2(gf)* allele. One group of alleles, *q179gf* and *e1941gf*, almost completely suppressed *fem-3(q20gf)* such that while most animals were self-fertile, some remained Mog (Table 6). Another group of *tra-2(gf)* alleles, *q103gf*, *q122gf*, *q244gf*, and *e2046gf*, completely suppressed *fem-3(q20gf)* such that all animals were self-fertile. A final group of *tra-2(gf)* alleles, *q101gf* and *e2020gf*, not only suppressed *fem-3(q20gf)* but were partially epistatic to it since some animals were females (Table 6).

When the strongest *fem-3(gf)* allele, *q95gf*, was used, it was fully epistatic to *tra-2(q122gf)* at 25° (Table 6). However, *q122gf* does increase the self-fertility of *q95gf* at 15° (data not shown). By contrast, *tra-2(e2020gf)* was able to partially suppress *q95gf* at 25° (Table 6). *e2020gf* was the strongest of the *tra-2(gf)* alleles with respect to suppression of *fem-3(q20gf* and *q95gf)*. This allele also shows the strongest feminizing effect on *XO* males (DONIACH 1986a).

TABLE 6

Interaction of *tra-2(gf)* with *fem-3(gf)* in *XX* animals[a]

Allele		Phenotype at 25°		
fem-3(gf)	*tra-2(gf)*	% Female	% Self-fertile	% Mog[b]
q20gf	*q179gf*	0	78	22 (n = 90)
q20gf	*e1941gf*[c]	0	91	9 (n = 85)
q20gf	*q103gf*[d]	0	100	0 (n = 92)
q20gf	*q122gf*[d]	0	100	0 (n > 200)
q20gf	*q244gf*	0	100	0 (n = 72)
q20gf	*e2046gf*[c]	0	100	0 (n = 72)
q20gf	*q101gf*	4	94	0 (n = 121)[e]
q20gf	*e2020gf*[c]	61	39	0 (n = 122)
q96gf	*q122gf*	0	100	0 (n = 81)
q95gf	*q122gf*[d]	0	0	100 (n > 200)
q95gf	*e2020gf*	0	71	29 (n = 56)

[a] From stocks homozygous for both *fem-3(gf)* and *tra-2(gf)* grown at 25°.

[b] Mog—masculinization of the germ line, see MATERIALS AND METHODS for a description of the phenotype and its scoring.

[c] See DONIACH (1986a) for a description of these *tra-2(gf)* alleles.

[d] Data from BARTON, SCHEDL and KIMBLE (1987).

[e] An additional 2% of animals produced no self-progeny. However, they were not Mog and contained sperm and oocytes of normal morphology in the normal positions.

Interaction of *fog-2* with *tra-2(gf)* (*XX*): There are two simple mechanisms by which *fog-2* might promote hermaphrodite spermatogenesis: *fog-2* could act as a negative regulator of *tra-2* and *tra-3*, or alternatively, *fog-2* could act independently and positively on the *fem* genes and *fog-1*, rendering them insensitive to the negative regulatory action of *tra-2* and *3* (see DISCUSSION; Figure 3, models 1 and 2). The loss-of-function phenotype of *fog-2* is essentially identical to the gain-of-function phenotype of *tra-2*. This suggests that the nature of the gain-of-function lesions of *tra-2(gf)* alleles can be used to distinguish between the two possible mechanisms of *fog-2* action: negative regulation of *tra-2*, or positive regulation of the *fem*s and *fog-1*. The nature of *tra-2(gf)* alleles can be tested by removing *fog-2* activity. If *fog-2* negatively regulates *tra-2*, one might expect that the defect in some *tra-2(gf)* alleles is insensitivity to this regulation. The phenotype of such *fog-2* insensitive *tra-2(gf)* alleles should not be affected (enhanced) by removal of *fog-2* activity. Additionally, the phenotype of other *tra-2(gf)* alleles may be affected (enhanced) by removal of *fog-2* activity even though *fog-2* negatively regulates *tra-2*. The defect in such *tra-2(gf)* alleles may be hyperactivity or an inappropriate interaction with the downstream *fem* genes and *fog-1* and yet they remain sensitive to *fog-2* activity. However, if *fog-2* and *tra-2* act independently, then the phenotype of all *tra-2(gf)* alleles should be affected (enhanced) by removal of *fog-2* activity. In this case, the defect in all the *tra-2(gf)* alleles may be hyperactivity or inappropriate

