CHROMOSOME REARRANGEMENTS IN *CAENORHABDITIS ELEGANS*

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ABSTRACT

A method for selecting unlinked duplications of a part of the *X* chromosome of *C. elegans* is described. Five such duplications have been identified. One of them, *Dp(X;V)1*, is translocated to linkage group *V*, where it suppresses crossing over along the left half of linkage group *V*. *Dp(X;V)1* homozygotes grow slowly and are sterile. The other four duplications are associated with chromosome fragments, as observed cytologically by fluorescence microscopy, and tend to be lost. Their frequency of loss is higher in strains homozygous for a mutation that promotes nondisjunction of *X* chromosomes. The recombination frequencies between two of these duplications and the *X* have been measured: the frequencies are at least 50 times less than for *X–X* recombination in the same region. The duplications may prove useful as balancers of recessive lethal mutations.

THE small, free-living soil nematode *Caenorhabditis elegans* consists of only about 600 cells, excluding the reproductive system, and is particularly well-suited to genetic manipulation (BRENNER 1974). It is therefore being used to carry out ultrastructural and mutational studies of development, particularly of the neuromuscular system (BRENNER 1973; EPSTEIN, WATERSTON and BRENNER 1974; WATERSTON, EPSTEIN and BRENNER 1974; WARD, *et al.* 1975; WARE, *et al.* 1975). It has been estimated that about 2,000 genes in *C. elegans* have indispensable functions and that these make up most of the organism's complementation units (BRENNER 1974). It should be feasible to identify virtually all of these genes by mutations. The mutants can then be studied anatomically and biochemically to try to discover the roles of the genes in development. Most mutations in *C. elegans* are recessive lethals, and in order to facilitate both the maintenance and the manipulation of recessive lethal mutations for complementation and mapping, it is essential that they be balanced in heterozygotes by genetically marked homologues. The value of such balancer chromosomes obviously depends on having a low frequency of recombination between the marker on the balancer and the recessive lethal mutation. It is for this reason that balancer chromosomes used in Drosophila research generally carry chromosome rearrangements, such as inversions, which act as crossover suppressors. In this paper we describe the first identification of chromosome rearrangements in *C. elegans* and show how they may be used as chromosome balancers.

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MATERIALS AND METHODS

Media and nematode strains: The media, growth of nematodes, and most of the mutant strains used are described by BRENNER (1974). N2 refers to the wild-type stock. Type alleles listed by BRENNER (1974) were used for all genes referred to in this paper except that alleles E151 and E324 were used for *unc-3* and *unc-23*, respectively, and alleles E1324 and E1281 were sometimes used for *dpy-7* and *dpy-8*, respectively. In addition, mutant E139, which was originally assigned to *unc-12*, has been found to be in the same gene as *unc-7* (E5) and has therefore been reassigned to *unc-7*. The *flu-4* (E1004) mutant, characterized by a bright blue fluorescence of its gut, was isolated by BABU (1974), who referred to it as fl-IV. The map positions of genes referred to in this paper belonging to *X* and linkage group *V* (LG*V*) are given in Figure 1. Various double mutants were made by standard methods (BRENNER 1974), although it is worth noting that the *unc-3/0*; *Dp(X;V)1/+* male (see RESULTS), which is much more fertile than an *unc-3/0* male, was used in the construction of the *unc-3 unc-9* double mutant.

Mating and counting procedures: All matings were carried out in small petri plates (48 mm diameter), with 3–4 young adult hermaphrodites and 1–12 males, depending on the experiment. Individual animals were picked with a platinum wire (0.2 mm diameter). The males were generally removed from the mating plate 1–2 days after the cross was set up. Hermaphrodites whose progeny were to be counted and classified according to phenotype were transferred daily during their egg-laying period. Adult progeny were scored 3–4 days later. Animals were grown at 20°. To avoid bias, all offspring on a plate were counted. Similarly, in those experiments where the fertility of wild-type progeny was to be tested, the parental hermaphrodites were allowed to lay eggs for a limited period on a plate. Then the fertility of all wild-type progeny arising on the plate was tested.

Irradiation of males and screening for duplications: A partially synchronized population containing many males was generated for X-irradiation: 20 N2 males and 12 N2 young adult hermaphrodites were put on a small petri plate seeded with bacteria. After one day, all of the adult worms were transferred to a new plate and then removed after 12 hr. The eggs and newly-hatched worms on the latter plate were incubated at 20° for a further 48 hr. The animals were then X-irradiated at a dose rate of 500 roentgens (r) per min using a Maximar Therapeutic machine: 220 kv, 15 ma, 1 mm Al filter. One day later males were picked off the plate and put

