NONDISJUNCTION MUTANTS OF THE NEMATODE *CAENORHABDITIS ELEGANS*

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ABSTRACT

The frequency of males (5*AA*; *XO*) among the self progeny of wild-type *Caenorhabditis elegans* hermaphrodites (5*AA*; *XX*) is about one in 500. Fifteen *him* (for "*h*igh *i*ncidence of *m*ales") mutations have been identified that increase this frequency by a factor of ten to 150, as a result of increased *X*-chromosome nondisjunction. The mutations define ten complementation groups, which have been mapped: nine are autosomal, and one sex linked. Most of the mutants are superficially wild type in anatomy and behavior; however, *him-4* mutants display gonadal abnormalities, and *unc-86* mutants, which have a Him phenotype, exhibit a variety of anatomical and behavioral abnormalities. All the mutants segregate fertile 3*X* hermaphrodite progeny as well as *XO* male progeny. Some produce large numbers of inviable zygotes. Mutants in all ten genes produce diplo-*X* and nullo-*X* exceptional ova, and in the four strains tested, diplo-*X* and nullo-*X* exceptional sperm are produced by 2*X* "transformed" males. It appears likely that most of the mutants have defects in both gamete lines of the hermaphrodite. *XO* males of *him* strains other than *him-4* and *unc-86* are similar to wild-type males in anatomy and behavior, and all produce equal or almost equal numbers of haplo-*X* and nullo-*X* sperm, and no diplo-*X* sperm. Male fertility is reduced to varying extents in all *him* mutants. In four of the strains, nondisjunction during oogenesis has been shown to occur at a reductional division, and in three of these strains, abnormalities in recombination have been demonstrated. One mutant also exhibits autosomal nondisjunction, but many of the others probably do not. Therefore, the *X* chromosome of *C. elegans* may differ from the autosomes in the mechanisms controlling its meiotic behavior.———3*X* hermaphrodites are shorter and less fertile than 2*X* hermaphrodites, and they produce many inviable zygotes among their self progeny: these are probably 4*X* zygotes. Haplo-*X* and diplo-*X* ova are produced in 2:1 ratio by 3*X* hermaphrodites. *him* mutations are expressed in these animals, increasing the frequency of self-progeny males and 2*X* hermaphrodites.

MUTANTS with alterations in the process of meiosis have been isolated in many organisms, ranging in complexity from fungi to higher plants (BAKER *et al.* 1976b). The most detailed investigations into meiosis have been made with Drosophila, where large numbers of meiotic mutants have been characterized

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(BAKER and HALL 1976), and a partial genetic dissection of meiosis has been possible. Many of these mutants affect not only meiotic processes but also mitotic chromosomal repair mechanisms (BAKER *et al.* 1976a).

The small nematode worm, *Caenorhabditis elegans*, is a simple metazoan with considersable potential for genetic studies (BRENNER 1974). This paper describes some mutants of *C. elegans* that exhibit increased nondisjunction of the sex (*X*) chromosome. *C. elegans* hermaphrodites have five pairs of autosomes and a single *X* chromosome. The hermaphrodite is self fertilizing, and normally the frequency of males in *C. elegans* cultures is very low: about 1 in 500. In some mutant cultures, this frequency is increased by a factor of up to 150. We call these *him* mutants (for "*h*igh *i*ncidence of *m*ales"), because the most obvious phenotype is the segregation of abnormally high numbers of males among the self progeny of mutant hermaphrodites.

It is likely that most or all of the increased nondisjunction in *him* mutants occurs during meiosis, so that it is interesting to compare these mutants with meiotic mutants isolated in other organisms. Little is known about meiosis in the phylum Nematoda, and some nematode species exhibit unusual meioses (NIGON 1965). *C. elegans*, unlike Drosophila, exhibits meiotic recombination in both sexes (BRENNER 1974).

The *him* mutants, besides helping our understanding of meiosis, are of interest for several reasons. First, *him* mutants provide a convenient source of males for genetic, anatomical, and biochemical purposes; males are otherwise seen only rarely or as the result of outcrosses. Second, some of the *him* mutants may segregate specific aneuploids, as well as males; such aneuploids might allow studies of gene dosage effects. Third, it is possible that some of these mutants may exhibit somatic nondisjunction (by analogy with mutants of Drosophila, such as claret nondisjunctional and paternal loss (HALL, GELBART and KANKEL 1976)); if so, the powerful method of mosaic analysis might be applied to the study of *C. elegans* (STERN 1968).

MATERIALS AND METHODS

The general techniques of *C. elegans* culture and genetics have been described (BRENNER 1974). It is important to realize that hermaphrodites can produce both self progeny by self-fertilization and cross progeny by fertilization of hermaphrodite ova with male sperm. Genetic markers must be used to distinguish the two classes of progeny.

When complete progeny scores were necessary, each parental hermaphrodite (or group of hermaphrodites) was transferred daily to a fresh culture plate, until no more progeny were produced; otherwise, late F_1 and early F_2 progeny could be confused. In the case of self-progeny scores, hermaphrodites were always picked as L4's (fourth-stage juveniles) to ensure parental virginity and the collection of all progeny. Large numbers of animals were generated in some of these experiments: these were usually counted by aspirating the worms from the culture plates with a drawn Pasteur pipette attached to a water aspirator. Inviable zygotes were scored by counting the number of eggs with refractile eggshells (HIRSH and VANDERSLICE 1976) that failed to hatch within 24 to 48 hr after being laid. Eggs normally hatch within 12 hr at 20° (BYERLY, CASSADA and RUSSELL 1976).

All experiments were carried out at 20°.

This paper conforms to the recently introduced standardized nomenclature for *C. elegans*

genetics (H. R. HORVITZ, unpublished). Allele numbers are bracketed, and the wild-type allele is indicated by (+), *e.g.*, *him-1*(+). Phenotypes are abbreviated as in bacterial genetics: a worm expressing a mutation in the gene *unc-65* has the phenotype Unc-65.

Strains: Genes and alleles utilized were as follows:

LGI : *unc-38*(*e213*), *him-1*(*e879*), *dpy-5*(*e61*), *unc-13*(*e51*), *him-2*(*e1065*), *unc-54*(*e190*).

LGII : *dpy-10*(*e128*), *unc-4*(*e120*), *him-9*(*e1487*), *rol-1*(*e91*), *unc-52*(*e444*).

LGIII : *dpy-1*(*e1*), *unc-32*(*e189*), *unc-86*(*e1416*, *e1507*), *unc-69*(*e587*), *unc-49*(*e382*), *tra-1*(*e1099*), *dpy-18*(*e364*).

LGIV : *dpy-9*(*e12*), *unc-17*(*e113*, *e245*), *dpy-13*(*e184*), *him-3*(*e1147*, *e1256*), *him-8*(*e1489*), *him-6*(*e1423*, *e1104*), *unc-30*(*e191*), *dpy-4*(*e1166*).

LGV : *unc-60*(*e677*), *him-7*(*e1480*), *unc-46*(*e177*), *dpy-11*(*e224*), *sma-1*(*e30*), *him-5*(*e1467*, *e1490*), *unc-76*(*e911*), *unc-51*(*e369*), *dpy-21*(*e428*, *e459*).

LGX : *dpy-3*(*e27*), *dpy-8*(*e130*), *lon-2*(*e678*), *dpy-7*(*e1324*), *him-4*(*e1266*, *e1267*), *unc-9*(*e101*), *unc-7*(*e5*), *dpy-6*(*e14*), *unc-18*(*e81*).

The gene *dpy-21* was previously incorrectly assigned to LGX (HODGKIN and BRENNER 1977).

We are indebted to P. BABU for the isolation of *e1065*, to DAVID BAILLIE for *e1104*, to him and DON RIDDLE for *e1266* and *e1267*, to JOHJI MIWA for *e1489*, and to MARTIN CHALFIE for *e1507*.

A simplified linkage map of *C. elegans* is shown in Figure 1.

Source of mutants: All but one of the mutants described in this paper were isolated fortuitously: mutant hermaphrodite stocks that had been established for other reasons were observed to segregate substantially higher frequencies of self-progeny males than the wild type. In each case, outcrossing and re-isolation of a homozygous *him* strain showed that the Him phenotype was caused by a single Mendelian factor. All the mutants were isolated after mutagenesis with EMS (ethyl methanesulfonate) (BRENNER 1974), except for the two *him-4* strains (isolated after treatment with ICR-191 (D. RIDDLE and D. L. BAILLIE, personal communication) and for *him-9* (isolated after treatment with acetaldehyde (J. HODGKIN, unpublished).

The *him-5* allele *e1490* was isolated as follows. Young adult hermaphrodites homozygous for *dpy-11* and *unc-76* (markers that flank *him-5*) were mutagenized (0.075 M EMS for four hours) and crossed with *him-5*(*e1467*) males. A total of about 2000 wild-type F_1 hermaphrodite progeny were obtained from these crosses and tested for male production in sets of about 50 worms per 9 cm plate. F_2 males were picked and crossed singly with *unc-17* or *dpy-13* hermaphrodites, and *dpy-11 unc-76* hermaphrodites re-isolated from the F_2 progeny of these crosses. One out of 15 fertile males tested in this way gave Dpy Him Unc F_2 progeny, and the *him* mutation *e1490* was shown to be an allele of *him-5*. The other 14 males were presumably spontaneous in origin, rather than *him* induced. It is unlikely that *e1490* is a re-isolate of *e1467* (as a result of gene conversion or double recombination) because it leads to higher frequencies of male production than does *e1467*.

Mapping and complementation: The *him* mutations are not as easy to map genetically as most *C. elegans* markers, because recognition of the Him phenotype requires progeny testing. However, homozygotes for *unc-86* and *him-4* have other phenotypes, and these genes were mapped using these phenotypes. Mutations in both these genes appear to be pleiotropic in effect.

Hermaphrodites homozygous for *unc-86* are behaviorally abnormal, as described below. The mutation *e1416* was assigned to LGIII on the basis of this phenotype, and more precisely located by means of the following three factor cross: a strain *unc-86* + *dpy-18*/+ *unc-69* + was constructed, and Unc-86 and Dpy recombinants picked (the phenotypes Unc-86 and Unc-69 are distinguishable). Nine of ten Dpy recombinants segregated Unc-69 animals in the next generation, whereas only one of five Unc-86 recombinants segregated Unc-86 Unc-69 animals in the next generation; therefore *unc-86* lies slightly to the left of *unc-69*. The Him phenotype and the Unc phenotype caused by the mutation *e1416* have never been observed to segregate independently; furthermore a second *unc-86* mutation, *e1507*, also has a Him phenotype. However, the heterozygote *e1416/e1507* is Unc but not Him (Table 1). This is probably a case of partial allelic complementation, since the two mutations fail to complement in other aspects of the complex phenotype (*e.g.*, behavior, brood size).

