DUPLICATIONS IN *CAENORHABDITIS ELEGANS*

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ABSTRACT

Thirteen chromosomal duplications, all unlinked to their linkage group of origin, have been identified following X-irradiation. Ten are *X*-chromosome duplications, of which six are half-translocations on three autosomomal linkage groups and four are free fragments. Five of the half-translocations are homozygous fertile and two are recognizable cytologically as chromosome satellites, both of which show some mitotic instability. The free-*X* duplications show varying tendencies for loss. Three appear not to overlap in extent previously identified free-*X* duplications. The fourth carries genes from linkage group *V*, as well as *X*. Three duplications of a portion of linkage group *II* were identified and found to be free and quite stable in hyperploids. Some of the free duplications tend to disjoin from the *X* chromosome in males. New *X*-chromosome map data are presented.

THE small free-living nematode, *Caenorhabditis elegans*, is a simple metazoan possessing many advantages for the genetic analysis of development and behavior (BRENNER 1974). If the full power of genetic analysis is to be exploited in *C. elegans*, however, certain tools of genetic research, such as those utilized for Drosophila over the past 60 years, need to be developed. One such tool is the chromosomal duplication. An *X*-chromosome duplication that is translocated to an autosome has been used, for example, to balance a number of *X*-linked lethals, steriles and deficiencies in *C. elegans* (MENEELY and HERMAN 1979). Duplications can also be used to vary the dosage of particular chromosomal segments to help identify genes of special interest (HALL and KANKEL 1976).

In a previous paper (HERMAN, ALBERTSON and BRENNER 1976), five unlinked *X*-chromosome duplications, all overlapping one region of the *X* chromosome of *C. elegans*, were identified. One was a half-translocation, homozygous sterile, and the other four were free duplications, all showing some tendency for loss. In this paper we report six new half-translocations, five of which are homozygous fertile. We also report four new free-*X* duplications, three of which appear not to overlap previously identified free fragments. Finally, we describe a procedure for identifying autosomal duplications and describe the properties of three duplications, all free, of a region of linkage group *II*.

MATERIALS AND METHODS

Strains, growth, nomenclature, and mating and counting procedures: N2 (wild type) and

mutant strains of *Caenorhabditis elegans* var. Bristol were obtained from the collection of S. BRENNER and grown as described by BRENNER (1974). The following genes and alleles were used. *LGI*: *dpy-5(e61)*, *unc-54(e190)*. *LGII*: *bli-2(e768)*, *dpy-10(e128)*, *unc-4(e120)*, *unc-53(e404)*, *unc-52(e444)*. *LGIII*: *dpy-18(e364)*. *LGIV*: *dpy-9(e12)*, *unc-17(e245)*, *dpy-13(e184)*, *dpy-4(e1166)*. *LGV*: *unc-60(e677)*, *unc-46(e177)*, *dpy-11(e224)*, *unc-23(e324)*, *sma-1(e30)*, *unc-42(e270)*, *unc-41(e268)*, *unc-39(e257)*, *unc-51(e369)*. *LGX*: *unc-1(e94)*, *dpy-3(e27)*, *unc-2(e55)*, *unc-20(e112)*, *unc-78(e1217)*, *dpy-8(e130)*, *lon-2(e678)*, *unc-6(e78)*, *dpy-7(e88)*, *unc-18(e81)*, *unc-10(e102)*, *dpy-6(e14)*, *unc-9(e101)*, *unc-84(e1410)*, *unc-3(e151)*, *unc-7(e5, e139)*.

Linkage relationships among these loci are shown in Figure 1, which has incorporated in it new map data cited in the RESULTS.

The nomenclature used in this paper for gene and allele names is in accord with a system recently proposed by H. R. HORVITZ (personal communication) after consultation with many *C. elegans* workers. Typical phenotypic abbreviations used in this paper, in agreement with the proposed nomenclature system, are Dpy non-Unc and Unc-3 (which is distinguishable from Unc-54). Also in accord with the proposed nomenclature system are our duplication names, an example being *mnDp8* or, optionally, *mnDp8(X;I)*, where *mn* is a prefix identifying our laboratory. Adoption of this format has led us to alter the names of the five duplications previously described (HERMAN, ALBERTSON and BRENNER 1976), so that, for example, *Dp(X;V)1* is hereafter referred to as *mnDp1* or *mnDp1(X;V)*.

Mating and counting procedures were as previously described (HERMAN 1978). Errors given in the tables are estimated standard errors of the means.

Identifying unlinked X *duplications*: Let *m* signify an *X*-linked recessive visible mutation and *Dp* signify a duplication. Partially synchronized N2 populations containing many males were X irradiated at a dose of 7,000 to 8,000 roentgens and crossed with *m/m* hermaphrodites by procedures previously described (HERMAN, ALBERTSON and BRENNER 1976). The progeny were screened for rare wild-type males, which were backcrossed to *m/m* hermaphrodites. The issuance of wild-type males (*Dp/m/O*) from the latter cross signified the transmission of an unlinked *X* duplication, since cross-progeny males normally inherit their one *X* chromosome from their mothers. *X*-linked mutants used were *dyp-3*, *unc-2*, *unc-20*, *dpy-8*, *unc-6*, *dpy-6*, and

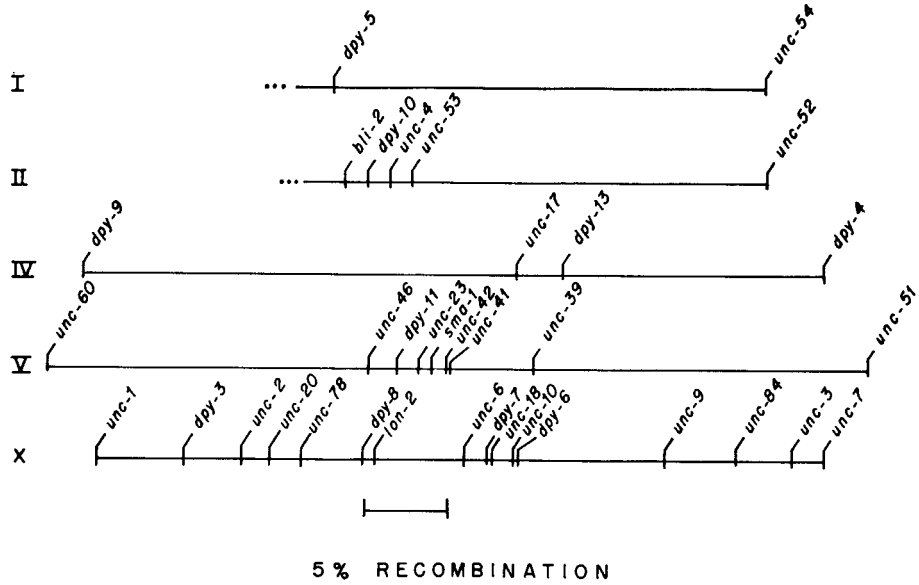

FIGURE 1.—Linkage relationships among loci used in this work. Markers not used are not shown.

