Gain-of-Function Mutations of *fem-3*, a Sex-Determination Gene in *Caenorhabditis elegans*

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Manuscript received July 17, 1986
Accepted September 12, 1986

ABSTRACT

We have isolated nine gain-of-function (*gf*) alleles of the sex-determination gene *fem-3* as suppressors of feminizing mutations in *fem-1* and *fem-2*. The wild-type *fem-3* gene is needed for spermatogenesis in *XX* self-fertilizing hermaphrodites and for male development in both soma and germ line of *XO* animals. Loss-of-function alleles of *fem-3* transform *XX* and *XO* animals into females (spermless hermaphrodites). In contrast, *fem-3*(*gf*) alleles masculinize only one tissue, the hermaphrodite germ line. Thus, *XX fem-3*(*gf*) mutant animals have a normal hermaphrodite soma, but the germ line produces a vast excess of sperm and no oocytes. All nine *fem-3*(*gf*) alleles are temperature sensitive. The temperature-sensitive period is from late L4 to early adult, a period just preceding the first signs of oogenesis. The finding of gain-of-function alleles which confer a phenotype opposite to that of loss-of-function alleles supports the idea that *fem-3* plays a critical role in germ-line sex determination. Furthermore, the germ-line specificity of the *fem-3*(*gf*) mutant phenotype and the late temperature-sensitive period suggest that, in the wild-type *XX* hermaphrodite, *fem-3* is negatively regulated so that the hermaphrodite stops making sperm and starts making oocytes. Temperature shift experiments also show that, in the germ line, sexual commitment appears to be a continuing process. Spermatogenesis can resume even after oogenesis has begun, and oogenesis can be initiated much later than normal.

THE development of a germ cell as a sperm or an oocyte provides a simple model system for the study of sex determination. There are several advantages to studying the sperm/oocyte decision in the nematode *C. elegans*. Efficient genetic selections have been devised for isolating germ-line sex-determination mutants (KIMBLE *et al.* 1986). The transparency of *C. elegans* permits the direct observation of gamete differentiation in living animals. Ultimately, molecules of interest can be assayed by microinjection into the germ-line syncytium (KIMBLE *et al.* 1982).

C. elegans can exist as either of two sexes, hermaphrodite or male. The two sexes differ morphologically and/or biochemically in all tissues examined. The ratio of *X* chromosomes to autosomes determines sex in *C. elegans* (MADL and HERMAN 1979). *XX* animals are self-fertilizing hermaphrodites. Each hermaphrodite has two ovotestes. In each ovotestis, first sperm and then oocytes are made (HIRSH, OPPENHEIM and KLASS 1976). *XO* animals are male. In males, only sperm are made in a single testis.

Genes necessary for the choice to develop as either a hermaphrodite or a male have been identified (Table 1) and placed in a regulatory hierarchy (HODGKIN 1986). Central to the work presented in this paper are the *fem* genes. The *fem* genes are required for male development in the *XO* soma and for spermatogenesis in both *XO* and *XX* germ lines. Loss-of-function mutations in either *fem-1*, *fem-2* or *fem-3* cause *XX* and *XO* animals to develop as females (NELSON, LEW and WARD 1978; DONIACH and HODGKIN 1984; KIMBLE, EDGAR and HIRSH 1984; HODGKIN 1986). A female is a spermless hermaphrodite. Females and hermaphrodites are morphologically identical in the soma. It is likely that the *fem* genes are regulated in the hermaphrodite to permit spermatogenesis in the germ line without masculinization of somatic tissues. Moreover, the *fem* genes must be regulated to limit masculinization of the *XX* germ line to the first germ cells that mature so that the hermaphrodite can switch from spermatogenesis into oogenesis.

This paper describes the phenotypic, genetic and developmental characterization of nine *fem-3*(*gf*) mutations. The results have implications both for the regulation of germ cell sexual phenotype *by fem-3* and for the regulation *of fem-3* that permits the short burst of spermatogenesis in the hermaphrodite germ line. In addition, the results suggest that sexual commitment in the germ line, in contrast to sexual commitment in the soma, is a continuing process.

MATERIALS AND METHODS

Maintenance: Worms were grown on agar-filled Petri dishes seeded with *E. coli* strain OP50 according to BRENNER (1974). All experiments were done at room temperature

TABLE 1

***C. elegans* sex determination genes**

Gene		Loss-of-function phenotype	
		Soma	Germ line
Wild type	*XX*	Female	Sperm, oocytes
	XO	Male	Sperm
fem-1[a],−2[b],−3[b]	*XX*	Female	Oocytes
	XO	Female	Oocytes
tra-1[c]	*XX*	Male	Sperm, oocytes[d]
	XO	Male	Sperm
tra-2[c]	*XX*	Incomplete male	Sperm
	XO	Male	Sperm
tra-3[c]	*XX*	Incomplete male	Sperm, oocytes[e]
	XO	Male	Sperm
her-1[f]	*XX*	Female	Sperm, oocytes
	XO	Female	Sperm, oocytes

[a] DONIACH and HODGKIN (1984).
[b] HODGKIN (1986).
[c] HODGKIN and BRENNER (1977).
[d] The extent of oogenesis is variable (KIMBLE and SCHEDL 1987).
[e] This paper.
[f] HODGKIN (1980).

(20–22°) unless otherwise noted.

Strains and nomenclature: *C. elegans*, var. Bristol (wild type designated N2), was obtained from S. BRENNER. All strains used in this study are derivatives of N2. Most mutations used in this study are listed by SWANSON, EDGLEY and RIDDLE, 1984. Mutations in genes involved in sex determination are referenced explicitly in the text. The following mutations were used:

LG II: *unc-4(e120)*, *tra-2(e1425)*, *tra-2(q122gf)*.

LG III: *unc-45(e286ts)*, *fem-2(b245ts)*.

LG IV: *unc-5(e53)*, *fem-1(hc17ts, e1991)*, *fem-3(e1996)*, *unc-24(e138)*, *daf-15(m81)*, *him-8(e1489)*, *dpy-20(e1282)*, *unc-30(e191)*, *tra-3(e1107)*, *eDf18*.

LG V: *him-5(e1490)*, *her-1(e1520)*.

A map giving the location of these mutations is shown in Figure 1.

Since both loss-of-function and gain-of-function alleles of *tra-2* and *fem-3* exhibit some dominance (TRENT, TSUNG and HORVITZ 1983; DONIACH 1986; HODGKIN 1986; this paper), we describe alleles as *gf* for gain-of-function and *lf* for loss-of-function, instead of using conventional abbreviations for dominant and recessive. The suffix *gf* is used both after a numerical allele designation (*e.g.*, *fem-3(q20gf)*) and as a freestanding suffix following a gene name (*e.g.*, *fem-3(gf)*. To avoid confusion between "el" and "one," the suffix *lf* is not used after a numerical allele designation but, rather, only as a freestanding suffix. All other genetic nomenclature conforms to guidelines of HORVITZ *et al.* (1979).

Isolation of *fem-3(gf)* alleles: Homozygous *fem-1(hc17ts)* or *fem-2(b245ts)* *XX* animals were raised at permissive temperature (15°). L4 animals were mutagenized with EMS (BRENNER 1974), picked six to ten per large Petri dish and placed at restrictive temperature (25°). Dishes were screened 4–7 days later for any F_2 progeny. To ensure independence of suppressor mutations, only one revertant was retained from any given Petri dish. The frequency at which *fem-3(gf)* mutations were obtained as dominant suppressors of *fem-1(hc17ts)* or *fem-2(b245ts)* was the same: 5×10^{-6} per haploid genome. Alleles *q20*, *q24*, *q60*, *q61*, *q95* and *q96* were isolated as dominant suppressors of *fem-1(hc17ts)*. Alleles *q22*, *q23* and *q66* were isolated as dominant suppressors of *fem-2(b245ts)*. Suppressor mutations in genes other than *fem-3* have been isolated using this scheme and will be described elsewhere.

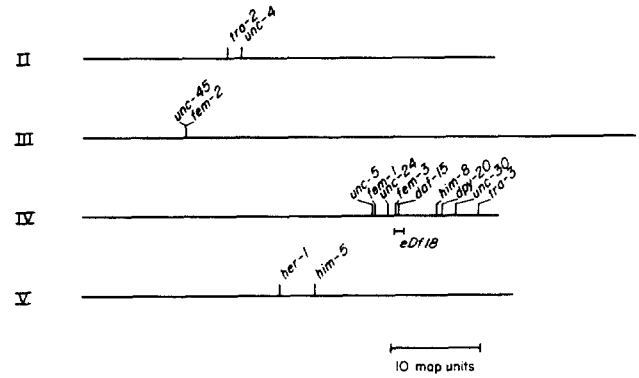

FIGURE 1.—Map positions of genes used in this study.

This selection selects for suppressors that result in a masculinization of the *XX* germ line. In theory, two types of masculinizing suppressors might be isolated by this selection: those that make the feminized *fem-1(hc17ts)* or *fem-2(b245ts)* *XX* mutant animals into self-fertile hermaphrodites and those that make them into males that can then mate with other females on the dish.

