GENETIC AND PHENOTYPIC CHARACTERIZATION OF ROLLER MUTANTS OF *CAENORHABDITIS ELEGANS*

GEORGE N. COX, JOHN S. LAUFER,[1] MEREDITH KUSCH AND ROBERT S. EDGAR

Thimann Laboratories, University of California, Santa Cruz, Santa Cruz, California 95064

Manuscript received June 4, 1979
Revised copy received February 8, 1980

ABSTRACT

Eighty-eight mutants of *C. elegans* that display a roller phenotype (a helically twisted body) have been isolated and characterized genetically and phenotypically. The mutations are located in 14 different genes. Most genes contain a number of alleles. Their distribution among the chromosomes appears nonrandom, with seven of the genes being located on linkage group II, some very closely linked. The phenotypes of the mutants suggest that there are five different classes of genes, each class representing a set of similar phenotypic effects: Left Roller (four genes), Right Roller (one gene), Left Squat (one gene), Right Squat (two genes) and Left Dumpy Roller (six genes). The classes of mutants differ with respect to a number of characteristics that include the developmental stages affected and the types of aberrations observed in cuticle structure. A variety of gene interactions were found, arguing that these genes are involved in a common developmental process. The presence of alterations in cuticle morphology strongly suggests that these genes are active in the formation of the nematode cuticle.

THE cuticle of the free-living soil nematode, *Caenorhabditis elegans*, is a potentially interesting model system for genetic and physiological studies of animal development. The cuticle is a highly complex extracellular structure elaborated by the underlying layer of hypodermal cells. It is shed four times during the animal's three-day development and a new cuticle is formed at each molt (BIRD 1971; ZUCKERMAN, HIMMELHOCH and KISIEL 1973; SINGH and SULSTON 1978).

Our efforts to develop this system for study involve morphological and chemical characterization of the cuticle and the isolation and analysis of mutants that appear to have alterations in cuticle structure. One such class of mutants, the roller mutants, are the subject of this report.

Roller mutants were first described by BRENNER (1974). The wild-type nematode moves in a sinusoidal wave pattern on the agar surface of a culture dish. Roller mutants rotate around their long axis and describe circular paths on the substrate. HIGGINS and HIRSH (1977) showed that this striking behavior is due to the fact that the whole animal, both its external cuticle and its internal organs (the ventral nerve cord and the body muscles), is helically twisted. They further

[1] Present address: Dept. of Molecular, Cellular and Developmental Biology, Univ. of Colorado, Boulder, Colorado 80309.

showed that some mutants have a left-handed helical twist, while others have a right-handed helical twist to their body. Circumstantial evidence suggested to these workers that the primary alteration in the roller mutants lay in the structure of the cuticle. Since the cuticle serves a skeletal function for the animal and is the point of attachment for the body muscle cells, this proposal seemed most plausible.

Following the study of HIGGINS and HIRSH, we have collected a large number of roller mutants. Genetic analysis has permitted us to identify 14 genes that can mutate to give a roller phenotype. Analysis of the phenotypes of the mutants has allowed us to group these genes into distinctive classes.

MATERIALS AND METHODS

Strains: *Caenorhabditis elegans* var. Bristol (wild-type strain designated N2) was originally obtained from SYDNEY BRENNER. The prefix SC is used to identify mutant strains isolated in our laboratory, except where noted below. Thirty-one roller mutants were sent to us by workers in other laboratories: mutants prefixed by the letter E were from the collection of SYDNEY BRENNER, SU1001 through SU1010 were from HENRY EPSTEIN, M38 and M39 were from DONALD RIDDLE, B238 from DAVID HIRSH, and SC26 and SC86 were from SAMUEL WARD and JAMES LEWIS, respectively. SC-M1, a strain containing *dpy-2(e8) rol-6(sc90) unc-4(e26)*, was also obtained from SYDNEY BRENNER.

Unless specifically noted otherwise, roller alleles used in crosses were those listed in Table 3. The following genes and alleles were also used in this study: *bli-4(e937)I*, *bli-2(e768)II*, *unc-4 (e120)II*, *unc-53(e404)II*, *bli-1(sc73)II*, *dpy-4(e1166)IV*, *dpy-11(e224)V*, *unc-42(e270)V*, *him-5(e1467)V* and *unc-76(e911)V*.

The genetic nomenclature used in this report conforms to the system agreed upon by most *C. elegans* workers. (H. R. HORVITZ, personal communication). Gene names consist of 3 lower-case italicized letters that are abbreviations for a general broad phenotype, *e.g.*, *rol* for roller, *dpy* for dumpy. An arabic numeral is used to distinguish different genes producing a similar phenotype. Alleles are bracketed. Dpy, Rol, and Rol-1 are examples of phenotypic abbreviations, *e.g.*, a roller mutant in the gene *rol-1* is said to express the phenotype Rol or, when ambiguous, Rol-1.

Genetic analysis: The general methods of strain culture, mutagenesis and genetic analysis were those described by BRENNER (1974). The following modification of the method of mutagenesis was used in some of our isolation experiments: A population (5,000 to 10,000) of roughly synchronized L3 and L4 juvenile animals was exposed to ethyl methanesulfonate (EMS, 0.05 M) for 4 hr. These animals were then placed on large petri dishes (100 mm) of NG agar (BRENNER 1974) seeded with *E. coli* OP50 and allowed to mature and lay *ca.* 1000 eggs per plate (100 animals per plate for 2 hr). At this time, the adults were gently rinsed off the plates, and the eggs, which stick to the agar surface, were allowed to hatch and develop. When mature, these F_1 animals were washed from the plates, counted and placed on new plates for a short period of time (30 to 60 min) such that each F_1 worm could lay only 2 to 10 eggs. These F_2 eggs were permitted to hatch and mature into adults, at which time roller mutants were picked by visual inspection of these plates. This method permits a relatively synchronous large population of F_2 animals to be examined simultaneously. Because each F_1 animal contributes only a very small proportion of the F_2, the likelihood of isolating mutant F_2 siblings is diminished, although not completely eliminated. It appears likely that a few sibling mutants were in fact isolated; these cases are pointed out in the text.

All mutants were backcrossed to N2 wild type before any genetic or phenotypic characterization was conducted. Complementation tests between the autosomal recessive mutants were performed as described by BRENNER (1974) by mating males heterozygous for one roller mutation to hermaphrodites homozygous for a different roller mutation and scoring the phenotype of the progeny males. Because many of the mutants exhibited a temperature-sensitive phenotype, these

tests were normally done at 25°. The temperature sensitivity of many of the sex-linked roller mutants facilitated complementation tests between them by allowing us to construct phenotypically wild-type hemizygous tester males to use in the complementation tests at 25°. Allelism between the sex-linked mutants was scored by the presence or absence of wild-type hermaphrodite progeny on cross plates containing mutant male progeny (the presence of F_1 male progeny indicating that matings had occurred).

Recombination frequencies between linked genes were determined as outlined by BRENNER (1974) by counting the self-progeny of hermaphrodites heterozygous for 2 markers in the *cis* configuration, *e.g.*, *unc-42 rol-4/++*. Hermaphrodites were transferred daily during the egg-laying period, and all progeny were scored. Mapping experiments were performed at 20°, except in cases of *sqt-3* and the semi-dominant mutation, *sc78*, for which crosses were made at 25°.

Light microscopy: A Zeiss microscope fitted with phase, polarizing and fluorescence (utilizing a neon lamp) optics was used in these studies.

Cuticle isolation: Adult worms were washed in M9/10 [M9 buffer (BRENNER 1974) diluted 1:9 with distilled water] and sonicated briefly (3 × 0.30 seconds) to puncture, but not disrupt, the cuticle. The damaged animals were centrifuged and washed several times in this low ionic strength buffer. In some experiments, the cuticles prepared in this manner were further washed in 1% sodium dodecyl sulfate (SDS) to remove any residual noncuticular material (unpublished observations).

Antibody preparation and use: Anti-cuticle antibodies were prepared by injecting adult cuticles (partially purified by sonication in low ionic strength buffer) into rabbits with complete Freunds adjuvant. Booster cuticle injections were given at monthly intervals and the rabbits were bled 7 to 10 days following each of the booster shots. Fluorescent nematodes were prepared by incubating live worms with a 10-fold dilution of this serum in phosphate buffered saline (0.145 M NaCl, 0.01 M sodium phosphate, pH 7.1) for 30 min, washing several times and then incubating an additional 30 min with a similarly prepared 10-fold dilution of fluorescein-labelled goat anti-rabbit antibody (Grand Island Biological Supply Company). Unbound antibody was removed by extensive washing in veronal saline buffer (10.3 g sodium veronal, 6.2 g sodium chloride, to 1 liter, pH 8.6) before samples were removed and mounted for observation in this medium. Nematodes remain alive through these procedures and seem to suffer no ill effects from the attached antibodies.

