MUTATIONS CAUSING TRANSFORMATION OF SEXUAL PHENOTYPE IN THE NEMATODE *CAENORHABDITIS ELEGANS*

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ABSTRACT

Ten mutations are described that transform genotypic hermaphrodites of the nematode *Caenorhabditis elegans* into phenotypic males. These fall into three autosomal complementation groups, termed *tra-1*, *tra-2*, and *tra-3*. Two alleles of *tra-1* produce almost complete transformation, to a fertile male phenotype; such transformed animals are useful for analyzing sex-linked genes. All alleles of *tra-1* and *tra-2* are recessive; the one known allele of *tra-3* is both recessive and maternal in effect. Where tested, both *XX* and *XXX* hermaphrodites are transformed into males, but *XO* males (true males) are unaffected by these mutations. It is suggested that these genes are actually involved in hermaphrodite development and have no role in male development.

THE genetic control of sexual differentiation has been investigated in many organisms. Much of this work has been concerned with the overall chromosomal control of sex determination, but in various animals single gene mutations have been described which lead to partial or complete transformation of sexual phenotype. Several cases of transformer genes have been found in mammalian systems, such as the autosomal recessive gene polled in goats (HAMERTON *et al.* 1969) and the autosomal dominant *Sxr* in mice (CATTANACH, POLLARD and HAWKES 1971). Some cases in human genetics have suggested the presence of such a gene, but no convincing proof has yet been obtained (DE LA CHAPPELLE 1972). These mammalian mutations transform genotypic females into phenotypic males or intersexes. Several genes in *Drosophila melanogaster* have similar effects: transformer (STURTEVANT 1945) and intersex (MEYER 1951); on the other hand, a mutation in a third gene (double sex) produces intersexual phenotypes when homozygous in either sex (HILDRETH 1965).

The present paper describes transformer mutations in another invertebrate, the small free living nematode *Caenorhabditis elegans*. Extensive genetic investigations have been carried out on *C. elegans*, mainly with the object of analyzing the genetic specification of the simple nervous system of this animal (BRENNER 1974). A variety of behavioral and nonbehavioral mutants have been isolated and characterized; the current genetic map includes about 190 complementation groups, distributed in six linkage groups (BRENNER, HORVITZ *et al.*, in preparation). The genetic system of *C. elegans* allows the ready detection of mutations

transforming hermaphrodites into males, and many have been found. Mutations causing the reverse transformation have not been found, but this kind of mutation is much harder to detect. The mutations described below are both technically useful and intrinsically interesting, for the light they may shed on sexual differentiation in *C. elegans*.

MATERIALS AND METHODS

The methodology of *C. elegans* culture and genetics has been described elsewhere (BRENNER 1974), but some points are repeated here, as they are particularly relevant to the present work. The animal has two sexes, self-fertilizing hermaphrodite and male. Males have five pairs of autosomes and one *X* chromosome (5 *AA*, *XO*). There is no *Y* chromosome. Males will mate with hermaphrodites, generating cross progeny. Most genetic mapping is done by crossing hermaphrodites with males and scoring the self progeny (F_2) of cross progeny hermaphrodites (F_1). When F_1 progeny are scored, extra genetic markers often have to be used to distinguish cross progeny from self progeny. Gene abbreviations used are *tra*: transformer; *unc*: uncoordinated; *dpy*: dumpy; *lon*: long; *him*: high incidence of males. Table 1 lists the mutants used.

Electron microscopy was carried out according to the procedures of WARD *et al.* (1975). Feulgen and Falk Hillarp stained preparations were prepared by DR. JOHN SULSTON and Ms. MARILYN DEW (SULSTON, DEW and BRENNER 1975) to whom we wish to express our gratitude. Most light microscopy was carried out on immobilized live worms, using a Zeiss Universal microscope with Nomarski optics (Figures 1–6, 8–11) or polarizing optics (Figure 7).

