THE GENETICS OF *CAENORHABDITIS ELEGANS*

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ABSTRACT

Methods are described for the isolation, complementation and mapping of mutants of *Caenorhabditis elegans*, a small free-living nematode worm. About 300 EMS-induced mutants affecting behavior and morphology have been characterized and about one hundred genes have been defined. Mutations in 77 of these alter the movement of the animal. Estimates of the induced mutation frequency of both the visible mutants and X chromosome lethals suggests that, just as in Drosophila, the genetic units in *C. elegans* are large.

HOW genes might specify the complex structures found in higher organisms is a major unsolved problem in biology. Many of the molecular mechanisms involved in gene expression in prokaryotic microorganisms have already been found to exist in a relatively unmodified form in eukaryotic cells. The genetic code is universal and the mechanism of protein synthesis is much the same in both kinds of organisms. There are, by contrast, great differences in the organization of the genetic material. The chromosomes of higher organisms are complex structures that contain histones and other proteins in addition to DNA and the genetic units are much larger than their counterparts in simple prokaryotes (JUDD, SHEN and KAUFMAN 1972). Although there are many theories suggesting how the extra DNA might be used for complex genetic regulation (BRITTEN and DAVIDSON 1969; GEORGIEV 1969; CRICK 1971), the problem is still opaque. We know very little about the molecular mechanisms used to switch genes on and off in eukaryotes. We know nothing about the logic with which sets of genes might be connected to control the development of the assemblages of different cells that we find in multicellular organisms.

These questions arise in a particularly acute form in elaborate structures like nervous systems. In one sense, all neurons resemble each other; they must be excitable, able to transmit electrical signals and produce and respond to chemical transmitters. They must be equipped with very similar, possibly commonly specified biochemical machinery. Yet, in another sense, they are all very different; cells are located at specific places and are connected to each other in definite ways. How is this complexity represented in the genetic program? Is it the outcome of a global dynamical system with a very large number of interactions? Or are there defined subprograms that different cells can get a hold of and execute for themselves? What controls the temporal sequences that we see in development?

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One experimental approach to these problems is to investigate the effects of mutations on nervous systems. In principle, it should be possible to dissect the genetic specification of a nervous system in much the same way as was done for biosynthetic pathways in bacteria or for bacteriophage assembly. However, one surmises that genetical analysis alone would have provided only a very general picture of the organization of those processes. Only when genetics was coupled with methods of analyzing other properties of the mutants, by assays of enzymes or *in vitro* assembly, did the full power of this approach develop. In the same way, the isolation and genetical characterization of mutants with behavioral alterations must be supported by analysis at a level intermediate between the gene and behavior. Behavior is the result of a complex and ill-understood set of computations performed by nervous systems and it seems essential to decompose the problem into two: one concerned with the question of the genetic specification of nervous systems and the other with the way nervous systems work to produce behavior. Both require that we must have some way of analyzing the structure of a nervous system.

Much the same philosophy underlies the work initiated by BENZER on behavioral mutants of Drosophila (for review, see BENZER 1971). There can be no doubt that Drosophila is a very good model for this work, particularly because of the great wealth of genetical information that already exists for this organism. There is also the elegant method of mosaic analysis which can be powerfully applied to find the anatomical sites of genetic abnormalities of the nervous system (HOTTA and BENZER 1972).

Some eight years ago, when I embarked on this problem, I decided that what was needed was an experimental organism which was suitable for genetical study and in which one could determine the complete structure of the nervous system. Drosophila, with about 10^5 neurons, is much too large, and, looking for a simpler organism, my choice eventually settled on the small nematode, *Caenorhabditis elegans*. Extensive work on the nutrition and growth of this and related nematodes had been done by DOUGHERTY and his collaborators (see DOUGHERTY *et al.* 1959), and there was a classical study of its sexual cycle by NIGON (1949). *C. elegans* is a self-reproducing hermaphrodite, each animal producing both sperm and eggs. The adults are about 1 mm in length and the life cycle for worms grown on *Escherichia coli* is 3½ days at 20°. It has a small and possibly fixed number of cells (about 600, excluding the reproductive system) of which about one-half are neurons. Occasionally cultures are found with a few males. Such males may be maintained by mating them with the hermaphrodites. NIGON (1949) found that males contained one less chromosome than the hermaphrodites and that the chromosome constitution is 5AA + XX in the latter and 5AA + XO in males. Using two strains of *C. elegans* differing in reproductive capacity at 25°, FATT and DOUGHTERY (1963) were able to show mendelian segregation of a single locus controlling heat tolerance. More recently, DION and BRUN (1971) studied two spontaneous mutants in the Bergerac strain of *C. elegans*.

Our work on this organism has been concentrated so far on two lines: the development of methods for determining the structure of the nervous system, which

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will be described elsewhere, and establishing the basic genetic features of *C. elegans*, which is the subject of this and the accompanying paper (SULSTON and BRENNER 1974). This paper reports the characterization of large number of mutants, mostly affecting behavior. About one hundred genes have been mapped onto six linkage groups. The methods used are given in some detail, mainly because hermaphrodite genetics has special technical problems.

MATERIALS AND METHODS

Media: 1. NG agar: 3 g NaCl, 2.5 Bactopeptone (Difco) and 17 g Bacto-agar (Difco) are dissolved in 975 ml distilled water. After autoclaving, 1 ml cholesterol in ethanol (5 mg/ml), 1 ml M $CaCl_2$, 1 ml M $MgSO_4$ and 25 ml M potassium phosphate buffer (pH 6.0) are added in order.

2. M 9 buffer: 6 g Na_2HPO_4, 3 g KH_2PO_4, 5 g NaCl and 0.25 g $MgSO_4{\cdot}7H_2O$ per litre.

3. S buffer: 0.1 M NaCl and 0.05 M potassium phosphate (pH 6.0).

4. Standard bacteriological media are used for growth and maintenance of bacterial strains.

Nematode strains: The nematode used in this work is the Bristol strain of *Caenorhabditis elegans*. It was originally sent by the late PROFESSOR E. C. DOUGHERTY as an axenic culture, but it was transferred to a strain of *Escherichia coli* B. After some passages on solid media, a culture was found which contained a large number of males. These males could be maintained by mating with hermaphrodites. From this stock, a hermaphrodite was isolated and its progeny used to establish two lines: one, a line of hermaphrodites propagating by self-fertilization; the other, a line with males. These are the founder stocks and carry the code name N2; all mutants have been isolated in these strains.

Maintenance of stocks: Stocks are maintained on NG plates seeded with OP50, a uracil-requiring mutant of *E. coli*, and incubated at 15°. 9 cm petri dishes are used and cultures require subculturing every 10 days or so. Male cultures are maintained by adding 6 or 7 males to a similar number of hermaphrodites on a seeded NG plate. Several of these stocks, staggered with respect to their subculturing, are held, so that active males are always available for crosses.

A uracil-requiring strain of *E. coli* is used to prevent overgrowth of the bacterial lawn. The medium has limited uracil, and the bacteria cannot grow into a thick layer which obscures the worms.