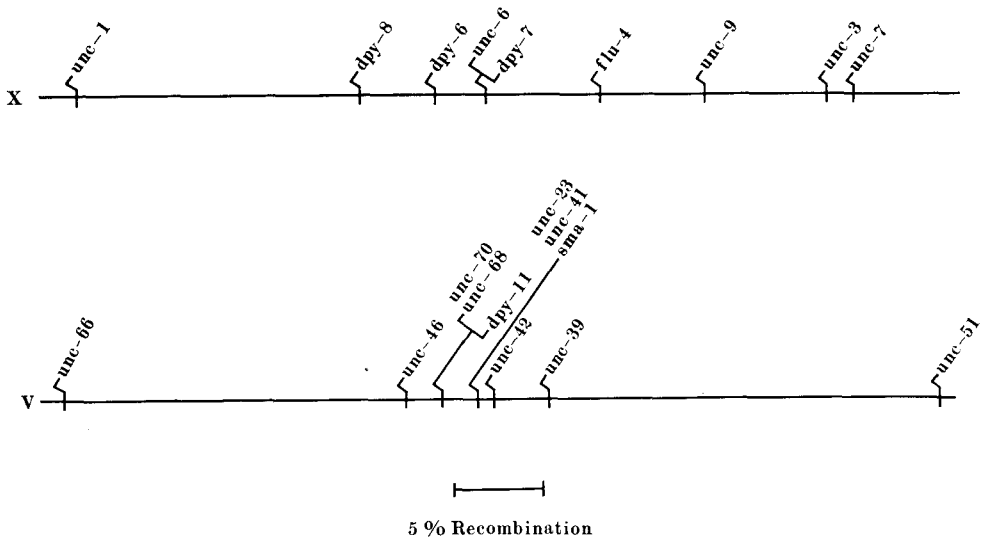

FIGURE 1.—Linkage maps of *X* and LG*V*, showing loci used in this work.

on small petri plates with young adult hermaphrodites homozygous for an X-linked uncoordinated mutant—3–4 hermaphrodites and 4 males per plate. The following day the males were removed and discarded, and the hermaphrodites were transferred to fresh plates. One day later the hermaphrodites were transferred once more. The progeny were screened for rare wild-type males. Uncoordinated males were counted in order to monitor both the efficiency of mating and the fraction of total males that were wild-type. All wild-type males found were crossed, one per plate, with 3 mutant hermaphrodites. These plates were seeded with bacteria spread in a small spot (10–15 mm diameter). Individual N2 males showed high fertility under these conditions: in 15 tests—5 each with *unc-1*, *unc-3* and *unc-7* (E5)—all gave cross progeny.

To give some indication of the effects of X-ray dose: 0.2% (13/6,666), 1.8% (58/3,164), and 3.0% (35/1,165) of the male progeny were wild-type when the above procedure was followed with the *unc-3* hermaphrodite after X-ray doses of 4,000 r, 6,000 r and 8,000 r, respectively. The fertility of the irradiated males dropped significantly between 6,000 r and 8,000 r, however, so that only about one-third as many outcross progeny were produced from the same number of mating plates after 8,000 r as after 6,000 r. Thus we have found that the most convenient doses are in the range of 6,000 r to 8,000 r.

Fluorescent staining with Hoechst 33258: Worms to be stained were either gently washed off the surface of the petri plate on which they were growing with a few ml of water and a Pasteur pipette or picked off the plate individually and put into distilled water. The worms were sedimented by centrifugation and resuspended in the following for at least 90 min: 6 parts ethanol, 3 parts acetic acid, 1 part chloroform. After removal of most of the fixing solution, worms were deposited, with a Pasteur pipette, onto slides that had previously been dipped into 1% bovine serum albumin and dried. After evaporation of the fixing solution the slides were dipped into 0.15 M NaCl, 0.03 M KCl, 0.01 M phosphate (pH 7) for 10 min, then Hoechst 33258 at 1 μg/ml for 5 min, and finally water, where they were kept until viewed in a Zeiss microscope with fluorescence attachment, using excitation filter UG1 and barrier filters 47 and —65.

RESULTS

Selection procedure: The rearrangements described in this paper were selected by the following procedure: young N2 males were X-irradiated and subsequently mated with hermaphrodites homozygous for an *X*-linked recessive marker giving uncoordinated movement. In the absence of X-irradiation, the male progeny of such a cross are virtually all uncoordinated. If, however, fertilization is accomplished by a nullo-*X* sperm carrying the wild-type allele of the uncoordinated gene either translocated to an autosome or on a free duplication, then a wild-type male may be produced—assuming that partial diploidy of the *X* chromosome does not prohibit male development. If such a male is backcrossed to the uncoordinated hemaphrodite, both wild-type and uncoordinated male progeny should be produced, assuming the appropriate meiotic segregations in the male parent are frequent and result in viable progeny.

By this strategy we have isolated five independent mutants carrying unlinked duplications of *unc-3*$^+$; *unc-3* is a recessive mutation that normally maps near the right end of *X* (Figure 1) and which confers a rather severe uncoordinated phenotype. The five mutants represent a small fraction of the total number of wild-type male progeny we picked out after mating X-irradiated males and uncoordinated hermaphrodites. The vast majority of the wild-type male offspring proved to be sterile upon backcrossing to *unc-3*, perhaps because of a gene dosage effect. Male fertility in Drosophila is very sensitive to duplication or transloca-