The organization and structure of the reproductive system in nematodes of many species including *C. elegans* have been extensively described [for example, see NIGON (1965)]. In *C. elegans*, the hermaphrodite has a double gonad and uterus, with a vulva opening ventrally in the mid region of the body. The male has a single testis, and complex genitalia in the tail, with

TABLE 1

Mutant strains

Linkage group	Gene name	Alleles (strain number)	Phenotype of homozygote
I	*him-1*	E879	high frequency of male progeny
	unc-13	E51	uncoordinated
II	*dpy-10*	E128	dumpy
	tra-2	E1047, E1093, E1094, E1095, E1098, E1209	hermaphrodites transformed into males
	unc-4	E120	uncoordinated
III	*unc-32*	E189	uncoordinated
	tra-1	E440, E1076, E1099	hermaphrodites transformed into males
	dpy-18	E364	dumpy
IV	*lin-5*	E1026	hermaphrodites have multiple vulvae
	unc-17	E876, E245	uncoordinated
	dpy-13	E184	dumpy (semidominant)
	unc-22	E66	uncoordinated
	dpy-4	E1166	dumpy
	tra-3	E1107	hermaphrodites have only male self-progeny
X	*lon-2*	E678	long
	dpy-6	E14	dumpy
	dpy-21	E459	hermaphrodite dumpy; male wild type
	dpy-22	E652	hermaphrodite dumpy; male near lethal

copulatory spicules, a copulatory bursa and fan, sensory rays, and associated nerves and muscles. The post anal part of this structure (referred to here as the bursa) is easily examined under the light microscope in live worms. The hermaphrodite tail is much simpler (compare Figures 1 and 2).

RESULTS

Isolation and mapping of mutants: Stocks of *C. elegans* mutants are normally maintained by means of pure homozygous cultures of hermaphrodites (which carry 2 *X* chromosomes); and males (which carry 1 *X* chromosome) are seen only rarely in such cultures (typically 0.15% of animals are male). When these males mate with hermaphrodites, half of the resulting progeny will be male, but unless precautions are taken to maintain a male stock, these males remain a small minority of the animals in a stock culture.

Higher frequencies of males are sometimes seen in the self progeny of mutant hermaphrodites, as a result of two effects. One is the induction of *him* mutations: these are mutations which increase the number of spontaneously occurring true males, by increasing meiotic nondisjunction of *X* chromosomes (HODGKIN *et al.*, in preparation). The other cause is the segregation of recessive transformer mutations: hermaphrodites heterozygous for such mutations will segregate 25% phenotypic males. We call these animals "pseudomales" because they can be shown to carry 2 *X* chromosomes (see below) and are therefore not male in karyotype. Many thousands of mutant hermaphrodites have been picked in the course of work on *C. elegans* (by the present authors, D. L. BAILLE, J. E. SULSTON, D. RIDDLE *et al.*), from both F_1 and F_2 generations after mutagenesis with EMS, and about 0.5% of these have carried transformed mutations. The first ten isolates have been analyzed: later isolates all resemble one or another of these first ten.

Transformer mutations cannot be maintained as homozygous stocks, and it

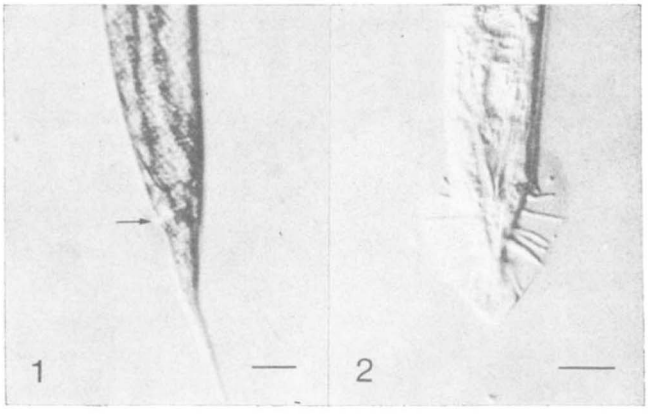

FIGURE 1 (left).—Wild-type hermaphrodite tail, lateral view: arrow indicates anus. All photographs were taken with Nomarski optics, except Figure 7, which was taken with polarizing optics. Scale bar = 25 microns in all photographs.

FIGURE 2 (right).—Wild-type male tail, dorsal view.

was therefore initially necessary to map the mutations, so that they could be maintained by propagating hermaphrodites heterozygous for the transformer gene and for a *trans* linked marker. Such hermaphrodites segregate approximately 2 wild: 1 marked: 1 transformed progeny at each generation, and only rarely is the mutation lost by recombination.

