GENETIC STUDIES OF UNUSUAL LOCI THAT AFFECT BODY SHAPE OF THE NEMATODE *CAENORHABDITIS ELEGANS* AND MAY CODE FOR CUTICLE STRUCTURAL PROTEINS

MEREDITH KUSCH* AND R. S. EDGAR

*Thimann Laboratory, University of California, Santa Cruz, CA 95064, and * Section of Genetics and Development, Cornell University, Ithaca, New York 14853*

Manuscript received November 13, 1985
Revised copy accepted April 3, 1986

ABSTRACT

In *Caenorhabditis elegans*, four loci (*sqt-1*, *sqt-2*, *sqt-3* and *rol-8*) in which mutations affect body shape and cuticle morphology have unusual genetic properties. (1) Mutant alleles of *sqt-1* can interact to produce animals with a variety of mutant phenotypes: left roller, right roller, dumpy and long. At least three mutant phenotypes are specified by mutations in the *sqt-3* locus. (2) Most alleles at these loci are either dominant or cryptic dominant (*i.e.*, are dominant only in certain genetic backgrounds). (3) Most alleles of these loci exhibit codominance. (4) Two putative null alleles of the *sqt-1* locus produce a wild-type phenotype. (5) Many alleles of these genes demonstrate unusual intergenic interactions that are not the result of simple epistasis: animals doubly heterozygous for mutations at two loci often display unexpected and unpredictable phenotypes. We suggest that these genetic properties might be expected of genes, such as the collagen genes, the products of which interact to form the animal's cuticle, and which are member genes of a gene family.

IN the nematode *Caenorhabditis elegans*, mutations that alter the animal's gross morphology have been identified in over 50 genes (BRENNER 1974; HIGGINS and HIRSH 1977; COX *et al.* 1980). Phenotypes produced by these mutations include dumpy, small, long, blister, right and left roller; in many cases mutants have demonstrable anatomical alterations in the extracellular cuticle (COX *et al.* 1980; M. KUSCH and R. S. EDGAR, unpublished observations). Since the *C. elegans* cuticle is composed primarily of small covalently cross-linked collagens (COX, KUSCH and EDGAR 1981; COX, STAPRANS and EDGAR 1981; EDGAR *et al.* 1982; POLITZ and EDGAR 1984), some of the identified morphological mutants are probably defective in genes coding for cuticle collagens.

Collagens have long stretches of triple helical structure formed by a repeating $(Gly\text{-}X\text{-}Y)_n$ amino acid sequence, in which the first amino acid is glycine and the X and Y positions are often proline or hydroxyproline (RAMACHANDRAN 1967; FESSLER and FESSLER 1978). In *C. elegans*, the collagen gene family is large, consisting of 40–150 genes that code for small (~30 kilodalton) pro-

teins (KRAMER, COX and HIRSH 1982; COX, KRAMER and HIRSH 1984). Many of these genes are regulated both temporally and quantitatively, and at least some are expressed at the time of cuticle synthesis, suggesting they code for cuticle proteins (POLITZ and EDGAR 1984; KRAMER, COX and HIRSH 1985; COX and HIRSH 1985). Although gene family members have substantial sequence differences, their protein products nevertheless have long stretches of similar repetitive sequences (KRAMER, COX and HIRSH 1982). Thus, protein products of different family members could potentially substitute for each other as they interact in building a cuticle.

So far, making a connection between genes identified by mutational alteration of gross morphology and collagen genes identified by molecular genetic techniques has not been possible. A few collagen genes have been localized on the genetic map using a combination of genetic and molecular methods (COX *et al.* 1985). However, general principles have not yet emerged to suggest which of the many genes affecting gross morphology are likely to be collagen genes.

We describe here a group of morphological mutants with unusual genetic properties. We suggest that these genetic properties might be expected of mutants with alterations in genes, such as the collagen genes, that code for structural proteins which interact together to form the cuticle.

MATERIALS AND METHODS

Strains and culture methods: *Caenorhabditis elegans* var. Bristol (N2 is the wild-type strain designation) and many strains used for mapping were obtained from and originally described by BRENNER (1974). The *Caenorhabditis* Genetics Center, supported by National Institutes of Health contract number N01-AG-9-2113 and by the Curators of the University of Missouri, also provided a number of previously described strains (SWANSON EDGLEY and RIDDLE 1984). Certain strains carrying duplications, deficiencies and rearrangements involving LG II were obtained from R. HERMAN and C. SIGURDSON, University of Minnesota. This paper uses standard *Caenorhabditis elegans* genetic nomenclature (HORVITZ *et al.* 1979).

LG I: *bli-4(e937)*, *dpy-5(e61)*, *dpy-14(e188)*, *dpy-24(s119)*, *unc-13(e51)*.

LG II: *bli-1(sc73)* (M. KUSCH and R. S. EDGAR, unpublished observation) *bli-2(e768)*, *dpy-2(e1359)*, *dpy-2(e489)*, *dpy-10(e128)*, *rol-1(e91)*, *rol-5(sc13)*, *rol-6(e187)*, *sqt-1(e1350)*, *sqt-2(sc3)*, *unc-4(e120)*, *unc-52(e444)* and *mnC1 dpy-10(e128) unc-52(e444)*. *mnC1 dpy-10 unc-52* balances the right arm of chromosome *II* and carries a crossover suppressor for this region of LG II (HERMAN 1978). A strain carrying a free duplication of the *sqt-1* region was used in some experiments and had the following genotype: *mnC1 dpy-10 unc-52/unc-4 unc-52/mnDp36 (II,f)* (HERMAN, MADL and KARI 1979). Deficiency strains covering the *sqt-1* region (SIGURDSON, SPANIER and HERMAN 1984) were genotypically *mnC1 dpy-10 unc-52/unc-4 Df* and contained the following deficiencies: *mnDf75*, *mnDf76*, *mnDf77*, *mnDf83*, *mnDf86*, *mnDf87*, *mnDf89* and *mnDf90*. The deficiencies all covered *rol-5* as well as *sqt-1* and complemented a newly identified *rol* locus, *rol-8*, described below.

LG III: *bli-5(e518)*, *dpy-1(e1)*, *dpy-17(e164)*, *dpy-17(e1345)*, *dpy-18(e364)*, *dpy-19(e1314)*, *dpy-19(e1259)*, *lon-1(e185)* and *unc-32(e189)*.

LG IV: *bli-6(sc16)*, *dpy-4(e1166)*, *dpy-9(e12)*, *dpy-13(e184)*, *dpy-13(e225)*, *dpy-20(el1282)* and *unc-24(e138)*.

LG V: *dpy-11(e224)*, *dpy-21(e428)*, *rol-3(e754)*, *rol-4(sc8)*, *sqt-3(sc63)*, *sqt-3(e24)* (J. HODGKIN, personal communiction) and *unc-42(e270)*.

X: *dpy-3(e27)*, *dpy-3(m39)*, *dpy-6(e14)*, *dpy-7(e88)*, *dpy-8(e130)*, *dpy-12(e182)*, *dpy-22(e652)* and *lon-2(e678)*.

General culture methods and genetic techniques were described previously (BRENNER 1974). Genetic crosses were performed at 20° unless an allele was temperature-sensitive (*ts*), in which case, crosses were done at 25° as well. Stocks were routinely maintained at 16°.

