CROSSOVER SUPPRESSORS AND BALANCED RECESSIVE LETHALS IN *CAENORHABDITIS ELEGANS*

ROBERT K. HERMAN

Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55108

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ABSTRACT

Two dominant suppressors of crossing over have been identified following X-ray treatment of the small nematode *C. elegans*. They suppress crossing over in linkage group *II* (LG*II*) about 100-fold and 50-fold and are both tightly linked to LG*II* markers. One, called *C1*, segregates independently of all other linkage groups and is homozygous fertile. The other is a translocation involving LG*II* and *X*. The translocation also suppresses crossing over along the right half of *X* and is homozygous lethal. *C1* has been used as a balancer of LG*II* recessive lethal and sterile mutations induced by EMS. The frequencies of occurrence of lethals and steriles were approximately equal. Fourteen mutations were assigned to complementation groups and mapped. They tended to map in the same region where LG*II* visibles are clustered.

THE nematode *Caenorhabditis elegans* is gaining favor as an organism in which to study the genetic basis of animal development and behavior, primarily because of its relative cellular simplicity (SULSTON and HORVITZ 1977) and its suitability for genetic analysis (BRENNER 1974). Most work with *C. elegans* is thus based on the idea that the detailed analysis of mutants, both at the cellular level and the molecular level, will prove to be an important approach in gaining an understanding of the genetic programs controlling development. Since many of the genes in *C. elegans* play indispensable roles in development, temperature-sensitive mutants are being utilized extensively (see, for example, EPSTEIN and THOMPSON 1974; HIRSH and VANDERSLICE 1976). Another approach is to make use of recessive lethal and sterile mutations, which of course must be maintained in heterozygous stocks. Each such heterozygote should be made distinguishable phenotypically from the homozygous wild type by using a suitably marked balancer. Furthermore, complementation and mapping of lethals and steriles would be greatly facilitated by using visibles that are tightly linked to the lethals. The most useful balancers, then, recombine as little as possible within the region they balance, which means they are often made to carry crossover-suppressing properties. In a previous paper (HERMAN, ALBERTSON and BRENNER 1976), unlinked duplications of a part of the *X* chromosome of *C. elegans* were identified, and their potential use as balancers was discussed. Indeed, one of these duplications, *Dp (X;V)1*, has now been used to balance a

number of *X*-linked recessive lethal and sterile mutations (MENEELY and HERMAN, unpublished results). In the work reported here, X-ray-induced crossover suppressors for linkage group *II* (LG*II*) were selected for directly, and the virtues of one such crossover suppressor as a balancer were explored by using it to balance a number of ethyl methanesulfonate (EMS)-induced recessive steriles and lethals, 14 of which were tested by complementation analysis and mapped.

MATERIALS AND METHODS

Strains and growth: N2 (wild type) and mutant strains of *Caenorhabditis elegans* var. Bristol were obtained originally from S. BRENNER and grown with *Escherichia coli* strain OP50 as food source by methods described by BRENNER (1974). Linkage relationships between the visible mutants used in this work are shown in Figure 1. The allele numbers of the mutant genes studied are given in BRENNER's Table 4 (1974), with the following exceptions: *unc-3(e151) X*, *dpy-4(e1166) IV*, and *unc-17(e933) IV*. Another mutant obtained from BRENNER was *him-1 (e879) I* which has a high rate of *X* nondisjunction, so that about 17% of the hermaphrodites' progeny are male.

A male stock of MN-H7 (see below) was established by shifting several young adult MN-H7 hermaphrodites from their normal growth temperature of 20° to 27.5° for 30 hr and

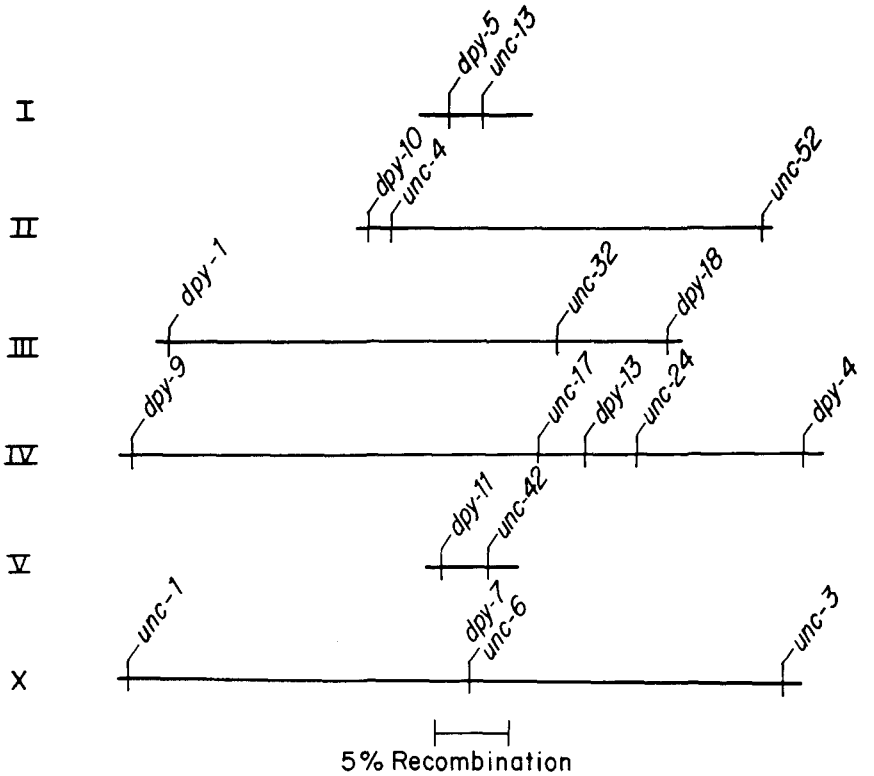

FIGURE 1.—Linkage relationships between loci used in this work. See BRENNER (1974) for a more extensive map. LG*I* and LG*V*, for example, are not relatively small linkage groups.

then back to 20°. This procedure markedly increased the occurrence of male progeny, which were picked and crossed back to MN-H7 hermaphrodites to initiate a male stock.