New suppressors (except *q95* and *q96*) were crossed twice to *fem-1(hc17ts)* or *fem-2(b245ts)* and reisolated on the basis of their suppressor phenotype. They were then crossed to N2 and reisolated on the basis of their Mog phenotype (for masculinization of the germ line, described in RESULTS). The mutations *q95* and *q96* were crossed directly to N2 twice.

The *fem-1(hc17ts)* mutation was removed from stocks by picking Unc Dpy recombinants either from the F_2 of heterozygotes of genotype *unc-5* + + +/+ *fem-1(hc17ts)* *fem-3(gf)* *dpy-20* (for *q20*, *q24* and *q60*), or from the F_2 of heterozygotes of genotype + *unc-24* + *daf-15* +/*fem-1(hc17ts)* + *fem-3(gf)* + *dpy-20* (for *q61*, *q95* and *q96*). Crosses were done at 15°, and the resulting stocks were checked at 25° to make sure they were homozygous for *fem-3(gf)*.

The *fem-2(b245ts)* mutation was excluded from stocks by making them homozygous for *unc-45*, a marker that is tightly linked to *fem-2*. Crosses were done at 15° and stocks were checked at 25° to make sure they were homozygous for *unc-45* and *fem-3(gf)*.

Mapping *fem-3(gf)* mutations: The temperature-sensitive Mog phenotype remaining after removal of *fem-1(hc17ts)* or *fem-2(b245ts)* was mapped with respect to *unc-24* and *daf-15* (see Table 2). These are the closest known flanking markers to *fem-3*. They map approximately one map unit apart (Figure 1). For two alleles, *q20* and *q23*, the suppression of *fem-1(hc17ts)* or *fem-2(b245ts)*, respectively, was mapped and gave results consistent with the *fem-3* map position (M. K. BARTON, unpublished results).

Reversion of *fem-3(gf)* mutations: Homozygous stocks of *fem-3(q20gf)* or *fem-3(q66gf)*, raised at permissive temperature (15°) were mutagenized and shifted to restrictive temperature (25°). Since either of these alleles in *trans* with a deficiency yields some fertile animals (see RESULTS), loss-of-function mutations in the *fem-3* gene will act as dominant suppressors of the Mog phenotype. The *fem-3* *(q20q90)* revertant was induced with gamma-rays (2500 r). The *fem-3(q66q99)* and *fem-3(q20q77)* revertants were induced with EMS. All three revertants failed to complement *fem-3(e1996)*. Twenty-four of 25 Dpy animals from the cross *fem-3(e1996)* *dpy-20*/+ + males × *fem-3(q20q77)* *dpy-20*

females were feminized. The remaining Dpy animal proved to be a recombinant. The analogous cross with *fem-3(q66q99)* gave 26 feminized Dpys of 27 Dpys; the remaining animal was a recombinant. For *fem-3(q20q90)*, 33 of 33 Dpy animals were feminized. Many suppressors of *fem-3(q20gf)* and *fem-3(q66gf)* were found using these selections, and at least some are unlinked to *fem-3*. Since most of these have not been analyzed, we have not yet determined the frequency with which *fem-3(lf)* alleles arise in this scheme.

Strategy for separating *fem-3(q20gf)* from a closely linked revertant: An attempt was made to separate *fem-3(q20gf)* from the closely linked revertant *q77* based on the ability of *fem-3(q20gf)* to act as a dominant suppressor of *fem-2(b245ts)* (see RESULTS). Animals of genotype *fem-2(b245ts)*; + *fem-3(q20q77) dpy-20/unc-24* + + were raised at permissive temperature (15°) and shifted to restrictive temperature (25°) as L4s. We then screened for fertile recombinants among the F_1 after 4–7 days.

Scoring the Mog phenotype: In all initial experiments the Mog phenotype was scored by Nomarski. It was found that Mog animals have a characteristic appearance under the dissecting microscope as well. They are noneggbearing adults that are fat with a clear outline, perhaps from accumulation of yolk. This phenotype is easily scored and has never been seen in wild-type worms. These criteria were used to score the Mog phenotype in later experiments. However, in instances where the penetrance of the Mog phenotype is not 100%, an occasional animal that is known to have laid some eggs shows the Mog phenotype when scored by the dissecting microscope. Examples of such situations are animals homozygous for weak alleles of *fem-3(gf)* at 25°, animals heterozygous for some alleles of *fem-3(gf)* at 25° and animals homozygous for the strong allele *q95* at 15°. When groups of animals displaying this leaky phenotype needed to be compared, the Mog phenotype was scored by cloning individual L4 animals to separate plates. The animal was then scored as Mog if it laid no eggs and if it exhibited the characteristic Mog phenotype as determined by dissecting microscope observation.

The Mog phenotype is dependent on temperature. Small changes in temperature (*e.g.*, a difference of 2°) can change the fraction of animals that are Mog. For this reason, when data between two experiments were compared, experiments were done simultaneously and in the same incubator.

Temperature shift experiments, shift up: Egg-laying hermaphrodites were placed on Petri dishes at 15°. Newly hatched L1 animals, less than 2-hr old, were picked from these plates at $t = 0$. Animals were shifted to 25° after 0-, 12-, 24-, 36-, 48-, 60-, 72-, 78- and 84-hr growth at 15°. (These times have been normalized to 25° time both in Figure 5 and in the text). The growth rate at 25° is approximately twice that at 16° (HIRSH and VANDERSLICE 1976). We have used the same approximation to standardize the times in these experiments. Animals were observed as adults using Nomarski optics, and the presence of oocytes was scored for each ovotestis. Animals at critical time points were observed by Nomarski 24–48 hr later for any possible changes.

Shift down: Egg-laying hermaphrodites raised at 15° were shifted to 25°. The progeny laid within the first 12 hr of egg laying at 25° were discarded to ensure that all of embryogenesis had taken place at 25°. Newly hatched L1 animals, less than 1-hr old, were picked at $t = 0$. Animals were shifted from 25° to 15° after 0-, 6-, 12-, 30-, 34-, 35-, 36- and 42-hr growth.

Double mutant experiments: *tra-2(lf)*. *XX* animals homozygous for an amber, putative null allele of *tra-2, e1425*, develop as pseudomales (HODGKIN and BRENNER 1977). These *XX* pseudomales look like *XO* males, except that tail structures are incompletely masculinized and mating behavior is absent. The germ line is indistinguishable from that of a wild-type male. Dpy Unc segregants from a mother of genotype *fem-3(q20gf) dpy-20/+ +; unc-4 tra-2/+ +* and raised at 25° were observed by Nomarski optics ($n = 30$). All showed a *tra-2* phenotype. The incomplete masculinization in *tra-2 XX* animals might provide a more sensitive background for detecting a somatic masculinization effect of *fem-3(gf)*. However, tail structures show no increased masculinization in the presence of *fem-3(q20gf)*, consistent with *fem-3(gf)* having no effect on the *XX* soma.

This experiment also uncovered a haploinsufficiency of *tra-2* in the germ line. *XX* animals heterozygous for *tra-2* and homozygous for *fem-3(gf)* are Mog even at 15°. Of 84 Dpy *XX* self-progeny from a *dpy-20 fem-3(q20gf)/+ +; tra-2(e1425)/+* mother, 53 were Mog. The other 31 were fertile and segregated no pseudomale progeny. Thus, the absence of one copy of *tra-2* eliminates the temperature sensitivity of *fem-3(q20gf)*.

tra-3. *XX* animals homozygous for an amber, putative null allele of *tra-3, e1107*, are pseudomales provided they come from a homozygous *tra-3* mother (HODGKIN and BRENNER 1977). Tail structures are incompletely masculinized. The germ line also shows incomplete masculinization: 44% ($n = 43$) of *tra-3* pseudomales raised at 25° make oocytes.

Homozygous *fem-3(q20gf) dpy-20 unc-30 tra-3 XX* pseudomales raised at 25° from a mother of genotype *fem-3(q20gf) dpy-20 unc-30 tra-3* make no oocytes ($n = 40$), only sperm. Tail structures remain incompletely masculinized. The absence of *tra-3* in the zygote reduces the temperature sensitivity of *fem-3(q20gf)*, but does not eliminate it. Nineteen of 28 Dpy Unc *XX* animals from a mother of genotype *fem-3(q20gf) dpy-20 unc-30 tra-3/fem-3(q20gf) dpy-20* + + are Mog at 15°.

her-1. *XO* animals homozygous for a putative null allele of *her-1, e1520*, are hermaphrodites (HODGKIN 1980). A strain of genotype *her-1(e1520) him-5*; *fem-3(q20gf)* was constructed to examine the phenotype of *XO* animals homozygous for these two mutations. All animals from this stock were Mog at 25°. No somatic masculinization was observed.