Phenotypes: With a number of the mutants in the *sqt* and *rol-8* loci, scoring phenotype is somewhat subjective as many weaker phenotypes are marginal, *i.e.*, they are not precisely wild type, but they are only slightly mutant. Therefore, phenotype definitions used in this study are listed below.

Dumpy: the animals are shorter and appear fatter than wild type; a weak dumpy appears slightly shorter than wild type and slightly fatter; a strong dumpy is an extremely short "piggy" animal that can barely "waddle."

Roller: the animals are helically twisted, such that their bodies roll over as they attempt to move; left rollers spin counterclockwise and right rollers spin clockwise; both leave circular tracks in a bacterial lawn. A weak left roller is an animal that only occasionally rolls or an animal that catches its nose on the surface when attempting to roll and stops, backs up and starts forward again with or without immediately turning its nose.

Long: Long animals appear longer than wild type, but body lengths were not generally measured.

Squat: Animals are dumpy when the mutation is homozygous, but they roll when the mutation is heterozygous with a wild-type allele.

Pseudo wild type: Most hermaphrodites homozygous for mutant *sqt-1* alleles have tail defects, such that the animal's tail is more blunt than normal or the animal appears to have a small ball attached to the tip of its tail. When no defects other than these tail aberrations were visible, mutant animals were classified as pseudo wild type.

Generation of new mutations and genetic methods: The left roller allele of *sqt-1*, *sc13*, was previously assigned to a different gene, *rol-5* (COX *et al.* 1980). Since recombination between *e1350* and *sc13* in 5000 animals was not found, COX *et al.* (1980) suggested that *rol-5* and *sqt-1* might represent a single locus. To further examine this idea, animals of genotype + + *e1350/unc-4 sc13* + were constructed, and their progeny were examined for recombinants. Parental animals are long, and an occasional animal roles left momentarily. The expected progeny from these animals are primarily dumpy (*e1350*), long, weak left roller (+ + *e1350/unc-4 sc13* +) and uncoordinated rollers (*unc-4 sc13*) in a 1:2:1 ratio. Left rollers (genotypically *unc-4 sc13/+ sc13*) are produced as a result of recombination between *unc-4* and *rol-5*, which are approximately 1 map unit apart; but right rollers could result only from a recombinant event between *e1350* and *sc13* (either + + +/+ + *e1350* or *unc-4* + +/+ + *e1350*). One right roller was found among 37,500 segregants examined. This recombinant did not segregate *unc-4* animals, suggesting that *sc13* is to the left of *e1350*, barring gene conversion effects.

Additional alleles of *sqt-1* were obtained by crossing males mutagenized with ethyl methanesulfonate (EMS), as described by BRENNER (1974), with hermaphrodites of genotype *sqt-1(e1350) unc-4*. The *unc-4* mutation allowed recognition of outcross progeny and marked the chromosome bearing the *sqt-1(e1350)* tester allele. Since *sqt-1(e1350)/+* animals are right rollers, new alleles are expected to be nonroller. Therefore, F_1 nonroller, non-uncoordinated hermaphrodites were cloned for further analysis, and only those clones that segregated no or very few right rollers among their progeny were saved. By this method, nine new *sqt-1* alleles were identified at a frequency of 1.5×10^{-4} (see Table 3). Two linked suppressors of the *sqt-1/+* phenotypes, *sc98* and *sc102*, were isolated and mapped to the left of *unc-4* (Table 1). As both suppressors failed to complement *rol-8(sc15)*, and mapped close to *sc15*, they have tentatively been assigned to the same gene, a newly identified *rol* locus on LG II (Table 1). While *rol-8* maps close to *rol-6*, the canonical alleles *rol-8(sc15)* and *rol-6(e187)* complement. A partial genetic map of LG II is shown in Figure 1.

TABLE 1

***rol-8* mapping data**

I. Two-factor crosses

Parental genotype	Recombinants (++)/total	90% confidence limits for map distance (*P*)[a]
(sc15 unc-4/+ +)	6/1238	0.42–1.9
(sc98 +/+ sqt-1(e1350))	5/1430	0.28–1.48
(sc102 +/+ sqt-1(e1350))	12/1260	1.1–3.1

II. Three-factor crosses

Parental genotype	Recombinant phenotype	Genotype of selected recombinants
dpy-10 unc-4/sc15	Dumpy	3 *(dpy-10 unc-4/dpy-10 sc15)*
	Dumpy	1 *(dpy-10 unc-4/dpy-10)*
dpy-10/sc15 unc-4	Roller	2 *(sc15 unc-4/sc15 +)*
sc98/unc-4 sqt-1(e1350)	Roller	3 *(+/unc-4 sqt-1)*
	Roller, uncoordinated	2 *(unc-4 +/unc-4 sqt-1)*
sc102/unc-4 sqt-1(e1350)	Roller	9 *(+/unc-4 sqt-1)*
	Roller, uncoordinated	3 *(unc-4/unc-4 sqt-1)*

[a] Ninety percent confidence limits for observed number of recombinants (R/2) derived from Poisson distribution. Map distances calculated as $P = 1 - \sqrt{1 - 2R}$, where R is recombinant frequency.

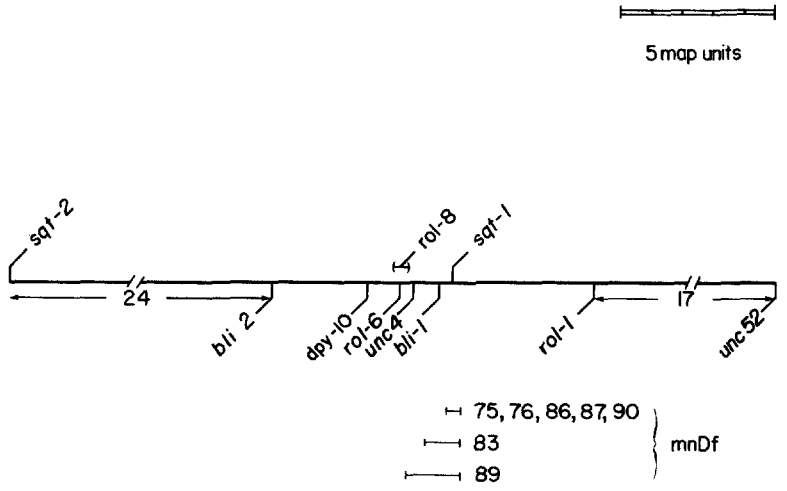

FIGURE 1.—Linkage map of LG II showing loci used in this study. Map is to scale, as indicated, except for the terminal intervals.

Mutations to be mapped were assigned to a linkage group using a reference *unc* on each chromosome, and map distances were determined as described by BRENNER (1974) from a standard marker *cis* to the unknown mutation. The *rol* mutations *sc15* and *sc42*, previously described (COX *et al.* 1980), were found by deficiency mapping not to reside at the *rol-5* locus. Seven other *rol-5* mutations were described by COX *et al.* (1980); these mutations also did not reside at the *sqt-1* locus, as determined by deficiency mapping. These other mutations have been lost. *sc15* defines the *rol-8* gene and is located on LG II (Table 1), whereas *sc42* has tentatively been assigned to *sqt-3* because

it is located on LG V and interacts with the other *sqt-3* alleles (see RESULTS). More precise mapping has been hindered by poor penetrance of *sc42*.