tion of *X* chromosome material (LIFSCHYTZ and LINDSLEY 1972). Many of the sterile males, while clearly not showing the phenotype of *unc-3*, were either sluggish in their movement or slow in development. In any case, on the assumption that these wild-type males do indeed carry duplications of some sort, their frequency of occurrence can be used as an assay for the induction of chromosome rearrangements; thus, one can monitor the relative effects of varying X-ray dose, age of males when irradiated, and the timing of subsequent mating on the incidence of these presumptive duplications. The details of the screening procedure, described in MATERIALS AND METHODS, were arrived at after some testing of these variables. Altogether, we picked 140 wild-type male progeny issuing from crosses of *unc-3* and irradiated N2 males. All but six were sterile. Of these, one gave only uncoordinated male progeny when backcrossed to *unc-3*, but the other five gave both wild-type and uncoordinated males. When *unc-1* was the hermaphrodite in crosses with irradiated N2 males, the fraction of male progeny that were wild-type was about one-eighth as many as for the case of *unc-3*. Six were picked and all were sterile. Finally, with *unc-6* as hermaphrodite, the incidence of wild-type males was roughly the same as for *unc-3*. Forty-five candidates were picked and all were sterile.

Maintenance of duplications: Each presumptive duplication has been maintained for the most part by continual backcrossing of the wild-type males to *unc-3*. Of course each such cross also produces wild-type hermaphrodites carrying the duplication, as well as uncoordinated hermaphrodites, which are indistinguishable from the progeny produced by self-fertilization. Indeed, in the case of each of our mutants, we have crossed wild-type hermaphrodites produced in this fashion with N2 males and obtained both uncoordinated and wild-type progeny, and, as expected, when the wild-type male progeny are crossed to *unc-3* they give rise to both wild-type and uncoordinated male offspring. This routine has been followed at least three times for each mutant without failure, suggesting that recombination between the duplications and *X* is not extensive. More stringent tests for detecting such recombination are described below for certain mutants.

The *Dp/unc-3/0* males are not as fertile as N2 males, and most crosses have been performed with 8–12 males per mating. Among the five duplication mutants, *Dp1* males are the most fertile and *Dp5* males are the least fertile.

Extents of duplications: The putative duplications so far described behave genetically in precisely the same manner as a suppressor of *unc-3*. If they are in fact duplications, then we may determine their extents by checking to see what other markers on *X* they will suppress. We illustrate this test as follows: *Dp1/unc-3/0* is crossed with *unc-9*. If *Dp1* carries *unc-9*$^+$, then some of the male progeny of the cross should be wild-type. Table 1 summarizes our results for all five duplications. In each case the suppressed mutations are limited to a contiguous set in the neighborhood of *unc-3*, as defined by the linkage map for *X* (Figure 1). This pattern of suppression is compatible with the supposition that unlinked, partial duplications are indeed responsible for the suppressions. Table 1 shows that the extents of the five duplications fall into just two classes: *Dp1*, *Dp2*, and *Dp5* belong to one class and *Dp3* and *Dp4* to the other.

TABLE 1

Extents of duplications

Duplication	Mutations suppressed	Mutations not suppressed
Dp1	*unc-3*, *unc-7*	*unc-9*, *dpy-7*, *dpy-8*, *unc-1*
Dp2	*unc-3*, *unc-7*	*unc-9*
Dp3	*unc-9*, *unc-3*, *unc-7*	*flu-4*, *dpy-7*, *unc-6*, *dpy-6*, *dpy-8*
Dp4	*unc-9*, *unc-3*, *unc-7*	*flu-4*, *dpy-7*, *unc-6*
Dp5	*unc-3*, *unc-7*	*unc-9*

On the progeny of Dp/unc-3/unc-3 hermaphrodites: As already noted, amongst the offspring produced by crossing *Dp/unc-3/0* with *unc-3* hermaphrodites are wild-type hermaphrodites which, assuming no recombination between the duplication and *X*, are *Dp/unc-3/unc-3*. A question to be considered at this point is whether these hermaphrodites segregate animals homozygous for the duplication. If so, and if they have normal viability, we might expect them to comprise one-third of the wild-type progeny and to segregate only wild-type (no uncoordinated) animals amongst their progeny. Table 2 shows, on the contrary, that none of the wild-type progeny of the duplication hermaphrodites segregates wild-type offspring only. Nearly one-third of the wild-type progeny of *Dp1/unc-3/unc-3* were sterile, however, and we shall in fact show below that most of these animals are homozygous for *Dp1*. These sterile animals are easy to recognize under the dissecting microscope because they grow more slowly than their sibs and never make a gonad.