These plates are the working stocks for genetical and other experiments. The canonical stocks of the mutants are held frozen in liquid nitrogen. Many experiments on long-term maintenance were carried out without much success. DR. J. SULSTON discovered that the worms could be stored in liquid nitrogen, provided that glycerol was present and that the initial freezing took place slowly. The standard method used is as follows: worms are washed off the surface of a petri dish culture using about 1.5 ml of S or M 9 buffer. To 1 ml of this suspension is added 1 ml of a 30% solution of glycerol in S buffer, and after mixing, four 0.5 ml aliquots are dispensed into small plastic tubes. These are placed in the holder provided with the Linde liquid nitrogen refrigerators at a level in the vapor phase giving a cooling rate of about 1°/min. After two hours or more, the tubes are mounted in canes and submerged in the liquid nitrogen. The next day, one of the four tubes is removed, thawed, and the contents poured on an NG plate. The plate is examined after a day to make sure that there are viable growing worms. The remaining three cultures are then stored: one in one refrigerator as a master stock, the other two in a different refrigerator as the canonical stocks. If, at any time, the last of these is used, it is immediately replaced so that the master stocks are only used in emergency. With the wild type and most mutants, it is mostly the early larval stages that survive freezing and thawing; eggs do not survive at all. This method has proved completely reliable.

Plate stocks can become contaminated with bacteria and moulds. Cultures may be rendered monoxenic in the following way: A culture containing many eggs is suspended in 1.5 ml M 9 buffer. 1.5 ml of 4% glutaraldehyde in M 9 buffer is added and the suspension allowed to stand at 4° for 4 hours. A few drops of a culture of *E. coli* is spread over a half sector of a 9-cm NG

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plate. The glutaraldehyde-treated suspension is briefly centrifuged, and the sediment taken up in 0.1 to 0.2 ml of M 9 buffer and applied to the edge of the uninoculated sector. If necessary, the plate is tilted so as to confine this to one side. The glutaraldehyde kills the worms and most contaminants but does not penetrate the eggs. After one day, these hatch and the larvae cross over to the bacterial lawn. The agar with the debris may then be removed.

Induction of mutation with ethyl methanesulphonate (EMS): The animals are washed off the plate in M 9 buffer, and to 3 ml of the suspension is added 1 ml of freshly prepared 0.2 M ethyl methanesulphonate in M 9 buffer (final concentration 0.05 M). The standard treatment is for 4 hours at room temperature. The suspension is then taken up into a pipette and the worms allowed to concentrate by sedimentation. 0.2–0.5 ml is dripped onto the surface of an NG plate to absorb the excess fluid. The worms move out and can then be picked to initiate clones.

Handling and observation of animals: Mass transfers of animals on plate cultures are carried out with paper strips. Single animals can be manipulated using a sharpened wooden stick or toothpick, sterilized by autoclaving. Observations of the plates are made using a dissecting microscope illuminated from below.

EXPERIMENTS AND RESULTS

Hermaphrodite genetics: Self-fertilizing hermaphrodites have many advantages for genetical analysis. It is likely that the clones established in the laboratory are of uniform genetical constitution since the animals are driven to homozygosity. Recessive mutants are easily isolated on both the autosomes and sex chromosome by the automatic segregation of heterozygous animals. The hermaphrodite, by itself, would be useless for genetical analysis; but the rare males, which can be maintained by mating with the hermaphrodite, permit the transfer of genetic markers from one hermaphrodite to another. When such a cross is carried out the progeny are of two types, those arising from self-fertilization and those produced by fertilization with male sperm, and measures must be taken to distinguish these. In general, nearly all genetical analysis is done by segregation of the hermaphrodite, and the male is used simply as a device to construct appropriately marked hermaphrodites.

Isolation of mutants: Ethyl methanesulphonate (EMS) is a potent mutagen in *C. elegans*, penetrating the animals readily. We consider a young adult individual treated with this agent. Mature sperm have already been produced and stored, and the ovary is manufacturing eggs. Any mutation produced in such an animal will not appear in the homozygous form in the progeny because there are no cells at this stage that can give rise to both sperm and eggs. If the mutagen acted only on non-replicating gametes, such as sperm or oocytes, then the mutations in the F_1 progeny would be independent. On the other hand, if it reacted with oogonia then clones of F_1 animals would occur, but they would still be heterozygotes. Such clones have been detected but there are also many single events suggesting that both kinds of gametes are susceptible to the mutagen. It can also be shown that primordial germ cells are susceptible to mutagenic action. Newly hatched larvae treated with EMS produce large clones of mutants in their progeny. The vast majority of these are heterozygous but homozygotes have been found occasionally, suggesting that the mutation occurred in a cell that gave rise to both sperm and eggs. Mutations can also be induced by EMS in the germ cells of males. Treated males are mated with hermaphrodites and the mutants are then isolated from the progeny of these crosses.

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In most of the experiments, mutants have come from the clones produced by mutagenized adults. Although the F_1 progeny are heterozygous for induced mutations, a detectable fraction are abnormal in appearance or movement. Such variants have been picked with the intention of isolating dominant or semidominant mutants, but in all cases these have produced wild-type progeny or segregated a coincidental recessive mutant with an unrelated phenotype. These animals are likely to be mosaics in which the EMS-induced mutations become fixed after fertilization and then only in cells that produce somatic structures. Semidominant mutants can, however, be isolated from the progeny of mutagenized young larvae; in this case the induced mutation becomes fixed during the development of the gonad in the treated parent.

Mutants with a dominant effect are rare in *C. elegans* and the vast majority of the mutants found are recessive. These emerge in the F_2 progeny, from the segregation of any F_1 heterozygotes. By this stage, the plate, initiated by a single parent, contains a very large number of individuals (up to 10^5), but not all of these have to be screened. One-quarter of the offspring of a heterozygous F^1 are homozygous and if the number of F_1 animals is a few hundred then only about a thousand of the F_2 need to be examined before the plate is discarded as containing no mutants. In practice, mutants are so abundantly produced by EMS that this does not arise, and in fact, it is often advantageous to remove the parent before it has laid all of its eggs to reduce the number of F_1 animals to about 50. 30 to 40 plates are used at a time, and only one mutant is ultimately selected from each plate. 550 mutants isolated in this way are dealt with in this paper; they comprise the M set. This mass isolation procedure is not altogether satisfactory. Since, at the time of picking, the plates contain large numbers of worms, mostly young, there is a bias against mutants that grow slowly or express their phenotypes fully only in the adult form. A more laborious but more accurate procedure is to pick the mutants from the segregants of F_1 progeny of mutagenized parents, putting single F_1 animals on separate plates. In practice, only 5 such F_1 clones are initiated from a given plate to ensure independent origin; any repeats found (which has happened only once) are considered members of a clone and are discarded. On such plates, one-fourth of the progeny of any heterozygous F_1 are homozygous mutants, and this widens the range of identifiable phenotypes. From 318 such plates, 69 mutants were isolated, comprising the S set.

Phenotypes of mutants: Most of the mutants have been selected by inspection. Although emphasis has been placed on mutants defective in movement to find genes controlling the structure or function of the nervous system, we have also collected mutants with morphological abnormalities and with differences in size and shape. It is our general practice not to classify the mutants by different names corrsponding to phenotype descriptions, but to put them into broad categories and then characterize them genetically. A general description of the phenotypes now follows, and special comments on the individual mutants can be found in Table 5.