One new *sqt-2* allele, *sc108*, resulted from screening 11,250 F_1 progeny of EMS-treated animals for rollers.

Homozygous males of some *sqt-1* alleles are fertile, mate well and were used in many crosses (see Table 5). When homozygous males were not available, males heterozygous with a wild-type chromosome were used, except for crosses requiring heterozygous *sqt-1(e1350)* or *sqt-2* males which are right rollers that cannot mate. For these crosses, nonroller males were generated by crossing *sqt-1(e1350)* or *sqt-2* hermaphrodites with males homozygous for one of the *sqt-1* suppressor alleles, usually *sc97* or *sc101*. In some crosses, homozygous *sqt-2(sc3)* males were used.

Complementation tests involving deficiencies were performed either with males generated from the deficiency strain or with males heterozygous for a wild-type chromosome and one chromosome of the deficiency strain. When results could have been ambiguous, progeny testing was used to confirm the presence (or absence) of deficiencies.

A duplication strain of genotype *mnC1 dpy-10 unc-52/unc-4 unc-52/mnDp36* carrying a duplication of the *sqt-1* region (D. C. SIGURDSON and R. K. HERMAN, personal communication) was used to construct animals carrying two wild-type alleles and one *sqt-1(e1350)* allele. The duplication covers *unc-52*, but not *dpy-10* or *unc-4* (HERMAN, MADL and KARI 1979). N2 males were mated with the duplication strain, and progeny males were crossed (one per plate) with two *unc-4 sqt-1(e1350)* hermaphrodites. All male progeny from crosses not segregating roller, uncoordinated progeny (*unc-4 sqt-1/unc-4 unc-52*) were right roller, suggesting that strains carrying two wild-type alleles and one *e1350* allele also had this phenotype. This inference was confirmed by cloning ten such right roller hermaphrodites, eight of which were *unc-4 sqt-1/mnC1*, and two of which produced dumpy segregants *mnC1/mnC1/mnDp36*.

The same duplication was used to construct animals carrying two copies of the recessive left roller *sqt-1(sc13)* and one copy of a wild-type allele. Wild-type males were mated with *sqt-1 unc-52* hermaphrodites, and these males were crossed to dumpy hermaphrodites of genotype *mnC1/mnC1/mnDp36*. Fifteen wild-type F_1 hermaphrodites were cloned, and their genotypes were determined by inspection of the F_2 for the presence of dumpy segregants (an assay for the presence of the duplication and the *mnC1* chromosome) and uncoordinated, nondumpy segregants (an assay for the *sqt-1 unc-52* chromosome, because *unc-52* is epistatic to *sqt-1(sc13)* presumably because animals are completely paralyzed). One-third (5 of 15) of the animals segregated both types and, thus, were of genotype *mnC1/sqt-1 unc-52/mnDp36*. These also segregated weak left rollers, indicating that a single copy of the $sc13^+$ allele is only partially effective in suppressing the left roller phenotype in animals carrying two copies of the *sc13* allele. That these weak left rollers carried the duplication was demonstrated by segregation of animals that displayed the *unc-52* phenotype. The presence of the *sqt-1(sc13)* mutation was demonstrated by complementation tests with *sqt-1(sc13)* males.

Temperature sensitivity (*ts*) studies: Experiments to determine if any *sqt* or *rol-8* alleles are *ts* were conducted to 16° and 25°. Most of the *sqt-1* alleles are not markedly *ts*; however, *sqt-1(e1350)* is much less dumpy at 25° than at 16° and appears to be less fertile at the higher temperature. One newly isolated allele of *sqt-1*, *sc101*, has a stronger left roller phenotype at 16°, whereas adults raised at 25° are long and curl up in spirals. Adults of *sqt-1(sc13)* are somewhat longer at the lower temperature. One *rol-8* allele, *sc102*, is *ts* for a long phenotype. Two *sqt-3* alleles, *sc63* and *e24*, are semidominant and *ts* (see Table 6 in RESULTS). Both alleles are homozygous dumpy at 25°, wild type at 20° and longer at 16° than at 20°. *e24* is sterile at 25° (COX *et al.* 1980).

RESULTS

Mutations at the squat loci produce a variety of mutant phenotypes: Three previously identified squat, *sqt*, genes reside on linkage groups II and V. Mu-

TABLE 2

Phenotypes produced by mutations at the squat and *rol-8* loci

Gene[a]	Representative alleles	Phenotypes	
		Mutant/mutant	Mutant/wild type
sqt-1 (II)	*e1350*	Dumpy[b]	Right roller
	sc101	Long	Wild type
	sc97	Wild type	Wild type
	sc13	Left roller	Wild type
sqt-2 (II)	*sc3*	Weak dumpy	Right roller
sqt-3 (V)	*sc63*	Dumpy *(ts)*[c]	Left roller *(ts)*
	e24	Severe dumpy *(ts)*	Weak dumpy *(ts)*
	sc8	Left roller	Wild type
rol-8 (II)	*sc15*	Left roller	Wild type
	sc98	Wild type	Wild type

[a] The linkage group on which each gene resides is given in parentheses.
[b] Phenotypes are described more fully in MATERIALS AND METHODS.
[c] *(ts)* denotes temperature-sensitive alleles that produce a mutant phenotype at the restrictive temperatures (25°) only. Animals homozygous for either *sc63* or *e24* are about 20% longer than wild type at 16°.

tations at the *sqt* loci produce phenotypes that include many of the morphological mutant phenotypes described by BRENNER (1974): dumpy, long, left roller and right roller (Table 2). The blister phenotype is the only major mutant morphological phenotype not produced by mutation of these genes. The canonical "squat" mutations produce a dumpy phenotype when homozygous, but a right roller (*sqt-1*, *sqt-2*) or left roller (*sqt-3*) phenotype when heterozygous, with wild type (COX *et al.* 1980). Bodies of rollers are helically twisted, causing nematodes to roll either clockwise (right roller) or counterclockwise (left roller) as they attempt to move forward.

The left roller allele of *sqt-1*, *sc13*, was previously assigned to a different gene, *rol-5* (COX *et al.* 1980). However, crosses described in MATERIALS AND METHODS indicate that *sc13* lies within 0.005 map units of the *sqt-1* allele, *e1350*. Along with complementation data described below, these results support the view that *sc13* and *e1350* are alleles of the same locus, *sqt-1*.