The other duplication hermaphrodites segregate very few sterile animals and no animals that segregate only wild-type offspring. It does not follow that homozygotes for these duplications are inviable, however, because of the data presented in Table 3. Table 3 shows, as expected, that one-quarter of the progeny of *Dp1/unc-3/unc-3* hermaphrodites are uncoordinated; but the other duplication hermaphrodites segregate more uncoordinated offspring than expected. If animals homozygous for these duplications were inviable, we might expect one-third of the progeny to be uncoordinated; but the mean proportions of uncoordinated progeny ranged from 0.38 to 0.48. Moreover, *Dp3/unc-3/unc-3* animals gave total numbers of progeny per parental hermaphrodite very close to that for N2 animals,

TABLE 2

On the progeny of Dp/unc-3/unc-3 *hermaphrodites*

Parent	Number of wild-type progeny picked	Number of sterile wild-type progeny	Fraction of fertile wild-type progeny that segregated wild-type offspring only
Dp1/unc-3/unc-3	117	32	0/85
Dp2/unc-3/unc-3	43	0	0/43
Dp3/unc-3/unc-3	40	1	0/39
Dp4/unc-3/unc-3	54	0	0/54
Dp5/unc-3/unc-3	49	1	0/48

TABLE 3

On the progeny of duplication hermaphrodites

Parent	Mean percentage uncoordinated animals amongst progeny*	Mean number of total offspring per hermaphrodite†
unc-3/unc-3+	24.0 ± 1.0	280 (10)
Dp1/unc-3/unc-3	25.5 ± 0.5	236 (10)
Dp2/unc-3/unc-3	48.0 ± 3.5	219 (13)
Dp3/unc-3/unc-3	45.0 ± 1.0	272 (14)
Dp4/unc-3/unc-3	38.0 ± 1.0	173 (14)
Dp5/unc-3/unc-3	41.0 ± 1.5	155 (13)
Dp2/unc-3/unc-3; him-1/him-1	62.5 ± 2.5	
Dp3/unc-3/unc-3; him-1/him-1	50.0 ± 1.5	
Dp4/unc-3/unc-3; him-1/him-1	46.0 ± 2.5	

* The percentage of uncoordinated animals in each brood sampled (minimum of 85 animals) was computed, and each such datum was given equal weight in computing mean percentages and standard deviations of the mean. The latter are given as error estimates. All values are given to the nearest 0.5%.

† The number of animals whose progeny were counted is given in parentheses.

suggesting that the high proportion of uncoordinated progeny was not due to the early death of many wild-type offspring. We suggest that the *Dp2*, *Dp3*, *Dp4*, and *Dp5* duplications tend to be lost during gametogenesis or shortly thereafter. This would mean that true duplication homozygotes could not persist because even an animal that arose by fusion of a *Dp*-containing egg and a *Dp*-containing sperm would, as a result of duplication loss, segregate some uncoordinated progeny.

To get some idea of the frequency of this presumed duplication loss in the egg line, we have counted numbers of uncoordinated and wild-type males arising from the mating of N2 males and *Dp3/unc-3/unc-3* hermaphrodites: of 789 male offspring, 655 (83%) were uncoordinated. By contrast, a similar experiment performed with *Dp1/unc-3/unc-3* hermaphrodites gave 303 uncoordinated animals out of 628 males (48%), which agrees satisfactorily with the expected frequency of 0.5. Loss in the sperm line of the hermaphrodite can only be derived indirectly: if the proportion of eggs contributing no *Dp3* is 0.83, then a nullo-*Dp3* frequency for sperm of 0.54 multiplied by 0.83 would give the observed proportion of offspring without a *Dp3*, 0.45. These rough estimates suggest that loss in the egg line is considerably higher than in the sperm line. Loss during spermatogenesis in males seems also to be low: there were 213 uncoordinated animals out of 396 males (54%) issuing from the mating of *Dp3/unc-3/0* with *unc-3* hermaphrodites; but the corresponding experiment with *Dp1* males gave a frequency of uncoordinated male progeny lower than expected: 226 out of 603 or 37%, so it is possible that male sperm with *Dp1* have a slight selective advantage or that *Dp1*, a half-translocation (see below), in the male gives rise to some aberrant meiotic segregations.

Hermaphrodites homozygous for a mutation called *him-1* (E879) have a high rate of *X* nondisjunction: 17–20% of their progeny are male, and they segregate

triple-*X* animals, which tend to be small (HODGKIN and BRENNER, unpublished results). The *him-1* mutation is recessive in this respect and maps in LG*I*. We have asked whether animals homozygous for *him-1* show enhanced loss of *Dp2*, *Dp3*, or *Dp4*. The mean percentages of uncoordinated animals amongst the progeny of duplication-containing *him-1* hermaphrodites are recorded in Table 3. Each of the three genotypes gave a significantly higher proportion of uncoordinated animals than the corresponding *him-1*$^+$/*him-1*$^+$ strain. The probability of loss of a particular *X* chromosome in a *him-1* strain, assuming each *X* is equally liable, is about 0.1. If we apply the same probability to the frequency of duplication loss and eliminate from consideration the proportion of progeny that would no longer carry the duplication even in the *him-1*$^+$ strain, then we conclude that *him-1* should increase the mean percentage of uncoordinated animals roughly 5–6% in the three duplication strains cited in Table 3. The results are consistent with this interpretation.

It is worth noting in connection with the data of Table 3 that the frequency of male production by hermaphrodites carrying any of the duplications was not significantly higher than for N2 animals (about 0.1%). This indicates that the duplications do not interfere with the normal disjunction of *X* chromosomes.