FIGURE 1.—A simplified linkage map of *C. elegans*, showing the position of almost all markers used in this study. The full known extent of each linkage group is shown, except for LGIII, which is about 50% longer than the other linkage group.

The gene *him-4* was mapped on the basis of the gonad eversion phenotype (see below), and shown to lie on the *X* chromosome (both alleles show approximately 15% recombination with *lon-2*). A heterozygote *dpy7* + *unc-9*/+ *him-4* + was constructed using transformed males

TABLE 1

Complementation tests between him *mutations*

him mutations tested	Hermaphrodite genotype	Number of broods counted	Average brood size	Percent males	Percent inviable zygotes
Complementing:					
e879	*him-1/+*	6	281	1.1	2.3
e1065, e879	*+ him-2/him-1 +; dpy-11/+*	3	240	0.4	1.1
e1489, e1147	*+ him-8/him-3 +; lon-2/+*	4	334	0.2	—
e1423, e1147	*+ him-6/him-3 +; dpy-11/+*	3	344	0.1	0.6
e1147, e1104	*him-3 +/+ him-6; lon-2/+*	2	329	0.0	0.2
e1256, e1489	*him-3 +/+ him-8; dpy-3/+*	4	350	0.2	0.7
e1256, e1423	*him-3 +/+ him-6; dpy-3/+*	4	340	0.2	0.7
e1256, e1104	*him-3 +/+ him-6; dpy-3/+*	4	324	0.2	1.2
e1423, e1489	*+ him-6/him-8 +; dpy-11/+*	2	321	0.0	0.5
e1489, e1104	*him-8 + unc-30/+ him-6 +*	3	313	0.2	1.3
e1480, e1467	*him-7 dpy-11 +/+ + him-5*	3	311	0.0	1.3
Noncomplementing:					
e1256, e1147	*him-3/him-3; dpy-3/+*	5	218	3.2	27.9
e1423, e1104	*him-6/him-6; sma-1/+*	6	158	4.8	23.3
e1490, e1467	*him-5 unc-76/him-5 +*	6	238	25.0	12.5
e1507, e1416	*unc-86 unc-69/unc-86 +*	6	136	0.0	0.2

Broods were scored as described in MATERIALS AND METHODS, with daily transfers of individual hermaphrodites. In almost all of these experiments, marked *him* hermaphrodites of one strain were crossed with unmarked *him* males of another, and cross progeny (heterozygotes) identified by the absence of the marker phenotype. In the listing of genotypes, the maternal homologue is written first throughout. In Tables 1 and 2, percent males and percent 3*X* hermaphrodites were calculated relative to total viable progeny; percent inviable zygotes was calculated relative to total zygotes produced.

(HODGKIN and BRENNER 1977), because neither *him-4/0* nor *dpy-7 unc-9/0* males will mate successfully. *lon-2 him-4* (*e1267*) hermaphrodites were crossed with *tra-1* transformed males, and transformed heterozygotes (genotype *tra-1*; *lon-2 him-4/+ +*) were obtained from the F_2 generation. These were crossed with *dpy-7 unc-9* hermaphrodites, and animals of the desired genotype were obtained from the F_2 progeny of this cross. Seven of 12 Dpy recombinants and four of 12 Unc recombinants segregated animals with everted gonads in the next generation; therefore, *him-4* lies between these markers. The Him phenotype and the gonad eversion phenotype have never been observed to segregate independently; furthermore an independently isolated mutation, *e1266*, causes the same two phenotypes in homozygotes. The two mutations fail to complement with respect to the gonad eversion phenotype: *unc-4*; *lon-2 him-4*(*e1267*) hermaphrodites were crossed with *tra-1; lon-2 him-4*(*e1266*)*/+ +* transformed males, and gonad eversion was observed in most (12 of 15) non-Unc Lon progeny. Complementation with respect to the Him phenotype could not be tested in this cross because the heterozygotes (*e1266/e1267*) were also heterozygous for *tra-1*, and therefore segregated 25% transformed males, masking any Him effect.

Other *him* genes were located by attempting to construct double mutants of each *him* isolate with standard markers for each linkage group. In all cases, one and only one double mutant was difficult to construct, indicating linkage. More precise locations were obtained by the following two- and three-factor crosses.

him-1 : one of two Unc recombinants, two of five Dpy recombinants from *unc-38 + dpy-5/+ him-1 +* gave F_2 males; all 13 Dpy segregants from *him-1 dpy-5/+ +* gave F_1 males.

him-2 : all six Dpy recombinants, none of six Unc recombinants from *dpy-5 unc-13* +/+ + *him-2* gave F_2 males; 13 of 15 Dpy segregants from *dpy-5 him-2*/+ + gave F_1 males.

him-3(*e1147*) : one of six Dpy recombinants, four of six Unc recombinants from *dpy-13* + *unc-30*/+ *him-3* + gave F_2 males.

him-5(*e1467*) : 13 of 15 Dpy recombinants, two of 15 Unc recombinants from *dpy-11* + *unc-76*/+ *him-5* + gave F_2males.

him-6(*e1423*) : four of six Dpy recombinants, one of six Unc recombinants from *dpy-13* + *unc-30*/+ *him-6* + gave F_2 males.

him-7 : some difficulties were encountered in mapping this marker; a map position was assigned based on the following data. A heterozygote *him-7* +/+ *dpy-11* was constructed, and 36 non-Dpy progeny were picked and progeny tested. Of those that were Him, two of seven were *dpy-11*/+; of those that were non-Him, 27 of 29 were *dpy-11*/+. Therefore *him-7* and *dpy-11* are linked (approximately 10% recombination). A strain *him-7* + *dpy-11* +/+ *unc-46* + *sma-1* was then constructed, and Unc and Sma recombinants picked. Of nine Unc that segregated Unc Dpy animals in the next generation, none gave Unc Dpy Him, whereas of nine Sma that segregated Dpy Sma in the next generation, at least eight gave Dpy Sma Him. Therefore, *him-7* lies to the left of *dpy-11*.

him-8 : None of 16 Unc segregants from *him-8* +/+ *unc-30* gave F_1 males, one of 16 gave F_2 males. Six of 16 Dpy recombinants, four of 15 Unc recombinants from *dpy-13* + *unc-30*/+ *him-8* + gave F_2 males.

him-9 : all seven Dpy recombinants, none of seven Unc recombinants from *dpy-10 unc-4* +/+ *him-9* gave F_2 males; also, one *dpy-10 unc-4 him-9*/+ + *him-9* recombinant was obtained from this heterozygote. One of two Rol Unc recombinants obtained from *unc-4 him-9* +/+ + *rol-1* segregated males. 15 of 16 Unc segregants from *unc-4 him-9*/+ + gave F_1 males.

These data have been used to assign the genes *him-1* to *him-9* and *unc-86*, to the positions shown in Figure 1. The relative order of the genes *him-3*, *him-6*, and *him-8* has not been determined, so that the arrangement shown (based on frequencies of segregation in the three factor crosses above) is tentative.

Complementation tests have been carried out between most pairs of linked *him* mutations; the results are shown in Table 1 and establish the assignment of 15 mutations to ten genes. These data and the mapping data show that all the mutations except for *him-1* are recessive with respect to male production. Heterozygotes for *him-1* segregate 0.5 to 1.0% males. The data in Table 1 are not sufficient to prove a significant increase in male production by *him-1*/+ hermaphrodites relative to the wild type, but in the course of experiments on recombination in *him* backgrounds, a total of six males in 6836 progeny was observed for the wild-type background, as compared to 49 in 7092 for the *him-1*/+ background. These frequencies are significantly different ($P < 0.001$). HERMAN (1978) has shown that an *X*-autosome translocation in *C. elegans* can lead to a dominant Him effect; it is conceivable that *him-1* is such a translocation. The absence of dominant effects in any of the other mutants argues against chromosomal aberration as a frequent cause of EMS-induced *him* mutants.

No striking anomalies were encountered in the course of mapping these mutations, which makes it more likely that they are all point mutants. No cases of segregation distortion (*i.e.*, deviation from expected Mendelian ratios of gametes (HARTL and HIRAIZUMI 1976)) were observed. However, a deficiency of *him-7* homozygotes in the progeny of *him-7*/+ hermaphrodites was observed in one set of experiments: only nine of 86 such progeny had a Him-7 phenotype, although 21 would have been expected.

RESULTS

Phenotypes: Generally, hermaphrodites homozygous for all the *him* mutations except *him-4* and *unc-86* are indistinguishable from wild type in terms of superficial anatomy and behavior. However, the composition of self-progeny broods produced by these hermaphrodites is very different from that of wild type

(Table 2). All the mutants segregate at least 1% males, as well as a smaller percentage of short animals that have been shown to be 3*X* hermaphrodites (*i.e.*, hermaphrodites with five pairs of autosomes and three *X* chromosomes); evidence for a 3*X* genotype is given below. Adult worms carrying one, two or three *X* chromosomes are shown in Figure 2: males (*XO*) and hermaphrodites (2*X* and 3*X*) are readily distinguished, but 2*X* and 3*X* hermaphrodites are sometimes hard to tell apart, particularly as juveniles.

Hermaphrodites of some *him* strains also lay large numbers of eggs that never hatch, although they appear to have been fertilized because they have refractile eggshells (HIRSH and VANDERSLICE 1976). These are scored as "inviable zygotes" in Tables 1 and 2 and elsewhere. The mutants that produce large number of inviable zygotes produce correspondingly low numbers of viable progeny. Populations of these strains (and to a lesser extent other *him* strains) also contain a small minority of animals with an ill-defined "sick" phenotype: these animals exhibit some combination of abnormal features such as small size, slow or uncoordinated movement, flaccidity, transparency, low viability and low fertility. Possibly these are autosomal aneuploids.