Mating, counting, and mapping procedures: Mating and counting procedures were generally as described by HERMAN, ALBERTSON and BRENNER (1976), except that the small petri plates used for matings and progeny counts were 35 mm in diameter (Falcon 1008).

Egg-hatching frequencies were determined as follows. Hermaphrodites were placed on small plates containing 10–15 mm diameter bacterial lawns and allowed to lay eggs for 3–5 hr, after which the animals were picked off, and the eggs, most of which were on the lawn, were counted under a dissecting microscope. The number of animals on the plate was counted 1–3 days later.

Several recombination distances were measured by counting progeny produced from hermaphrodites that were *cis* heterozygotes (+ +/ab), as described by BRENNER (1974), except that instead of counting all of the progeny, I usually counted only the A, B, and AB phenotypes. If c is defined as the ratio of the numbers of A plus B animals to the numbers of A plus B plus AB, then it is easy to show that the recombination frequency, $p = 1-\sqrt{1-c/(2-c)}$. The measurement of c follows the binomial distribution, for which symmetric 95% confidence limits were tabulated (MAINLAND, HERRERA and SUTCLIFFE 1956). The corresponding confidence limits for p were then calculated. Since the mapping of LG*II* lethal mutations was done in *him-1*/+ stocks, the effect of *him-1* heterozygosity on LG*II* recombination frequencies was checked. Neither the *unc-4–dpy-10* nor the *unc-4–unc-52* recombination frequencies were significantly different in the *him-1*/+ strain versus *him-1*+.

*Irradiation and screening for suppression of crossing over on LG*II: A partially synchronized population containing many *dpy-10 unc-52/unc-4* hermaphrodites was produced in the following way. First, *dpy-10 unc-52/unc-4* stocks were maintained by picking six wild-type progeny every four days and putting each on a fresh plate. Because of frequent crossing over between *dpy-10* and *unc-52*, only 65% of the animals picked had the parental genotype. These were easily identified by inspecting the phenotypes of their progeny. The phenotype of *unc-52* adults, which I call paralyzed, is a completely flaccid body, which is easily distinguished from the uncoordinated phenotype of *unc-4*. Three days before X irradiation, 20 egg-laying, wild-type progeny from a *dpy-10 unc-52/unc-4* stock were put singly onto plates for 10 hr, after which they were transferred, two per plate, to 10 small, seeded plates. The animals were removed after 12 hr of egg-laying. Four of the latter plates, chosen on the basis that both parents proved to have been the *dpy-10 unc-52/unc-4* genotype, were X irradiated with 7,500r (roentgens) at a rate of 450r per min. The irradiated animals were 49–61 hr old, the ages being measured from the time the embryos were laid as eggs by the hermaphrodite parent.

Five to eight progeny from each of 221 irradiated wild-type hermaphrodites were plated singly and their progeny inspected. The goal was to find plates containing wild-type and paralyzed dumpy animals (and probably *unc-4* animals as well) but few dumpy or paralyzed animals. From a total of 1,472 plates screened, in two runs, 423 gave no or too few progeny to permit classification, 391 gave no paralyzed dumpy progeny, and 650 gave many paralyzed or dumpy animals, or both, in addition to paralyzed dumpy and wild-type animals. The eight candidates remaining were quickly pared to the best three and subsequently to the two showing the strongest crossover suppression. These two stocks were maintained by picking wild-type progeny every generation. After backcrossing by the procedures described under RESULTS to reduce the probability of the stocks carrying extraneous mutations or rearrangements, the stocks were designated MN-H7 and MN-H8 (MN as a laboratory prefix and H standing for heterozygote) and maintained as before. Samples of each have also been stored in liquid nitrogen (BRENNER 1974).

Mutagenesis with EMS: EMS mutagenesis was by the procedures described by DUSENBERY, SHERIDAN and RUSSELL (1975), using 47 mm Millipore type SC filters for washing the animals before and after exposure to EMS. In the run with MN-H7, young adult hermaphrodites not containing eggs were picked out after mutagenesis, and only four F_1 progeny of any one mutagenized parent were picked and their *unc-4* progeny screened. In the run with MN-H9 (defined in RESULTS), egg-laying adults were picked, but they were transferred to fresh plates

on the day following mutagenesis so that their first day's progeny could be ignored. Five to eight F_1 progeny of each mutagenized parent were picked and their progeny screened.

RESULTS

*Identification of crossover suppressors for LG*II: Table 1 gives the frequencies at which the various offspring of *dpy-10 unc-52/unc-4* hermaphrodites appeared. Apart from a slight deficit in the appearance of the paralyzed phenotype, the observed frequencies agreed with the frequencies expected on the basis of the known map distances. The production of dumpy progeny and paralyzed progeny, which comprised 21% of the animals, obviously requires crossing over between *dpy-10* and *unc-52*. MN-H7 and MN-H8 are stocks derived from animals that were selected after X irradiation of *dpy-10 unc-52/unc-4* on the basis that they segregated paralyzed dumpy animals at roughly normal frequency but yielded many fewer paralyzed progeny and dumpy progeny than their unirradiated parent. I shall refer to the features of the genetic material that confer these crossover-suppressing properties to strains MN-H7 and MN-H8 as *C1* and *C2*, respectively.