Dpl *is on linkage group* V: *Dpl1/unc-3/0* males were mated with five double mutants, each homozygous for *unc-3* and a dumpy mutation (*dpy-5*, *dpy-10*, *dpy-18*, *dpy-13*, *dpy-11*) mapping near the middle of an autosomal linkage group. Wild-type hermaphrodite progeny were picked, and the phenotypes of their progeny were scored, ignoring the small sterile animals. In four cases the phenotypic ratios of wild-type:uncoordinated:dumpy:uncoordinated dumpy were close to 6:3:2:1, respectively, as expected if *Dp1* is not linked to the particular dumpy markers used. In the case of *dpy-11*, however, out of 6,912 offspring only 34 recombinants were found: 22 dumpy and 12 uncoordinated animals. Thus *Dp1* is on LG*V* and we hereafter denote it as *Dp*(*X*;*V*)*1*. We next ask about the genotypes of the recombinants. The *Dp*(*X*;*V*)*1*-bearing chromosome must have been involved in all of the events that produced the recombinants, but its partner in crossing over in each case could have been either its *V* homologue or an *X* chromosome. Considering the dumpy recombinants first, Table 4 shows that if an *X* is involved in the recombination, then an *X* chromosome carrying *unc-3*$^+$ will be produced. This chromosome could, as a result of its recombination with the *Dp*(*X*;*V*)*1*-chromosome, be inviable in homozygous form; but, its presence or absence can be detected in any case by mating each dumpy recombinant with N2 males and selecting wild-type hermaphrodite offspring, whose genotypes can be deduced from the phenotypic ratios of their progeny, as shown in Table 4.

All 22 dumpy recombinants were mated with N2 males, and in each case 12 wild-type hermaphrodite offspring were picked and put on separate plates. The phenotypic ratios of the progeny of these animals were determined. In all 22 cases, two phenotypic ratios were found: one ratio was 9:3:3:1 and the other was invariably 11:1 for the ratio of wild-type to uncoordinated animals, with less than 1% fertile dumpy or uncoordinated dumpy segregants. Approximately 20% of the segregants were sterile dumpy animals, however. These animals were

TABLE 4

On the genotypes of dumpy recombinants segregated by unc-3/unc-3; Dp(X;V)1 dpy-11+/dpy-11

Pairing partner of *Dp(X;V)1*-chromosome	Recombinant genotype	Genotypes of wild-type hermaphrodite offspring produced by crossing recombinants with N2 males	Phenotypic ratios of segregants, neglecting recombination and neglecting sterile animals:wild-type:uncoordinated: dumpy:uncoordinated dumpy
LG*V*	*unc-3/unc-3; Dp(X;V)1 dpy-11/dpy-11*	*unc-3/unc-3+; dpy-11/dpy-11+*	9:3:3:1
		unc-3/unc-3+; Dp(X;V)1 dpy-11/dpy-11+	11:1:0:0
X	*unc-3/unc-3+; dpy-11/dpy-11*	*unc-3/unc-3+; dpy-11/dpy-11+*	9:3:3:1
		unc-3+/unc-3+; dpy-11/dpy-11+	3:0:1:0

easy to distinguish from the others: their growth was retarded so they had a characteristic small dumpy appearance, and they never developed gonads. From these results we first conclude that all 22 dumpy animals were generated by recombinational events involving the *Dp(X;V)1*-chromosome and its *V* homologue. Secondly, we conclude that the slowly developing sterile animals noted earlier (Table 2) are homozygous for *Dp(X;V)1*.

The 12 uncoordinated recombinants can be analyzed more easily. If an *X* chromosome is involved in the recombinational event generating an uncoordinated animal, then the resulting genotype would be: *unc-3/unc-3*; *Dp(X;V)1 dpy-11+/dpy-11*, where the duplication now carries the *unc-3* allele. Assuming, as before, that this animal cannot segregate fertile *Dp(X;V)1* homozygotes, then more than 99% of its fertile uncoordinated progeny will segregate dumpy (uncoordinated) offspring. On the other hand, if the *Dp(X;V)1*-chromosome recombined with *V*, then the resulting uncoordinated recombinant genotype would be: *unc-3/unc-3*; *dpy-11+/dpy-11*. One-third of the uncoordinated offspring of this animal would not segregate dumpy animals.

For each of the 12 uncoordinated recombinants, 10 uncoordinated offspring were picked and put on separate plates. In all 12 cases at least 2 of the 10 animals (up to as many as 7 out of 10) segregated uncoordinated progeny exclusively. We conclude that all of the uncoordinated recombinants tested, as was true for the dumpy recombinants, were produced by events involving the *Dp(X;V)1*-chromosome and its *V* homologue.