The ten mutations were found to map to three different chromosomes. Three (E440, E1076, E1099) are linked to linkage group III (LGIII), and are maintained as heterozygotes over the marker *dpy-18* (E364); as shown below, they are allelic. Six (E1047, E1093, E1094, E1095, E1098, E1209) are linked to LGII, and are maintained as heterozygotes with the markers *dpy-10* (E128) or *unc-4* (E120); some, and almost certainly all, are allelic. One (E1107) is linked to LG IV, and is maintained as a heterozygote with *dpy-13* (E184). This last isolate differs from the other nine in being maternal in effect: that is, no pseudomales are seen in the F_1 progeny of hermaphrodites heterozygous for the gene, but 25% of the F_1 hermaphrodites produce only pseudomale self progeny.

The precise location of the LG III locus (termed *tra-1*) was found by measuring the linkage to *dpy-18*, using the *tra-1* allele E440. The progeny of seven animals, genotype *tra dpy*/++ were scored, and the numbers of 1109 wild, 333 *tra dpy*, 13 *tra* and 14 *dpy* were obtained, implying linkage of 1.5% (since the frequency of 27/1469 is given by $(2p\text{-}p^2)/2$ for linkage p). *tra-1* was shown to lie to the left of *dyp-18* by constructing a hermaphrodite of genotype *unc-32(E189)* ++/+ *tra dpy* and picking F_1 *dpy* (recombinant) progeny; these segregated both *unc dpy* and *tra dpy* animals in the next generation, so the order must be as shown.

The LG II locus (termed *tra-2*) was found to lie between the two closely linked genes *dpy-10* and *unc-4;* hermaphrodites of genotype *dpy-10* + *unc-4*/+ *tra-2* (*E1098*) + segregated both *unc tra* and *dpy tra* progeny in the F_2 generation. *Dpy tra* animals were much rarer, so it is assumed that the *tra-2* locus is closer to *dpy-10* than to *unc-4*.

The LG IV locus (*tra-3*) is hard to map precisely because of the delay in the expression; it was shown to lie to the right of the marker *unc-17* (E876) and probably to the right of *unc-22* (E66) by examining the F_3 progeny of a hermaphrodite *unc-17 unc-22*+/++ *tra-3* and finding many *unc-17* pseudomales but no *unc-22* pseudomales. Linkage to *dpy-13* was measured by picking 63 dpy^+/dpy^+ progeny from hermaphrodites of genotype *dpy*+/+ *tra* and finding that 45 produced only male progeny, *i.e.*, were of genotype *tra/tra*, while 18 did not. The dpy^+/dpy^+ animals could be distinguished from the dpy^+/dpy heterozygotes because *dpy-13* (E184) is semidominant. In such a *trans* self cross, the frequency of 18/63 is given by $2p\text{–}p^2$ for linkage p; hence *tra-3* lies 15% from *dpy-13*, close to *dpy-4* (E1166). No recombinants have been found between *tra-3* and *dpy-4* in a stock *dpy-4* +/+ *tra-3*, therefore the two genes are provisionally assigned to the same position.

Phenotypes: The *tra-1* strains E440, E1099 and E1076 have distinguishable pseudomale phenotypes. E440 and E1099 both have wild-type male genital (bursal) morphology when examined at 400× (Figure 3) and both exhibit mat-

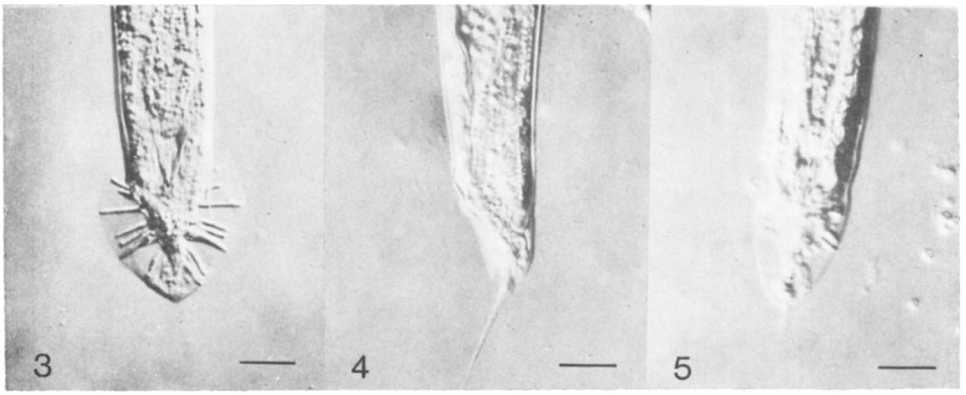

FIGURE 3 (left).—*tra-1* pseudomale tail (*E440/E440*), dorsal view. *E1099/E0199* is morphologically identical.