RESULTS

Origin of mutants

All 88 of the roller mutants analyzed in this study arose after treatment with EMS, and isolation procedures were used to enhance the likelihood that the isolates arose by independent mutational events. Some of the E mutants and most of the SC mutants were isolated as displaying an adult roller phenotype at 25° so as to permit the recovery of temperature-sensitive mutants, *i.e.*, mutants that would exhibit the roller phenotype at 25°, but not at 16°, the two growth extremes for *C. elegans* (HIRSH and VANDERSLICE 1976).

Most of the mutants arose as F_1 segregants from mutagen-treated animals and, with only a few exceptions, proved to be recessive in phenotype. As would be expected, roller mutants that were isolated from among the F_1 progeny of mutagen-treated animals proved to be dominant. Interestingly, nearly all of these animals were found to belong to a special class of dominant mutants that we term "squat"; their peculiar characteristics are described in a later section.

One mutant, *rol-6(sc90)*, was extracted by recombination from SC-M1, a strain that also contains *dpy-2(e8) unc-4(e26)*. The presence of *sc90* within

this strain had previously led to the incorrect assignment of *rol-6* and *dpy-2* alleles to the same gene (BRENNER 1974). Since *sc90* does not confer a visible phenotype, it can be identified only by its failure to complement other alleles of *rol-6*.

Complementation analyses and gene assignments

Recessive mutants: Complementation tests revealed that many of the new roller mutants were alleles of genes previously identified by BRENNER (1974) as capable of mutating to give a roller phenotype: *rol-1*, *rol-3*, *dpy-2* and *dpy-7*. In the case of the latter two genes, some alleles confer a dumpy roller phenotype (a short plump animals that rolls). *rol-2*, a roller gene described by BRENNER (1974), has been determined to be the same as *dpy-2* (R. HERMAN, personal communication). Some of the remaining roller mutations were found to be alleles of the genes *dpy-3*, *dpy-8*, *dpy-10* and *dpy-15* (BRENNER 1974) for which only nonroller dumpy alleles had previously been isolated. The remaining recessive mutants served to identify three genes not previously described. We have named these genes *rol-4*, *rol-5* and *rol-6*. Evidence on the location of these genes is presented in Tables 1 and 2. Intragenic complementation was observed with certain alleles of *rol-5*. This was the only instance where this phenomenon was observed among recessive alleles of roller genes.

Dominant mutations: In addition to the squat mutants described separately below, four other roller mutants were found to be dominant or semi-dominant. SC6 and SC7 are both semi-dominant right rollers. While animals homozygous for *sc6* and *sc7* are strong rollers, heterozygotes roll occasionally and catch their heads on the agar surface, signs of a weak roller phenotype. These mutations appear to be alleles of *rol-6* since the *trans* double heterozygotes with the recessive *rol-6* allele, *e187*, display a strong roller phenotype.

Another right roller, SU1006, is a strong dominant mutant, the heterozygote having a phenotype indistinguishable from the homozygote. This mutation has tentatively been assigned to the *rol-6* gene only on the basis of recombination data since allelism tests with this mutant are not interpretable. *su1006* gives less than 0.025% recombination with *e187*.

TABLE 1

Two-factor crosses

Gene	Allele	Linkage group	Marker used	R*	$p \times 100$†
rol-4	*sc8*	V	*unc-42*	55/1930	2.9 ± 0.7
rol-5	*sc13*	II	*dpy-10*	76/2986	2.6 ± 0.6
			unc-4	14/820	1.7 ± 0.9
rol-6	*e187*	II	*dpy-2*	70/8866	0.8 ± 0.2
sqt-1	*su1005*	II	*unc-4*	34/1944	1.8 ± 0.6
sqt-2	*sc64*	II	*unc-4*	230/1367	18.5 ± 2.0
sqt-3	*sc84*	V	*unc-42*	28/1232	2.3 ± 0.8

* R represents the number of recombinants among total progeny examined.

† The recombination frequency, p, was calculated according to the equation, $p = 1 - \sqrt{1 - 2R}$ (BRENNER 1974). 95% confidence limits for each p are provided.

TABLE 2

Three-factor crosses

Gene*	Parental genotype†	Recombinant phenotype	Results
rol-4	*dpy-11 unc-42* / + + *rol-4*	Dpy	5/5 segregated *rol-4*‡
		Unc	0/3 segregated *rol-4*
rol-5	*dpy-10 rol-5* / + + *unc-53*	Rol-5	0/10 segregated *unc-53*
rol-6	*dpy-2 rol-6* / + + *unc-4*	Rol-6	0/6 segregated *unc-4*
	+ *rol-6* / *bli-2* +	Unc	16/16 segregated *bli-2*
sqt-1	*dpy-2 unc-4* / + + *sqt-1*	Unc§	11 (+*unc*+/*dpy unc*+)
		Rol Unc	0 (+*unc sqt*/*dpy unc*+)
sqt-2	+ *dpy-2* / *sqt-2* +	Unc§	1 (++*unc*/+ *dpy unc*)
		Rol Unc	9 (*sqt*+*unc*/+*dpy unc*)
sqt-3	*dpy-11 unc-42* / + + *sqt-3*	Unc§	6 (+*unc*+/*dpy unc*+)
		Rol Unc	0 (+*unc sqt*/*dpy unc*+)

* Roller alleles used in these experiments were as listed in Table 1, except that *sc63* was used for *sqt-3*.

† Genes are shown in inferred order.

‡ Since *dpy-11* was found to be epistatic to *rol-4*, the presence of *rol-4* was confirmed by complementation tests with the *dpy-11 rol-4* segregants.

§ The roller phenotype of animals heterozygous for squat mutations allowed determination of recombinant genotypes without need for progeny testing.

The semi-dominant mutation, *sc78*, was mapped to linkage group II, approximately 3.0 map units to the left of *unc-4*. Since recombination values in *C. elegans* increase with temperature (ROSE and BAILLIE 1979), this number probably represents an overestimate of the true distance between these mutations, for it was obtained at 25° rather than 20°. Animals homozygous for *sc78* possess a dumpy roller phenotype at 20° and 25°, whereas animals heterozygous for *sc78* display a roller phenotype only at 25° and appear wild type at 20° or below. *sc78* has been tentatively assigned to the *dpy-2* rather than the *dpy-10* gene because the *trans* heterozygote, *sc78/dpy-2(sc38)*, is a strong roller at 20°, whereas *sc78/dpy-10(sc48)* heterozygotes appear indistinguishable from *sc78*/+ animals. The semi-dominance of *sc78* permits the use of the above heterozygotes to measure the distances between *sc78* and the *dpy-2* and *dpy-10* genes, since all segregants from the double heterozygotes are rollers at 25° except for wild-type recombinants. To date, neither double heterozygote has yielded wild-type recombinants [0/4100 for *sc78/dpy-2(sc38)* and 0/2700 for *sc78/dpy-10(sc48)*]; therefore, *dpy-2* and *dpy-10* must be very closely linked to *sc78* and to each other.

Squat mutants: While squat heterozygotes display a strong roller phenotype, homozygous adults do not display any rolling behavior or other indication of a helically twisted body. As will be described later, however, some juvenile stages of certain homozygous squat animals are strong rollers.

Squat mutants can be grouped into two categories based on whether they roll left or right as heterozygotes. All of the squat mutants that roll right as heterozygotes had mutations that mapped to linkage group II; seven of them mapped approximately 1.5 map units (mean value for all alleles) to the right of *unc-4* and were designated alleles of *sqt-1*, while the other seven mapped approximately 18.4 map units (mean value for all alleles) to the left of *unc-4* and were presumed to be alleles of a second squat gene, *sqt-2* (see Tables 1 and 2). Allelism tests support our assignment of the right squat mutations to only two genes. Since the roller phenotype of heterozygous squat males interferes with their ability to mate, homozygous squat males were generated through the construction of double mutants of *sqt-1(sc1)* and *sqt-2(sc3)* with *him-5(e1467)* and used for the complementation tests. *him* mutations cause an increased frequency of males in hermaphrodite populations, presumably through increasing the incidence of nondisjunction of the *X* chromosome (HODGKIN, HORVITZ and BRENNER 1979). These tests showed that worms heterozygous for two squat mutations that had the same map location displayed a squat phenotype, the phenotype expected if the two mutations were allelic and failed to complement, while worms heterozygous for two squat mutations from different map locations displayed a strong roller phenotype, the phenotype characteristic of squat heterozygotes.

