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THE EFFECT OF GAMMA RADIATION ON RECOMBINATION FREQUENCY IN CAENORHABDITIS ELEGANS

By

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ABSTRACT

Treatment with ionizing radiation is known to cause chromosome breakage in many organisms including Caenorhabditis elegans. In addition, gamma radiation increases recombination frequency in Drosophila melanogaster. In order to investigate the generality of radiation-induced recombination, I have undertaken to study the effect of gamma radiation on recombination frequency in C. elegans. This is the first study to describe radiation-induced recombination in C. elegans.

Radiation doses of 2K rads increased recombination frequency in the dpy-5 unc-13 interval of LGI approximately two-fold. The amount of the increase was affected by the developmental stage of gonads at the time of radiation treatment and by radiation dose. X-chromosome nondisjunction was also increased by radiation treatment. A high frequency of the recombinant progeny produced with radiation treatment were sterile unlike their nonrecombinant siblings.

When parameters affecting recombination frequency are held constant, chromosomal regions within the gene clusters of the meiotic map increased most after radiation treatment. However, none of the increases were of the magnitude described for centric heterochromatin in Drosophila.

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INTRODUCTION

Gamma-radiation is an ionizing radiation known to cause chromosomal breaks in many organisms resulting in chromosomal rearrangements such as deletions and translocations. In addition to creating chromosomal rearrangements, ionizing radiation is known to increase crossing-over in Drosophila melanogaster (Muller, 1925). The effect of radiation on recombination frequency in Drosophila melanogaster has been extensively studied (Muller, 1925; Patterson and Suche, 1934; Whittinghill, 1951; Roberts, 1969; Mglinets, 1972,1973; Haendle, 1979; Tattersall, 1981). In order to investigate the generality of this effect with regard to other organisms, I have studied the effect of gamma-radiation on recombination frequency in Caenorhabditis elegans. In C. elegans it has been demonstrated that ionizing radiation generates chromosomal rearrangements (Rosenbluth, Cuddeford and Baillie, 1985). However, prior to the work presented in this thesis, the effect of gamma-radiation on recombination frequency in C. elegans has not been reported.

Genetic studies in D. melanogaster have demonstrated a correlation between the centromeric heterochromatin and radiation-induced map expansion (increased recombination frequency). Very large increases in recombination frequency have been observed primarily in regions composed of centric heterochromatin (Tattersall, 1981). The magnitude of the increase was at least 2 to 6-fold in heterochromatin as compared to increases from 0 to 1.5-fold in euchromatic regions.

Since radiation has been shown to alter recombination primarily in the centric heterchomatin region of D. melanogaster chromosomes, it might be possible to detect regions of centric heterochromatin in C. elegans chromosomes using radiation-induced map expansion. For example, one could attempt to study whether radiation dramatically increases recombination in regions of C. elegans chromosomes as it does in regions of heterochromatin in D. melanogaster. The genetic map of C. elegans, which is based on meiotic recombination frequencies, shows clusters of genes on the autosomes. That is, most of the genes which were identified in Brenner's (1974) original screen are clustered within a region of a few map units on each autosome. See Appendix 1. For example, on Linkage Group (LG) I there are 30 genes located in the unc-11 to unc-13 interval, an interval of 4 map units; compared to 3 genes located in the adjacent unc-35 to unc-11 interval, an interval of 12 map units. The dpy-5 unc-13 region described in this thesis is in the LG I °gene cluster'. Brenner (1974) suggested that these clusters were the result of suppressed recombination frequency in defined chromosomal regions. In D. melanogaster, the area of centromeric heterochromatin is known to be a region of low recombination frequency relative to the amount of DNA (Yeomans, 1979; Grell, 1978).

Studies on C. elegans mitotic chromosomes suggest that there is no single site for spindle attachment (Albertson and Thomson, 1982) suggesting the possible absence of centric heterochromatin in this organism. The meiotic chromosomes are

however too small to study cytologically. Although some aspects of the descriptive cytology of chromosomes in C. elegans have been studied (Nigon and Brun, 1955; Albertson and Thomson, 1982; Abi-Rached and Brun, 1979; Goldstein, 1982; Goldstein and Slaton, 1982), the overall structure of the C. elegans chromosomes is poorly understood at this time. Since the amount of recombination induced with radiation seems to be proportional to the amount of DNA present (rather than related to the amount of meiotic recombination), studying the effects of radiation on recombination frequency across a C. elegans chromoscme might provide insight into the architecture of these chromosomes. The work reported here is an attempt to characterize aspects of chromosomal organization using ionizing radiation.

Having a method for determining the relative amounts of DNA present in different chromosomal regions is important with regard to understanding the organization of genes in C. elegans chromosomes. On the other hand, the study of meiotic recombination is itself intrinsically interesting. In this regard C. elegans provides a model system in which to study aspects of meiotic recombination. This organism is well suited for such studies since generally it is a good experimental system and since specifically there exists an enhancer of meiotic recombination.

Caenorhabditis elegans is an excellent experimental genetic system for a number of reasons and has become the model organism of choice for many types of studies: it has a short generation time (3.5 days at 20°C); it has a large number

of offspring (each adult wild-type hermaphrodite produces 250-300 offspring); and it has a small genome size (300 map units and 8×10^7 bp of DNA). In addition, C. elegans is easy to maintain in the laboratory and has the advantage that genetic stocks can be frozen in liquid nitrogen. Since C. elegans is a self-fertilizing hermaphrodite, populations tend to become homozygous (genetically identical individuals). Males are produced spontaneously by X-chromosome nondisjunction (Hodgkin, Horvitz and Brenner, 1979). Hermaphrodites have five pairs of autosomes and one pair of sex chromosomes (XX), whereas males have five pairs of autosomes and only one X-chromosome (XO). Genetic analysis relies on the existence of males, since the genetic markers from independently isolated mutant hermaphrodites can be transferred to the following generation when males are crossed to hermaphrodites.

A strain (Rec-1) has been isolated and characterized which has a three-fold increase in meiotic recombination (Rose and Baillie, 1979b; Rose, 1980; Rattray and Rose, unpublished results). In contrast to the effect of radiation on recombination frequency in D. melanogaster, Rec-1 in C. elegans shows increased recombination frequencies throughout the genome (Rose and Baillie, 1979b; Rose, unpublished data). Therefore, recombination frequency may be increased in Rec-1 through different mechanisms or alternatively, C. elegans chromosomes may have a different organization than D. melanogaster chromosomes.

The existing map of C. elegans genes is based on

recombination between genes along each linkage group. In order to standardize the conditions for measuring recombination frequencies, Rose and Baillie (1979a) have studied some factors affecting recombination. They found that recombination frequency increased with temperature and decreased with parental age and suggested that in order to standardize mapping procedures controlled temperatures of 20°C and scoring of all the progeny be adopted. Similarly in order to make comparisons of the effect of radiation on recombination across different chromosomal regions, it is necessary to standardize the conditions under which radiation treatment is applied.

The initial objective of the research reported in this thesis was to examine the effect of ionizing radiation on recombination frequency within the cluster of Linkage Group I. Since recombination with regard to temperature and parental age across the dpy-5 unc-13 interval has been well characterized (Rose and Baillie, 1979a), this region was chosen to characterize factors affecting the increase.

The goals of this research were to determine:

1) if the dpy-5 unc-13 region of the C. elegans genetic map would show increased recombination frequency after radiation treatment,
2) the parameters affecting the amount of the increase;
3) if the amount of the increase varied with region;
4) if the magnitude of the increase was comparable to that seen in the centric heterochromatin in D. melanogaster after radiation treatment

MATERIALS AND METHODS

I. NEMATODE STRAINS AND CULTURE CONDITIONS.

Wild-type Caenorhabditis elegans var. Bristol, strain N2 and mutant strains (BC) were obtained from the stock collection at Simon Fraser University (Table 1). KR strains were constructed here from strains obtained either from the Medical Research Council, Cambridge, England or the Caenorhabditis Genetics Center, University of Missouri, Columbia.

The strains were maintained and mated on petri plates containing nematode growth medium (NGM) streaked with OP50 (Brenner, 1974). OP50 is a uracil-requiring mutant of Escherichia coli, used to prevent overgrowth of the bacterial lawn. The medium contains limited uracil so that the bacteria cannot overgrow and obscure the worms.

Crosses were carried out either on 10x35 mm or 10x60 mm petri plates. Since temperature and parental age affect recombination frequency in C. elegans, all experiments were performed at a standard temperature of 20°C and all the progeny of an individual were scored as recommended by Rose and Baillie (1979a).

Early experiments involving the dpy-5 unc-13 interval were done using the BC 26 strain (Table 3). Subsequent experiments used BC 415.

Table 1

List of Double Mutant Strains Used in this Study

Genotype	LG	Alleles		Strain Name
dpy-5 unc-13	I	e61	e51	BC 26
dpy-5 unc-13	I	e61	e450	BC 415
dpy-5 dpy-14	I	e61	e188	BC 196
dpy-14 unc-13	I	e188	e450	BC 69
dpy-5 unc-11	I	e61	e47	BC 260
bli-3 unc-35	I	e579	e259	KR 286

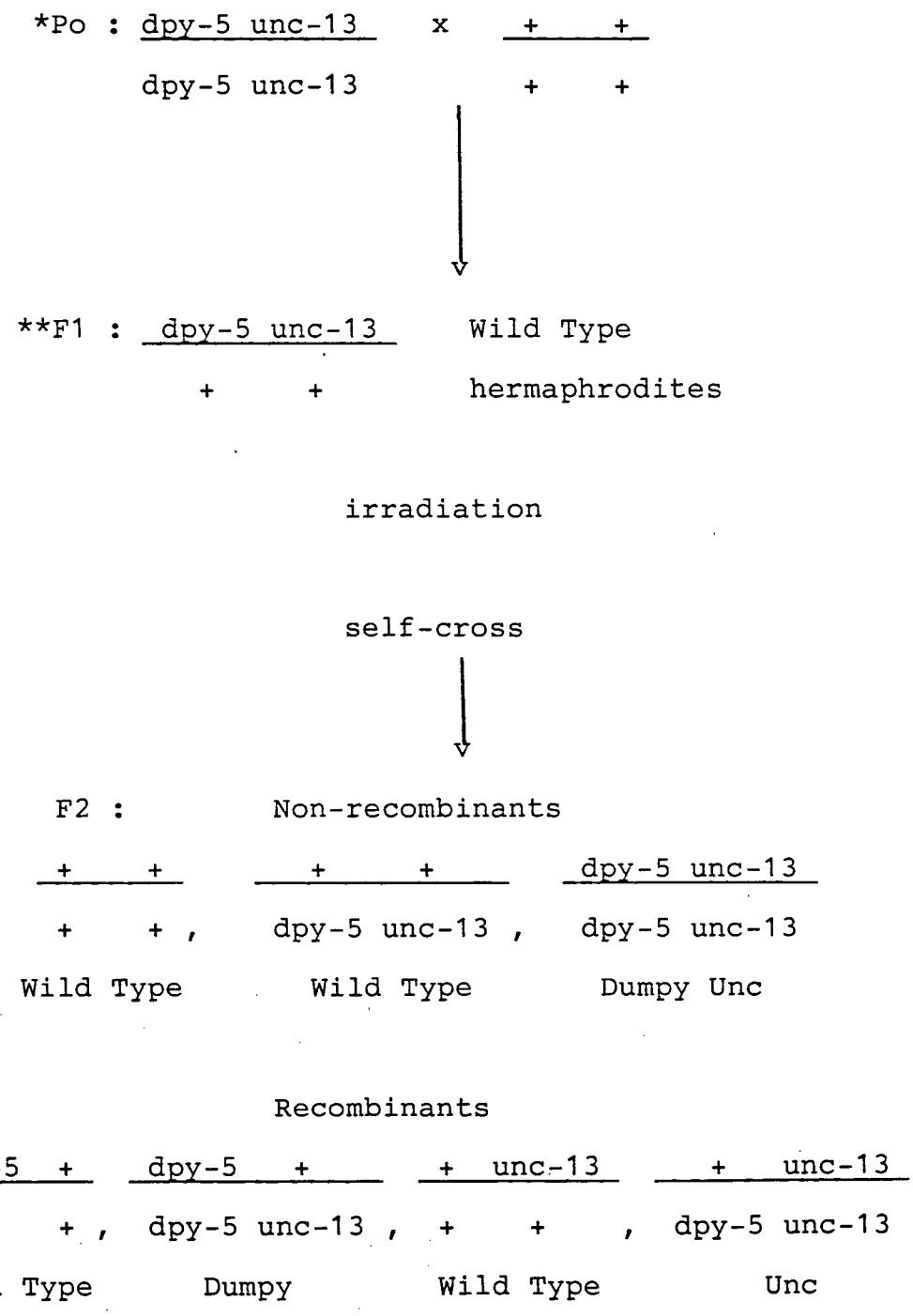
II. MATING PROTOCOL AND RADIATION TREATMENT.

Caenorhabditis elegans is a self-fertilizing hermaphrodite. Individual doubly mutant hermaphrodites (Table 1) were crossed to wild-type males. The mated hermaphrodites produced two types of progeny, those resulting from self-fertilization and from fertilization with male sperm.

In order to generate appropriate heterozygous hermaphrodites, mating was carried out by placing 20-25 homozygous mutant hermaphrodites with 30-40 wild-type males on a 60mm petri plate (Figure 1). 24 hours later mated double mutant hermaphrodites were transferred to fresh plates. Approximately 3 days later, the heterozygous progeny (F1 generation) of the cross began laying eggs.