FIGURE 4 (middle).—*tra-1* pseudomale tail (*E1076/E1076*), lateral view.

FIGURE 5 (right).—*tra-1* pseudomale tail (*E1099/E1076*), dorsal view. The small spherical objects to the right of the animal are free spermatozoa.

ing behavior. About 1 in 20 E440 pseudomales is capable of mating successfully to produce a few cross progeny (all hermaphrodite); more than a third of E1099 pseudomales mate successfully, though they still produce many fewer progeny than true males. Feulgen-stained preparations show that the gonads frequently develop abnormally in both E440 and E1076 pseudomales, as intersexual structures, which probably explains the inefficiency of mating. E1076 pseudomales are more abnormal in phenotype than the other two types, exhibiting only vistigial bursa structures (Figure 4). Mating behavior seems to be absent.

The mutations at the *tra-2* locus fall into two phenotypic classes. Pseudomales of one type (E1047, E1093, E1094, E1095, E1098) have abnormal bursal morphology, with variable reductions in the structure of the bursal fan and sensory rays, and abnormalities (sometimes duplication) of the copulatory spicules (Figures 6 and 7). A pseudomale of the other type (E1209) has a more vestigial bursa, and a vestigial vulval structure develops two-thirds of the way down the body (roughly where the vulva develops in the hermaphrodite) (Figures 8 and 9). Feulgen stained preparations show considerable gonadal defects in all these mutants; in the case of E1209, the gonad has two reflexed arms, as in the hermaphrodite. None of these *tra-2* pseudomales ever exhibits mating behavior.

E1107, the single isolate at the *tra-3* locus, has no effect on the progeny of *tra-3*/+ hermaphrodites: the *tra-3* animals appear to be entirely wild-type hermaphrodites. The self progeny of these animals, however, uniformly resemble pseudomales of the E1209 (*tra-2*) type, with a vestigial bursa and a vestigial vulva (Figure 11). An attempt was made to demonstrate that this structure is vulval by constructing a double mutant of E1107 and E1026 [a mutant which causes multiple differentiation of vulva-like structures in the adult hermaphrodite (HODGKIN 1974)], but the transformed E1026 animals grew very poorly and produced only one vulval structure, or died before maturity. The gonad of

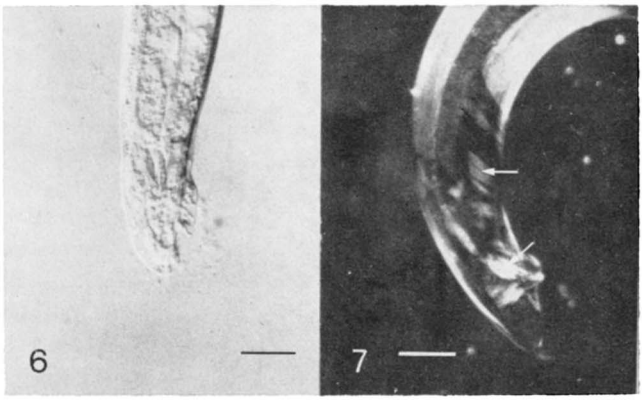

FIGURE 6 (left).—*tra-2* pseudomale tail (*E1095/E1095*), dorsal view.

FIGURE 7 (right).—*tra-2* pseudomale tail (*E1098/E1098*), lateral view under polarizing optics. Upper arrow indicates diagonal muscles; lower arrow indicates abnormal spicules.

E1107 pseudomales appears to differ from that of E1209 in being larger. In both cases the adult transformed animals often burst open at the site of the vestigial vulva.

It is of interest to know what effect these mutations have on the development of the sexual nervous system. This part of the nervous system is found only in the male worm, and develops late in larval life, during the fourth larval stage (HODGKIN 1974). There are several features of the male nervous system that can be examined fairly easily; one is the complexity of the posterior ventral cord, as measured by the number of processes seen in electron micrographs of transverse sections through this cord, and a second is the appearance of the catecholamine containing cells in the male tail (SULSTON, DEW and BRENNER 1975). These cells can be specifically stained by means of the Falk-Hillarp