The six remaining squat mutants all roll left when heterozygous, and these mutations were localized to linkage group V, approximately 2.9 map units (mean value for two alleles) to the right of *unc-42* (see Tables 1 and 2) and are assumed to define a third squat gene, *sqt-3*. Since each of these mutations was temperature sensitive, we were able to perform complementation tests in the standard way as described for autosomal recessive mutants. In all cases, animals heterozygous for two different left squat mutations displayed a squat phenotype, indicating that the mutations were probably allelic.

While the complementation results described above are consistent with the notion that squat mutations that map to the same location are allelic with one another, other interpretations are possible. Since both heterozygotes display a mutant roller phenotype, these phenotypes might have an additive effect in the double heterozygote, producing a squat phenotype. Therefore, three should be considered a minimum estimate for the number of squat loci identified by these mutations.

As will be described, *rol-5* acts as a dominant suppressor of *sqt-1*. Therefore, it is possible to use the *trans rol-5* +/+ *sqt-1* heterozygote to measure the recombination frequency between these two genes, since right roller progeny of these animals can arise only through recombination. Of 5300 such progeny examined, none have been right rollers, indicating that *rol-5* and *sqt-1* are less than 0.04 map units apart.

The results of the genetic analysis of our collection of roller mutants are summarized in Table 3. The 88 roller mutants define 14 different genes, including six genes not previously identified. The location of each of these genes is depicted in Figure 1. It should be noted that, for more than half of these genes, alleles exist that do not display a roller phenotype.

TABLE 3

Genes that contain roller alleles

Gene	Linkage group	Handedness	Reference allele	Number of alleles	Number nonroller alleles	Number *ts* alleles	Number *cs* alleles
rol-1	II	left	*e91*	2	0	1	0
rol-3	V	left	*e754*	1	0	0	0
rol-4	V	left	*sc8*	3	0	2	0
rol-5	II	left	*sc13*	18*	0	7	2†
rol-6	II	right	*e187*	8	1	0	0
dpy-2	II	left	*e8*	14‡	0	3	0
dpy-3	*X*	left	*e27*	9	3	6	0
dpy-7	*X*	left	*e88*	5‡	1	3	0
dpy-8	*X*	left	*e130*	7‡	4	4	0
dpy-10	II	left	*e128*	9‡	7	1	0
dpy-15	V	left	*e24*	2	1	1	0
sqt-1	II	right	*sc1*	7§	0	0	0
sqt-2	II	right	*sc3*	7§	0	0	0
sqt-3	V	left	*sc63*	6§	0	6	0

* Some of these mutants are suspected of being repeated isolates of the same initial mutated strain. There appear to be a minimum of 10 different mutants.

† Two mutants are cold sensitive, manifesting a more severe mutant phenotype at 16° than at 25°.

‡ Four mutants described by BRENNER (1974) are included, but were not examined by us; *dpy-2(e961)*, *dpy-2(e260)*, *dpy-7(e88)*, *dpy-8(e130)*. Six alleles of *dpy-10* are also included, but not examined.

§ Homozygous adults do not roll. In some cases certain larval stages roll. Adult heterozygous do roll.

Table 4 compares the distribution of roller genes among the chromosomes with the genes that give an uncoordinated phenotype and presumably affect the neuromuscular system. While all roller genes are not organized into tight clusters, they do appear to be less randomly distributed among the chromosomes than are the uncoordinated genes, with a large proportion of the genes on linkage group II, some of them very closely linked.

Phenotypic differences among the roller mutants

Handedness: Individual roller mutants roll either to the right (in a clockwise direction when moving forward) or to the left (counterclockwise). No exception to this rule has been found for countless worms examined. All of our mutants have been characterized as either right-handed or left-handed. All mutants defective in the same gene show the same handedness. Table 3 shows which of the genes gives rise to right-handed and which to left-handed rollers.

Development of rollerness: During its development to an adult, *C. elegans* goes through several juvenile stages terminated by a molt at which the entire cuticle is shed and a new cuticle is formed. These juvenile stages are designated L1, L2, L3 and L4. Under starvation conditions, L2 animals will molt to produce a developmental alternative to the L3, the dauer larva, which can resist starvation

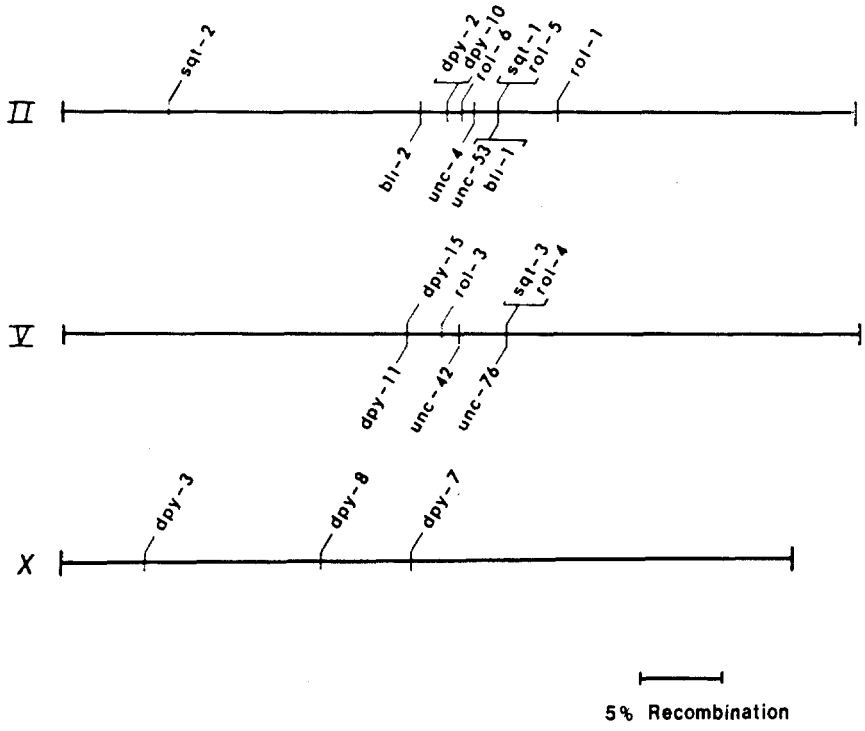

FIGURE 1.—Map location of roller genes. Marker genes used for mapping are indicated below the lines. Separate locations are shown only for genes that have been unambigously ordered.

for long periods of time (CASSADA and RUSSELL 1975; KLASS and HIRSH 1976). In the presence of food, the dauer molts to an L4 animal and so returns to the regular developmental pathway.

We have examined most of our mutants with regard to the visible phenotypes of the different developmental stages. Some sample results are shown in Table 5. In general, mutants defective in the same gene tended to have very similar

TABLE 4

Distribution of roller and uncoordinated genes among linkage groups

Linkage group	Roller genes	Uncoordinated genes*
X	3	13
I	0	16
II	7	3
III	0	11
IV	0	13
V	4	14
	14	70

* Data derived from BRENNER (1974).