unc-3. From the runs using *unc-3*, 203 wild-type male progeny, comprising 1.4% of the male progeny screened, were picked. For the other six mutants, the number of wild-type males picked ranged from 16 to 39, corresponding to 0.2 to 0.7% (weighted average = 0.3%) of the male progeny. The vast majority of these wild-type males were sterile. Indeed, even the fertile ones were generally not nearly as fertile as N2 males. One duplication of *unc-20*+, two of *dpy-8*+, one of *unc-6*+, and 21 of *unc-3*+ were recovered.

Wild-type hermaphrodites of genotype *Dp/m/m* were picked to establish temporary duplication stocks, which were maintained by picking single wild-type self-progeny each generation. These stocks were put through the following backcrossing regime. Wild-type male progeny from the mating of *Dp/m/m* and N2 males were crossed with *m/m*, from which wild-type hermaphrodite progeny were picked. This procedure was then repeated to establish our standard *Dp/m/m* stocks.

The genetic extent of each duplication with respect to other *X*-linked visible markers was usually checked by simply crossing *Dp/m/O* males with various mutant hermaphrodites and screening for the presence or absence of wild-type males among the male cross-progeny. This procedure was modified in the case of *mnDp30* because of the high frequency of loss of this duplication and because *mnDp30*-bearing males were not very potent. Once we had shown that *mnDp30* covered both *dpy-8* and *unc-6*, all the other markers were checked in double mutants; for example, *dpy-3* was checked as follows: *mnDp30/unc-6/O* was mated with *dpy-3 unc-6* hermaphrodites, and either the cross-progeny were screened for the presence of non-Unc males to see whether or not they were Dpy, or wild-type hermaphrodites were picked to see whether or not they segregated Dpy non-Unc progeny (*mnDp30/dpy-3 unc-6/dpy-3 unc-6*). This procedure was also used for most markers in the case of *mnDp32*.

It should be noted that in these genetic tests the duplications behave like dominant suppressor mutations, and the extent of each duplication is assessed by determining which mutations are suppressed by it. The supposition that duplications of wild-type alleles are in fact responsible for the suppressions is supported by the suppression patterns, which generally show that a contiguous set of markers is suppressed (see RESULTS). Moreover, in several cases duplications are implicated cytologically.

The procedure for assaying linkage between an *X* duplication and an autosomal marker is illustrated as follows. *mnDp8/unc-3/O* and *dpy-5 I*; *unc-3 X* were crossed. Wild-type hermaphrodite progeny were picked and their self-progeny were counted. The frequency of recombination between *mnDp8* and *dpy-5* was calculated from the frequency of non-Dpy Unc plus Dpy non-Unc animals (BRENNER 1974). Recombination between the duplications and the *X* chromosome appears to be negligible (see RESULTS). In certain cases only one of the recombinant phenotypes was distinguishable from nonrecombinant types; for example, only Unc-3 non-Unc-54 recombinants can be recognized among the progeny of *mnDp8/unc-54*; *unc-3* because *unc-54* is epistatic to *unc-3*.

Identifying unlinked duplications of unc-52+ II: *C1* is a dominant crossover suppressor specific for *LGII* (HERMAN 1978). It is tightly coupled to *dpy-10* and *unc-52* on *LGII* and segregates independently of all other linkage groups. Nearly 100% of the eggs laid by *C1* heterozygotes hatch to give viable and fertile adults, including *C1* homozygotes, but rare recombinant chromosomes are homozygous inviable. Thus, *C1* appears to be an intrachromosomal rearrangement. From a *C1 dpy-10 unc-52/unc-4* stock, a spontaneous wild-type male was recovered and used to establish a *C1 dpy-10 unc-52/unc-4* stock containing many males. The continued presence of males was insured by periodically setting up wild-type male by wild-type hermaphrodite crosses.

C1 dpy-10 unc-52/unc-4 males, 48 to 62 hours old as measured from the time of egg laying, were X-irradiated at a dose of 7,500 roentgens and subsequently mated with *unc-4 unc-52* hermaphrodites. Rare wild-type animals among the progeny were picked. Wild-type males were backcrossed to *unc-4 unc-52* to obtain wild-type hermaphrodites. Out of several candidates, three of independent origin were retained for further study. Two of these gave progeny ratios similar to those reported in Table 5. The third stock segregated only wild-type, Unc-52 (*unc-52* is epistatic to *unc-4*) and Unc-4 progeny, but carried a *dpy-10 unc-52* chromosome, as judged by

the results of crosses with *C1 dpy-10 unc-52/unc-4* males; the *dpy-10 unc-52* chromosome apparently incurred a recessive lethal mutation from the X-irradiation. All three stocks were "cleaned up" in the following way: Unc-4 segregants from the wild-type stocks were crossed with *C1 dpy-10 unc-52/unc-4* males, and wild-type male progeny were picked and crossed with *unc-4 unc-52*. Wild-type hemaphrodite progeny were picked. This procedure, which replaces both *LGII* chromosomes, was then repeated, and the resulting stocks, all *Dp/C1 dpy-10 unc-52/unc-4 unc-52*, were used in all experiments reported in the RESULTS.

Chromosome staining: Young adult hermaphrodites were put on a bacteria-free agar plate for a few minutes and then picked and placed in a small drop of water on a slide. The animals were squashed under a No. 1 cover glass; the slide was placed on dry ice for 30 sec; and the cover glass was popped off. The material on the slide was gently heated for a few seconds and then placed in Carnoy's fixing solution (6 parts ethanol, 3 parts chloroform, 1 part acetic acid) for at least 30 min. The slide was rinsed first in 50% ethanol; next in 0.15 M NaCl, 0.03 M KCl, 0.01 M potassium phosphate buffer, pH 7; then placed in 1 μg per ml of Hoechst 33258 for 20 min; and finally in water until viewed with either a Zeiss GFL microscope equipped for reflected light fluorescence or a Zeiss RA microscope equipped for transmitted light fluorescence. In each case exciter filter BG-12 and barrier filter 50 were used.