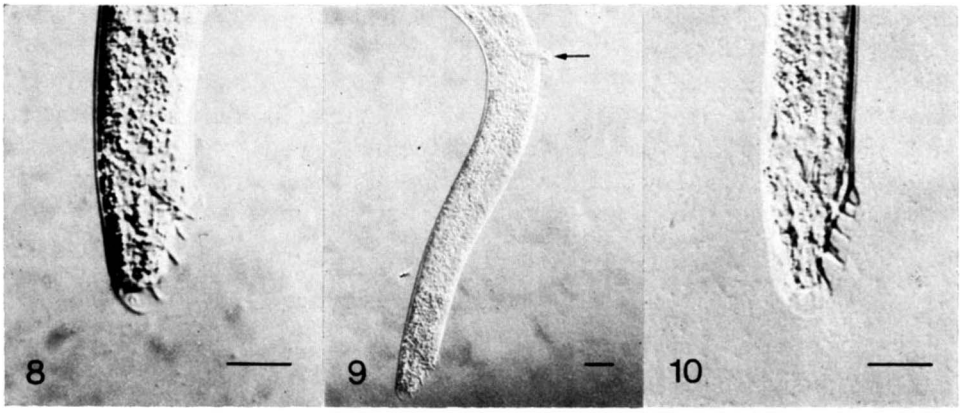

FIGURE 8 (left).—*tra-2* pseudomale tail (*E1209/E1209*), dorso-lateral view.

FIGURE 9 (middle).—*tra-2* pseudomale posterior body (*E1209/1209*), dorso-lateral view, showing vestigial vulva (arrow).

FIGURE 10 (right)—*tra-2* pseudomale tail (*E1095/E1209*), dorsal view.

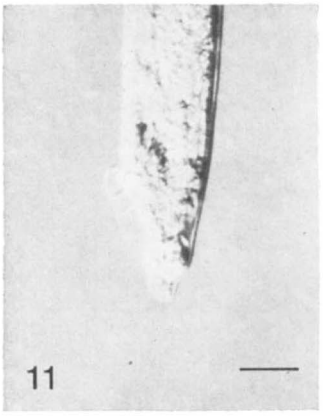

FIGURE 11.—*tra-3* pseudomale tail (*E1107/E1107*), dorso-lateral view.

procedure (FUXE and JONSSON 1973) and examined in whole mounts under the light microscope. Table 2 summarizes a comparison of wild-type male, wild-type hermaphrodite, and various pseudomales with respect to these features. It is obvious from the data that the extra male nervous system is developing to some extent even in E1107 pseudomales, but the variability of this structure, as judged by the appearance of the catecholamine cells, is considerable in pseudomales of E1076 and all *tra-2* alleles. Another feature peculiar to the male nervous system is the set of extra cephalic sensory structures (WARD *et al.* 1975); these were looked for, and found, in serial sections through the heads of *tra-1* (E440) and *tra-2* (E1209) pseudomales. Finally, there is a set of muscles in the

TABLE 2

Development of the sexual nervous system in normal and transformed animals

Genotype and sex	Number of processes seen in posterior ventral nerve cord (± standard deviation)	Appearance of posterior catecholamine nervous system
Wild-type:		
adult hermaphrodite (n=14)	46 ± 5	absent
adult male (n=9)	75 ± 5	normal : 3 pairs of bursal cell bodies and extensive ganglion fluorescence.
young L4 male (n=6)	53 ± 5	developing
E1076/E1076 : *tra-1* pseudomale	64, 66, 67	variable : some individuals close to normal. Often only most posterior cell bodies visible.
E1095/E1095 : *tra-2* pseudomale	not examined	variable : some individuals close to normal.
E1209/E1209 : *tra-2* pseudomale	65, 71, 74, 74	variable : almost all individuals deficient.
E1107/E1107 : *tra-3* pseudomale	73	not examined

most posterior quarter of the adult male body, which is conspicuous under polarizing optics (Figure 7). These muscles are not seen in the hermaphrodites or larval males, but were seen in pseudomales of all 10 transformer genes.

Complementation: Complementation of *tra* mutations cannot be done as simply as with other mutations of *C. elegans* (by crossing hermaphrodites homozygous for one mutation with males heterozygous for another mutation, and examining the phenotype of the F_1 males), because of the difficulty of distinguishing cross progeny males from self progeny pseudomales. For complementation of mutations at the *tra-1* locus, the pseudomales of E1099 can be utilized. Crosses of hermaphrodites with E1099 pseudomales do not produce F_1 males, as there are no nullo-*X* sperm from the pseudomale parent; therefore the appearance of cross progeny pseudomales in the progeny of such a cross shows that the hermaphrodite carried a mutation at the *tra-1* locus. Hermaphrodites of genotype *tra-1 (E440)/+*, *unc-17/unc-17* and *unc-32 tra-1 (E1076)/unc-32 +* were constructed and crossed with E1099 pseudomales. In both cases F_1 non-*unc* pseudomales were observed, showing that E440 and E1076 fail to complement E1099. The phenotype of the *E1099/E440* animals was not closely examined, but the phenotype of the *E1099/E1076* pseudomales was examined, and these pseudomales were seen to resemble the E1076 phenotype more closely than the E1099 phenotype (Figure 5).