Additional mutant alleles of *sqt-1* were detected in complementation screens, as non-right roller heterozygous segregants from crosses of mutagenized N2 males with *sqt-1(e1350) unc-4* hermaphrodites (see MATERIALS AND METHODS). Three classes of alleles were found (Table 3): those that, when heterozygous with *e1350*, give (1) a wild-type phenotype (five alleles), (2) a dumpy phenotype (three alleles) and (3) a long, weak left roller phenotype (one allele). One new squat-type allele (an allele that produces a dumpy phenotype when homozygous, but a roller phenotype when heterozygous, with wild type), *sc104*, was isolated by this screening procedure. Recombination between these alleles and

TABLE 3

***sqt-1* alleles**

Alleles	Phenotypes		Recombinants[a]
	Mutant/mutant	Mutant/*e1350*	
Previously identified			
e1350[b]	Dumpy	Dumpy	
sc13[c]	Left roller	Long, weak left roller	1/42,000[d]
sc23	Left roller	Wild type	
New[e]			
sc97	Wild type	Wild type	0/15,722
sc99	Wild type	Wild type	0/7,500
sc107	Wild type	Wild type	0/1,250
sc100	Wild type	Dumpy	0/1,870
sc103	Wild type	Dumpy	0/5,750
sc104	Dumpy	Dumpy	0/6,550
sc101	Long	Long, weak left roller	0/360

[a] Recombinants were scored as right rollers (*i.e.*, *e1350*/+ animals) segregating from animals heterozygous for *e1350* and the mutant allele.

[b] Six other squat-type alleles were described by COX *et al.* (1980), but four have since been lost.

[c] Nine other *rol-5* alleles were described by COX *et al.* (1980). Only *sc13* and *sc23* are *sqt-1* alleles (see RESULTS).

[d] This figure includes the 5000 animals scored by COX *et al.* (1980).

[e] Two additional alleles have not been extensively tested, but when heterozygous with *e1350*, the resulting animals are wild type.

sqt-1(*e1350*) was not detected (Table 2) Surprisingly, no new left roller alleles comparable to *sc13* were identified by this method.

In addition to new *sqt-1* alleles detected in the complementation screens, two mutations (*sc98* and *sc102*) linked to but not at the *sqt-1* locus were identified as dominant suppressors of the *e1350*/+ right roller phenotype. These mutations give either a wild-type (*sc98*) or a weak left roller (*sc102*) phenotype when homozygous. As shown in Table 1 and described in MATERIALS AND METHODS, *sc98* and *sc102*, along with the recessive left roller allele, *sc15*, which these mutations fail to complement (see Table 7 below), map approximately 2 map units to the left of *sqt-1* and may identify a fourth locus, *rol-8*.

Comparable screens for new alleles of *sqt-2* or *sqt-3* have not been done. So far, for *sqt-2*, only squat-type alleles have been isolated. However, several allelic types have been found at the *sqt-3* locus: *ts* squat alleles, a *ts dpy* allele and left roller alleles.

Most alleles at the squat loci are dominant: All the squat-type alleles at the three *sqt* loci are codominant with wild type in that heterozygotes are rollers. Except for *sqt-3*(*e24*), which is a semidominant *ts* dumpy, all other identified mutations at these loci appear recessive to wild type. However, many of these apparently recessive alleles behave as dominants in certain genetic backgrounds

TABLE 4

Effect of *dpy-12(e182)* genetic background on alleles specifying morphological phenotypes

Allele test (m)	Genotype			
	dpy-12$^+$		*dpy-12(e182)*	
	m/m +/+	*m*/+ +/+	*m/m dpy/dpy*	*m*/+ *dpy/dpy*
Wild type	+[a]	+	D	D
sqt-1				
e1350	*D*	*RR*	ND	D,wLR
sc13	*LR*	+	D,LR	D,LR
sc23	*LR*	+	D,LR	D
sc97	+	+	D,LR	D,LR
sc99	+	+	ND	D,LR
sc100	+	+	D	D
sc103	+	+	D	D
sqt-2				
sc3	D	RR	ND	D,LR
sqt-3				
sc8	LR	+	ND	D
sc42	LR	+	ND	D,LR
sc63[b]	D	LR	ND	D,LR
rol-8				
sc15	LR	+ ND	D	D
Other loci				
rol-1(e91)	LR	+	ND	D
rol-3(e754)	LR	+	ND	D
rol-6(e187)	RR	+	D,RR	D
lon-1(e185)	Lon	+	ND	D

[a] Phenotypes were determined when each allele listed was homozygous *(m/m)* and heterozygous *(m/+)* in either a wild-type background *(dpy-12*$^+$*)* or in a *dpy-12(e182)* background. Phenotypes in this and subsequent tables are abbreviated as follows: +, wild type; D, dumpy; wD, weak dumpy; xD, strong dumpy; LR, left roller; wLR, weak left roller; RR, right roller; Lon, long; ND, not determined.

[b] The phenotypes for strains carrying *sc63* were determined at 25°.

and therefore should be considered *cryptic dominants*. For example, *sc13*, a recessive left roller allele of *sqt-1*, is dominant to its wild-type allele in animals homozygous for *dpy-12(e182)*: animals of genotype *sqt-1(sc13)/+; dpy-12/dpy-12* are dumpy left rollers, rather than simply dumpy (Table 4). One or, in some cases, two alleles of 21 *dpy* genes (see MATERIALS AND METHODS) were examined, and alleles of nine genes (*dpy-2, dpy-3, dpy-7, dpy-8, dpy-9, dpy-10, dpy-12, dpy-14* and *dpy-17*) provide a phenotypic context in which *sc13* behaves as a dominant.

"Dumpy-dependent" dominance has not been examined with all apparent recessive alleles at the *sqt* loci; however, two pseudo wild-type alleles of *sqt-1*, *sc97* and *sc99* are dominant left rollers in a *dpy-12* background, and two other

TABLE 5

Phenotypes of *sqt-1* homozygous and heteroallelic strains

sqt-1 allele	*e1350*	*sc13*	*sc97*	*sc99*	*sc101*	*sc100*	*sc103*	*Df*	+
e1350	D[a]	Lon,wLR	+	+	Lon	D	D	D	RR
sc13		LR	LR	LR	LR	LR	LR	LR	+
sc97[b]			+	+	+	+	+	+	+
sc99[b]				+	+	+	+	wD	+
sc101[b]					Lon,wLR	+	+	wD	+
sc100[b]						+[c]	+	wD	+
sc103[b]							+[c]	wD	+

[a] Abbreviations: see footnote to Table 4. *Df*, stands for deficiency of the *sqt-1* region (see MATERIALS AND METHODS). These results were found with all deficiencies tested, including *mnDf87*, which deletes markers on both sides of *sqt-1*.

[b] Homozygous males mate well and were used in most crosses involving these strains.

[c] Homozygous males have a weak dumpy phenotype.

pseudo wild-type alleles, *sc100* and *sc103*, which we believe are null alleles (see below), do not behave as dominants (Table 4). "Dumpy-dependent" dominance is allele-specific, as *sqt-1* and *sqt-3* each contain two recessive left roller alleles, and only one allele from each locus, *sc13* and *sc42*, respectively, is dominant in a *dpy-12* background. *sqt-1*(*sc23*) and *sqt-3*(*sc8*) do not show dumpy-dependent dominance.

A *dpy-12* genetic background also affects the phenotype of the squat-type alleles, *sqt-1*(*e1350*) and *sqt-2*(*sc3*). In a wild-type background these alleles, when heterozygous with their wild-type alleles, both produce a right roller phenotype. However, in a *dpy-12* genetic background, both alleles, when heterozygous, produce a left roller phenotype. Since many *dpy* genes contain alleles that produce a homozygous dumpy left roller phenotype, conceivably, *dpy-12* animals were physically constrained to roll only left, not right. This is not the case, however, since mutants homozygous for *both dpy-12*(*e182*) and a right roller mutation, *rol-6*(*e187*), have a dumpy right roller phenotype. Reference alleles of the other *rol* genes identified in *C. elegans*, *rol-1*(*e91*), *rol-3*(*e754*), *rol-6*(*e187*) and *rol-8*(*sc15*), are not dominant in a *dpy-12* context.