TABLE 5

*Developmental manifestations of roller mutant phenotypes**

Class	Gene	Mutant	L1	L2	L3	L4	Adult	Adult (16°)	Dauer	Comments
wild		N2	++†	+	+	+	+	+	+	
LR	*rol-1*	E91	+	+	+	+	3/+	3/+	+/1	
		SC22	+	+	+	+	3/1	1/+	+	ts
	rol-3	E754	+	+	+	+	2/+	2/+	+	
	rol-4	SC8	+	+	+	3/+	3/+	3/+	2/+	
		B238	+	+	+	+	3/+	+	+	ts
	rol-5	SC13	+	+	2/+	3/+	3/+	3/+	3/+	
		SC33	+	+	+	+	3/+	+	+	ts
LDR	*dpy-2*	E8	+	+	+/1	+/3	1/3	1/3	+	
		SC38	+	+	+/1	2/2	2/3	+/1	+/1	ts
	dpy-3	E27	+	+	+/2	+/2	+/3	+/3	+/1	nonroller dumpy
		SC26	+/2	+/2	4/2	4/2	4/3	3/3	+/2	
	dpy-7	SC27	+	+	+/1	+/2	3/3	+/1	+/1	ts
		E1324	+	+	+/3	+/3	+/3	+	ND‡	ts nonroller dumpy
	dpy-8	E1281	+	+	+	+/2	+/3	+/1	+	ts nonroller dumpy
		SC44	+	+	+/1	2/1	3/2	+	+	ts
	dpy-10	SC48	+	+	+/1	+/2	1/3	3/2	—	
		SC30	+	+	+	+	1/1	+	ND‡	ts
	dpy-15	E24	+/4	+/4	+/4	+/4	+/4	+	ND‡	*ts* sterile
		SC43	+/1	+/1	+/1	+/1	1/2	1/1	+/1	
RR	*rol-6*	E187	+	+	3/+	3/+	3/+	3/+	3/+	
		SU1006	+	+	4/+	4/+	4/+	4/+	4/+	
LS	*sqt-3*	SC63	+/3	+/3	+/2	+/2	+/2	1/+	+/3	ts
		SC63/+	+	+	+	+	3/+	+	+	ts
		SC80	+/3	+/3	+/3	+/2	+/2	1/+	+/3	ts
		SC80/+	+	+	+	+	3/+	1/+	ND‡	ts
RS	*sqt-1*	SC1	+	+	3/+	+/1	+/1	+/2	3/+	
		SC1/+	+	+	3/+	3/+	3/+	3/+	3/+	
		SU1005	+	+	3/+	+/1	+/1	+/1	3/+	
		SU1005/+	+	+	3/+	3/+	3/+	3/+	3/+	
	sqt 2	SC3	+	+	3/+	+	+	+/1	3/+	
		SC3/+	+	+	3/+	3/+	3/+	3/+	3/+	
		SC14	+	+	3/+	+/1	+/1	+/1	3/+	
		SC14/+	+	+	3/+	3/+	3/+	3/+	3/+	

* Phenotypes are of animals grown at 25°, except for the adult (16°) column.

† The number to the left of the slash refers to the roller phenotype, while the number to the right of the slash refers to the dumpy phenotype. The number indicates the severity of the mutant phenotype, with (+) being similar to wild type and (4) being the most severe.

‡ Not determined.

phenotypes with regard to the onset of the roller phenotype, the severity of the roller phenotype, and the presence or absence of a secondary dumpy phenotype. However, a comparison of the phenotypes of mutants in different genes revealed a number of differences. On the basis of these differences and other phenotypic characterizations to be described below, we have chosen to group the genes into the following classes:

Left Roller: Class LR mutants (genes *rol-1*, *rol-3*, *rol-4* and *rol-5*) are all left-

handed rollers. None of the mutant animals exhibit a significant dumpy phenotype. In general, mutant animals do not display the roller phenotype until the L4 or adult stage, and dauer larvae do not roll. The *rol-5* gene contains three exceptional alleles. Animals carrying these alleles also roll as L3's and as dauers. One allele of *rol-4* also displays the roller phenotype in the dauer stage. Many mutants (10 of 24 examined) exhibit a temperature-sensitive phenotype. One gene, *rol-5*, has alleles that produce a cold-sensitive phenotype since mutant animals display a stronger roller phenotype at 16° than at 25°.

Left Dumpy Roller: Mutants of the class LDR (genes *dpy-2*, *dpy-3*, *dpy-7*, *dpy-8*, *dpy-10* and *dpy-15*) are also left-handed rollers, which, except for *dpy-3(sc26)*, do not display roller behavior until the L4 or adult stage. Nearly all alleles, however, also confer a dumpy phenotype that is manifest at an early stage of development, sometimes the L1 or L2 stage. Dauer larvae do not roll, but often exhibit a mild dumpy phenotype. Some alleles of these genes give a straight nonroller dumpy rather than a dumpy roller phenotype. Many mutants are temperature sensitive (18 of 36 examined). For these mutants, both the dumpy and roller phenotypes are greatly diminished by growing the animals at 16°. At 20°, several of the temperature sensitive animals, *e.g.*, *dpy-3(sc11)*, display a non-dumpy roller phenotype similar to that of the LR class mutants.

Right Roller: Mutants of the class RR (the *rol-6* gene) are right-handed rollers that do not display a significant dumpy phenotype. None of the isolates (eight alleles) is temperature sensitive. Mutant animals start to exhibit rolling behavior at the L3 stage, and dauer larvae also roll. As mentioned earlier, animals carrying *sc90*, the *rol-6* allele extracted from SC-M1, do not roll and appear indistinguishable from wild type at all stages of development.

Left Squat: Class LS consists of mutants of the *sqt-3* gene. All six isolates are temperature sensitive as both homozygotes and heterozygotes. At 25°, homozygotes start life as severely deformed dumpy-like animals that become more like wild type in later development. Heterozygotes are not dumpy, but display a strong left roller phenotype in the adult stage. At 16°, adult homozygotes possess a mild roller phenotype.

Right Squat: Class RS consists of mutants of the right squat genes, *sqt-1* and *sqt-2*. When homozygous, alleles of both genes confer roller phenotypes on L3's, but not on any other regular stage. Some alleles of both genes develop a mild dumpy (squat) phenotype in the L4 and adult stages. All mutants roll strongly as dauers. Surprisingly, whereas *sqt-2* L4's and adults derived from L3's are nonrollers, we observed that *sqt-2*, and sometimes *sqt-1*, L4's and adults that develop from dauer larvae are strong rollers. Animals heterozygous for mutations of both genes exhibit a strong roller phenotype, which begins at the L3 stage and continues through the L4 and adult stages. Dauer larvae of heterozygotes also roll strongly. None of the isolates (14 alleles) is temperature sensitive.

A summary of these observations concerning the classification of roller mutants is given in Figure 2.

Cuticle morphology: The morphology of the *C. elegans* cuticle has been examined by several workers (CASSADA and RUSSELL 1975; SINGH and SULSTON

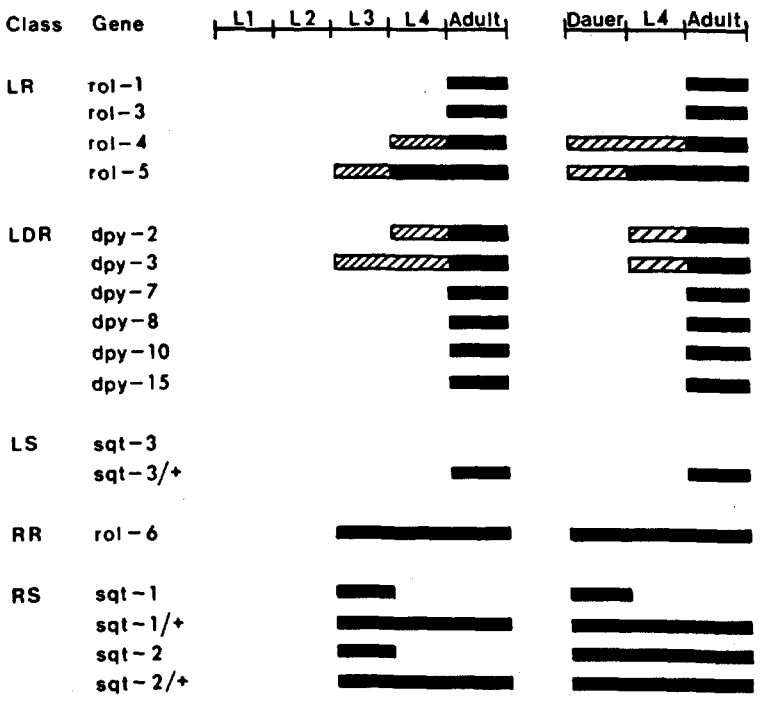

FIGURE 2.—Developmental expression of the roller phenotype at 25°. Genes are grouped according to classes, and a typical developmental sequence for each gene is shown graphically. Juvenile or adult stages that display a roller phenotype are indicated by solid bars, while slashed bars are used in cases where only a few exceptional alleles of a gene display this phenotype.