RESULTS

Isolation of *fem-3(gf)* alleles: Nine mutations have been isolated as dominant suppressors of either *fem-1(hc17ts)* or *fem-2(b245ts)*. The *fem-1(hc17ts)* and *fem-2(b245ts)* mutations feminize *XX* and *XO* animals (NELSON, LEW and WARD 1978; KIMBLE, EDGAR and HIRSH 1984): at restrictive temperature *XX* animals make no sperm and thus develop as females (spermless hermaphrodites), instead of self-fertilizing hermaphrodites; *XO* animals are incompletely feminized in both the soma and the germ line. Since putative null alleles of *fem-1* and *fem-2* result in complete feminization of *XO* animals (DONIACH and HODGKIN 1984; HODGKIN 1986), both *fem-1(hc17ts)* and *fem-2(b245ts)* must retain some residual activity at restrictive temperature. The suppressor mutations suppress the *XX* female phenotype of *fem-1(hc17ts)* or *fem-2(b245ts)*. The suppressed *XX* animals develop as self-fertile hermaphrodites. Where tested (alleles *q20, q24, q60, q61,*

q66), the suppressor mutations fail to suppress the *XO* somatic feminization phenotype of *fem-1(hc17ts)* or *fem-2(b245ts)*. The suppression of *fem-1(hc17ts)* and *fem-2(b245ts)* is thus restricted to the germ line.

The suppression is not specific to *fem-1(hc17ts)* or *fem-2(b245ts)*. Suppressors of *fem-1(hc17ts)*, *q20*, *q24*, *q60 and q96*, were tested for suppression of *fem-2(b245ts)*. *XX* animals homozygous for any one of these *fem-3(gf)* mutations and homozygous for *fem-2(b245ts)* are fertile (25°). Conversely, a suppressor of *fem-2(b245ts)*, *q66*, was tested for suppression of *fem-1(hc17ts)*. *XX* animals heterozygous or homozygous for *q66* and homozygous for *fem-1(hc17ts)* are fertile at restrictive temperature (25°).

Evidence that these suppressor mutations are gain-of-function alleles of *fem-3*: When crossed away from the feminizing mutations that they suppress, all nine suppressors masculinize the *XX* germ line (the Mog phenotype, for masculinization of the germ line, is described in detail in the next section). Their effect on the hermaphrodite germ line is opposite to that of loss-of-function alleles of *fem-3* [referred to as *fem-3(lf)*]. *XX* animals homozygous for *fem-3(lf)* make only oocytes. *XX* animals bearing the suppressor mutations make only sperm. These germ-line masculinizing mutations have been shown to be alleles of *fem-3* by the criteria of mapping, reversion and a *cis/trans* test. Because these alleles are dominant and because they revert to *fem-3(lf)*, we conclude they are gain-of-function alleles of *fem-3* [referred to as *fem-3(gf)*].

A comparison of three-factor map data for the *fem-3(gf)* alleles and the reference allele *fem-3(e1996)*. (HODGKIN 1986) is shown in Table 2. All map to the same position on chromosome *IV*.

We obtained revertants of two of the putative *fem-3(gf)* alleles—*q20*, a suppressor of *fem-1(hc17ts)* and *q66*, a suppressor of *fem-2(b245ts)*. The revertants, *q20q90*, *q20q77* and *q66q99*, fail to complement *fem-3(e1996)*, map to the *fem-3* locus (Table 2) and feminize *XX* and *XO* animals. The alleles *q20q77* and *q66q99* are weak—some *XX* animals make some sperm—similar in phenotype to weak *fem-3(lf)* alleles found by HODGKIN (1986).

The strongest revertant allele, *fem-3(q20q90)*, is similar to the putative null allele *fem-3(e1996)* both in its weak haploinsufficiency and its maternal absence effect (Table 3). The deletion *eDf18* (HODGKIN 1986) shows an even weaker haploinsufficiency than either *fem-3(e1996)* or *fem-3(q2090)* (Table 3). If *eDf18* deletes all of *fem-3*, this suggests that some residual product in *fem-3(e1996)* and *fem-3(q20q90)* may interfere with the wild-type product in the heterozygote (*i.e.*, *fem-3(e1996)*/+ and *fem-3(q20q90)*/+).

The phenotypes of animals heterozygous for both *fem-3(q20gf)* and a loss-of-function allele of *fem-3* were compared in *XX* animals that carried these mutations either in *cis* or in *trans*. The *cis* heterozygotes, *q20q90*/+ +, are either Fem or fertile (Table 3), but never Mog. In contrast, the *trans* heterozygotes, *q20/e1996*, are either Mog or fertile, but never Fem: 12 of 74 *XX* animals of genotype *q20/e1996* are Mog, the rest are fertile. Had the phenotype of the double heterozygote been due to the additive effects of mutations in two genes, it should have been independent of their configuration. Instead, the phenotype is different in the *cis* and *trans* heterozygotes. This result supports the idea that the suppressor mutations are gain-of-function alleles of *fem-3*. Note that the same *fem-3(lf)* allele could not be used in *cis* and in *trans* in this experiment.

Finally, an attempt was made to separate *fem-3(q20gf)* from its closely linked revertant, *q77*, by intragenic recombination. The strategy for this separation was based on the ability of *fem-3(q20gf)* to act as a dominant suppressor of *fem-2(b245ts)* at 25°. Fertile recombinants were sought among the progeny of *XX* animals of genotype *fem-2(b245ts)*; + *fem-3(q20q77lf)* *dpy-20/unc-24* + +. None were found among approximately 3×10^6 animals. Assuming *q20* only suppresses *fem-2(b245ts)* when in combination with *fem-3(+)*, this corresponds to a map distance of <0.0002, which is within the bounds of known intragenic distances in *C. elegans* (MOERMAN and BAILLIE 1979; ROSE and BAILLIE 1980; WATERSTON, SMITH and MOERMAN 1982). Indeed, this distance is so small that either *q77* or *q20* may be a rearrangement, and

TABLE 2

Three-factor map data

Allele	No. of Unc non-Daf recombinants carrying *fem-3(gf* or *lf)*[a]: Total no. of Unc non-Daf recombinants (%)
fem-3(q22gf)	25/30 (83)
fem-3(q23gf)	27/30 (90)
fem-3(q20gf)	25/30 (83)
fem-3(q66gf)	27/30 (90)
fem-3(q24gf)	25/30 (83)
fem-3(q60gf)	24/29 (83)
fem-3(q61gf)	21/28 (75)
fem-3(q96gf)	27/29 (93)
fem-3(q95gf)	23/30 (77)
fem-3(e1996)	55/65 (85)[b]
fem-3(q20q90)	28/30 (93)
fem-3(q20q77)	27/30 (90)
fem-3(q66q99)	23/26 (88)

[a] Unc non-Daf recombinants segregating from a heterozygote of genotype + *fem-3(gf* or *lf)* + *dpy-20/unc-24* + *daf-15* + were picked. The recombinants were then scored for whether they carried a mutant allele of *fem-3* by examining their self-progeny. For the *fem-3(gf)* alleles, recombinants were picked at 15° and shifted to 25° as adults. Their progeny were scored at 25°.

[b] Data for reference allele pools data published by HODGKIN [1986; 25 of 27 Unc non-Daf recombinants carried *fem-3(e1996)*] and data generated in our laboratory [30 of 38 Unc non-Daf recombinants carried *fem-3(e1996)*].

TABLE 3

Effect of maternal and zygotic genotypes on feminization of the XX germ line by different *fem-3(lf)* alleles

Allele	% female[a] [maternal(M) and zygotic (Z) genotyes with respect to *fem-3(lf)*]			
	M(+/+),*Z*(+/−)[b]	*M*(+/−),*Z*(+/−)[c]	*M*(−/−),*Z*(+/−)[d]	*M*(+/−),*Z*(−/−)[e]
fem-3 (e1996)	4% (n = 120)[g]	7% (n = 219)[g]	12% (n = 219)[g]	100% (n = 149)
fem-3 (q20q90)	3.5% (n = 115)[i]	8% (n = 144)	10%(n = 144)[i]	100% (n = 148)
eDf18	0.6% (n = 340)	1.3% (n = 232)[h]		
fem-3 (+)	0% (n = 282)[f]			

[a] Animals were cloned to individual plates as L4s and scored as female if they produced no progeny and if they showed the characteristic morphological female phenotype.

[b] Dpy progeny from the cross + *fem-3(lf) dpy-20*/+ + + male × *unc-24* + *dpy-20* hermaphrodite or wild-type progeny from the cross + *eDf18* +/+ + *dpy-20* male × *unc-24* + *dpy-20* hermaphrodite. Self-fertile animals were checked to make sure they were not recombinants by examining their self-progeny.

[c] Wild-type self-progeny from a + *fem-3(lf)* + *dpy-20/unc-24* + *daf-15* + mother, or wild-type self-progeny from a + *eDf18* +/*unc-24* + *dpy-20* mother. Self-fertile animals were checked to make sure that they were not recombinants by examining their self-progeny.

[d] *fem-3(lf)* dpy-20/+ + progeny from the cross N2 male × *fem-3(lf) dpy-20* female.

[e] Dpy self-progeny from a + *fem-3(lf)* + *dpy-20/unc-24* + *daf-15* + mother. Self-fertile Dpy animals that were found proved to be recombinants.

[f] Animals were of genotype *unc-24* + *dpy-20*/+ + *dpy-20*.

