A GENETIC ANALYSIS OF THE RIGHT ARM OF LINKAGE GROUP IV OF CAENORHABDITIS ELEGANS , WITH EMPHASIS ON THE sDf2 REGION

by

Leo Arthur Maria Donati

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NAME: Leo Arthur M. Donati

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EXAMINING COMMITTEE:

Chairman Dr. J.E. Rahe

Dr. D.L. Baillie, Senior Supervisor

Dr. A.T. Beckenbach

Dr. B.M. Honda

Dr. A.M. Rose, UBC

Dr. D.G. Holm, UBC

R.E. Rosenbluth

Dr. S. Wood, Department of Medical Genetics, UBC, Public Examiner

Date approved Sept 18 1985

Title of Thesis/Project/Extended Essay

A genetic analysis of the right arm of linkage group IV of Caenorhabditis elegans with emphasis on the sDf2 region.

Author: _____
(signature)

Leo A.M. Donati
(name)

Sept 18/85
(date)

ABSTRACT

Fifty-four ethylmethane sulfonate (EMS) induced lethals in the region recombinationally suppressed by the translocation nT1(IV;V) were analyzed. The lethals were mapped either to linkage group IV or linkage group V and for those on linkage group IV their right-left position relative to unc-22 was determined. All the EMS-induced lethals mapped to the left of unc-22 . Three of these lethals mapped within the deficiency sDf2 . That no lethals mapped to the right of unc-22 suggests that this region is gene sparce; this region comprises twenty-five percent of the nT1(IV;V) crossover suppressed area on linkage group IV . In order to assess the possibility of isolating lethal mutations to the right of unc-22 , the nT1 balancer screen was used to make a preliminary assessment of the differences in the mutational spectra of EMS-, gamma-ray-, and interstrain-crossing-induced lethal mutations.

Another goal of this study was to develop and validate a method for estimating the number of essential genes in the nematode Caenorhabditis elegans . Previous estimates of essential gene number in C. elegans have ranged from 2000 to 5700 genes. The deficiency sDf2 lies within the region recombinationally suppressed by nT1 . The number of

essential genes within the sDf2 region originally estimated at 32 (Rogalski, 1983), has been reestimated at 36. Assuming all genes are equally mutable, The fraction of lethal genes mutated in sDf2 should be roughly equal to the fraction of lethals mutated in the total region defined by nTl . Knowing that nTl comprises approximately one-seventh of the genome, we can calculate the number of essential genes in C. elegans . The novel method employed in this study suggests that there are approximately 4500 (the 95% confidence limits being 1,379-21,984) genes with non-dispensable functions in C. elegans .

A large portion of the sDf2 and surrounding region has been cloned. It will soon be possible to make a direct comparison of the genetic and molecular maps.

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INTRODUCTION

Much of our understanding of gene regulation and expression has been revealed through analysis of prokaryotes. While prokaryotes afford us the opportunity to dissect relatively simple biological systems, they do not aid us in unraveling the mysteries behind complex multicellular eukaryotes. As a rule, all cells in a multicellular organism possess the same genetic information. It is thought that differentiated tissues and cell types are the result of the regulation of the expression of genes in different cells at different times in development (Garrels 1979).

In order to understand why some genes are expressed while others are not, it is advantageous to assess the distribution of genes relative to one another. An understanding of how the genome is organized is required in order to understand gene regulation and expression. Such an approach has been fruitful in the study of prokaryotic systems. The work of Jacob and Monod (1961) for example, relied on an understanding of gene organization and how it affected gene regulation.

In the case of viruses it is within the realm of the

researcher to sequence the entire genome, or a large portion of it. Such an analysis allows the direct determination of the number of genes in an organism, and their spatial arrangement. Viruses are notorious for arranging their genes in such a manner as to conserve space. The bacteriophage ϕX174, for instance, contains an overlapping gene (Barrell et al. 1976). Gene regulation and expression in viruses is intricately tied into the spatial organization of the genetic information.

Caenorhabditis elegans is a useful organism for the study of gene organization. The C. elegans genome has been estimated to contain between 2000 and 6000 (Brenner 1974; Moerman and Baillie 1979; Rogalski 1983) genes which specify the development and the function of approximately 1000 cells that are differentiated into nerve, muscle, hypodermis, intestine, and gonad. The nervous system consists of 300 cells, most of which are unique both in their structure and function. The development and structure of the C. elegans nervous system has been studied extensively over the past few years (Sulston and Horvitz 1977; Chalfie et al. 1983; Chalfie 1984).

There are a number of reasons for undertaking the genetic analysis of C. elegans (Brenner,1974):

1) a small genome size;

2) a short generation time of 3.5 days at 20 degrees celsius;

3) a large burst size with a wild-type hermaphrodite producing about 200-300 progeny;

4) a small size and an ease of culturing;

5) an ability to freeze stocks in liquid nitrogen.

C. elegans generally reproduces as a self-fertilizing hermaphrodite (both ova and sperm are produced by hermaphrodites). This mode of reproduction provides several advantages for genetic analysis: (a) populations tend to become homozygous and, therefore, it is easy to isolate clones; (b) new mutations will become homozygous on their own in one generation. This alleviates the necessity to conduct brother-sister matings as is the case with Drosophila; (c) mutant stocks may be established from single individuals; (d) many morphologically defective mutants which would not normally be able to mate due to defects in movement, are fertile since fertilization occurs internally.

Since hermaphrodites are not capable of cross-fertilization, males are used in order to introduce different mutations into stocks. Diploid hermaphrodites have five pairs of autosomes and two X chromosomes. Males on the other hand have five pairs of autosomes and only one X chromosome. Males arise spontaneously via X -chromosome

non-disjunction at a frequency of about Ø.1% (Hodgkin et al., 1977). This frequency alters with temperature (Rose and Baillie 1979). These males are capable of copulataing with and cross-fertilizing hermaphrodite ova, and thus may be maintained in large numbers.

The C. elegans haploid genome contains approximately 8 X $1Ø^7$ base pairs of DNA (Sulston and Brenner 1974). A major focus of a number of researchers is the generation of a restriction map of the entire C. elegans genome. This restriction map is being integrated with the genetic map by researchers concentrating on specific intervals. Usually, the number of genes and the gene products of the genes, within a given interval are unknown. Therefore, it is important to know the number of genes in the area being studied and in the genome as a whole.

Gene saturation experiments allow the determination of the number of genes in a given region. In gene saturation experiments, the researcher attempts to recover the total number of genes in a defined region (Rose and Baillie 198Ø; Rogalski et al. 1982; Meneely and Herman 1979; Rogalski 1983). It is useful to know the approximate number of lethals in a region prior to beginning such an experiment. If one knows the total number of essential genes in the genome and if one assumes the gene density to be similar

throughout the genome, a rough estimate of the number of genes in an area may be made. Since lethal genes are thought to comprise the largest class of genes, the number of essential genes should be close to the total number of genes in the genome. Knowing the approximate number of lethals in the region being studied will allow the researcher to estimate the sample size needed to saturate the region of interest.

Another eukaryotic organism in which saturation mapping has played a large role is Drosophila (Chovnick et al. 1977; Judd et al. 1972;Mohler and Pardue 1984; Perrimon et al. 1984) . Realizing the importance of knowing the number of genes in the genome, Drosophila geneticists devised a myriad of schemes for assessing essential gene number, early in the study of this organism (Bishop 1974).

Gowen and Gay (1933), using X-ray mutagenesis, attempted to measure gene number by dividing the mutation rate per locus into the mutation rate per chromosome. Demerec (1934) also used this method. Based on Demerec's data, Bishop (1974) calculated the rate of mutation of the average X chromosome locus to be 7.5×10^{-5} after 2500r X-ray-irradiation. He then arrived at a figure of 920 loci for the X chromosome.

Herskowitz (1959) analyzed X-ray-induced mutations of

the white locus. He argued that changes to the w allele were a result of chromosome breakage. By dividing the observed rate of X chromosome breakage by the number of mutagenic events at the white locus, he arrived at an estimate of 726 genes on the X chromosome. There are faults in this reasoning; not all X chromosome breakage results in a mutagenic event and not all mutations in the white locus can be resolved as chromosome breakage.

An alternative method to determining the number of genes in Drosophila relies on the polytene chromosomes. The work of Judd et al. (1972) and Hochman (1978), where regions of the genome were dissected genetically and compared to the polytene map, suggest that there is a one to one correspondence between the gene and the polytene band. Both the light microscope and the electron microscope yield the same polytene banding pattern (Sorsa and Sorsa, 1973). The X chromosome contains approximately 1000 polytene bands; note that this is close to the estimates presented above. If this is extended to all the chromosomes, the entire Drosophila genome must contain 5000 to 6000 genes (Bishop 1974).

In C. elegans the number of essential genes has been estimated by Brenner (1974) as 2000. He used a method analogous to that of Gowen and Gay (1933), except that he

used ethylmethane sulfonate as a mutagen and he based his mutation rate per locus on the average forward induction rate of visible genes. Unfortunately, his visible gene data were biased towards large mutagenic targets. For instance, Brenner obtained 21 isolates of unc-22(IV) and 23 isolates of dpy-1 , but he recovered only 1 representative of dpy-5 and no alleles of dpy-4 .

Rogalski has estimated the number of genes in C. elegans to be in the order of 5700 (Rogalski 1983). Her calculation is based on saturation mapping of the unc-22(IV) region. She calculated that the gene density in her region is 22 genes per map unit. This region is defined by the deficiency sDf2 , which is approximately 1.5 map units in size.

The primary objective of this study was to estimate the number of essential genes in C. elegans . This was accomplished by isolating a random set of ethylmethane sulfonate induced lethals in the region recombinationally suppressed by the translocation nT1(IV;V) . The translocation nT1(IV;V) has been characterized by Ferguson and Horvitz (1985). As a heterozygote it causes pseudolinkage between unc-5(IV) and dpy-11(V) and suppresses crossing-over on the right-half of linkage group IV between the markers unc-17 and dpy-4 , and on the left-half of

linkage group IV . The fraction of lethals isolated within the deficiency sDf2(IV) was determined. The deficiency sDf2 lies within the nTl balanced region. If we assume that the fraction of lethal genes within the region defined by sDf2 that were 'hit' is equivalent to the fraction of genes within nTl that were 'hit', the total number of essential genes in nTl and subsequently in the genome can be calculated.

MATERIALS AND METHODS

Nematode strains and culture conditions

All strains of Caenorhabditis elegans employed in this study were maintained on petri plates containing nematode growth medium (NGM) streaked with Eschericia coli strain OP5Ø as described by Brenner (1974). Unless stated otherwise, all mutants were derived from the wildtype Bristol strain, N2. Mutations designated with an "s" number were isolated at Simon Fraser University. The wildtype N2 strain and most of the other mutations were originally obtained from either the MRC stock collection in Cambridge, England or the Caenorhabditis Genetics Center at the University of Missouri, Columbia, MO (table 1). BC121Ø (unc-31dpy-4/nT1(VI) ; +/nT1(V))was constructed by R. Rosenbluth using the strain MT1ØØØ that had been received from R.H. Horvitz at MIT. BC1261 was derived from the wildtype Bergerac strain, BO. Stocks were maintained at 15 degrees celsius. The nicotine selection technique of Moerman and Baillie (1981) was used to distinguish individuals heterozygous for unc-22 . Individuals homozygous or heterozygous for unc-22 twitch in a 1%

nicotine solution while wild type individuals contract and lie rigid. The genetic nomenclature follows the recommendations of Horvitz et al. (1979).