1978; COX, KUSCH and EDGAR, in preparation) and also that of the closely related species, *C. briggsae* (ZUCKERMAN, HIMMELHOCH and KISIEL 1973). The adult cuticle consists of two layers separated by a space that is believed to be fluid-filled (BIRD 1971) .The two layers are joined by columnar "struts" that are arranged in regular transverse double rows. The outer cuticle layer is indented at these rows to produce pleated-appearing annulae. The cuticle contains over a thousand such annulae. Running along the lateral sides of the animal are alae composed of three closely spaced tread-like projections. Embedded within the inner cuticle layer are two fiber layers that spiral along the animal in opposite directions. These features of cuticle anatomy are depicted in Figure 3.

While the cuticles of the various juvenile forms have not been studied in detail, it is clear that their morphologies differ from one another and from that of the adult cuticle. The L1 and dauer cuticles have alae, while the other juvenile stages do not. Furthermore, the L1 and dauer alae differ in form from one another and from the alae of the adult. The internal anatomies of the cuticles differ as well. For all stages except the adult, the cuticle apparently is composed of only one layer.

We have examined whole animals and isolated cuticles of representative roller mutants by indirect immunofluorescence and phase-contrast microscopy to see if

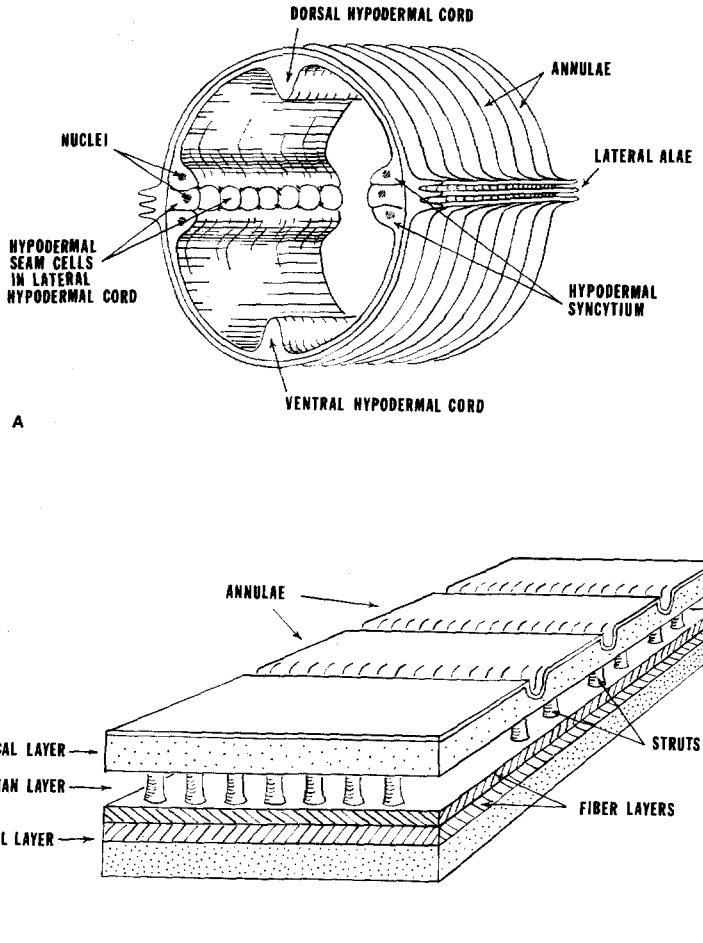

FIGURE 3.—Diagrammatic sketch of the adult *C. elegans* cuticle. (a) Cross-section of an adult nematode showing the general organization of the cuticle and underlying hypodermis (after BIRD 1971). (b) Magnified view of the internal anatomy of the cuticle. Neither figure is drawn to scale.

any morphological features of the cuticle were altered as a consequence of defects in roller genes. Where possible, two or more mutants representing each gene were examined. The results of this survey are given in Table 6.

Alae: The number of helical twists that the alae make about the animal given in Table 6 is a rough estimate, based on the inspection of 10 to 20 animals. The degree of helical twist seemed to vary slightly between animals of the same genotype and, for some mutants, may increase with age. Nevertheless, there seemed to be reproducible differences between mutants in the degree of helical twist, and these differences correlated with the number of helical twists to the body wall muscles (Figure 4) and with the severity of the roller phenotype, as presented in Table 5. Those mutants that were considered strong rollers were found to have their alae twisted by a full turn or more. Those mutants classified

TABLE 6

*Cuticle morphology of roller mutants**

Class	Gene	Mutant	Helicity†	Alae‡	Annulae§	Struts‖
wild		N2	0	+	+	+
LR	*rol-1*	E91	1/2	+	+	+
		SC22	1/2	+	+	+
	rol-3	E754	1/4	+	+	+
	rol-4	SC8	1/2	+	1	+
		B238	1/2	+	+	+
	rol-5	SC13	1/2	+	+	+
		SC33	1/2	+	+	+
LDR	*dpy-2*	E8	1	1	2	1
		SC38	1/2	1	3	2
	dpy-3	E27	0	+	3	2–3
		SC26	2	1	3	2–3
	dpy-7	SC27	1/4	1	2	2
		E1324	0	1	3	2–3
	dpy-8	E1281	0	1	3	2–3
		SC44	1/2	1	3	2–3
	dpy-10	SC48	1/4	1	2	3
		SC30	1/4	+	2	2
	dpy-15	SC43	1/4	1,2,3	1	3
RR	*rol-6*	E187	1	+	+	+
		SU1006	2	2	1	+
LS	*sqt-3*	SC63	0	+	2	3
		SC63/+	1/2	+	+	+
		SC84	0	2,3	2	3
		SC84/+	1/4	+	+	+
RS	*sqt-1*	SC1	0	1,2	3	3
		SC1/+	1	2	1	1
		E1350	0	1,2	3	3
		SU1005/+	1	2	1	1
	sqt-2	SC3	0	+	3	3
		SC3/+	1	+	1	1
		SC14	0	+	3	3
		SC14/+	1	+	1	1

* Data are for adult animals grown at 25°.

† Refers to the number of complete turns the alae make along the length of the animal.

‡ Refers to the condition of the alae: + = wild type, 1 = beaded, 2 = loops present, 3 = interruptions present.

§ Refers to the condition of the annulae: + = wild type, 1 = slightly deranged, 2 = very deranged, 3 = only smooth or rough surfaces present.

‖ Refers to the appearance of the struts: + = wild type, 1 = somewhat irregular or compressed rows, 2 = random arrangement, 3 = no struts, only "gray" areas observed.

as nonrollers showed no helical twist. Most mutants that were left-handed rollers appeared to have a twist of about one-half turn, while all right-handed rollers had their cuticles twisted by at least one full turn.

The cuticles of roller animals remain twisted into helices after the worm's cellular contents are removed by sonication. For some mutants the isolated cuticle is even more severely helically twisted than is the intact animal, suggesting that