Since the radiation treatment to adult worms did not show any effect on recombination, gamma-radiation treatment of approximately 2K rads was applied to the experimental parents at the 12-hr prior to egg-laying stage. Both the experimental (treated) and control (untreated) plates were carried to the cobalt-60 source (Gammacell, Atomic Energy of Canada), so that identical temperature conditions were maintained during their absence from the incubator. F1 heterozygous wild-type hermaphrodites were picked from each set of plates. These hermaphrodite individuals were maintained at 20°C and transferred to fresh plates every 12 hours, thus generating °12-hr broods'. The F1 hermaphrodites were transferred until they were exhausted of fertilized eggs.

Figure 1. Mating protocol for generating offspring which carry recombinant chromosomes.



* Po: Parental individuals.

**F1: First generation progeny.

III. MEASURING RECOMBINATION FREQUENCY.

a. Identification of Recombinants.

In this study, the recombinant phenotypes are "Dumpy" (shorter than the wild types), "Uncoordinated" (detectable defect in the normal pattern of movement), and "Blistered" (blistered cuticle) (Table 2). The number of "Dumpy" progeny was used to determine recombination frequency in most experiments. Since the Dpy-5 recombinants from the outcross involving Dpy-5 are more viable than Unc-13s, or Unc-11s (Rose, unpublished data), a more accurate estimate of recombinant events was calculated by doubling the number of Dpy-5s. In an analogous way, Bli-3 recombinants were used for calculation of recombination frequencies. All Dpy-5 or Bli-3 recombinants were progeny tested to confirm that they were the expected genotype.

b. Calculation of Recombination Frequency.

Recombination frequency was determined in the following way. The ratio of wild-type hermaphrodites to double mutant hermaprodites (e.g. Dpy Unc, Dpy Dpy, Bli Unc) is expected to be 3 to 1, so the total number of offspring was calculated as 4/3 the number of wild types plus more viable recombinants (Rose, 1979a). Since the double mutants have reduced viability compared to wild type, this is a more accurate estimate of the total number of progeny than scoring wild-types plus Dpy Uncs or

Table 2

Description of Recombinants Observed in the Experiments

Recombinants	LG	Allele	Phenotype
Dpy-5	I	e61	short, decreased length to width
Dpy-14	I	e188	short, swollen medially
Unc-13	I	e450	severely uncoordinated
Bli-3	I	e579	blistered cuticle, and darker and fatter than Wild-Type
Unc-35	I	e259	moderately uncoordinated

Bli Uncs. All the F2 phenotypes were scored and the recombination frequency was calculated by applying the formula $p = 1 - \sqrt{1 - 2R}$, where R = the fraction of observed recombinants over total progeny (Brenner, 1974; Figure 2).

The 95% confidence intervals (C.I.) were obtained by using the formula C.I. = $1.96\sqrt{npq}$, where n = the total number of progeny, p = recombination frequency, and q = 1 - p. If the number of recombinants was less than 15, Poisson statistics were used to calculate the confidence intervals (Stevens, 1942). Assuming that the recombination events follow a binomial distribution, the Poisson distribution is a good approximation of the binomial when the events are rare.

IV.MEASURING RADIATION DOSE RESPONSE.

In order to determine the effect of radiation dose on recombination frequency, three different dosages were applied. The dpy-5 unc-13 and dpy-5 dpy-14 intervals were chosen for this experiment. Dpy-5 recombinants from dpy-5 unc-13 / + + and dpy-5 dpy-14/ + + hermaphrodites were scored. Males were scored as an estimate of the frequency of X-chromosome nondisjunction (Hodgkin, 1977; Rose and Baillie, 1979a).

V. MEASURING F1 STERILITY.

In order to investigate the effect of gamma-radiation on sterility of the F2 offspring of treated individuals, progeny testing of Dpy-5 recombinants and wild-type non-recombinant siblings, was performed. All Dpy-5 recombinants from untreated

Figure 2. Method for calculating recombination frequencies (from Rose, 1980).

The number of recombinant progeny (Dumpy and Uncoordinated)	= 2 x (number of more viable recombinants)
The total number of progeny	= 4/3 x (number of Wild Types + number of more viable recombinants)
The fraction of observed recombinants, R	= $\frac{2 \times \text{number of Dumpies}}{\text{total progeny}}$

The frequency of recombination, $p = 1 - \sqrt{1 - 2R}$, where

$2R$ = the fraction of progeny carrying recombinant chromosomes

$1 - 2R$ = the fraction of progeny not carrying a recombinant chromosome

$\sqrt{1 - 2R}$ = the frequency of non-recombinant gametes

$1 - \sqrt{1 - 2R}$ = the frequency of recombinant gametes

(control) and treated (experimental) were placed individually on fresh plates. If they produced no self-fertilized progeny, they were scored as a sterile. Furthermore, approximately the same number of wild-type hermaphrodites were also isolated and examined in order to investigate the sterility of non-recombinant progeny.

VI. MEASURING X-CHROMOSOME NONDISJUNCTION.

a. Determination of Nondisjunction Frequency.

The sex of the free-living soil nematode Caenorhabditis elegans is determined by the ratio of X chromosomes to autosomes. Normal hermaphrodites are 5 AA XX (X:A =1.0), whereas males are 5AA XO (X:A = 0.5) (Hodgkin, Horvitz and Brenner, 1979). Hermaphrodites normally produce hermaphroditic (XX) offspring by self-fertilization. Males (XO) are generally produced by spontaneous X-chromosome nondisjunction or chromosome loss (Hodgkin, 1977; Rose and Baillie, 1979a). The number of males were scored as a measure of X-chromosome nondisjunction.

b. Chromosome Loss or Nondisjunction.

In addition to measuring the frequency of males, it is necessary to confirm whether the presence of males is a

consequence of X-chromosome nondisjunction or the loss of X-chromosomes. In C. elegans, 1X diploids are males, 2X diploids are hermaphrodites, 3X diploids are short hermaphrodites, and 4X diploids are inviable (Hodgkin, Horvitz and Brenner, 1979). As an indication of X-chromosome nondisjunction, the occurrence of 3X hermaphrodites has been used. Trisomic-X individuals were collected and the segregation of self progeny was observed.

VII. DEVELOPMENTAL STAGES.

a. Effect on recombination frequency.

It is of interest to know whether the radiation-induced increase in recombination frequency is stage-specific. To investigate this, it is necessary to consider the age of the worms at the time of irradiation. The dpy-5 unc-13 interval was used for this experiment. The worms were treated with 2000 rads of gamma-radiation at three different stages of the life cycle: 1) 36-hr prior to egg-laying, 2) 24-hr prior to egg-laying, and 3) 12-hr prior to egg-laying.

b. Cytology of gonads.

The structure of the adult gonad of wild type worms in both the treated and untreated condition has been studied using

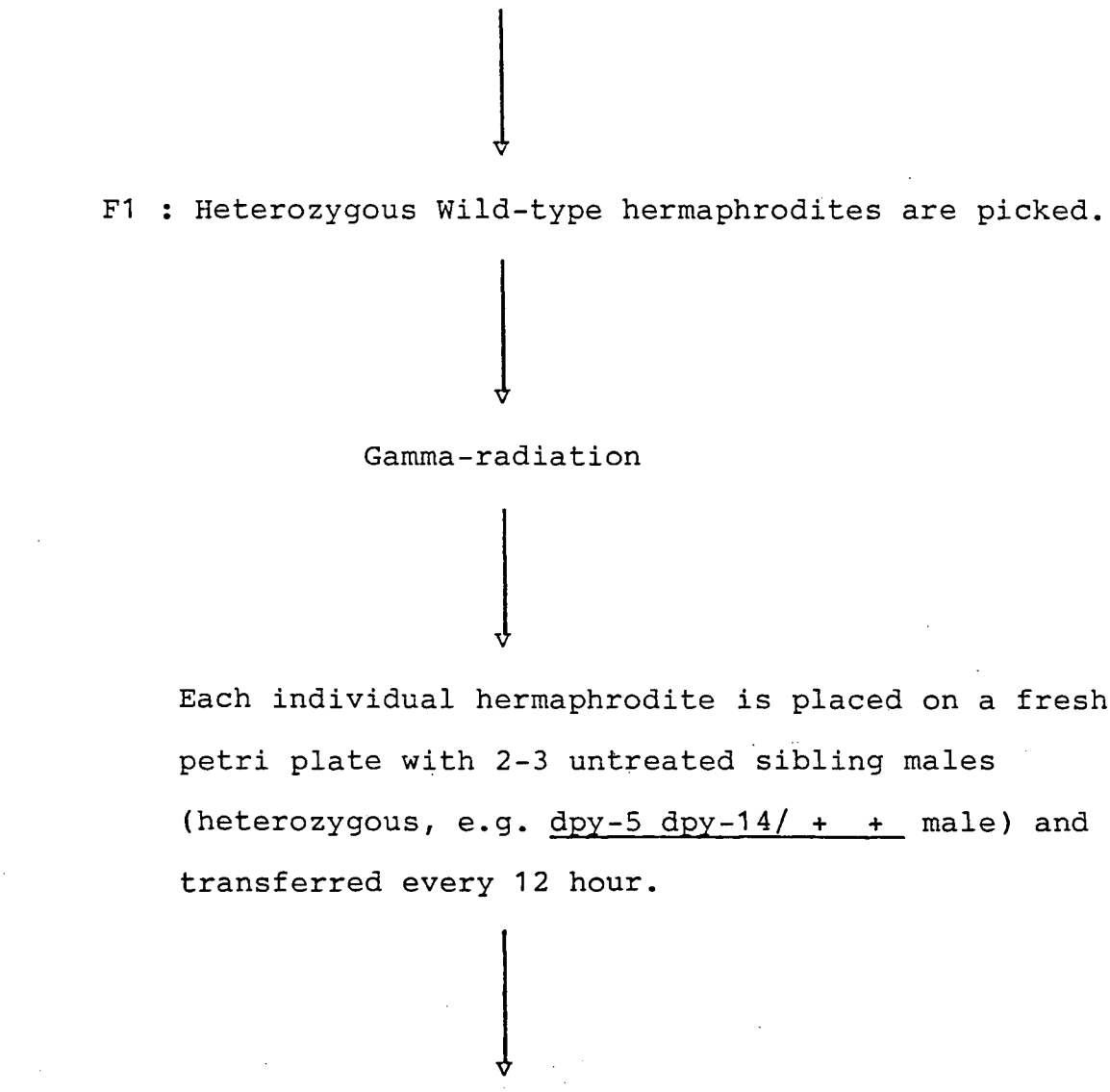
whole worms. The technique of Sulston and Horvitz (1977) for mounting live worms for observation was used. Approximately 3 to 5 live worms were placed on a thin agar pad on a microscope slide. A coverslip was placed on top to trap the worms between the agar surface and the coverslip in a drop of M9 buffer. The mature worms were heat-killed, since accurate measurements could not be made because of their movement. The primordial gonadial cells (p cells) of the reproductive system at three different developmental stages were observed using Nomarski-interference microscopy.

c. Effect on spermatogenesis or oogenesis

Since spermatogenesis and oogenesis take place in the same gonad in C. elegans, it was necessary to investigate whether radiation affects recombination frequency through its effect on the sperm or the oocytes in the self-fertilizing hermaphrodites. In order to determine if the sperm were affected, it was also necessary to evaluate the recombination events in hermaphrodite oocytes. The double mutant hermaphrodites were mated to wild type males and transferred to fresh plates on the following day as described in section 2 of Materials and Methods.

After treatment with gamma-radiation, F1 individual wild-type hermaphrodites were placed on 10x60 mm petri plates with 2-3 untreated sibling males (shown in Figure 3). Treated hermaphrodites were transferred with untreated sibling males at

Figure 3. Method for measuring recombination frequency in hermaphrodite oocytes.



12-hour intervals until male sperm were exhausted. It has been reported that male sperm are used preferentially (Ward and Carrel, 1979). By scoring recombinant males, the recombination frequency in hermaphrodite sperm was excluded. Therefore, it is possible to detect whether the effect of radiation on recombination is occurring during spermatogenesis or oogenesis.

VIII. CHROMOSOMAL REGIONALITY.

In order to evaluate if the effect of radiation on recombination frequency is specific for the gene cluster, its effect on a region at the tip of the chromosome was also measured. The bli-3 unc-35 interval on the left end of Linkage Group I was chosen for this purpose. The recombination frequency was measured in the same manner as previously described.

RESULTS

I. EFFECT OF RADIATION ON RECOMBINATION FREQUENCY.

Heterozygous hermaphrodites were gamma-irradiated and individuals of the same age isolated in order to observe and characterize the effect of radiation on recombination frequency. The results for the dpy-5 unc-13 interval are presented in Table 3. In the absence of gamma-irradiation, the frequency of recombination in this region was 0.021. After treatment with 2000 rads of gamma-radiation the recombination frequency increased about two-fold ($p = 0.039$).

a. Brood Analysis.