Most alleles of the *sqt* loci exhibit codominance: Phenotypes of animals heterozygous for two alleles of a *sqt* locus are often unpredictable and are best considered to result from codominance. A striking example of this phenomenon is seen with squat-type alleles that generate a dumpy phenotype when homozygous, but a roller phenotype when heterozygous, with a wild-type allele. Codominance suggests that both mutant (*e1350*) and wild-type (*sqt-1*$^+$) alleles produce gene products. This notion is supported by results from crosses with nematodes carrying a duplication for the *sqt-1* region (see MATERIALS AND METHODS). Animals of genotype +/+/*e1350* were found to be right rollers, equivalent to +/*e1350* animals.

Numerous examples of codominance are found with both *sqt-1* (Table 5) and *sqt-3* alleles (Table 6). For example, although *sqt-1*(*sc13*) is recessive and gives a wild-type phenotype when heterozygous with a wild-type allele, animals

TABLE 6

Phenotypes of *sqt-3* homozygous and heteroallelic strains at 20° and 25°

sqt-3 allele	*sc8*	*sc42*	*sc63*	*e24*	+
At 20°					
sc8	LR[a]	+	wLR	+	+
sc42		LR	LR	wLR	+
sc63			+	wD	+
e24				+	+
At 25°					
sc8	LR	+	+	wD	+
sc42		LR	wLR	wD,wLR	+
sc63			xD	xD	LR
e24				xD	wD

[a] Phenotype abbreviations: see footnote to Table 4.

TABLE 7

Phenotypes of *rol-8* homozygous and heteroallelic strains

rol-8 allele	*sc15*	*sc98*	*sc102*	+
sc15	LR[a]	LR	LR	+
sc98		+	wLR	+
sc102			wLR	+

[a] Phenotype abbreviations: see footnote to Table 4.

of genotype *sc13/sc13/+* (see MATERIALS AND METHODS) are weak left rollers. This finding, along with cryptic dominance discussed earlier, is additional evidence for the presence of *sc13* gene product. Furthermore, when *sc13* is heterozygous with a squat-type allele, *e1350*, the resulting animal is neither wild type nor right roller: it is a long, weak left roller. Another cryptic dominant, *sqt-1(sc97)*, is a pseudo wild-type allele that gives a wild-type phenotype when heterozygous with *e1350*. However, when *sc97* is heterozygous with the apparent recessive left roller allele *sc13*, animals are left rollers. Other examples of *sqt-1* allelic interactions are listed in Table 5.

Alleles of the *sqt-3* locus also demonstrate codominance (Table 6). For example, *sqt-3(sc8)/sqt-3(sc63)* animals are wild type at 25°. This result is unexpected because *sc8* is a recessive allele and *sc63/+* nematodes are left rollers at this temperature. Many of the *sqt-3* mutations identified are temperature-sensitive. At 20°, squat-type alleles, such as *sc63*, seem identical to wild type until they are made heterozygous with other alleles. For example, when *sc63* is heterozygous with the recessive left roller allele, *sqt-3(sc42)*, a left roller phenotype is produced. At 25°, however, the left roller phenotype generated by these two alleles is weaker than at 20°.

Complementation patterns for alleles of *rol-8* are shown in Table 7. Not enough alleles have been isolated to determine if this locus shows codominance. *rol-8(sc98)* is a pseudo wild-type allele, and *rol-8(sc102)* is a weak left roller

allele that is recessive to wild type, but animals *trans*-heterozygous for *sc98* and *sc102* are weak left rollers.

Null alleles of the *sqt-1* locus produce a wild-type phenotype: In experiments designed to identify null alleles of *sqt-1*, only two of the nine new alleles had complementation properties similar to deficiency strains and therefore expected of a null. The complementation characteristics of the two putative null alleles, *sc100* and *sc103*, are shown in Table 5: these mutations behave in a manner similar to *sqt-1* deficiencies characterized by SIGURDSON, SPANIER and HERMAN (1984). This result can best be seen with the strongest mutant alleles of the *sqt-1* locus, *sc13* and *e1350*. Animals heterozygous for one of these alleles and for *sc100* or *sc103* have the same or similar phenotypes as animals heterozygous for these alleles and a deficiency. However, *sc100* may have some residual activity because animals heterozygous for *sc100* and a deficiency of this region are slightly more dumpy than the *sc100* homozygotes. Hermaphrodites homozygous for the putative null alleles are essentially wild type in morphology, although males are slightly dumpy.

Further support for the notion that these two alleles are nulls comes from the observation that (1) neither allele is a cryptic dominant in the *dpy-12* test system, and (2) neither allele shows intergenic dominant suppression or enhancement effects when combined with other squat or *rol-8* alleles (see below).

Intergenic interaction: We have found numerous examples of intergenic interaction involving mutations at the squat and *rol-8* loci; animals doubly heterozygous for mutations at two of the loci often display unexpected phenotypes. The most striking instances are intergenic noncomplementation: animals heterozygous for two recessive mutations nevertheless manifest a mutant phenotype.

Based on complementation results, COX *et al.* (1980) identified a gene, *rol-5*, containing many recessive left roller alleles, now assigned to *sqt-1*. Some, but not all, heterozygous combinations of these mutations gave a mutant (left roller) phenotype. Using deficiency mapping we have found that only two of the alleles originally assigned to *rol-5* actually residue at the *sqt-1* locus (*sc13* and *sc23*). The remainder are located elsewhere in the genome, but were classified as *rol-5* alleles because of failure to complement *rol-5*(*sc13*). Two of these alleles have been mapped. One, *rol-8*(*sc15*) is on linkage group II approximately 2 map units to the left of *sqt-1* (Table 1; Figure 1). The other, *sc42*, is a left roller on linkage group V and may reside at the *sqt-3* locus, as it interacts with several alleles of this locus (Table 5).

Further examples of intergenic noncomplementation between recessive alleles of *sqt-1*, *rol-8* and *sqt-3* are to be found in Tables 8 and 9. *sqt-1*(*sc13*) and *rol-8*(*sc98*) give a weak left roller phenotype when both are heterozygous over wild type, as do *sqt-1*(*sc101*) and *rol-8*(*sc15*) when heterozygous (Table 8). All of the *sqt-3* alleles are recessive at 20°, yet fail to complement certain of the *sqt-1* (*sc13*, *sc101*) or *rol-8* (*sc98*, *sc102*) recessive alleles at this temperature (Table 9). At 25°, *sqt-3*(*sc42*) is recessive and fails to complement the *sqt-1* alleles, *sc13* and *sc101*, and the *rol-8* allele, *sc102*. In all cases, a left roller or weak left roller phenotype results.