C1 *is coupled to* dpy-10 *and* unc-52, *and* C2 *is coupled to* unc-4: The original *C1*-bearing and *C2*-bearing stocks were crossed to N2 males. Wild-type male progeny were then crossed to *unc-4* hermaphrodites and to *dpy-10 unc-52* hermaphrodites. Single wild-type hermaphrodite progeny were picked and their offspring checked. Obviously, the animals of interest were those that carried both a *dpy-10 unc-52* chromosome (or chromosomes) and an *unc-4* chromosome, with one of the two coming from a crossover-suppressed stock. The presence of *C1* (or *C2*) was then easily checked by looking at the frequency of appearance of dumpy and paralyzed animals. The results showed that *C1* was invariably coupled to *dpy-10* and *unc-52* and that *C2* was invariably coupled to *unc-4*. Moreover, in each case the crossover-suppressing property was dominant, as expected from the manner in which the original stocks were selected. Hermaphrodites

TABLE 1

Progeny of dpy-10 unc-52/unc-4 *hermaphrodites*

Strain	Progeny phenotypes*				
	Wild type	Uncoordinated (unc-4)	Dumpy paralyzed	Dumpy	Paralyzed (unc-52)
Unirradiated parent	.415	.240	.135	.121	.090
MN-H7	.505	.252	.241	.001	.001
MN-H8	.697	.131†	.168	.003	.001

* Total progeny examined were 2,718, 3,027, and 1,524 for the unirradiated parent, MN-H7, and MN-H8, respectively.

† None of these animals developed beyond an early larval stage.

carrying *C2* displayed two additional characteristics: low brood sizes (because they gave many inviable zygotes—see below) and high incidences (about 3%) of male progeny. One *C2*-bearing stock produced in the way just described was designated MN-H8. For *C1*, the procedure was repeated and a resulting *C1*-bearing animal was used to initiate the stock called MN-H7. As already noted, the phenotypic frequencies of the offspring produced by MN-H7 and MN-H8 are given in Table 1. Dumpy and paralyzed offspring constitute only 0.2% and 0.4% of the progeny of MN-H7 and MN-H8, respectively.

C2 *is a translocation involving LG*II *and* X: Males arising spontaneously on MN-H8 plates were picked and mated with five different double mutant hermaphrodites: each was homozygous for *dpy-10* and one additional marker, a representative from each of the linkage groups besides *II*: *unc-13 I*, *unc-32 III*, *unc-24 IV*, *unc-42 V* and *unc-6 X*. Wild-type hermaphrodites were picked and their dumpy, uncoordinated, and uncoordinated dumpy progeny were counted. In all but one case, the ratio of uncoordinated dumpy animals to dumpy plus uncoordinated plus uncoordinated dumpy animals was roughly 1/8, the value expected if the chromosome represented by the *unc* marker is not involved in a translocation with *II*. The exceptional case involved *unc-6 X*. Table 2 shows that animals heterozygous for *unc-6 X* and *dpy-10 II* and carrying *C2* gave extremely few recombinant progeny homozygous for either *unc-6* or *dpy-10* only. This means that *dpy-10*$^+$ and *unc-6*$^+$ were tightly coupled, and therefore both must be tightly coupled to *C2*; hence, *C2* is a translocation involving LG*II* and *X*.

Let the two parts of *C2* be called $C2^{II}$ and $C2^{X}$, and suppose the disjunction of $C2^{II}$ and a normal *II* is independent of the disjunction of $C2^{X}$ and a normal *X*. This is equivalent to saying that alternate and adjacent-1 segregations are equally probable and that adjacent-2 segregations are negligible. Since *dpy-10* and *unc-6* recombine so little with *C2*, they are useful markers of the normal *II* and *X*, respectively. Figure 2 then illustrates what gametes and zygotes would be expected from *C2 unc-4/dpy-10 unc-6* hermaphrodites. One further assumption has been made: unbalanced gametes, carrying $C2^{II}$ but not $C2^{X}$, or *vice*

TABLE 2

On the segregation of C2 *with respect to* X*-linked markers*

Parent	Progeny				
	Wild type	unc-4 larvae	Uncoordinated* dumpy	Dumpy	Uncoordinated*
C2 unc-4 II/dpy-10 II unc-6 X	664	104	173	0	0
C2 unc-4 II/dpy-10 II unc-3 X	678	88	137	2	0
C2 unc-4 II/dpy-10 II unc-1 X	316	72	†	†	93

* These classes of uncoordinated animals refer to *unc-6*, *unc-3*, and *unc-1*, each of which is clearly distinguishable phenotypically from the *unc-4* type. No adult *unc-4* animals were found.
† The sum of these two phenotypes, which were sometimes difficult to distinguish, was 126.

Sperm / Eggs	$C2^{II}C2^{X}$	*dpy-10 unc-6*	$C2^{II}$ *unc-6*	*dpy-10* $C2^{X}$
$C2^{II}C2^{X}$	*unc-4* Larvae	W	†	†
dpy-10 unc-6	W	DU	†	†
$C2^{II}$ *unc-6*	†	†	†	W
dpy-10 $C2^{X}$	†	†	W	†

FIGURE 2.—Postulated segregation pattern from *C2 unc-4 II/dpy-10 II unc-6 X* hermaphrodites. Abbreviations: W=wild type, DU=dumpy uncoordinated (*unc-6*), †=inviable zygote.

versa, are functional and can produce viable zygotes. Zygotes carrying $C2^{II}$ or $C2^{X}$ only must be inviable, however, since so few *unc-6* or *dpy-10* animals were recovered. Furthermore, 30 wild-type hermaphrodite progeny of *C2 unc4/dpy-10 unc-6* hermaphrodites were picked and progeny tested. All segregated wild-type and uncoordinated dumpy progeny. This means that $C2^{II}/C2^{II}$; $C2^{X}$/*unc-6* and $C2^{II}$/*dpy-10*; $C2^{X}/C2^{X}$ zygotes were also inviable. Thus, none of the genotypes represented in Figure 2 as inviable were in fact recoverable.