Successive 12-hr broods were routinely collected. In each case, the recombination frequency for each brood was calculated individually (Materials and Methods, section 3b). Figure 4 summarizes the changes in recombination frequency for each brood after radiation treatment. The frequency of recombination for untreated heterozygotes decreased characteristically with parental age. Treated individuals showed no increase in the first 12-hr brood, but did show increases in recombination frequency in the later broods. Radiation treatment produced a peak at the second brood (B). However, the effect of radiation on recombination frequency did not appear to be as

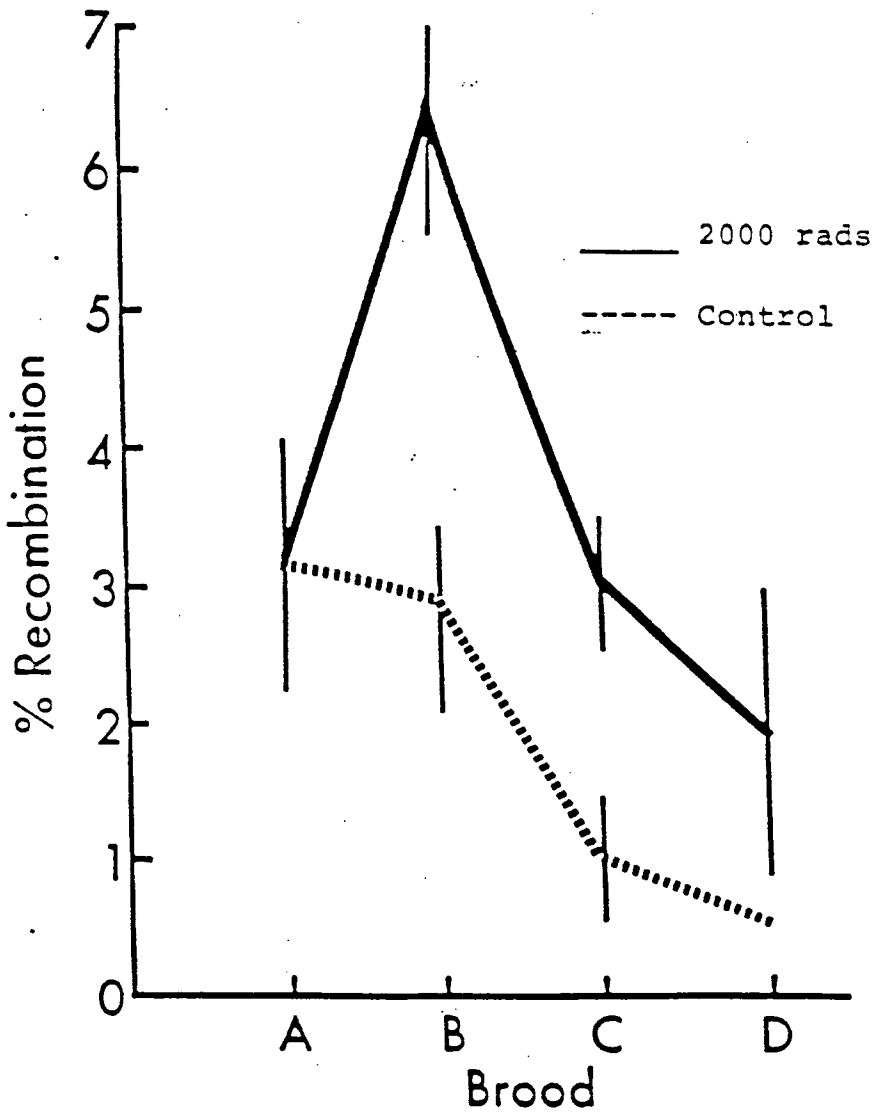
TABLE 3

‹Effect of radiation on recombination frequency in dpy-5 unc-13 ›.

Stage: 12-hr prior to egg-laying.

12-hr BROOD	PROGENY	RECOMBINANTS	[p]x100	95% C.I.
2000 rads, N = 52				
a	1333	40	3.1	(2.0-4.2)
b	2347	142	6.5	(5.0-8.0)
c	2800	84	3.1	(2.0-4.2)
d	1275	24	1.9	(1.0-2.8)
TOTAL	7755	290	3.9	(3.0-4.8)
CONTROL, N = 26				
a	1051	34	3.4	(2.0-4.8)
b	1656	46	2.9	(2.0-3.8)
c	1767	18	1.0	(0.4-1.6)
d	317	2	0.6	(0.01-1.32)
TOTAL	4791	100	2.1	(1.6-2.6)

Figure 4



Effect of radiation on recombination frequency for dpy-5 unc-13 strain at 12 hr prior to egg-laying stage.

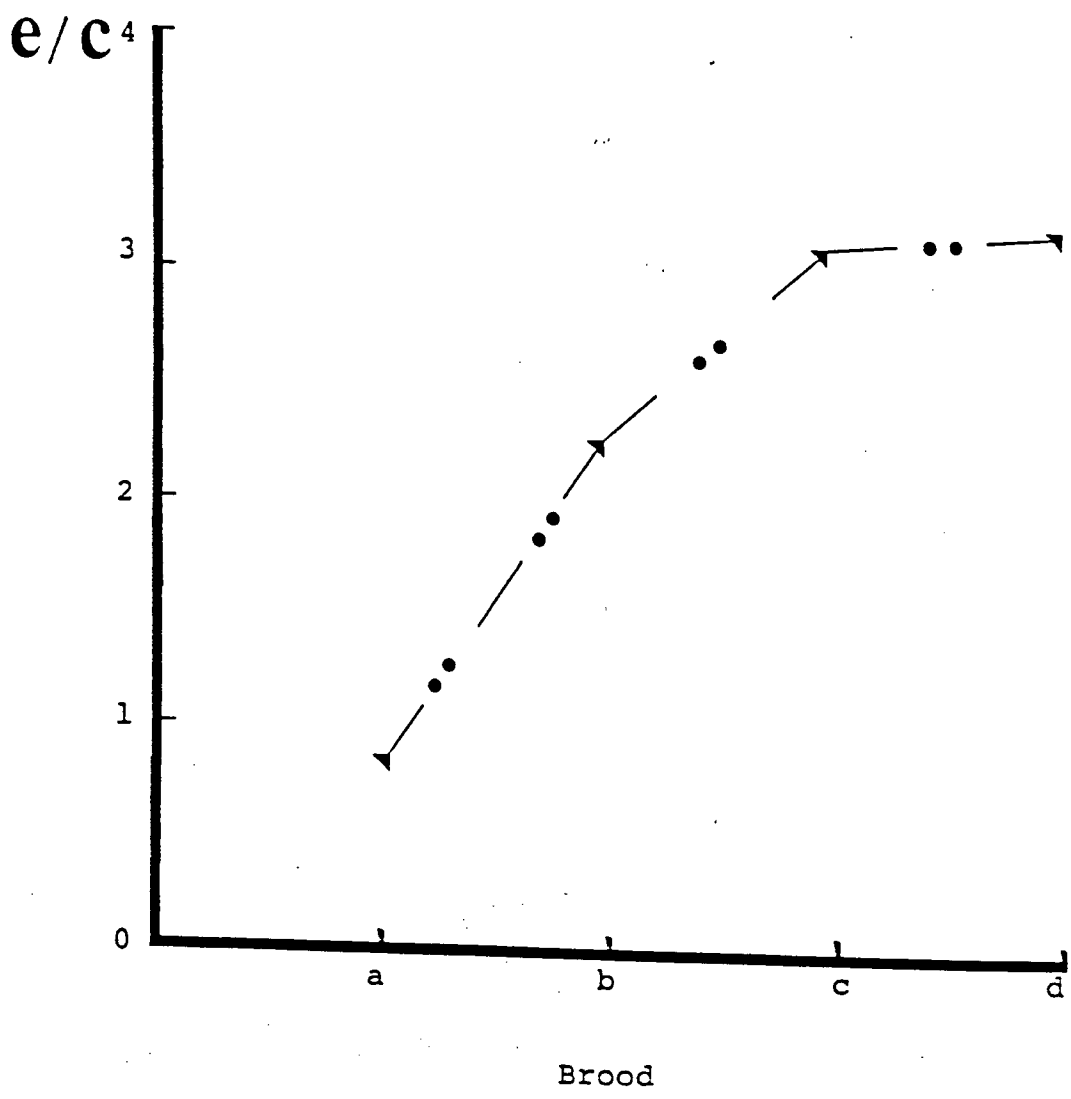
pronounced when the recombination frequencies for all the broods were averaged.

In order to determine whether the effect of radiation on recombination is continuous with parental age, the experimental to control ratio of recombination frequency was calculated. Figure 5 shows a continuous effect of radiation on recombination events with parental age.

b. Radiation Dose Response.

Experiments measuring dose response were done as described in the Materials and Methods, section 4. Table 4 shows the dose response for recombination frequency in the dpy-5 unc-13 interval. The recombination frequencies differ at the three doses used, 1, 2 and 4K rads. A linear increase in recombination frequency was observed for the doses examined. Above 1K rads there was also a decrease in progeny number. When 4K rads was applied, recombination frequency increased more than two-fold compared to untreated individuals. However, the high dose resulted in a reduced number of progeny. It might be possible that the radiation can cause embryonic lethality. Therefore, the optimal dose for the experiments appeared to be 2K rads.

Figure 5



Ratio of Experimental to Control for recombination frequency in dpy-5 unc-13 strain.

TABLE 4

‹Radiation dose response›.

Stage : 12-hr prior to egg-laying.

DOSES	N*	PROGENY	PROGENY/ PARENT	RECOMBINANTS	NDJ**	[p]	95% C.I.
dpy-5 unc-13:							
CONTROL	26	4791	184	100	1.0	2.1	(1.7-2.5)
1 Krads	10	2120	212	64	2.0	3.1	(2.3-3.9)
2 Krads	52	7755	149	290	5.9	3.9	(3.2-4.6)
4 Krads	25	3491	139	168	5.7	4.9	(4.1-5.5)
dpy-5 dpy-14:							
CONTROL	10	2578	257	46	0.5	1.8	(1.3-2.3)
2 Krads	16	2650	165	58	3.6	2.2	(1.6-2.8)
4 Krads	16	2398	149	78	3.5	3.3	(2.5-4.0)

* N = Number of parent.

**NDJ =Number of wild-type males per 1000 progeny.

c. Developmental Stages.

i. Time of Treatment and Recombination Frequency.

Since oogenesis begins during the larval stages and continues into adulthood past the onset of egg laying (Abi-Rached and Brun, 1975; Hirsh, Oppenheim and Klass, 1976), the effect of radiation treatment through the oogenic stages can be examined to determine whether the radiation sensitivity changes with time of treatment. These experiments were done using dpy-5 unc-13/ + + individuals at three different stages: 12-hr, 24-hr, and 36-hr prior to the beginning of egg-laying (described in Materials and Method, section 7). When the hermaphrodites were treated 12-hours prior to egg-laying, a two- to three-fold increase in recombination frequency was observed through the egg-laying period. However, when the hermaphrodites were treated 36-hours prior to egg-laying, approximately a five-fold increase in recombination frequency was observed (shown in Table 5). The increase was observed in the B brood (shown in Figure 6). Therefore, it seems that a very radiation-sensitive stage exists 36-hours before the first egg is laid.

In order to determine if this stage effect would occur for different regions, the dpy-5 dpy-14 interval was studied. The results are presented in Table 6. As Figure 7 shows, gamma-radiation treatment at 36-hr prior to egg laying also caused a dramatic increase in recombination.

TABLE 5

<Stage experiment (I)>

Region: dpy-5 unc-13

Dose : 2K rads.

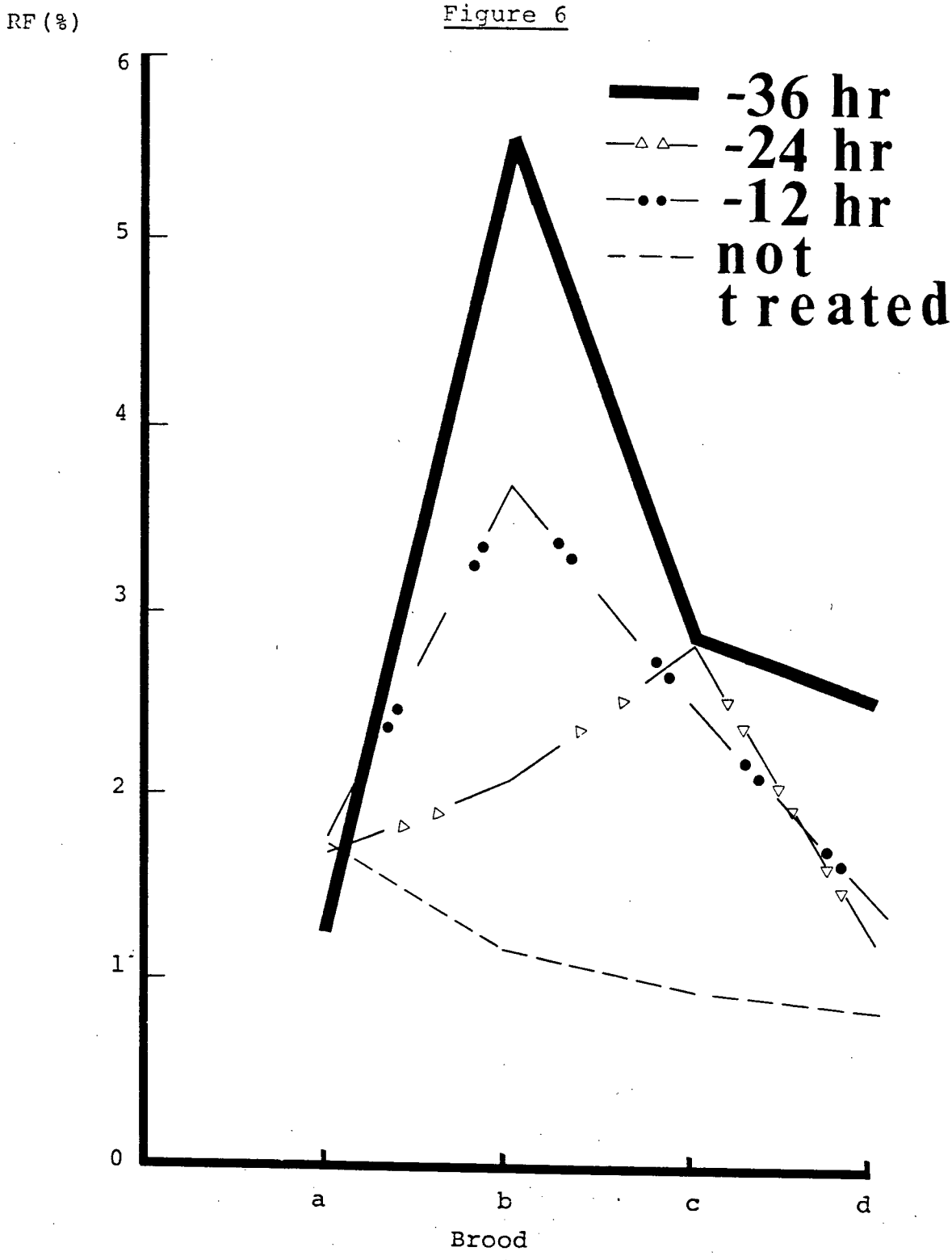
	[P] per brood					
*STAGE±BROOD**	a	b	c	d	[p]	95% C.I.
-36hr	1.25	5.54	2.91	2.53	3.1	(2.39-3.98)
	(271)	(641)	(482)	(542)	(1936)	
-24hr	1.66	2.06	2.90	1.14	1.88	(1.33-2.48)
	(296)	(599)	(430)	(686)	(1624)	
-12hr	1.87	3.68	2.49	1.42	2.42	(1.77-2.91)
	(413)	(684)	(377)	(649)	(2123)	
NOT TREATED	1.76	1.14	0.92	0.86	1.00	(0.67-1.31)
	(521)	(897)	(694)	(755)	(2867)	

* Hours before egg-laying.