Uncoordinated mutants: The wild type (Figure 1a) displays a smooth sinuous movement on the agar surface. It is important to realize that the motion of the body is confined to the dorsoventral plane, and that, on plates, the animals are

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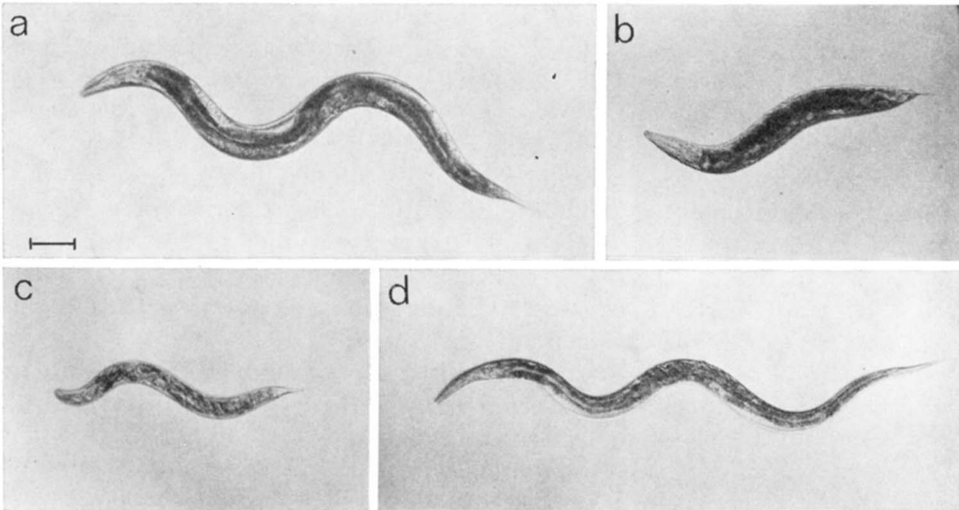

FIGURE 1.—Photomicrographs of *C. elegans* and some of its mutants. a: wild type, b: dumpy (*dyp-1*), c: small (*sma-2*), d: long (*lon-1*). The scale is 0.1 mm.

lying on their sides. The head can move in all directions, but surface tension restrains it to the surface as well. The animals can reverse with the same wave-like motion. Reverse motion can be stimulated either by tapping the surface of the plate in front of the animal or by touching the tip of its head. Any mutant that shows any detectable defect in this normal pattern of behavior is called "uncoordinated". As may be expected, this covers a very wide range of phenotypes from paralysis to quite small aberrations of movement. Although there are mutants with strikingly singular properties, most of the phenotypes are very difficult to describe. In general, the pulsating pharyngeal movements are not affected in the mutants, even in severely paralyzed animals. Paralysis of the pharynx would probably be lethal since the animals would be unable to feed. In some paralyzed and semiparalyzed mutants the vulva is also affected, and eggs are not laid. Progeny hatch inside the parent and ultimately devour it. Much of the animal's capacity for and control of motion is dispensable, because it does not require active males for propagation and because laboratory conditions can supply adequate sources of food.

Roller mutants: The body of the animal rotates around its long axis as the animal moves. The effect is to force the animal to move in a circle, and such mutants are easily recognized by the craters they inscribe in the bacterial lawn. When such animals reverse, the hand of rotation becomes opposite. Careful observation with a series of mutants shows that they all have the same hand of rotation of the body but that the way they move in circles on the plate differs, and this appears to be controlled by the movement of the head. In liquid media it can be shown that wave propagation in roller mutants is helical, rather than planar. In most of the mutants the phenotype is clearly expressed only in the adult form.

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Dumpy and small mutants: These animals are shorter than the wild type; dumpy mutants (Figure 1b) have the same diameter, but the small mutants (Figure 1c) are thinner and at least one seems to be an accurately scaled-down form of the wild type. Some of the dumpy mutants are also rollers. In many of the mutants the phenotype becomes expressed only in the later stages of growth, whereas others have altered larvae as well.

Long mutants: These mutants are longer and thinner than the wild type (Figure 1d).

Blistered mutants: In such mutants, fluid-filled blisters appear on the cuticle. Often the entire cuticle separates and the resulting single blister can squeeze the animal to death. This phenotype is expressed only in adults, the earlier larval stages appearing quite normal.

Abnormal mutants: In these mutants there are clear-cut aberrations in the morphology of the animal. They comprise a large and heterogeneous range of phenotypes on which not much work has, as yet, been done. Many of these abnormal mutants have variable phenotypes, and the mutations show low penetrance. One common variable abnormal phenotype is an animal with a notched head. All clones of such mutants contain animals that range from an apparently normal phenotype to very severe notching.

The distribution of phenotypes in the M and S sets of mutants is shown in Table 1. Most of the mutants fall into the major phenotypic classes, except for six in the M set, labelled residual. These have special properties and will be described elsewhere.

TABLE 1

Phenotypes, linkage and summary of mapping for M and S mutants

		Autosomal		Sex-linked		Unassigned		
Set	Phenotype	Located	Not located	Located	Not located	Dominant	Other	Total
M	Uncoordinated	173	39	41	59	9	43	364
	Dumpy and small	71	8	5	24	2*	0	110
	Long	5	0	4	1	0	0	10
	Roller	2	2	0	1	2	0	7
	Blistered	8	0	0	0	1	0	9
	Abnormal	0	17	0	3	0	24	44
	Residual	..	..	..	..	..	..	6
		259	66	50	88	14	67	550
S	Uncoordinated	14	4	1	9	2	16	46
	Dumpy and small	11	0	0	1	0	0	12
	Long	0	0	0	0	1	0	1
	Roller	1	1	0	1	0	0	3
	Blistered	0	0	0	0	0	0	0
	Abnormal	0	1	0	1	0	5	7
		26	6	1	12	3	21	69

* Recessive lethals.

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Characterization of the mutants: Many of the mutants picked turn out to be sterile and are discarded. Some do not breed true, producing mixed progeny. These are of two types: dominant mutants and mutants with variable penetrance. These can be distinguished by picking 8 to 12 individuals onto separate plates and observing their progeny. Some dominant mutants persist as heterozygotes. They segregate ⅓ wild-type animals and are thus either lethal when homozygous or have a closely linked independent lethal mutation. If any mutant segregates a second, independent mutant, the double is picked and the two mutations are separated later by recombination.

Most mutants with variable phenotypes are not further analyzed. The remainder are then crossed with wild-type males. The presence of males in the progeny indicates that mating was successful. The mutants are classified according to the rules given in Table 2. The distinction between dominance and semidominance can be a matter of fine judgment, but there are definitely cases in which the heterozygote cannot be distinguished from the parental homozygote. For these, no assignment of linkage can be made and they are included in the unassigned class in Table 1. It is also at this stage in the analysis that mutants are found with phenotypes that cannot be rapidly or reliably distinguished from wild type on plates containing both. Such marginal mutants are withdrawn and these, together with the mutants with variable phenotypes, account for most of the mutants labelled as unassigned in Table 1.

Very occasionally, the rules given in Table 2 break down. For example, among the residual mutants there are two which were initially classified as autosomal recessives but which later turned out to be sex-linked with no expression in the male.

Genetic complementation: Many of the mutants studied here have such severe defects in movement that males carrying such mutations in the homozygous form cannot mate efficiently with hermaphrodites. Autosomal recessives are, however, easily tested for complementation, by using heterozygous males. A mutant hermaphrodite (m_1/m_1, say) is mated with wild-type males. The heterozygous offspring males ($+/m_1$) are then crossed with tester hermaphrodites (m_2/m_2). In the resulting progeny only males are scored, since no distinction can be made between hermaphrodites produced by self-fertilization and those produced by fertilization with a male sperm carrying a non-complementary mutation. Of

TABLE 2

Classification of mutants: progeny of cross with wild-type males

Phenotype of progeny ♀	Phenotype of progeny ♂	Type
wild	wild	autosomal recessive
wild	mutant	sex-linked recessive
intermediate	intermediate	autosomal semidominant
intermediate	mutant	sex-linked semidominant
mutant	mutant	dominant

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these males, one-half must be wild type with genetic constitution $+/m_2$ since the male contributes one wild-type allele. The other half are m_1/m_2 males, and if these have the mutant phenotype then the two mutations do *not* complement each other. If all of the males are wild type then the two mutants complement.