interaction with the downstream *fem* genes and *fog-1*. As described in detail below, the seven *tra-2(gf)* alleles tested are all sensitive to *fog-2* activity: all showed an enhanced *XX* germ-line feminization phenotype when *fog-2* was removed. This does not allow one to distinguish between either model (see above, and see DISCUSSION), since both models predict a class of *tra-2(gf)* alleles that are sensitive to *fog-2* activity.

The interaction of *tra-2(q179gf)* and *fog-2(q71)* was examined by constructing a homozygous male/female strain. The *tra-2(q179gf)* single mutant is incompletely penetrant; 73% of *XX* homozygotes are female while the remaining 27% are self-fertile (see MATERIALS AND METHODS). Enhanced germ-line feminization was observed when *tra-2(q179gf); fog-2(q71)* was examined as 100% of *XX* animals were female ($n > 250$). This indicates that the incomplete penetrance (residual spermatogenesis) of *q179(gf)* is dependent on *fog-2* activity.

For the remaining *tra-2(gf)* alleles which have the same fully penetrant phenotype as *fog-2*, the interaction was tested in a *fem-3(gf)* background. In this background, all *fem-3(q20gf); fog-2* double mutants are self-fertile (Table 5), and most *tra-2(gf); fem-3(q20gf)* double mutants are self-fertile (Table 6). One then tests whether *tra-2(gf); fem-3(q20gf)* becomes further feminized (female) when *fog-2* activity is removed in the triple mutant. Since the *tra-2(gf); fem-3(gf); fog-2* triple mutants are, in most cases, female (see below), the phenotype could not be assessed in animals from triple homozygous mutant strains. Instead, the germ-line phenotypes of *fem-3(gf)* Unc Dpy homozygotes segregating from a *unc-24 fem-3(gf) dpy-20/+; tra-2(gf)/+; fog-2/+* hermaphrodite were scored (see MATERIALS AND METHODS). Among the segregants, one-sixteenth (6.25%) are the triple mutant homozygotes. The phenotypes of these *fem-3(gf)* Unc Dpy homozygous segregants are shown in Table 7. Note that since *tra-2(+)* and *fog-2(+)* are present in the mother, progeny with a Mog phenotype will be observed.

The five *tra-2(gf)* alleles (*q103gf, q122gf, q244gf, e2046gf,* and *q101gf*) showed enhanced feminization when *fog-2* activity was removed as females were observed among the segregating triple mutants (Table 7). For the *tra-2(gf)* alleles (*q122gf, e2046gf, q103gf* and *q244gf*), doubles with *fem-3(q20gf)* were 100% self-fertile (Table 6). In the segregating triple mutants for these four *tra-2gf* alleles, more than one-sixteenth of the animals were female (Table 7). Since about three-sixteenths of the *fem-3(gf)* segregants were female, not only were the *tra-2(gf); fog-2* homozygotes female, but some animals homozygous for either *fog-2* or *tra-2gf* and heterozygous for the other must also have been female. The *tra-2(q101gf); fem-3(q20gf)* double has a low percentage of females (4%, Table 6). Yet, among the segregants of *fem-3(q20gf)/+; tra-2(q101gf)/+; fog-2/+*, 22% of the *fem-3(q20gf)* segregants were female. Enhanced feminization was even observed in triple mutant segregants when *fem-3(q95gf)* was used. *fem-3(q95gf)* is epistatic to both *tra-2(q122gf)* (Table 6) and *fog-2(q71)* (Table 5). The enhanced feminization phenotype indicates that these five *tra-2(gf)* alleles are sensitive to *fog-2* and that the spermatogenesis observed in *tra-2(gf); fem-3(q20gf)* double mutants (Table 6) is a consequence of *fog-2(+)* activity. In the absence of *fog-2* activity in the triple mutant, spermatogenesis was not observed. When triple mutant female segregants were crossed with N2 males, all *XO* male progeny were wild type. This is in contrast to the maternal absence effect observed in *XO* progeny of *fem-3(lf)* females. Thus, even though *tra-2(gf); fog-2* mutations abolish *fem-3(gf)* activity (animals are female not Mog), this is not equivalent to the absence of *fem-3* activity in a *fem-3(lf)* female.