In order to map *Dp(X;V)1* on LG*V*, the dumpy offspring from one of the dumpy recombinants described above were mated with N2 males; and wild-type male progeny (*unc-3/0*; *Dp(X;V)1 dpy-11/dpy-11+*) were selected. These males were crossed with the paralyzed mutants *unc-66* and *unc-51*, which map near the two ends of LG*V*. Wild-type hermaphrodite offspring were picked. Those carrying *Dp(X;V)1 dpy-11* were recognized on the basis that they segregated small, sterile dumpy progeny. That wild-type animals were isolated shows that the *Dp(X;V)1*-chromosome carries both *unc-66+* and *unc-51+*. Sixty-five wild-type hermaphrodite progeny from *Dp(X;V)1 unc-66+ dpy-11/unc-66 dpy-11+* were picked and put individually on small plates. Sixty-four proved to have the same genotype as their parent since they segregated both small, sterile dumpies and paralyzed animals (as well as wild-types). Only one animal carried a recombinant chromosome: it segregated paralyzed animals but no small, sterile dumpies. Thus, *Dp(X;V)1* reduced the recombination frequency between *dpy-11* and *unc-66* (normally about 21%) to roughly 1/130. On the other hand, recombination between *dpy-11* and *unc-51* is little affected by the presence of *Dp(X;V)1*. Fifty wild-type hermaphrodite progeny from *Dp(X;V)1 dpy-11 unc-51+/dpy-11+ unc-51* were picked and put individually on small plates: 23 segregated both small, sterile dumpies and paralyzed animals, 14 segregated small, sterile dumpies but no paralyzed animals, 8 segregated paralyzed animals but no small, sterile dumpies, and 5 segregated wild-type progeny only. Thus, the recombination frequency between *dpy-11* and *unc-51*, where *Dp(X;V)1* is cis to *dpy-11*, is roughly 32/100, which agrees with the normal value of about 28%.

Recombination frequencies between *DP(X;V)1* and *unc-66* and *Dp(X;V)1* and *unc-51* were also measured more directly. A total of 3,536 offspring of *unc-3/unc-3*; *Dp(X;V)1 unc-66*$^+$*/unc-66* animals were screened, and no recombinants were found. That *unc-3* was in fact present in the parents was checked by mating them with N2 males. As expected, both uncoordinated (of the *unc-3* type) and wild-type male progeny were produced. From *unc-3/unc-3*; *Dp(X;V)1 unc-51*$^+$*/unc-51* hermaphrodites 91 *unc-3* type recombinants were found, corresponding to 27% recombination. Finally, amongst the progeny of *unc-3/unc-3*; *Dp(X;V)1 unc-42*$^+$*/unc-42* animals, five recombinants of the *unc-42* type (whose phenotype is less severe than that of *unc-3*) were found, corresponding to a recombination frequency of about 0.5%.

In summary, *Dp(X;V)1* shows no (< 0.1%) recombination with a left end marker *unc-66*, shows only about 0.5% recombination with middle markers *dpy-11* and *unc-42*, and shows about 25–30% recombination with a right end marker *unc-51*.

LGV genes present on the Dp(X;V)1*-containing chromosome*: Results already described have shown that the *Dp(X;V)1*-chromosome carries *unc-66*$^+$, *dpy-11*$^+$, *unc-42*$^+$ and *unc-51*$^+$. Since it is possible that a portion of the LGV chromosome was deleted when *Dp(X;V)1* was attached to its new linkage group, we have tested for the absence of other LG*V* markers. This was done by crossing *unc-3/0*; *Dp(X;V)1/+* males with various mutants and checking the phenotypes of the resulting male offspring, just as in the usual complementation test (BRENNER 1974). This was done with the following mutants: *sma-1*, *unc-46*, *unc-39*, *unc-41*, *unc-23*, *unc-70* and *unc-68*. The *Dp(X;V)1*-chromosome proved to carry all of the corresponding wild-type alleles.

Dp2, Dp3, Dp4, *and* Dp5 *are associated with chromosome fragments*: No linkage could be detected between the duplications *Dp2*, *Dp3*, *Dp4*, or *Dp5* and *dpy5*, *dpy-10*, *dpy-18*, *dpy-13*, or *dpy-11*. These duplications are therefore either free or attached to sites only very weakly linked to the midpoints of any autosomes. In view of both this result and the anomalous segregation ratios already noted (Table 3), we decided to look at the oocytes of hermaphrodites carrying these duplications to see if chromosome fragments might be observed cytologically. To our surprise, such fragments were clearly apparent under fluorescence microscopy after staining fixed animals with Hoechst 33258 (Figure 2). The fragments have been found in animals carrying *Dp2*, *Dp3*, *Dp4*, and *Dp5* but not in N2 animals nor in animals harboring *Dp(X;V)1*. (We have been unable to distinguish the karyotype of animals carrying *Dp(X;V)1* from that of N2 animals.) The N2 karyotype of oocytes shows 6 chromosomes of roughly equal size, corresponding properly to the number of linkage groups (BRENNER 1974). The 6 chromosomes are presumed at the stage shown in Figure 2, just prior to fertilization, to be bivalents in the first meiotic prophase, the remaining stages of meiosis occurring after fertilization (NIGON and BRUN 1955). The fragments are much smaller than the normal chromosomes and when observed, can almost always be seen to be present in addition to the full complement of 6 chromosomes. Recall that a brood of animals generated by a *Dp/unc-3/unc-3* hermaphrodite contains both