RESULTS

Initial characterization of X-chromosome duplications: The *X*-chromosome duplications were tentatively classified as free or translocated on the basis of self-progeny counts from *Dp/m/m* hermaphrodite parents. Hermaphrodites of this genotype with free-*X* duplications have invariably given frequencies of wild-type progeny lower than Mendelian expectation, apparently because of a tendency for loss of the free duplication; whereas translocated duplications have segregated normally (HERMAN, ALBERTSON and BRENNER 1976; and see below). Many new duplications of the *unc-3* region were classified as free by this criterion. But since four free duplications of the *unc-3* region had been identified earlier (HERMAN, ALBERTSON and BRENNER 1976), all of those recovered in this work, with the exception of *mnDp26*, were discarded, some after confirming that some of the oocytes of duplication-bearing hermaphrodites did indeed show fluorescent chromosome fragments when stained with Hoechst 33258.

The genetic extents of the *X*-chromosome duplications were determined with respect to 16 *X*-linked visible markers, and the results are given in Figure 2. Also included in Figure 2 are three duplications, now called *mnDp1(X;V)*, *mnDp2 (X;f)*, and *mnDp3(X;f)*, whose genetic extents were partially characterized previously (HERMAN, ALBERTSON and BRENNER 1976).

The X-chromosome genetic map: The *X*-chromosome linkage map shown in Figure 1 is an extension of the map published by BRENNER (1974). Several of the new map positions were obtained by making use of the *X*-chromosome duplications reported here. For example, the gene order *unc-2 unc-20 unc-78 dpy-8* was derived from the results of three three-factor crosses, each of which was facilitated by *mnDp33*. In the first, *mnDp33/unc-78/O*, a wild-type male able to copulate (in contrast to an *unc-78* male), was crossed with *unc-2 dpy-8* hermaphrodites. Wild-type hermaphrodite progeny were picked. We were interested in those, half of the total, that did not carry *mnDp33*. They were distinguished on the basis of their progeny ratios. Animals without *mnDp33* gave

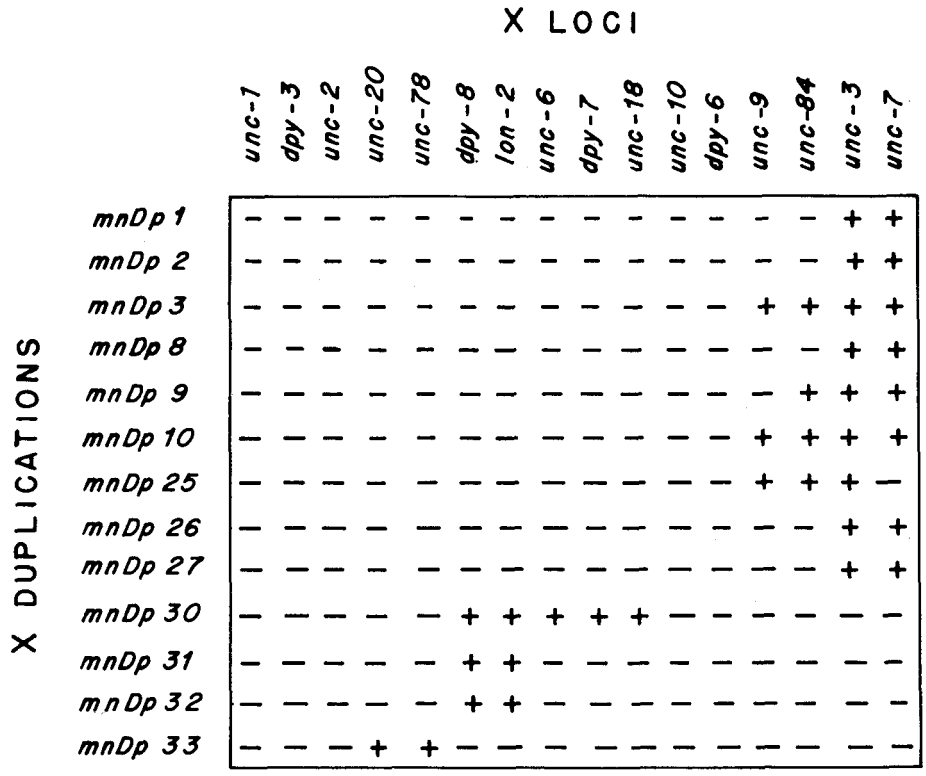

FIGURE 2.—Genetic extents of various *X* chromosome duplications. The *X* loci are arranged in their map order. + signifies that a locus was suppressed and — signifies that it was not suppressed. *mnDp2*, *mnDp3*, *mnDp26* (which also carries *LGV* genes, see text), *mnDp30*, *mnDp31*, and *mnDp32* are free duplications. The other duplications are translocated to autosomes.

one-fourth Unc-78 progeny, whereas animals with *mnDp33* gave one-twelfth Unc-78 progeny. Eleven Dpy non-Unc recombinants were picked from the progeny of *unc-78/unc-2 dpy-8* hermaphrodites. Six of the 11 gave about 50% Dpy Unc progeny, some Unc-78 and some Unc-2. The other five gave about 25% Dpy Unc progeny, all Unc-2. This implies that *unc-78* lies between *unc-2* and *dpy-8*, and we symbolize the result as follows: *unc-2*(5/11)*unc-78*(6/11)*dpy-8*. By analogous procedures, we also obtained the following results: *unc-2* (2/11) *unc-20*(9/11)*dpy-8* and *unc-20*(4/17)*unc-78*(13/17)*dpy-8*.

The gene order *lon-2 unc-6 dpy-7 unc-18 dpy-6 unc-3* follows from the results of nine other three-factor crosses. Two were similar to those just described except that *mnDp30*-bearing males were used to facilitate introduction of the *trans* marker; the results were: *lon-2*(23/27)*unc-6*(4/27)*dpy-6* and *unc-6* (12/16)*dpy-7*(4/16)*dpy-6*. The results of the other crosses must be symbolized differently, and we therefore illustrate with an example. We crossed *lon-2* males with *unc-6 dpy-7* hermaphrodites and picked wild-type hermaphrodites. Among the progeny of these animals, we picked six Unc non-Dpy recombinants. None segregated Lon self-progeny. This result by itself implies that the *trans* marker

lon-2 lies either on the side of *unc-6* opposite that of *dpy-7* or on the same side as *dpy-7*, but relatively close to *unc-6*. We symbolize this result as follows: *(lon-2 unc-6)dpy-7*—which is the same as *dpy-7(unc-6 lon-2)*. Two analogous results, using *lon-2* males, were: *(lon-2 unc-6)dpy-6* and *(lon-2 unc-18)dpy-6*. Similarly, *mnDp30*-bearing males were used to obtain the following results: *(unc-6 dpy-7) unc-18* and *lon-2(unc-6 dpy-6)*, and *mnDp1* males were used to obtain these results: *unc-6(dpy-6 unc-3)* and *unc-6(dpy-7 unc-3)*.