TABLE 7

Interaction of *fog-2* with *tra-2(gf)* in a *fem-3(gf)* background

Allele			Phenotype of segregants[a]		
fog-2	*fem-3(gf)*	*tra-2(gf)*	% Female	% Self-fertile	% Mog
q71	*q20gf*	*q179gf*	0	71	29 (n = 166)
q71	*q20gf*	*e1941gf*	0	68	32 (n = 196)
q71	*q20gf*	*q103gf*	16	59	25 (n = 104)
q71	*q20gf*	*q122gf*	17	62	21 (n = 99)
q71	*q20gf*	*q244gf*	20	49	31 (n = 104)
q71	*q20gf*	*e2046gf*	16	63	21 (n = 117)
q71	*q20gf*	*q101gf*	22	52	26 (n = 98)
q71	*q95gf*	*q122gf*	14	1	84 (n = 125)

[a] All segregants are *XX* animals homogyzous for *unc-24 fem-3(gf) dpy-20* and are homozygous, heterozygous or wild-type for *tra-2(gf)* and for *fog-2*. All at 25°, see text for details.

Females were not observed when the *tra-2(gf); fem-3(q20gf); fog-2(q71)* triple mutants were assessed using *tra-2(q179gf* or *e1941gf)* (Table 7). The phenotype of *tra-2(e1941gf)* was shown to be sensitive to *fog-2* activity by examining a strain homozygous for *tra-2(e1941gf); fem-3(q20gf); fog-2(q71)* (see MATERIALS AND METHODS). The triple mutant strain was 100% self-fertile ($n > 212$). This represents an enhanced feminization compared to the *tra-2(e1941gf); fem-3(q20gf)* double mutant which was 91% self-fertile and 9% Mog (Table 6).

Interaction of *fog-2* with *tra-2(gf)* (*XO*): In an attempt to reveal an effect of *fog-2* on the *XO* male germline (or somatic) phenotype, male/female strains were constructed that were homozygous for both *fog-2(q71)* and *tra-2(q122gf)* or *tra-2(q179gf)*. *XO* males were examined in the course of the construction and in the final strains. Double mutant males were found to be indistinguishable from *q122gf* or *q179gf* males alone. Thus, in contrast to the additive effect observed in *XX fog-2(q71); tra-2(q122gf)* animals in a

fem-3(gf) background, or *fog-2(q71)*; *tra-2(q179gf)* alone (see above), there is not an additive effect for *tra-2(gf)* and *fog-2* in the germ line of *XO* males.