Hermaphrodites homozygous for *him-4* have a defect causing frequent eversion through the vulva of one or both arms of the gonad, so that adults often appear to have burst open at the vulva. Consequently, brood sizes are small, and it is not possible to count the number of fertilized eggs with any accuracy because many

TABLE 2

Self progeny broods of hermaphrodites

him genotype	Number of complete broods counted	Average brood size	Percent males	Percent 3*X* herm.	Percent inviable zygotes
wild type	8	330 ± 34	0.3	0.04	0.8
him-1	11	209 ± 34	20.6	5.6	5.8
him-2	6	217 ± 56	2.0	0.9	14.6
him-3(e1147)	6	353 ± 29	3.5	1.1	1.4
him-3(e1256)	9	45 ± 23	10.9	1.2	70.9
him-4(e1266)	30	19	7.6	—	—
him-4(e1267)	30	22	6.0	—	—
him-5(e1467)	6	198 ± 24	16.4	3.2	17.6
him-5(e1490)	7	217 ± 30	32.9	6.7	14.1
him-6(e1423)	10	43 ± 15	15.3	5.8	78.4
him-6(e1104)	6	184 ± 44	5.0	1.1	16.4
him-7	6	182 ± 32	2.9	1.0	34.1
him-8	6	302 ± 37	36.7	6.4	0.8
him-9	6	209 ± 24	4.5	1.8	15.9
unc-86(e1416)	6	104 ± 11	2.2	1.1	3.5
unc-86(e1507)	12	89 ± 29	2.4	0.8	2.0

Data obtained and presented as in Table 1. Brood sizes are quoted as mean ± standard deviation. The *him-4* broods were counted in sets of ten; *him-4* inviable zygotes were not counted, and *him-4* 2*X* and 3*X* hermaphrodites were counted together.

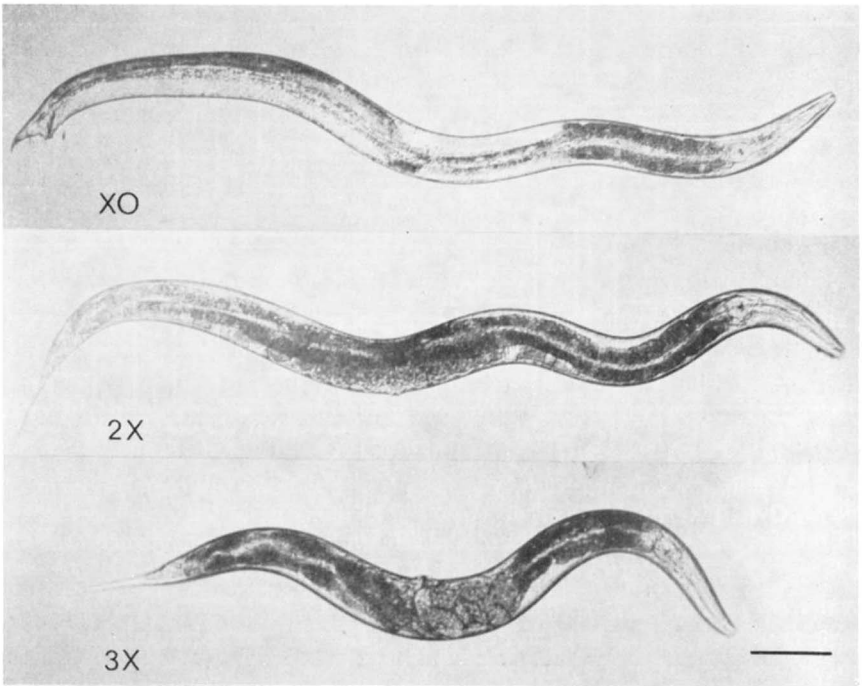

FIGURE 2.—Adult *him*(+) worms with five pairs of autosomes and one (*XO male*), two (2*X* hermaphrodite) or three (3*X* hermaphrodite) *X* chromosomes. The *him*(+) 3*X* strain was obtained by crossing *him-1*; *lon-2* +/+ *dpy-6* hermaphrodites with wild-type males: a 3*X* animal of genotype *him-1*/+; *lon-2* +/+ *dpy-6*/+ + was obtained, and all marked chromosomes segregated in subsequent generations. The scale bar is 0.1 mm.

of the eggs are never laid. Examination of mutant hermaphrodites by Nomarski microscopy did not reveal any obvious explanation for the gonad eversion.

unc-86 hermaphrodites segregate males and 3*X* hermaphrodites, but the most obvious feature of these animals is behavioral: *unc-86* animals are sluggish, insensitive to light touch, and retain eggs. Detailed examination has revealed other abnormalities affecting specific aspects of the normally invariant neuroanatomy (unpublished observation), cell lineages (SULSTON and HORVITZ 1977), and distribution of biogenic amines as revealed by formaldehyde-induced fluorescence (SULSTON, DEW and BRENNER 1975 and personal communication).

The male animals segregated by *him* strains have also been examined; all strains except *him-4* and *unc-86* produce males with superficially wild-type anatomy and behavior. The fertility of males was tested by crossing with marked (*dpy-11 V*) hermaphrodites under standard conditions and scoring cross progeny. These data (Table 3) show that the males are *XO* males (in the germ line at least) because they produce approximately equal numbers of haplo-*X* and nullo-*X* sperm. Only one mutant, in fact, produces a gamete ratio significantly different from 1:1, and in this case (*him-7*) the deviation is small. Additional evidence for *XO* karyotype was obtained by constructing double mutants of

TABLE 3

Size and sex ratio of broods sired by him *males*

Male genotype	Cross progeny Herm.	Male	Percent of wild type fertility	χ^2 for sex ratio relative to 1:1	
wild type	1065	1073	100	0.03	
him-1	12	18	1	1.20	
him-2	249	253	23	0.03	
him-3(e1147)	806	778	74	0.50	
him-3(e1256)	53	62	5	0.70	
him-4(e1266)	0	0	0	—	
him-4(e1267)	0	0	0	—	
him-5(e1467)	652	683	62	0.72	
him-5(e1490)	594	586	55	0.05	
him-6(e1423)	332	316	31	0.40	
him-6(e1104)	406	412	38	0.04	
him-7	302	388	32	10.70	($P < 0.005$)
him-8	88	100	9	0.77	
him-9	945	961	89	0.13	
unc-86(e1416)	79	75	7	0.11	
(Larger experiments)					
him-1	316	308	—	0.10	
him-7	1986	2182	—	15.95	($P < 0.001$)
unc-86(e1416)	219	227	—	0.14	

The numbers in the upper part of the table were obtained from crosses under standard conditions: six L4 *dpy-11* hermaphrodites were placed in a small (1 cm) spot of bacteria on a small (5 cm) NGM plate and six L4 males added. 24 hr later the males were removed and the hermaphrodites transferred daily to fresh plates until no more cross progeny were produced. The numbers in the lower part of the table were obtained from crosses using larger numbers of parents, and longer mating periods; they also include the data from the crosses under standard conditions.

several *him* mutations with *dpy-21 V*. This gene is expressed in 2*X* hermaphrodites or 2*X* males, but not in *XO* males (HODGKIN and BRENNER 1977). The double mutants of *dpy-21* with *him-1*, *him-4(e1267)*, *him-5(e1467*, *e1490)* and *him-8* all behaved as expected: the hermaphrodites were Dpy but the self-progeny males are not. Proof of *XO* genotype in *him-4* males could not otherwise be easily obtained because these males are completely sterile.

The data show that all mutant males are less fertile than wild type, in that they sire smaller numbers of progeny. This is not surprising in the case of *unc-86* males, because of other aspects of the phenotype. In another case of very low fertility (*him-1*), the deficit does not seem to be due to low sperm production, because males contain normal or nearly normal numbers of sperm. The complete sterility of *him-4* males was explained when the gonads of these animals were examined microscopically, either after Feulgen staining or by using Nomarski optics on live worms (SULSTON and HORVITZ 1977): the testis in these males never forms a proper connection with the cloaca, and frequently grows in a disorganized manner. Despite this, spermatogenesis seems to be normal, and apparently mature sperm often accumulate in the pseudocoelom throughout the

animal. Also, both the genital apparatus (apart from the vas deferens) and mating behavior seem normal. The cause of the low fertility of the other *him* strains is not known, though evidence for sperm abnormality has been obtained for some of them (see below). In other cases secondary mutations could be responsible.

X-chromosome nondisjunction in oogenesis: A plausible explanation for the production of excess males in *him* strains is an increase in *X*-chromosome nondisjunction during gametogenesis, leading to nullo-*X* and diplo-*X* gametes. Exceptional ova (*i.e.*, gametes produced by the egg line) of these types can be detected in crosses with wild-type males if the hermaphrodite carries a sex-linked marker. Therefore, a series of *him* strains carrying a sex-linked marker (*dpy-3* or *lon-2*) and an autosomal marker (*unc-4 II*) were constructed, and hermaphrodites of these strains were crossed with wild-type males. The *unc* marker allows distinction between self progeny (Unc) and cross progeny (non-Unc). Under the conditions used, complete outcrossing was almost always observed.

In the crosses with *unc*; *dpy* hermaphrodites, nullo-*X* ova are detected by the presence of patroclinous wild-type males (*him*/+; *unc*/+; +/*O*) and diplo-*X* ova by the appearance of matroclinous Dpy hermaphrodites (*him*/+; *unc*/+; *dpy*/*dpy*) or 3*X* hermaphrodites (*him*/+; *unc*/+; *dpy*/*dpy*/+). The last two classes of animal are sometimes very similar in phenotype, and are therefore scored together. In the other cross, with Unc Lon hermaphrodites, all three classes of hermaphrodite cross progeny (Lon, 2*X* and 3*X* hermaphrodites) are distinguishable, but the distinction between the two types of male (matroclinous Lon and patroclinous wild) is occasionally difficult, so that the estimates of frequency for nullo-*X* ova are less reliable in these crosses.

The results of these crosses are summarized in Tables 4 and 5. The data show that all the *him* strains tested produce both types of exceptional ova at detectable frequencies (with the exception of diplo-*X* ovum production by *unc-86*; relatively few progeny were obtained from this cross). Production of nullo-*X* ova by the wild type has also been demonstrated, and confirmed in crosses with other sex-linked markers (one wild male to 1765 wild hermaphrodites using *dpy-6* as a marker; one to 1663 using *unc-18* as a marker). These events presumably account for some or all of the rare males segregated by *him*(+) hermaphrodites.