** 12 hr broods.

( )Number of progeny.

Figure 6



Effect of radiation on recombination frequency for dpy-5 unc-13 strain at different developmental stages.

ii. Cytology of Gonads.

The number of primordial gonadal cells (p cells) was counted for the three different developmental stages that were treated with gamma-radiation. For each stage untreated siblings were examined cytologically. At the 36-hr prior to egg-laying stage, approximately ten primordial gonadal cells were observed. At 24-hr prior to egg-laying, 50-55 primordial gonadal cells were observed. At the 12-hr prior to egg-laying stage, the number of p cells was too many to count. My experiments were carried out at 20°C whereas those of Kimble and Hirsh (1979) were done at 25°C, so that the developmental time in hours did not correspond exactly with their published work (Table 7). No detectable morphological difference was found between treated and untreated individuals when examined immediately after treatment.

TABLE 6

‹Stage experiment (II)›.

Region: dpy-5 dpy-14

Dose : 2K rads.

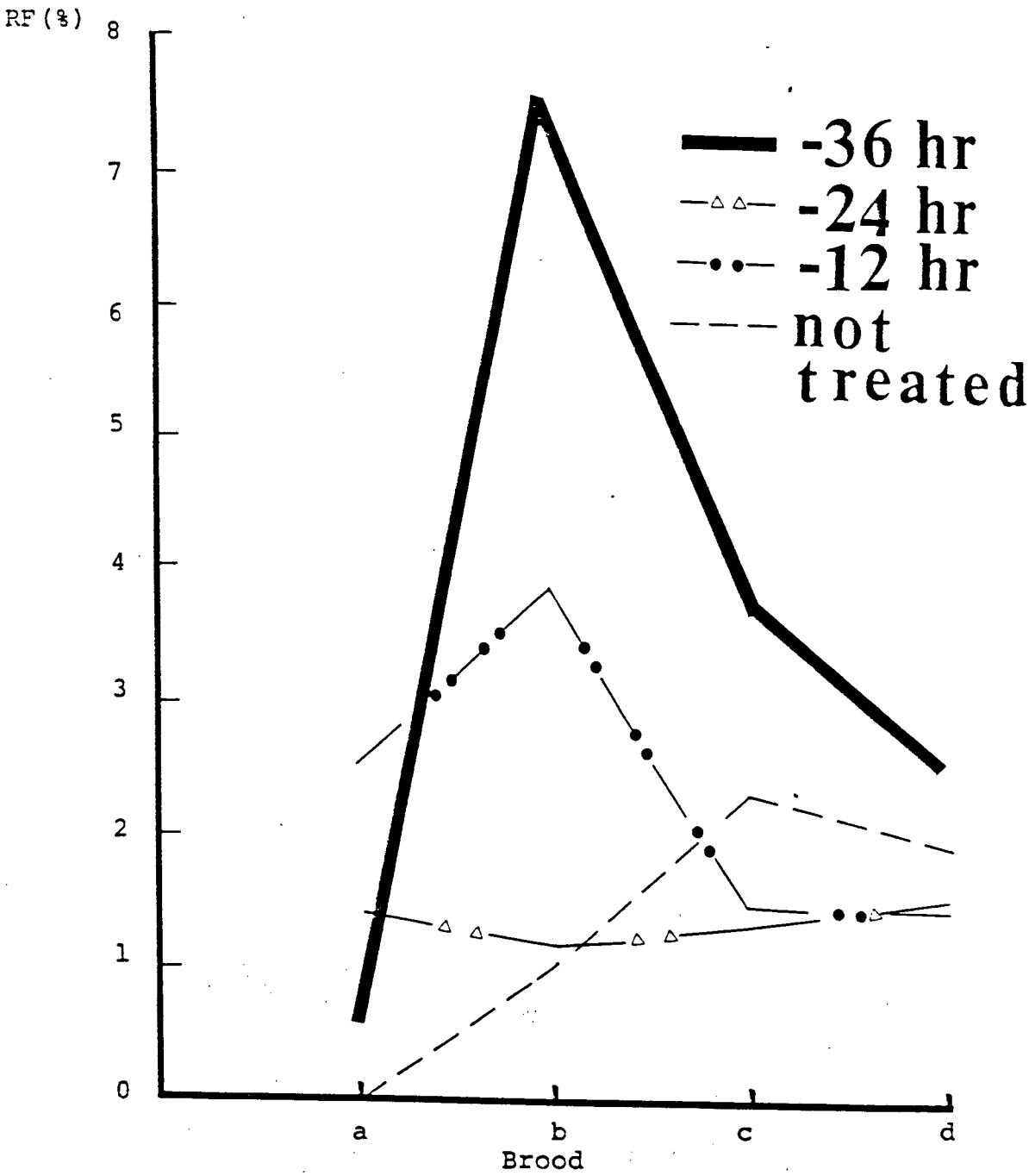
	[p] per brood					
*STAGE±BROOD**	a	b	c	d	[p]	95% C.I.
-36 hr	0.66	7.54	3.69	2.56	3.47	(2.42-4.51)
	(196)	(259)	(243)	(345)	(1043)	
-24 hr	1.37	1.14	1.20	1.42	1.28	(0.72-1.83)
	(480)	(861)	(405)	(507)	(2253)	
-12 hr	2.50	3.90	1.40	1.30	2.10	(1.25-2.54)
	(300)	(456)	(398)	(746)	(1900)	
NOT TREATED	0	1.05	2.37	2.03	1.92	(1.39-2.46)
	(120)	(445)	(365)	(590)	(1075)	

* Hours before egg-laying.

**12-hr broods.

( )Number of progeny.

Figure 7



Effect of radiation on recombination frequency for dpy-5 dpy-14 at different developmental stages.

TABLE 7

‹Development of the reproductive system of C. elegans›.

	Hours at 25 degrees celsius	
	0	Hatch
	0-6	4 primordial gonadal cells (p cells)
L1	6-11	6 cells ‹--- 36-hr prior to egg-laying
L2	11.5	First larval molt
	12-13	10 p cells
	14-18	30 p cells
L3	18.5	Second larval molt
	20-22	60 p cells. Hypodermal cells enlarge. ‹---24-hr prior to egg-laying
	24-26	120 p cells
L3	26	Gonad begins 180² turn
L4	26	Third larval molt
	28-32	Hypodermal cells form vulva and vagina
	32-35	Sperm formation in proximal arm; ‹---12-hr prior to egg-laying
		Spermathecae formation.
L4	35.5	Fourth larval molt
Adult	35-36	Oocytes appear
	37	Fertilized eggs present
	45-46	Egg laying begins

Retyped from the results reported by Kimble and Hirsh (1979).

iii. Effect on Spermatogenesis or Oogenesis.

To investigate whether the increased recombination frequencies occurred during spermatogenesis or oogenesis, sperm in treated hermaphrodites were replaced by introducing the sperm from untreated sibling males (the Materials and Methods, section 8). The male outcross progeny resulted from fertilization of treated oocytes by untreated sperm. The number of self-cross males would be negligible. The recombination events in the hermaprodite sperm are not scored, if only male progeny were scored.

When events in hermaphrodite sperm are excluded, there is less increase in recombination frequency after radiation treatment (shown in Table 8). If all of the observed increases in the self-cross experiments were the result of radiation on spermatogenesis, one would expect no increase in recombination frequency with untreated male sperm. Since some increase occurred, this increase must be due to an affect of radiation on oogenesis. Since the amount of the increase is reduced some affect on hermaphrodite spermatogenesis may also be occurring.

d. Sterility of Recombinants.

Dumpy, Uncoordinated, and Blistered recombinants from the experiments were tested to confirm that they were true recombinants as well as to investigate their sterility. The

TABLE 8

‹Effect on spermatogenesis or oogenesis›.

Region: dpy-5 unc-13

Stage : 36-hr prior to egg-laying.

Dose : 2K rads.

			[p]	95% C.I.
OUTCROSS:				
CONTROL, N = 17:	WILD-TYPE MALES	909		
	RECOMBINANT MALES	35	2.85	(2.05-3.56)
2000 rads, N = 18:	WILD-TYPE MALES	1167		
	RECOMBINANT MALES	54	3.41	(2.47-4.24)
SELF-CROSS:				
CONTROL, N = 13:	TOTAL PROGENY	3721		
	RECOMBINANTS	37	1.00	(0.67-1.31)
2000 rads, N = 10:	TOTAL PROGENY	1820		
	RECOMBINANTS	58	3.1	(2.39-3.98)

data is recorded in Table 9. In untreated individuals, all the recombinants were fertile. However, some fraction of recombinants recovered from the treated individuals were found to be sterile. From the data which were collected at 12-hr intervals, the highest sterility was observed in the B brood (shown in Figure 8). This coincides with the highest peak of recombination frequency caused by gamma-radiation. Therefore, radiation appears to cause genetic damage resulting sterility accompanied by an increase in recombination frequency.

In order to determine whether the effect of radiation on sterility is associated with the recombination event, the non-recombinant wild-type progeny from treated individuals were also collected and examined. All of the wild-type progeny hermaphrodites tested were found to be fertile. Therefore it appears that the radiation-induced sterility is associated with the recombination event.

II. EFFECT OF RADIATION ON X-CHROMOSOME NONDISJUNCTION.

a. Rate of Nondisjunction.

The effect of radiation on X-chromosome nondisjunction was observed (Table 10). The number of wild-type males was scored as a measure of X-chromosome nondisjunction.

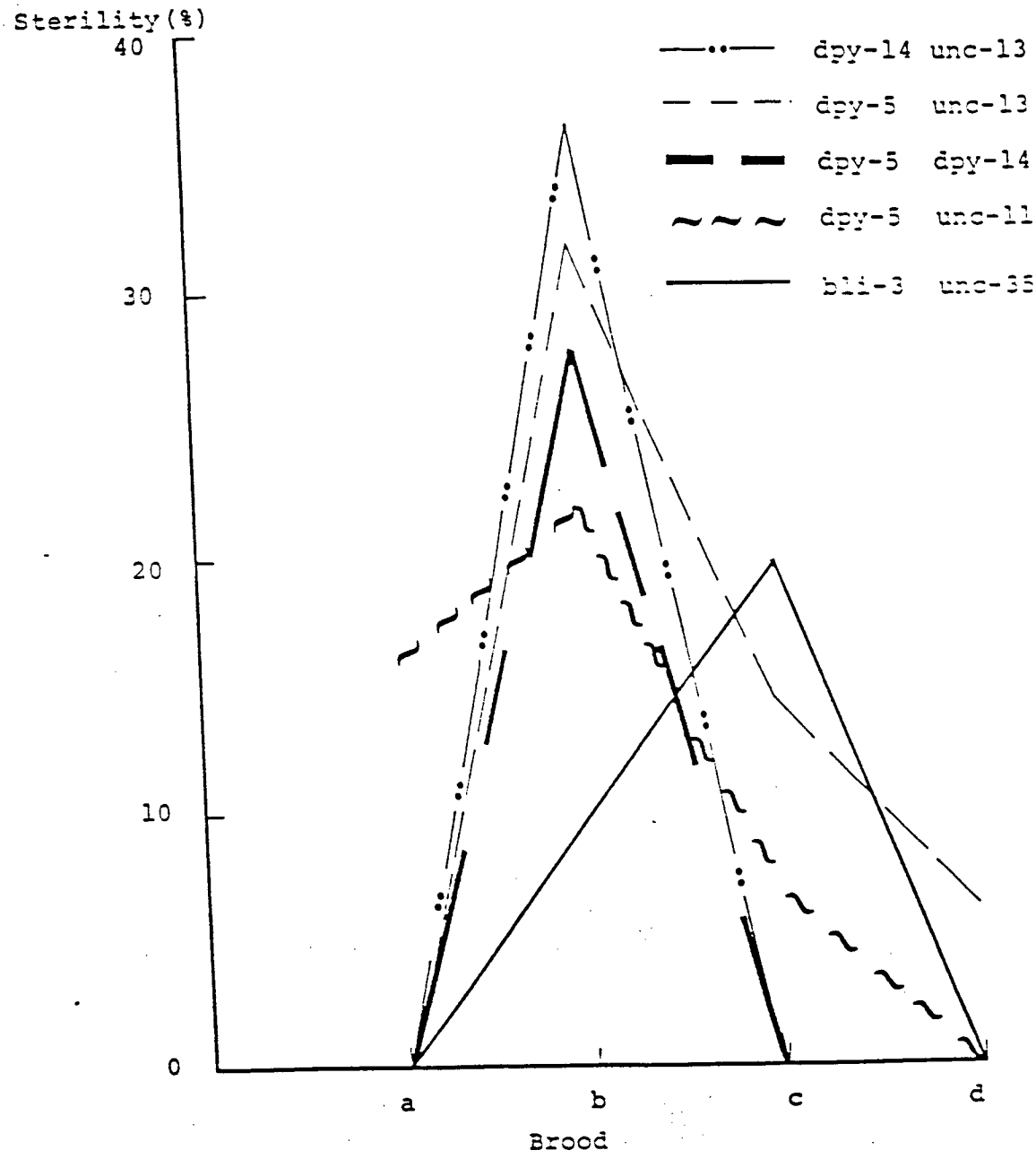
TABLE 9

<Effect of radiation on sterility of recombinants>.