Figure 2 predicts that only 6/16 of the zygotes will be viable. This prediction has been satisfied by measurements of egg-hatching frequencies, which showed that only one-third of the eggs laid by MN-H8 hatched (Table 3). The unhatched eggs were encased in the hard shell that characterizes zygotes as opposed to unfertilized oocytes (HIRSH and VANDERSLICE 1976); furthermore, their early

TABLE 3

Egg-hatching frequencies and brood sizes of dyp-10 unc-52/unc-4 *hermaphrodites*

Strain	Egg-hatching frequency	Mean brood size*
Unirradiated parent	165/169	302(9)
MN-H7	146/147	216(14)
MN-H8	172/506	76(10)

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

cleavages appeared to be normal. Figure 2 also predicts that the ratios of translocation homozygotes: translocation heterozygotes: no translocation types should be 1:4:1. The translocation homozygotes were represented by *unc-4* animals. They were all arrested at an early larval stage. The predicted ratios agree well with the progeny counts given in Tables 1 and 2 if one assumes that some of the small *unc-4* larvae, which were difficult to count in any case, did not develop far enough to be counted.

The expected genotype for a spontaneous MN-H8 male is *C2^{II}/dpy-10 unc-4*$^+$ *unc-52*; *C2^X/0*. If such a male were crossed with an *unc-4* hermaphrodite, the only viable zygotes receiving *unc-4*$^+$ would have been produced by nullo-*X* sperm and would therefore be male; that is, all wild-type progeny should be male. This is the result that has been found.

C2 is an effective crossover suppressor for the right half of *X* since *unc-3*, which normally maps about 20 map units to the right of *unc-6*, showed very tight linkage to *dpy-10* also (Table 2). On the other hand, *unc-1 X*, which is about 23 map units to the left of *unc-6*, was only loosely linked to *dpy-10* (Table 2). It is of interest to translate the last line of data in Table 2 into a recombination frequency, *p*, between *unc-1* and *C2^X*. It follows from the assumptions already adopted that there are 4 noncrossover gametes, each occurring at frequency $(1\text{-}p)/4$: *unc-1* or *C2^X unc-1*$^+$ segregating with *C2^{II}* or *dpy-10*. Similarly, there are 4 crossover gametes, each occurring at frequency $p/4$: *unc-1*$^+$ or *C2^X unc-1* segregating with *C2^{II}* or *dpy-10*. An 8-by-8 Punnett square can be constructed, and it can then be shown that the number of *unc-1* animals divided by the sum of the members of wild-type plus uncoordinated dumpy plus dumpy animals equals $4p/5\text{-}4p^2/5$. On this basis the data of Table 3 give 0.23 and 0.50 as the 95% confidence limits for *p*.

C1 *is not a translocation*: Table 3 shows that the egg-hatching frequency from MN-H7 is virtually 100%. Furthermore, Table 1 shows that the ratios of *C1* homozygotes: *C1* heterozygotes: wild-type homozygotes (corresponding to paralyzed dumpy, wild-type, and uncoordinated, respectively) were very close to 1:2:1. These results make it seem unlikely that *C1* is a translocation; however, they would be consistent with *C1* being a translocation if, for example, alternate segregations were somehow strongly favored (and if crossing over in the interstitial regions between centromeres and translocation breakpoints were rare). I have therefore investigated the segregation of *C1* with respect to markers on the other linkage groups, as shown in Table 4. Seven different *dpy* markers were chosen for their wide coverage of the genome. Double homozygous mutants, carrying each *dpy* marker with *unc-4*, were crossed with *C1*-bearing males to produce the parents listed in Table 4. The very low incidence of paralyzed progeny from all seven parental stocks listed in Table 4 confirmed the presence of *C1* in each.

If *C1* and a particular *dpy* marker segregated independently, then a *C1 dpy-10 unc-52/unc-4*; *dpy/*+ parent would give, neglecting the rare LG*II* crossovers, ratios of wild type: paralyzed dumpy: uncoordinated: dumpy: uncoordinated dumpy progeny of 6:4:3:2:1, respectively. On the other hand, if *C1* were com-

TABLE 4

On the segregation of C1 *with respect to other linkage groups*

Parent genotype	Progeny phenotypes*						r† (95% confidence limits)
	W	PD	U	D	UD	P	
C1 dpy-10 unc-52/unc-4; dpy-5/+	232	143	125	83	47	2	.14-.24
C1 dpy-10 unc-52/unc-4; dpy-1/+	171	107	98	54	28	1	.11-.22
C1 dpy-10 unc-52/unc-4; dpy-18/+	268	162	119	79	43	0	.13-.23
C1 dpy-10 unc-52/unc-4; dpy-9/+	191	140	109	57	37	1	.13-.24
C1 dpy-10 unc-52/unc-4; dpy-13/+	168	88	62	54	16	1	.07-.19
C1 dpy-10 unc-52/unc-4; dpy-11/+	244	171	122	84	42	1	.13-.22
C1 dpy-10 unc-52/unc-4; dpy-7/+	270	206	130	94	53	1	.15-.24

* The symbols stand, in the order given, for wild-type, paralyzed dumpy, uncoordinated (*unc-4*), dumpy, uncoordinated dumpy, and paralyzed (*unc-52*) animals.