The frequency of nullo-*X* ova can be compared with the frequency of self-progeny males segregated by the same strains in the absence of outcrossing. If nondisjunction occurs only in the egg line, then these two frequencies should be the same, but if nondisjunction also occurs in the sperm line, then the progeny male frequency should be higher. In some cases, nondisjunction varies with age; in order to make the comparisons accurate, nullo-*X* ova scores have to be terminated at the point corresponding to the exhaustion of sperm in the production of self progeny. Hermaphrodites produce more ova than sperm, so that more progeny are produced in an outcross than in a self cross.

When this correction is applied, most of the *him* strains do not produce enough nullo-*X* ova to account for all male production, implying that nondisjunction also

TABLE 4

Production of exceptional ova by unc-4 II; dpy-3 X *hermaphrodites crossed to wild-type males*

him genotype	Self progeny (Unc) Dpy herm.	Dpy males	Percent males	Cross progeny (non-Unc) Regular Wild herm.	Dpy males	Exceptional Dpy herm., 3X herm.	Wild males	Percent diplo-X ova	Percent nullo-X ova	Corrected percent nullo-X ova
wild type	1636	1	0.06	1289	1297	0	0	0.0	0.0	0.0
him-1	347	81	18.9	506	511	9	22	0.8	4.1	7.5
him-3(e1147)	1043	36	3.3	652	661	26	32	1.9	4.6	2.3
him-5(e1467)	698	190	21.4	512	519	28	55	2.4	9.5	12.5
him-6(e1423)	402	44	9.9	462	439	34	48	2.9	9.1	9.4
him-7	1135	39	3.3	913	881	2	4	0.1	0.4	0.6
him-8	1005	634	38.7	480	481	170	357	9.2	38.7	38.3
him-9	1707	82	4.6	620	641	12	14	0.9	2.2	2.2
unc-86(e1416)	484	6	1.2	260	266	0	2	0.0	0.8	0.8

The self-progeny columns list total progeny of ten *unc-4*; *dpy-3* hermaphrodites of each *him* genotype, all ten being scored together. The cross-progeny columns list the cross progeny (non-Unc) obtained from parallel experiments in which *unc-4*; *dpy-3* hermaphrodites were crossed with wild-type males. The high-frequency *him* mutants (*him-1*, *him-5*, *him-6* and *him-8*) were crossed and scored singly (one hermaphrodite crossed with five wild-type males for 24 hr); the other mutants in sets of three or four hermaphrodites (crossed with ten wild-type males for 24 hr). Percentage of diplo-*X* ova was calculated as 100 × 0.5 (Dpy hermaphrodites and 3*X* hermaphrodites) ÷ (wild-type males + wild-type hermaphrodites + 0.5 (Dpy hermaphrodites and 3*X* hermaphrodites)). Percentage of nullo-*X* ova was calculated as 100 × (wild-type males) ÷ (same denominator). Corrected percentage was calculated in the same way, but with the exclusion of progeny produced by old hermaphrodites, as explained in the text.

occurs in the sperm line. The differences are significant ($P < 0.05$) in the case of *him-1*, *him-2*, *him-4*, *him-5*, *him-7* and *him-9*. In the case of *him-3* and *unc-86*, numbers were small. The crosses with *him-6* and *him-8* gave high frequencies of nullo-*X* ova, sufficient to account for all male production. However, other experi-

TABLE 5

Production of exceptional ova by unc-4 II; lon-2 X *hermaphrodites crossed to wild-type males*

him genotype	Self progeny (Unc) Lon herm.	Lon 3X herm.	Lon males	Percent males	Cross progeny (non-Unc) Regular Wild herm.	Lon males	Exceptional Lon herm.	Wild 3X herm.	Wild males	Percent diplo-X ova	Percent nullo-X ova	Corrected percent nullo-X ova
wild type	1895	0	2	0.1	1126	1098	0	0	2	0.0	0.1	0.1
him-1	1040	67	203	15.5	805	782	11	9	48	1.0	5.6	7.7
him-2	1790	8	51	2.8	1772	1779	11	8	26	0.4	1.4	1.5
him-4(e1267)	131	1	8	5.7	246	250	2	4	5	1.6	2.0	1.8
him-5(e1467)	1031	70	225	17.0	677	646	25	20	65	2.6	8.5	8.9
him-6(e1423)	450	24	60	11.2	302	302	8	10	19	3.0	5.7	6.4
him-8	1393	370	981	35.8	554	572	92	75	379	7.4	37.6	37.6

Data obtained and presented as in Table 4, except that *unc-4*; *lon-2* hermaphrodites were used. Percentage of diplo-*X* ova was calculated as 100 × (wild-type 3*X* hermaphrodites) ÷ (wild-type hermaphrodites + wild-type males + wild-type 3*X* hermaphrodites). Percentage of nullo-*X* ova was calculated as 100 × (wild-type males) ÷ (same denominator). Corrected percentage was calculated in the same way, but with the exclusion of progeny produced by old hermaphrodites, as explained in the text.

ments (see below) indicate that both *him-8* and *him-8* can have effects on spermatogenesis. It is likely that all these mutants are affected in both gamete lines.

Tables 4 and 5 also indicate that the frequencies of nondisjunction change with the age of the animal in some of the *him* mutants, because the corrected nullo-*X* ova frequencies are not always the same as the total frequencies. Thus, nondisjunction decreases with the age of the animal in strains *him-1* and *him-5(e1467)*, whereas it rises for *him-3(e1147)*. As might be expected, the corresponding frequencies of self-progeny males also change with age, falling for *him-1* and *him-5(e1467)* and rising for *him-3(e1147)*. These differences are significant (comparing the first 48 hr of egg laying with the subsequent 72 hr; χ^2 values of 20.21 (*him-1*), 6.32 (*him-5(e1467)*), and 35.73 (*him-3(e1147)*) were observed for progeny male frequencies, using the data summarized in Table 2). The other *him* mutants show less marked changes with age. NIGON and BRUN (1955) observed differences in the appearance of meiotic figures between young and old hermaphrodites.

Diplo-*X* ova are invariably rarer than nullo-*X* ova, which is consistent with the observation that *him* strains produce more males (*XO*) than 3*X* hermaphrodites. In the crosses with Unc Lon hermaphrodites (Table 5), 3*X* hermaphrodite progeny are observed at frequencies only slightly lower than those of matroclinous Lon progeny, which shows that the 3*X* animals can be reliably recognized and do not differ greatly from 2*X* hermaphrodites in viability. Nondisjunction should lead to equal numbers of nullo-*X* and diplo--*X* gametes, so that there may be some inviability in diplo-*X* ova or also some complete loss of *X* chromosomes.

HERMAN, ALBERTSON and BRENNER (1976) observed that the rate of loss of free-*X* chromosome duplications is increased in *him-1* homozygotes.

X-chromosome behavior in spermatogenesis: The frequency of nullo-*X* ova produced by *him* hermaphrodites is not sufficient to explain all male production in most of these strains, which implies that *him* mutations affect spermatogenesis as well as oogenesis. Spermatogenesis in the hermaphrodite cannot be directly examined genetically, but spermatogenesis in the male can be. Three tests were made to see if there are detectable abnormalities in *X*-chromosome behavior in male spermatogenesis.

First, the data of Table 3 show that males of all but one of these strains produce equal numbers of haplo-*X* and nullo-*X* sperm, as do wild-type males. In the exceptional case, *him-7*, there is a slight excess of nullo-*X* sperm (52 nullo-*X*: 48 haplo-*X*). Therefore, if there is a mechanism to prevent loss of the unpaired *X* chromosome in meiosis in the male, it seems that the *him* mutations have little or no effect on it.

Second, crosses were made to try to detect diplo-*X* sperm production by *XO* males. Such sperm could be produced by *X*-chromosome nondisjunction at the second meiotic division, assuming a conventional meiosis, or by mitotic nondisjunction. In these crosses, *him*; *lon/O* males were crossed with *him-1*; *him-5 (e1467)unc-76* hermaphrodites. These hermaphrodites produce about 30%

nullo-*X* ova, because the two *him* mutations are partly additive in effect. Fertilization of a nullo-*X* ovum by a diplo-*X* sperm from the Lon male should result in the appearance of a patroclinous Lon hermaphrodite, which would be readily detected. Lon males of nine *him* genotypes (*him-1*, *him-2*, *him-3(e1147)*, *him-5(e1467)*, *him-6(e1423)*, *him-7*, *him-8*, *him-9*, *unc-86(e1416)*) were tested in this way, and in each experiment about 100 to 200 patroclinous Lon males were counted. However, no Lon hermaphrodites were observed, except for a single individual sired by a *him-1* male (total cross-progeny counts for this experiment were 281 wild hermaphrodites, 266 wild males, one Lon hermaphrodite, 139 Lon males and 16 3*X* hermaphrodites). Thus, diplo-*X* sperm occur infrequently (frequency less than 0.005) in the gametes produced by *XO* males of any of these strains.

Third, crosses were made using transformed *him* males: these animals carry two *X* chromosomes, but are phenotypically fertile males because they are homozygous for the gene *tra-1 (e1099) III* (HODGKIN and BRENNER 1977). Nondisjunction of *X* chromosomes in these males is detectable because all regular sperm carry one *X* chromosome; diplo-*X* and haplo-*X* sperm are exceptional.

Males of the appropriate *him*; *tra* genotypes were obtained from strains of genotype *him*; *unc*+/+*tra* (using *unc-69* or *unc-49* as balancers for *tra-1*: these genes map about four recombination units to the left of *tra-1*). These strains were constructed by crossing *him*; *unc* hermaphrodites with *tra-1* males and picking single F_2 *unc*+/+*tra* hermaphrodites. Those homozygous for the *him* gene were recognized by the segregation of Unc males (*XO*) in addition to non-Unc males (*XO* and 2*X*). This criterion is difficult to apply in the case of low-frequency *him* mutants, because the Unc males will be rare; therefore only four *him* strains of this type were constructed. Single non-Unc male progeny, many of which will be *tra*; *him* 2*X* males, were picked from each strain and crossed separately with marked (*unc-17 IV*) hermaphrodites. 2*X* males mate poorly even under optimal conditions, so that many crosses had to be carried out. Only those males siring predominantly hermaphrodite offspring were considered; the others sired many males and were therefore *XO* males. Many of the hermaphrodites sired by the putative *tra* 2*X* animals were progeny tested to confirm the presence of *tra-1* and the absence of *unc-49* or *unc-69*. All 3*X* hermaphrodite offspring were progeny tested and confirmed.