DISCUSSION

Sex determination in the hermaphrodite germ line: The *C. elegans* hermaphrodite gonad makes sperm first and then oocytes. This raises two major questions about the control of sex determination in the hermaphrodite germ line. How is male germ-line development (spermatogenesis) initiated within a female soma? and how is the switch from male to female germ-line development (oogenesis) achieved? Sex determination in the hermaphrodite germ line is not specified during early development (BARTON, SCHEDL and KIMBLE 1987). Throughout larval and adult life, a mitotic stem cell population generates meiotic germ cells for the continued production of gametes (HIRSH, OPPENHEIM, and KLASS 1976; KIMBLE and WHITE 1981). By manipulating temperature and thereby the state of a temperature sensitive gain-of-function allele of *fem-3*, either spermatogenesis or oogenesis can be induced after the L4 stage, independent of the type of gametogenesis that occurred previously (BARTON, SCHEDL and KIMBLE 1987). Therefore, the choice between spermatogenesis and oogenesis appears to be made continuously in a population of uncommitted germ cells late in development.

***fog-2* regulates the sex determining genes to initiate male development in a female soma:** The results of this study indicate that the *fog-2* locus is normally required for initiation of spermatogenesis in hermaphrodites. *XX* animals homozygous for a loss-of-function allele of *fog-2* are transformed from self-fertile hermaphrodites to females, whereas *XO fog-2(lf)* mutant males are indistinguishable from wild-type males. Therefore, *fog-2* is required for spermatogenesis in *XX* hermaphrodites but not in *XO* males. Since the sexual fate of the germ line but not the soma is transformed in *fog-2* mutants, *fog-2* is a germ-line-specific sex determination gene. Further, *XO* mutant hermaphrodites, homozygous for *her-1*, are transformed into females in the absence of *fog-2* (a *her-1 fog-2* double mutant). Therefore, *fog-2* has a hermaphrodite-specific mutant phenotype, rather than an *XX* specific one. Thus, *fog-2* does not appear to be controlled by the ratio of *X* chromosomes to autosomes. The germline- and hermaphrodite-specific properties of the *fog-2* mutant phenotype are expected for a gene that regulates the onset of spermatogenesis in hermaphrodites, but does not specify spermatogenesis *per se*.

In contrast to *fog-2*, the *fem* genes, *fem-1* (DONIACH and HODGKIN 1984), *fem-2* and *fem-3* (HODGKIN 1986) and *fog-1* (DONIACH 1986b; M. K. BARTON, personal communication) promote spermatogenesis in both *XX* and *XO* animals, whether hermaphrodite or male. Also in contrast to *fog-2*, the *fem* genes and *fog-1* are essential for specification of spermatogenesis. No sperm are made in animals that lack any of the *fem* genes or *fog-1* in any genetic background tested to date. Thus, spermatogenesis does not occur in double mutants with *fem-1(lf)*, *fem-2(lf)*, *fem-3(lf)*, or *fog-1* plus any of the *tra* genes (DONIACH and HODGKIN 1984; HODGKIN 1986; DONIACH 1986b; M. K. BARTON, personal communication) or plus any of several other germline masculinizing mutations (T. SCHEDL and M. K. BARTON, unpublished results). The essential nature of the *fem* genes and *fog-1* in the germ line places their regulatory activity most proximate to the genes that direct male germ cell differentiation.

fog-2 appears to be the regulatory gene that activates the *fem* genes and *fog-1* in the hermaphrodite germ line to achieve spermatogenesis. Positive regulation of *fem-3* by *fog-2* is indicated by the phenotypes of double mutants with *fog-2* and *fem-3*. Thus, *fog-2(lf)* enhances *fem-3(lf)*, but it suppresses *fem-3(gf)*. How *fem-1*, *fem-2*, *fem-3*, and *fog-1* interact to direct spermatogenesis is unknown, and therefore they will be considered here as a group. The regulatory action of *fog-2*, which may be indirect or direct (see below, Figure 3), needs only to affect the activity of one of these genes or gene products. Since *fog-2* mutants show no maternal effect, regulation by *fog-2* is likely to be zygotic.