GENOTYPE	*DOSE(STAGE)	a	b	c	d
dpy-5 unc-13	2R(12hr)	0/36 = 0	26/132 = 0.20	11/79 = 0.14	0/43 = 0
dpy-5 unc-13	4R(12hr)	1/28 = 0.04	28/ 76 = 0.37	13/41 = 0.32	0/23 = 0
	Control	1/29 = 0.03	0/ 34 = 0	0/26 = 0	0/11 = 0
dpy-5 dpy-14	2R(12hr)	0/ 3 = 0	3/ 22 = 0.14	0/16 = 0	0/17 = 0
dpy-5 dpy-14	4R(12hr)	0/ 9 = 0	6/ 34 = 0.18	1/20 = 0.05	1/15 = 0.07
	Control	0/10 = 0	0/ 19 = 0	0/ 9 = 0	0/ 8 = 0
dpy-5 unc-13	2R(36hr)	0/ 4 = 0	8/ 26 = 0.31	3/20 = 0.15	1/18 = 0.05
dpy-5 dpy-14	2R(36hr)	0/ 3 = 0	5/ 18 = 0.28	0/ 8 = 0	0/ 5 = 0
dpy-14 unc-13	2R(36hr)	0/ 2 = 0	4/ 11 = 0.36	0/ 5 = 0	0/ 6 = 0
	Control	0/ 2 = 0	0/ 3 = 0	0/ 2 = 0	0/ 0 = 0
dpy-5 unc-11	2R(36hr)	1/ 6 = 0.17	3/ 14 = 0.21	1/14 = 0.07	0/ 6 = 0
	Control	0/ 7 = 0	0/ 8 = 0	0/ 8 = 0	0/ 1 = 0
bli-3 unc-35	2R(36hr)	0/ 9 = 0	1/10 = 0.1	2/10 = 0.2	0/ 8 = 0
	Control	0/22 = 0	0/22 = 0	1/22 = 0.05	0/27 = 0

* DOSE : 2R = 2K rads, 4R = 4K rads.

Figure 8



Effect of radiation on sterility of recombinants at 36 hr prior to egg-laying stage.

After treatment with 2K rads of gamma-radiation, the fraction of males observed increased dramatically. The result is summarized in Figure 9 which shows the number of males per thousand wild-type hermaphrodites after irradiation. The frequency of X-chromosome nondisjunction can be used as a means of confirming the developmental synchrony of treated individuals. That is, only data from experiments in which the nondisjunction peaks coincided were compared to study regional effects.

In order to determine whether the males were a consequence of X-chromosome nondisjunction, trisomic hermaphrodites from F1 heterozygous individuals were collected and progeny tested. Individual trisomic hermaphrodites segregated only 3X and 2X hermaphrodites (3X : 2X = 2 : 1). The number of 3X hermaphrodites was almost equivalent to that of males observed.

The dose response for X-chromosome nondisjunction was also studied (Table 4). It appeared to increase with increasing doses examined.

b. Stage Effect on Nondisjunction.

The effect of radiation on nondisjunction frequency at different developmental stages was studied (Table 11). The peak of nondisjunction occurred in C brood when individuals were treated 36-hr prior to egg-laying (Figure 10) compared to aB brood peak following a minus 12-hr treatment.

TABLE 10

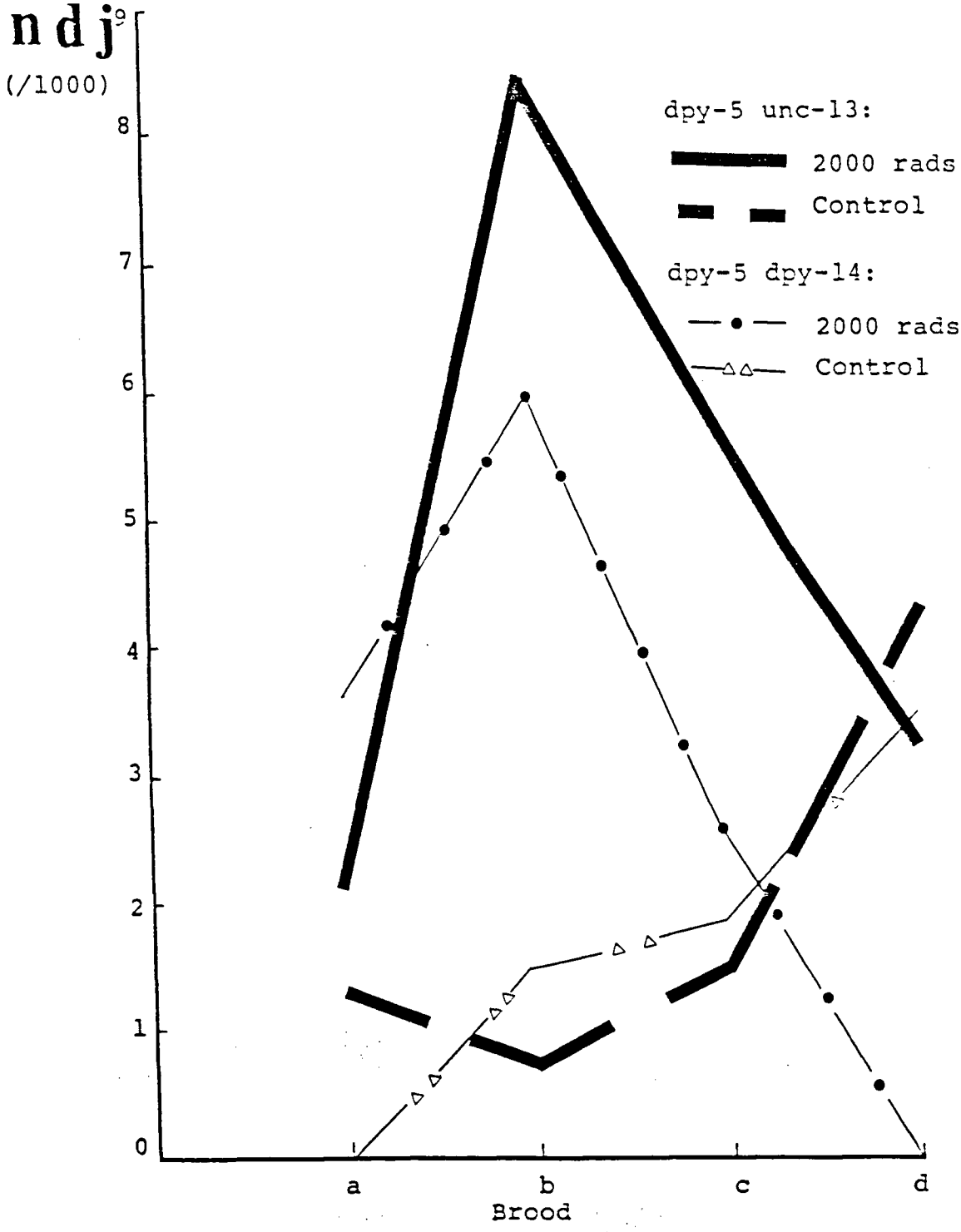
‹Effect of radiation on non-disjunction frequency›.

Stage: 12-hr prior to egg-laying.

Dose : 2K rads.

	Number of males per 1000			
GENOTYPE±BROOD	a	b	c	d
dpy-5 unc-13				
CONTROL:	1/763 = 1.3	1/1208 = 0.8	2/1312 = 1.5	1/236 = 4.2
2000 rads:	2/967 = 2.1	14/1654 = 8.5	10/2037 = 4.9	3/938 = 3.2
dpy-5 dpy-14				
CONTROL:	0/381 = 0	1/ 673 = 1.5	1/ 540 = 1.9	1/306 = 3.4
2000 rads:	1/278 = 3.6	3/ 497 = 6.0	1/ 352 = 2.8	0/292 = 0

Figure 9



Effect of radiation on non-disjunction frequency at 12 hr prior to egg-laying stages.

III. COMPARISON OF RECOMBINATION AND NON-DISJUNCTION

In order to investigate the gamma-radiation sensitive brood for recombination and X-chromosome nondisjunction, the ratio of experimental (treated) to control (untreated) data were plotted (Table 12 and Figure 11). The ratios of experimental to control showed a continuous increase with parental age in recombination frequency, whereas a peak in X-chromosome nondisjunction was observed in the B brood .

IV. CHROMOSOMAL REGIONALITY.

In order to determine if recombination and X-chromosome nondisjunction increased in other regions of the chromosome with radiation treatment, several intervals were chosen and compared: 1) clustered intervals (dpy-5 dpy-14, dpy-14 unc-13, dpy-5 unc-13), 2) an interval adjacent to a cluster (dpy-5 unc-11), and 3) an interval at the left end of the chromosome (bli-3 unc-35). See map in Appendix 2

a. Recombination in the Clustered Region.

The well characterized clustered region between dpy-5 and unc-13 was chosen for my research. This clustered region between dpy-5 and unc-13 is subdivided into two intervals, dpy-5 dpy-14 and dpy-14 unc-13. Results for the intervals are

TABLE 11

‹Effect of radiation on non-disjunction frequency at different stages›.

Region: dpy-5 unc-13

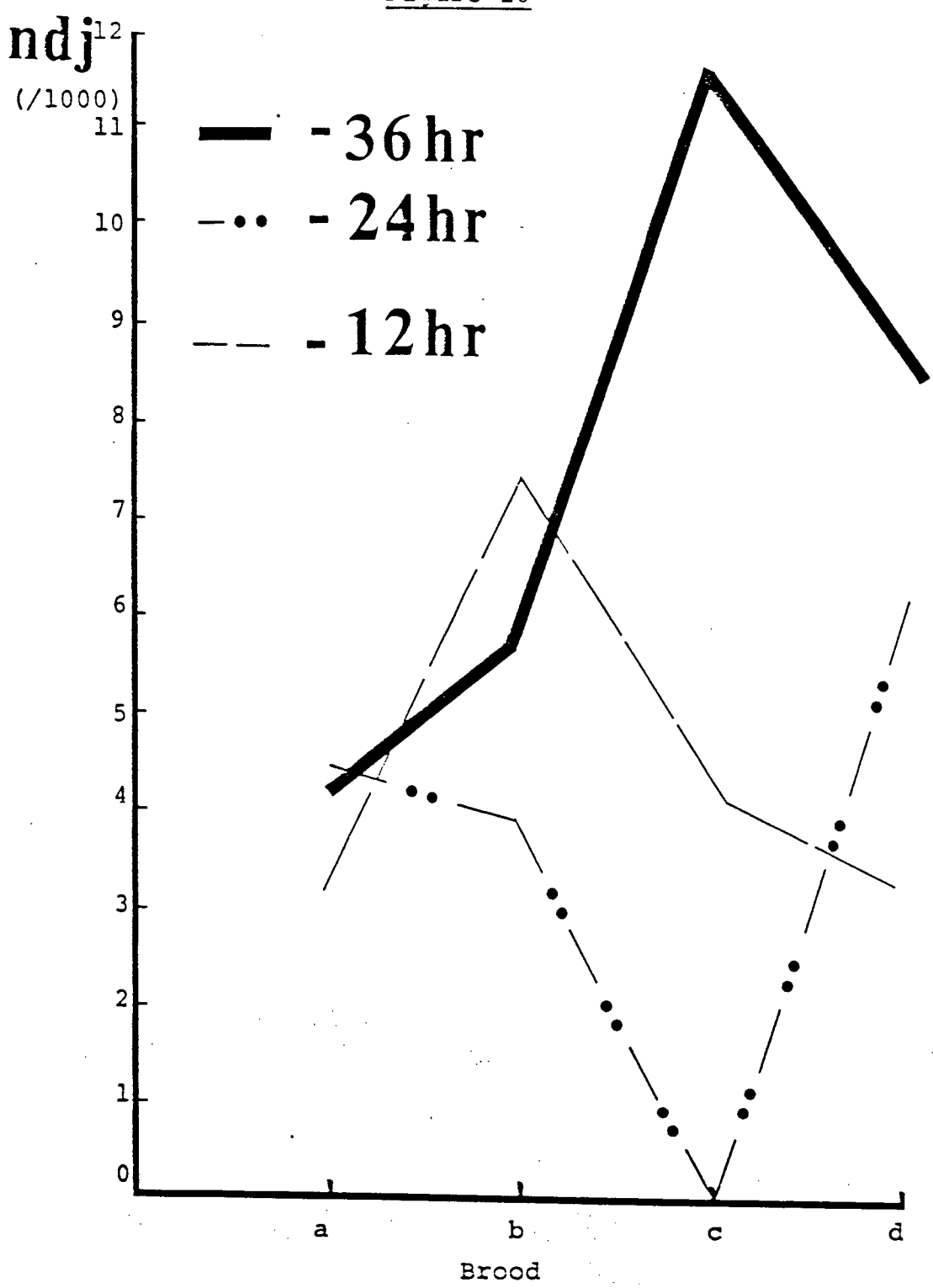
Dose : 2K rads.