Complementation of alleles at the *tra-2* locus entails the use of a sex-linked marker in the maternal parent, to distinguish pseudomale progeny from cross progeny males. The marker used was *dpy-6* (E14); a strain of genotype *tra-2 (E1095) +/+ unc-4, dpy-6/dpy-6* was constructed and hermaphrodites were crossed with true males heterozygous for various other *tra-2* mutations. The appearance of non-*dpy* pseudomales in the F_1 indicated lack of complementation between the mutation and E1095. In this way, E1209, E1094, and E1095 were shown not to complement each other, and in an analogous cross, E1047 and E1093 were shown not to complement. It is assumed that all six mutations at this locus, and others that have been found since, belong to the same gene. The phenotype of the *E1095/E1209* pseudomales was examined: these animals resembled the E1029 phenotype, with vestigial vulvae, rather than the E1095 phenotype (Figure 10).

For both *tra-1* and *tra-2*, therefore, heterozygotes of alleles of different phenotypic classes have a phenotype which resembles the more intersexual type. This implies that the more extreme the transformation, the more hypomorphic the allele.

Demonstration of XX *karyotype in pseudomales*: Hermaphrodites crossed with pseudomales of E440 or E1099 produce only hermaphrodite cross progeny, so these pseudomales are gonadally *XX*. It remains possible that the soma could be *XO*, and for pseudomales of *tra-2* and *tra-3*, both gonad and soma could be *XO*.

These possibilities were tested by constructing hermaphrodites heterozygous for transformer genes and for sex-linked markers, and scoring progeny. One animal of genotype *tra-1 (E1099)/+*, *lon-2+/+dpy-6* (*lon-2* (E678) and *dpy-6* (E14) are closely linked sex-linked markers) gave pseudomale progeny with

phenotypes 16 wild: 6 *lon*: 9 *dpy*, which would not have been observed if *X* loss or *X* inactivation were occurring in the pseudomales (as this would lead to a deficiency of wild, *i.e.*, *lon-2* +/+ *dpy-6* pseudomales). Similarly an animal of genotype *tra-2(E1095)*/+, *lon-2* +/+ *dpy-6* gave pseudomale progeny with phenotypes 9 wild: 7 *lon*: 3 *dpy*. A corresponding experiment for *tra-3* (E1107 has not been carried out.

The fact that these pseudomales are phenotypically male but carry two *X* chromosomes has two useful consequences. Firstly, there are mutations in at least two sex-linked genes which have markedly different effects on the two sexes ["chauvinist" mutants (HODGKIN 1974)]. Examination of the phenotypes expressed in pseudomales shows that the difference is due to the different *X* dose in the two sexes, not to sexual differentiation *per se*. One type of mutation, exemplified by E459 (*dpy-21*) has dumpy expression in hermaphrodites but no expression in males. Animals of genotype *tra-1(E440)*/+, *dpy-21/dpy-21* were constructed and found to segregate many *dpy* pseudomales and very few wild-type males (presumably *XO* males). The other type of mutation, exemplified by E652 (*dpy-22*), has *dpy* expression in hermaphrodites but near lethal expression in males (many males die as larvae). The progeny of *tra-1 (E1099)*/+, *dpy-22*/+ hermaphrodites were examined, and 1/16 were found to be *dpy* pseudomales which were clearly less affected than *dpy*/— males.

The second important consequence is that these pseudomales can be used to carry out complementation tests on severely defective sex-linked mutations. About 1/3 of sex-linked mutations cannot be complemented easily because hemizygously marked males are so uncoordinated or dumpy that they never mate successfully. However, a pseudomale of E1099 (*tra-1*), heterozygous for one of these mutations does not express the mutation, so complementation tests can be made and double mutants constructed. This technique has led to considerable improvement in the genetic map of the *X* chromosome.

Effect of X dosage on expression of tra *mutations:* The previous data refer to the phenotypes of *tra* mutations in *XX* animals. It is of interest to know what effect these mutations have on *XO* and *XXX* animals.