The positions of *unc-10*, *lon-2*, and *unc-84* were determined by S. BRENNER, J. HODGKIN and H. R. HORVITZ (personal communication). The map distances drawn in Figure 1 are based on the results of many two-factor crosses, done mostly by S. BRENNER, which have been recorded in a map notebook by H. R. HORVITZ (personal communication).

Translocated X-*chromosome duplications*: For five duplications—*mnDp8*, *mnDp9*, *mnDp10*, *mnDp25*, and *mnDp27*—about one-fourth of the self-progeny of *Dp/unc-3/unc-3* hermaphrodites were Unc-3, and among the wild-type progeny about one-third were duplication homozygotes: they gave only wild-type self-progeny. The *mnDp33/unc-20/unc-20* hermaphrodites gave one-third Unc-20 self-progeny and no viable duplication homozygotes. Of 85 eggs laid by *mnDp33/unc-20/unc-20* animals, 82 hatched, but only 60 animals reached adulthood: the other 22, presumably *mnDp33* homozygotes, died as young (L1 and L2) larvae.

Four duplications—*mnDp8*, *mnDp9*, *mnDp10*, and *mnDp25*—mapped very near *unc-54* (Table 1), which, apart from a very closely linked lethal (R. WATERSTON, personal communication), is the right-most marker on *LGI*. The published map (BRENNER 1974) shows *unc-59* to the right of *unc-54*, but more recently it has been shown to map to the left of *unc-54* (H. R. HORVITZ, personal communication). The map distance from each duplication to *unc-54* was obtained by screening about 185 broods for Unc-3 non-Unc-54 recombinants. About 80 animals were screened per brood. Most broods sampled in this way showed no recombinants, and a small fraction showed one, but three broods in the case of *mnDp10* and three in the case of *mnDp25* gave clusters of recombinants, with an average of 5.3 recombinants per cluster. (No clusters were found in the cases of *mnDp8* and *mnDp9*.) We conclude that mitotic loss of *unc-3*$^+$ can occur from *mnDp10* and *mnDp25*. The clustered recombinants were included in the data of Table 1, and since at least some of the unclustered recombinants may

TABLE 1

Linkage of four X-*chromosome duplications to* LGI *loci*

Duplication	Percent recombination between duplication and *LGI* locus	
	dpy-5	*unc-54*
mnDp8	16.8 ± 1.1	≤ 0.2
mnDp9	18.8 ± 1.1	≤ 0.2
mnDp10	22.5 ± 1.7	≤ 0.4
mnDp25	16.8 ± 1.2	≤ 0.4

also have been generated mitotically, the map distances given in the table represent upper limits. None of the four duplications on *LGI* seemed to have much effect on recombination in the *dpy-5* to *unc-54* interval, which normally corresponds to about 26 map units: recombination between *dpy-5* and the four duplications was in the range 17% to 23% (Table 1). *mnDp27* is on *LGII*. It showed 2.7% recombination with *bli-2*, 3.6% with *dpy-10* and the surprisingly high value of 50% with *unc-52*. A three-factor cross gave the following results: *mnDp27* (12/16) *bli-2* (4/16) *dpy-10*. Finally, *mnDp33* gave 23% recombination with *dpy-9 IV*, 12.5% with *dpy-4*, 2.5% with *dpy-13*, and 0.2% with *unc-17*. These values, all corrected for the inviability of *mnDp33* homozygotes, agree very well with the *LGIV* map (Figure 1) and indicate that the insertion of *mnDp33* in *LGIV* has very little effect on recombination frequencies.

All six translocated duplications have been investigated cytologically by scanning oocytes by fluorescence microscopy after staining with Hoechst 33258. Four duplication stocks were indistinguishable from wild type, but *mnDp10* and *mnDp25* stocks at the stage of diakinesis shown in Figure 3 showed satellites associated with one bivalent: two satellites were apparent in homozygotes and just one in heterozygotes.

In view of the evidence noted above for mitotic loss of *unc-3*$^+$ from *mnDp10* and *mnDp25*, we would expect to observe instability in the homozygotes for these duplications. Indeed, roughly 1 to 2% of the progeny of *mnDp10/mnDp10*; *unc-3/unc-3* segregated some Unc-3 progeny: we recovered eight such animals, all of independent origin, in the transfer of several hundred animals over the past two years. We inspected the oocytes of three Unc-3-segregating lines derived in this way from the homozygous *mnDp10* stock and observed the single satellites characteristic of *mnDp10* heterozygotes. The Unc-3 segregants were viable and

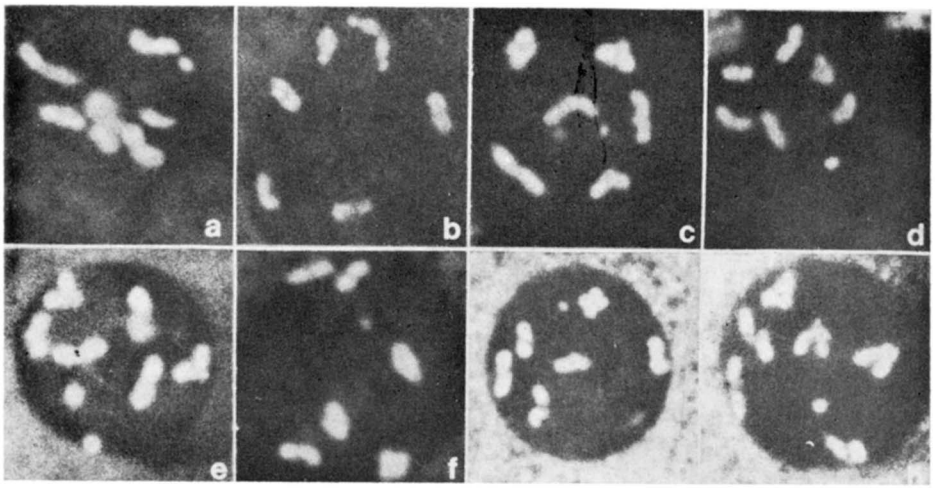

FIGURE 3.—Fluorescence microscopy of oocytes stained with Hoechst 33258. (a) *mnDp10* heterozygote. (b) *mnDp10* homozygote. (c) *mnDp25* homozygote. (d) *mnDp26*. (e) *mnDp26*, showing two fragments. (f) *mnDp30*. (g) *mnDp32*. (h) *mnDp35*. Magnifications range between 2100× and 2500×.

fertile. In one case we looked at oocytes from a homozygous stock recovered from one of these heterozygous lines, and as expected, observed the double satellite again. We have also recovered Unc-3-segregating animals from strains homozygous for *mnDp25* or *mnDp9*, although the frequencies appeared to be lower than in the case of *mnDp10*.