The pooled data from a number of crosses are given in Table 6, which shows that nondisjunction occurs in spermatogenesis of all four *him* mutants tested. As in oogenesis, more nullo-*X* gametes are produced than diplo-*X* gametes. The fre-

TABLE 6

Production of exceptional gametes by 2X (transformed) males

him genotype	Cross progeny (non-Unc) 2*X* herm.	Males	3*X* herm.	Percent nullo-*X* sperm	Percent diplo-*X* sperm
wild type	1229	3	0	0.2	0.0
him-1	250	28	18	9.5	6.1
him-5(e1467)	54	16	9	20.3	11.4
him-6(e1423)	69	6	2	7.8	2.6
him-8	266	34	10	11.0	3.2

Progeny (non-Unc) sired by *tra-1* 2*X* males crossed singly with *unc-17* hermaphrodites.

quencies of nondisjunction are similar to those observed for oogenesis, (Tables 4 and 5) except in the case of *him-8*: here the sperm line seems to be less affected than the egg line.

Origin of inviable zygotes: Some of the *him* strains produce many inviable zygotes (Table 2), which occur at too high a frequency to be explained by the fertilization of nullo-*X* ova by nullo-*X* sperm and of diplo-*X* ova by diplo-*X* sperm. Crosses were performed to determine whether these inviable zygotes arose from defects in the egg line or in the sperm line, or in both. In order to test the egg line, *him*; *dpy-11* hermaphrodites were constructed, and the frequency of inviable zygotes produced after crossing was compared with the frequency among self progeny. Under the conditions of mating used, virtually complete displacement of the endogenous hermaphrodite sperm by male sperm was observed. If the zygote inviability were due partly or wholly to abnormalities in the endogenous sperm, then the cross-progeny frequency should be lower. The results (Table 7) show different patterns in the different mutants: complete rescue is observed for *him-7*, but no rescue at all for *him-2*. The other mutants show partial rescue.

The sperm line in hermaphrodites cannot be tested directly, but the sperm line in males can be. Wild-type and *him* males were crossed with *dpy-11* hermaphro-

TABLE 7

Production of inviable zygotes from abnormal ova

him genotype		Self progeny (Dpy)	Cross progeny (non-Dpy)	Inviable zygotes	Percent inviable zygotes
wild type	self	519	0	8	1.5
	cross	1	1272	31	2.4
him-2	self	792	0	395	33.3
	cross	0	624	293	32.0
him-3(e1256)	self	51	0	379	88.1
	cross	1	214	794	78.7
him-6(e1423)	self	345	0	814	70.4
	cross	0	927	330	26.3
him-7	self	722	0	375	34.2
	cross	22	1513	21	1.3
him-9	self	479	0	102	17.6
	cross	6	1260	91	6.7

Three or four *dpy-11* hermaphrodites of each *him* genotype were picked as single L4's and allowed to produce progeny for four days, either without crossing (self) or after mating with five wild-type males for 24 hr (cross). Under these conditions of crossing, almost no self progeny are produced. Percentage of inviable zygotes was calculated as 100 × (inviable zygotes) ÷ (total live progeny + inviable zygotes).

dites, and the frequency of inviable zygotes (relative to total cross progeny) sired by these males was determined (Table 8). *him-2* and *him-9* males appear to produce normal sperm, since they sire no more inviable zygotes than do wild-type males, but the other three mutants sire substantial numbers of inviable zygotes. Two of the mutants therefore display complementary defects in these tests: *him-2* is abnormal only in the egg line, *him-7* is abnormal only in the sperm line.

It is possible that this abnormality of the *him-2* strain is not related to the *him* genotype, but is caused by another mutation. One reason for suspecting that the two phenomena are not related is that the frequency of *him-2* inviable zygotes rises markedly with temperature, but the male frequency does not (at 15°, 20° and 25°, the inviable zygote percentages observed were 12.5, 14.6 and 37.7, respectively, while the corresponding male percentages were 3.9, 2.0 and 3.2). This explanation will not suffice for the infertility of *him-3* and *him-5* mutants, because alleles of these genes fail to complement in both aspects of the phenotype.

Sperm from *him* males that sire inviable zygotes must carry dominant lethal factors of some kind. Since nematode sperm are small in size relative to ova, these factors are probably chromosomal in nature. A plausible explanation is gamete aneuploidy resulting from autosomal nondisjunction.

Autosomal nondisjunction: Nondisjunction of autosomes is more difficult to detect than nondisjunction of *X* chromosomes. It is likely that the five possible monosomics are all inviable, and the same may be true for the five trisomics. A search for monosomics was carried out by crossing *him-1 dpy-5* or *him-1*; *unc-17* hermaphrodites with males heterozygous for autosomal markers (*dpy-5 I*, *dpy-10 II*, *sma-2 III*, *unc-17 IV* or *dpy-11 V*). Each linkage group was tested separately: 2000 to 5000 cross progeny were counted in each case without finding any that expressed the paternal marker.

It is possible to detect autosomal nondisjunction events if they occur in both gamete lines, *i.e.*, if a disomic sperm fertilizes a nullisomic ovum, or *vice versa*. For the reasons given in the previous section, *him-3(e1256)* and *him-6(e1423)* are candidates for mutants with autosomal nondisjunction in both sperm and egg

TABLE 8

Production of inviable zygotes from abnormal sperm

Genotype of male parent	Cross progeny (non-Dpy)	Inviable zygotes	Percent inviable zygotes
wild type	255	2	0.8
him-2	321	7	2.1
him-3(e1256)	135	117	46.4
him-6(e1423)	209	34	14.0
him-7	147	49	25.0
him-9	259	4	1.5

Two *dpy-11* hermaphrodites (L4 or young adult) were crossed with eight to ten males of each *him* genotype for 24 or 48 hr and then transferred to a fresh culture plate. Production of inviable zygotes and cross progeny in the next 24 hr was scored; self progeny were not scored. Percentage of inviable zygotes was calculated as 100 × (inviable zygotes) ÷ (live cross progeny + inviable zygotes).

lines. Therefore, a strain of genotype *him-6 unc-30 IV*; *dpy-11 V* was constructed, and hermaphrodites of this strain crossed with *him-6* males, using *him-6(e1423)* in both parents. Self progeny will be Dpy Unc and cross progeny non-Dpy non-Unc, but if LGIV or LGV nondisjoin independently in both hermaphrodite and male, with the production of functional disomic ova and nullisomic sperm, then Unc non-Dpy or Dpy non-Unc progeny will be observed. A total of 1126 cross progeny were counted, of which six were Unc non-Dpy (two hermaphrodites, four males) and five were Dpy non-Unc (two hermaphrodites, three males). Three of the exceptional hermaphrodites (one Unc, two Dpy) segregated progeny consistent with the expected genotypes (*him unc/him unc*; *dpy/+* and *him unc/him +*; *dpy/dpy*); one Unc hermaphrodite was sterile. A control cross *unc-30*; *dpy-11* hermaphrodites with wild-type males produced 2537 wild-type cross progeny and no Dpy non-Unc or Unc non-Dpy exceptions.

The frequency of exceptional progeny in the *him-6* cross was about 0.5% for both LGIV and LGV exceptions. Experiments with the other autosomes suggested that these may nondisjoin at a lower rate: in crosses of *dpy-5 I*; *unc-4 II*; *him-6 IV* hermaphrodites with *him-6* males, 875 cross progeny were obtained, of which 1 was Unc but none was Dpy. Crosses of *unc-32 III*; *him-6 IV*; *sma-1 V* hermaphrodites with *him-6* males yielded 1043 cross progeny, of which one was Unc and one was Sma.

Pooling these data for autosomal nondisjunction gives an average frequency of 0.21% per autosome for exceptional euploid progeny produced by the fertilization of disomic ova by nullisomic sperm. The corresponding frequency for LGX can be calculated using the exceptional gamete frequencies deduced in Tables 4 and 6. Only 2*X* progeny are considered in this calculation, in order to make it comparable with the autosomal case. Taking p (the frequency of diplo-*X* ova) = 0.029, q (the frequency of nullo-*X* ova) = 0.091, r (the frequency of diplo-*X* sperm) = 0.026, and s (the frequency of nullo-*X* sperm) = 0.078, then the fraction of 2*X* progeny produced by fertilization of diplo-*X* ova by nullo-*X* sperm is $ps / (ps + qr + (1-p-q)(1-r-s)) = 0.29\%$. The rate of autosomal nondisjunction therefore appears to be similar to the rate of *X* chromosome nondisjunction.

It seems reasonable to conclude that the low fertility of *him-6(e1423)* is due largely to autosomal nondisjunction: if all five autosomes nondisjoin at about the same rate as the *X* chromosome (15%), then gametes with the normal number of autosomes will result from only 44% of meioses ($0.88^5 = 0.44$), and only 25% of zygotes will be euploid ($0.44^2 + 10 \times 0.075^2 = 0.25$). The same argument may apply to *him-3(e1256)*, but analogous experiments have not been carried out with this strain.

If this argument is correct, then the four mutants with the highest rates of *X*-chromosome nondisjunction, *him-1*, *him-5* (both alleles) and *him-8*, cannot exhibit comparably high rates of autosomal nondisjunction, because they produce relatively few inviable zygotes. This suggests that the meiotic behavior of the *X* chromosome is to some extent under independent control.

Time of nondisjunction: Some crosses were carried out to determine whether the nondisjunction observed in *him* strains occurs at an equational or a reductional division. In these experiments, *him* and wild-type hermaphrodites heterozygous for two sex linked markers and homozygous for *unc-4* (genotype *unc-4 II*; *dpy-6* + *X*/+ *lon-2 X*) were crossed with wild-type males. If nondisjunction occurs at an equational division in the absence of exchange between the markers, then diplo-*X* ova carrying two identical chromosomes (*dpy/dpy* or *lon/lon*) will result, and these will be detected as Dpy or Lon hermaphrodites if fertilized by nullo-*X* sperm from the male. On the other hand, if nondisjunction occurs at a reductional division in the absence of exchange between the markers, no Dpy or Lon hermaphrodites will be seen in the cross progeny. Fertilization by haplo-*X* sperm will lead to 3*X* hermaphrodites in both cases, but progeny testing of these animals allows distinction between those deriving from equational nondisjunction (*dpy/dpy/*+ and *lon/lon/*+) and those deriving from reductional nondisjunction (*dpy* +/+ *lon*/+ +). Four of the *him* mutants were tested in this way. The results (Table 9) show that all four exhibit detectable reductional nondisjunction with respect to these markers in oogenesis, but essentially no equational nondisjunction. It should be noted that equational exceptions (such as the single Lon hermaphrodite recorded in Table 9) do not necessarily imply equational nondisjunction of the centromere, unless the marker (in this case *lon-2*) is tightly linked to the centromere. The Lon exception could have arisen from exchange between the markers, or between *lon-2* and the centromere, followed by appropriate reductional nondisjunction of the centromeres.