For *tra-1*, the *XO* phenotype was examined using the linked mutations *tra-1* (E1076), *dpy-18* and *unc-32*. The crosses shown in Figure 12 were made. The *unc* progeny of the last cross (*unc tra* +/++ *dpy*♀♂ × *unc tra* +/++ *dpy*♂♂) were examined and clearly fell into two classes: all were male but 34 had the E1076 phenotype while 39 had a wild-type male phenotype, with respect to the bursal morphology. The former class are presumed to be *unc tra/unc tra*, *XX* (self and cross progeny) while the latter class are presumed to be *unc tra/unc tra*, *XO* (cross progeny). Examination at 160× confirmed that the second class had wild-type genitalia, although the *unc* background makes it difficult to say that they were wholly wild. Unfortunately, a *cis*-linked recessive marker has to be used in this cross to identify the *tra-1* homozygotes, because no dominant marker is available. E1076 was used because the other two *tra-1* alleles lead to wild-type bursal phenotype, even in pseudomales.

Similar crosses were made for *tra-2*, using the LGII markers *tra-2* (E1098),

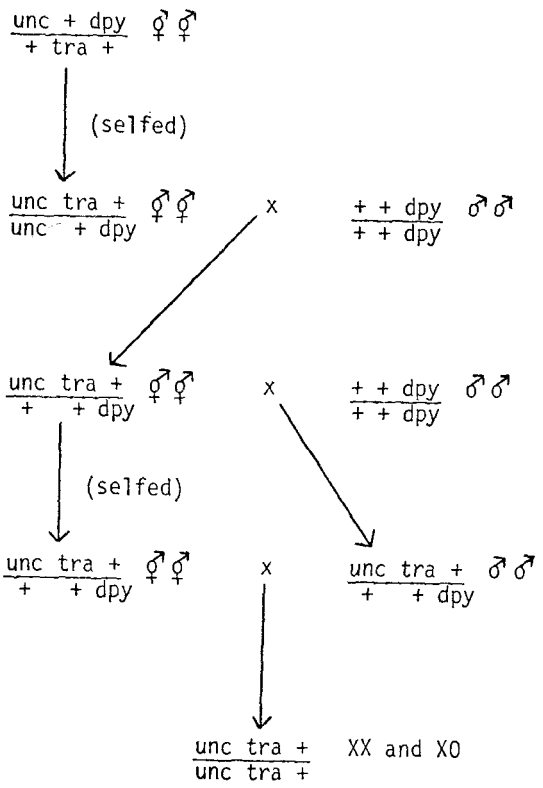

FIGURE 12.—Crosses generating homozygous *tra-1* cross-progeny. *unc* = *E189* (*unc-32*, III); *tra* = *E1076* (*tra-1*, III); *dpy* = *E364* (*dpy-18*, III). An extra generation was included in order to confirm genotypes.

dpy-10 and *unc-4*. E128 (*dpy*) males have not been observed to mate successfully, so *dpy*/+ males were used instead. The final cross (*dpy* ++/+ *tra unc* ♀♀ × + *tra unc*/+++♂♂ and *dpy* ++/+++♂♂) produced many *unc* progeny, all of which appeared male, but of 38 examined at 160×, 28 had abnormal bursae, while 10 had normal bursae. Therefore *tra-2* (E1098), like E1076, has little effect on *XO* males. Given these results, it is not surprising that *tra-1*, *tra-2* double homozygotes have a wild phenotype when E1099 is used as the *tra-1* allele. Hermaphrodites with LGII genotype *dpy-10* + *unc-4* / + *tra-2 (E1098)*+ were crossed with E1099 pseudomales, and wild-type progeny picked. Those that segregated both wild and *unc* pseudomales in the next generation must have had the genotype *tra-2 unc* / ++, + / *tra-1*. Three such worms were obtained, and 59 *unc* progeny were examined at 160×. All but one of these were male in phenotype; 43 had the bursal morphology of E1098, the *tra-2* allele, but 15 had bursae of apparently wild-type morphology, as expected if *tra-1* is epistatic to *tra-2*. In another cross, an animal of genotype *tra-2 unc-4* / ++, *tra-1 dpy-18* / ++ was obtained, so the *tra-1 tra-2* progeny were unambiguously identified by an *unc dpy tra* phenotype. These animals had the bursal mor-

phology of the *dpy tra* siblings, not the *unc tra* siblings. Therefore, *tra-2* (E1098) has little or no expression in the presence of homozygous *tra-1* (E1099). Other combinations of *tra* alleles have not been investigated.