This method cannot be used for sex-linked mutants. For these, tester strains are constructed containing another mutant, suitably chosen to allow the progeny of any cross to be distinguished from those produced by selfing. For example, in the case of sex-linked uncoordinated mutants, doubles are constructed with an autosomal dumpy mutant. The sex-linked mutant to be tested is crossed with wild-type males and the resulting hemizygous males are then crossed with the double. Large numbers of such males must be used because they express the defect and mate very poorly. These crosses are then examined for non-dumpy hermaphrodites, which can only be produced by mating. If these are uncoordinated then the two mutations are non-complementing, whereas the presence of wild-type progeny signifies complementation. Complementation tests on sex-linked mutants are difficult and there are some mutants in which the movement defect is so severe as to prevent these strains from being used as donors. Only a limited number of the sex-linked mutants isolated have been studied.

Location of mutants on linkage groups: As the number of complementation groups increased, allocation of new mutants involved more experiments, even if the testers were judiciously selected by phenotype. It became more effective first to locate the mutant on a linkage group and then to test it only with linked mutants. Sex-linked mutants are, of course, detected directly at the time of the initial backcross; special methods are required for the autosomal mutants. Consider the segregation of two recessive alleles *a* and *b* in the *trans* configuration. Table 3A shows the distribution of phenotypes in the progeny. If the mutants are unlinked, then the recombination frequency, p, is 0.5, and the phenotypes will show the classical 9:3:3:1 segregation ratios. Linked mutants can segregate by recombination, but the exact pattern will depend on the recombination frequencies in each of the germ lines in the hermaphrodite. An initial experiment with two distant sex-linked markers yielded the doubly recessive homozygote (AB in Table 3A) in the progeny of the *trans* heterozygote). This proved that recombination occurs in *both* germ lines and additional experiments, reported below, show that the recombination frequencies are approximately the same in both.

The *trans* configuration is used to identify the linkage group of a new mutant. Heterozygotes are constructed containing the mutant *trans* to tester mutants on the different linkage groups. The phenotype of the tester is chosen such that the double homozygote can be distinguished from both of the parents. Thus uncoordinated mutants are crossed with dumpy testers and morphological mutants with uncoordinated testers. The double heterozygotes are constructed as follows: The mutant (a/a) is crossed with wild-type males and the heterozygous male offspring $(+/a)$ then mated with tester hermaphrodites (b/b). The wild-type hermaphrodites produced by this cross are of two types, $++/+b$ and $a+/+b$. Five of these are picked onto separate plates and allowed to produce progeny. Clones that segregate only one of the parental types are discarded. The remaining

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TABLE 3

Patterns of segregation of phenotypes from doubly heterozygous hermaphrodites

A: *trans* heterozygote $a+/+b$

Sperm		Eggs: Parental $1-p$ $a+$	Parental $1-p$ $+b$	Recombinant p ab	Recombinant p $++$
Parental $1-p$	$a+$	A	W	A	W
	$+b$	W	B	B	W
Recombinant p	ab	A	B	AB	W
	$++$	W	W	W	W

B: *cis* heterozygote $++/ab$

Sperm		Eggs: Parental $1-p$ $++$	Parental $1-p$ ab	Recombinant p $a+$	Recombinant p $+b$
Parental $1-p$	$++$	W	W	W	W
	ab	W	AB	A	B
Recombinant p	$a+$	W	A	A	W
	$+b$	W	B	W	B

a and *b* are mutant recessive alleles and A and B their corresponding phenotypes.
W is wild type phenotype.

plates are then inspected for the occurrence of the double homozygote. In practice this is scored as the ratio AB/A, which is p^2. This is a very sensitive test for linkage; the ratio is 0.25 for unlinked mutants and is sharply reduced for linked mutants. Since most of the mutants studied are concentrated into clusters (see later) linkage is often signified by the absence of the AB class and it is not necessary to count progeny.

With these methods 285 autosomal recessive mutants have been allocated to complementation groups, 259 of the M set and 26 of the S set (Table 1). Most of the mutants that have not been allocated have phenotypes that are difficult to score in crosses with other markers either because of variable expression or because the double cannot be readily distinguished from one of the parents. For example, some uncoordinated mutants have phenotypes which are obscured in a dumpy background so that the dumpy uncoordinated double is very similar to the dumpy tester. Table 1 also shows that relatively fewer of the sex-linked mutants have been allocated because of the difficulties of performing the crosses. The sample is biased toward mutants which can mate and, as will be pointed out later, many of the genes characterized by a single mutant rely for their distinction on mapping results rather than on exhaustive complementation tests.

Table 4 shows the distribution of the M and S set mutants. The 259 M autosomal mutants define a total of 77 genes, 56 *unc*, 14 *dpy* and *sma*, 1 *lon*, 2 *rol* and

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TABLE 4

Occurrences of EMS mutants in mapped genes

Linkage group	Gene	Reference mutant	Number of isolates		Comments
			M	S	
I	*bli-3*	E767	1		
	unc-35	E259	1		
	unc-56	E403	2		
	unc-11	E47	2		
	unc-40	E271	1		
	unc-57	E406	2		
	unc-38	E264	4	1	Tetramisole-resistant
	unc-63	E384	2		Tetramisole-resistant
	dpy-5	E61	2		
	dpy-14	E188	1		Larvae abnormal
	unc-14	E57	5	1	Small, paralyzed body
	unc-37	E262	1		
	unc-15	E73	1		Paralyzed; defect in body muscle cells
	unc-55	E402	2		
	unc-13	E51	17		Paralyzed; pharyngeal movement irregular
	unc-21	E330	1		
	unc-29	E193	2		Tetramisole-resistant
	unc-54	E190	5		Paralyzed; defect in body muscle cells
	unc-59	E261	1		
II	*rol-2*	E489	1		
	bli-2	E768	4		
	dpy-2	E8	8		All alleles have roller phenotype
	dpy-10	E128	6	1	
	unc-4	E120	5		
	bli-1	E769	2		
	unc-53	E404	1		
	rol-1	E91	1		
	unc-52	E444	2		Progressive dystrophy of body musculature
III	*unc-45*	E286	1		Slow moving; defect in body muscle cells
	dpy-1	E1	20	3	
	dpy-17	E164	3	1	Larvae abnormal
	sma-4	E729		1	Semidominant
	sma-3	E491	2		
	unc-16	E109	1		
	lon-1	E185	5		
	sma-2	E502	3		
	unc-32	E189	1		
	unc-36	E251	3		
	unc-47	E307	3		
	unc-69	E587	2		
	unc-50	E306	2		
	unc-49	E382	3	1	
	dpy-18	E364		2	
	unc-71	E541	1		