These mutants are therefore similar to the majority of Drosophila meiotic mutants (BAKER and HALL 1976), which exhibit abnormalities at the reductional meiotic division (meiosis I) but not at the equational division (meiosis II). It is not known which of the two meiotic divisions in *C. elegans* is reductional, since there are some peculiarities in the appearance of meiotic figures (NIGON and BRUN 1955) that make interpretation difficult.

TABLE 9

Tests for equational versus *reductional nondisjunction*

	Cross progeny (non-Unc)							
him genotype	Wild herm.	Wild males	Wild 3*X* herm.	Dpy herm.	Dpy males	Lon herm.	Lon males	Progeny tested and confirmed 3*X* herm.
wild type	1310	77	0	0	671	0	600	—
him-1	951	116	14	0	468	0	462	13
him-5(e1467)	397	45	18	0	189	0	188	18
him-6(e1423)	972	107	28	0	504	1	456	21
him-8	880	619	118	0	412	0	413	25

Single hermaphrodites of genotype *unc-4 II*; *dpy-3* + *X*/+ *lon-2 X* were picked as L4's and crossed with five wild-type males for 24 hr. Many putative 3*X* cross progeny were picked and progeny tested (last column), either by scoring self progeny or (more usually) by crossing with wild-type males and scoring male progeny. All gave progeny consistent with the *X*-chromosome genotype *dpy-3* +/+ *lon-2*/+ +. Recombinant male progeny cannot be scored in this cross, because one class (wild type) cannot be distinguished from wild-type patroclinous males, and the other class (*dpy-3 lon-2*/*O*) cannot be distinguished from *dpy-3*/*O* males.

All 77 3*X* hermaphrodites that were progeny tested in these experiments (Table 9) gave progeny consistent with a genotype *dpy* +/+ *lon*/+ +. This suggests that nondisjoining *X* chromosomes are nonrecombinant, as is seen in nondisjunction in the recombination defective mutants of Drosophila (BAKER and HALL 1976). However, the distance between *dpy-6* and *lon-2* is not great in the wild type (9%) and it is further reduced in some of the *him* mutants (see next section). Therefore an analogous experiment was performed using the markers *dpy-8* and *unc-7*, which are less tightly linked (28% in the wild type; 16% in *him-5* homozygotes). Hermaphrodites of genotype *him-5 V*; *dpy-8*+ *X*/+*unc-7 X* were crossed with wild type males, and 44 3*X* hermaphrodite cross progeny were obtained. These animals were progeny tested by crossing with wild-type males and counting the resulting male progeny. Of the 3*X* hermaphrodites, 43 gave progeny consistent with the genotype *dpy* +/+ *unc*/+ + (total male progeny counts: 757 wild, 625 Unc, 604 Dpy and 134 Dpy Unc) and one with the genotype *dpy unc*/+ +/+ + (male progeny counts: 30 wild, three Unc, three Dpy and 13 Dpy Unc). This single animal suggests that nondisjoining chromosomes in *him-5* animals can be recombinant, albeit rarely. Both recombinant chromosomes appear to have been recovered in this animal. If exchange and nondisjunction were independent, then identifiable recombinant 3*X* progeny would arise from three-fourths of the exchange tetrads. The frequency of exchange tetrads is 32%, assuming 16% linkage for these two markers in a *him-5* background (Table 10). Therefore, the expected number of recombinant 3*X* progeny is 11 out of 44 (0.75 × 0.32 × 44), as opposed to the one out of 44 observed.

Effects on recombination: A number of preliminary experiments were carried out to examine the effects of *him* mutations on recombination. Data for intervals

TABLE 10

Effect of him *genotype on linkage on LGX*

	Segregation from *dpy-3 lon-2 X*/+ +			Segregation from *lon-2 unc-7 X*/+ +		
him genotype	Frequency of Lon recombinants	Percent linkage	χ^2 relative to wild type	Frequency of Lon recombinants	Percent linkage	χ^2 relative to wild type
wild type	60/1374	9.2	—	153/1288	27.6	—
him-1/+	70/1357	10.9	0.77	185/1226	37.0	4.82*
him-1/him-1	24/790	6.3	2.28	44/716	13.1	15.39***
	(4/144)	(5.6)	—	(11/154)	(14.3)	—
him-5/+	74/1267	9.6	0.05	162/1415	26.4	0.11
him-5/him-5	17/727	3.4	10.24**	54/752	15.6	10.33**
	(4/217)	(3.7)	—	(19/183)	(20.8)	—
him-8/+	84/1422	12.6	3.22	163/1520	24.4	0.83
him-8/him-8	4/1068	0.7	36.77***	15/980	3.1	80.46***
	(13/690)	(3.8)	—	(18/515)	(7.0)	—

Three to nine hermaphrodites of each genotype were picked as L4's and their total self progeny scored. Scores are given as (Lon hermaphrodites)/(total hermaphrodites). Scores in brackets are (Lon males)/(total males). *, **, *** indicate significance at 5, 1, and 0.1% levels, respectively, for differences between mutant and wild-type scores. The *him-5* allele was *e1467*.

TABLE 11

Effect of him *genotype on linkage on LG*I, *LG*II, *LG*III

him genotype	Segregation from *dpy-5 unc-54 I/+ +* Frequency of recombinants(a)	Percent linkage	χ^2 relative to wild type	Segregation from *dpy-10 unc-52 II/+ +* Frequency of recombinants(b)	Percent linkage	χ^2 relative to wild type	Segregation from *dpy-1 unc-32 III/+ +* Frequency of recombinants(a)	Percent linkage	χ^2 relative to wild type
wild type	130/812	27.9	—	188/1295	24.9	—	220/1169	21.0	—
him-1/+	145/927	27.1	0.04	———	—	—	265/1324	22.5	0.46
him-1/him-1	161/836	35.0	2.46	186/1100	29.8	2.18	272/1225	25.4	3.33
him-5/him-5	132/709	33.5	1.49	161/984	28.6	1.24	238/1007	27.6	5.96*

Three to nine L4 hermaphrodites of each genotype were picked and their total self progeny scored. Scores in columns marked (a) are (Unc non-Dpy progeny + Dpy non-Unc Progeny) / (total progeny). Scores in columns marker (b) are (Dpy progeny) / (total non-Unc progeny). In the latter cases, the Unc and Unc Dpy worms have reduced viability and are therefore not included in the final scores. *, **, *** as inTable 13. The *him-5* allele was *e1467*.

TABLE 12

Effect of him *genotypes on linkage on LG*IV, *LG*V

him genotype	Segregation from *dpy-9 unc-17 IV/+ +* Frequency of recombinants(a)	Percent linkage	χ^2 relative to wild type	Segregation from *unc-60 dpy-11 V/+ +* Frequency of recombinants(b)	Percent linkage	χ^2 relative to wild type	Segregation from *dpy-11 unc-51 V/+ +* Frequency of recombinants(a)	Percent linkage	χ^2 relative to wild type
wild type	360/1413	30.0	—	105/777	22.9	—	398/1539	30.5	—
him-1/+	318/1214	31.0	0.13	97/1030	15.3	6.65**	———	—	—
him-1/him-1	440/2213	22.4	12.24***	59/419	24.0	0.06	———	—	—
him-5/+	———	—	—	103/831	22.7	0.39	398/1521	31.0	0.03
him-5/him-5	268/1677	17.5	34.03***	28/471	9.4	15.76***	298/1038	34.7	1.86
him-8/+	———	—	—	79/911	14.0	9.02**	210/833	29.6	0.09
him-8/him-8	———	—	—	106/982	17.8	2.67	324/1124	34.9	2.11

Data obtained and presented as in Table 11.

on the *X* chromosome are summarized in Table 10; data for autosomal intervals in Tables 11 and 12. Exhaustive analysis of recombination in *him* mutants would have been arduous and was not attempted. No extensive study of recombination in *C. elegans* has yet been carried out, so that little is known about environmental and genotypic effects on meiotic parameters. Quantitative crosses involving more than two linked markers are difficult in *C. elegans* because of the similarity of many of the marker phenotypes (predominantly Dpy and Unc), and because of epistatic interactions between many markers. Scoring of crosses in *him* backgrounds is difficult because some phenotypes are difficult to distinguish in 3*X* hermaphrodites. Finally, it is possible that some of the differences listed in Tables 10 to 12 are due to other factors, such as extraneous EMS-induced mutations, rather than to the *him* mutations themselves.

The following conclusions seem justified: *him-1*, *him-5*, and *him-8* all reduce recombination on the *X* chromosome when homozygous. *him-1* and *him-5*, and probably *him-8*, have less effect on autosomal intervals tested, and may in fact increase recombination in some of these intervals. This could be the result of an interchromosomal effect (LUCCHESI 1976), though such an effect has yet to be demonstrated in *C. elegans*. The interval on the left of LGV (*unc-60* to *dpy-11*) and the interval on LGIV (*dpy-9* to *unc-17*) seem to be more sensitive to the *him* genotype than the four other autosomal intervals tested. Heterozygote effects are apparent in some of the data for *him-1*/+ and *him-8*/+ backgrounds.

Linkage measurements for the *X* chromosome in *him* animals were all based only on hermaphrodite counts, for the sake of consistency with other linkage measurements. Similar values are observed in self-progeny male counts (data bracketed in Table 10). Most of the *X* chromosomes in these males must have arisen from meioses in which *X* chromosomes disjoined normally, yet recombination on these chromosomes was reduced. Therefore, these *him* mutations appear to have effects on meiosis beyond simply increasing nondisjunction.