Table 1

List of existing strains used.

genotype	Strain Name
dpy-4(e1166)	CB1166
dpy-20(e1282)dpy-26(n199)/nT1(IV);+/nT1(V)	CB3608
unc-22(s7)	BC0023
unc-22(s727)	BC1261
unc-31(e169)	CB0169
unc-22(s7)unc-31(e169)	BC0171
unc-26(e345)dpy-4(e1166)	BC1235
unc-31(e169)dpy-4(e1166)	BC0020
unc-31(e169)dpy-4(e1166)/nT1(IV) ; +/nT1(V)	BC1210
sDf2(IV)/+	BC0390
sDf7(IV)/+	BC0439
sDf8(IV)/+	BC0440
let-56(s168)unc-22(s7)/+	BC0906
let-59(s49)unc-22(s7)/+	BC0961
let-60(s59)unc-22(s7)/+	BC0916
let-61(s65)unc-22(s7)/+	BC0917

let-62(s175)unc-22(s7)/+	BCØ934
let-63(s17Ø)unc-22(s7)/+	BCØ918
let-64(s216)unc-22(s7)/+	BCØ954
let-65(s694)unc-22(s7)/+	BC1121
let-69(s684)unc-22(s7)/+	BC11Ø9
let-7Ø(s689)unc-22(s7)/+	BC1115
let-71(s692)unc-22(s7)/+	BC1119
let-72(s695)unc-22(s7)/+	BC1122
let-73(s685)unc-22(s7)/+	BC1111
let-74(s697)unc-22(s7)/+	BC1124
let-91(s678)unc-22(s7)/+	BC11Ø3
let-92(s677)unc-22(s7)/+	BC11Ø2

Construction of new strains

A hermaphrodite strain employed throughout most of this study has the genotype unc-22(s7)unc-31(e169)(IV)/nT1(IV); + (V)/nT1(V) . This strain was constructed from the strains dpy-20(e1282)dpy-26(n199)(IV)/nT1(IV);+(V)/nT1(V) and the homozygous strain unc-22(s7)unc-31(e169) . Wild type males were mated to unc-22unc-31 homozygotes. Heterozygous males from the cross were picked and mated to dpy-20dpy-26/nT1 hermaphrodites. Hermaphrodites of the genotype unc-22unc-31/dpy-20dpy-26 and unc-22unc-31/nT1 were picked in a 1% nicotine solution. Individuals heterozygous for unc-22 twitch in a 1% nicotine solution. Those stocks which segregate wildtype, unc-22unc-31 , and nT1 individuals were maintained. The phenotype of nT1 homozygotes can be described as scrawny, uncoordinated, and vulvaless. From one of these stocks a single wild type hermaphrodite was picked as the progenitor of the strain.

A male strain, unc-22(s7)unc-31(e169)/nT1 , was constructed by Raja Rosenbluth through a different protocol (personal communication). Homozygous hermaphrodites of the genotype unc-22(s7)unc-31(e169) were mated with unc-31(e169)dpy-4(e1166)(IV)/nT1(IV);+(V)/nT1(V) males.

Wildtype males were selected and mated to sibling wild type hermaphrodites. These individuals all being of the genotype unc-22unc-31/nT1 .

The balanced strain sDf8(IV)/nT1(IV);+(V)/nT1(V) was constructed by mating sDf8/+ hermaphrodites with wildtype males. Since sDf8 deletes unc-22 (figure 1) it was possible to pick unc-22 heterozygote males in a 1% nicotine solution and mate them to the unc-22unc-31/nT1 hermaphrodite strain. Wildtype hermaphrodites which had twitched in nicotine but produced no Unc-22 progeny were selected. The stock was derived from a single hermaphrodite.

The sDf2(IV)/nT1(IV);+(V)/nT1(V) was contructed by Raja Rosenbluth. All other double mutant strains used for this analysis were constructed by Don Moerman or Teresa Rogalski.

Isolation of EMS induced unc-22-linked lethal mutations

All lethals generated by EMS were isolated by a modification of the procedure described by Rosenbluth et al. (1983); the translocation nT1(IV;V) was employed as a balancer in the place of the translocation eT1(III;V) . Young egg laying hermaphrodites heterozygous for nT1 and carrying a normal chromosome IV marked with unc-22(s7)unc-31(e169) , were treated with Ø.Ø12M EMS for

four hours and placed individually on separate petri plates. These individuals were transferred to new plates at 24 hour intervals until they ceased egg production. When mature, the wildtype F1 progeny of these individuals were placed on separate plates and allowed to lay eggs until exhausted. No more than 15 progeny were used from any one treated worm. After three to four days these plates were examined for the presence of individuals homozygous for the marked chromosome IV . If egg laying individuals of this type were seen, the plate was discarded since no lethal was present in the crossover suppressed region. If either no mature individuals homozygous for the marked chromosome were seen or if these individuals were present but failed to lay eggs, these plates were retained. Several wild type hermaphrodites were set from each of these putative lethal bearing strains and the progeny of these individuals once again inspected for the presence of egg laying homozygotes. Only lines which continued to give no egg laying homozygous hermaphrodites were specified as lethals for the purpose of this study.

Isolation of gamma-induced unc-22-linked lethal mutations

The procedure is the same as that outlined for the

isolation of EMS-induced unc-22 -linked lethal mutations. 1500R gamma-irradiation was used in the place of 0.012M EMS. A small sample of unc-22 unc-31 homozygous F2's were also tested to see if any maternal lethals had been induced (see screening for gamma-induced maternal lethals, this section).

Positioning mutations by recombination mapping

Since the balancer employed in this study suppresses recombination on both the right hand end of chromosome IV and also the left portion of chromosome V and causes pseudolinkage between these regions (Ferguson and Horvitz 1985), putative lethal bearing strains were first mapped crudely to one of the two chromosomes in the following manner. Hermaphrodites from each lethal bearing strain were mated to several N2 (wild type) males on separate petri plates and individual outcross hermaphrodites were selected from amongst their progeny. These individuals twitched in a 1% nicotine solution (an indication of heterozygosity for unc-22) and they failed to segregate nT1 homozygotes in their progeny. Three of these individuals from each lethal bearing strain were set on separate plates and transferred at 24 hour intervals over a 48 hour period. The progeny of these worms were scored for the expected phenotypes.

Clearly, individuals bearing a lethal only on the left hand end of chromosome V will give a normal percentage of egg laying individuals homozygous for the chromosome IV markers employed. Individuals of this type were not analyzed further in this study. The remaining individuals, those segregating the marked chromosome in reduced numbers, were analyzed (using three-factor mapping) as to their map position relative to the markers used. For example, lethals located recombinationally to the the left of unc-22 on the standard map (see figure 1), will give both double mutant progeny (Unc-22 Unc-31) and Unc-31 individuals, but no Unc-22 individuals. Similarly, lethals which lay to the right of unc-22 will segregate the double mutant and Unc-22 recombinants. Those lethals which lie between the markers will segregate both Unc-22 and Unc-31 individuals, but no Unc-22 Unc-31 individuals. The mapping function used throughout this study is a modified version of that used by Brenner (1974; Rogalski et al. 1982; Rogalski 1983). A derivation of this formula is presented in appendix 2. Lethals which mapped within 5 map units to the left of unc-22 or anywhere to the right of this marker were used in this study.

Positioning mutations by deficiency mapping

Lethal bearing strains were complementation tested by mating males heterozygous for a lethal mutation, let-x unc-22 unc-31/+ ,to hermaphrodites carrying an unc-22 deficiency, sDf-2/nT1 or sDf7/+ . Three to four hermaphrodites were mated to 1Ø to 12 males. Whenever possible, these hermaphrodites and males were mass transferred twice at four hour intervals. Finally, hermaphrodites were placed on individual plates with three males each. They were allowed to lay eggs for 24 to 48 hours, depending on the fecunditity of the stock.

The plates were screened for the presence of adult male and hermaphroditic "twitcher" (Unc-22) progeny. If present, the twitcher hermaphrodites were placed on a plate and allowed to develop. If these hermaphrodites developed into fertile adults, then the lethal is outside the bounds of the deficiency. When the lethal failed to complement the deficiency, an attempt was made to record the effective lethal phase of the let-x unc-22 unc-31/sDf-y heterozygote.

Identification of lethal complementation groups

The procedure followed that described by Rogalski (1983). Males having the genotype let-a(sx) unc-22(s7)/++

(with or without unc-31 present) were generated from one strain and crossed with heterozygous hermaphrodites of another lethal strain, let-b(sy) unc-22(s7) unc-31(e169)/nT1(IV);+/nT1(V) . If outcrossing was confirmed by the presence of wild type males, then the plates were screened for the presence of twitcher males and females. When present, twitcher hermaphrodites were placed on plates and tested for fertility. The presence of fertile egg laying twitcher hermaphrodites denotes complementation.

Determining the effective lethal phases of the mutations

All of the EMS-induced lethals where characterized with respect to the stage at which development was blocked. For each strain, five to ten homozygous twitcher larvae of the genotype let-a(sx) unc-22(s7) unc-31(e169) were picked from stock plates and placed onto a petri plate. These larvae were left at 20 degrees celsius for two to four days. They were then heat killed by placing the tip of a hot soldering iron near them in the agar. The larvae were measured using an ocular micrometer. The lengths of the blocked larvae were compared with the unc-22 growth curve of Rogalski et al. (1982). The effective lethal phases of some

gamma-induced lethals as well as a number of N2/BO hybrid lethals was also determined.

Isolation of N2/BO hybrid lethal mutations

The lethals in this screen were generated on a Bergerac chromosome and balanced over the Bristol translocation nT1 . The strain unc-22(s727) was generated by Teresa Rogalski (personal communication) by .Ø25M EMS mutagenesis of the Bergerac strain. Homozygous unc-22(s727) Bergerac hermaphrodites were mated to unc-22(s7) unc-31(e169)/nT1(IV);+/nT1(V) Bristol males. Wild type hermaphrodites from this cross were set one per plate and screened for the absence of fertile Unc-22 progeny. Those lines which did not segregate any twitcher hermaphrodites were retested by placing one to three phenotypically wild type hermaphrodites on a plate and ascertaining whether or not they segregated any twitcher progeny. Only strains which failed to segregate twitcher hermaphrodites after several generations of testing were maintained.

This protocol was also used to ascertain if heat shock resulted in a higher rate of spontaneous lethal mutations in the unc-22(s727) strain. In these experiments, the parental

unc-22(s727) hermaphrodites were heat shocked for one hour at 33 to 35 degrees celsius prior to mating.