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TABLE 4—Continued

Linkage group	Gene	Reference mutant	Number of isolates M	S	Comments
	bli-5	E518	1		
	unc-25	E156	3		
	unc-64	E246	1		
	unc-67	E713		1	
IV	*dpy-9*	E12	2		
	unc-33	E204	2	1	Paralyzed
	unc-17	E113	2		One allele lannate-resistant
	dpy-13	E184	5		Semidominant
	unc-77	E625	1		
	dpy-16	E225	1		
	unc-28	E15	1		Semidominant
	unc-5	E53	9		
	unc-8	E49	1		
	unc-44	E362	2	3	Small paralyzed
	unc-24	E138	2		
	unc-43	E408	2	1	
	unc-31	E169	7	1	Slow movement
	unc-22	E66	21		Twitching superimposed on normal movement
	unc-26	E205	9		
	unc-30	E191	6		
V	*unc-66*	E677	1		Paralyzed; defect in body muscle cells
	unc-60	E723		1	Paralyzed; defect in body muscle cells
	unc-34	E315	2		
	unc-62	E644	1		
	unc-46	E177	2	1	
	dpy-15	E24	1		Larvae abnormal
	unc-70	E524	1		
	unc-68	E540	3	1	
	dpy-11	E224	9	3	Same alleles are extreme dumpies
	unc-23	E25	5		Progressive dystrophy of head musculature
	unc-41	E268	5		
	rol-3	E754		1	
	sma-1	E30	8		Larvae have shortened round heads
	unc-42	E270	3		
	unc-65	E351	2		
	unc-61	E228	1		
	unc-39	E257	1		
	unc-51	E369	3	1	Paralyzed
X	*unc-1*	E94	12	1	
	dpy-3	E27	1		
	unc-2	E55	3		
	unc-20	E112	1		Temperature-sensitive
	dpy-8	E130	2		One allele temperature-sensitive
	lon-2	E678	4		
	dpy-6	E14	1		
	unc-6	E78	3		

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TABLE 4—Continued

Linkage group	Gene	Reference mutant	Number of isolates M	S	Comments
	dpy-7	E88	1		Roller
	unc-18	E81	1		Paralyzed
	unc-27	E155	1		
	unc-19	E174	1		
	unc-10	E102	3		
	unc-58	E665	2		Semidominant; animal shakes
	unc-9	E101	3		
	unc-12	E139	3		
	unc-7	E5	2		
	unc-3	E95	6		

The numbers of isolates in the M and S set are shown for genes on each of the linkage groups. The genes are in rough map order. Distinctive phenotypic characters, which are easy to describe, are summarized under comments.

4 *bli*. As may be expected from the average of 3.4 mutants per gene, there are frequent independent recurrences of mutants which do not complement each other, and many of the genes are characterized by more than one independent isolate. The 50 sex-linked mutants studied distribute over 18 genes, 13 *unc*, 4 *dpy* and *sma* and 1 *lon*. Although the sample is biased the distribution is not very different from that of the autosomal mutants. Most of the S set mutants occur in genes already defined by the M set. Even in the small sample of 26 mutants studied there are three instances, *dpy-1* III, *unc-44* IV, and *dpy-11* V, each with 3 independent recurrences. However, the S set includes mutants which define 5 additional genes, 2 *unc*, 2 *dpy* and *sma*, and 1 *rol*. This probably reflects the difference in the conditions under which mutants are picked; the isolation methods in the S set allow a wider range of phenotypes to be discerned. Mutants in *unc-44* IV are a striking example. Three were found in the S set and only two in the M set, where we might have expected about thirty. This mutant is a small paralyzed animal which grows slowly, and is easily obscured on crowded plates. One can show this by paying particular attention to this phenotype when picking mutants, and later mutant collections not included in the persent set have yielded many more alleles of this gene. Among these there are also repeats of some of the genes previously defined only by an S-set mutant, in particular *sma*-4 III.

It is our practice to define a gene only when it has been mapped (see below). The 96 genes characterized in Table 4 do not exhaust the number of complementation groups. Thus we know that the three unallocated roller mutants shown in Table 1 are not only different from all the defined roller mutants but are also different from each other by complementation tests. There are, therefore, three more *rol* genes, but they have not been defined because of difficulties in mapping them. The same is true of the abnormal mutants. Complementation tests have been done on a subset of these and the results are shown in Table 5. There are two complementation groups for the eight notched head mutants. The two bent head mutants fall into a single group. These mutants cannot be easily mapped because

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TABLE 5

Distribution of a subset of EMS variable abnormal mutants

Class	Phenotype	Reference mutant	Number of isolates	
			M	S
1	Notched head, variable	E2	6	0
2	Notched head, variable	E96	2	0
3	Bent head, variable	E611	2	0
4	Defective head, variable	E108	1	0
5	Twisted body, variable	E697	1	1

of their variability, but the results again show that independent recurrences of mutants in the same gene are common.

Mapping of mutants: To explain how mutants are mapped in the hermaphrodites, we will assume that the frequency of recombination in both germ lines is the same; this assumption will be justified later. The *trans* configuration ($a+/+b$) cannot be easily used to measure recombination between a pair of recessive mutants. As Table 3A shows, the only recombinant phenotype that can be directly scored is the double recessive homozygote, AB, and its frequency is a function of p^2. Such crosses exaggerate linkage and are insensitive measures of p. Mapping requires the *cis* configuration ($++/ab$), but before such heterozygotes can be constructed the double must first be obtained. Examination of Table 3A shows that the animals with either of the parental phenotypes, A say, are of two types: parental homozygotes $a+/a+$ and recombinant heterozygotes, $a+/ab$. The fraction of the latter is $2p(1-p)/((1-p)^2+2p(1-p))$ or approximately $2p$ for small p. The doubles are isolated as segregants from these animals. Either A or B animals are picked, usually six to a plate, and their progeny examined for doubles. Doubles of mutants separated by about 1% recombination can readily be isolated as segregants by picking 50 to 100 animals, and doubles from mutants more closely linked than this have been obtained by using larger numbers. There are, however, other ways of constructing very close doubles, and one method is discussed later.

Once the double mutant has been isolated the cis double heterozygote is constructed simply by crossing it with wild-type males. Table 3B shows that among the segregants the recombinant phenotypes A and B are obtained with total frequences of $(4p(1-\text{p})+2p^2)/4$, that is as a more *linear* function of p. In practice, all the animals on such plates are scored wherever possible and the frequency of recombination, $p=1-\sqrt{1-2R}$, where R is the fraction of recombinant phenotypes. In cases where all the phenotypes cannot be distinguished from each other or where there are differences in viability, recombination can be calculated from the measurement of any one recombinant class and either of the parental types.

Usually a thousand or more animals are counted in such crosses, removing the animals from the plate as they are counted. If necessary, crosses can be counted over 24- or 36-hour periods to adjust for the age distribution of the animals and

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to prevent any difference in growth rates of the mutants from biasing the results. It is quite laborious to count such crosses, and with very closely linked mutants the recombination distances are likely to be unreliable since only a few recombinants were detected. For more distant mutants the results are less subject to statistical fluctuations.

Sex-linked recessive mutants are mapped in the same way, although the construction of double mutants is much more difficult. However, there are some sex-linked mutants which produce good males such as *lon*-2. A few temperature-sensitive mutants have been used as well. In these, males produced at the permissive temperature of 15° have almost wild-type behavior and will mate successfully. The crosses are scored at a higher temperature to display the phenotype.

Some semidominant mutants have been mapped using the same methods. However, if the wild-type phenotype can be unambiguously distinguished from the intermediate phenotype then recombination frequencies can be obtained from segregation of the *trans* heterozygote. In the heterozygote $a+/+b$, if a is semidominant then one can detect all of the segregants which do *not* contain this allele; this class contains $+b/+b$ animals which are parental, and $+b/++$, and $++/++$ which are recombinant. In this case, p is given by $1-\sqrt{1-R}$, where R is the recombinant fraction.