TABLE 8

Phenotypes of strains showing intergenic noncomplementation (*sqt-1* alleles vs. *sqt-2* and *rol-8* alleles)

sqt-1 genotype	*sqt-2* genotype			*rol-8* genotype		
	+/+	*sc3*/+	*sc108*/+	*sc15*/+	*sc98*/+	*sc102*/+
+/+	+[a]	RR	RR	+	+	+
e1350/+	RR	RR	RR	RR	+	+
sc13/+	+	+	+	LR	wLR	+
sc97/+	+	RR/+[b]	RR/+[b]	+	+	+
sc99/+	+	+	+	+	+	+
sc101/+	+	+	+	wLR	+	+
sc100/+	+	RR	RR	+	+	+
sc103/+	+	RR	RR	+	+	+

[a] Abbreviations: see footnote to Table 4.

[b] Heterozygotes between *sc97* and the two *sqt-2* alleles were right roller or wild type. The phenotype was somewhat dependent on age of the adult in *sc108*/*sc97* heterozygotes. Young, newly molted animals were right rollers, but became wild type when they were a day older at 20°. Age dependence of phenotype was not determined with *sc3*/*sc97* animals.

12

TABLE 9

Phenotypes of animals showing intergenic noncomplementation (*sqt-3* vs. *sqt-1*, *sqt-2* and *rol-8*)

	sqt-3 genotype[a]							
	20°				25°			
Genotype	+/+	*sc42*/+	*sc63*/+	*e24*/+	+/+	*sc42*/+	*sc63*/+	*e24*/+
+/+	+	+	+	+	+	+	LR	wD
sqt-1[b]								
e1350/+	RR[c]	RR	+	Lon	RR	RR	+	xD
sc13/+	+	wLR	LR	LR	+	LR	LR	LR
sc101/+	+	+	LR	wLR	+	wLR	LR	LR
sqt-2								
sc3/+	RR	wLR	+	+	RR	+	LR	wD
sc108/+	RR	RR	+	+	RR	RR	+	+
rol-8								
sc15/+	+	+	+	+	+	+	LR	wLR
sc98/+	+	+	wLR	+	+	+	LR	wLR,wD
sc102/+	+	+	LR	wLR	+	wLR	LR	wLR

[a] The recessive left roller allele *sqt-3(sc8)* was tested in heterozygous combinations with all alleles listed here and with the other *sqt-1* alleles listed in Table 3, and no examples of intergenic interactions were found.

[b] No intergenic interactions were found between the *sqt-1* alleles, *sc97*, *sc99*, *sc100*, *sc103*, and the *sqt-3* alleles listed, or *sqt-3(sc8)*.

[c] Abbreviations: see footnote to Table 4.

Other cases of unexpected intergenic interactions were found in combinations of a dominant, such as *sqt-1(e1350)*, with a recessive, such as *rol-8(sc98)* (Table 8). In this instance, a wild-type phenotype, rather than the expected

right roller phenotype, was produced. It is also remarkable that *e1350/+*; *e24/+* is long. Other cases of unexpected allelic interactions are to be found in Table 9. For example, the semidominant dumpy allele, *sqt-3(e24)*, interacts with recessive *sqt-1* alleles, *sc13* and *sc101*, and with recessive *rol-8* alleles, *sc15* and *sc102*, to give animals that are left rollers.

Many of these results can be described as due to dominant enhancer or suppressor mutations that, in many cases, have recessive morphological phenotypes. Thus, *sqt-3(sc42)* has a recessive left roller phenotype, but can be thought of as a dominant enhancer of *sqt-1(sc13)* (or vice versa). Similarly, *rol-8(sc98)* has no mutant morphological phenotype when homozygous, but can be described as a dominant suppressor of the *sqt-1(e1350)/+* right roller phenotype. The dominant gene interactive effects found for alleles that have either no obvious phenotype or are recessive to wild type provide further support for the notion that most of these alleles produce mutant gene products contributing to the cuticle's morphology. The *sqt-1* alleles, *sc100* and *sc103*, do not display dominant modifier effects and on other grounds appear to be null alleles.

Additional dominant modifier genes might be identified through further study since we found numerous dominant suppressors of the right roller phenotype of *sqt-1(e1350)/+* animals in experiments designed to isolate new *sqt-1* alleles (see MATERIALS AND METHODS). We have investigated dominant modification using either *sc13* or *e1350* as heterozygous tester alleles with a number of mutations of already identified genes that specify morphological phenotypes. We tested one and sometimes two alleles of 24 dumpy genes; *sqt-3(e24)* is the only dumpy mutation that is a dominant modifier of these *sqt-1* alleles. No dominant interactions were found with reference alleles of the three other *rol* genes, five blister genes (*bli-3* was not tested) or the two *lon* genes.

It should be noted that, due to the unusual gene interaction effects and the fact that most mutations described here are dominant or cryptic dominant in phenotype, lack of complementation between mutations cannot be used to assign mutations to genes or genetic loci. Gene assignment must be based solely on recombination. However, only in the case of alleles of *sqt-1* (Table 3) have systematic mapping crosses been done. Some mutations that, for convenience, were tentatively assigned to *rol-8* and *sqt-3* may, in fact, represent mutations at several linked loci, all of which display the unusual characteristics described above.

DISCUSSION

Mutational analyses of gene structure have been derived almost exclusively from studies of genes that code for enzymes (*e.g.*, β-galactosidase, tryptophan synthetase), and mutant phenotypes directly reflect an enzymatic activity that often can be assayed quantitatively. Such gene products have one or a few highly specific binding sites and often an exact conformation that must be maintained for biological activity. As a consequence, when a mutation occurs, a quantitative effect on phenotype is usually found: the enzyme's activity is often eliminated (amorphic or null mutations), sometimes decreased (hypo-

morphic mutations) or, more rarely, increased (hypermorphic mutations). As a rule, mutations that give rise to amino acid substitutions will produce gene products that are either amorphic or hypomorphic, and recessive.

The mutations we have assigned to the four loci, *sqt-1*, *sqt-2*, *sqt-3* and *rol-8*, do not show these characteristics. (1) Alleles of a single locus, such as *sqt-1*, can interact to specify a wide variety of morphological phenotypes: long, dumpy, left roller, right roller. (2) Most mutations are either dominant or cryptic dominant (*i.e.*, behave as dominant in some genetic backgrounds). (3) Most mutations produce unpredictable codominant heterozygous phenotypes. (4) Two alleles of *sqt-1*, *sc100* and *sc103* appear by various criteria to be null alleles. They both produce a wild-type phenotype when homozygous. (5) Unusual intergenic interactions are found between alleles at these loci, *e.g.*, noncomplementation between apparent recessives. We suggest that these properties might be expected of genes, the products of which are structural proteins that interact to form the cuticle, and which are member genes of a gene family.

Mutational studies of structural protein genes (PROCKOP *et al.* 1979; KEMPHUES *et al.* 1979; MORRIS, LAI and OAKLEY 1979; RAFF and FULLER 1984) suggest that such genes may, in fact, display these unusual genetic properties. Mutations in genes that code for structural proteins, such as the cuticle collagens, will produce recognizable phenotypic effects resulting in changes of form, rather than quantifiable enzyme activities. Collagen has long stretches of triple helix and large numbers of cross-linking sites; exact molecular configurations may not be essential for incorporation of mutant proteins into a structure such as the cuticle.