Screening for gamma-induced maternal lethals

This screen was derived from the screen employed to isolate gamma-induced unc-22 -linked lethals. From plates which segregated fertile unc-22unc-31 F2 homozygotes, 3 unc-22unc-31 homozygotes were picked per strain and placed on a plate. A total of 45 lines were tested. If these homozygotes gave rise to progeny, the progeny were again tested in the same manner. Lines which did not give rise to progeny in the first generation were deemed adult steriles. Those which gave rise to blocked progeny were F2 lethals and those which gave blocked progeny in the next generation were called F3 lethals (this terminology has been proposed by Heschl, Kaan, and Baillie, personal communication).

Isolation and characterization of deficiencies isolated by precomplementation

Two strains were used, unc-31(e169) dpy-4(e1166) and unc-26(e345) dpy-4(e1166) . Gillian Wild isolated the set of unc-31 and dpy-4 deficiencies by using the unc-31 dpy-4

strain. Wild type males were irradiated with 1500R gamma-radiation and mated to an appropriately marked homozygous strain. The hermaphrodites were mass mated overnight and then placed individually on plates with a number of males. Their progeny were screened for the presence of the appropriate mutant phenotype. Only individuals showing the appropriate mutant phenotype and subsequently indicating the presence of a tightly linked lethal were analyzed for their extent as deficiencies. The deficiencies were subsequently balanced over nT1 . The deficiency bearing strains, for instance sDf21/unc-31 dpy-4 were mated to unc-31 dpy-4/nT1 males. Wildtype hermaphrodites were picked from the progeny of this cross. A stock was established from a wildtype hermaphrodite which segregated Wildtype and homozygous nT1 individuals, but no Unc-31 Dpy-4's. The putative deficiencies from this screen were then tested by outcrossing hermaphrodites from each deficiency strain against a battery of males which were heterozygous for different chromosome IV visible mutations. The outcross progeny from each of these matings were scored in order to determine whether the particular deficiency in question showed pseudoallelism with any markers other than the one employed in its selection.

Screening the unc-22(s727) Bergerac strain for visible mutations

In order to determine if the unc-22(s727) Bergerac strain gave rise to an unusually high rate of spontaneous visible mutations, 100 L4 to early adult hermaphrodites were placed on separate petri plates and allowed to lay eggs until exhausted, at 20 degrees celsius. Five days later, the plates were screened for visible mutations. The worms were allowed to grow for one more generation. The plates were slightly crowded and therefore a detailed analysis of this generation was not possible.

RESULTS

Isolation of EMS-induced lethal mutations

By screening the progeny of 943 F1 individuals arising from EMS treated PØ's for lethals induced in the region which is recombinationally suppressed by the translocation nT1 , 68 lethals were identified initially. Fifty-six of these lethals were chosen at random for analysis. Two were lost due to poor fecundity. The frequency of induction of Ø.Ø12M EMS-induced mutations in the nT1 suppressed region in my sample is $7.5 \times 1Ø^{-2}$ (table 2).

Table 2

Results of screening 943 F1 individuals arising from EMS treated PØ's for lethal mutations

ose of EMS used	Number of F1's screened	Number of lethals inside sDf2	Number of lethals outside sDf2	Number of lethals synthetic	Mutation rate in nT1 region
Ø.Ø12M	943	3	21	2	7.5×10^{-2}

Positioning EMS-induced lethals by recombination mapping

Of 68 lethals identified originally, 56 were chosen at random for analysis. Of these, 26 mapped to linkage group IV . The remaining 30 are putative linkage group V lethals. All of the mutations on linkage group IV mapped to the left of unc-22 . While mapping, two strains segregated uncoordinated non-Unc-22 individuals; these strains carry putative synthetic lethals. Table 3 summarizes the three-factor mapping results of the linkage group IV EMS-induced lethals. The two synthetic lethals have been ommitted. The entire set of EMS lethals with the exception of s886 , s887 , s888 , and s744 ,are included in figure 1. Four EMS-induced lethals (let-303(s761), let-304(s747), let-305(s762) , and let-306(s759))were assigned lethal names based on the mapping data.

Table 3

Summary of EMS induced lethal mutations

Mutation	left-right position *	phenotypes Wild	U22U31	U22	U31	map units from unc-22 **
s730	left	1003	41	0	2	6.06(3.93-8.74)
let-93(s734)	left	1827	4	0	3	.33(.09-.84)
s735	left	1338	16	0	3	1.78(.83-3.11)
s736	left	140	11	0	1	11.52(5.57-21.80)
s737	left	322	4	0	3	1.84(.50-4.78)
s744	left	399	2	0	4	.74(.09-2.71)
s745	left	640	5	0	4	1.16(.68-1.79)
s746	left	504	13	0	2	3.83(2.02-6.64)
let-304(s747)	left	399	15	0	3	5.55(2.12-9.11)
s748	left	352	8	0	19	3.21(1.37-6.45) ***
s749	left	217	4	0	1	2.74(.74-7.18)
s750	left	887	9	0	4	1.51(.69-2.89)
let-94(s751)	left	1263	6	0	1	.71(.26-1.55)
let-95(s753)	left	490	4	0	1	1.22(.33-3.15)
s754	left	500	15	0	1	4.46(2.00-7.98)

Table 3 continued

Mutation	left-right position *	phenotypes Wild	U22U31	U22	U31	map units from unc-22 **
s755	left	715	11	Ø	1	2.3Ø(1.13-4.15)
s758	left	1253	43	Ø	3	5.1Ø(3.34-7.27)
let-3Ø6(s759)	left	19Ø	17	Ø	3	12.99(6.Ø7-22.8Ø)
s76Ø	left	155Ø	8	Ø	3	.77(.33-1.53)
let-3Ø3(s761)	left	7Ø7	16	Ø	1	3.37(1.56-5.91)
let-3Ø5(s762)	left	115	8	Ø	2	1Ø.11(4.23-21.15)
s886	left	155	16	Ø	12	14.11(7.57-23.34) ***
s887	left	271	29	Ø	7	15.35(8.91-23.93)
s888	left	277	3Ø	Ø	1	15.87(9.31-24.6Ø)

* position relative to unc-22

** 95% confidence intervals are presented in brackets. The confidence limits were calculated either by using the formula $1.96 \cdot \sqrt{Npq}$ or with Steven's table (Stevens 1942).

*** these strains showed unusually high numbers of unc-31 recombinants.

Figure 1. Map of lethals and deficiencies on linkage group IV. EMS-, Gamma, and N2/BO hybrid lethals on the right arm of LG IV are depicted.

figure 1

EMS, GAMMA, AND N2/BO HYBRID LETHALS ON THE RIGHT ARM OF LG IV

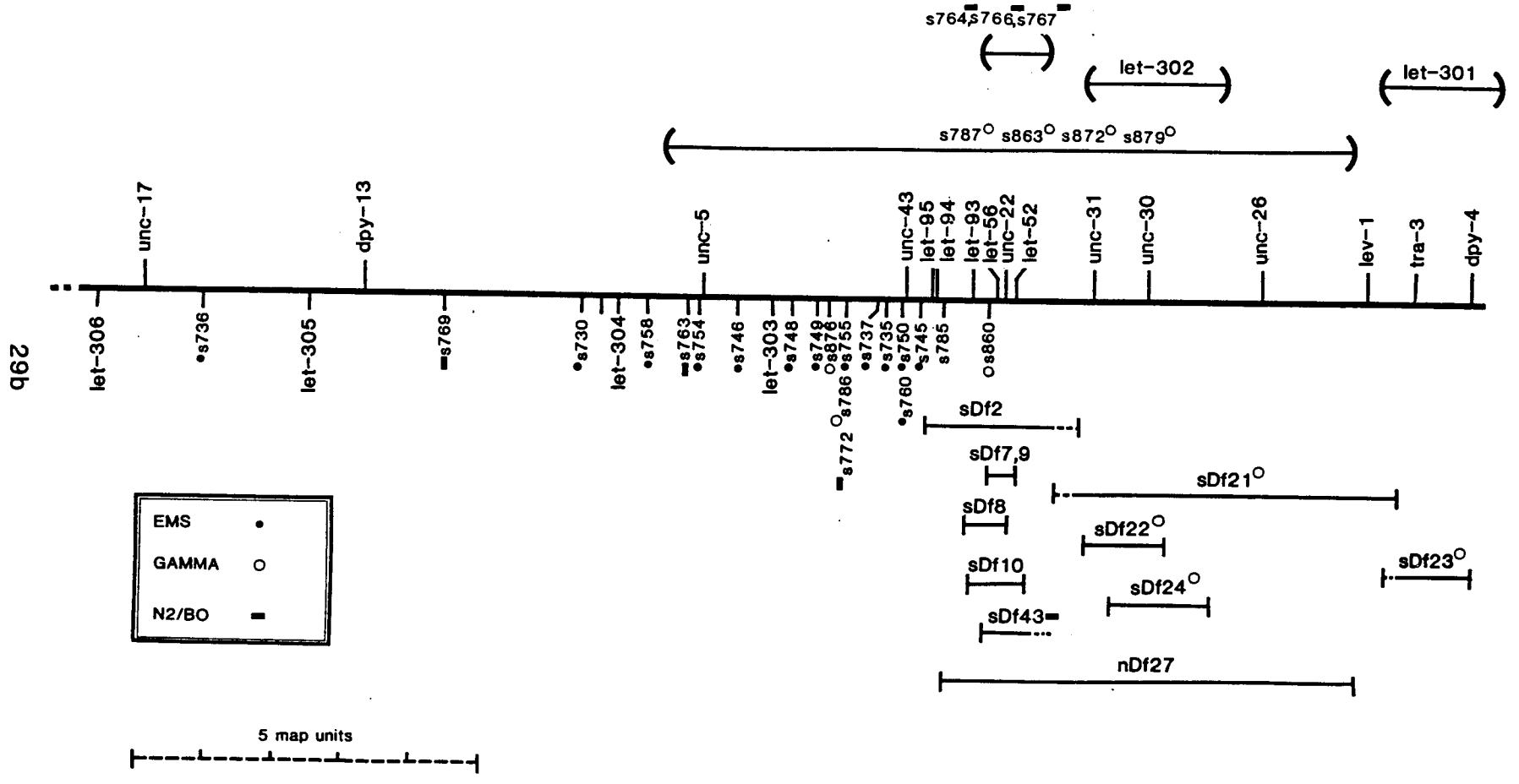
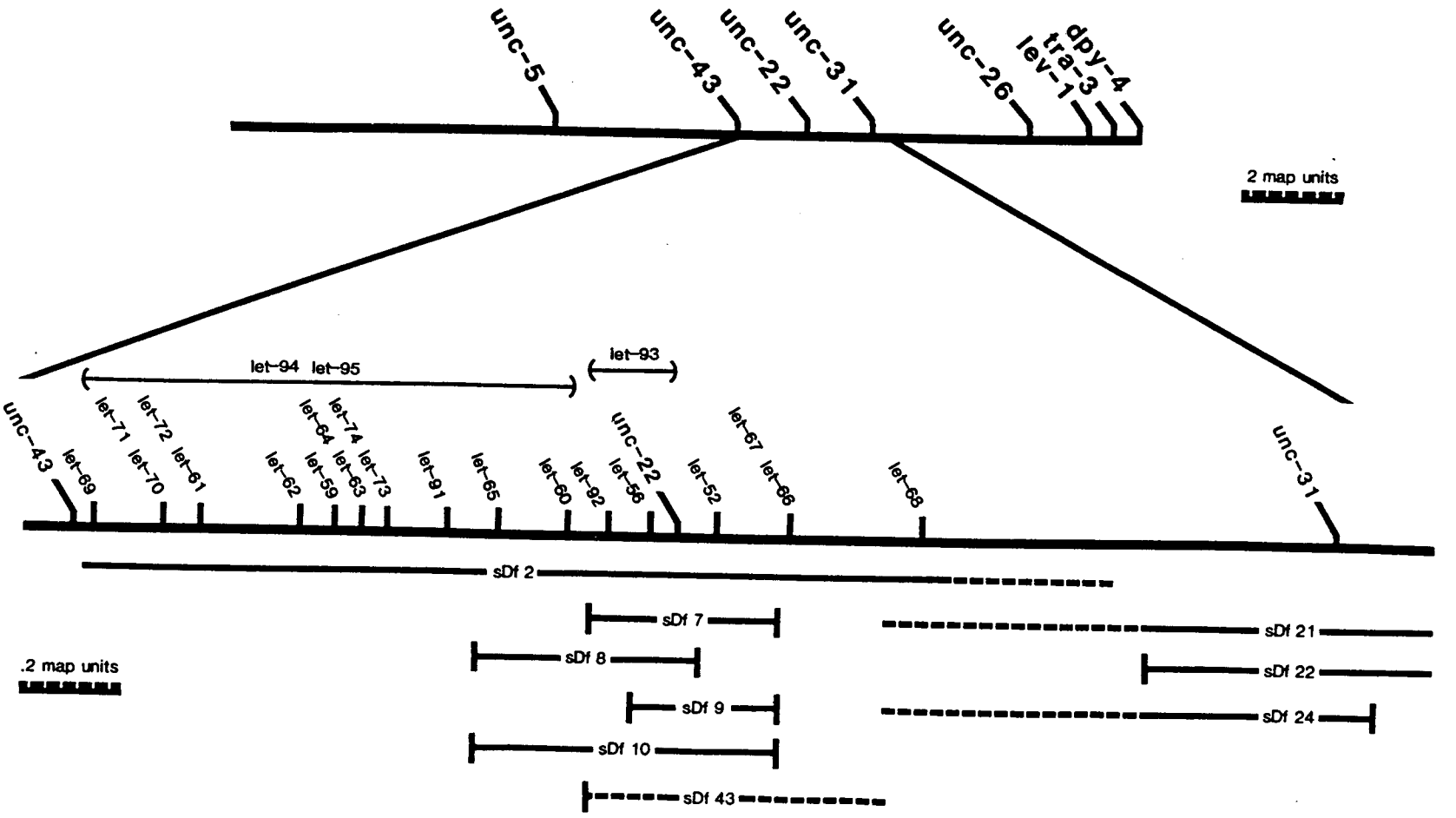