† r is defined as the ratio of the number of uncoordinated dumpy animals to the sum of the numbers of uncoordinated plus dumpy plus uncoordinated dumpy animals.

pletely linked to the *dpy* marker, then the respective ratios would be 2:1:0:0:1. A sensitive measure of linkage is the ratio of the number of uncoordinated dumpy progeny to the sum of the numbers of uncoordinated plus dumpy plus uncoordinated dumpy progeny. Let this ratio be r. For no linkage, $r = 1/6$ and for complete linkage $r = 1$. All seven parents in Table 4 gave values of r not significantly different from 1/6. To be more specific, it can be shown that the upper confidence limits given in Table 4 in every case correspond to at least 40% recombination between *C1* and each *dpy* marker. This result, plus the expectation that a translocation might well suppress crossing over in the region where it resides (ROBERTS 1970; HERMAN, ALBERTSON and BRENNER 1976), makes it seem unlikely that *C1* is linked to any linkage group besides II. All of the evidence taken together indicates that *C1* is probably an intrachromosomal rearrangement, presumably an inversion or transposition.

C1 *is homozygous fertile*, C2 *is homozygous inviable*: Fifteen paralyzed dumpy progeny of MN-H7, which I infer were homozygous for *C1*, were picked: all were fertile, with an average brood size of 7. By comparison, 15 paralyzed dumpy progeny of the unirradiated *dpy-10 unc-52/unc-4* parent gave an average brood size of 8. The *C2* homozygotes, however, were arrested at an early larval stage. This recessive lethality has not been separated from *unc-4* since no adult *unc-4* animals have been obtained. Whether the defect is conferred by some aspect of *C2* itself or by a second X-ray-induced alteration, inseparable from *C2* owing to the suppression of crossing over, has not been determined. The defect appears to be on the $C2^{II}$ chromosome and not $C2^{X}$ because spontaneous wild-type male progeny of MN-H8 (and of $C2^{II}$/*dpy-10*; $C2^{X}$/*unc-6*) were viable (and fertile).

Crossing over involving C1*-bearing chromosomes generally gives aberrant products*: Twenty-nine recombinant chromosomes produced by MN-H7 have been checked and none could be made homozygous. This was done as follows. Twenty dumpy and nine paralyzed progeny of MN-H7 were picked. All 29 exhibited very low fertility, but at least 20 fertile descendants of each animal were checked as to whether or not they segregated paralyzed dumpy descendants. All did; that is, each fertile dumpy animal gave both dumpy and paralyzed dumpy descendants and each fertile paralyzed animal gave both paralyzed and paralyzed dumpy descendants. I conclude that all 29 crossover products tested were homozygous lethal despite the fact that the chromosomes participating in the crossing over were originally homozygous viable. This result is clearly consistent with the supposition that *C1* is a rearrangement which gives rise to crossover products bearing duplications and deficiencies.

C1 *and* C2 *do not suppress crossing over everywhere*: We have already seen that crossing over between *unc-1* and $C2^x$ occurs at relatively high frequency. Crossing over in both LG*IV* and LG*V* in the presence of *C1* has been measured, and the results are given in Table 5. As before, the presence of *C1* was confirmed when 1/4 of the progeny were paralyzed dumpy and only 0.1% were paralyzed. Interchromosomal effects of rearrangements on crossing over are common in Drosophila (SCHULTZ and REDFIELD 1951; LUCCHESI 1976), but clearly *C1* had little interchromosomal effect on crossing over between the markers investigated.

Recessive lethal and sterile mutations balanced by C1: The objective in isolating crossover suppressors is to use them to balance recessive lethal and sterile mutations. *C1* seemed a good candidate for such a role, so I set out to use it to balance LG*II* recessive lethal and sterile mutations coupled to the visible marker *unc-4*, which could then be used in complementation testing (see below). Two stocks (one run of each) were exposed to EMS: MN-H7 and a related stock: *him-1*; *C1 dpy-10 unc-52/unc-4*, which I call MN-H9. In each run, wild-type hermaphrodite progeny of mutagenized animals were picked and put on separate plates, and their uncoordinated progeny were screened. Two classes of mutants

TABLE 5

Interchromosomal effect of C1 *on crossing over*

Parent	Recombination frequency (95% confidence limits)
unc-17 dpy-4/+	0.16-0.21
C1 dpy-10 unc-52/+; unc-17 dpy-4/+	0.18-0.24
unc-42 dpy-11/+	0.017-0.030
C1 dpy-10 unc-52/+; unc-42 dpy-11/+	0.023-0.038

were distinguished. The first class, embryonic and larval lethals, was characterized by the absence of adult uncoordinated hermaphrodite progeny from F_1 animals. When adult uncoordinated progeny from F_1 animals were present, 8–10 were picked and put on a plate to see if they gave offspring. Those that did not were tentatively assigned to the second class, steriles. Both classes of mutants were propagated by picking single wild-type hermaphrodite progeny of F_1 animals. The screening tests were repeated, and those stocks passing both tests were maintained as before. In this way 102 mutant stocks were established: 52 embryonic and larval lethals and 50 steriles. These mutants comprised 23% of the fertile F_1 animals from mutagenized MN-H7 and 8% of the fertile F_1 animals from MN-H9. These high frequencies suggest that some of the *unc-4* chromosomes, in fact, carried more than one mutation.

Verifying lethals and cleaning up stocks: A set of embryonic and larval lethals was chosen for further analysis: all 13 mutants in this class derived from MN-H9, which were therefore randomly chosen. Hermaphrodites with the putative genotype *him-1*; *C1 dpy-10 unc-52/unc-4 let*, where *let* stands for a recessive lethal mutation, were crossed with *him-1*; *C1 dpy-10/unc-4* males. Mature *unc-4* progeny (*him-1*; *unc-4 let/unc-4 let*$^+$) were produced, indicating that the mutant stock still carried the *unc-4* gene. Uncoordinated hermaphrodites were picked and crossed back to *him-1*; *C1 dpy-10 unc-52/unc-4* males. Wild-type hermaphrodite progeny were picked and placed individually onto small plates. We expect that half of these animals will again not segregate adult uncoordinated progeny. This proved to be true for all 13 mutants. This result indicates that all 13 lethals were indeed coupled to the *unc-4* marker; furthermore, the procedure provided a new stock of each mutant that had been through two backcrosses, giving each mutant stock an unmutagenized balancer and reducing the likelihood that ancillary mutations introduced by the EMS treatment were still present.