The cuticle is the exoskeleton of *C. elegans* and therefore determines the nematode's shape. Cuticles of squat-type animals, such as *sqt-1(e1350)*, are very deranged. At the light microscope level, aberrant annuli and lateral alae are visible (COX *et al.* 1980); at the electron microscope level, aberrant structures are found within cuticles that have been cut in cross-section (M. KUSCH and R. S. EDGAR, unpublished observations). These results suggest that the affected genes may code for structural protein components of the cuticle. Collagens, the major cuticle proteins, appear to be synthesized as relatively small primary gene products that are later cross-linked to form larger molecules. From *in vitro* translation of collagen mRNA, POLITZ and EDGAR (1984) concluded that at least 30 different small collagen molecules are synthesized before the animals' last molt when they become adults. The collagen gene family in *C. elegans* is large, containing up to 150 genes, many of which may specify different cuticle proteins (COX, KRAMER and HIRSH 1984).

So far, mutation of the *sqt-1* locus can produce animals of all *C. elegans* mutant morphologies previously described, except blister; *sqt-3* and *rol-8* each contain at least three allelic types. This variety of mutational alteration in animal morphology could well arise from changes in structure of the cuticle's elemental building blocks. Genes coding for cross-linking enzymes, etc., could affect the cuticle's morphology, but the resulting mutant phenotypes might be expected to differ from one another in severity, rather than overall shape.

With a structure, such as the cuticle, a large portion of each protein or

collagen molecule is in close contact with other cuticle proteins and is covalently attached at a number of sites. As a consequence, many single-site mutations probably would have little or no effect on the resulting cuticle, because most of them would not deform the proteins sufficiently to provoke a detectable shape change. However, those mutations that do produce altered morphologies would likely be dominant. The presence of a very defective protein will often lead to deformation of the structure, even in the heterozygote. For example, mutational changes that alter cross-linking sites or protein length would lead to a mutant phenotype even in the presence of wild-type collagens.

Many alleles we identified are dominant only in a mutant background: we have termed them "cryptic" dominants. A number of the *sqt-1* alleles behave as dominant left roller mutations when they are in backgrounds homozygous for *dpy-12(e182)*. Nine *dpy* genes provide a context in which *sqt-1(sc13)* is a dominant left roller allele. Although other cryptic dominant *sqt* alleles were not examined in other *dpy* backgrounds, it may be that a number of the *sqt* alleles will have unusual dominance characteristics in these *dpy* backgrounds. Interestingly, all *dpy* genes that contain dumpy roller alleles (*dpy-2*, *dpy-3*, *dpy-7*, *dpy-8* and *dpy-10*; Cox *et al.* 1980) permit expression of cryptic dominance. Possibly, on further examination, all the *dpy* genes that allow expression of cryptic dominance may also be found to have alleles that produce dumpy left rollers. These characteristics may be unique to a specific class of *dpy* genes involved primarily in cuticle morphology. The products of these *dpy* genes could be intimately associated with those of the *sqt* genes, such that the perturbation caused by a mutant *sqt* gene product, even in the presence of allelic wild-type products, would be enough to cause animals to roll in addition to being dumpy.

Codominance of alleles might be expected for structures that incorporate two mutant allelic forms of a protein; the final product, the cuticle, may well have a morphology different from that generated by either allelic form alone. Animals heterozygous for a squat-type allele and its wild-type allele have a phenotype different from either homozygote (that is, the phenotype is neomorphic), suggesting that the *sqt* and wild-type gene products interact. For these genes, in general, the phenotypes obtained when two alleles fail to complement are unpredictable and may be various mutant forms or wild type. These results are not readily explained by a linear phenotypic hypomorph-to-hypermorph gradient, as would be expected if these alleles coded for an enzyme activity.

Null mutations could be relatively rare in genes coding for structural proteins, whereas they are expected to be common with other kinds of genes. With collagen or other cuticle structural proteins, it is probable that most mutant forms of the proteins will be incorporated into the cuticle as long as the mutation permits export of the product into the cuticle space. Therefore, null mutations would have to abolish production of a protein altogether, not merely eliminate a biological activity. In addition, aberrant soluble proteins are efficiently degraded, whereas aberrant cuticle proteins are probably retained within the cuticle space.

Of the 16 alleles of *sqt-1* described in this study and in Cox *et al.* (1980), only two, *sc100* and *sc103*, have complementation properties similar to those of *sqt-1* deficiencies and are therefore putative null alleles. None of the other alleles are amorphs by this criterion. Although we have not demonstrated conclusively that *sc100* and *sc103* are, indeed, null alleles, this notion is supported by two other findings: (1) neither allele is a cryptic dominant in a *dpy-12* background, and (2) neither allele produces animals of unexpected phenotype in studies of intergenic interactions with *sqt-2*, *sqt-3* or *rol-8*. If, in fact, *sc100* and *sc103* are null alleles, these two findings suggest that both cryptic dominance and intergenic interaction result from incorporation of mutant proteins into the cuticle.

The putative null alleles display an almost wild-type phenotype when homozygous. GREENWALD and HORVITZ (1980) suggested that genes identified by dominant mutations and exhibiting a wild-type null phenotype are member genes of a gene family in which other member genes can replace the deleted gene product. This hypothesis has been confirmed for the actin gene family in *C. elegans* (LANDEL *et al.* 1984). By these criteria, *sqt-1* is a member of a gene family, perhaps the collagen family. It does not follow, however, that the other genes (*rol-8*, *sqt-2* and *sqt-3*) shown to interact with *sqt-1* are members of the same gene family.

The three squat loci and *rol-8* exhibit unexpected interallelic interactions, interactions that are not the result of simple epistasis. Most striking was the finding that recessives at these loci (some of which are cryptic dominants) fail to complement although they are distantly linked or unlinked. Indeed, this failure to complement led to an initial misclassification of some of these mutations as alleles. We suggest that animals of unexpected phenotype resulted from interaction of two heterozygous, nonallelic mutations, because the mutations produce aberrant gene products that interact in assembly of a common structure—in this case, the cuticle. This interpretation is supported by the finding of unexpected phenotypes for double heterozygotes involving a dominant allele at one locus and a recessive at the other.

Looking for such nonepistatic gene interactive effects may provide a strategy for identifying further genes coding for proteins that collaborate in forming the cuticle. The crucial test for determining group membership is the phenotype of double heterozygotes, at least one of which must be recessive: (1) dominant enhancement is seen when animals heterozygous for recessive alleles at two loci produce mutant phenotypes; (2) dominant suppression or modification is observed when one gene is dominant and the presence of a heterozygous recessive allele of another locus alters the expected dominant phenotype. (In this test, cryptic dominant mutations are considered recessive alleles if they are recessive to wild type in a wild-type background.) Since extragenic dominant suppressors were frequently found in experiments designed to isolate new *sqt-1* alleles, a number of such genes may exist. This strategy could potentially identify collagen gene family members and also genes that code for other, as yet unidentified, cuticle components.

Examples of similar interallelic interactions have been found with tubulin

genes in Drosophila (RAFF and FULLER 1984) and Aspergillus (MORRIS, LAI and OAKLEY 1979; GAMBINO, BERGEN and MORRIS 1984) and in Drosophila flight muscle genes (DEAK *et al.* 1982). Another case of failure of recessives at different loci to complement (ATKINSON 1985) has also been interpreted as due to interaction of mutant gene products to form multimeric structures.