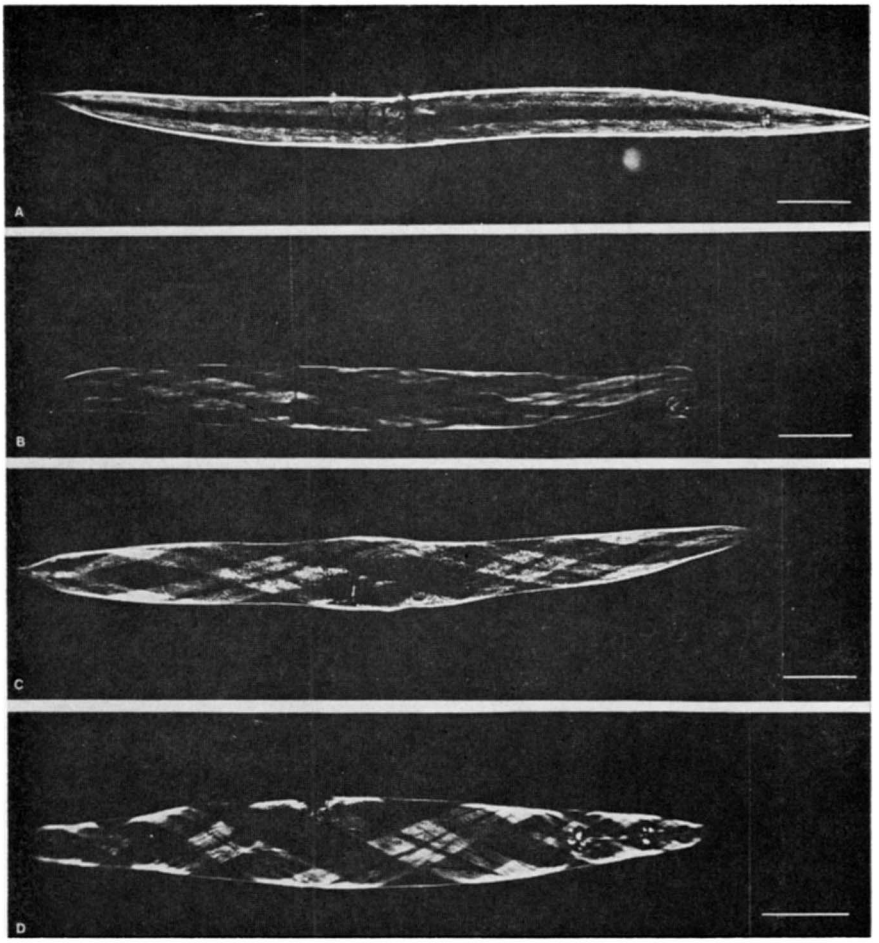

FIGURE 4.—Polarized light micrographs of roller mutants. (a) N2 wild type. The highly birefringent muscle bundles are arranged parallel to the long axis of the worm in contrast to those of roller mutants that display characteristic spirals; (b) *rol-1(e91)*; ½ helical twist. (c) *rol-6 (e187)*; 1½ helical twist. (d) *dpy-3(sc26)*; 2¼ helical twist. Bar = 100 microns.

the turgor pressure of the animal acts partially to unwind an inherent twist in the cuticle structure.

The alae of the LR mutants showed no other morphological defects. However, most of the mutants in the other roller classes had aberrant-appearing alae (Figure 5). The alae of the LDR mutants appeared "beaded", indicating many minor interruptions. Loops were sometimes observed in the alae of the RR mutants but were very common in homozygous LS and RS mutants. One LDR mutant, *dpy-15(sc43)*, also frequently exhibited alae loops and in addition often possessed large gaps in its alae.

Annulae: The annulae of roller animals were generally present in the wild-type orientation, perpendicular to the worm's long axis, and thus were no longer

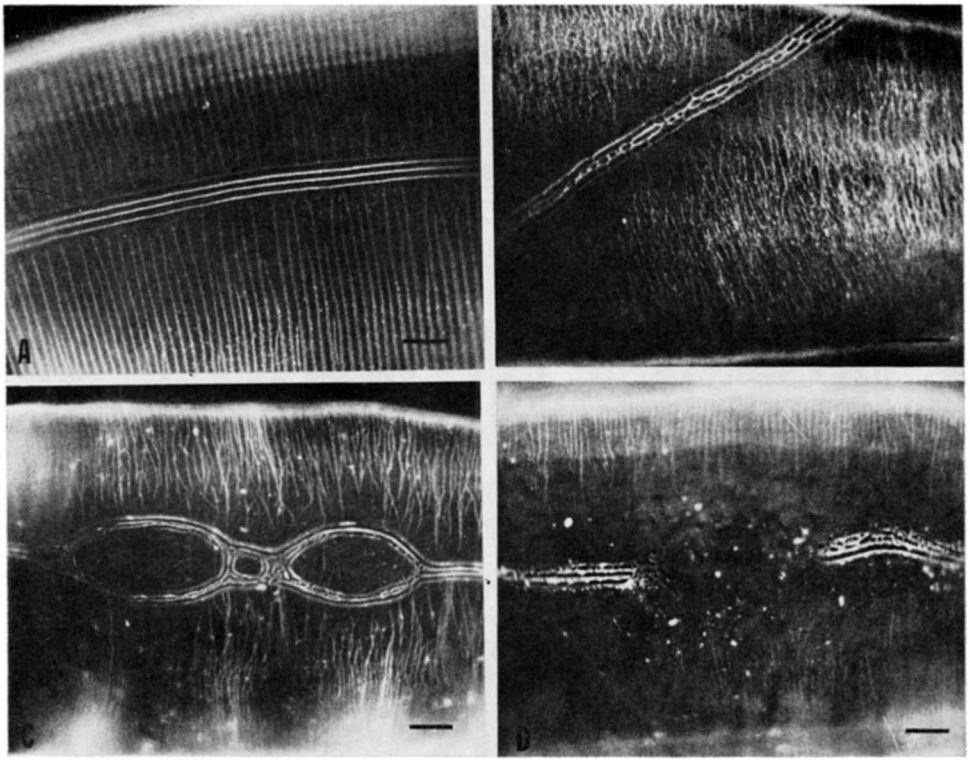

FIGURE 5.—Roller mutant cuticle defects. Live adult nematodes were incubated with rabbit anti-cuticle antibodies followed by treatment with fluorescein-conjugated goat anti-rabbit antibodies and observed by fluorescence microscopy. (a) N2 wild type. The annulae are patterned into regular transverse rows perpendicular to the lateral alae, which appear as four continuous bright lines parallel to the long axis of the worm; (b) *dpy-3(sc26)*. The annulae are severely deranged, and the lateral alae have a "beaded" appearance. Also note that the alae and annulae are no longer perpendicular; (c) *sqt-3(sc84)*. The annulae are highly irregular and loops are present in the lateral alae; (d) *dpy-15(sc43)*. Gap present in the lateral alae. Bar = 5 microns.

at right angles to the helically twisted alae. The structure of the annulae of LR and RR mutants showed a regular appearance comparable to wild type. However, the annulae of the LDR, LS and RS mutants were either highly irregular in appearance or absent (Figure 5). The surface cuticle of the severely afflicted animals often displayed loose folds and creases resembling the skin of a rhinoceros. The annulae of heterozygous LS and RS mutants appeared relatively normal.

Struts: There seemed to be a strong correlation between the nature of the annulae defects and aberrations in the patterning and formation of the struts. All mutants with normal annulae displayed normal strut patterns as well. However, mutants with irregular annulae had struts arranged in irregular rows. Those mutants with no annulae had struts arranged in a random, dense array or had no visible struts but rather diffuse "dark" areas in the cuticle, suggesting perhaps

that strut material was present in the area between the two cuticle layers but not organized into discrete columns.

Birefringence: The isolated cuticle of adult *C. elegans* is birefringent when viewed with plane polarized light (COX, KUSCH and EDGAR, in preparation). The direction of this birefringence is at right angles to the long axis of the worm and is believed to be due to the sublayers of spiralling fibers present in the inner layer of the adult cuticle, as has been shown to be the case for the cuticle birefringence of the related nematode, Ascaris (BIRD and DEUTSCH 1957). Each of the fiber layers of the Ascaris cuticle spirals along the worm at an angle of 70 degrees relative to the worm's long axis (BIRD and DEURSCH 1957; HARRIS and CROFTON 1957). Since these fiber layers spiral in opposite directions, they cross each other at an angle of 140 degrees. This orientation would produce a net birefringence in the transverse direction, as we observed for *C. elegans*.

We have surveyed isolated cuticles from all mutants listed in Table 6 for their birefringent characteristics. All seemed similar to wild type, except perhaps for several LDR mutants that seemed to display somewhat less cuticle birefringence than wild type. Cuticle preparations from LS mutants reproducibly resulted in the production of some cuticle fragments that lacked birefringence. Conceivably, these fragments could derive from the outer cuticle layer only, since this layer displays little if any birefringence.

Gene interactions

We have examined the phenotypes of a number of double mutants to study the interactions of genes of the various roller classes with each other and with two other classes of potential cuticle-defective mutants, the dumpy and blister mutants (BRENNER 1974). Blister mutants are so named because of fluid-filled swellings that appear on their cuticles. Some sample results of these studies are shown in Figure 6.

All of the representative mutants from the different roller classes were found to suppress strongly blister formation in double mutants. Small, isolated, splotchy blisters were usually present on the cuticles of double mutants carrying LR, LS and RR mutations, while complete epistasis was observed with the LDR and RS mutations. Rolling behavior was generally unaffected in the double mutants.

Two dumpy genes, *dpy-4* and *dpy-11*, have been extensively studied. Neither of these genes contains roller alleles. In nearly all instances, these genes were found to be epistatic to roller mutants of all classes. Double mutants of *dpy-4* with *dpy-2*, *rol-5*, *rol-6*, *sqt-1*, *sqt-3* and *sqt-3*/+ all displayed a similar, very dumpy phenotype, indicating that these roller genes act as enhancers of *dpy-4*. The *dpy-4;sqt-3* double mutant is extremely dumpy and does not reproduce.