[g,h,i] Results indicated with the same letter are significantly different from one another [P < 0.05; z-test (FREUND 1973)].

therefore it might be impossible to get recombinants between *q20* and *q77*.

Phenotype of animals homozygous for *fem-3(gf)*, the Mog phenotype: Figure 2 shows a wild-type hermaphrodite ovotestis. Sperm can be seen in the proximal arm of the ovotestis. These are followed by large oocytes. In the distal arm is a granular core. The nuclei that line this core will become incorporated into oocytes. As the oocytes mature, they travel down the proximal arm. They are fertilized by sperm in the spermatheca.

The gain-of-function alleles of *fem-3* masculinize the *XX* germ line. Figure 3A shows a photomicrograph of an ovotestis dissected from an *XX* animal homozygous for *fem-3(q20gf)* and raised at 25°. Sperm fill the entire proximal arm of the ovotestis and often extend well into the distal arm. [These cells are technically spermatids (S. WARD, personal communication), but will be referred to throughout as sperm for simplicity.] The enlarged granular core that typically forms in the distal arm of the *XX* ovotestis is absent. Instead, primary spermatocytes are found in the distal arm of the *XX fem-3(gf)* ovotestis; this indicates that spermatogenesis is continuing. For comparison, an ovotestis from an *XX* animal homozygous for *fem-3(q20q90lf)* is shown in Figure 3C. No sperm are made. Oocytes can be seen throughout the proximal arm of the ovotestis, and the enlarged granular core indicative of oogenesis is present in the distal arm.

Since loss-of-function alleles of *fem-3* feminize the *XO* soma as well as the *XX* and *XO* germ lines, gain-of-function alleles of *fem-3* might have been expected to masculinize the soma of *XX* animals. However, no masculinization of somatic structures has been observed in *XX* adults homozygous for any of the nine *fem-3(gf)* alleles and raised at 25° (examination by Nomarski optics).

Table 4 summarizes the phenotypes of *XX* animals carrying the various alleles of *fem-3(gf)* at permissive (15°) and restrictive (25°) temperatures. All nine alleles of *fem-3(gf)* are generally fertile at 15° and Mog at 25°. The exceptions are two weak alleles, *q22* and *q23*, and one strong allele, *q95*. Thus, even at 25°, some *XX* animals homozygous for *q22* or *q23* are fertile (Table 4). Conversely, even at 15°, some *XX* animals homozygous for *q95* are Mog (Table 4).

An effect of *fem-3(gf)* activity on brood size can be seen at permissive temperature for several alleles (Table 4). The brood size of a hermaphrodite is the number of self-progeny she produces. Since brood size is limited by the number of sperm she makes (WARD and CARREL 1979), it is also a minimum estimate of the number of functional sperm made. Table 4 shows the brood sizes of *XX* animals homozygous for the different alleles of *fem-3(gf)* at 15°. Animals homozygous for *q23*, *q20*, *q66*, *q24*, *q60* or *q61* all have a larger brood size than *fem-3*(+) animals. Thus, these mutations cause the hermaphrodite to make more sperm even at permissive temperature. Three alleles, *q22*, *q96* and *q95*, do not show such an increase. The brood size of *q22*, a weak allele, does not differ greatly from that of the control. The alleles *q96* and *q95* are the two strongest alleles. They both produce smaller broods because some animals or ovotestes are Mog at permissive temperature and because, occasionally, a region of excess sperm and nonfunctional germ cells obstructs the passage of oocytes so that fertilization cannot occur.

Males segregating from *fem-3(gf) dpy-20; him-5* stocks were examined to see if the *fem-3(gf)* mutations

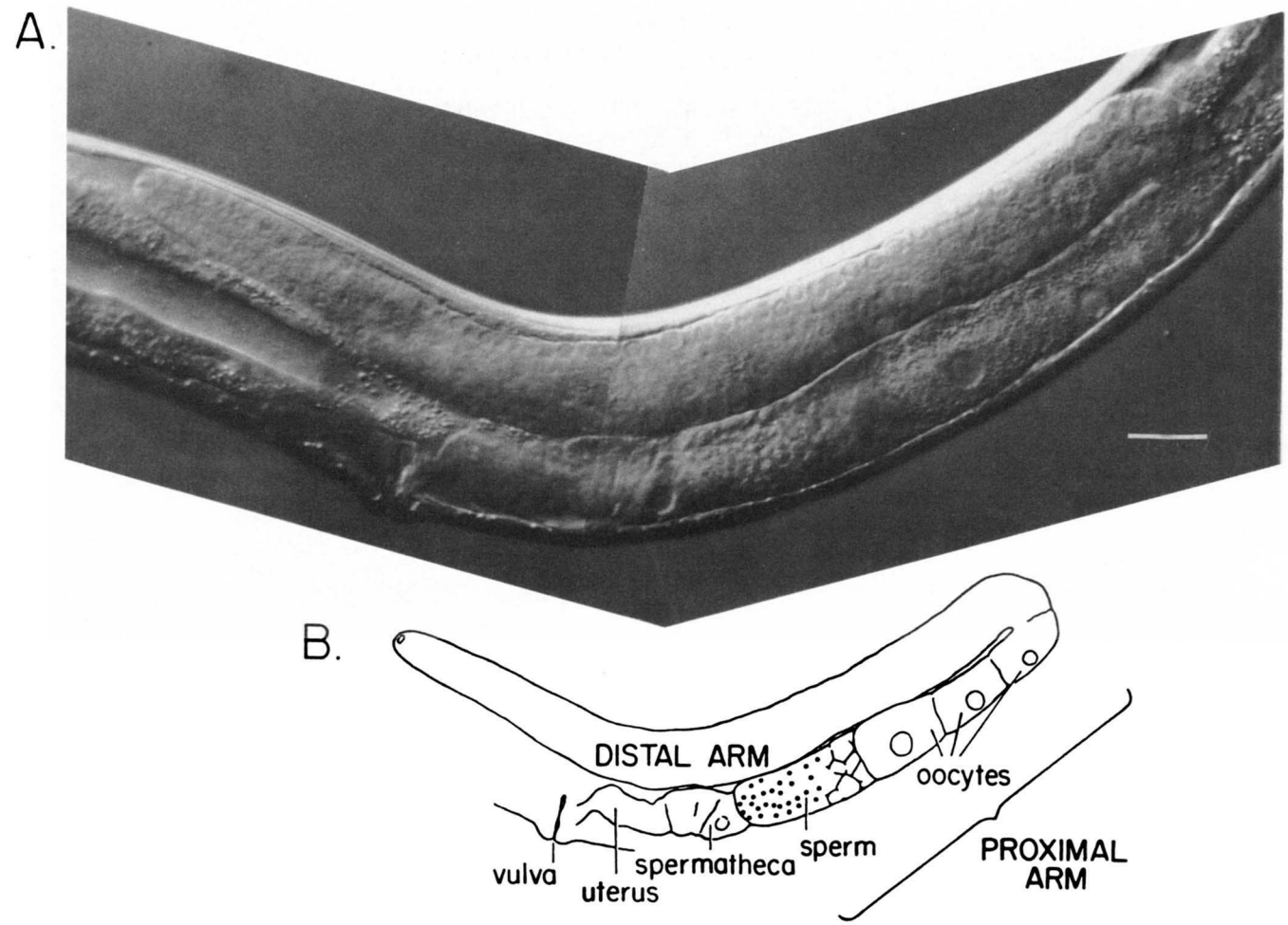

FIGURE 2.—Wild-type hermaphrodite ovotestis. A, Nomarski optics; bar = 20 μm. B, Diagram of ovotestis in (A). Sperm are present in the spermatheca. These are followed by oocytes in the proximal arm. An enlarged granular core not seen in this focal plane extends throughout the distal arm of the gonad. Anterior is to the left in this figure.

had any effects on males. With one exception, *XO* animals homozygous for alleles of *fem-3(gf)* and raised at 25° appear to be unaffected. These males are capable of mating and show normal morphology at the level of Nomarski microscopy. The exception is *q22*. Twenty-three percent (six of 20) of males homozygous for *q22* showed some morphological evidence of feminization (abnormal tail morphology similar to *tra-2 XX* animals and/or morphological evidence of yolk). At 15°, 20 of 20 *XO* animals from the same stock showed no signs of feminization. The gain-of-function mutation, *q22*, may disable the *fem-3* gene slightly at the same time that it disrupts its regulation in the hermaphrodite.

Phenotype of *XX* animals heterozygous and hemizygous for *fem-3(gf)*: The different alleles of *fem-3(gf)* vary in their degree of dominance (Table 4). No Mog animals were seen among 50 *q22*/+ heterozygotes raised at 25°. In contrast, 100% of *q95*/+ animals were Mog at 25°. The remaining alleles lie in between these two extremes.