Figure 2. Essential genes and deficiencies in the sDf2 region. The breakpoints of the deficiencies sDf21, sDf22, and sDf24 are also shown.

figure 2

Essential genes and deficiencies in the sDf2 region



Positioning EMS-induced mutations by deficiency mapping and complementation

The EMS-induced lethals which mapped within five map units of *unc-22* were complementation tested against *sDf2* . Three mutations, *s734* , *s751* ,and *s753* failed to complement *sDf2* . These three mutations were complementation tested against known lethals in the *sDf2* region and against each other (table 4). They define three new genes, *let-93* , *let-94* ,and *let-95* , respectively. The essential genes and deficiencies in *sDf2* are displayed in figure 2.

Table 4

Complementation of EMS-induced lethals with known lethals in sDf2

	let-93	let-94	let-95
sDf2	-	-	-
sDf7	-	+	+
let-61	ND	+	+
let-62	ND	+	+
let-59	ND	+	+
let-63	ND	+	+
let-64	ND	+	+
let-65	ND	+	+
let-60	ND	+	+
let-56	+	ND	ND
let-92	+	ND	ND
let-69	ND	+	+
let-71	ND	+	+
let-70	ND	+	+
let-72	ND	+	+
let-73	ND	+	+
let-74	ND	+	+
let-91	ND	+	+

Isolation of gamma-induced lethal mutations

Of 906 F1's screened, 45 gamma-induced lethal mutations were recovered. The 25 most fertile strains were chosen for analysis. Of these, 14 were on linkage group IV and 11 were assumed to be on linkage group V . Two of the gamma induced lethals mapped within the deficiency sDf2 . The frequency of induction of 1500R gamma-induced lethal mutations in the nT1 suppressed region is 5.0×10^{-2} in this study. Table 5 summarizes this data.

Table 5

Results of screening 906 F1 individuals arising from 1500R gamma-irradiation treated P0's for lethal mutations

Dose of irradiation	Number of F1's screened	Number of lethals on LG IV inside sDf2	outside sDf2	Mutation rate in nT1 region
1500R	906	2	12	5×10^{-2}

Positioning gamma-induced lethal mutations by recombination mapping and complementation testing

The 14 gamma-induced lethal mutations which mapped to linkage group IV were 3 factor mapped relative to unc-22 (table 6). There are eight gamma-induced lethals within five fic ve map units of unc-22. Of these, 3 failed to segregate Unc-22 or Unc-22 Unc-31 recombinants. One of these, s881, no longer appears to carry the unc-22(s7) allele. The mutation s860 fails to complement sDf2, while the mutation s879 complements this deficiency. The lethal s785 which has been positioned 1.16 map units to the left of unc-22 also fails to complement s751. These results are summarized in table 6.

Table 6

Summary of gamma-induced mutations mapping within seven map units of unc-22

Mutation	left-right position	Phenotypes WT	U22U31	U22	U31	map units from unc-22
s785	left	637	5	Ø	4	1.16(.82-4.Ø5)
s786	left	796	13	Ø	1	2.25(1.29-4.21)
s787		1133	38	Ø	Ø	4.99(3.17-7.28)
s86Ø		2ØØ	Ø	Ø	Ø	Ø.ØØ(Ø.ØØ-2.81)
s863		832	18	Ø	Ø	3.3Ø(1.62-5.61)
s864		315	13	Ø	Ø	6.13(3.22-1Ø.74)*
s872		195	2	Ø	Ø	1.53(.18-5.66)
s876		397	7	Ø	Ø	2.63(1.Ø5-5.39)
s879		2ØØ	Ø	Ø	Ø	Ø.ØØ(Ø.ØØ-2.81)
s881		111	Ø	Ø	Ø	Ø.ØØ(Ø.ØØ-5.12)**

* complementation results are not straightforward

** does not carry unc-22(s7)

Isolation and positioning of deficiencies isolated by precomplementation

Four deficiencies, sDf21 , sDf22 , sDf23 ,and sDf24 were recovered from the unc-31 dpy-4 precomplementation experiment. Three of these deficiencies were isolated on the basis of their failure to complement unc-31 ,(sDf21 , sDf22 , and sDf24) and one was recovered due to its pseudoallelism with dpy-4 (sDf23). The extent of these deficiencies is presented in figure 1.

One deficiency, sDf21 , originally behaved as though it extended from unc-31 to lev-1 . The strain was then balanced over nT1 as described in Materials and Methods. Subsequently, it came to my attention that dpy-4(e1166) failed to complement this strain. Jonathan Hodgkin (personal communication) has shown that sDf21 complements tra-3 ,located between lev-1 and dpy-4 . The simplest explanation is that dpy-4(e1166) crossed onto the deficiency bearing chromosome, while the stock was being balanced. The deficiency has been outcrossed to wild type males in an attempt to recover dpy-4 recombinants. I failed to observe any recombinants in a sample of 900 F2's.

Determining the effective lethal phases of the mutations

The EMS-induced lethal homozygotes mapping to linkage group IV were characterized with respect to their effective lethal phase. All terminal phenotypes were exhibited from egg lethal to adult sterile (table 7 and figure 3). In the cases where the lethal was within sDf2 , the effective lethal phase of the let-x/sDf2 is also reported.

Eight percent of the lethals are egg lethals, 25 percent are early larval blockers, 21 percent arrest at the mid larval stage, and 29 and 17 percent block at the late larval and adult sterile stages respectively.

Table 7

Effective lethal phases of the EMS-induced lethal mutations

Mutation	length at arrest	terminal phenotype
s729	na	synthetic
s730	.460mm	mid larval
s731	.780mm	late larval
s732	.657mm	late larval(leaky)
s733	.270mm	early larval
s734	.464mm	mid larval
s734/sDf2	.500mm	mid larval
s735	.688mm	late larval
s736	>1.0mm	adult sterile
s737	>1.0mm	adult sterile
s744	lost	egg lethal
s745	.428mm	mid larval
s746	.950mm	adult sterile
s747	.614mm	mid larval
s748	.417mm	mid larval
s749	.366mm	early larval
s750	na	egg