Another mutant (mutation number *mn21*) derived from MN-H9 was also processed in the manner just described. In this case homozygous mutants did not die but were transformed into males or pseudomales, which are presumably *XX*; that is, male but not hermaphrodite *unc-4* segregants were plentiful (roughly 1/4 of the progeny) and this was true even when a *him-1*$^+$ gene was present. The fertility of the transformed males has not been tested because *unc-4* males are generally too uncoordinated to copulate. Based on its map position (see below), *mn21* is probably an allele of *tra-2*, which has been studied by HODGKIN and BRENNER (1977) and KLASS, WOLF and HIRSH (1976), but the appropriate complementation test has not been done.

The efficacy of *C1* as a balancer was checked by screening for uncoordinated animals amongst all adult progeny of mutant stocks. The results are shown in Table 6, which shows that the frequency of uncoordinated adults in all cases was low, with an average of 0.26%. The frequency would be even lower if expressed per zygote, since 1/4 of the zygotes did not reach adulthood. The frequencies were still somewhat higher than expected, however, until it was discovered that the frequency of appearance of dumpy and paralyzed recombinants in MN-H9

TABLE 6

Lethals balanced by C1

Mutation	Fraction of adult progeny that were unc-4
let-19(mn19)	4/1105
let-20(mn20)	0/1006
tra-?(mn21)	1/1084*
let-22(mn22)	5/1077
let-23(mn23)	3/1342
let-24(mn24)	3/1027
let-25(mn25)	3/1066
let-26(mn26)	4/1229
let-27(mn27)	0/1338
let-28(mn28)	9/1335
let-29(mn29)	6/1649
let-30(mn30)	2/1134
let-31(mn31)	0/1027
let-32(mn32)	3/1120

* *unc-4* males (plentiful in this case) were not counted.

stocks (53/2944) was about 5–10 times higher than for MN-H7, which has retained its frequency of about 0.2% recombinant types during regular transfers over more than 10 months. The difference in recombination frequency between MN-H7 and MN-H9 may be related to the *him-1* genes, but I have not pursued this point.

Complementation testing: The homozygosity for *him-1* in the mutant stocks made complementation testing straightforward. Wild-type males appearing on stock plates of one mutant line were easily recovered and crossed with wild-type hermaphrodites from another mutant stock. The appearance of adult *unc-4* animals indicated that the two mutations complemented. All 91 possible pairwise combinations of the 14 mutants listed in Table 6 were tested, with the result that every mutation complemented every other, and the 13 lethals were assigned separate *let* gene names. Tests for allelism with LG*II* visibles have not been done.

Mapping lethals: Wild-type *him-1*; *C1 dpy-10 unc-52/unc-4 let* males carry-

ing each of the lethal mutations (and the *tra-?* mutation) listed in Table 6 were mated with *dpy-10* hermaphrodites. Wild-type hermaphrodite progeny (*unc-4 let/dpy-10*) were picked and screened for *unc-4* types. Let the recombination frequency between *unc-4* and *let* be p, so that the *unc-4 let*$^+$ gametic frequency is $p/2$. The appropriate Punnett square then gives $p/2\text{-}p^2/4$ for the frequency of the *unc-4* phenotype among zygotes. But ¼ of the zygotes do not reach adulthood because they are homozygous for *let*. Hence, the frequency of appearance of uncoordinated animals amongst adult progeny, R, is $2p/3\text{-}p^2/3$, which gives $p = 1 - \sqrt{1 - 3R}$, which for small p reduces to $p = 3R/2$. The presence of *dpy-10 trans* to *unc-4* and *let* does not affect the analysis given. It maps so close to *unc-4* that virtually none of the homozygous *unc-4* progeny are also homozygous for *dpy-10*. But the *dpy-10* marker is obviously useful for three-factor ordering of *let* mutants, as outlined by BRENNER (1974) for visibles: if the *let* gene is on the *dpy-10* side of *unc-4*, then a certain proportion of the *unc-4 let*$^+$ recombinant chromosomes will also carry *dpy-10*, the proportion decreasing as the distance distal to *dpy-10* increases; and the presence of *dpy-10* is signified by the segregation of dumpy progeny from an uncoordinated recombinant.

Figure 3 summarizes the results of mapping 13 embryonic and larval lethals and the transformer. It is interesting that the mutants tend to cluster in the region of *dpy-10* and *unc-4*, as do the visibles (BRENNER 1974). The position of *let-28* could not be determined with respect to *dpy-10* because of their large separation; therefore *unc-52* was used. In addition, *dpy-10 unc-52/unc-4 let-28* animals were produced, and 1,821 of their progeny were classified according to phenotype. The results confirmed the relative map position of *let-28*, confirmed the previously measured map distance for *unc-4* to *let-28*, and gave a map distance for *let-28* to *unc-52*. I cannot be sure that all of the mapped mutants are single mutants. *let-28* was mapped from both sides and behaved as a single