The phenotype of *XX* animals heterozygous for *fem-3(gf)* was compared with the phenotype of *XX fem-3(gf)* hemizygotes to learn more about the mechanism of dominance of these alleles (Table 4). For alleles *q22*, *q20*, *q66*, *q24*, *q60*, *q61* and *q96*, a greater percentage of Mog animals was found among hemizygous animals (*fem-3(gf)/eDf18*) than among heterozygous (*fem-3(gf)*/+) animals. This difference is statistically significant ($P < 0.05$; *z*-test, FREUND 1973) in the cases of *q20*, *q66*, *q24*, *q60* and *q61*. These results suggest that the presence of the wild-type *fem-3* allele alleviates the effect of *fem-3(gf)*. Since the Mog phenotype is exacerbated by removing a wild-type copy of *fem-3*, the simple hypothesis that *fem-3(gf)* acts to increase the activity or amount of *fem-3* must be rejected.

Attempts to reveal a maternal effect of *fem-3(gf)*: No maternal effect has been observed for *fem-3(gf)* (Table 5). Several tests were performed in an effort to detect one. First, *fem-3(gf)*/+ heterozygotes from a mother that was wild type for *fem-3* were compared with *fem-3(gf)*/+ heterozygotes from a mother homozygous for *fem-3(gf)*. For the three alleles tested, no difference in the proportion of Mog animals was seen between these two groups (Table 5). Thus, the *fem-3(gf)* product inherited by the zygote does not

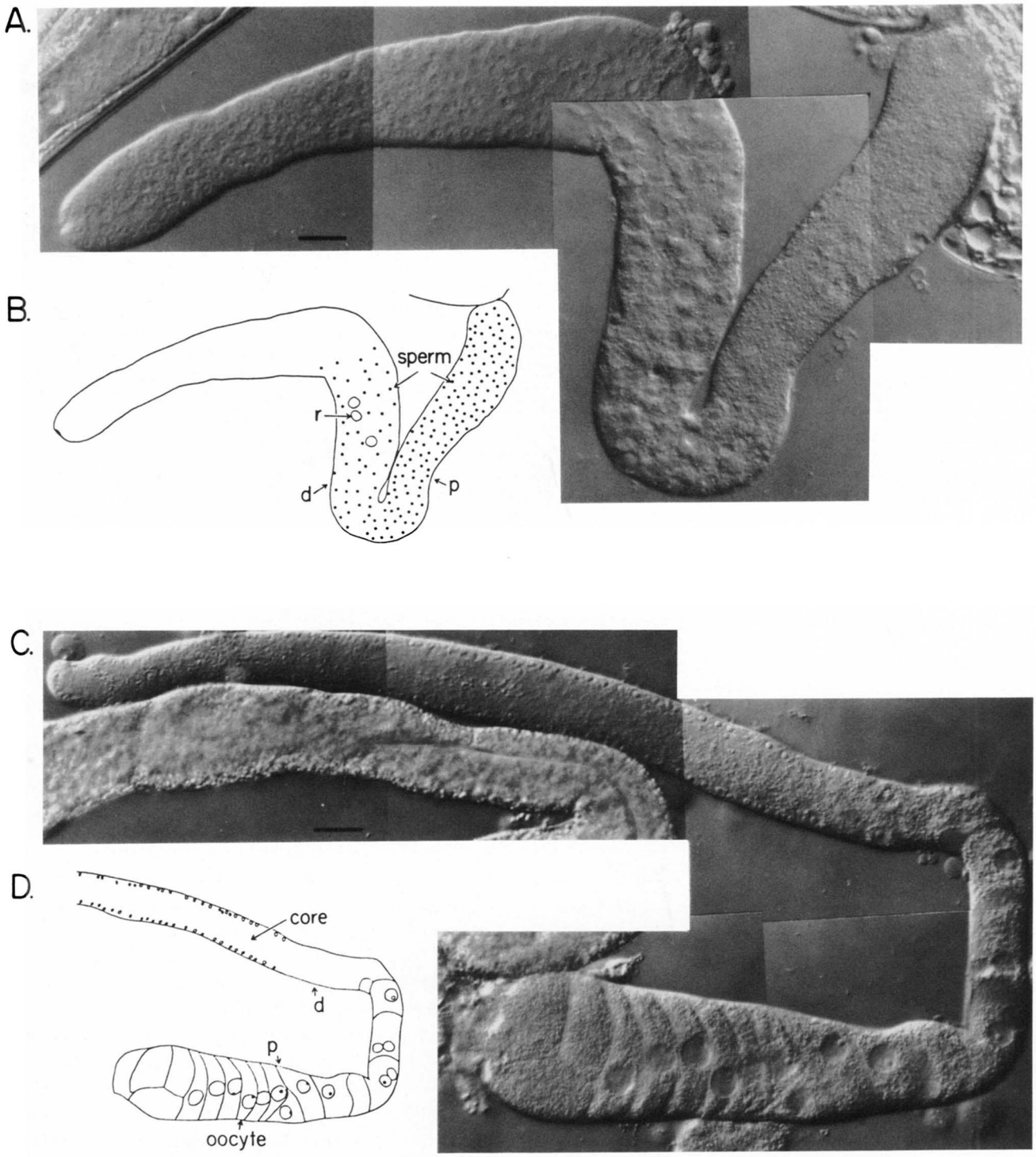

FIGURE 3.—A and B, *fem-3(gf)* phenotype. An ovotestis dissected from an *XX* homozygous *fem-3(q20gf)* animal raised at restrictive temperature. A, Nomarski optics; bar = 10 μm. B, Diagram of ovotestis in (A). The proximal (p) arm is packed with spermatids (sperm). Both sperm and residual bodies (r) are observed in the loop region and distal (d) arm of the ovotestis. We estimate that there are roughly 5000–10,000 sperm in this ovotestis. C and D, *fem-3(lf)* phenotype. An ovotestis dissected from an *XX* homozygous *fem-3(q20q90)*, *M*(+)*Z*(−) animal. C, Nomarski optics; bar = 10 μm. D, Diagram of ovotestis in (C). The proximal arm is packed with oocytes, no sperm are present. Note granular core.

detectably increase the fraction of *fem-3(gf)* heterozygotes that are Mog. Second, *XX* animals which were wild type for *fem-3* and segregating from a *fem-3(gf)*/+ mother were scored (Table 5). The *fem-3*(+) chromosome was marked with *unc-24*. The number of Unc Mogs seen was consistent with these being recombinants of genotype *unc-24 fem-3(gf)*/*unc-24* +. Thus, the *fem-3(gf)* product inherited maternally by these *fem-3*(+) animals does not detectably masculinize their germ lines. Third, no rescue of *fem-3(e1996) XX* ani-

TABLE 4

Phenotypes of *XX* animals carrying the various alleles of *fem-3(gf)* at permissive (15°) and restrictive (25°) temperatures

	15°		25°		
Allele[a]	Brood size *fem-3(gf)* / *fem-3(gf)*	% Mog *fem-3(gf)* / *fem-3(gf)*	% Mog *fem-3(gf)* / *fem-3(gf)*	% Mog[b,c] *fem-3(gf)* / *fem-3(+)*	% Mog[b,d] *fem-3(gf)* / *eDf18*
fem-3(+)	360 (n = 10)	0 (n > 200)	0 (n > 200)		
fem-3(q22gf)	373 (n = 11)	0 (n > 100)	94(b) (n = 72)	0 (n = 50)	6 (n = 52)
fem-3(q23gf)	445 (n = 9)	0 (n > 100)	78(b) (n = 78)	2 (n = 49)	0 (n = 59)
fem-3(q20gf)	454 (n = 36)	0 (n > 100)	100 (n > 200)	15 (n = 47)	41 (n = 54)
fem-3(q66gf)	409 (n = 10)	0 (n > 100)	100 (n > 200)	40 (n = 48)	68 (n = 47)
fem-3(q24gf)	493 (n = 9)	0 (n > 100)	100 (n > 200)	44 (n = 50)	79 (n = 48)
fem-3(q60gf)	424 (n = 10)	0 (n > 100)	100 (n > 200)	53 (n = 47)	74 (n = 50)
fem-3(g61gf)	489 (n = 10)	0 (n > 100)	100 (n > 200)	54 (n = 48)	80 (n = 50)
fem-3(q96gf)	311 (n = 11)	0 (n > 100)	100 (n > 200)	89 (n = 47)	98 (n = 47)
fem-3(q95gf)	50 (n = 10)	80(b) (n = 10)	100 (n > 200)	100 (n = 100)	100 (n = 38)

[a] All alleles, including wild type, are marked with *dpy-20*.

[b] Individual animals were cloned into separate plates. An animal was scored as Mog if it laid no self-progeny and if it showed the characteristic Mog phenotype by the dissecting microscope.

[c] *unc-24 fem-3(gf) dpy-20*/+ + + progeny from the cross N2 male × *unc-24 fem-3(gf) dpy-20* hermaphrodite.

[d] Wild-type progeny from the cross + *dpy-20/eDf18*; *him-5*/+male × *unc-24 fem-3(gf) dpy-20* hermaphrodite.