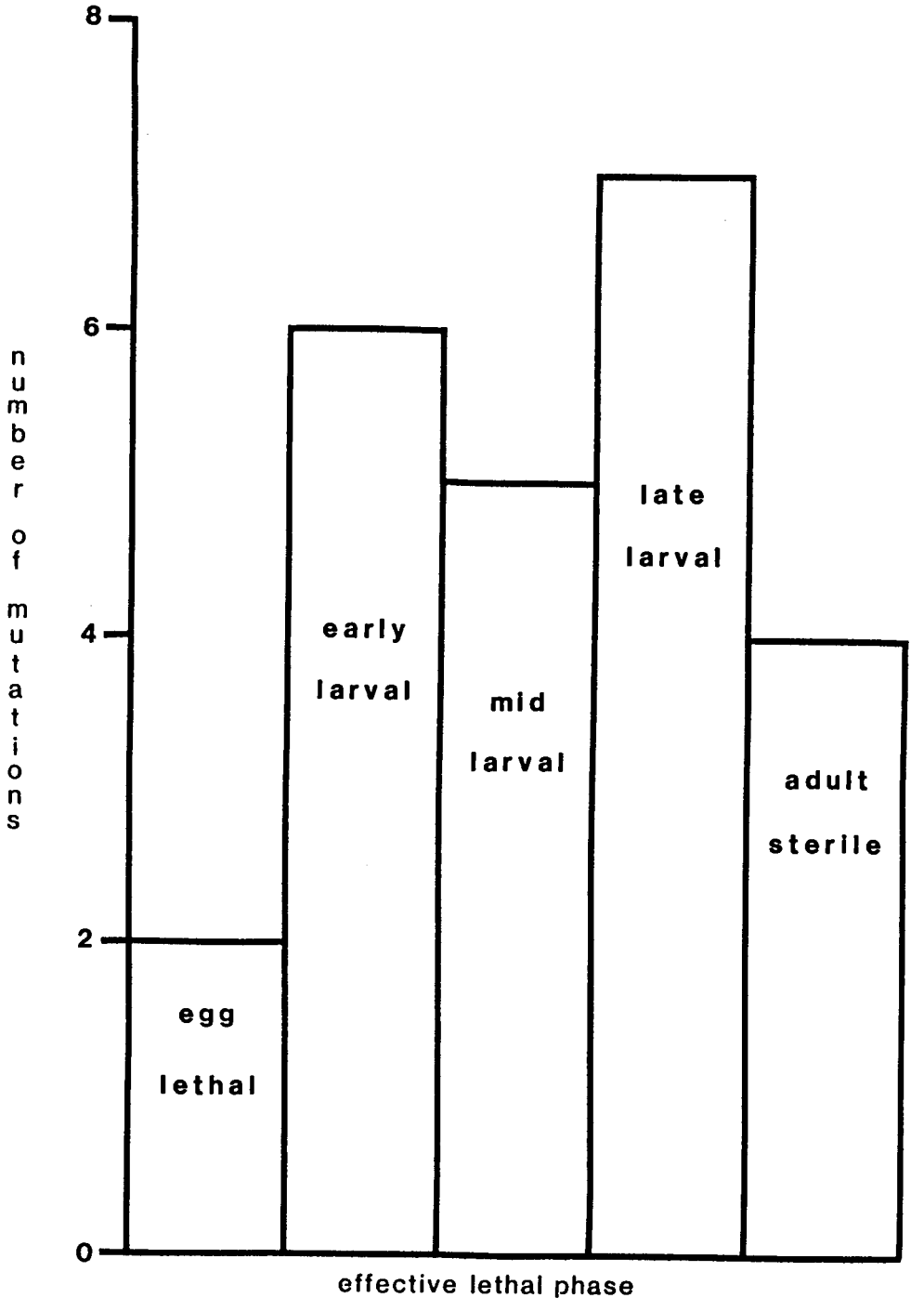
Table 7 continued

mutation	length at arrest	terminal phenotype
s751	.640mm	late larval
s751/sDf2	na	early larval
s752		synthetic
s753	.927mm	late larval to sterile
s753/sDf2	.900mm	late larval to sterile
s754	.350mm	early larval
s755	.740mm	late larval
s758	lost	early larval
s759	.640mm	late larval
s760	.300mm	early larval
s761	.427mm	early larval
s762	>1.0mm	adult sterile

Figure 3. Distribution of the effective lethal phases of the EMS-induced lethal mutations.

figure 3

Distribution of the effective lethal phases of the EMS-induced lethal mutations



Screening for gamma-induced maternal effect lethal mutations

This screen was a continuation of the gamma-induced lethal screen. From plates which gave adult Unc-22 Unc-31 individuals, unc-22 unc-31 homozygotes were set and examined over two generations for the presence of maternal effect lethals. One adult sterile was recovered. 3 slow developing lines were recovered, but later discarded as they did not carry a lethal.

Isolation of N2/BO hybrid lethals

A total of 25 unc-22 -linked lethal mutations were generated by the two protocols described in Materials and Methods. The heat shock protocol yielded 18 lethal mutations out of 272 individuals screened. The non heat shock protocol yielded 6 lethals from a total of 265 F1's screened. The frequency of induction of lethals is 6.6X 10^{-2} and 2.2 X 10^{-2} respectively. Table 8 summarizes these results.

Table 8

Results of screening for interstrain-crossing-induced lethal mutations

Protocol	Number of F1's screened	Number of lethals	Mutation Rate in nT1 region
control	265	6	2.2×10^{-2}
heat shock	272	18	6.6×10^{-2}

Positioning the N2/BO hybrid lethals by recombination and deficiency mapping

Eight of the 2Ø N2/BO hybrid lethals were recombinationally mapped (table 9). Six mapped to linkage group IV and two mapped to linkage group V . One of the linkage group V lethals (s8ØØ) mapped outside the region recombinationally suppressed by eT1 and the other, s743 is a deficiency, sDf31 , of unc-6Ø . Of the 6 linkage group IV lethals one, s765 failed to complement let-52 and sDf8 and therefore, is a deficiency, sDf43 . Two additional lethals, s885 and s766 were found to be on linkage group IV since they failed to complement sDf43 .

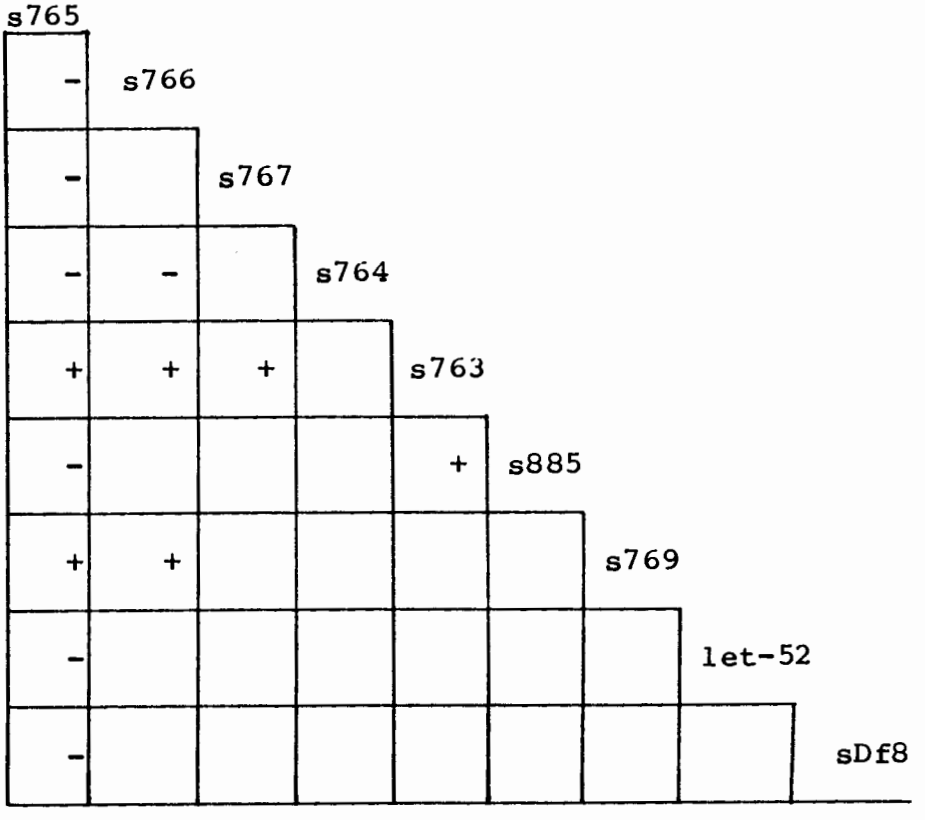
Table 1Ø summarizes complementation data obtained for 7 of the 8 linkage group IV lethals. It should be noted that s766 and s767 , both of which failed to complement sDf43 , were derived from the same PØ as sDf43 . Furthermore, s766 fails to complement s764 as does sDf43 . It is possible, therefore that sDf43, s766 , and s767 represent a single mutational event.

Table 9

Summary of interstrain-crossing lethals

Mutation	Phenotypes		map distance
	WT	U22	from unc-22
sDf31(V)		NA	
s800(V)		NA	
s763	845	26	4.58(2.68-7.19)
s764	382	0	0.00(0.00-1.46)
sDf43	762	0	0.00(0.00-0.73)
s767	1178	0	0.00(0.00-0.47)
s769	203	11	8.03(3.93-14.9)
s772	348	3	1.29(0.27-3.82)

Table 1Ø
Interse complementation of interstrain-crossing-induced lethal mutations



Screening the unc-22(s727) Bergerac strain for spontaneous visible mutations

One hundred individuals were screened to investigate the frequency of induction of recessive visible mutations generated in the previous generation. No visible mutations were observed, however it is of interest that the average brood size of these individuals was 10, compared with close to 300 for wildtype Bristol hermaphrodites. The next generation was scanned for visible mutations, but none were seen.

DISCUSSION

In this study, a system employing the translocation nT1 has been developed for the recovery and maintenance of lethals on three regions of linkage group IV which are currently being analyzed at the molecular and genetic level. It also allows the determination of the relative number of genes in two regions which are currently being analyzed molecularly. Further, this work presents the development and validation of a novel means for determining the number of essential genes in C. elegans .

The region of the C. elegans genome which was being analyzed in this study is defined by the translocation nT1(IV;V) . It is in the order of 40-45 map units, or approximately 1/7 of the genome. More specifically, my interest lies in the distribution of lethals on linkage group IV , primarily in the unc-43 to dpy-4 interval. Two deficiencies lie within this interval. The sDf2 region is approximatley 1.5 map units in size. It extends one map unit to the left of unc-22 , but does not include unc-43 , and at least 0.5 map units to the right of unc-22 and complements unc-30 and unc-31 . The sDf2 region is the most intensively analyzed region in C. elegans (Rogalski 1983). The sDf21 region extends at least from unc-31 to lev-1 .

This is an area of approximately five map units. The unc-43 to dpy-4 region is currently being cloned in this laboratory.

One goal of this study has been to develop and test a novel method for estimating the number of essential genes in C. elegans . The method employed is dependent on:

1) the extent of recombinational suppression of the translocation nT1(IV;V) ;

2) the total number of genes recovered over nT1(IV;V) and the number of genes falling within the deficiency sDf2 . Therefore, these factors must be addressed before an estimate of essential gene number can be made.

In addition, since the screening procedure allows the recovery of lethals in both the sDf2 and the sDf21 regions, it is possible to compare the distribution of lethals in these two regions. Don Moerman and Teresa Rogalski (Moerman 1981; Rogalski et al. 1982; Rogalski 1983) found that the number of genes within sDf2 and to the right of unc-22 were considerably less than the number of genes within the deficiency and to the left of unc-22 . Since the unc-43 to dpy-4 region is being cloned, it is of interest to know if the low gene density observed to the right of unc-22 is regional, or if it extends through the sDf21 region. The relative gene density of the sDf2 and the sDf21 regions is addressed in the discussion.

The nT1 screen was also used in order to make a preliminary assessment of the mutational spectra of EMS-, gamma-, and interstrain crossing-induced lethal mutations. It was hoped that these alternative mutagens might allow the recovery of lethal mutations to the right of unc-22 . A portion of the discussion is devoted to the interpretation of these experiments.

The region recombinationally suppressed by the translocation nT1(IV;V)

The distribution of the EMS lethals in linkage group IV recovered over nT1 ,is consistent with the results of Ferguson and Horvitz (1985). The two lethals furthest to the left of unc-22 map 15.8 and 15.3 map units away from unc-22 . This suggests that the suppression extends a few map units to the left of unc-17 and corresponds to a balanced region of about 21-22 map units on linkage group IV covered by nT1 (s733 to dpy-4). One lethal which mapped approximately 22 map units from unc-22 was eventually discarded as it gave rise to a fertile Unc-22 Unc31 individual on a stock plate.

No EMS lethals were recovered to the right of unc-22 . Evidence that nT1 does suppress recombination all the way to

the right-hand tip of the chromosome comes from: 1) the fact that Ferguson and Horvitz (1985) found that recombination is suppressed from unc-17 to dpy-4 ; 2) the fact that four deficiencies isolated by precomplementation (see results) map to the right of unc-22 . These are maintained over nT1 with no crossing over (Donati et al. 1984).