	Number of males per 1000			
*STAGE±BROOD**	a	b	c	d
-36hr	1/239 = 4.18	2/342 = 5.84	6/507 = 11.83	2/234 = 8.55
-24hr	2/445 = 4.49	2/504 = 3.96	0/306 = 0	2/327 = 6.12
-12hr	1/318 = 3.14	3/401 = 7.48	3/715 = 4.19	2/631 = 3.17

* Hours before egg-laying.

** 12-hr broods.

Figure 10



Effect of radiation on nondisjunction frequency for dpy-5 unc-13 strain at different developmental stages.

TABLE 12

<Comparison of recombination and non-disjunction>.

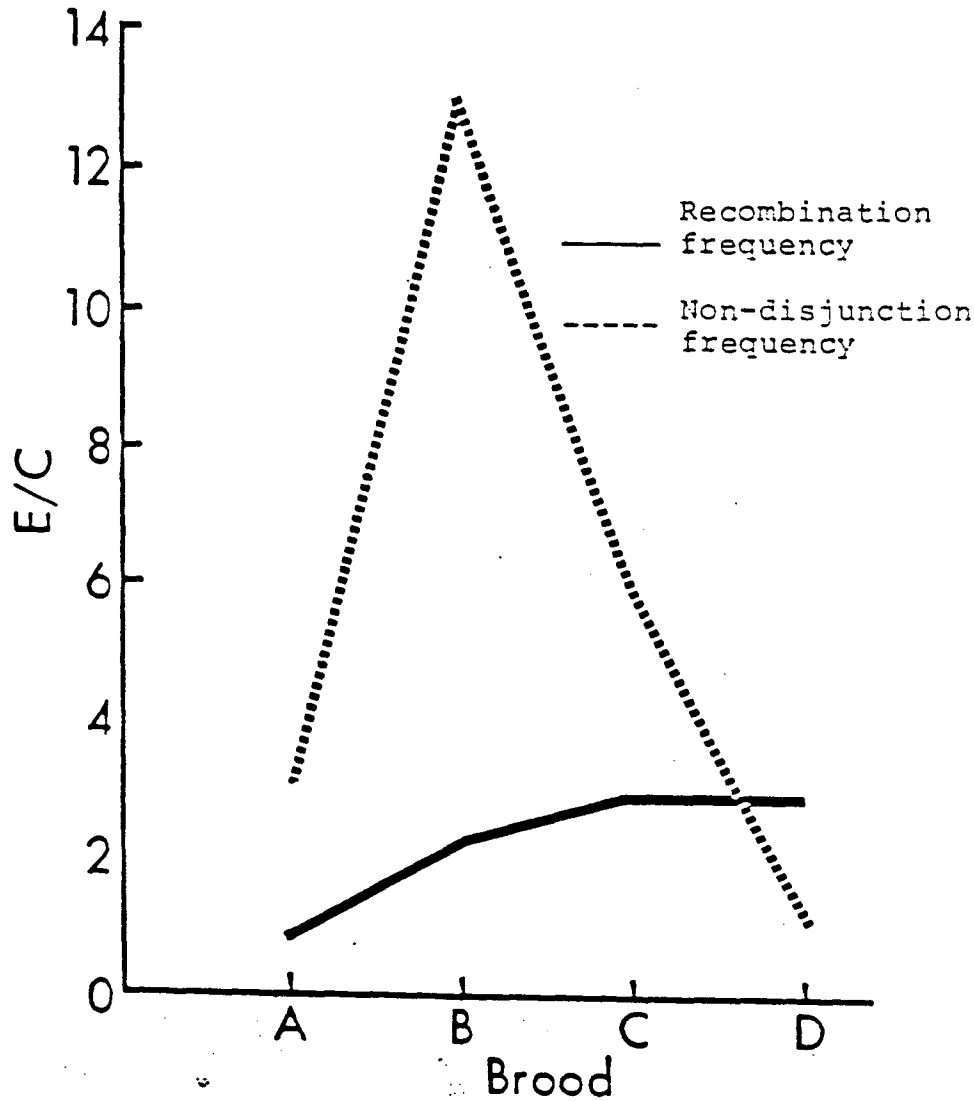
Region: dpy-5 unc-13

Stage : 12-hr prior to egg-laying.

Dose : 2K rads.

	Experimental/Control			
BROOD	a	b	c	d
Recombination Frequency	0.91	2.24	3.10	3.17
Non-disjunction Frequency	2.73	12.80	6.18	1.22

Figure 11



Comparison of the effect of radiation on the frequency of recombination and nondisjunction for dpy-5 unc-13 strain at 12 hr prior to egg-laying stage.

recorded in Table 13. As Figure 12 summarizes, the maximum magnitude of increase is observed in the dpy-14 unc-13 interval. Increases in recombination frequency by radiation treatment seems to be approximately the same in both the dpy-5 dpy-14 interval and the dpy-5 unc-13 interval.

b. Recombination in a Region Adjacent to the Cluster.

These experiments in the clustered region between dpy-5 and unc-13 prompted a further study on a contiguous region. The dpy-5 unc-11 interval was chosen for this purpose and showed a little less of an increase in recombination frequency than in the clustered region (Table 14). A similar brood effect is observed (Figure 13).

c. Recombination at the Left-end of the Chromosome.

It is clear that the above observations have significance for an understanding of the effect of radiation on recombination frequency: gamma-radiation has an increased effect on recombination frequency in the clustered region of LG I. It is, then, important to test a region farther apart from this cluster. The bli-3 unc-35 interval from the left end of LG I was studied. The regional responses described are recorded in Table 15. The bli-3 unc-35 interval seems to show almost no response to a radiation treatment.

TABLE 13

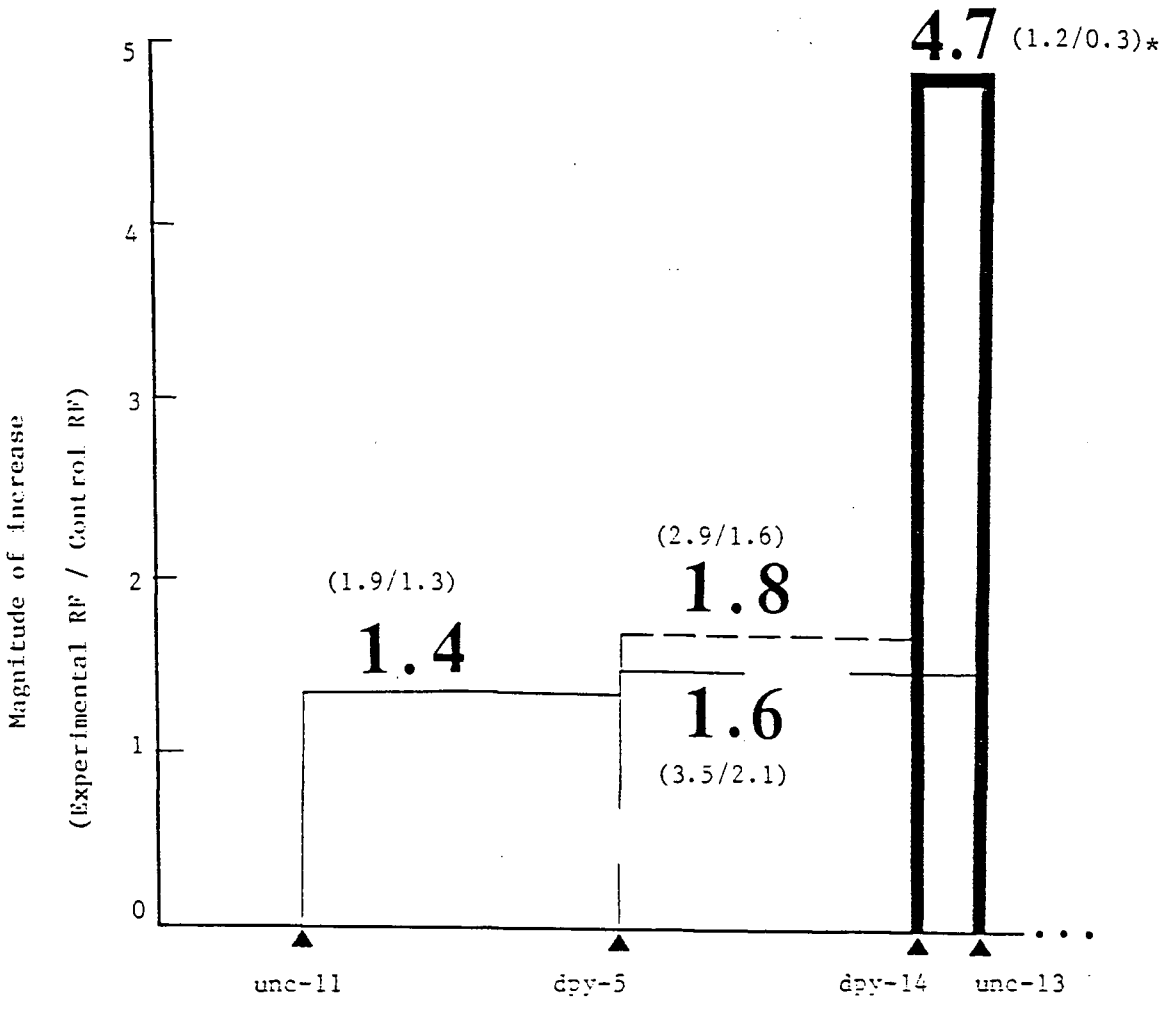
‹Effect of radiation on recombination in the clustered regions›.

Stage: 36-hr prior to egg-laying.

Dose : 2K rads.

GENOTYPE		*N	WTS	DPYS	UNCS	[p]	95% C.I.
dpy-5 unc-13	CONTROL	26	4691	61	39	2.11	(1.61-2.62)
	2K rads	18	1447	29	39	3.46	(2.60-5.81)
dpy-5 dpy-14	CONTROL	10	2532	23	19	1.64	(1.23-1.65)
	2K rads	9	861	20	14	2.92	(1.92-3.84)
dpy-14 unc-13	CONTROL	17	2340	4	3	0.26	(0.07-0.66)
	2K rads	13	1092	9	14	1.23	(0.67-1.79)

* N = Number of parents.



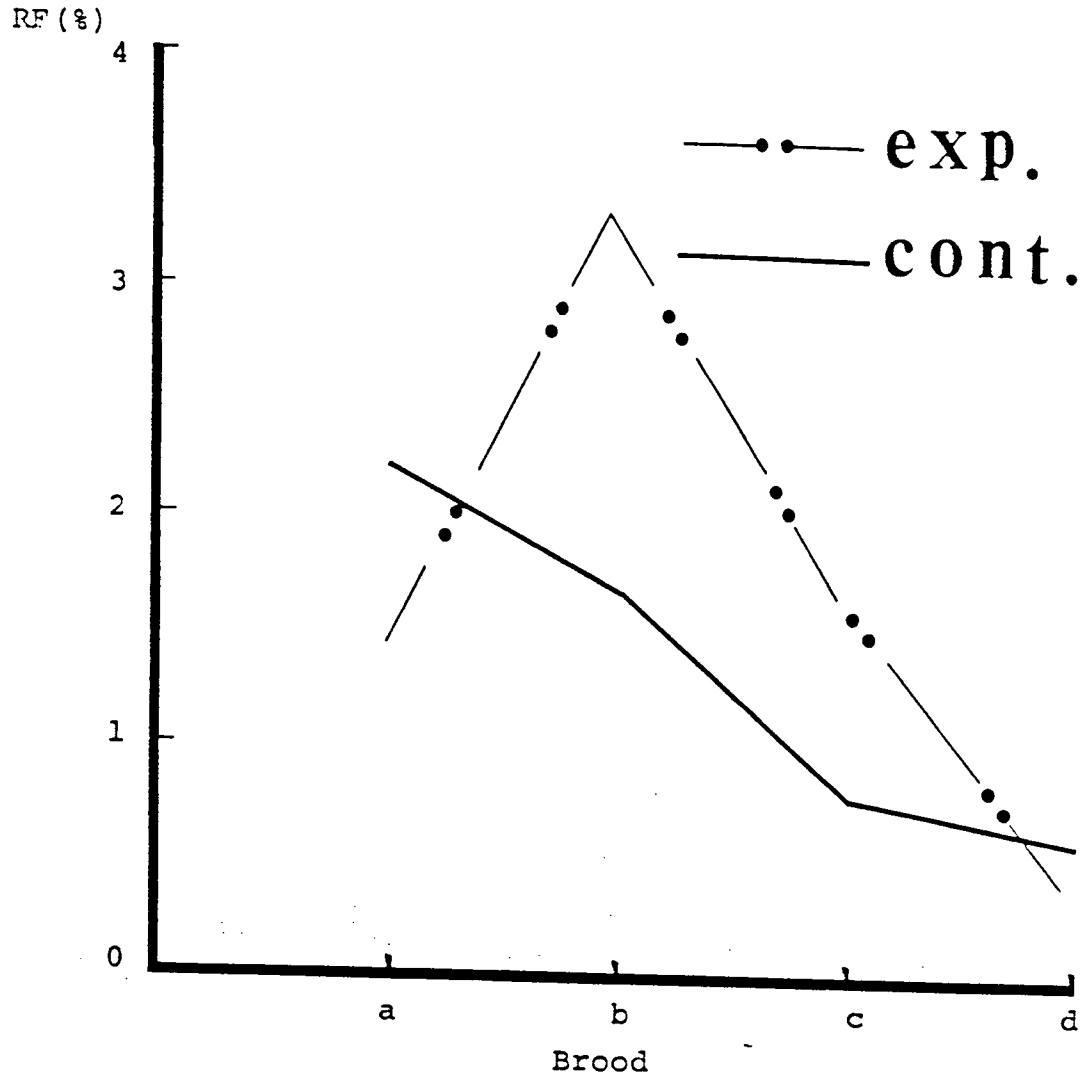
*(Experimental/Control) value.