TABLE 5

Apparent lack of a maternal effect for *fem-3(gf)*

	% Mog			% Fem
	M(+/+),*Z*(*gf*/+)[a]	*M*(*gf*/*gf*),*Z*(*gf*/+)[b]	*M*(*gf*/+),*Z*(+/+)[c]	*M*(*gf*/−),*Z*(−/−)[d]
fem-3(q23gf)	0% (n = 50)	2% (n = 49)	1% (n = 114)	100% (n = 92)
fem-3(q66gf)	40% (n = 50)	40% (n = 48)	3% (n = 111)	99% (n = 102)[e]
fem-3(q95gf)	100% (n = 137)	100% (n = 100)	1% (n = 115)	100% (n = 100)

[a] + *fem-3(gf) dpy-20/unc-24* + +; *him-5*/+ progeny from the cross *fem-3(gf)dpy-20*; *him-5* male × *unc-24* hermaphrodite.

[b] Data from Table 4, included here for comparison.

[c] Unc self-progeny from a + *fem-3(gf) dpy-20/unc-24* + + mother.

[d] Unc self-progeny from a + *fem-3(gf) dpy-20/unc-24 fem-3(e1996)* + mother. All fertile Uncs proved to be recombinants.

[e] One animal was Mog; this was most likely a recombinant.

mals segregating from a *fem-3(gf)/fem-3(e1996)* mother has been seen (Table 5). Maternally inherited *fem-3(gf)* is not sufficient to allow the *fem-3(−)* individuals to make sperm. In conclusion, *fem-3(lf)* mutations show a maternal *absence* effect, but the converse, a maternal *presence* effect of *fem-3(gf)* has not been detected. All experiments in this section were done at 25°.

The temperature-sensitive period of *fem-3(gf)*: Temperature shift experiments were done to learn the time of development at which the mutant activity of *fem-3(gf)* could direct spermatogenesis in the hermaphrodite germ line. An intermediate strength allele, *q20*, was chosen for these temperature shift experiments because at permissive temperature (15°) all homozygotes are fertile and at restrictive temperature (25°) all homozygotes are Mog. In all cases, hr refers to hours after hatching normalized to 25° time. In *fem-3(q20gf) dpy-20 XX* animals raised at 15°, spermatogenesis begins during the late L4 stage (32–34 hr), and the first sign of oogenesis, appearance of an enlarged granular core in the distal arm of the ovotestis, is observed soon after the molt into adult (40 hr).

All animals shifted from permissive to restrictive temperature prior to 36 hr were Mog in both ovotestes (Figure 4). When animals were shifted to restrictive temperature at or after 36 hr, the proportion of ovotestes that made both sperm and oocytes increased sharply (56% when shifted at 36 hr; 95% when shifted at 42 hr). Additionally, in a separate experiment, single *fem-3(gf) dpy-20* animals were shifted to restrictive temperature in late L4 and observed individually over time. Of those ovotestes that made sperm and then began oogenesis, 54% (17 of 31) switched back into spermatogenesis. Such a phenotype has never been observed in *fem-3(q20gf)* homozygotes raised entirely at permissive temperature.

The shift from restrictive to permissive temperature yielded complementary results (Figure 4). Animals shifted down as late as 32 hr make the number of sperm typical of *q20* homozygotes raised at permissive

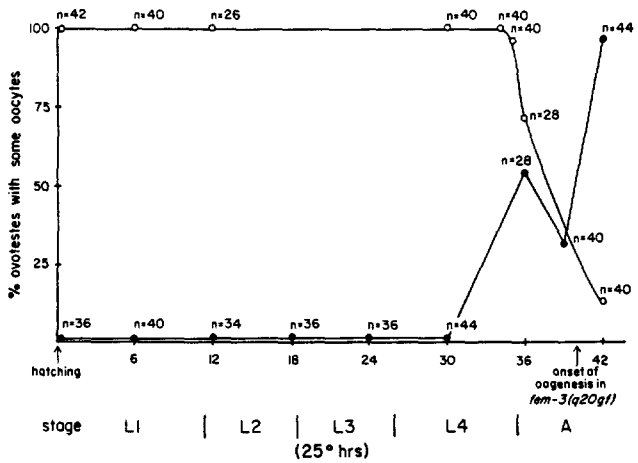

FIGURE 4.—Temperature-sensitive period of *fem-3(gf)*. Percentage of ovotestes with oocytes is plotted *vs.* the time at which the animal was shifted. –O–O– shifts from restrictive to permissive temperature; –●–● shifts from permissive to restrictive temperature. 0 hr is hatching. All time points have been translated into 25° hours (HIRSH and VANDERSLICE 1976).

temperature and then switch into oogenesis. Animals shifted at 36 hr make significantly more sperm than the typical number for *q20* (sperm are packed midway up the proximal arm). Of such ovotestes, 71% eventually make oocytes. In animals shifted to permissive temperature at 42 hr, even more sperm are made (sperm are packed all the way to the loop between proximal and distal arms). About 15% of ovotestes in the group shifted at 42 hr ultimately switch into oogenesis. These oocytes can be fertilized and give rise to progeny.

These temperature shift experiments reveal a late (36- to 42-hr temperature-sensitive period that closely preceeds and overlaps the first signs of oogenesis (Figure 4). The overlap may reflect the variability in the time of onset of oogenesis—at 40 hr only 60% of *fem-3(q20gf) dpy-20* animals raised at 15° actually show the enlarged granular core indicative of the onset of oogenesis (n = 13). Thus, the presence of *fem-3(gf)* 25° activity during this period causes spermatogenesis to occur at the expense of oogenesis. Even at the last time point taken (42 hr), a shift from restrictive to permissive temperature had an effect on the phenotype of the germline; therefore, the temperature-sensitive period may extend past 42 hr.

The interaction of *fem-3(gf)* with other sex determination genes: The phenotypes of animals homozygous for both *fem-3(gf)* and a mutation in one of the other sex determination genes were examined to learn how *fem-3(gf)* interacts with these other genes or their products. The phenotypes of most of these double mutants (*i.e.*, *fem-3(q20gf)* in combination with *tra-2(lf)*, *tra-3(lf)* or *her-1(lf)*; see MATERIALS AND METHODS) simply confirm the role of *fem-3* in the regulatory hierarchy that HODGKIN (1986) has proposed based on double mutants using *fem-3(lf)* alleles.

Two double mutant combinations have yielded novel information pertinent to hermaphrodite spermatogenesis and will be discussed in depth here. These are the *fem-1 fem-3(gf)* and the *tra-2(gf); fem-3(gf)* combinations.

To provide the background necessary for understanding these experiments, the proposed regulatory roles of *tra-2* and the *fem* genes are first outlined. (For simplicity, the word "gene" is used throughout to refer to the gene or its product.) The *fem-1*, *fem-2* and *fem-3* genes are necessary for both male somatic development and spermatogenesis in males and for spermatogenesis in hermaphrodites (NELSON, LEW and WARD 1978; DONIACH and HODGKIN 1984; KIMBLE, EDGAR and HIRSH 1984; HODGKIN 1986). The *tra-2* and *tra-3* genes are thought to negatively regulate the *fem* genes in the *XX* soma, thus preventing inappropriate masculinization (DONIACH and HODGKIN 1984; HODGKIN 1986). However, in the hermaphrodite germ line the *tra-2* gene is thought to be transiently repressed (DONIACH 1986), thus allowing the *fem* genes to be "on" and a burst of spermatogenesis to occur.

XX animals homozygous for an amber, putative null allele of *fem-1*, *e1991*, are feminized—they produce only oocytes and no sperm (DONIACH and HODGKIN 1984). When derived from a homozygous *fem-1(e1991)* mother, 100% of homozygous *fem-1(e1991)* *XX* animals are feminized. When derived from a heterozygous mother (genotype *fem-1(e1991)*/+), 20% of homozygous *fem-1(e1991)* *XX* animals are rescued, *i.e.*, make both sperm and oocytes. Thus, *fem-1* shows a maternal rescue effect (DONIACH and HODGKIN 1984).

When the mother is homozygous for *fem-1(e1991)*, the *XX* double mutant, *fem-1(e1991) fem-3(q20gf)*, is a female. The oocytes it makes can be fertilized and can give rise to progeny. This is true at both 15° and 25°. Thus, *fem-1* is epistatic to *fem-3(gf)*; no sperm are made in the absence of *fem-1* wild-type product.