The coefficient of coincidence (*C*, the ratio of the observed frequency to the expected frequency of double crossovers) has been used as a diagnostic parameter in the characterization of Drosophila meiotic mutants (CARPENTER and SANDLER 1974). We therefore tried to obtain an estimate of coincidence for the *X* chromosome by looking for Lon males among the progeny of hermaphrodites of genotype *dpy-3 lon-2 unc-7*/+ + +, either after crossing with wild type males or in the self progeny of *him* hermaphrodites. However, no Lon males were observed, even in the wild-type background: 2002 male progeny were counted in crosses of *him*(+) heterozygous hermaphrodites with wild-type males, and none was Lon. It is possible that one or two Lon males might have been mis-scored as wild, but even so the number would be far less than the 25 expected (taking *dpy-lon* linkage as 9%, and *lon-unc* as 28%). Also, no Lon males were found among the self-progeny males of four *him*; *dpy-3 lon-2 unc-7*/+ + + strains (0 of 125 for *him-1*, 0 of 165 for *him-5(e1467)*, 0 of 49 for *him-6(e1423)*, and 0 of 498 for *him-8*). At least one autosome does have a measurable *C* value, however: crosses of *dpy-9 unc-17 dpy-4 IV* hermaphrodites with *dpy-9 unc-17 dpy-4*/+ + +

males produced eight Unc non-Dpy (*i.e.*, + *unc* +/*dpy unc dpy*) in approximately 1000 cross progeny; the predicted number (taking *dpy-9—unc-17* linkage as 24% and *unc-17—dpy-4* as 15%) was 18. Further experiments on double recombination have not been carried out, because most of the possible experiments are difficult to carry out. It would be intriguing if high interference were confined to the *X* chromosome (or, alternatively, to the hermaphrodite) in this organism.

Other effects of him *mutations*: Mutations affecting chromosome behavior may possibly also exhibit increased sensitivity to radiation or to mutagens, (BAKER *et al.* 1976a) and may display mutator activity (GREEN 1976). Radiation and mutagen sensitivity have not been tested extensively in any of the *him* strains. With regard to mutator activity, one of the *him* strains (*him-1*) was observed to segregate at least four spontaneous mutants in the course of this work (including alleles of *bli-1 II* and *sma-2 III*), a rate apparently higher than that of the wild-type or the other *him* strains. A specific test for mutator activity by *him-1* was therefore made by comparing reversion frequencies of two severely uncoordinated mutants in wild-type and *him-1* backgrounds. The mutants used were *unc-17(e245) IV* and *unc-58(e665) X*; revertants of these strains are easily found after mutagenesis with EMS (or other mutagens). Revertants of *e245* arise by the induction of dominant suppressor mutations at any of several different loci unlinked to *unc-17*, whereas revertants of *e665* arise by mutations at the *unc-65* locus or close to it (HODGKIN 1974). Twenty 9-cm plates each of *unc-17(e245)* and of *him-1*; *unc-17(e245)* were grown to starvation; no revertant (non-Unc) animals were observed on these plates, which contained a total population of about 10^6 animals in each case. Forty-five 9-cm plates of *unc-58 (e665)* and of *him-1*; *unc-58(e665)* were grown to starvation and three revertants were obtained from each set, out of a total population of about 4×10^6 in each case. Thus, no increase in reversion frequency under the influence of *him-1* was observed for either of these mutants, so that it seems unlikely that *him-1* has any significant mutator activity.

As mentioned in the introduction, we hoped that some of the mutants would exhibit mosaicism as a result of mitotic chromosome loss. However, we have no proven assay for mosaicism as yet, and it is not even known if a gynandromorph (*i.e.*, an animal some of whose cells were *XO* and some *2X*) would have a recognizable phenotype. Many *him* animals heterozygous for a variety of sex-linked markers were constructed in the course of this work, but none was demonstrably mosaic. However, the markers used may not have been cell autonomous in their expression. Moreover, cell lineages in *C. elegans* are short (SULSTON and HORVITZ 1977), so that mosaics within a tissue may be prohibitively rare. Conceivably, cytochemical markers may allow a more organized approach to this problem in the future.

X *chromosome behavior in* 3X *hermaphrodites*: The short animals segregated by *him* strains were assumed to be *3X* hermaphrodites in the experiments described above, and all the data are consistent with this karyotype. A further

proof of the presence of three X chromosomes in the short animals was obtained by constructing hermaphrodites carrying three differently marked X chromosomes (Table 13, first row).

This was achieved by crossing *him-1 I*; *unc-18 X* hermaphrodites with *him-1 I*; *dpy-6 X* males, to give a fairly stable heterozygous line (*him-1*; *dpy-6*+/+*unc-18*). Short non-Dpy non-Unc animals from this line were crossed with *him-1 I*; *lon-2 X* males, and the short progeny were picked singly and allowed to generate self progeny. One produced all three mutant phenotypes (Dpy, Unc, and Lon) in the next generation, and from this a triply marked line (genotype *him-1*; *lon* + +/+ *dpy*+/+ + *unc*) was established and maintained for six generations by picking single short animals at each generation and progeny testing them. Animals of all seven possible triple heterozygous genotypes were observed; pooled counts for the seven classes are given in Table 13. About 60 progeny were counted from each animal, but these counts are not very accurate because the shortness of the 3X animals interferes with the recognition of Dpy and Lon phenotypes, and *vice versa*. A total of 23 animals were observed that segregated all three mutant phenotypes in numbers consistent with the proposed 3X karyotype.

In the absence of X chromosome loss, one might expect that 3X hermaphrodites would segregate self progeny in the ratio of one 2X : two 3X : one 4X, but these ratios were not observed (Table 14). The data show that 2X hermaphrodites account for almost half the progeny in *him*(+) animals, and more than half in all the *him* strains tested. Many inviable zygotes are also produced. The ratios observed in the wild type can be roughly explained by assuming that gametes with one and two X chromosomes are produced in a ratio of 2:1, and that 4X animals die before hatching. This will generate progeny ratios of four 2X hermaphrodite : four 3X hermaphrodite : one 4X inviable zygote (as compared to the observed ratios of 4:4.2:1.6).

Production of exceptional ova by 3X hermaphrodites was assayed as in Table 5 by crossing *unc-4 II*; *lon-2 X* 3X hermaphrodites (both *him*(+) and *him*) with wild-type males. The data (Table 15) show that haplo-X and diplo-X ova are produced in a 2:1 ratio, as predicted. Therefore, a deficiency of diplo-X ova occurs as in 2X hermaphrodites. The data (Tables 14 and 15) also show that *him* muta-

TABLE 13

Self-progeny broods of marked 3X hermaphrodites

Assumed X-chromosome genotype	Number of broods scored	Wild herm.	Wild males	Wild 3X herm.	Unc herm.	Unc males	Dpy herm.	Dpy males	Lon herm.	Lon males
lon + +/+ *dpy* +/+ + *unc*	23	471	1	559	95	4	80	6	103	7
lon + +/*lon* + +/+ *dpy* +	7	143	0	164	0	0	26	1	116	6
lon + +/+ *dpy* +/+ *dpy* +	8	119	0	145	0	0	124	2	23	3
lon + +/*lon* + +/+ + *unc*	4	70	0	68	13	4	0	0	53	5
lon + +/+ + *unc*/+ + *unc*	9	130	0	139	140	4	0	0	30	2
+ *dpy* +/+ *dpy* +/+ + *unc*	5	89	0	105	16	0	95	2	0	0
+ *dpy* +/+ + *unc*/+ + *unc*	5	63	0	72	63	3	22	1	0	0
ambiguous	17									

See text. Ambiguous animals produced too few progeny to be typed with certainty, or carried recombinant chromosomes. The Dpy, Unc, and Lon hermaphrodite counts include both 2X and 3X hermaphrodites.

TABLE 14

Self-progeny broods of 3X hermaphrodites

him genotype	Number of broods counted	Average brood size	Percent males	Percent 3X herm.	Percent inviable zygotes
wild type	6	181	0.1	51.3	16.5
him-1	5	115	3.1	49.0	22.6
him-2	5	92	1.1	42.5	43.8
him-3(e1147)	5	113	0.4	45.3	26.9
him-3(e1256)	5	25	4.0	44.0	89.9
him-4(e1266)	7	5	2.7	29.7	—
him-4(e1267)	6	30	4.5	36.9	—
him-5(e1467)	5	77	5.7	32.5	39.3
him-6(e1423)	6	14	1.2	34.1	—
him-6(e1104)	6	83	1.2	32.1	44.2
him-7	5	74	1.6	46.5	50.2
him-8	5	158	11.4	35.4	11.4
unc-86(e1416)	4	33	0.0	43.8	12.2

Data obtained and presented as in Table 2. The *him-4* data refer only to fertile 3*X* hermaphrodites, so that the mean *him-4* brood sizes are overestimates (unlike those in Table 2). The *him*(+) 3*X* strain was obtained as described in the legend to Figure 2.

tions are expressed even in 3*X* hermaphrodites, since increased numbers of nullo-*X* ova and self-progeny males are produced by *him* 3*X* hermaphrodites as compared to *him*(+) 3*X* hermaphrodites.

One of the crosses mentioned above in the experiments on time of nondisjunction allows a measurement of *X* chromosome recombination in 3*X* hermaphrodites. In this cross, *him-5*/+; *dpy-8* +/+ *unc-7*/+ + animals were crossed with wild-type males, and 134 Dpy Unc male progeny were found among 2120 male progeny. If we assume that the three possible *X* chromosome bivalents form independently in 3*X* hermaphrodites, then this frequency is given by $p/6$, for linkage p, and $p = 37.9\%$. This value is higher than that observed in a control

TABLE 15

Production of exceptional ova by unc-4 II; lon-2 X 3X *hermaphrodites crossed to wild-type males*

	Cross progeny (non-Unc)						
	Regular		Exceptional				
him genotype	Wild herm.	Lon males	Lon herm.	Wild 3X herm.	Wild males	Percent diplo-X ova	Percent nullo-X ova
wild type	205	208	118	116	0	36.1	0.0
him-1	138	140	71	63	0	31.3	0.0
him-5(e1467)	104	115	69	51	9	31.1	5.5
him-6(e1423)	76	74	42	38	3	32.5	2.6
him-8	214	222	102	95	61	25.7	16.5

Data presented as in Table 5. All hermaphrodites were crossed and scored singly. Self-progeny scores were not made. The *him*(+) 3*X* strain was obtained from among the *unc-4*; *lon-2* 3*X* F_2 progeny of a cross *unc-4*; *him-5*(*e1467*); *lon-2* 3*X* hermaphrodites with wild type males.

experiment: in a cross of *him-5*/+; *dpy-8* +/+ *unc-7* with wild-type males, 79 Dpy Unc males appeared among 594 male progeny, giving $p = 26.6\%$. Therefore, *X*-chromosome recombination is not reduced in 3*X* animals, and may in fact be enhanced.