The nT1(IV;V) region may be slightly larger than the eT1(III;V) region described by Rosenbluth et al.(1983; Rosenbluth et al. 1985). The eT1 crossover suppressed region is approximately 40 map units in size.The lethal s800 was recovered by the nT1 screening protocol. It was located on linkage group V . Subsequent attempts to balance this strain over eT1(III;V) were unsuccessful, that is the lethal crossed off when placed over eT1 (Rosenbluth, personal communication). This suggests that s800 lies within the nT1 suppressed region but outside the eT1 suppressed region. Further, nT1 appears to suppresses recombination in the interval unc-42 to sma-1 while eT1 does not (Rosenbluth, personal communication). Another line of evidence that nT1 may be slightly larger than eT1 is that the induction rate for 0.012M EMS induced lethals with the nT1 screen is 7.2 X 10^{-2}. This is slightly higher than that obtained for eT1 (6.6 X 10^{-2}) by Rosenbluth et al. (1983). However, this line of reasoning assumes that both the nT1 and the eT1

recombinationally suppressed regions are equally mutable.

Essential genes in the nT1(IV;V) crossover suppressed region

It is important that each mutation induced for the purpose of determining the number of essential genes in *C. elegans* , affects only one gene. The alkylating agent ethylmethane sulfonate (EMS) was used in this study because it is thought to produce primarily point mutations. The main targets for alkylation in DNA are the nitrogen atoms of the bases (N-7 of guanine, N-1 and N-3 of adenine, and the N1 position of the pyrimidines). It is believed that the main cause of the mutagenic effect of EMS is caused however, by the alkylation of the O-6 of the guanine molecule. The O-6 alkykl derivatives of guanine have the double-bond distribution of the enol-form of the normal base, and hence have hydrogen-bonding properties similar to those of adenine, promoting G:C to A:T transitions. In *E. coli* there is evidence that EMS does produce primarily G:C to A:T transitions (Coulondre and Miller 1977).

The dosage of EMS is also important. On the recommendation of Rosenbluth et al. (1983), Ø.Ø12M EMS was used. This dosage is lower than those used by others and

therefore lowers the possibility of inducing multiple mutations in the nT1 region in any one strain.

Of the 56 EMS lethal strains analyzed, 26 mapped to likage group IV . Of these, two appeared to carry synthetic lethals. When these two strains were outcrossed, they gave rise to some Uncoordinated recombinants. The phenotype of these two Uncs might be described as lethargic and dumpy.

All of the remaining 24 unc-22 -linked lethal mutations mapped to the left of unc-22 (see table 3). It should be noted that in the case of the mutations s886 and s748 an unussually high numbeer of Unc-31 recombinants were seen. One possible explanation is that an additional uncoordinated mutation was induced in these strains. Since these two strains were amongst the first analyzed, it is also conceivable that the majority of "Unc-31's" were actually Wildtypes. Only four mutations were assigned lethal names on the basis of their recombinational separation. These are let-303(s761), let-304(s747), let-305(s762) and let-306(s759). It is interesting that no lethals were recovered to the right of unc-22 , a region comprising approximately 25 percent of the nT1 region on linkage group IV .

The linkage group IV lethals which mapped five map units or closer to unc-22 were deficiency mapped against sDf2 .

Three of the lethal mutations mapped within sDf2 , based on recombination and deficiency mapping. The complementation tests with the previously identified genes within sDf2 suggest that these mutations represent three new genes, designated let-93(s734) , let-94(s751) , and let-95(s753) . One lethal, s744 which was recombinationally positioned at Ø.74 map units from unc-22 was lost before it could be complementation tested against sDf2 . The induction rate of lethal mutations in the sDf2 region in this study is approximately 3.2 - 4.2 X $1Ø^{-3}$. This is approximatley one half the induction rate observed by Rogalski (1983), who used Ø.Ø25M EMS.

it is interesting that three new lethals were recovered in the sDf2 region. This region has been estimated to contain 32 essential genes (Rogalski, Moerman, and Baillie 1983; Rogalski 1983; Rogalski and Baillie 1985) 61% of which were thought to be recovered. The probability of isolating three new lethals in this interval is approximately six percent. It is most probable that the original estimate of the number of essential genes in the sDf2 region was an underestimate. An estimate based on a Poisson analysis of the data, incorporating the three new lethals, suggests that there are approximately 36 essential genes in the sDf2 region, 24 of which have been recovered.

The number of essential genes in C. elegans

To date, the main method for approximating the number of genes in C. elegans has involved extrapolation from gene saturation mapping. Such studies give approximate results. However, they are dependent on the gene density of the given region being studied. The unc-22(IV) region and the unc-15(I) region have been estimated to contain approximately 28 and 22 genes per map unit respectively (Rogalski 1983). The larger region of the X -chromosome studied by Meneely and Herman (1979) contains 7 genes per map unit. Estimates of essential gene number based on the gene density in these regions would be approximately 8000, 6000 and 2000 respectively.

Another method for estimating the number of genes in C. elegans involves Sulston and Brenner's (1974) data on the amount of DNA in the C.elegans haploid genome. Assuming we knew the size of the average gene in terms of map units, we could determine the maximum number of genes the DNA could code for. The data of Rogalski (1983) estimates the unc-22 gene to be in the order of 0.01 - 0.02 map units in size (Rogalski 1983). Assuming that unc-22 is an average size gene, although it probably is not as it is a large target

with respect to EMS, we could make a gene number calculation. This would yield 15,000 to 30,000 genes.

A third method which has been employed by Brenner involves the determination of the induction rate of lethals on the X -chromosome in conjunction with the mutation rate per locus derived from data on visible loci. To determine the mutation rate per locus, Brenner (1974) averaged his data for the induction of visible mutations. However, it should be noted that some loci mutate at a much higher rate than others. He arrived at a value of about 2,000 genes with indespensable functions. He comments that this appears to be a surprisingly small number (Brenner 1974). Brenner's estimate was revised by Baillie (unpublished results), whose measurement of X-linked lethals was higher than Brenner's and whose mutation rate per locus was lower than Brenner's. Baillie arrived at an estimate of 4000 essential genes in the genome (estimate taken from Moerman and Baillie 1979). The 1979 estimate is in close agreement with that of the present study.

A number of assumptions have been made in the preceding schemes. Not all the following assumptions relate to each scheme:

1) Gene density is similar in all regions of the genome.
2) All genes are of a similar size.

3) All genes mutate at a similar rate.

4) The mutation rate of lethals is similar to that of visible mutations.

In this study, a novel method of determining the number of essential genes in C. elegans is employed. EMS induced lethals (putative point mutations) are recovered over the region recombinationally suppressed by the translocation nT1(IV;V) . This region corresponds to an area approximately one seventh the size of the total genome. The deficiency sDf2 falls within this region. The number of genes in the sDf2 region has been estimated to be in the order of 36 genes. After isolating a large number of lethals in the nT1 region, the fraction of essential genes mutated within sDf2 is determined. Out of the sample of 54 lethal mutations analyzed in detail, three mapped within sDf2 . During this time, no recombinants were seen on the stock plates. Therefore, 3/36 of the genes in sDf2 were 'hit'. This also implies that 3/36 of the genes in the nT1 region were isolated. It should be noted here, that it is unlikely that any of the 54 mutations are outside the nT1 suppressed region. All 54 lethal strains were maintained on plates from six months to two years with no loss of the associated lethal due to crossing over. Therefore, there are approximately (36/3 X 54) 650 genes in the region

covered by nT1 . Since the approximate extent of nT1 is known, this analysis can be extended to the entire genome to yield (36/3 X 54 X 7) 4536 essential genes. The 95% confidence limits calculted using the Stevens tables (Stevens 1942) are 1,379 - 21984). It should be noted that if the lethal s744 ,which was lost, did map within sDf2 and was not allelic to the three other lethals the genome size estimate would be approximately 3402 (1,329 - 12,484) essential genes.

The method employed in this study to determine the number of essential genes in C.elegans has a number of advantages:

1) Lethals are screened for over a large expanse of the genome (approximately 40 map units corresponding to the region recombinationally suppressed by the translocation nT1). This allows both regions of low and high gene density to be assessed.

2) Gene density does not affect the calculation of indispensable gene number. For instance, if three genes are isolated in the sDf2 region, the estimate of genome number is 32/3 X 7 X 54 =4000. If on the other hand the region had contained only 10 genes, we would have expected to isolate one mutation in this region so that 10/1 X 7 X 54 =4000. Therefore, gene density of the region being analyzed does

not play a role in these calculations.

3) There is no dependence on the target size of genes. Clearly, genes mutate at different rates. For example, let-56(IV) has four alleles while let-52(IV) has only one known isolate. These genes, defined by their respective alleles, were recovered in the same screens (Rogalski, Moerman, and Baillie 1982; Rogalski 1983). In order to eliminate bias introduced by genes which are large mutagenic targets, the lethal mutations mapping within sDf2 were complementation tested against one another.All three muations are non-allelic. Any gene which had been mutated more than once would have been counted as a single isolate.

A comparison of the gene density of the sDf2 and sDf21 region

The EMS-induced lethals can also be used to compare the gene density of the sDf2 and sDf21 regions. This is of interest since the unc-43 to dpy-4 region of linkage group IV is currently being cloned in this laboratory. Out of 24 lethals isolated on linkage group IV , three mapped to the left of unc-22 and within the deficiency sDf2 . The remaining 21 lethals mapped to the left of unc-22 and outside sDf2 . None of the EMS lethals mapped to the right

of unc-22 , in the sDf21 region. This suggests that there are at least three times as many genes in the sDf2 region compared to the sDf21 region. This is anomalous since the sDf21 region is five map units in size while sDf2 comprises only 1.5 map units . On the basis of recombinational size, one would predict to see approximately three times as many genes in the sDf21 region compared to the sDf2 region.

Brenner noted that visible genes tended to map in a cluster (Brenner 1974). On linkage group IV , the visible genes cluster just to the left of unc-22 ; Brenner's original set of visible mutations display this (Brenner 1974). Lethal mutations also appear to cluster in the same region as visible genes (Herman 1978; Rogalski 1983). It should be noted that a large number of the EMS lethals isolated in this screen cluster just outside sDf2 , and three are within sDf2 . This is in agreement with the presence of a lethal cluster in this region. Two visible mutations have also been remapped within sDf2 by Jeff Way; mec-3 and him-8 (Jeff Way, personal communication). If the region to the left of unc-22 and defined by sDf2 is indeed a part of the linkage group IV cluster, then the observed gene density of the sDf2 region most likely is higher than that of the genome as a whole.

While no EMS-induced lethals were recovered in the sDf21 region in this study, there are at least two genes in this area. These are defined by the deficiencies sDf21 , sDf22 , sDf23 ,and sDf24 . The first lethal (let-301) corresponds to the region covered by sDf21 , sDf22 ,and sDf24 . The second lethal (let-302) is defined by sDf23 . Since sDf23 and sDf21 have not been tested to see if they overlap, these two deficiencies may share this lethal in common.