Recombination between *mnDp10* and the *X* chromosomes appears to be rare. Of more than 1,000 self-progeny of *mnDp10*/+; *unc-9 unc-3*, no Unc-9 non-Unc-3 recombinant was found, and of over 2,500 self-progeny of *mnDp10*/+; *unc-3 unc-7*, no Unc-7 non-Unc-3 recombinant was found. We considered it possible that recombination in males would be more likely than in hermaphrodites because of the presence of a single *X* chromosome in males, but of more than 1,700 cross-progeny from the mating of *mnDp10*/+; *unc-9 unc-3*/*0* and *unc-9 unc-3*, only one Unc-9 non-Unc-3 recombinant, a hermaphrodite, was found, and of over 1,600 cross-progeny from the mating of *mnDp10*/+; *unc-3 unc-7*/*0* and *unc-3 unc-7*, no Unc-7 non-Unc-3 recombinant was found.

Free-X *duplications*: Four *X*-chromosome duplications—*mnDp26*, *mnDp30*, *mnDp31*, and *mnDp32*—have been found by fluorescence microscopy of oocytes to be associated with free chromosome fragments (Figure 3), which tend to be lost. *mnDp26* was noticeably larger cytologically than the other three duplications, which were quite small. The frequencies of loss in the ovum lines were measured in the following way. Hermaphrodites derived from *Dp*/*m*/*m* zygotes were mated with N2 males, and the mutant and wild-type male progeny were counted. Table 2, which includes the translocated duplication *mnDp8* for comparison, gives the results. All four duplications showed some tendency for loss, *mnDp30* and *mnDp32* the greatest and *mnDp26* the least. This result makes it difficult to demonstrate whether or not *Dp*/*Dp*/*m*/*m* animals are viable, since they might be expected to segregate mutant progeny, albeit at a lower frequency than would *Dp*/*m*/*m* animals. We have evidence on this point for *mnDp26* only. About 20% of the self-progeny of *mnDp26*/*unc-3*/*unc-3* hermaphrodites developed more slowly than their sibs, remained smaller than their sibs as they matured, gave many fewer progeny, and gave a smaller fraction of Unc-3 progeny. Microscopic inspection of such animals showed oocytes with two chromosome fragments (Figure 3).

We measured the frequency of duplication loss in the male sperm line by

TABLE 2

X-chromosome duplication loss through ovum line

Genotype	Percent nullo-*Dp* ova*
mnDp8/+; *unc-3*/*unc-3*	46 ± 1
mnDp26/*unc-3*/*unc-3*	59 ± 3
mnDp30/*unc-6*/*unc-6*	95 ± 1
mnDp31/*dpy-8*/*dpy-8*	67 ± 3
mnDp32/*dpy-8*/*dpy-8*	94 ± 1

* Measured as percent mutants among male cross-progeny after mating with N2 males.

TABLE 3

X-chromosome duplication loss through male sperm line

Cross	Percent nullo-*Dp* sperm*
mnDp8/+; unc-3/O × *dpy-5 I; unc-3 X*	48 ± 2
mnDp26/unc-3/O × *dpy-10 II; unc-3 X*	43 ± 2
mnDp30/unc-6/O × *dpy-10 II; unc-6 X*	87 ± 3
mnDp31/dpy-8/O × *unc-4 II; dpy-8 X*	67 ± 1
mnDp32/dpy-8/O × *unc-4 II; dpy-8 X*	95 ± 3

* Measured as percent *X*-linked mutants among cross-progeny. The autosomal markers served to distinguish cross-progeny from self-progeny.

mating *Dp*-bearing males with double mutant (autosomal and *X*-linked) hermaphrodites. The results, again with *mnDp8* included for comparison, are given in Table 3 and are similar to those observed for the ovum line: *mnDp30* and *mnDp32* showed the greatest loss and *mnDp26* the least (no detectable loss in this case).

We have been unable to detect recombination between any of the free duplications and the *X* chromosome. Of 1,800 progeny of *mnDp26/unc-3 unc-7/unc-3 unc-7* hermaphrodites and of 1,900 cross-progeny from *mnDp26/unc-3 unc-7/0* × *unc-3 unc-7*, none were Unc-7 non-Unc-3 recombinants. And of the 1,600 progeny of *mnDp30/dpy-8 unc-6/dpy-8 unc-6* hermaphrodites, there were no non-Unc Dpy or Unc non-Dpy recombinants.

Finally, we were interested in the segregation of *X*-chromosome duplications during male spermatogenesis. The results given in Table 4 indicate that *mnDp26* tends to segregate from the *X* chromosome during meiosis in males. Two-thirds of the *Dp*-bearing sperm that were successful in fertilization were nullo-*X*. This effect is probably not attributable to differences in sperm fertility because the reciprocal relation also held: among those fertilizing sperm not carrying a duplication, less than one-third were nullo-*X*. (The overall ratio of nullo-*X* to *X* sperm was close to 1:1.) Some of the other duplications, particularly *mnDp31* and *mnDp10*, may also show this effect, but not to the same degree as *mnDp26*.

mnDp26 *carries some* LGV *markers*: As already noted, *mnDp26* has shown less loss than any other free-*X* duplication we have studied, including the four previously reported (HERMAN, ALBERTSON and BRENNER 1976) and several other duplications of the *unc-3* region identified during this work but not reported on in detail here. We were therefore prompted to study *mnD26* further. We found, to our surprise, that it appears to carry several genes from *LGV*. We cannot say, however, that their presence is responsible for the low frequency of loss of *mnDp26*.