When the mother is heterozygous for *fem-1(e1991)*, the *XX* double mutant, *fem-1(e1991) fem-3(q20gf)* is nearly always fertile (Table 6). Thus, *fem-3(q20gf)* has increased the rescue of *fem-1(e1991)* homozygotes by maternally inherited wild-type *fem-1* product from 20 to 99%. This increase in the maternal rescue might have been due to the interaction of *fem-3(q20gf)* and the *fem-1* gene in the mother, *e.g.*, *fem-3(q20gf)* might act to increase the amount of *fem-1* product inherited by the zygote. Alternatively, *fem-3(q20gf)* might be acting on the maternally inherited wild-type *fem-1* product in the zygote. To distinguish between these two possibilities, *XX* animals of genotype *fem-1(e1991)* *fem-3(q20gf)/fem-1(e1991)*+ from a mother of genotype *fem-1(e1991) fem-3(q20gf)*/+ + were compared with *XX* animals of the same genotype (*fem-1 (e1991) fem-3(q20gf)/fem-1(e1991)*+) from a mother of genotype *fem-1(e1991)*/+ (Table 6). The same proportion

TABLE 6

Potentiation of *fem-1* maternal rescue by *fem-3(q20gf)*

Source of progeny	Genotype of progeny	Genotype of mother	% fertile Dpys
$\frac{fem\text{-}1}{+}$ $\frac{dpy\text{-}20}{+}$ ♀ (self)	$\frac{fem\text{-}1}{fem\text{-}1}$ $\frac{+}{+}$ $\frac{dpy\text{-}20}{dpy\text{-}20}$	$\frac{fem\text{-}1}{+}$ $\frac{+}{+}$	20% (n = 100)
$\frac{+}{unc\text{-}5}$ $\frac{fem\text{-}1}{+}$ $\frac{fem\text{-}3(gf)}{fem\text{-}3(gf)}$ $\frac{dpy\text{-}20}{+}$ ♀ (self)	$\frac{+}{+}$ $\frac{fem\text{-}1}{fem\text{-}1}$ $\frac{fem\text{-}3(gf)}{fem\text{-}3(gf)}$ $\frac{dpy\text{-}20}{dpy\text{-}20}$	$\frac{fem\text{-}1}{+}$ $\frac{fem\text{-}3(gf)}{fem\text{-}3(gf)}$	99% (n = 109)
$\frac{fem\text{-}1}{+}$ $\frac{dpy\text{-}20}{+}$ ♂ × $\frac{fem\text{-}1}{+}$ $\frac{fem\text{-}3(gf)}{+}$ $\frac{dpy\text{-}20}{+}$ ♀	$\frac{fem\text{-}1}{fem\text{-}1}$ $\frac{fem\text{-}3(gf)}{+}$ $\frac{dpy\text{-}20}{dpy\text{-}20}$	$\frac{fem\text{-}1}{+}$ $\frac{fem\text{-}3(gf)}{+}$	60% (n = 29)
$\frac{fem\text{-}1}{+}$ $\frac{fem\text{-}3(gf)}{+}$ $\frac{dpy\text{-}20}{+}$ ♂ × $\frac{fem\text{-}1}{+}$ $\frac{dpy\text{-}20}{+}$ ♀	$\frac{fem\text{-}1}{fem\text{-}1}$ $\frac{fem\text{-}3(gf)}{+}$ $\frac{dpy\text{-}20}{dpy\text{-}20}$	$\frac{fem\text{-}1}{+}$ $\frac{+}{+}$	60% (n = 17)
$\frac{fem\text{-}1}{+}$ $\frac{fem\text{-}3(gf)}{+}$ $\frac{dpy\text{-}20}{+}$ ♀ (self)	$\frac{fem\text{-}1}{fem\text{-}1}$ $\frac{fem\text{-}3(gf)}{fem\text{-}3(gf)}$ $\frac{dpy\text{-}20}{dpy\text{-}20}$	$\frac{fem\text{-}1}{+}$ $\frac{fem\text{-}3(gf)}{+}$	100% (n = 106)

All experiments were done at 15°.

of animals was rescued in the two groups. Thus, the interaction between *fem-3(q20gf)* and the maternally inherited *fem-1* wild-type product occurs zygotically and must therefore be posttranscriptional with respect to *fem-1*. This experiment also shows that the number of *fem-1* mutant animals rescued when only one copy of *fem-3(q20gf)* is present is intermediate between that in animals with no or two copies (Table 6), indicating that, in this situation as well as by itself, *fem-3(q20gf)* is semidominant.

The nearly complete maternal rescue of *XX fem-1(e1991) fem-3(q20gf)* homozygotes from a *fem-1(e1991) fem-3(q20gf)/+ fem-3(q20gf)* mother reported above is seen at 15°. At 25° only 38% (28 of 73) of *XX fem-1(e1991) fem-3(q20gf)* homozygotes from a *fem-1(e1991) fem-3(q20gf)/+ fem-3(q20gf)* mother are self-fertile; the others make only oocytes. Thus, rescue by maternal *fem-1(+)* is increased by *fem-3(gf)* at 25°, but it is increased even more at 15°. This is an unexpected result since the masculinizing effect of *fem-3(gf)* is stronger at 25°.

Gain-of-function alleles of *tra-2* feminize the germ line of *XX* animals but do not affect *XO* animals (DONIACH 1986; T. A. ROSENQUIST and T. SCHEDL, unpublished results). As described above, DONIACH (1986) has suggested that *tra-2* is turned off transiently in the hermaphrodite germ line to allow the *fem* genes to direct spermatogenesis. The *tra-2(gf)* mutations appear to be defective in this modulation, hence the *fem* genes are not active.

The construction of double mutants of various *fem-3(gf)* alleles and a *tra-2(gf)* allele, *q122* (T. SCHEDL, unpublished results) reveals a balancing of masculinizing and feminizing activity in the germ line (Table 7). The *tra-2(q122gf)* allele is similar to the strong *tra-2(gf)* allele, *e2020* (DONIACH 1986). A weak and an intermediate allele of *fem-3(gf)*, *q23* and *q20*, when homozygous in combination with *tra-2(gf)* give a fertile hermaphrodite at 25°. A stronger allele of *fem-3(gf)*, *q95*, when heterozygous in combination with *tra-2(gf)* also yields self-fertile animals, but a portion are Mog. Animals homozygous for both *fem-3(q95gf)* and *tra-2(q122gf)* are all Mog.

TABLE 7

Phenotypes of *tra-2(gf)fem-3(gf)* double mutants

Genotype	% Feminized	% Self-fertile	% Mog
$\frac{tra\text{-}2(gf)}{+}$; $\frac{+}{+}$	100	0	0 (n > 200)
$\frac{tra\text{-}2(gf)}{tra\text{-}2(gf)}$; $\frac{+}{+}$	100	0	0 (n > 200)
$\frac{tra\text{-}2(gf)}{tra\text{-}2(gf)}$; $\frac{fem\text{-}3(q23gf)}{fem\text{-}3(q23gf)}$	0	100	0 (n = 66)
$\frac{tra\text{-}2(gf)}{tra\text{-}2(gf)}$; $\frac{fem\text{-}3(q23gf)}{+}$	97	3	0 (n = 102)
$\frac{tra\text{-}2(gf)}{+}$; $\frac{fem\text{-}3(q20gf)}{fem\text{-}3(q20gf)}$	0	100	0 (n= 105)
$\frac{tra\text{-}2(gf)}{tra\text{-}2(gf)}$; $\frac{fem\text{-}3(q20gf)}{fem\text{-}3(q20gf)}$	0	100	0 (n = 108)
$\frac{tra\text{-}2(gf)}{tra\text{-}2(gf)}$; $\frac{fem\text{-}3(q20gf)}{+}$	81	19	0 (n = 118)
$\frac{tra\text{-}2(gf)}{tra\text{-}2(gf)}$; $\frac{fem\text{-}3(q95gf)}{fem\text{-}3(q95gf)}$	0	0	100 (n = 123)
$\frac{tra\text{-}2(gf)}{tra\text{-}2(gf)}$; $\frac{fem\text{-}3(q95gf)}{+}$	0	80	20 (n = 66)

All experiments were done at 25°.

DISCUSSION

***fem-3* is a regulatory gene:** The *fem-3* gene plays a key role in the decision of a germ cell to develop as a sperm or an oocyte in *C. elegans*. Normally, hermaphrodites first make sperm and then oocytes. In this paper, we describe gain-of-function (*gf*) alleles of the *fem-3* locus that masculinize the hermaphrodite germ

line resulting in continuous production of sperm (the Mog phenotype—for masculinization of the germ line). In contrast to gain-of-function alleles of *fem-3*, loss-of-function alleles of *fem-3* feminize the germ line resulting in continuous production of oocytes (HODGKIN 1986). Thus, gain-of-function and loss-of-function mutations of *fem-3* cause opposite sexual transformations of the germ line.

Many switch genes have gain-of-function and loss-of-function alleles that confer opposite phenotypes (*e.g.*, *cII* in lambda, JONES and HERSKOWITZ 1978; *Sex-lethal* in Drosophila, CLINE 1978; *lin-12* in *C. elegans*, GREENWALD, STERNBERG and HORVITZ 1983). By this criterion, *fem-3* appears to be a *bona fide* regulatory gene, the state of which determines whether an oocyte or a sperm will be made. Gain-of-function and loss-of-function mutations that cause opposite sexual transformations have been found for other loci in the sex determination pathway (*her-1*, TRENT, TSUNG and HORVITZ 1983; *tra-1*, HODGKIN 1983; *tra-2*, DONIACH 1986). So far, of the three *fem* genes, only *fem-3* is represented by both types of mutation.

A control of *fem-3* that limits its effects in hermaphrodites to the germ line: The *fem-3(gf)* phenotype is limited to a single tissue—only the *XX* germ line is masculinized. In contrast, the *fem-3(lf)* phenotype affects all tissues—both somatic and germ-line tissues of *XO* animals are feminized (HODGKIN 1986). Why do the *fem-3(gf)* alleles masculinize only the *XX* germ line? In wild-type hermaphrodites, the action of *fem-3(+)* is also limited to the germ line: *fem-3* activity is needed for a short burst of spermatogenesis. Thus, some control must exist in hermaphrodites that restricts the effect of *fem-3* activity to the germ line. We suggest that this control is still functional in *fem-3(gf)* animals.