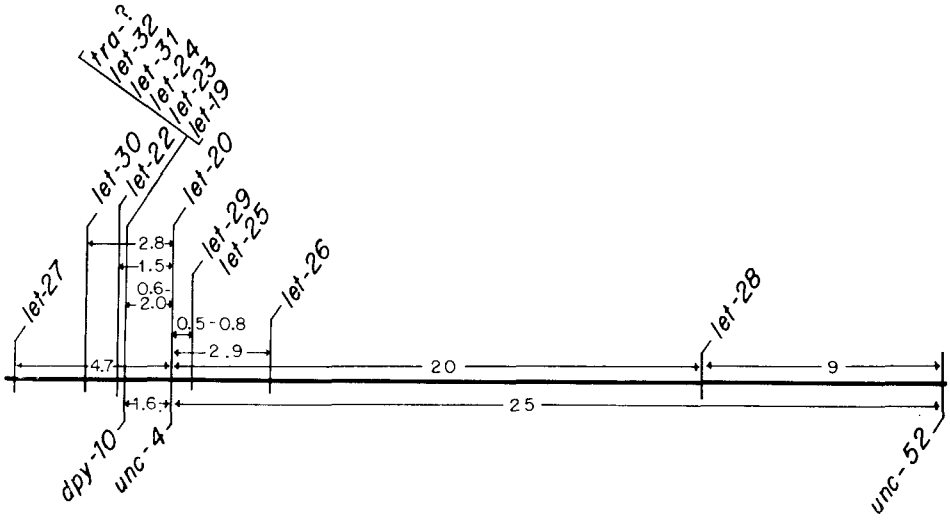

FIGURE 3.—Map of LG*II* lethals. Map units are percent recombination.

mutant. But any of the nine mutants that mapped to the left of *unc-4*, for example, could carry a lethal to the left (but not the right) of the mapped mutation and still be consistent with the mapping data.

Mutant phenotypes: The phenotypes of *let* homozygotes have not yet been studied in any detail. Among the 13 mapped lethals, only two seem to be embryonic, all of the others giving small, uncoordinated and arrested larvae. Among the set of steriles, some lay eggs (that do not hatch) and some do not. All of the sterile mutants were tested to see if mutant oocytes could be rescued by mating with wild-type males. Several could and not necessarily those from egg-layers, but the efficiency of rescue was variable: for a few mutants the rescue invariably occurred with the production of many outcross progeny. For other mutants only a few (and sometimes no) offspring were produced in any one cross. Most of the male-rescuable steriles were also mated with males heterozygous for the same mutation carried by the sterile to see if uncoordinated animals issued from the cross. Specifically, *unc-4 ste* hermaphrodites, which are sterile as self-fertilizing hermaphrodites, were crossed with *C1 dpy-10 unc-52/unc-4 ste* males. Wild-type offspring were produced, as expected, since the oocytes were previously shown to be rescuable by wild-type sperm, but the question was whether or not *unc-4* progeny were also produced. For at least 3 mutants, they were. This result suggests that the ste^+ function missing in each of these 3 mutants is essential in the parental animal for producing functional sperm, but that the sperm need not actually carry the ste^+ gene to function.

DISCUSSION

This work demonstrates the feasibility of recovering crossover suppressors in *C. elegans* by direct selection following X-ray treatment. Two dominant crossover suppressors, each suppressing crossing over in linkage group *II*, were identified and characterized. One, called *C2*, was shown to be a translocation involving LG*II* and *X*. The precise nature of the translocation is unknown: it could be reciprocal or nonreciprocal, and if nonreciprocal, it could be a piece of *II* inserted in *X*, or *vice versa*. If we refer to the two parts of *C2* as $C2^{II}$ and $C2^{X}$, then $C2^{II}$ represents either a deficiency on *II*, an insertion (or attachment) of a piece of *X* into *II*, or both. $C2^{X}$ is, of course, complementary to $C2^{II}$. It is clear from the results that viable animals carry either one copy each of $C2^{II}$ and $C2^{X}$, two copies of each (these animals die as larvae, however) or no copies of each. This is the expected result since other combinations would have duplications and deficiencies.

Both the egg-hatching frequency and the progeny ratios from heterozygous *C2* hermaphrodites were accounted for by assuming that alternate and adjacent-1 segregations were about equally frequent, that adjacent-2 segregations were rare, and that duplication-deficiency gametes were functional. These conditions are satisfied by typical translocations in Drosophila (ROBERTS 1976). In the interest of accuracy, perhaps it is worth noting that it is not essential to assume that alternate and adjacent-1 segregations were equally frequent. If there were no crossing over in an interstitial segment (between centromere and translocation

breakpoint), then alternate segregation would give two types of gametes and adjacent-1 segregation would give two other types of gametes. However, if a crossover did occur in an interstitial segment, then all 4 types of gametes would be produced regardless of whether the segregation that followed was alternate or adjacent-1. Therefore, a bias toward either alternate or adjacent-1 segregation could have been present but obscured by frequent crossing over in an interstitial segment (see BURNHAM 1962). It is easy to predict the effect of adjacent-2 segregations: for example, on the assumption that adjacent-2, alternate, and adjacent-1 segregations were equally frequent, one can draw a 6-by-6 Punnett square and show that the egg-hatching frequency should be 22% and the ratios of C2 heterozygotes:C2 homozygotes:homozygous wild-type would be 6:1:1. Neither prediction fits the results. The presumption that duplication-deficiency gametes (bearing $C2^{II}$ but not $C2^{X}$ or bearing $C2^{X}$ but not $C2^{II}$) were functional seems inescapable and worth emphasizing. The conclusion is not surprising, however, since the haploid phase for the ovum in *C. elegans* is extremely short-lived (HIRSH, OPPENHEIM and KLASS 1976), and as for sperm, those of Drosophila are notoriously indifferent to the genetic material they do or do not carry (LINDSLEY and GRELL 1969).

C2 translocation homozygotes die as larvae. Translocation homozygosity is frequently lethal for flies, and the lethality may have any of three causes: mutation of genes at the points at which chromosomes are broken, independent recessive lethal mutations at another locus of the chromosome, and a position-effect of the rearrangement (ROBERTS 1976). Any of these is possible in the case of *C2*. All that can be said is that *C2* carried all the *X*-linked genes needed for male development in a functional state because $C2^{II}$/normal *II*; $C2^{X}/0$ animals were fertile males.