Gamma-irradiation induced unc-22 linked lethals

Gamma-induced lethal mutations were isolated in order to make a preliminary assessment of the differences between the distribution of EMS- and gamma-induced lethals on linkage group IV . Fourteen gamma-induced mutations mapped to linkage group IV . Of the 14 gamma-induced lethals, only two were located within sDf2 . One, s785 fails to complement let-94(s751) . The lethal s785 has been complementation tested against the six most leftward lethals within sDf2 . It has complemented these. It is possible that s785 is either an internal deletion of s751 or that it is a very small deficiency. The second lethal, s860 , maps tightly to unc-22 . It has not yet been complementation

tested against the known lethals in sDf2 . The lethal s879 maps tightly to unc-22 , however it complements sDf2 .

Two lethals did not give straight forward complementation results. One lethal, s881 appears to have lost the diagnostic unc-22(s7) allele and behaves as a wild type; this is a putative duplication carrying strain. Another lethal, s864 ,still appears to carry the unc-22(s7) allele however, when outcrossed to unc-22(s7)/+ males only a few Unc-22's are seen amongst the progeny. This is a putative large deficiency.

Maternal effect lethals in the nT1 region

Fifty non-lethal bearing strains from the gamma-irradiation screen were tested for the presence of maternal effect lethals. No maternal effect lethals were recovered. This is not surprizing since one would expect gamma-irradiation to produce deletions. It would be more appropriate to screen for EMS-induced maternal lethals. It would be most interesting if the region to the right of unc-22 is populated by maternal lethals, considering there were no standard (F1)lethals recovered in the region, in this study.

There is evidence that the maternal lethals may cluster

in a manner similar to that of the visible and essential lethals (Philomena Kann, personal communication). It will be interesting to see if maternal lethals do in fact cluster in the same regions as visible and lethals, if they do cluster at all.

Nature of the N2/BO hybrid lethals

A set of hybrid lethals was generated by two protocols; the protocols differing in the presence or absence of a heat shock step. The frequency of induction of lethals in the control (non-heat shock) protocol is 2.2×10^{-2} for the nT1 region. For the heat shock protocol the frequency is 6.6×10^{-2}. The spontaneous mutation rate in Bristol strain hermaphrodites as assessed by Rosenbluth et al. (1983) is 6.6×10^{-4} for the eT1 region which coveres approximately the same number of map units as eT1 . The control experiment shows an increase of approximately 30 fold over the spontaneous lethal mutation rate in the Bristol strain. The heat shock experiment shows a 3-fold increase over the control. Even the control protocol yields an increase over the spontaneous rate in the order of 30 fold.

Researchers have attempted to induce hybrid dysgenesis

in C. elegans . The key trait they were looking for was dysgenic sterility. In P-M hybrid dysgenesis in Drosophila, the offspring of crosses between P and M strains exhibit dysgenic traits including male recombination, gene mutation, chromosome breakage, and dysgenic sterility. These traits are seen when P males are mated to M females, but not in the reciprocal cross. In C. elegans , Bergerac males do not mate efficiently. Since Bergerac exhibits the traits of a P-like strain, it is not possible to duplicate the cross expected to produce hybrid dysgenesis, that is between Bergerac males and Bristol hermaphrodites. When Liao et al. (1983) attempted to induce hybrid dysgenesis in C. elegans , they used a "Bergerac" strain which gave rise to fertile males. They were unsuccessful at inducing hybrid dysgenesis.

If hybrid dysgenesis does occur in C. elegans perhaps it does not take the form of P-M hybrid dysgenesis. In Drosophila, in hybrid dysgenesis involving the offspring of crosses between Q strains and M strains exhibit some dysgenic traits including male recombination, gene mutation, and chromosome breakage, but not dysgenic sterility (Engels and Preston 1981; Simmons et al., 1980).

Since male recombination normally occurs in C. elegans it cannot be studied as a dysgenic trait. Gene mutation and chromosome breakage, however can be studied. In the present

study, hybrid lethals were recovered. Two of these showed evidence of chromosome breakage: lethals s743 and s765 have been shown to be deficiencies, sDf31(V) and sDf43(IV) respectively. There is another feature of hybrid dysgenesis which appears evident and that is the clustering of mutations. One $P_{\emptyset}$ gave rise to four mutations, three of which behave as though they may be allelic, suggesting they arose as a result of a premeiotic event.

There is a major difference between the N2/BO hybrid phenomenon and hybrid dysgenesis and that is the time of action. The N2/BO phenomenon manifests itself one generation prior to hybrid dysgenesis.

The mutagenic events must occur at an early stage in development, probably between fertilization and the production of gametes. Heat shock appears to enhance the phenomenon and the premeiotic mutations occurred in the heat shock protocol.

That this phenomenon is due to hybrid dysgenesis has not been proven. It is possible that the unc-22 strain itself has a high spontaneous mutation rate (see discussion of the unc-22(s727) strain). Eide and Anderson (1985) have observed that the DH424 strain of C. elegans gives rise to a large number of spontaneous rearrangements six generations after it had been crossed to the Bristol strain.

If the mutations generated by interstrain-crossing are due to the insertion and excision of transposable elements, they will prove invaluable for the molecular analysis of the unc-43 to dpy-4 region of linkage group IV . That there appears to be site specificity for this region is quite promising. Site specificity has been observed for hybrid dysgenesis in Drosophila (Simmons et al. 1984a; Simmons et al. 1984b) On this line, it is interesting that Moerman and Waterston (1984) have isolated a mutator strain which results from active Tcl elements. It should be possible to molecularly "tag" genes in the unc-43 to dpy-4 region by screening for unc-22 -linked lethal mutations in this strain. The basic method has been described for Drosophila by Bingham et al. (1981). This will allow cloning and identification of lethals whose gene products have not yet been determined.

On the unc-22(s727) strain

Although no visible mutations were observed when screening the unc-22(s727) homozygous strain, it should be noted that the brood size of each hermaphrodite was on the average 10 progeny. While visible mutations were not observed, the low brood size suggests that this strain is

highly inviable, possibly due to the generation of spontaneous mutations. These spontaneous mutations might be due to the insertion and/or excision of transposable elements.

Merits and dissadvantages of the nT1 screening system

The nT1 screening system employed in this study provides a means of isolating a large number of linkage group IV lethals. One of the objectives of this research was to isolate EMS induced lethals throughout the nT1 recombinationally suppressed region in order to arrive at an estimate of the number of essential genes in C. elegans . To this end, the nT1 system is most advantageous.

Where the goal of a researcher is to isolate lethals in a specific region of linkage group IV , the nT1 system should not be used in the screening step. The time and effort required to analyze and eliminate unwanted lethal mutations can be prohibitive. For example, if the goal of this work were to isolate lethals in the unc-22 to dpy-4 interval, no lethals would have been recovered, yet 56 strains would have been isolated, maintained, and analyzed. However, since nT1 is an effective balancer for the right-half of linkage group IV , lethals isolated in the

unc-43 to dpy-4 region can be subsequently balanced by nT1 allowing maintenance to occur with relative ease.

On a number of occassions, the nT1 balanced strains have appeared to break down. Two unc-22 -linked EMS induced lethals balanced over nT1 no longer twitch in nicotine. Further, these strains now are phenotypically wild type. Due to the vulvaless nature of nT1 , it is not possible for the nT1 homozygotes to have taken over. The simplest explanation is that when transfering the stocks, somehow a wild type individual or egg contaminated the plate. Since the wild types are healthier and grow at a faster rate than both nT1 homozygotes and the lethal heterozygotes, and since these stocks are often mass transfered, the wild type individuals could take over. Therefore, utmost care should be taken when transfering nT1 balanced strains.

Summary and Prospects

As indicated previously one aim of this analysis was to develop and validate a novel method for estimating the number of essential genes in C. elegans . The method employed involved the determination of the number of mutations falling within the deficiency sDf2 ,a region which

has previously been extensively analyzed (Rogalski and Baillie (Rogalski, Moerman, and Baillie 1983; Rogalski 1983; Rogalski and Baillie, in press). In general, the recombination distance and deficiency mapping agreed, with the exception of one lethal which mapped within sDf2 but complemented this deficiency. It is most interesting that the three mutations falling within sDf2 define three new genes. Since about 2/3 of the essential genes in the sDf2 region have already been identified. The probability of recovering three new genes is $(1/3)^3$ (4%).

This analysis has also allowed a direct comparison between the regions defined by sDf2 and by sDf21 . While the sDf21 region is recombinationally approximately three to four times the size of sDf2 , it appears to contain only about 1/3 as many genes.

The unc-43 to dpy-4 interval is currently being cloned in this laboratory (Baillie and Beckenbach, personal communication). Several hundred kilobase pairs of DNA have already been isolated and positioned by the use of Bristol/Bergerac recombinants.

The Bristol/Bergerac interstrain-cross lethals may prove very valuable to the molecular analysis of the unc-43 to dpy-4 region. Two of these lethals have been shown to be deficiencies. These strains can be used directly for

mapping Restriction Fragment Length Differences (RFLD's). In the case of sDf43 , it has been maintained over nT1 without any crossing over occurring on the right-arm of linkage group IV . Therefore, RFLD's which map within sDf43 will display the banding pattern characteristic of the Bristol strain. RFLD's mapping outside the deficiency would give rise to the joint Bristol and Bergerac banding patterns.

Another line of research in this lab involves the identification of coding sequences in the unc-43 to dpy-4 region (Bird, Prasad and Baillie, personal communication). This involves identifying highly conserved sequences between the two species C. briggsae and C. elegans . It will be most interesting to see whether the region to the right of unc-22 contains a large or a small amount of DNA and to see whether this region is gene sparse or if it contains cryptic genes(genes whose phenotype is not discernable without the use of a special selective system). An example of a cryptic gene might be a gene whose product is involved in drug resistance. The phenotype of an individual with a mutation in such a gene would be Wildtype, unless it was subjected to the antibiotic). This region might also contain a set of repeated genes (either tandemly repeated or dispersed throughout the genome), with the ability of replacing one

another. An example of such a set of genes would be the heat shock genes (Mohler and Pardue 1984).

Proposals for future research

There are a number of projects which should be undertaken. Some of these are:

1) The isolation of specific lethals of the unc-43 to dpy-4 region. The nT1 screen should not be used. Rather, unc-22 unc-31 hermaphrodites should be mutagenized with Ø.Ø12M EMS and mated to wild type males. Wild type F1's should be set up and their progeny screened for the absence of the diagnostic chromosome. The putative lethals can subsequently be balanced over nT1 . This will avoid stock maintenance of loosely linked lethals.

2) The use of sDf19(IV)/nT1(IV);+/nT1(V) to screen for gamma induced deficiencies of unc-22 and the surrounding area.

3) The isolation of a set of overlapping deficiencies in the unc-43 to unc-22 region and the unc-22 to dpy-4 region. I would recommend that these be isolated via pseudocomplementation or by the protocol discussed for EMS lethals above. Isolating deficiencies over nT1 by screening for lethals requires a great deal of manpower. Interstrain

cross lethals may be another method of isolating Bergerac deficiencies, these being powerful both at the genetic and the molecular level.

4) The sample size used to determine the number of essential genes in C. elegans should be increased.

5) The essential gene number should be calculated by using the data from another region of the genome. For instance linkage group I or linkage group V , both regions which are being intensivley analyzed at this time (Howell and Rose, personal communication; Rosenbluth et al., personal communication).