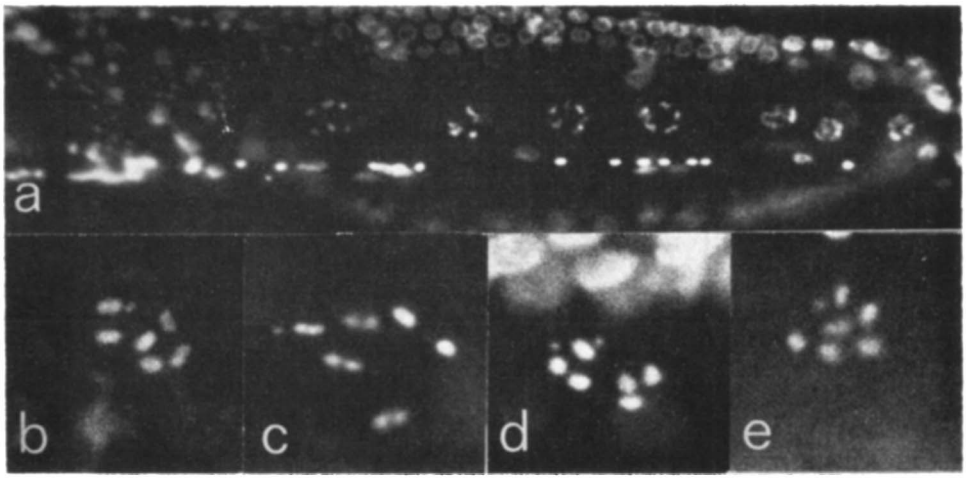

FIGURE 2.—Fluorescence microscopy of oocytes stained with Hoechst 33258. a: N2, showing one arm of two-armed, reflexed gonad. Tightly packed nuclei at top of picture are oocyte precursors. Faint dots at left are sperm. As oocytes develop, they gradually join a single file (horizontal row through middle of picture) that moves toward the sperm, where they are fertilized. The 3 or 4 unfertilized oocytes of each arm nearest the sperm are in diakinesis and are the ones we have screened for the presence of fragments. Oocyte maturation divisions occur after fertilization. (The horizontal row of small, bright nuclei below the oocytes is part of the ventral nerve cord.) b: Oocyte from *Dp2* animal. c: *Dp3* oocyte. d: *Dp3* oocyte showing two fragments. e: *Dp4* oocyte. Magnification in a: ×680. Magnification in b–e: ×1700.

wild-type animals, which presumably contain the duplication, and uncoordinated animals, which presumably do not. From such broods we have picked out wild-type and uncoordinated animals individually and treated them for fluorescence microscopy. The fragments were found in wild-type animals but not in the uncoordinated animals. We have screened wild-type animals one at a time counting the number of oocytes with distinct chromosomes at the stage shown in Figure 2 and the number of oocytes clearly containing a fragment. These data are summarized in Table 5, which shows that about 50% to 80% of the oocytes in the duplication mutants contained clearly discernable fragments. That significantly more than half of the oocytes in *Dp3* and *Dp4* animals exhibit the fragment cytologically supports the contention that these cells are pre-anaphase I. The question then arises as to why the other 20% to 50% of the oocytes showed

TABLE 5

Cytological screening of oocytes for the presence of chromosome fragments

Duplication	Number of animals screened	Number of oocytes screened	Number of oocytes showing a fragment
Dp2	18	95	50
Dp3	30	108	82
Dp4	9	29	23
Dp5	21	71	41

TABLE 6

Distribution of fragments in pairs of neighboring oocytes

Duplication mutant	Both oocytes showing fragments		One oocyte showing a fragment		Neither oocyte showing a fragment	
	Observed	Expected*	Observed	Expected	Observed	Expected
Dp2	21	15	20	32	23	17
Dp3	43	39	20	27	8	5
Dp4	11	11	5	6	2	1
Dp5	18	11	5	19	14	7

* Expected numbers were calculated on the assumption that fragments were randomly distributed (one degree of freedom for each set of 3 numbers).

no fragments: this seems too high a fraction to be explained simply by their being obscured by other chromosomes. We suggest that the existence of many oocytes not showing fragments is related to the premeiotic loss of *Dp2*, *Dp3*, *Dp4*, and *Dp5*. It is of interest in this connection to point out that the oocytes with and without discernible fragments were not randomly situated with respect to each other in the gonads of these mutants. In Table 6 we have tabulated data on all of the pairs of neighboring oocytes we have observed. The table shows that there is a tendency for oocytes of the same type to be next to one another. (The probability that the deviations from the expected numbers are due solely to chance is < 0.001.) The simplest interpretation of this result is that neighboring oocytes tend to have a common ancestral cell in which the duplication was lost; that is, premeiotic loss generates a clone of oocytes in which the duplication is absent. Rare but clear cases of oocytes containing 6 bivalents and 2 fragments (Figure 2) also suggest that mitotic nondisjunction of duplications can occur.