Figure 1 shows nine markers of *LGV*. The only modifications of BRENNER's (1974) published map are the placement of *unc-41* (RIDDLE and BRENNER 1978) and *unc-23*, which we ordered by a three-factor cross, with the following results: *dpy-11* (21/35)*unc-23*(14/35)*sma-1*. Nine double mutants were constructed: each of the nine *LGV* markers shown in Figure 1 with *unc-3*. Each of the double-

TABLE 4

Segregation of X-chromosome duplications in males with respect to the X chromosome

Male genotype	Percent males among *Dp*-bearing cross-progeny*	Percent males among nullo-*Dp* cross-progeny*
Free duplications:		
mnDp26/unc-3/O	69 ± 2	26 ± 3
mnDp31/dpy-8/O	58 ± 2	37 ± 2
Half-translocations:		
mnDp8/+; unc-3/O	52 ± 2	45 ± 2
mnDp9/+; unc-3/O	56 ± 3	44 ± 3
mnDp10/+; unc-3/O	56 ± 4	37 ± 4
mnDp25/+; unc-3/O	52 ± 3	45 ± 3
mnDp27/+; unc-3/O	51 ± 3	46 ± 3

* Males were mated with hermaphrodites that carried the same *X*-linked mutation as the male plus a convenient autosomal marker (*dpy-5 I, dpy-10 II,* and *dpy-11 V* were used with *unc-3 X,* and *unc-4 II* was used with *dpy-8 X*) to distinguish cross-progeny from self-progeny.

mutant hermaphrodites was crossed with *mnDp26/unc-3/0* males, and wild-type hermaphrodites were picked.

We shall first consider the results for *unc-60* and *unc-51*. Each of these mutants is severely paralyzed and epistatic to *unc-3*. Wild-type hermaphrodites issuing from the cross of *mnDp26/unc-3/O* and *unc-60; unc-3* segregated wild-type, Unc-3, and Unc-60 progeny. Some of these wild-type progeny segregated only wild-type and Unc-60 offspring. The genotype of the wild-type animals is *unc-60/unc-60; mnDp26/unc-3/unc-3*, which means that *mnDp26* suppresses *unc-60*. We confirmed that these animals carried *unc-3* by mating them with N2 males: Unc-3 male progeny were produced, as expected. We can also conclude that in the mating of *mnDp26/unc-3/O* with *unc-60; unc-3*, the fertilizing sperm that carried *mnDp26* also carried a normal *unc-60*$^+$ chromosome, otherwise the wild-type hermaphrodite progeny would not have given Unc-3 self-progeny. The results for *unc-51* were the same as for *unc-60* in every detail.

We next consider the results for *unc-46*, *unc-23*, *unc-42*, *unc-41*, and *unc-39*. The phenotypes of these mutants are less severe than that of *unc-3*, and *unc-3* is epistatic to them. By virtually the same procedure as was described above for *unc-60*, we generated animals with the following genotype: *unc-46/unc-46; mnDp26/unc-3/unc-3*. They showed the Unc-46 phenotype: therefore *mnDp26* does not carry *unc-46*$^+$. Moreover, when *mnDp26/unc-3/O* was crossed with *unc-46; unc-3*, wild-type but no Unc-46 progeny were produced; hence, as before, the fertilizing sperm that carried *mnDp26* also carried a normal *unc-46*$^+$ chromosome. The results for *unc-42*, *unc-41*, and *unc-39* were the same as for *unc-46* in all respects. In the case of *unc-23*, however, *unc-23/+; mnDp26/ unc-3/unc-3* animals did not produce Unc-23 progeny; hence we conclude that *mnDp26* suppresses *unc-23*.

Finally we consider *dpy-11* and *sma-1*, the phenotypes of which can be recognized in an *unc-3* background. Here it was easier to show that *dpy-11/dpy-11;*

mnDp26/unc-3/unc-3 was wild type and *sma-1/sma-1*; *mnDp26/unc-3/unc-3* was Sma. In summary, *mnDp26* appears to carry one marker for each end of *LGV*, *unc-60*$^+$ and *unc-51*$^+$, and two near the middle, *dpy-11*$^+$ and *unc-23*$^+$, but does not carry certain intervening markers; and *mnDp26* does not substitute for a normal *LGV* chromosome, but is present in addition to the normal diploid complement. The latter conclusion is also supported by the cytology of *mnDp26*, as already described. Other markers not suppressed by *mnDp26* include *dpy-5 I*, *dpy-10 II*, *dpy-18 III*, *dpy-9 IV*, *dpy-13* IV and fourteen loci on *LGX* (Figure 2).

Free autosomal duplications: We have used *C1*, an *LGII* crossover suppressor described previously (HERMAN 1978), in a scheme for the selection of X-ray-induced unlinked duplications of parts of *LGII* (see MATERIALS AND METHODS) and have identified three independently derived duplications of *unc-52*$^+$, all of which have been maintained in stocks of the following genotype: *Dp/C1 dpy-10 unc-52/unc-4 unc-52*. All three duplications are free and are readily apparent by fluorescence microscopy of oocytes stained with Hoechst 33258 (Figure 3). The progeny ratios for each duplication stock are given in Table 5. We have picked more than 150 wild-type progeny from each duplication-bearing stock, and all had the same genotype as their parent—as judged by their progeny ratios. One implication of this result is that duplication homozygotes are inviable. We have in fact found that the egg-hatching frequencies are nearly 100% in all three cases but that about 20% of the larvae, presumably the duplication homozygotes, die at an early stage. Another implication of the result that all wild-type progeny of duplication-bearing stocks give the same progeny ratios as the parent is that none of the three duplications carries either *unc-4*$^+$ or *dpy-10*$^+$.

The duplication stocks are selected continually for hyperploidy, but we can ask whether any of the duplications can interfere with normal *LGII* disjunction such that viable hypoploids—a duplication with only one *LGII* chromosome—are produced. Unc-4 segregants of each duplication stock were mated with *C1 dpy-10 unc-52/unc-4* males, and among several hundred cross-progeny for each duplication, no *Dp/C1 dpy-10 unc-52* animals, which would have been Dpy, were found. We conclude that either the duplications have little effect on *LGII* disjunction during oogenesis or the hypoploids are inviable.