The activity of *fog-2* is opposite to that of *tra-2* in regulating the onset of hermaphrodite spermatogenesis. Not only do loss-of-function alleles of *fog-2* and *tra-2* have opposite effects on the sexual phenotype of the germ line, but also *fog-2(lf)* and *tra-2(gf)* have equivalent effects on that phenotype. The phenotypes of both *fog-2(lf)* and *tra-2(gf)* are similarly restricted to hermaphrodites (either *XX* or *XO*), and both *fog-2(lf)* and *tra-2(gf)* suppress *fem-3(gf)* to generate self-fertile hermaphrodites.

Figure 3 presents two models by which *fog-2* may promote hermaphrodite spermatogenesis. In model 1, regulation is indirect: *fog-2* promotes spermatogenesis by negatively regulating *tra--2* and *tra-3*. The role of *tra-2* and *tra-3* as negative regulators of the *fem* genes and *fog-1* is well documented (DONIACH and HODGKIN 1984; HODGKIN 1986; DONIACH 1986b; M. K. BARTON, personal communication). Further, based on the phenotype of gain-of-function alleles of *tra-2*, DONIACH (1986a) has suggested that the activity of *tra-2* may be modulated in the hermaphrodite germ line to achieve spermatogenesis. In model 2, regulation is direct: *fog-2* acts positively on the *fem* genes and *fog-1*, while independently, *tra-2* and *tra-3* act negatively on the *fem* genes and *fog-1*. In both models, oogenesis is represented as a default state that takes place in the absence of spermatogenesis,

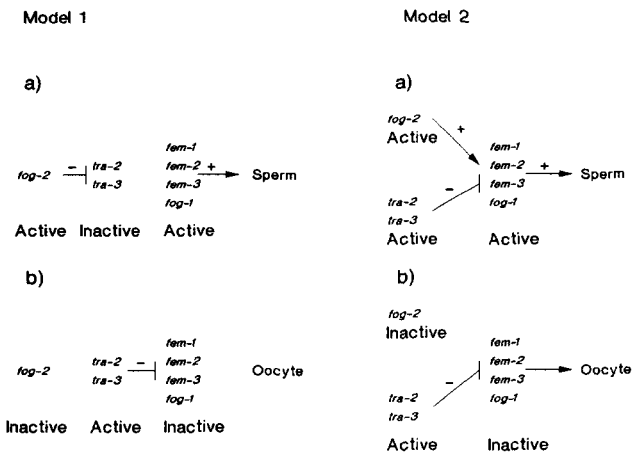


FIGURE 3.—Two models for the genetic control of hermaphrodite germ-line sex determination in *C. elegans*. The interactions between genes in the two models are either negative (——|) or positive (→) and determine whether a gene (or its product) is active or inactive. For both models, the hierarchy of regulatory interactions exists in two sequential states which specify the hermaphrodite germ-line pattern of first sperm and then oocytes. In the first state (a), the three *fem* genes and *fog-1* are active, and uncommitted germ cells develop as sperm. In the second state (b), the three *fem* genes and *fog-1* are inactive, and uncommitted germ cells develop as oocytes. Models 1 and 2 differ primarily in the manner in which *fog-2* acts as a positive regulator of spermatogenesis [part (a)]. In model 1, positive regulation of spermatogenesis by *fog-2* is indirect, by acting as a negative regulator of *tra-2* and *3*. In model 2, the regulation is direct, *fog-2* acts positively on the *fem* genes and *fog-1* rendering them insensitive to the independent negative action of *tra-2* and *tra-3*. Maternally contributed *fem*(+) would, in both models, contribute to the net amount of masculinizing activity to be regulated. In part (b) of both models, oogenesis represents a default state that takes place in the absence of spermatogenesis; oogenesis occurs when the *fem* genes and *fog-1* are inactivated as a result of *tra-2* and *tra-3* activity (DONIACH and HODGKIN 1984; HODGKIN 1986; DONIACH 1986b; M. K. BARTON, personal communication). This is presumably a consequence of *fog-2* becoming inactive; however, the mechanism by which the switch from spermatogenesis to oogenesis is effected is unknown (see text). It should be recognized that the interactions shown in models 1 and 2 are formalisms based on genetic experiments and do not imply molecular mechanisms of regulation. Further, since there is no evidence for physical interactions between these genes/products, it is possible that the regulation occurs through other unidentified elements. Finally, since gain-of-function mutations may change the mode of action of a gene/product in unpredictable ways, one must be cautious about conclusions reached using them.

because no genes essential to specification of oogenesis (as the *fem* genes and *fog-1* are essential to specification of spermatogenesis) have been identified to date.