DISCUSSION

We have demonstrated the existence of a number of genes (*him-1* to *him-9* and *unc-86*) that affect the stability of the *X* chromosome in *C. elegans*. We examined 15 mutations, and most of their properties are summarized in Table 16. Not all mutants were put to all tests, but enough data were accumulated to show that each mutant exhibits a distinctive set of properties. Mutations in each of the ten genes have been shown to cause excess production of exceptional ova carrying either none or two *X* chromosomes and, consequently, the production of unusual numbers of *XO* males and 3*X* hermaphrodites in self progeny. It is likely that the same effects occur in the sperm line of mutant hermaphrodites: at least some of the mutations cause increased *X*-chromosome nondisjunction in the sperm line of 2*X* (transformed) males. However, the behavior of the unpaired *X* chromosome in *XO* males appears to be normal or almost normal in all of the mutants. In the four cases tested, nondisjunction occurs at a reductional division in oogenesis. Recombination was examined in three of these mutants and shown to be significantly altered for some intervals, particularly on the *X* chromosome. Nondisjoining *X* chromosomes appear to be generally nonrecombinant. These observations show that at least some of the mutants affect meiosis.

Autosomal nondisjunction has been demonstrated in one mutant, *him-6 (e1423)*, but some of the other mutants are probably normal or almost normal in this respect, because they produce relatively few inviable zygotes. However, almost all produce more inviable zygotes than would be expected if zygotic lethality were due only to the production of nullo-*X* and 4*X* zygotes. One exception to this rule is *him-8*: if the exceptional gamete frequencies listed in Table 16 are used, then the predicted frequency of inviable zygotes is 4.4%, as opposed to 0.8% observed. Possibly the sperm line in hermaphrodites is less affected than the sperm line in 2*X* males, in which case the predicted frequency would be lower.

The *him* mutants producing the highest frequencies of males (*him-1*, *him-5* and *him-8*) all produce inviable zygotes at a much lower rate than that expected if the autosomes were nondisjoining at the same rate as the *X* chromosome. This suggests that these mutants have a specific or preferential effect on the *X* chromosome. The data on recombination in these mutants, while incomplete, tend to support this conclusion. Therefore, there are differences between the control of the autosomes and the control of the *X* chromosome during meiosis. Another difference between the *X* chromosome and the autosomes is seen in the genetic map. The *X* chromosome does not have a pronounced gene cluster, whereas all the autosomes do. This difference is more obvious in the current map (H. R. HORVITZ, unpublished) than in the original map (BRENNER 1974). Also, as noted in this paper, interference may be higher on the *X* chromosome than on the autosomes.

TABLE 16

Summary of properties of him *mutants*

Gene allele	Relevant tables	*him-1 I*	*him-2 I*	*him-3 IV*		*him-4 X*		*him-5 V*		*him-6 IV*		*him-7 V*	*him-8 IV*	*him-9 II*	*unc-86 III*	
	—															
	1	*e879*	*e1065*	*e1147*	*e1256*	*e1266*	*e1267*	*e1467*	*e1490*	*e1423*	*e1104*	*e1480*	*e1489*	*e1487*	*e1416*	*e1507*
Hermaphrodite effects:																
Percent wild-type fertility	2	63.3	65.8	107.0	13.6	5.8	6.7	60.0	65.8	13.0	55.8	55.2	91.6	63.3	31.6	27.0
Percent inviable zygotes	2	5.8	14.6	1.4	70.9	—	—	17.6	14.1	78.4	16.4	34.1	0.8	15.9	3.5	2.0
Percent lethal ova	7	—	32.0	—	78.7	—	—	—	—	26.3	—	1.3	—	6.7	—	—
Percent males	2	20.6	2.0	3.5	10.9	7.6	6.0	16.4	32.9	15.3	5.0	2.9	36.7	4.5	2.2	2.4
Percent nullo-*X* ova	4,5	7.6	1.5	2.3	—	—	1.8	10.7	—	7.9	—	0.6	37.9	2.2	0.8	—
Percent 3*X* hermaphrodites	2	5.6	0.9	1.1	1.2	—	—	3.2	6.7	5.8	1.1	1.0	6.4	1.8	1.1	0.8
Percent diplo-*X* ova	4,5	1.0	0.4	0.8	—	—	0.9	3.1	—	2.2	—	0.1	8.6	0.9	0	—
Percent w.t. recombination (LGX)	10	58	—	—	—	—	—	47	—	—	—	—	9	—	—	—
Percent w.t. recombination (LGI-LGV)	11,12	109	—	—	—	—	—	97	—	—	—	—	96	—	—	—
Nature of *X* nondisjunction	9	—*	—	—	—	—	—	—*	—	—*	—	—	—*	—	—	—
Autosomal nondisjunction	—	—	—	—	—	—	—	—	—	yes	—	—	—	—	—	—
Male effects:																
XO ♂ Autosomal nondisjunction	—	—	—	—	—	—	—	—	—	yes	—	—	—	—	—	—
XO ♂ Percent wild type fertility	3	1.4	23.5	74.1	5.4	0	0	62.4	55.2	30.3	38.3	32.3	8.8	89.1	7.2	—
XO ♂ Percent lethal sperm	8	—	2.1	—	46.4	—	—	—	—	14.0	—	25.0	—	1.5	—	—
XO ♂ Percent nullo-*X* sperm	3	49.4	50.4	49.1	53.9	—	—	51.2	49.7	48.8	50.4	52.4	53.2	50.4	50.9	—
XO ♂ Percent diplo-*X* sperm	—	0.4	0	0	—	—	—	0	—	0	—	0	0	0	0	—
XX ♂ Percent nullo-*X* sperm	6	9.5	—	—	—	—	—	20.3	—	7.8	—	—	11.0	—	—	—
XX ♂ Percent diplo-*X* sperm	6	6.1	—	—	—	—	—	11.4	—	2.6	—	—	3.2	—	—	—

* Reductional.

Data are expressed as in the relevant tables: thus percent inviable zygotes, percent lethal ova and percent lethal sperm are calculated relative to total zygotes, not total viable progeny. "Lethal ova" and "lethal sperm" refer to gametes carrying dominant lethal factors. Percent nullo-*X* ova and percent diplo-*X* ova are calculated using corrected counts (see legend to Table 4). Where two values were obtained, the average is taken. Percent of wild-type recombination (LGX) is calculated as the average of all intervals tested on LGX; percent of wild-type recombination (LGI-LGV) is the average of all autosomal intervals tested.

Mechanisms for independent control of the *X* chromosome in *C. elegans* may have evolved in order to modulate the degree of outbreeding in populations of this organism (HEDGECOCK 1976), and also in order to handle the unpaired *X* chromosome during meiosis in the male. However, none of the *him* mutants has much effect on the behavior of the unpaired *X*.

A few other conclusions about meiosis in *C. elegans* can be drawn from the present study. The fact that the *him* mutants appear to affect both gamete lines of the hermaphrodite (and in some cases the sperm line of the male as well) suggests that the control of meiosis is basically the same in all gametogenesis in this organism. A bias is detectable in some of the mutants, however: *him-8* seems to have more severe effects on oogenesis, and *him-7* on spermatogenesis. The experiments on the crossover status of nondisjoining chromosomes suggest that exchange may be necessary for proper disjunction in *C. elegans*, as in many other organisms, and hence that *C. elegans* does not have a secondary system for disjunction like distributive pairing (GRELL 1976), but more data are needed. The observations on *X*-chromosome behavior in 3*X* hermaphrodites show that the extra *X* chromosome is easily lost in these animals (presumably during meiosis), and that the *him*(+) gene products are needed to control *X* chromosome behavior in 3*X* hermaphrodites. It remains to be seen whether any of the five other trisomics are viable.

In other organisms, meiotic mutants usually have effects over the whole chromosome complement: *him-6(e1423)* appears to be a mutant of this type, and *him-3(e1256)* is a good candidate for another. The low-frequency *him* mutants (*him-2*, *him-4*, *him-7*, *him-9* and *unc-86*) could also have general effects, but it would be difficult to demonstrate this in view of their weak phenotypes. Chromosome-specific mutants such as *him-1*, *him-5* and *him-8* are unusual. One such mutant has been reported in the plant Hypochoeris (PARKER 1975); it is not known whether the mutation involved is linked to the chromosome concerned or not. Work on Schizophyllum and other fungi has revealed the existence of "fine control" meiotic mutations that are region-specific in action: some are linked to the region affected, and some are not (STAMBERG and KOLTIN 1973). In Drosophila, meiotic mutants with specific effects on chromosome *2* have been described by GETHMAN (1974). The closest parallel to these *him* mutants is provided by *mei-1* (VALENTIN 1973), an autosomal recessive that affects only the *X* chromosome. The differences between the genetic systems of Drosophila and *C. elegans* vitiate detailed comparisons.

From a technical point of view, some of the *him* mutants should be useful for the production of large quantities of males for biochemical purposes, particularly *him-5(e1490)* and *him-8*. Either of the *him-5* alleles is useful for male production for genetic purposes, because the males are produced at reasonably high frequency and have higher fertility than most of the other *him* males. Sometimes a low-frequency *him* mutant is more convenient, for example in mutant hunts (mutants are difficult to find in high-frequency *him* cultures because the high degree of outcrossing reduces the expression of recessive mutations). Strains

carrying *him-2* were used successfully in a hunt for male-specific mutants (HODGKIN 1974), but *him-6(e1104)* or *him-9* might be more convenient for this purpose.

It is certain that more *him* mutants will be isolated as studies of *C. elegans* continue, and one of the purposes of this work is to indicate ways in which *him* mutants and other meiotic mutants can be characterized. A new high-frequency *him* mutant (*him-10(1511) III*) has already been isolated (M. CHALFIE, personal communication), and it is likely that this class of mutant is far from saturation. *C. elegans* may prove to be a favorable organism for the study of meiosis. Although its genetics is obviously less sophisticated than that of Drosophila, it is superior to that of most other metazoa and has a number of particularly attractive features. Furthermore, much of the anatomy of wild-type *C. elegans* has been described at the electron microscope level, so that ultrastructural investigations are facilitated. In addition, a biochemical approach may be possible because the majority of the nuclei in adults of either sex belong to the germ line (HIRSH, OPPENHEIM and KLASS 1976; KLASS, WOLF and HIRSH 1976) and are either undergoing meiosis or are destined for meiosis. Collection of biochemical quantities of meiotic material may therefore be feasible.

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