The method of analysis proposed depends on the assumption that the frequency of recombination is the same in both germ lines. Actually it would not matter very much if it was different; one would still get a valid recombination measure but it would be related to the geometrical mean of the two values. In order to test whether it is the same, recombination frequency must be measured in only one of the germ lines. This can be done for sex-linked markers in eggs. If appropriately marked hermaphrodites are crossed with males, then the progeny males inherit the X chromosome of the egg and the fraction of recombinants is exactly the recombination frequency. The results of two such experiments are shown in Table 6. In the first experiment, the markers used, *lon-2* and *unc-6* are both recessive, and IH shows the results for segregation from the *cis* heterozygote. In IX, the *trans* heterozygote was crossed with wild-type males and only progeny males were scored. The recombination values obtained are not significantly different. In cross 2, *unc-58* is semidominant and segregation of the *trans* heterozygote was analyzed in IH. Although there is some difference between the two values it is in the opposite direction from that of cross 1 and probably within statistical error. Since the values obtained by segregation of hermaphrodites are the same as that found in male progeny of crosses, the recombination frequencies in the two germ lines in the hermaphrodite cannot be too different. Notice that these experiments say nothing about recombination in the sperm line in males: one experiment has been carried out which shows that recombination also occurs in these cells.

Characterized genes have been mapped using segregation from *cis* heterozygotes. The method is restricted to mutants with different phenotypes. One must be able to distinguish the double from at least one of the single mutants, if not both. All possible two-factor crosses therefore cannot be carried out. Table 7

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TABLE 6

Comparison of recombination frequencies in two lines of the hermaphrodite

Cross		Score			Percent recombination
1 H	Segregation of ++/*lon-2 unc-6* ♀	all ♀	wild	822	
			unc long	231	
			unc	34	
			long	34	6.3 ± 1.7
1 X	++ ♂ by *lon-2* +/+ *unc-6* ♀	♂	unc	270	
			long	283	
			wild	24	
			unc long	25	7.9 ± 1.4
2 H	Segregation of *lon-2* +/+ *unc-58* ♀	non-unc ♀	long	387	
			wild	133	13.6 ± 1.7
2 X	++ ♂ by *lon-2* +/+ *unc-58* ♀	non-unc ♂	long	421	
			wild	43	9.3 ± 1.8

TABLE 7

Recombination between dpy *and* unc *genes on different linkage groups*

Linkage group	*dpy-*	Mutant	*unc-*	Mutant	W	D	U	UD	Percent recombination
X	6	E14	6	E78	1308	24	25	394	2.8
	6	E14	3	E95	826	97	110	213	18.3
I	5	E61	13	E51	1771	28	—	—	2.4
	5	E61	13	E312	865	12	—	—	2.1
	5	E61	54	E190	582	106	—	—	26.7
	5	E61	54	E843	632	133	—	—	30.8
III	1	E1	32	E189	1090	149	163	261	21.0
	1	E745	32	E189	808	114	123	177	21.8
IV	13	E184	17	E113	757	14	—	—	2.7
	13	E184	17	E245	1270	26	—	—	3.0
	13	E184	17	E464	990	20	—	—	3.0
	13	E184	30	E191	623	32	—	—	7.7
	13	E184	30	E318	773	45	—	—	8.6

The phenotypes—W = wild, D = dumpy, U = uncoordinated and UD = uncoordinated dumpy—were scored in the progeny of *cis* heterozygotes (++/*dpy unc*). In some of the crosses only the wild and dumpy animals were counted.

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shows a sample of the results for a set of uncoordinated and dumpy mutants on different linkage groups. In the cases where all four phenotypes have been scored there is an agreement with the expected 3:1 ratio for segregation of the recessive alleles. The table also allows a comparison to be made between independent crosses using different alleles of the same genes; the deviations that are found are not alarmingly large.

The recombination distances estimated from two-factor crosses are in many cases insufficient to establish the map order of mutants. To order genes unambiguously, three-factor crosses, using linked outside markers, must be carried out. Table 8 shows the general principle of the method used. In outline, a heterozygote is constructed of a double mutant $a\ b$ trans to a third mutant, c, by crossing $+/c$ males with homozygous $a\ b/a\ b$ hermaphrodites. The animals are allowed to segregate, and recombinants picked onto separate plates. Wherever possible both the A and B class of recombinants are used. Each recombinant must be homozygous for a or b, but is likely to contain the parental $a\ b$ genotype if the mutants are reasonably closely linked. In any case, the structure of the recombinational heterozygote is deduced from the phenotypes of its segregants. Table 8 shows how the c marker is expected to distribute, in the first case, where it is outside a and b and, in the second case, where it lies between them. If the three mutants used have different phenotypes then the plates can be scored directly by inspection. Quite often, however, two of the mutants have similar phenotypes and the segregants can only be typed by counting. For example, if b and c are both dumpy mutants and a is an uncoordinated mutant, then the distinction between the $+a+/+ab$ and the $ca+/+ab$ recombinant heterozygotes must be made

TABLE 8

Three-factor crosses: segregants of recombinant heterozygotes

Heterozygote	Recombinant phenotype		Predominant genetic structures	Phenotypes of segregants		
$\frac{+\ a\ b}{c\ +\ +}$	A		$\frac{+\ a\ +}{+\ a\ b}$	A	AB	
	B		$\frac{c\ +\ b}{+\ a\ b}$	B	AB	CB
$\frac{a\ +\ b}{+\ c\ +}$	A		$\frac{a\ +\ +}{a\ +\ b}$	A	AB	
		and	$\frac{a\ c\ +}{a\ +\ b}$	A	AB	AC
	B		$\frac{+\ +\ b}{a\ +\ b}$	B	AB	
		and	$\frac{+\ c\ b}{a\ +\ b}$	B	AB	BC

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by scoring the ratio of uncoordinated to dumpy uncoordinated segregants. This is 3:1 in the first case and 1:1 in the second. As the map distance between the mutants increases the recombinant heterozygotes can include other classes and a large number must be studied to obtain unequivocal results.

Three-factor crosses are a good source of new double mutants, and very close doubles may be constructed in this way using recombination in one interval to select for the class containing the desired recombination event.

The genetic map of the mutants is shown in Figure 2. For completeness' sake the map also includes genes in which mutants have been isolated either spontaneously or after ^{32}P decay (P. BABU and S. BRENNER, unpublished) but which are not represented in the present set of EMS mutants. There are six linkage groups. Wherever possible, terminal markers have been crossed with markers on other linkage groups and in all cases recombination values close to 50% have been obtained. There is a strong tendency for mutant sites to be concentrated in one region of a linkage group to form clusters. Once this was realized, it became easy to locate new mutants on a linkage group by crossing them with an appropriate marker in the cluster. On the other hand, this property makes it diffi-