From the same crosses described in the previous paragraph, we obtained a measure of duplication loss through the ovum line by counting wild-type and

TABLE 5

Self-progeny of LGII *duplication stocks*

Stock	Phenotypic ratios*				
	Wild type	Unc-52	Unc-4	Dpy	Unc-52 Dpy
mnDp34/C1 dpy-10 unc-52/unc-4 unc-52	0.31	0.28	0.16	0.14	0.11
mnDp35/C1 dpy-10 unc-52/unc-4 unc-52	0.31	0.24	0.15	0.18	0.12
mnDp36/C1 dpy-10 unc-52/unc-4 unc-52	0.28	0.30	0.18	0.13	0.11

* Total progeny examined were 1485, 930, and 1330 for the *mnDp34*, *mnDp35*, and *mnDp36* stocks, respectively. *unc-52* is epistatic to *unc-4*. No Unc-4 Dpy offspring were found.

TABLE 6

Loss of LGII *duplications in ovum line*

Genotype	Percent nullo-*Dp* ova*
mnDp34/unc-4 unc-52/unc-4 unc-52	58 ± 2
mnDp35/unc-4 unc-52/unc-4 unc-52	64 ± 4
mnDp36/unc-4 unc-52/unc-4 unc-52	58 ± 2

* The Unc-4 hermaphrodites were mated with *C1 dpy-10 unc-52/unc-4* males, and the fraction of nullo-*Dp* ova was measured as the ratio of Unc-52 males to wild-type males plus Unc-52 males. In a control cross, *unc-4/unc-4 unc-52* hermaphrodites were mated with *C1 dpy-10 unc-52/unc-4* males, and the percent ova not containing *unc-52*+ was 49 ± 2.

Unc-52 male progeny. The results, which are given in Table 6, indicate that in all three cases about 40% of the ova inherit a duplication. Self-progeny ratios from *Dp/unc-4 unc-52/unc-4 unc-52* animals, corrected for the inviability of duplication homozygotes, in conjunction with the results of Table 6, indicate that the duplication is transmitted to roughly 50% of the hermaphrodite sperm. With the foregoing information we can satisfactorily account for the observed progeny ratios reported in Table 5 for the duplication stocks.

We now demonstrate that each duplication suppresses *unc-53 II*. We shall use *mnDp34* as an example, but the same results were obtained for *mnDp35* and *mnDp36*. *C1 dpy-10 unc-52/unc-4* males were crossed with *mnDp34/unc-4 unc-52/unc-4 unc-52*, and the resulting wild-type male progeny were crossed with *unc-52 unc-53* hermaphrodites. Wild-type hermaphrodites were picked and allowed to self-fertilize. In those broods that contained Unc-52 Dpy animals, no Unc-53 animals were observed, and wild-type hermaphrodites were picked. Some proved not to carry the *C1 dpy-10 unc-52* chromosome and were therefore *mnDp34/unc-53 unc-52/unc-53 unc-52*. We conclude from the fact that these animals were wild-type that *mnDp34* carries *unc-53*+. Since *unc-52* is epistatic to *unc-53*, we checked to see that *unc-53* was present in the latter strain by mating with *unc-53/*+ males: Unc-53 progeny were produced, as expected.

The segregation of *mnDp34*, *mnDp35*, and *mnDp36* during male spermatogenesis appears not to be random with respect to the segregation of the *X* chromosome. Table 7 indicates that two-thirds of the *Dp*-bearing sperm were nullo-*X*.

TABLE 7

Segregation of LGII *duplications in males with respect to the* X *chromosome*

Male genotype	Percent males among *Dp*-bearing cross-progeny*
mnDp34/C1 dpy-10 unc-52/unc-4 unc-52	71 ± 2
mnDp35/C1 dpy-10 unc-52/unc-4 unc-52	67 ± 3
mnDp36/C1 dpy-10 unc-52/unc-4 unc-52	71 ± 2

* The males were mated with *unc-4 unc-52* hermaphrodites and all *Dp*-bearing progeny were counted: wild type and Unc-4. There were no significant differences between wild-type and Unc-4 sex ratios. In a control cross, *C1 dpy-10 unc-52/unc-4* males were mated with *unc-4 unc-52* hermaphrodites, and the percent males among Unc-4 progeny was 47 ± 2.

DISCUSSION

In this paper we have characterized 13 new duplications, all selected following X-irradiation to be unlinked to their linkage group of origin. A summary of some of the properties of these duplications, and three others previously identified (HERMAN, ALBERTSON and BRENNER 1976), is given in Table 8. As in the previous work, a considerable fraction of the duplications recovered were free. We presume that the free duplications must have a centromere; furthermore, work with Drosophila supports the idea that newly broken chromosome ends must be capped by telomere material to be stable (ROBERTS 1976). The free-*X* duplications pose a problem as to the nature and location of the *X*-chromosome centromere. As far as we are aware, *mnDp2*, *mnDp3*, *mnDp30*, *mnDp31*, and *mnDp32* are composed of *X*-chromosome material only; yet the genetic evidence suggests that *mnDp2* and *mnDp3* have no *X*-chromosome material in common with the other duplications. It is of course possible that some of the duplications picked up centromeric material, from either the *X* or another chromosome, by multiple-break events that have gone undetected genetically. Additional breaks would also have to be postulated for the capping of broken ends. *mnDp26* is the only duplication for which the genetic evidence clearly indicates multiple breaks. It appears to carry, in addition to a portion of the *X* chromosome, at least three separate segments of *LGV*: markers from each end and the middle. It also shows less tendency for loss and appears distinctly larger cytologically than the other *X*-chromosome duplications. Another possibility to be noted is that the *X* chromosome (and perhaps the autosomes as well) may be polycentric, as are the germ line chromosomes of the horse parasitic nematode *Parascaris equorum*, or may have a diffuse centromere, examples of which, also said to be holocentric, can be found in both plant and animal kingdoms (JOHN and LEWIS 1965). A feature of these nonlocalized centric systems is that chromosome fragments, whether spontaneous or induced, tend to move normally at mitosis and meiosis. Evidence on the nature of *C. elegans* centromeres is lacking. We have looked at the chromosomes of oocytes formed at various times after X-irradiation of young hermaphrodites and have often seen fragments present, but there are usually only one or two fragments per oocyte. Perhaps an electron microscopic investigation of the attachment of spindle fibers to chromosomes would shed light on this question (COMINGS and OKADA 1972).