The interactions between *fog-2* and either *tra-2* or *tra-3* have been investigated in double mutants. The phenotype of *tra-2(lf); fog-2(lf)* is the same as that of *tra-2(lf)* alone: sperm are made continuously in a masculinized soma. Similarly the phenotype of *tra-3(lf); fog-2(lf)* is essentially the same as that of *tra-3(lf)* alone: some sperm are made in a masculinized soma. Thus, in the absence of *tra-2* or *tra-3* activity, *fog-2* activity is not necessary to initiate spermatogenesis. One interpretation of the epistasis of *tra-2(lf)* and *tra-3(lf)* over *fog-2* is that *fog-2* acts to regulate *tra-2* and *tra-3* (model 1). In the absence of *tra-2* or *tra-3* activity, *fog-2* is no longer required for negative regulation. An alternative interpretation is that *fog-2* acts positively, rendering the *fem*'s and *fog-1* insensitive to the negative action of *tra-2* and *tra-3* (model 2). In the absence of *tra-2* or *tra-3*, counteraction by *fog-2* is no longer required.

Models 1 and 2 might have been distinguished by examination of interactions between *fog-2* and *tra-2(gf)* mutations. If *fog-2* negatively regulated *tra-2* (model 1), a class of *tra-2(gf)* alleles would be predicted that is insensitive to *fog-2* regulation. Such *tra-2(gf)* alleles would be unaffected by removal of *fog-2* activity. *tra-2(gf)* alleles were tested in an attempt to find a class that is unaffected by removal of *fog-2*. However, all seven *tra-2(gf)* alleles tested were sensitive to the state of *fog-2*.

There is currently no evidence that favors one of the two models presented in Figure 3 over the other. It might be argued from the complete epistasis of the mutant phenotypes of *tra-2* and *tra-3* over that of *fog-2* that model 1 is more likely. However, in support of model 2 is the apparent balancing of germ-line masculinizing and feminizing activities observed in double mutants. Thus, whereas *fog-2(lf)* and *tra-2(gf)* mutants are feminized and *fem-3(gf)* mutants are masculinized, most *fog-2; fem-3(gf)* and *tra-2(gf); fem-3(gf)* double mutants are self-fertile hermaphrodites. Further, *fog-2(lf)* is partially suppressed when *tra-2*(+) feminizing activity is reduced in *tra-2(lf)*/+ double mutants.

It is likely that the activity rather than the synthesis of *fem-3* is regulated by *tra-2* and *fog-2*. The presence or absence of *fem-3* in the hermaphrodite/female germ line can be deduced by examination of the sexual phenotype of her progeny. In particular, the *XO* progeny of mothers homozygous for *fem-3(lf)* are feminized; therefore, *fem-3* maternal product is required for normal sex determination of the embryo. However, no feminization has been observed among *XO* progeny of single mutant [*fog-2* or *tra-2(gf)*], double mutant [*tra-2(gf); fog-2*], or triple mutant [*tra-2(gf); fem-3(gf); fog-2*] mothers. Instead, the sexual phenotype of these progeny is normal. Therefore, functional maternal product of *fem-3* must be contributed to these embryos, even though it has not directed spermatogenesis in the mother's germ line.