The interactions of the various roller mutant classes with each other were found to be more variable, although certain patterns did emerge. When either dumpy or dumpy roller alleles of LDR genes were combined with mutations from the other roller classes, the double mutant typically displayed a left dumpy roller phenotype. We interpret this result to mean that the left dumpy roller phenotype is the most severe phenotype for the LDR genes and that the pheno-

		Class	BLI		DPY		LDR		RR	RS	
		Gene	bli-1	bli-2	dpy-4	dpy-11	dpy-2	dpy-10	rol-6	sqt-1	sqt-1/+
Class	Gene	Phen.	B	B	D	D	LDR	D	RR	Sqt	RR
LR	rol-1	LR	LRwB	LRwB	D	LDR	LDR	wLDR	wRR	*	*
	rol-3	LR	LRwB	LRwB	D	*	*	LDR	Sqt†	*	*
	rol-4	LR	LRwB	LRwB	D	D	D	D	wLR	Sqt	wLR
	rol-5	LR	LRwB	LRwB	sD	D	D	LDR	LR	*	*
RR	rol-6	RR	RRB	RRwB	sD	D	LDR	*	—	Sqt	RR
LS	sqt-3	Sqt	SqtwB	SqtwB	sD	D	D	LDR	Sqt	Sqt	*
	sqt-3/+	LR	wLRwB	wLRwB	sD	D	*	LDR	wLR	Sqt	wLR
RS	sqt-1	Sqt	Sqt	Sqt	sD	D	D	D	Sqt	—	—
	sqt-1/+	RR	RRwB	RRwB	D	D	LDR	LDR	RR	—	—
LDR	dpy-2	LDR	LDR	*	sD	D	—	*	LDR	D	LDR
	dpy-10	D	*	D	*	*	*	—	*	D	LDR
DPY	dpy-4	D	D	D	—	*	sD	*	sD	sD	D
	dpy-11	D	DwB	D	*	—	D	*	D	D	D

FIGURE 6.—Phenotypic characteristics of double-mutant adults at 25°. The following phenotype abbreviations are used: LR, left roller; RR, right roller; LDR, left dumpy roller; Sqt, squat; B, blister; D, dumpy. A (w) preceding a phenotype indicates partial suppression, *i.e.*, wB signifies a weak or partially suppressed blister phenotype. An (s) preceding a phenotype indicates a stronger or enhanced phenotype. *sc38* was used to construct all *dpy-2* double mutants. All other roller alleles used in crosses were as listed in Table 3, except that *sqt-1(su1005)* was used to construct the *rol-6 sqt-1* double mutant.

* Not determined.

† These animals were right rollers as L3's, but were squattish nonrollers as L4's and as adults.

type of the weaker dumpy alleles of these genes is enhanced through interaction with other roller mutants. We conclude, therefore, that LDR mutations are epistatic to the other roller mutant classes.

Our result with *dpy-10(e128);rol-3(e754)* differs from that of HIGGINS and HIRSH (1977), who reported that the double mutant displays a dumpy rather

than a dumpy roller phenotype. These authors were unable to reextract *rol-3* from their double mutant, however, suggesting that they had not successfully isolated it to begin with. When our double mutant was crossed to wild type, 88 rollers out of 639 total F_2 progeny were obtained. Considering the weak roller phenotype and less than 100% penetrance of *rol-3(e754)*, we believe this value to be within experimental error of the 3/16 ratio expected from the segregation of two unlinked recessive mutations.

Most combinations of left (LR and LS/+) with right (RR and RS/+) roller mutants were found to suppress each other. Double mutants were either nonrollers or very weak left rollers. Those animals that did roll weakly usually did so only in the adult stage.

Homozygous squat mutants were found to be epistatic to both left and right roller mutants, although only a few left roller genes were examined. The RS;LS double mutant was also squat, resembling the RS mutant at 16° and the LS mutant at 25°.

Several instances of dominant intergenic suppression by recessive roller mutations were encountered when trying to construct certain double mutants. The right rolling behavior of *sqt-1* and *sqt-2* heterozygotes and of *rol-6* homozygotes was found to be suppressed when these animals were also heterozygous for *rol-5(sc13)*. Suppressed animals were either nonrollers or very weak left rollers. The cuticles of several nonroller *sqt-2* +/+ *rol-5* animals were examined by indirect immunofluorescence and were found to be very similar to wild type, as opposed to the severely deranged cuticle morphology of *sqt-2* homozygotes, supporting the notion that the effect observed was due to suppression rather than enhancement of the squat phenotype. Three alleles of *sqt-2*, two alleles of *sqt-1* and one allele of *rol-6* have been tested with *rol-5*, and all were found to be suppressed.

The other instance of dominant intergenic suppression involved *rol-4(sc8)* and *sqt-3*. Whereas *sqt-3*/+ animals are strong left rollers, *sqt-3* +/+ *rol-4* animals display a nonroller or very weak left roller phenotype. Since these animals are not squat, we assume that this effect is due to suppression. Five alleles of *sqt-3* have been tested with *rol-4*, and all are suppressed.

In cross-suppression tests, *rol-5(sc13)* produced no discernible effect on the left roller phenotype of *sqt-3* heterozygotes, and *rol-4(sc8)* had no effect on the right roller phenotype of *sqt-1* and *sqt-2* heterozygotes or *rol-6* homozygotes. Mutations in three other roller genes, *rol-6(e187)*, *rol-3(e754)* and *rol-1(e91)*, have been tested for similar dominant suppressor effects on squat heterozygotes, but none have been observed, although mild cases of suppression may have been overlooked. No other alleles of *rol-4* or *rol-5* have been examined in similar crosses.

DISCUSSION

We have identified 14 genes of *C. elegans* that can mutate to give a roller phenotype. Several alleles exist for all but one of these genes, suggesting that their number is near saturation. Most of the mutants studied were originally

selected on the basis that they showed an adult roller phenotype; however, many also display roller or dumpy phenotypes in certain juvenile stages. The stage specificity in the expression of these phenotypes indicates that the cuticles of the adult and various juvenile forms differ from one another. This conclusion is reinforced by electron microscopic studies (CASSADA and RUSSELL 1975; SINGH and SULSTON 1978; our unpublished results) and biochemical studies (unpublished results), which have demonstrated considerable variation in both the ultrastructure and protein composition of the different stage cuticles. It is possible, therefore, that other roller genes exist that specifically affect only certain juvenile stages and not the adult. Genes of this type may have been overlooked in this study, especially those that might affect only the very early juvenile stages.

The 14 genes identified fall into five general phenotypic classes: Right Roller (RR), Right Squat (RS), Left Roller (LR), Left Squat (LS) and Left Dumpy Roller (LDR). Mutations in genes within a class produce similar phenotypes and presumably affect similar processes. Light microscopy studies support this conclusion. The various roller mutant classes exhibit different cuticle defects, but genes within a class exhibit very similar defects. Overall, LS and RS mutants are the most severely afflicted, possessing abnormal struts, annulae and alae, Roller heterozygotes of these animals appear similar in phenotype to LR and RR mutants, respectively, which display very minor, if any, cuticle abberations other than helically twisted alae. Mutants of the class LDR have severe annulae and strut defects, but milder alae defects. The close correlation observed between the severity of the annulae defects and the degree of distortion of the strut patterns suggest that these two types of aberrations may be causally related. However, it should be noted that L3 and L4 juveniles of many of these mutants also display annulae defects, but struts are not observable in juvenile stage cuticles (unpublished observations).

SINGH and SULSTON (1978) have demonstrated that the hypodermal seam cells are responsible for the formation of the adult alae. Ablation of juvenile seam cells with a laser microbeam or disruption of the hypodermal cell lineages by genetic means results in adult animals with large gaps in their alae. That similar gaps exist in the lateral alae of *dpy-15(sc43)* animals suggests that this gene may affect the divisions and/or migrations of these cells. The extreme dumpiness and resultant sterility of another *dpy-15* allele, *e24*, would support this conclusion. Alae loops may correspond to misaligned seam cells, but cytological studies would be needed to verify this.