ROBERTS (1970) selected directly for dominant crossover suppressors in Drosophila after X-ray treatment and found that over two-thirds of those recovered were translocations. Thus, it is not surprising that one of the two crossover suppressors recovered in the work reported here was a translocation. ROBERTS concluded that the crossover reductions observed in translocation heterozygotes were not due to elimination of crossover strands in aneuploid segregants. He therefore postulated that the reductions were due to the prevention of synapsis in the regions of the breakpoints. The reduction in crossing over for a given chromosome arm was most pronounced when the breakpoint for that arm occurred at a position one-third to one-half of the way in from the tip. In metacentric chromosomes, crossing over in the unrearranged arm is little affected (ROBERTS 1976). This suggests that the *X* chromosome of *C. elegans* may have its centromere near *unc-6*, which maps near the middle of the linkage group. Crossing over between either *unc-6* or *unc-3* (which maps near the right end of X) and $C2^{X}$ was extremely rare, whereas crossing over between *unc-1* (a left-end marker) and $C2^{X}$ was at least as high as is normally found between *unc-1* and *unc-6*.

X-autosomal translocations in Drosophila can show high frequencies of *X* nondisjunction (CHANDLEY 1965). *C2* appears to have this effect also, as the spon-

taneous appearance of male progeny from *C2* heterozygotes, which is due to the loss of an *X*, was roughly 30 times higher than normal.

The other crossover suppressor, *C1*, is almost certainly not a translocation. First, *C1* heterozygotes did not produce duplication-deficiency gametes: the egg-hatching frequencies were close to 100% and the progeny ratios of *C1/C1*:*C1*/+:+/+ were about 1:2:1. Furthermore, *C1* segregated independently of markers on all linkage groups but *II*. The obvious suggestion is that *C1* is an intrachromosomal rearrangement, such as a transposition or an inversion.

Cytological confirmation of the nature of *C1* or *C2* awaits technical advance, and certain questions, particularly pertaining to *C1*, remain open. One question involves the mechanism of crossover suppression by *C1*. The nearly 100% hatchability of eggs from *C1* heterozygotes can be taken to suggest that crossing over does not give rise to lethal products but instead occurs at reduced frequency, presumably because of synaptic difficulties. On the other hand, dicentric chromatid ties produced as a result of single crossovers within a paracentric inversion are in some organisms selectively eliminated from forming functional gametes in either the female (STURTEVANT and BEADLE 1936) or, less commonly, the male germ line (for review see JOHN and LEWIS 1965). The difficulty with this interpretation for *C1*, however, would be in understanding why the rare recombinants that were recovered were not double crossovers, which would have been homozygous viable. Indeed, the absence of double-crossover products out of about 15,000 progeny also suggests that crossing over is infrequent. This is not a conclusive point, however, because it is possible that both *dpy-10* and *unc-52* are very close to rearrangement breakpoints, making the required double crossovers rare.

Another question about *C1* that remains open involves the disjunction of *C1* and its normal homologue. Chiasma formation may be sufficiently frequent outside the *dpy-10-unc-52* segment to insure normal disjunction. On the other hand, in some organisms, such as Drosophila females (see GRELL 1976), special mechanisms exist to insure normal disjunction in the absence of crossing over. In many other organisms, however, loss of chiasma formation leads to nondisjunction (JOHN and LEWIS 1965). It remains to be seen whether or not *C. elegans* has a means for insuring disjunction in the absence of crossing over.

As to the nature of *C1*, the most likely possibilities seem to be either a pericentric inversion or a transposition of material near *dpy-10* to near *unc-52* (or *vice versa*). Either sort of rearrangement can be an effective crossover suppressor (ROBERTS 1976), and in each case single crossovers in the rearrangement heterozygotes give rise to duplication-deficiency products.

I showed that *C1* is a useful balancer for a number of recessive lethals on LG*II*. Out of 13 lethals randomly chosen, no two were affected in the same gene. This suggests that a large number of indispensable functions could be balanced by *C1*.

The ease with which LG*II* lethals were recognized leads to the suggestion that *C1* would be useful in evaluating the efficacy of various mutagens and conditions of mutagenesis of *C. elegans*. It is considerably easier to assay for *C1*-balanced

lethals, for example, than it is to assay for sex-linked lethals (BRENNER 1974). The high frequency at which lethals were recovered suggests that it would be wise to use lower concentrations of EMS to reduce the frequency of double mutations. Another modification in the procedure might also be recommended: *unc-4* hermaphrodites could be mutagenized and mated with *C1 dpy-10 unc-52/ unc-4* males. Wild-type hermaphrodite progeny would be picked and screened for lethals linked to *unc-4*. This procedure insures that the balancer and at least one homologue for every linkage group are not mutagenized.

Very few lethals were embryonic. This is not surprising since temperature-sensitive mutations often show a strong maternal effect (VANDERSLICE and HIRSH 1976). It was somewhat surprising, however, that the incidence of steriles was about equal to the incidence of lethals. It will be interesting to see whether the steriles represent a set of genes largely distinct from the lethals or whether they might often be leaky alleles of lethals. An interesting class of steriles included animals that by genetic tests were sperm defective.

BRENNER (1974) found that the visible mutants tended to map in clusters, one cluster per autosomal linkage group. The 13 lethals that were mapped in this work tended to map in the LG*II* cluster. One might argue that lethals mapping away from the cluster were selected against because they were not well-balanced by *C1*, but not all of the lethals mapped in the cluster, and lethals near *unc-52* should have been well balanced, but the closest lethal to *unc-52* was 9 map units away. Thus, it seems likely that the clustering of mutations on LG*II* is a characteristic of lethals as well as visibles.

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Corresponding editor: A. CHOVNICK