Recombination involving Dp3 *or* Dp4 *and* X: Since *Dp3* and *Dp4* both include *unc-9+* as well as *unc-3+*, which are about 6 map units apart, it is possible to manage a fairly sensitive test for recombination between *Dp3* or *Dp4* and the *X* chromosomes. The results are given in Table 7. Since the phenotype of the *unc-3 unc-9* double mutant is close to that of *unc-3*, only the recombinant with the phenotype of *unc-9*, which involves a less severe uncoordinatedness than does *unc-3*, could be detected. No recombinants were found in over 3,600 progeny, combining the data for *Dp3* and *Dp4*. To show that the *unc-9* allele was still present in the stocks, we picked an uncoordinated segregant from each stock, mated them with N2 males, picked wild-type hermaphrodite offspring, and

TABLE 7

On recombination of Dp3 *or* Dp4 *with* X

Parents	Total number of offspring inspected	Number of *unc-9* type recombinants
unc-9 unc-3/unc-9+ unc-3+	933	28
Dp3/unc-9 unc-3/unc-9 unc-3	2,025	0
Dp4/unc-9 unc-3/unc-9 unc-3	1,601	0

looked for *unc-9* type recombinants. Recombinants in each case were found and their frequency of appearance was about 3%, as expected (Table 7). We conclude that recombination between *Dp3* or *Dp4* and the homologous region of the *X* chromosome is 50 or more times rarer than recombination between *X* chromosomes in the same region.

DISCUSSION

We have identified two sorts of chromosome rearrangement in *C. elegans*. Both involve duplications of parts of the *X* chromosome near the right end; and in both cases the duplications are not *X*-linked. One duplication, *Dp(X;V)1* is translocated to LG*V*. The data are consistent with its being attached near the left end of *V*: it is extremely tightly linked to the left-most marker, *unc-66*. The presence of *Dp(X;V)1* severely suppresses crossing over along the left half of LG*V*. Heterozygous translocations in Drosophila do frequently suppress crossing over (ROBERTS 1970), but it is also possible that an additional rearrangement, such as an inversion, could be involved in the attachment of *Dp(X;V)1* to LG*V*.

The other duplications we have identified are all associated with chromosome fragments, as seen cytologically by fluorescence staining of oocytes. Both from the phenotypic ratios of offspring produced by duplication heterozygotes and from the cytology of oocytes of duplication-containing animals, we conclude that *Dp2*, *Dp3*, *Dp4*, and *Dp5* are susceptible to loss. Moreover, the cytological results indicate that this loss can occur premeiotically. The simplest interpretation of the results is that these duplications are autonomous and tend to be lost during nuclear division. Grossly deleted Drosophila chromosomes undergo meiotic loss (LINDSLEY and SANDLER 1958), as do univalents in a variety of plants and animals (SANDLER and BRAVER 1954). Moreover, telocentric partial chromosomes (which can be produced from univalents during meiosis) are somatically unstable (e.g., RHOADES 1940; STEINITZ-SEARS 1966). There may be specially evolved mechanisms controlling the segregation of *X* chromosomes in *C. elegans* hermaphrodites, since it is by loss of an *X* that a male is produced. The high rate of duplication loss might therefore be due to the absence of some portion of the *X* chromosome critical to the normal control of *X* segregation. Supporting the view that *Dp2*, *Dp3*, and *Dp4* are autonomous are our results with the *him-1* (E879) mutation, which appears to promote nondisjunction of *X* chromosomes selectively (HODGKIN, unpublished results): the frequencies of loss of *Dp2*, *Dp3*, and *Dp4* (*Dp5* was not tested) in strains homozygous for *him-1* were higher than in the corresponding *him-1*$^+$ strains.

Our principal reason for looking for chromosome rearrangements, as noted in the introduction, was to obtain chromosome balancers for maintaining recessive lethal mutations in heterozygotes. *Dp(X;V)1* may prove useful in this respect as a balancer for the left half of LG*V*: it strongly suppresses crossing over in this region, and the homologous chromosome is not easily lost because animals homozygous for *Dp(X;V)1* are sterile and morphologically distinct. Recombination between *Dp(X;V)1* and *X* appears also to be very infrequent; therefore, *Dp(X;V)1* may also be a good balancer for the portion of *X* that it duplicates.

The other duplications may also be used as balancers for the regions of *X* they duplicate. *Dp3* and *Dp4*, the duplications for which recombination could be looked for easily, certainly satisfy the criterion for a good balancer that recombination between balancer and the region it balances should be low. The absence of stable duplication homozygotes is also a desirable property. A potential difficulty with these duplications as balancers, however, is their tendency to be lost. Frequent losses during somatic development could obviously reduce the value of the duplications as balancers of recessive lethal mutations. On the other hand, it is possible that such losses could be useful for producing mosaic animals. Unfortunately, there are no markers presently available in the duplicated region to test this idea.

The duplications may find other uses as well. *Dp(X;V)1* has already been used to map *X*-linked markers with respect to *unc-3*: the *unc-3/0*; *Dp(X;V)1/+* male, which mates very much better than an *unc-3/0* male, was used to transmit *unc-3* to mutant hermaphrodites. Finally, we suggest the possibility of the duplication fragments finding a use in the fractionation of the *C. elegans* genome. The fragments are clearly much smaller than any of the normal chromosomes, so it is possible they could be separated from the rest of the genome on this basis.

The scheme we used to select for duplications gave us many male candidates, the vast majority of which were sterile; but on the assumption that most of the candidates carried chromosome rearrangements, we defined conditions for the effective induction of rearrangements by X-irradiation of males. The effectiveness of other mutagens in this regard could be tested by the same procedure.

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