There are several ways in which *fem-3* could be controlled in wild-type hermaphrodites so that only the germ line and not the soma is masculinized. The time of action, level and/or localization of active *fem-3* could be regulated to achieve this germ-line-specific masculinization. The time of action of *fem-3* could be regulated such that *fem-3* is on after the somatic tissues have made a sexual choice but before the germ cells have been committed to male or female development. Alternatively, if the threshold for masculinization of the germ line were lower than that for the soma, a low level of *fem-3* might be sufficient for spermatogenesis to occur but insufficient for somatic masculinization. Finally, the action of *fem-3* could be localized to the germ line or to another tissue that directs the sexual differentiation of the germ line. Consistent with this last possibility, it should be noted that *fem-3* does show a maternal effect (HODGKIN 1986; this paper), suggesting that its product is present in the germ line, at least in mature oocytes.

A control of *fem-3* that permits the switch from spermatogenesis to oogenesis in the hermaphrodite germ line: In wild-type hermaphrodites, sperm are made first and then oocytes. The wild-type switch from spermatogenesis to oogenesis implies a control of *fem-3* activity in which its spermatogenesis-promoting activity is negatively regulated to achieve a switch to oocyte production. The mutant phenotype of the *fem-3(gf)* alleles suggests that it is *fem-3* or its product that is negatively regulated to permit the onset of oogenesis. In *fem-3(gf)* hermaphrodites, sperm are made continuously; oogenesis never begins. The temperature-sensitive period of the Mog phenotype is late L4 to early adulthood, just before the onset of oogenesis. We suggest that the *fem-3(gf)* mutations interfere with a negative regulation of the *fem-3* gene or its product that normally stops spermatogenesis. Since one of the controls of *fem-3* (negative regulation to permit oogenesis) appears to be mutated without affecting the other (limitation of its effect to the hermaphrodite germ line), the molecular basis for the two controls may be different.

Two genes, *tra-2* and *tra-3*, behave genetically as negative regulators of *fem-3* in most or all tissues (HODGKIN 1986). The activity of one of these genes, *tra-2*, appears to be modulated to permit hermaphrodite spermatogenesis (DONIACH 1986). Above, we suggested that the activity of *fem-3* is modulated to stop hermaphrodite spermatogenesis. It is plausible that *tra-2* is the negative regulator that inactivates *fem-3* to permit oogenesis. Alternatively (or in addition), a germ-line-specific regulator may exist that negatively regulates *fem-3* in the germ line.

Speculations on the molecular basis of the *fem-3(gf)* change: How do the *fem-3(gf)* mutations escape the negative control of *fem-3* that allows oogenesis in the hermaphrodite germ line? One model might have been that the *fem-3(gf)* mutations cause an increase in the amount or activity of *fem-3* and, in so doing, allow *fem-3* to escape its negative regulation. Such a change predicts that *fem-3(gf)/fem-3(Df)* animals should be less Mog than *fem-3(gf)/fem-3(+)* animals; however, just the opposite was observed. Thus, the presence of a wild-type allele in the heterozygote ameliorates the mutant phenotype. Therefore, these *fem-3(gf)* alleles do not act to increase the amount of *fem-3*. Furthermore, the wild-type *fem-3* competes with *fem-3(gf)*.

The temperature sensitivity of all nine *fem-3(gf)* alleles and the fact that they all show the same germ-line masculinization suggest that they all affect the same functional part of *fem-3*. This temperature sensitivity suggests that they may change the structure of the *fem-3* protein. If so, the *fem-3(gf)* mutations might damage a site in the *fem-3* protein necessary for recognition by a negative regulator. The varying

strengths of the nine alleles suggest that this domain can be altered in a graded manner.

***fem-3* is unique among the *fem* genes:** Each of three *fem* genes is required for specification of male development (NELSON, LEW and WARD 1978; KIMBLE, EDGAR and HIRSH 1984; DONIACH and HODGKIN 1984; HODGKIN 1986). Each shows essentially the same loss-of-function phenotype, and each gives the same results in epistasis experiments (DONIACH and HODGKIN 1984; HODGKIN 1986). The *fem* genes appear to be regulated negatively by *tra-2* and *tra-3*, and they appear to regulate *tra-1* negatively in the somatic tissues and to promote spermatogenesis in the germ line.

Several features of *fem-3* are unique among the *fem* genes. These include the late temperature-sensitive period (for *lf*, HODGKIN 1986; for *gf*, this paper), the maternal absence effect (HODGKIN 1986; this paper), the haploinsufficiency (HODGKIN 1986; this paper) and the existence of gain-of-function alleles.

Interactions among *fem-1*, *fem-2* and *fem-3*: How do the three *fem* genes interact to promote spermatogenesis? First, *fem-3(gf)* enhances *fem-1*(+) in its maternal rescue of *fem-1*(−) progeny from a *fem-1*(−)/+ mother. This potentiation can occur in the zygote and thus must be posttranscriptional with respect to *fem-1*. Second, *fem-3(gf)* can suppress the self-sterility of either *fem-1(ts)* or *fem-2(ts)* mutants. These *ts* alleles of *fem-1* and *fem-2* have residual activity even at restrictive temperature (NELSON, LEW and WARD 1978; DONIACH and HODGKIN 1984; KIMBLE, EDGAR and HIRSH 1984; HODGKIN 1986). In a number of systems, suppressor mutations have been used to identify genes which have products that physically interact (JARVIK and BOTSTEIN 1975; MORRIS, LAI and OAKLEY 1979). Two properties are often observed for such suppressor mutations: (1) suppression is allele-specific, and (2) the phenotype of the suppressor mutation alone is similar to the phenotype of the original mutation. Neither of these properties is observed for the *fem-3(gf)* suppressors. This suggests that suppression does not occur by a physical interaction that specifically compensates for the original defects in *fem-1(hc17ts)* and *fem-2(b245ts)*. Rather, the interactions of *fem-3(gf)* with *fem-1*, both with the wild-type and a temperature-sensitive allele, suggest that *fem-3(gf)* compensates for either a lower amount of *fem-1* or less active *fem-1*. A similar argument can be made for the interaction of *fem-3(gf)* with *fem-2(b245ts)*.

The *fem-3(gf)* mutation cannot bypass the requirement for *fem-1* activity. In the absence of maternally inherited *fem-1*(+) product, *fem-1*(null) is epistatic to *fem-3(gf)*—no sperm are made. This result indicates that *fem-1* is absolutely required for spermatogenesis; however, this result does not indicate a functional order for *fem-1* and *fem-3*. The *fem-1* gene might act as a necessary positive regulator of *fem-3*. Conversely, the *fem-3* gene might act as an obligate positive regulator of *fem-1*.

Conclusions and implications for sex determination in the germ line: The gain-of-function alleles of *fem-3* support the idea that *fem-3* is a key regulatory gene that directs spermatogenesis in the germ line. Based on the mutant phenotype and late temperature-sensitive period of the *fem-3(gf)* alleles, we suggest that *fem-3* is normally inactivated to stop spermatogenesis in the hermaphrodite germ line and permit oogenesis.

In contrast to sex determination in the soma, the sexual commitment of germ cells is a continuing process. Throughout larval and adult life, germ cells are continuously generated from a stem cell population (KIMBLE and WHITE 1981). In the experiments reported here, spermatogenesis could be induced after the onset of oogenesis, and oogenesis could be induced even if spermatogenesis had been permitted to continue after the normal switch to oogenesis. Therefore, a pool of uncommitted, immature germ cells must exist. These germ cells become committed to spermatogenesis or oogenesis as they mature. The switch from spermatogenesis to oogenesis is not irreversible or necessarily restricted to a particular time of development.

We are grateful to JONATHON HODGKIN and TABITHA DONIACH for sharing unpublished data and strains. We thank JULIE AHRINGER, PHIL ANDERSON, JUDITH AUSTIN, MICHAEL HOFFMAN, TOM ROSENQUIST and MARVIN WICKENS for critical comments on the manuscript. PETER OKKEMA, DAVID CROUCH and JULIE AHRINGER helped in isolating suppressors and SANDRA MAPLES provided excellent technical assistance. We are indebted to LARRY JOHNSON for help with statistical analysis. This work was made possible by support from Public Health Service grant GM31816 and by a Basil O'Connor Starter Research Grant no. 5-514 from the March of Dimes Birth Defects Foundation to J.K., and by a Public Health Service grant GM09554 to T.B.S. M.K.B. is a National Science Foundation predoctoral fellow; J.K. is a recipient of Public Health Service Research Career Development Award HD00630. Some nematode strains used in this study were provided by the Caenorhabditis Genetics Center, which is supported by contract number NO1-AG-9-2113 between the National Institutes of Health and the Curator of the University of Missouri.

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Communicating editor: R. K. HERMAN