The phenotype of *tra-3 XO* animals is also that of a normal male: this was shown by crossing hermaphrodites homozygous for *dpy-13* (E184) and *tra-3* (E1107) with males heterozygous for *tra-3*. Cross progeny counts of 120 wild hermaphrodites: 231 wild males: 127 *tra-3* pseudomales were obtained, *i.e.*, a ratio of 1:2:1, indicating that heterozygous and homozygous *tra-3* have no effect on *XO* animals. The same experiment shows that the defect in eggs produced by *tra-3 / tra-3* hermaphrodites can be corrected by sperm carrying *tra-3*+.

The expression of transformer mutations in *XXX* animals was investigated by constructing animals carrying *tra* genes and *him-1*, a mutation which causes *X*-chromosome nondisjunction and hence high frequencies of self progeny males together with a few self progeny *XXX* hermaphrodites, which can usually be identified by their small size (HODGKIN *et al.*, in preparation). For *tra-1*, a strain *him / him, tra / +* was constructed by first making the strain *unc-13 / unc-13, tra-1 (E440) / +* and crossing hermaphrodites with E879 (*him-1*) males. The *unc-13* marker (which is closely linked to *him-1*) is included to avoid confusion between *him* homozygotes and *tra* heterozygotes, both of which segregate 25% males. Progeny of this cross which segregated both males and *unc* must have been *him +/+ unc, tra / +*, and hermaphrodites were obtained from the progeny of such animals which segregated no *unc* animals and many males (more than 30%) as well as small (*XXX*) animals. The parents must therefore have been *him / him, tra / +*. Several smalls were picked, and two of these segregated males in the next generation; scores of 51 wild, 17 male. 45 small, 16 small male were obtained. Small males had never previously been seen in the self progeny of *XXX* animals, and it is assumed that these were *XXX* pseudomales. The bursal morphology of these pseudomales is slightly more abnormal than that of the *XX* pseudomales, but this may be merely a result of the smallness of these animals.

For *tra-2, him-1 / him-1, tra-2 (E1094) / +*, *XXX* animals were obtained in a similar fashion; three segregated 85 wild, 37 male, 70 small, 28 small male. The small males grew poorly but were undoubtedly transformed in sexual phenotype.

DISCUSSION

The transformer mutations are useful and interesting for several reasons. Firstly, *XX* pseudomales which will mate successfully are technically very useful for working with sex-linked mutations.

Secondly, the alleles of *tra-2* and *tra-3*, and the *tra-1* allele E1076, exhibit abnormal development and function of the sexual nervous system. This part of the nervous system differs from the rest of the nervous system in developing late on in larval life, and analysis of the developing sexual nervous system (and other male specific structures) is possible. It may be useful to analyze the abnormal patterns of development in these mutants.

Thirdly, the transformer mutations are of intrinsic interest, because they provide some information about the control of sexual differentiation in *C. elegans*. It is significant that they are all autosomal, indicating that genes controlling sexual development are not confined to the *X* chromosome. Also, all of the mutations analyzed so far have the following features in common: they are recessive; they transform both *XX* and *XXX* animals into pseudomales; and they have no effect on *XO* males. In addition, where 2 alleles of a transformer gene differ in phenotype, the heterozygote resembles the less male phenotype. These results can be explained on the assumption that the nematode egg develops as a male unless the tra^+ alleles are active. Thus, the transformer genes may only be involved in hermaphrodite development, not in male development at all. If they were involved in male development, *i.e.*, if the *tra* mutations were constitutives of some sort, then one might expect them to be semidominant, or to affect development of *XO* males, or to exhibit the more male phenotype in heterozygotes of different alleles. None of these effects is observed; however, the arguments in favor of the first interpretation are not compelling.

Finally, a temperature sensitive allele of one of these genes can be used to investigate the time and degree of commitment to one type of sexual development. It is possible that such mutations occur naturally in stocks of *C. elegans*, as intersexual types have been observed to result from heat shock (NIGON 1965). They may also occur naturally in some mosquito species (ANDERSON and HORSFALL 1963). Subsequent to these experiments, a t.s. *tra* mutation (an allele of *tra-2*) was isolated in *C. elegans* and used to investigate male development by means of temperature shift experiments (KLASS, WOLF and HIRSH 1976).

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