6) The sample size of the interstrain crossing experiment should be increased. A number of screens or a large scale screen will be necessary due to the poor fecundity of the Bergerac unc-22 stock. The remaining lethals isolated in the first four screens should be characterized. It will be interesting to determine the fraction of lethals which are rearrangements.

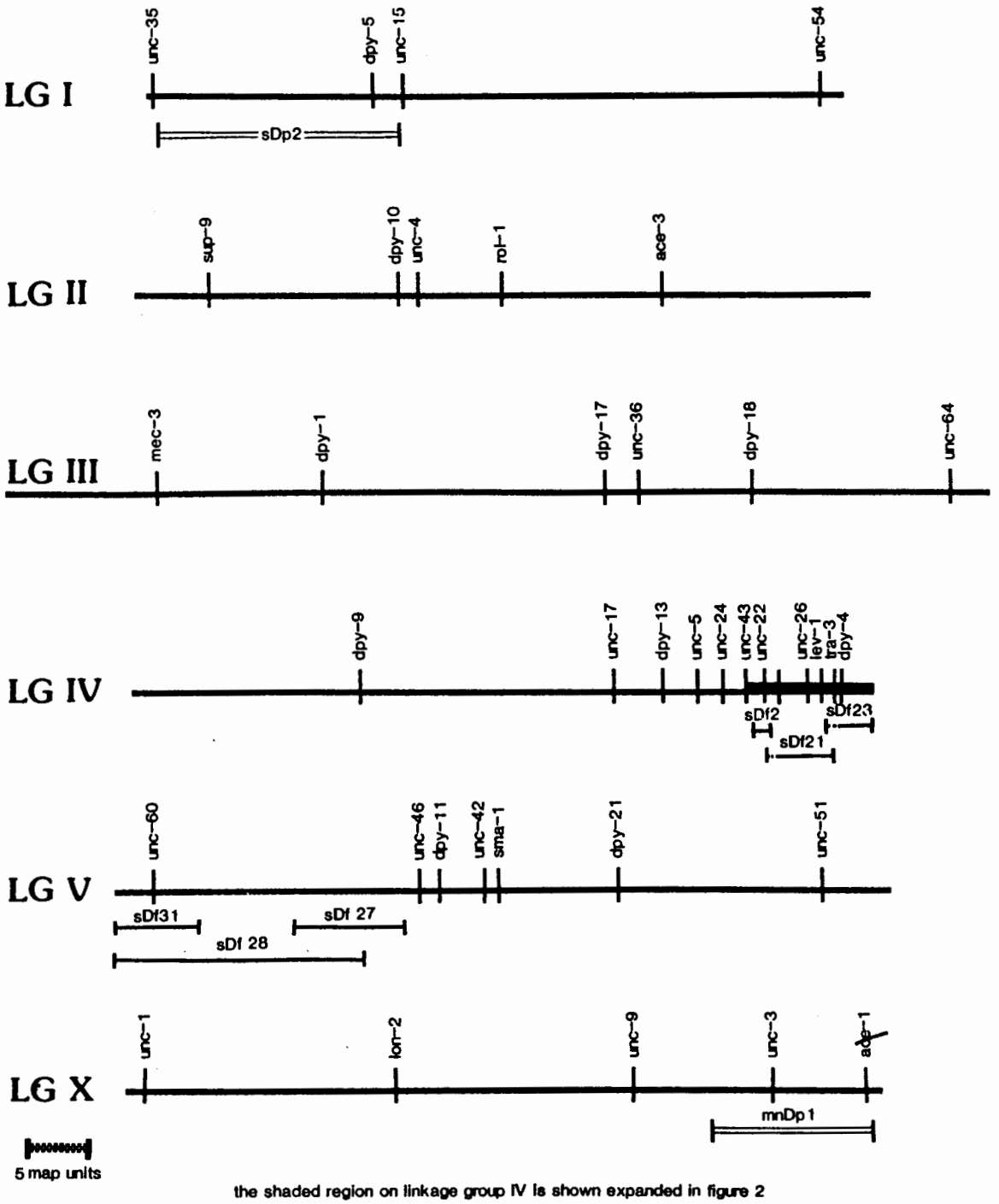
7) The isolation of a linkage group IV balancer which suppresses crossing-over between the markers unc-43 to dpy-4 . This would allow for the effective recovery of balanced lethals throughout this interval.

Appendix 1

An Abbreviated Map of the *C. elegans* Genome

figure 4

Abbreviated map of the C. elegans genome



the shaded region on linkage group IV is shown expanded in figure 2

APPENDIX 2

Following is the derivation of the mapping formula which was used throughout this study. The derivation of the basic mapping formula is depicted in the Punnet Square. The visible recombinant individuals are circled.

	1-p/2 ab	1-p/2 ++	p/2 a+	p/2 +b
ab	ab ab	++ ab	a+ ab	+b ab
++	ab ++	++ ++	a+ ++	+b ++
a+	ab a+	++ a+	a+ a+	+b a+
+b	ab +b	++ +b	a+ +b	+b +b

$$R = \frac{\text{number of recombinant offspring}}{\text{total number of progeny}}$$

p = recombination frequency between a and b

R = frequency of visible recombinant progeny

$= 1/4p(1-p) + 1/4p(1-p) + 1/4p(1-p) + 1/4p(1-p) + 1/4p^2 + 1/4p^2$

$R = p(1-p) + 1/2p^2$

$R = p - 1/2p^2$

$\emptyset.5p^2 - p + R = \emptyset$

solve for p by using the quadratic formula

$p = 1 - \sqrt{1 - 2R}$

To determine the distance of <u>let-a(sx)</u> from <u>unc-22</u> in the case of <u>let-a(sX)unc-22(s7)unc-31(e169)</u> ,we regard the wild type category to be comprised of both the phenotypically Wild Type and the phenotypically Unc-31 individuals. Since 1/4 of all the progeny die, the number of total progeny is 4/3(WT + Unc-31 + Unc-22Unc-31). The phenotypically Unc-22Unc-31 recombinants comprise only 1/2 of the total number of recombinants. Therefore, the number of recombinants is 2(Unc-22).

Finally,

$p = 1 - \sqrt{1 - 2\ (2\ \text{Unc-22Unc-31}/\ (4/3(\text{WT})))}$

$p = 1 - \sqrt{1 - 2\ (3\ \text{Unc-22Unc-31}/\ (2(\text{WT})))}$

$p = 1 - \sqrt{1 - 3(\text{Unc-22Unc-31})/\text{WT}}$

APPENDIX 3

List of Strains Carrying Mutations Generated in this Study

Genotype	BC #
EMS-induced mutations	
unc- (s729)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	14Ø4
let- (s73Ø)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1273
let- (s886)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1862
let- (s887)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1865
let- (s888)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1868
let-093(s734)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1291
let- (s735)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1292
let- (s736)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1293
let- (s737)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1294
let- (s744)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	NA
let- (s745)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	14Ø1
let- (s746)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	14Ø2
let-3Ø4(s747)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1863
let- (s748)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1864
let- (s749)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1541
let- (s75Ø)unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1866

let-094	(s751) unc-22(s7) unc-31(e169) IV/nT1; + V/nT1	1450
unc-	(s752) unc-22(s7) unc-31(e169) IV/nT1; + V/nT1	1867
let-095	(s753) unc-22(s7) unc-31(e169) IV/nT1; + V/nT1	1451
let-	(s754) unc-22(s7) unc-31(e169) IV/nT1; + V/nT1	1870
let-	(s755) unc-22(s7) unc-31(e169) IV/nT1; + V/nT1	1400
let-	(s756) unmapped	1397
let-	(s757) unmapped	1398
let-	(s758) unc-22(s7) unc-31(e169) IV/nT1; + V/nT1	1399
let-306	(s759) unc-22(s7) unc-31(e169) IV/nT1; + V/nT1	1403
let-	(s760) unc-22(s7) unc-31(e169) IV/nT1; + V/nT1	1563
let-303	(s761) unc-22(s7) unc-31(e169) IV/nT1; + V/nT1	1869
let-305	(s762) unc-22(s7) unc-31(e169) IV/nT1; + V/nT1	1559

Gamma-induced mutations

let-	(s785) unc-22(s7) unc-31(e169) IV/nT1; + V/nT1	1871
let-	(s786) unc-22(s7) unc-31(e169) IV/nT1; + V/nT1	1850
let-	(s787) unc-22(s7) unc-31(e169) IV/nT1; + V/nT1	1567
let-	(s788) unc-22(s7) unc-31(e169) IV/nT1; + V/nT1	1839
let-	(s790) unmapped	1851
let-	(s791) unmapped	1845
let-	(s792) unmapped	1533
let-	(s793) unmapped	1537
let-	(s794) unc-22(s7) unc-31(e169) IV/nT1; + V/nT1	1852
let-	(s795) unmapped	1853

let- (s796)	unmapped	1538
let- (s797)	unmapped	1534
let- (s798)	unmapped	1840
let- (s799)	unmapped	1539
let- (s860)	unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1449
let- (s861)	unmapped	1841
let- (s863)	unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1545
let- (s864)	unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1546
let- (s865)	unmapped	1855
let- (s867)	unmapped	1535
let- (s868)	unmapped	1842
let- (s869)	unmapped	1856
let- (s870)	unmapped	1846
let- (s871)	unmapped	1857
let- (s872)	unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1858
let- (s873)	unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1859
let- (s874)	unmapped	1860
let- (s875)	unmapped	1843
let- (s876)	unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1540
let- (s878)	unmapped	1536
let- (s879)	unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1861
let- (s880)	unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1844
let- (s881)	unc-22(s7)unc-31(e169) IV/nT1; + V/nT1	1553

let- (s882)	unmapped	1847
let- (s883)	unmapped	1848
let- (s884)	unmapped	1849

N2/BO Interstrain-crossing-induced lethals

unc-22(s727)IV/nT1; sDf31(s743)V/nT1	BC	1379
let- (s885)unc-22(s727) IV/nT1; + V/nT1		1443
let- (s763)unc-22(s727) IV/nT1; + V/nT1		1444
let- (s764)unc-22(s727) IV/nT1; + V/nT1		1445
sDf43 (s765)unc-22(s727) IV/nT1; + V/nT1		1446
let- (s766)unc-22(s727) IV/nT1; + V/nT1		1447
let- (s768) unmapped		1556
let- (s767)unc-22(s727) IV/nT1; + V/nT1		1448
let- (s769)unc-22(s727) IV/nT1; + V/nT1		1854
let- (s77Ø) unmapped		1564
let- (s771) unmapped		183Ø
let- (s772)unc-22(s727) IV/nT1; + V/nT1		1566
let- (s773) unmapped		1831
let- (s774) unmapped		1832
let- (s775) unmapped		1558
let- (s776) unmapped		1833
let- (s777) unmapped		1834
let- (s778) unmapped		1565
let- (s779) unmapped		1835

let- (s780)	unmapped	1836
let- (s783)	unmapped	1838
unc-22(s727)IV/nT1; let- (s800) V/nT1		1419
let- (s782)	unmapped	1542
let- (s775)	unmapped	1558
let- (s781)	unmapped	1837

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