TABLE 14

‹Effect of radiation on recombination in dpy-5 unc-11›.
Stage: 36-hr prior to egg-laying.

12-hr BROOD	PROGENY	RECOMBINANTS	[p]	95% C.I.
2000 rads, N = 10				
a	450	6	1.34	(0.27-2.40)
b	440	14	3.23	(1.53-4.83)
c	906	14	1.56	(0.74-2.35)
d	469	6	1.29	(0.26-2.30)
TOTAL	2265	40	1.85	(1.48-2.59)
CONTROL, N = 15				
a	325	7	2.17	(0.57-3.74)
b	502	8	1.60	(0.50-2.69)
c	953	8	0.84	(0.26-1.42)
d	127	1	0.78	(- -2.32)
TOTAL	1907	24	1.27	(0.76-1.76)

Figure 13



Effect of radiation on recombination frequency for dpy-5 unc-11 strain.

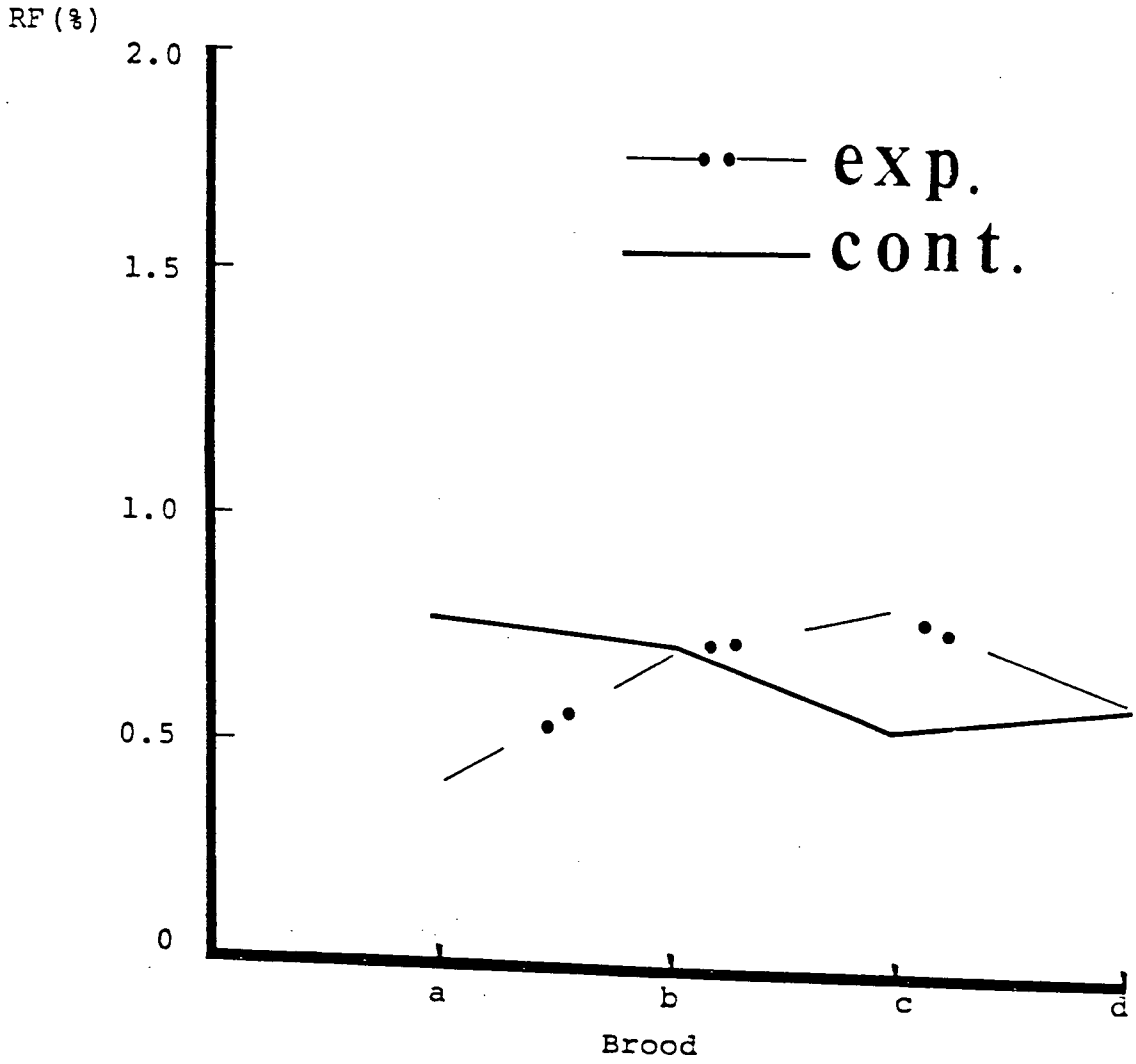
TABLE 15

‹Effect of radiation on recombination in bli-3 unc-35›.

Stage: 36-hr prior to egg-laying.

12-hr BROOD	PROGENY	RECOMBINANTS	[p]	95% C.I.
2000 rads, N = 28				
a	1117	9	0.82	(0.28-1.33)
b	774	10	1.30	(0.49-2.09)
c	611	10	1.65	(0.63-2.65)
d	680	8	1.18	(0.36-2.38)
TOTAL	3174	37	1.17	(0.79-1.54)
CONTROL, N = 43				
a	1462	22	1.52	(0.88-2.13)
b	1652	22	1.34	(0.78-1.89)
c	2160	22	1.02	(0.59-1.44)
d	2396	27	1.13	(0.70-1.55)
TOTAL	7671	93	1.22	(0.97- 1.46)

Figure 14



Effect of radiation on recombination frequency for bli-3 unc-35 strain.

d. Summary Regarding Recombination at Different Intervals

In summary, data on the effect of gamma-radiation on recombination frequency over the left portion of LG I were collected, and the magnitude of increase was compared in each interval (Table 16). Regional differences in the relative increases of recombination frequency seemed to be apparent in the magnitude of the 2K rads/Control values. The dpy-14 unc-13 interval appeared to increase the most after radiation treatment. The bli-3 unc-35 interval in a non-clustered region did not increase after treatment.

e. Non-disjunction curves for different strains.

The frequencies of X-chromosome non-disjunction were also compared for different strains. Table 17 and Figure 15 summarize the results. It appears that the effect of radiation on X-chromosome nondisjunction occurs at the same developmental stage for different mutant strains. This confirms that the parental hermaphrodites were treated at the same developmental stage.

TABLE 16

<Summary>.

REGION	BROOD WITH HIGHEST INCREASE OF RECOMBINATION	RECOMBINATION CONTROL	RECOMBINATION 2000 rads	RATIO OF 2K rads/Control
dpy-5 unc-13	b	2.11	3.46	1.64
dpy-5 dpy-14	b	1.64	2.92	1.78
dpy-14 unc-13	b	0.26	1.23	4.73
dpy-5 unc-11	b	1.27	1.85	1.46
bli-3 unc-35	c	1.22	1.17	0.96

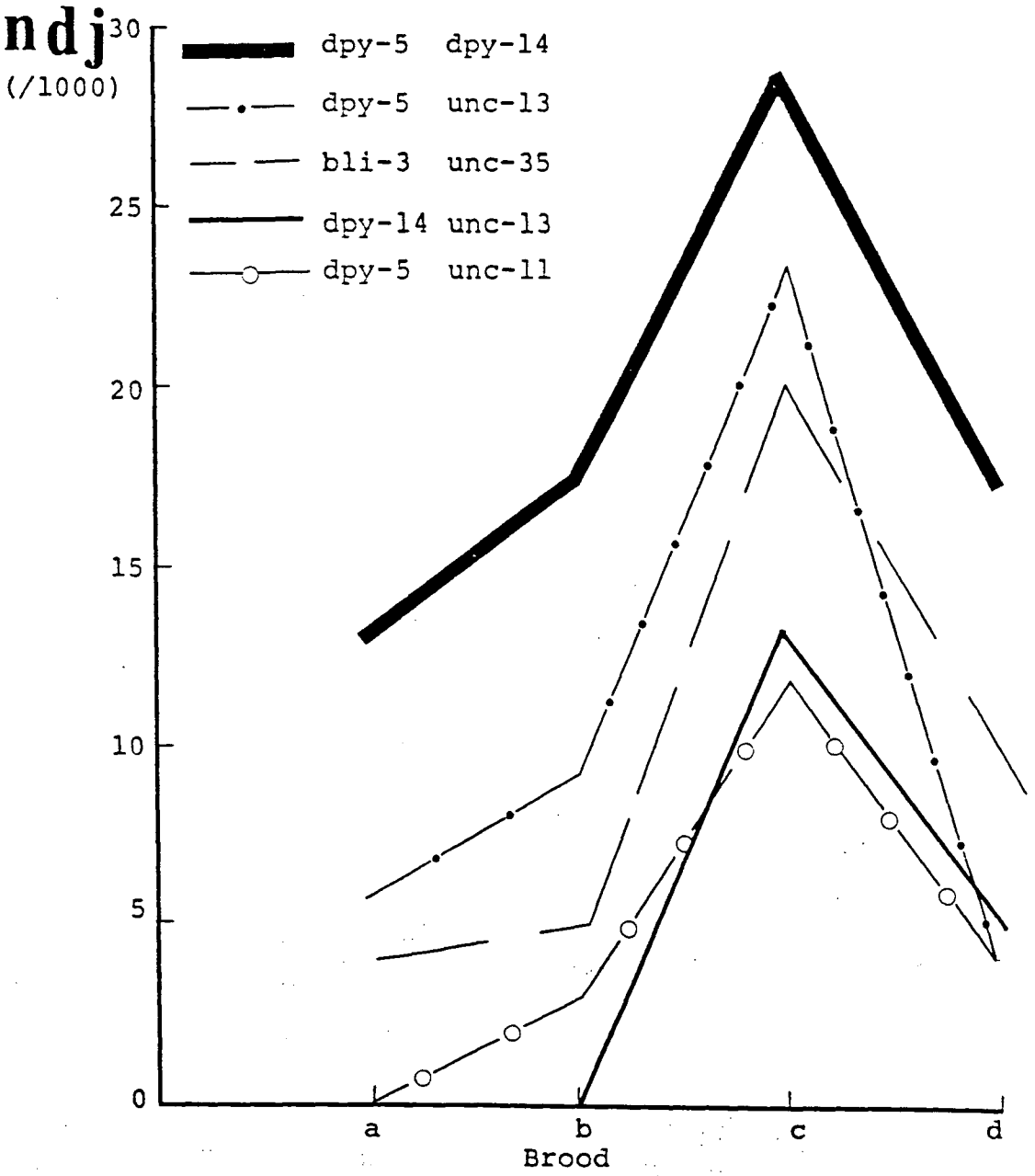
TABLE 17

‹Effect of radiation on non-disjunction frequency at different regions›. Stage: 36-hr prior to egg-laying.

GENOTYPE±RROOD	a	b	c	d
dpy-5 unc-13	1/158 = 6*	3/349 = 9	10/432 = 23	1/281 = 4
dpy-5 dpy-14	3/226 = 13	3/177 = 17	8/283 = 28	3/175 = 17
dpy-14 unc-13	0/ 78 = 0	0/362 = 0	4/302 = 13	2/350 = 5
dpy-5 unc-11	0/333 = 0	1/320 = 3	8/671 = 12	2/498 = 4
bli-3 unc-35	3/831 = 4	3/573 = 5	9/451 = 20	2/498 = 4

* Fraction of wild type progeny that were male converted to number of males per 1000.

Figure 15



Effect of radiation on non-disjunction frequency for different regions.

DISCUSSION

The effect of gamma radiation on recombination frequency for intervals of Linkage Group I in C. elegans has been studied. Recombination frequency increased approximately two-fold across the dpy-5 unc-13 interval after treatment with 2K rads of gamma radiation. This thesis is the first description of the effect of gamma radiation on recombination frequency in this organism.

Several factors affecting the magnitude of the increase have been characterized. Recombination frequency increased more with higher doses of radiation. However, the increase in recombination frequency with increasing dose was accompanied by a reduced average number of progeny in radiation-treated individuals. Thus, because gamma radiation also affects fecundity, a dose of 2K rads was chosen as the optimal dose for my experiments. In order to determine whether the effect of radiation on recombination is specific for a certain stage of oogenesis, a brood analysis was performed for every 12-hr interval. In this self-fertilizing hermaphrodite, spermatogenesis is completed prior to the onset of oogenesis which continues throughout the adult life of the hermaphrodite. Thus, the progeny from each 12-hr brood are the result of female gametes which were at different developmental stages at the time of treatment. The earliest (A) brood was most mature when they were treated. The results presented in Table 3 and Figure 4 show the greatest increase in the second (B) brood. There was

no increase in the first (A) brood, and as in the untreated individuals, decreases in recombination frequency were seen in later broods. The A brood progeny resulted from eggs that were post-meiotic at the time of treatment. Thus, the lack of increase in recombination frequency in the first brood is most likely because the oocytes sampled in the first brood have already completed recombination events prior to treatment with radiation. Because spermatogenesis occurs prior to oogenesis and the mature sperm are used throughout all the broods, general increases throughout all the broods would have been expected if increases in recombination frequency after radiation treatment were the result of an effect on spermatogenesis. These experiments demonstrate therefore that treatment with radiation caused an increase in recombination frequency in oocytes that are in a meiotic stage which occurs between 12 and 24 hours prior to egg-laying. The sensitive stage is possibly (but not necessarily) prophase of meiosis I (the actual event of recombination).