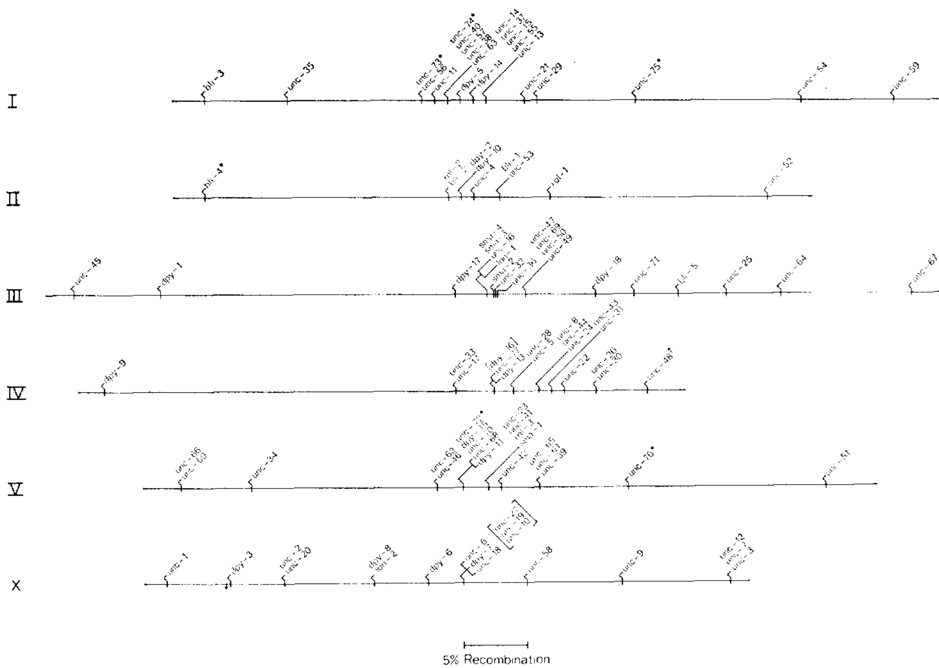

FIGURE 2.—Genetic map of *C. elegans*. The locations are shown only for mutants that have been unambiguously ordered. Two genes joined to the same location means that no recombination has been detected between them (< 0.5%). In other cases, the genes are known to be closely linked but the internal order has not been determined. The map position of bracketed markers is only approximately known. All the genes are represented by EMS-induced mutants, except for those marked * which were isolated after ^{32}P decay (BABU and BRENNER, unpublished results), and for the site marked †, which is a spontaneous mutant. Four of the genes with a blistered phenotype (*bli-1* to *bli-4*) were mapped by MR. JONATHAN HODGKIN.

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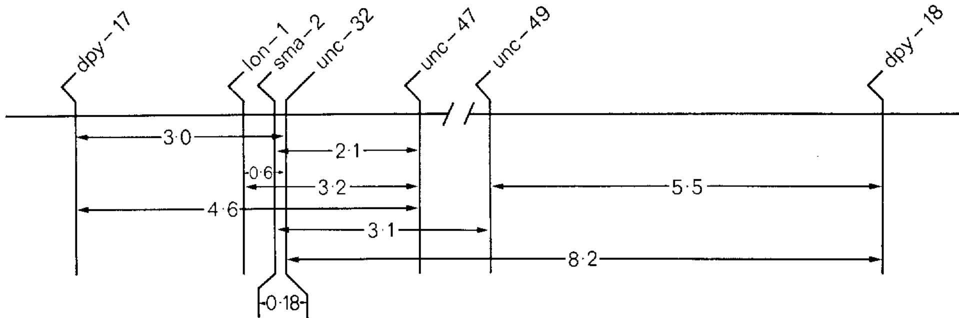

FIGURE 3.—Two-factor recombination values for a region of linkage group III. With the exception of *unc*-47 and *unc*-49, all of the sites have been ordered independently by three-factor crosses.

cult to order mutants within the cluster and for many of the closely linked uncoordinated mutants all that has been done is to show that they are on one or the other side of a dumpy marker. The metric of the map is taken from selected two-factor crosses. In Figure 3, part of the map of linkage group III is shown in more detail. Most of the mutants have been ordered independently by three-factor crosses and the map shows that the additivity of map distance over this region is satisfactory.

Lethal mutants: It is not possible to estimate lethal mutation rates using wild-type strains. Even the classical sex-linked lethal assay cannot be used because animals produced by self-fertilization cannot be distinguished from those produced by mating. However, once genetic markers become available this experiment could be done. One such experiment is briefly described. S17 is a double mutant, *sma-2* III *lon-2* X; it has a small phenotype since *sma-2* is epistatic to *lon-2*. When this mutant is crossed with wild-type males, three kinds of progeny are found: small hermaphrodites resulting from selfing, and wild-type hermaphrodites and long males produced by mating. S17 was treated with EMS in the standard way and single young adults placed on a large number of separate plates. After a few days, one F_1 progeny was picked from each plate and mated with 2 to 3 wild-type males. The numbers of wild-type hermaphrodites and long males were then counted. Plates with less than 25 of these were discarded and a sex-linked lethal was scored when the males were less than 50% of the cross progeny. In 74 crosses 20 sex-linked lethals were detected giving the crude ratio $R = 0.270$. The frequency of induced lethals per X chromosome is $1 - \sqrt{1 - R} = 0.15$, which also corrects for double events.

Drug-resistant mutants: The ease with which large numbers of nematodes can be obtained and handled suggested the possibility of using selective methods for the isolation of mutants. About one hundred compounds including metabolic analogs, antibiotics, and neuropharmacological agents were screened. Most are without effect on the worms, probably because they are not absorbed. Two of the drugs tested proved sufficiently interesting to warrant an attempt to isolate resistant mutants. The results are briefly described below.

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Lannate is a trivial name for $CH_3NHCO.ON{:}\ C(CH_3)SCH_3$, an oxime ester which is a potent inhibtor of acetyl-cholinesterase. At a concentration of 10^{-4} M it paralyzes the nematodes producing hypercontraction, cessation of pharyngeal movement and eventual death. To select for resistant mutants, F_2 progeny were collected from mutagenized animals grown on standard NG plates and placed on NG plates containing 10^{-4} M lannate. Most of the animals died and after a few days the survivors were examined for mutants with improved movement. Many of these were only marginally resistant on retesting, but there was one class of mutants with a definite phenotype. These were clearly resistant, but when transferred and grown on normal plates were found to be severely uncoordinated. In fact, movement is better on the drug-containing plate, so that these mutants are probably better described as lannate-dependent than resistant. There were seven independent mutants with these properties and they all failed to complement each other in tests where the uncoordinated phenotype was scored. Further tests showed that the mutants were in the gene *unc-17* IV, and failed to complement both of the mutants isolated in this gene in the standard way. These mutants have different phenotypes; one E113, is larger and less uncoordinated than the other, E464, which resembles the lannate-resistant set. When tested on lannate plates, E464 was found to be resistant, while E113 is not. This gene is being studied further.

Tetramisole is a recently discovered broad spectrum anthelmintic (THIENPOINT *et al.* 1966). It has interesting effects on *C. elegans*, inducing a hypercontraction of the body and paralysis of the pharynx. A reasonable surmise is that it acts as an acetylcholine agonist since the paralysis resembles that produced by lannate. At 10^{-4} M the animals are semiparalyzed and hypercontracted but can still feed and lay eggs. To look for resistant mutants, 20 mutagenized young adults were placed on one side of a large NG plate containing 10^{-4} M tetramisole. Both the F_1 and F_2 progeny were examined for mutants with improved movement; such animals migrate more quickly across the plate than the sensitive wild type, which helps to detect them.

Among the F_1 animals, individuals were found which appeared to be partially resistant. These were less hypercontracted and moved better than the wild type. Four of these were picked from different plates to ensure independence, and all were found to segregate twitchers, a phenotype characteristic of mutants in *unc-22* IV. That all were mutants in this gene was confirmed by complementation tests. As may be expected, heterozygotes of twitchers isolated by direct picking turn out to be partially tetramisole-resistant. Although the twitching phenotype is fully recessive in the heterozygote, the mutant gene can be detected by the altered response to the drug.