The cuticles of roller animals maintain, and in some cases increase, their helicity following isolation from the worm's cellular contents. This finding indicates that the structural components of the cuticle are stably assembled, rather than being twisted after assembly, into a helically distorted pattern as a consequence of mutations in these genes. This conclusion is supported by the observation that roller mutants typically commence (or cease as in the case for RS mutants) rolling in the middle of a molt when the new cuticle is being laid down. The presence of two helically wound fiber layers in the inner layer of

the adult cuticle provides a possible structural basis for how this distortion might occur. Roller mutations may affect specifically one or the other of these spiralling layers. A change in the orientation of one layer would result in an altered plane of symmetry, introducing a left-handed helical twist to the cuticle, while perturbations in the other layer would create a right-handed helical twist. Since one of the fiber layers adjoins the struts, one might imagine that this is the layer that also can, when altered, give rise to strut aberrations.

It was hoped that alterations in the organization of the fiber layers in the mutants might be revealed through birefringence studies of isolated cuticles. While no apparent differences from wild type were observed, our studies were not sufficiently sensitive to detect small changes in birefringence Birefringence measurements of a more sensitive, quantitative nature might indeed reveal a pattern to support the notion just proposed.

The existence of both left and right roller genes suggests that these two groups of genes might affect symmetrical processes. If this were the case, then one would expect left and right roller genes to exhibit similar phenotypic properties. They do not. Right roller mutations are found in only three genes, all of which contain many dominant alleles. None of these mutations confers a dumpy phenotype and none is temperature sensitive. In contrast, 11 left roller genes have been identified, but in only one is there a large proportion of dominant alleles. Many of the left roller genes (7 of 11) have alleles that also confer a dumpy phenotype. Most strikingly, nearly half (34 of 76) of the left roller mutations are temperature sensitive. These differences lead us to believe that the functions specified by many of the left roller genes are qualitatively different from those specified by the right roller genes. Left roller genes that we have grouped into the LDR class have no right roller counterparts and presumably do not function in a symmetrical way with any right roller genes. The epistasis of LDR to RR and RS mutations supports this conclusion. The partial, and in some cases full, suppression of rolling behavior observed in certain combinations of mutants from the remaining roller classes (*e.g.*, *RR-LR*, *RR-LS*/+) is compatible with the notion that at least some of these genes perform symmetrical functions, but the differences in phenotypes displayed by mutants in these genes would argue against such an interpretation. This lack of phenotypic symmetry might be expected if these genes affect the different cuticle fiber layers as proposed above, for while the two fiber layers are symmetrically disposed, they are adjacent to the presumably interact with different nonfiber portions of the cuticle, *e.g.*, the struts.

The high proportion of *ts* alleles among left roller mutations deserves further comment. Temperature-sensitive mutants are usually thought to arise as a consequence of an amino acid substitution that renders the affected protein thermolabile. Such mutations are expected to form a small subclass of all genetic alterations in a gene. The fact that nearly half of the left roller mutants, representing 10 out of 11 genes of this type, are temperature sensitive suggests the need for a different explanation of the temperature-sensitive phenotype. We propose that one or more of the wild-type proteins in the developmental system

affected by the left roller genes is marginally sensitive to elevated temperature. As a consequence, mutations in any gene that perturbs the system may now render the system as a whole temperature sensitive. On this notion, the protein responsible for the temperature-sensitive phenotype may not be the one genetically altered.

We have identified dominant or semi-dominant roller mutations in five genes of *C. elegans*: *dpy-2*, *rol-6*, *sqt-1*, *sqt-2* and *sqt-3*. For the latter three genes, all known alleles exhibit a dominant roller phenotype. Animals heterozygous for deletions of *dpy-2* and *rol-6* are phenotypically wild type (R. HERMAN, personal communication); therefore, the dominant nature of mutations in these genes is not due to their being null, but rather to their producing a qualitatively altered gene product. The isolation of deletions for *sqt-1*, *sqt-2* and *sqt-3* should allow us to determine if this is the case for the dominant mutations in these genes as well. Mutations exhibiting visible gene-dosage effects are known to occur in genes coding for structural proteins, *e.g.*, myosin heavy chain (MACLEOD *et al.* 1977), paramyosin (WATERSTON, FISHPOOL and BRENNER 1977), β-tubulin (KEMPHUES *et al.* 1979), and for proteins involved in complex aggregates such as ribosomes (DAVIES and NOMURA 1972). Certain bacterial regulatory mutations ,*lac* operon O^c and I^s mutations, for instance (JACOB and MONOD 1961), are also known to give dominant phenotypic effects, but mutations of this sort would be expected to represent only a small fraction of the alleles for a particular gene. It is tempting to speculate that these genes represent loci coding for several of the structural proteins present in the *C. elegans* cuticle.

The epistatic and suppressive interactions observed between the various roller, dumpy and blister mutants argue for these genes being involved in a common developmental process. The results support a graded series of epistatic interactions: Dpy > LDR > LS and RS > LR and RR > Bli. The developmental basis for this hierarchical pattern is not known; however, the types of cuticle defects displayed by the different mutant classes suggests that this pathway represents a pattern of structural interdependence rather than a series of sequential enzymatic steps. The fact that mutations in each of these genes give rise to structural defects in the cuticle supports our belief that these genes are active in the formation of the nematode cuticle. It is possible, however, that each of the observed cuticle defects could be reflections of lesions in cellular processes not directly related to cuticle formation, as is the case for bristle shortening of bobbed mutants of *Drosophila* (WEINMANN 1972). The stage specificity in the expression of these mutant phenotypes renders this alternative less likely. Direct proof must await biochemical demonstration that certain of the structural components of the cuticle are altered as a consequence of mutations in these genes.

The dominant suppression observed with *rol-5(sc13)* and *rol-4(sc8)* argues strongly for a functional interaction between these genes and the genes that they suppress. *rol-5(sc13)* appears to interact specifically with *sqt-1*, *sqt-2* and *rol-6*; all are right roller or right squat genes that contain many dominant alleles. All four of these genes are located on linkage group II; *rol-5* maps extremely close to *sqt-1*. On the other hand, *rol-4(sc8)* seems to be a dominant suppressor specific

for *sqt-3*, a left squat gene that also contains many dominant alleles. These two genes map very close to each other on linkage group V, although we have not been able to measure their relative locations directly. The map positions of these genes are not random and lend further support to the notion that they represent interacting gene sets. Neither *rol-5(sc13)* nor *rol-4(sc8)* have been observed to suppress mutations in other classes of genes, such as the *unc* genes. Suppression, therefore, appears to be gene-specific rather than allele-specific and presumably does not result from an alteration in the protein synthesizing machinery, *e.g.*, *via* nonsense suppressor tRNAs. Determination of the mechanism responsible for the suppression observed with these mutants must await further biochemical and genetic analysis.

The failure to detect recombination between *rol-5* and *sqt-1* raises the possibility that these two genes may actually represent a single locus that can be mutated to produce two distinctly different phenotypes. The dominant suppression of *sqt-1* by *rol-5* could then be explained by interallelic complementation, a phenomenon that we have observed between certain alleles of *rol-5*. A similar situation may exist for *rol-4* and *sqt-3* as well. This reasoning, however, cannot explain the dominant suppression of *sqt-2* and *rol-6* by *rol-5*, since these genes are located at quite different positions on linkage group II.

HIGGINS and HIRSH (1977) reported several instances of non-Mendelian segregation of roller mutations, suggesting the possibility that some roller genes might not be chromosomal. However, all of the 88 mutations we studied behaved in a Mendelian fashion, including two that appeared to give aberrant segregation in the study of HIGGINS and HIRSH (1977), the segregation of *dpy-10(e128)*/+; *rol-3(e754)*/+.

We observed that adult *sqt-2* animals derived from L3 juveniles displayed a squat phenotype, whereas adult *sqt-2* animals arising from dauer larvae manifested a roller phenotype. This was a most surprising observation, for it had been generally assumed that adults rising from L3 juveniles or from dauer larvae were indistinguishable. This is clearly not the case. Since the cuticle is shed twice between these stages, it is unlikely that this difference is due to residual cuticle differences between the L3 and the dauer larva. This observation suggests that future published observations on the adult form should be careful to specify if the adult was derived from the L3 or the dauer larva.

We wish to thank ALISON PILLSBURY for substantial contributions to this work. Also, we wish to acknowledge JOAN POLITZ and WENDY CRUM who carried out some of the genetic experiments reported on here. This work was supported by grants PCM 76-11481 and PCM 78-09439 from the National Science Foundation and 5-SO7RR07135-08 from the Public Health Service.

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Corresponding editor: A. CHOVNICK