These interallelic interactions are, in fact, indistinguishable from the allelic interactions we have found. As a consequence, map location is the only genetic method that can be reliably utilized to define a locus. In many cases we are unable to distinguish recombinant phenotypes; thus, our mapping information is quite incomplete. Quite possibly, more than four loci are represented by the mutations studied here.

We have looked for evidence that *sqt-1* is a collagen gene through 2D gel electrophoresis of adult cuticle proteins isolated from animals carrying 11 independent *sqt-1* alleles, including the two putative null alleles. No striking differences between mutant and wild-type cuticle proteins were found, although small differences were observed (M. KUSCH and R. S. EDGAR, unpublished observations). This result might be expected if two or more gene products were sufficiently similar to substitute for each other. Consequently, other methods will be needed to demonstrate conclusively that these genes code for collagens. We are presently attempting to isolate transposon, Tc*1* (EIDE and ANDERSON 1985), insertion mutants of *sqt-1* to allow identification of this gene for cloning.

R.S.E. wishes to thank R. HORVITZ for support and encouragement, since these studies were initiated in his laboratory during a sabbatical leave. We wish to acknowledge the contributions of EVAN ASHKIN who carried out some of the experiments reported here. This work was supported by grants from the National Institutes of Health (GM28311-03) and the National Science Foundation (DCB-8409866).

LITERATURE CITED

ATKINSON, K. D., 1985 Two recessive suppressors of *Saccharomyces cerevisiae cho1* that are unlinked but fall in the same complementation group. Genetics **111:** 1–6.

BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

COX, G. N., S. CARR, J. M. KRAMER and D. HIRSH, 1985 Genetic mapping of *Caenorhabditis elegans* collagen genes using DNA polymorphisms as phenotype markers. Genetics **109:** 513–528.

COX, G. N. and D. HIRSH, 1985 Stage-specific patterns of collagen gene expression during development of *Caenorhabditis elegans*. Mol. Cell. Biol. **5:** 363–372.

COX, G. N, J. M. KRAMER and D. HIRSH, 1984 Number and organization of collagen genes in *Caenorhabditis elegans*. Mol. Cell. Biol. *4:* 2389–2395.

COX, G. N., M. KUSCH and R. S. EDGAR, 1981 Cuticle of *Caenorhabditis elegans*: its isolation and partial characterization. J. Cell Biol. **90:** 7–17.

COX, G. N., J. S. LAUFER, M. KUSCH and R. S. EDGAR, 1980 Genetic and phenotypic characterization of roller mutants of *Caenorhabditis elegans*. Genetics **95:** 317–339.

COX, G. N., S. STAPRANS and R. S. EDGAR, 1981 The cuticle of *Caenorhabditis elegans*. II. Stage-specific changes in ultrastructure and protein composition during postembryonic development. Dev. Biol. **86:** 456–470.

DEAK, I., P. R. BELLAMY, M. BIENZ, Y. DUBUIS, E. FENNER, M. GOLLIN, A. RAHMI, T. RAMP, C. A. REINHARDT and B. COTTON, 1982 Mutations affecting the indirect flight muscles of *Drosophila melanogaster*. J. Embryol. Exp. Morphol. **69:** 61–81.

EDGAR, R. S., G. N. COX, M. KUSCH and J. C. POLITZ, 1982 The cuticle of *Caenorhabditis elegans*. J. Nematol. **14:** 248–258.

EIDE, D. and P. ANDERSON, 1985 Transposition of Tc*1* in the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **82:** 1756–1760.

FESSLER, J. H. and L. I. FESSLER, 1978 Biosynthesis of procollagen. Annu. Rev. Biochem. **47:** 129–162.

GAMBINO, J., L. G. BERGEN and N. R. MORRIS, 1984 Effects of mitotic and tubulin mutations on microtubule architecture in actively growing protoplasts of *Aspergillus nidulans*. J. Cell Biol. **99:** 830–838.

GREENWALD, I. S. and H. R. HORVITZ, 1980 *unc-93-(e1500)*: a behavioral mutant of *Caenorhabditis elegans* that defines a gene with a wild-type null phenotype. Genetics **96:** 147–164.

HERMAN, R., 1978 Crossover suppressors and balanced lethals in *Caenorhabditis elegans*. Genetics **88:** 49–65.

HERMAN, R. K., J. E. MADL and C. K. KARI, 1979 Duplications in *Caenorhabditis elegans*. Genetics **92:** 419–435.

HIGGINS, B. J. and D. HIRSH, 1977 Roller mutants of the nematode *Caenorhabditis elegans*. Mol. Gen. Genet. **150:** 63–72.

HORVITZ, H. R., S. BRENNER, J. HODGKIN and R. HERMAN, 1979 A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol. Gen. Genet. **175:** 129–133.

KEMPHUES, K. J., R. A. RAFF, T. C. KAUFMAN and E. C. RAFF, 1979 Mutation in a structural gene for a β-tubulin specific to testes in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **76:** 3991–3995.

KRAMER, J. M., G. N. COX and D. HIRSH, 1982 Comparisons of the complete sequences of two collagen genes from *Caenorhabditis elegans*. Cell **30:** 599–606.

KRAMER, J. M., G. N. COX and D. HIRSH, 1985 Expression of the *Caenorhabditis elegans* collagen genes *col-1* and *col-2* is developmentally regulated. J. Biol. Chem. **260:** 1945–1951.

LANDEL, C. P., M. KRAUS, R. WATERSTON and D. HIRSH, 1984 DNA rearrangements of the actin gene cluster in *Caenorhabditis elegans* accompanying reversion of three muscle mutants. *J. Mol. Biol.* **180:** 497–513.

MORRIS, N. R., M. H. LAI and C. E. OAKLEY, 1979 Identification of a gene for β-tubulin in *Aspergillus nidulans*. Cell **16:** 437–442.

POLITZ, J. C. and R. S. EDGAR, 1984 Overlapping stage-specific sets of numerous small collagenous polypeptides are translated *in vitro* from *Caenorhabditis elegans* RNA. Cell **37:** 853–860.

PROCKOP, D. J., K. I. KIVIRIKKO, L. TUDERMAN and N. A. GUZMAN, 1979 The biosynthesis of collagen and its disorders. N. Engl. J. Med. **301:** 13–23, 77–85.

RAFF, E. C. and M. T. FULLER, 1984 Genetic analysis of microtubule function in *Drosophila*. pp. 293–304. In: *Molecular Biology of the Cytoskeleton*, Edited by G. G. BORISY, D. W. CLEVELAND and D. B. MURPHY. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

RAMACHANDRAN, G. N., 1967 Structure of collagen at the molecular level. pp. 103–183. In: *Treatise on Collagen, I,* Edited by G. N. RAMACHANDRAN. Academic Press, New York.

SIGURDSON, D. C., G. J. SPANIER and R. K. HERMAN, 1984 *Caenorhabditis elegans* deficiency mapping. Genetics **108:** 331–345.

SWANSON, M. M., M. L. EDGLEY and D. L. RIDDLE, 1984 The nematode *Caenorhabditis elegans*. pp. 286–299. In: *Genetic Maps 1984*, Vol. 3, Edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Communicating editor: R. K. HERMAN