Treatment of hermaphrodites at different developmental times has shown that radiation applied prior to meiosis can have an affect on recombination frequency in the sensitive B brood. However not all developmental stages are sensitive to radiation treatment (with regard to recombination frequency). The experiments were performed at three different developmental stages. When hermaphrodites were treated with radiation 24-hr prior to egg-laying no significant increase in recombination frequency was observed. It may be that at this time in the

development of the gonad the DNA is insensitive to radiation damage. The maximum affect of radiation was produced by treating the hermaphrodites 36 hours prior to egg-laying (see the Results section I-c-i; Tables 5 and 6). The difference in control values between Table 3 and Table 5 might be due to a strain effect since the first experiment (Table 3) was done using the e51 allele (BC 26 strain) and the latter experiments using e450 (BC 415). The amount of increase relative to the control is the same in both cases even though all the recombination frequencies are reduced when the BC 415 strain was used. Thirty-six hours prior to egg-laying is an early pre-meiotic stage prior to a time of extensive cell division and DNA replication in the gonads (Nelson, Lew and Ward, 1978; Kimble and Hirsh,1979). Presumably, chromosomal breaks caused at this stage are not directly responsible for the increased recombination because these cells will have to go through many more cell divisions before entering meiosis. Base-pair modifications which are known to be caused by radiation could occur and since many of these alterations are unstable, chemical changes in the DNA at later stages might cause nicks or breaks which could become substrate for recombination events. Other types of explanations are feasible. For example radiation may have a stimulatory affect on recombination enzymes. Currently however there is no evidence that this occurs.

When hermaphrodites are treated 36-hrs prior to egg-laying, an increase in recombination frequency was observed in the B brood just as when radiation was applied 12-hr prior to

egg-laying. These results suggest that induced lesions produced by radiation at earlier (premeiotic) stages are carried through to later (meiotic) stages. Although the nature of these lesions is not known, this provides a reasonable explanation for the greater increase in recombination frequency at the -36 hour stage. In D. melanogaster, studies on the correlation between the radiation-sensitive period and the developmental stages of the male have been reported (Bonner and Luning, 1950; Luning, 1952). My observations support those made in Drosophila that more chromosome damage can be induced in cells in earlier meiotic stages than in mature stages.

Radiation treatment at -36 hours appears to affect spermatogenesis as well as oogenesis. Rose and Baillie (1979a) have demonstrated that in C. elegans, as in D. melanogaster (Stern, 1926; Schultz and Redfield, 1951), recombination frequency decreases with maternal age. In C. elegans the decrease in recombination frequency with successive broods must be a reflection of changing recombination frequency in oogenesis since spermatogenesis is completed prior to oogenesis. After treatment with radiation, although recombination frequency decreases with age it remains elevated above the control values. In order to evaluate whether this elevation in the later broods was the result of an affect of radiation on spermatogenesis, outcrossing of treated hermaphrodites to untreated males was done. In these experiments the radiation treated sperm in hermaphrodites were replaced with untreated male sperm (see the Results, section I-c-iii) which are used

preferentially by the hermaphrodite. Fertilization with male sperm (XO) results in 50% male progeny which were counted and used to calculate recombination frequency (a combination of recombination events in the hermaphrodite oocyte and the male sperm but excluding recombination events in hermaphrodite sperm). The data is reported in Table 8. An increase in recombination frequency was observed with male sperm in both the treated and untreated individuals. This increase in recombination frequency was suspected to be a result of the higher recombination frequency which has been observed in males (Rose, unpublished data). After treatment with radiation, recombination frequency increased in both self-crossed and out-crossed hermaphrodites. The magnitude of increase is greater in treated self-crossed hermaphrodites than in the treated hermaphrodites crossed to untreated males. Thus, radiation is affecting oogenesis and may be having some affect on hermaphrodite spermatogenesis.

The effect of radiation on X-chromosome nondisjunction was investigated. At 20°C, approximately 1 male per 700 wild-type progeny was observed for the controls, as previously reported (Hodgkin, 1977; Rose and Baillie, 1979a). As well, X-nondisjunction increased with maternal age and temperature in C. elegans (Rose and Baillie, 1979a). In D. melanogaster, temperature (Grell, 1971, 1973) and maternal age (Tokunaga, 1970) effects on nondisjunction have have been reported. I observed an increase in the rate of X-chromosome nondisjunction with radiation treatment (Table 4). The data presented shows a

linear increase with increasing dose.

In order to compare radiation-induced nondisjunction and recombination events, Experimental/Control values were plotted for each brood (Figure 11). As previously discussed recombination frequency is increased over more than one brood. Nondisjunction, on the other hand, has a definite peak in one of the broods.

This peak in nondisjunction differed when different developmental stages were treated. The greatest increase appeared in C brood for the 36-hr prior to egg-laying stage and in B brood for the 12-hr prior to egg-laying stage. These results were somewhat unexpected (Table 11). One might expect that those individuals that were treated earlier would have an earlier peak in nondisjunction or that the peak would occur in the same brood regardless of the time of treatment as was seen for the greatest increase in recombination frequency. The reason for the observed result is not known, possible a different mechanism exists for the pre-meiotic radiation-induced nondisjunction than for the meiotic.

Since the brood in which the peak nondisjunction appeared varied with the time of treatment, I have used the brood peak as means of confirming that synchronized populations were treated with radiation in separate experiments. In order to do so, the non-disjunction curves were plotted for the different mutant strains used in separate experiments (Table 17).

In order to determine whether radiation affected the

fecundity of the progeny of treated individuals, all the recombinant individuals recovered from the experiments were progeny tested. As many as 30% of these recombinants were found to be sterile in the affected broods after radiation treatment (Table 9). However, none of the non-recombinants tested were sterile. Thus gamma-radiation appeared to cause sterility predominantly in recombinant individuals. Furthermore, the stage of maximum increase in recombination frequency by radiation treatment coincides with that of maximum sterility of recombinants: all the intervals with the greatest increase in recombination frequency at B brood show the maximum sterility at the same developmental stage (Tables 9 and 16). Comparable results were previously observed in Drosophila (Ives, 1960): the recombination frequency reached its highest level in the same meiotic stage at which the greatest negative radiation effect on fecundity occurred.

One of the goals in this thesis was to investigate the regional response to radiation treatment in C. elegans. In D. melanogaster, a regional response in recombination frequency had been demonstrated previously (Tattersall, 1981): a dramatic maximum increase in recombination frequency was observed in a region of centric heterochromatin. Brenner (1974) suggested that the autosomal gene clusters in C. elegans were a consequence of recombination suppression in a defined region of a chromosome. Since clustered regions in C. elegans may be comparable to regions of centric heterochromatin in D. melanogaster with regard to the reduced recombination frequency,

one might expect a similar regional response to radiation treatment in C. elegans. I have studied the clustered region between dpy-5 and unc-13. In order to evaluate the effect of chromosomal regionality with regard to radiation-induced recombination, an interval from the non-clustered region (bli-3 unc-35) on Linkage Group (LG) I was also studied. As can be seen in Table 16, the largest increase in recombination frequency was observed in the dpy-14 unc-13 region, which happens to coincide with the region of highest gene density on LG I. In contrast, no effect on recombination frequency was observed with radiation treatment in the bli-3 unc-35 interval, which coincides with a gene poor region. Although alternate explanations are possible, this finding supports the proposal of Brenner (1974) that the gene clusters are a consequence of recombination suppression.

Rec-1 is a meiotic recombination enhancer known to cause an increased recombination frequency throughout the whole genome. It expands the map uniformly. In contrast, °map expansion' by radiation treatment is not proportional to the meiotic map, that is, gamma-radiation produces regional differences. Therefore, gamma-radiation and rec-1 are probably acting through different mechanisms to cause increases in recombination frequencies.

The regional response to radiation treatment in C. elegans is comparable to that of D. melanogaster. However, the magnitude of the increase in the clustered region in C. elegans was not comparable to the magnitude of increase described for

the centric heterochromatin in D. melanogaster. A more extensive study encompassing the entire chromosome seems to be necessary to search for a region with properties of centric heterochromatin.

This work has demonstrated for the first time that gamma-radiation increases recombination frequency in C. elegans. Factors affecting the magnitude of the increase have been investigated. The amount of increase varied with different chromosomal regions presumably reflecting more accurately the amount of DNA than does the meiotic map. In comparison, the recombination enhancer, Rec-1 apparantly increases recombination according to meiotic rules. The observed sterility associated with the radiation-induced recombination is worth further investigation and may be a reflection of extensive DNA damage in the region of the recombination event.

PROPOSALS FOR FURTHER RESEARCH

A number of experiments, which are proposed for further study from the results of my research, are listed below.

1. Investigation of the nature of F1 sterility with regard to linked lethal events.

2. Extension of the investigation to the rest of the chromosomes to search for a region with the properties of centric heterochromatin.

3. Investigation of different methods for synchronizing the time of radiation treatment (e.g. dauer larvae, etc.).

4. Analysis of shorter brood interval (e.g. 3-hr or 4-hr), to determine the effect of radiation on spermatogenesis.

5. Analysis of the interaction between rec-1 and gamma-radiation.

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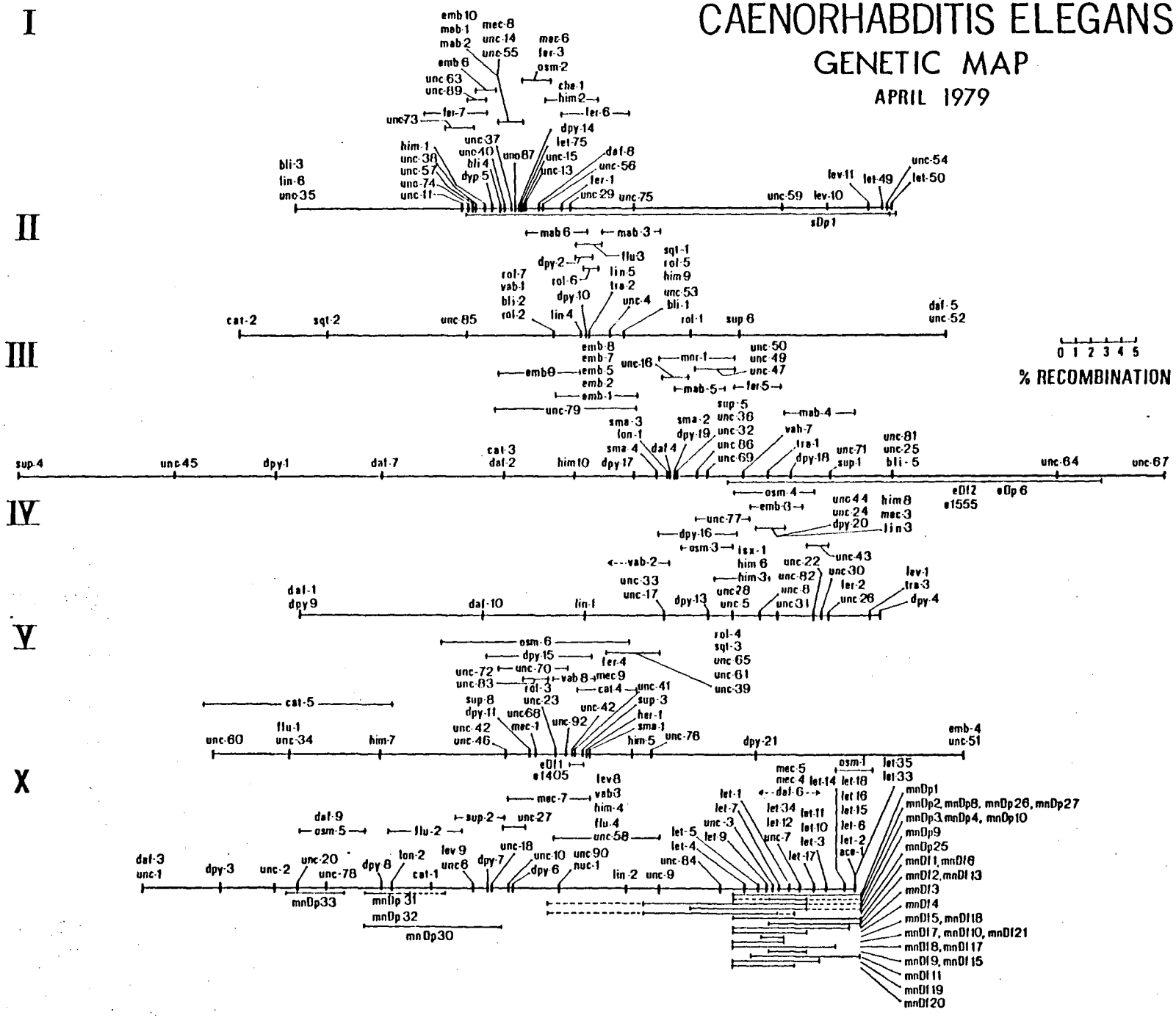
APPENDIX 1:


CAENORHABDITIS ELEGANS
GENETIC MAP
APRIL 1979
I
II
III
IV
V
X
% RECOMBINATION
0 1 2 3 4 5
sDp1
eDf2
eDp6
e1555
eDf1
e1405
mnDp1
mnDp2, mnDp8, mnDp26, mnDp27
mnDp3, mnDp4, mnDp10
mnDp9
mnDp25
mnDf1, mnDf6
mnDf2, mnDf13
mnDf3
mnDf4
mnDf5, mnDf18
mnDf7, mnDf10, mnDf21
mnDf8, mnDf17
mnDf9, mnDf15
mnDf11
mnDf19
mnDf20
mnDp33
mnDp31
mnDp32
mnDp30

APPENDIX 2:

RADIATION-INDUCED MAP EXPANSION