It was suggested for the free-*X* duplications studied previously (HERMAN, ALBERTSON and BRENNER 1976) that some duplication loss occurs premeiotically, since a considerable fraction of oocytes screened did not show cytologically discernable fragments. It was therefore suggested that such free duplications might be useful in producing genetic mosaics. The cytology of *mnDp30*, *mnDp31*, and *mnDp32* suggested that they too may be lost premeiotically, but none of these fragments carry markers for which this idea can presently be tested. *mnDp26*, however, covers *unc-60 V*; and *mnDp34*, *mnDp35*, and *mnDp36* cover *unc-52 II*. *unc-60* and *unc-52* mutants have greatly reduced birefringence of body muscle cells (BRENNER 1974; R. WATERSTON, personal communication). We therefore

TABLE 8

Summary of properties of duplications

Duplication	Isolated by suppression of	Other mutations suppressed	Cytology	Genetic linkage	Duplication homozygote	Other comments
mnDp1	*unc–3 X*	*unc–7 X*	wild type	*unc–60 V*	sterile	reduced recombination on *LGV*
mnDp2	*unc–3 X*	*unc–7 X*	free		?	
mnDp3	*unc–3 X*	*unc–9, unc–84, unc–7 X*	free		?	
mnDp8	*unc–3 X*	*unc–7 X*	wild type	*unc–54 I*	fertile	
mnDp9	*unc–3 X*	*unc–84, unc–7 X*	wild type	*unc–54 I*	fertile	
mnDp10	*unc–3 X*	*unc–9, unc–84, unc–7 X*	satellite	*unc–54 I*	fertile	subject to mitotic loss
mnDp25	*unc–3 X*	*unc–9, unc–84*	satellite	*unc–54 I*	fertile	subject to mitotic loss
mnDp26	*unc–3 X*	*unc–7 X; unc–60, dpy–11, unc–23, unc–51 V*	free (large)		weakly fertile	some disjunction from *X* chromosome in males
mnDp27	*unc–3 X*	*unc–7 X*	wild type	*bli–2 II*	fertile	
mnDp30	*unc–6 X*	*dpy–8, lon–2, dpy–7, unc–18 X*	free		?	frequently lost
mnDp31	*dpy–8 X*	*lon–2 X*	free		?	
mnDp32	*dpy–8 X*	*lon–2 X*	free		?	frequently lost
mnDp33	*unc–20 X*	*unc–78 X*	wild type	*unc–17 IV*	inviable	
mnDp34	*unc–52 II*	*unc–53 II*	free		inviable	
mnDp35	*unc–52 II*	*unc–53 II*	free		inviable	
mnDp36	*unc–52 II*	*unc–53 II*	free		inviable	

used polarization microscopy (EPSTEIN, WATERSTON and BRENNER 1974) to look at the progeny of *unc-60* or *unc-52* mutants carrying an appropriate duplication to see if we could find animals that were mosaic with respect to birefringence of muscle cells. None was found, but unfortunately all four of the free duplications used were quite stable, as judged by the cytology of oocytes, so that they must rarely be lost at mitosis, if at all. In addition, *unc-52* and *unc-60* may not be cell autonomous. Moreover, there are only 95 body muscle cells, 81 present in a young L1 animal and 14 added post-embryonically (SULSTON and HORVITZ 1977), so that the production of a reasonable fraction of mosaic animals might require duplications giving such a high rate of mitotic loss that they would be difficult to identify and maintain in the first place, unless a way could be found to increase their mitotic loss, say by changing the genetic background or giving a heat shock or dose of X irradiation. We have not investigated possible factors affecting the frequency of free duplication loss.

mnDp26 tended to segregate from the *X* chromosome during meiosis in males. The frequencies of *mnDp26*, nullo-*X* sperm and nullo-*mnDp26*, *X* sperm were twice the frequencies of *mnDp26*, *X* and nullo-*mnDp26*, nullo-*X*. The same effect was apparent in the case of the three free duplications from *LGII*: about 70% of the duplication-bearing sperm were nullo-*X* (nullo-*Dp* sperm were not assayed). This nonrandom assortment of heterologues may be analogous to the well-studied phenomenon in Drosophila oogenesis whereby nonexchange chromosomes, whether homologous or not, tend to segregate from each other (GRELL 1976).

Five of the six translocated duplications are homozygous viable, and four of these are attached at or near the right end of *LGI*. Indeed, *mnDp10* and *mnDp25* were manifest cytologically at a particular stage of oogenesis as chromosome satellites, and both showed some mitotic instability. This behavior is reminiscent of the instability of certain quasiterminal duplications in Neurospora: in several stocks reversion to an apparently normal euploid condition is invariably accomplished by breakage of the translocated segment at the interchange point (NEWMEYER and GALEAZZI 1977). It is interesting that the frequency of deletion of these duplications in Neurospora is enhanced by certain mutations that affect both meiosis and sensitivity to ultraviolet light (NEWMEYER and GALEAZZI 1978). SANDLER and SZAUTER (1978) have recently reported an analogous phenomenon in Drosophila: in premeiotic gonia of females homozygous for recombination-defective meiotic mutants, a y^+ region translocated to the fourth chromosome is lost, leaving an apparently normal fourth chromosome. We have not investigated the effects of various meiotic mutants (HODGKIN, HORVITZ and BRENNER 1979) of *C. elegans* on the stability of *mnDp10* or *mnDp25*.

mnDp33 is a translocated *X*-chromosome duplication that is homozygous inviable. Its formation required at least three breaks: one at each end of the duplication itself and one near *unc-17* IV, where the duplication was inserted. The presence of *mnDp33* has very little effect on recombination between the duplication-bearing chromosome and its normal homologue on either side of the

duplication. This is quite unlike *mnDp1(X;V)*, which suppresses crossing over along the left half of *LGV* (HERMAN, ALBERTSON and BRENNER 1976).

Some of the *X*-chromosome duplications proved useful in mapping *X*-linked genes. Many hemizygous mutant males are effectively sterile, but duplication-bearing males were used to transmit X-linked mutations covered by the duplication. The construction of many double X-linked mutants and the setting up of several three-factor crosses were thus facilitated.

We expect that duplications will be used to vary gene dosage. For example, a mutation called *ace-1*, which results in the absence of a particular species of acetylcholinesterase, maps near the right end of the *X* chromosome, where it is covered by some of the duplications reported here, and the enzyme activity per animal has been varied by using duplications to vary the dosage of that region (R. RUSSELL, personal communication). We have also used some of the *X*-chromosome duplications in a study of sex determination in triploids (J. MADL and R. HERMAN, unpublished), following the studies of DOBZHANSKY and SCHULTZ (1943) with Drosophila. Indeed, it would be desirable to have available duplications covering the entire genome. The procedures demonstrated in this work suggest that such a goal is feasible.

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