When the F_2 animals were examined, twitchers were found in abundance. In addition to these there were other resistant mutants with normal body length and vastly improved movement. Fourteen independent mutants of this type were picked. When grown on standard plates eleven showed an uncoordinated phenotype, whereas three were nearly normal. The first set could be complemented by scoring for the uncoordinated phenotype, the remaining mutants were tested on tetramisole plates. As shown in Table 9, all the mutants with a normal pheno-

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TABLE 9

Properties of tetramisole-resistant mutants

Gene	Phenotype	Number of isolates	Equivalence
tmr-1	normal	3	
tmr-2	uncoordinated	3	*unc-38* I
tmr-3	uncoordinated	7	*unc-63* I
tmr-4	uncoordinated	1	*unc-29* I

type were in the same gene, whereas the uncoordinated mutants fell into three different complementation groups. These were found to be genes already identified by uncoordinated mutants picked by direct inspection. Not only did the tetramisole-resistant mutants have the same uncoordinated phenotype as the other alleles, but these, in turn, when tested were found to be tetramisole-resistant. The basis for resistance is at present unknown, but the *tmr* mutants are also resistant to another drug, pyrantel tartrate, which has the same effect as tetramisole, but is less potent, producing the characteristic hypercontraction at 10^{-3} M.

DISCUSSION

The experiments reported in this paper show that *C. elegans* is a favorable organism for genetical analysis. Apart from the general advantages of small size and a rapid life cycle, the sexual system of self-fertilization makes it easy to isolate recessive mutants on all chromosomes. Since propagation of the animal does not depend on mating, stocks can be produced from homozygous mutants with severely defective phenotypes. In a bisexual strain these would be inviable. Unlike Drosophila, nematodes do not have a rich repertoire of external features for mutant selection, but this has not prevented the isolation of visible mutants. Indeed, it has had the effect of focusing selection on the behavioral characteristics of the animals and uncoordinated mutants form the largest class of mutants. Methods have been developed for complementing and mapping mutants, and the difficulties with sex-linked mutants can now be overcome by the recent discovery of a class of transformer mutants that are effective XX males (J. Hodgkin and S. Brenner, unpublished results).

Only two comments need be made about the genetic map. The six linkage groups correspond satisfactorily with the haploid number of chromosomes found by Nigon (1949). The strong tendency for the clustering of mutant sites on chromosomes cannot, at the moment, be explained. It is unlikely that this corresponds to some functional association since the clusters include mutants with all phenotypes. One possibility is that the clusters are produced by a lower frequency of recombination in a defined region of a chromosome—for example, near the centromere—but more work is required to determine the exact distribution. This is particularly true for the X chromosome where the clustering is not so well defined, but the mutants studied are a selected and incomplete sample.

The most striking feature of the results is the small number of genes in which the observable mutants occur. Table 10 shows how the 258 autosomal mutants

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TABLE 10

Distribution of recurrences of autosomal mutants

Recurrences	Number of genes
1	27
2	21
3–4	11
5–8	12
>8	6

distribute over the 77 defined genes. This is a far from random distribution and is probably biased by the efficiency with which different mutants are picked up by direct inspection. Nevertheless, the results strongly suggest that for the level of phenotype recognition used, the spectrum must be nearly saturated. This does not mean that there are no more mutants to be found. In no sense can the characterized uncoordinated mutants be said to exhaust the set of genes specifying the nervous system, and more precise methods of detection would certainly uncover many more genes. Indeed there already exists a large number of mutants which are erratic in their movement but which have not yet been characterized. On the other hand, for those mutants with a distinctive and perhaps extreme phenotype, we can say that the subset is nearly exhausted. Thus there is only one gene in which mutations occur to produce twitching animals, since all of the 21 mutants are found in one complementation group, assigned *unc-22* IV.

What is surprising is that even though the genes identified represent a single and possibly small subset, their forward induced rates of mutation are very high. In the S set, among 318 F_1 studied, 69 segregated mutants, and 26 of these were autosomal mutants that could be located (Table 1). Since each F_1 could yield mutants induced on either of the parental homologs, the total forward induced rate of mutation for this subset of classifiable mutants is $\frac{1}{2} \times 26/318$ or 4.1%. We conservatively assume that these mutants are samples of the larger group of M mutants, which provides 77 genes of this class. This allows us to estimate that the average forward mutation rate induced by EMS under the conditions used is about 5×10^{-4} per gene.

As shown in the accompanying paper, the unique sequence component of the DNA of *C. elegans* is 6.7×10^7 base pairs (SULSTON and BRENNER 1974). This could code for about 6.7×10^4 average polypeptide chains, taking 10^3 base pairs as the average coding length required. Given that the genes studied are representative of all the genome, then after treatment with EMS each diploid complement would have received a total of 34 effective mutational hits which would be lethals if all the DNA was used to code for indispensable functions. It is immediately obvious that this cannot be true; it would mean that each visible mutation is accompanied by about six linked lethal mutations, and since the mutants are isolated as segregants we should never have been able to isolate homozygous mutants at all. This argument is reinforced by the measurements of the rate with which lethals are induced on the sex chromosome. The induced lethal frequency is 0.15 per X chromosome, and if the lethal genes do not differ from the subset

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of visible mutants this would be given by 300 genes with an average induced forward rate of 5×10^{-4}. Extending this to the six chromosomes of *C. elegans* leads to an estimate of about 2,000 genes with indispensable functions in this organism. This is a surprisingly small number and is at least one order of magnitude less than that expected from the coding potential of the DNA.

There are, of course, trivial explanations for these results. It could be argued that the bulk of the DNA is in a specific structure which protects the bases from the action of chemical mutagens, or effectively repairs the mutational lesions. Such explanations are not easy to eliminate, but what is striking is that our results on the nematode are very similar to those found in Drosophila. Work by JUDD *et al.* (1972) and others (HOCHMAN 1971; LIFSCHYTZ 1971) has shown that there is a one-to-one correspondence between genetic complementation units units and the chromomeres on the giant salivary chromosomes. On the average, each gene would then contain about 20,000 base pairs of DNA. Our estimate in *C. elegans* would not be far off this value. As mentioned previously, the reason for the large size of the genetic units in higher organisms is unknown. At the moment, we cannot tell whether the mutants we have found are in coding sequences or in elements concerned with regulation. Further genetical analysis might help to resolve these problems.

We have already begun to analyze the mutants described in this paper. Some of the uncoordinated mutants have distinctive abnormalities in the nervous system. For example, the dorsal nerve cord is absent in several independent *unc-5* IV mutants, while mutants in *unc-30* IV have a specific lesion affecting a subset of neurons in the ventral nerve cord (S. BRENNER, unpublished results). It appears likely that many of the uncoordinated phenotypes are specific developmental mutants of the nervous system, altering the establishment of proper connections. Others may, of course, affect the working of a correctly wired system; but our studies are not yet deep enough to reveal this class. As might have been expected, the uncoordinated mutants include animals with defective body musculature (Table 5). In some of these, the regular structure is absent in the body muscle cells, while others seem to be dystrophic in character (H. EPSTEIN, R. WATERSON and S. BRENNER, unpublished results).

Many other kinds of mutants can and have been isolated in *C. elegans* in this laboratory. The ease of handling of the nematode coupled with its small genome size suggests that it is feasible to look for mutants in all of the genes to try to discover how they participate in the development and functioning of a simple multicellular organism.

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