***fog-2* and the switch from spermatogenesis to oogenesis:** The switch from spermatogenesis to oogenesis requires that the *fem* genes and *fog-1* no longer direct spermatogenesis. Regulation of *fem-3* appears to be key to the sperm/oocyte switch, because gain-of-function alleles of *fem-3* no longer make the switch (BARTON, SCHEDL and KIMBLE 1987). In addition, the

tra-2 gene appears to be required for the switch, because in the absence of *tra-2*, sperm are made continuously (HODGKIN and BRENNER 1977). Given the proposed role of *fog-2* in the onset of spermatogenesis, it is plausible that *fog-2* must be negatively regulated to switch to oogenesis. In both models 1 and 2 (Figure 3), inactive *fog-2* permits *tra-2* and *tra-3* activity to mediate the switch to oogenesis.

XX animals possessing *fem-3(gf)* make sperm continuously; no oocytes are seen. The double mutant, *fem-3(gf); fog-2*, makes sperm and then oocytes. Therefore, *fem-3(gf)* bypasses the need for *fog-2* to initiate spermatogenesis. Yet, in a *fem-3(gf)* genetic background, *fog-2(+)* prevents the switch to oogenesis. However, an inactive *fog-2* gene reinstates the switch. Perhaps *fog-2* remains "on" (*e.g.*, active, stable) in *fem-3(gf)* hermaphrodites, and it must become inactive to make oocytes. If so, then perhaps there is a regulatory feedback loop that normally inactivates *fog-2* and it is defective in *fem-3(gf)* animals.

Conclusions and speculations: Sex determination in the germ line, production of sperm or oocytes, depends on the state of activity of the *fem* genes and *fog-1*. In hermaphrodites, these genes must first be active to direct male germ-line development, and then inactive to permit female germ-line development. The choice between spermatogenesis and oogenesis occurs in uncommitted germ cells that are present in the germ line even in the adult. Regulation of the *fem* genes and *fog-1* depends on the activities of *tra-2*, *tra-3*, and *fog-2*. The *fog-2* locus, a previously undescribed sex determination gene, is required for production of sperm in hermaphrodites, whereas *tra-2* and *tra-3* regulate sex determination in both somatic and germline tissues. We propose that *fog-2*, a germline- and hermaphrodite-specific sex determination gene, acts to regulate the sex determination hierarchy between two alternate states: active *fog-2* leads to active *fem* genes and *fog-1* and spermatogenesis, and inactive *fog-2* leads to inactive *fem* genes and *fog-1* and oogenesis. The mechanism by which the switch from spermatogenesis to oogenesis occurs, however, remains unknown.

It has been argued by HODGKIN (1987), based on the prevalence of male/female reproduction among nematodes, that hermaphroditism in *C. elegans* is likely to be a secondary specialization of an ancestral female sex. Given that the somatic tissues of *C. elegans* are extremely similar to those of females of *C. remanei* (SUDHAUS 1974; T. SCHEDL, unpublished) and *Panagrellus* (STERNBERG and HORVITZ 1981; 1982), it is possible that the evolution of self-fertile hermaphrodites in nematodes involved changes in the germ line but not in the soma. The mechanism by which the sex determining genes could be regulated to achieve a hermaphrodite germ line would be simplified if the products of the masculinizing sex determination genes (*e.g.*, the *fem* genes) were already present in the ancestral female germ line to direct male development in the embryo. In *C. elegans*, at least, all three *fem* genes do have maternal effects (DONIACH and HODGKIN 1984; HODGKIN 1986). These maternal *fem* products in the female germ line must be negatively regulated to prevent them from directing spermatogenesis and thereby to permit oogenesis. Perhaps the *fog-2* gene evolved to alleviate the negative regulation of the *fem* products briefly to permit some spermatogenesis in the otherwise female germline. One plausible ancestor of the *fog-2* gene is *her-1*, a sex determination gene that negatively regulates *tra-2* and *tra-3* in response to the ratio of *X* chromosomes to autosomes.

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