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X Chromosome Dosage and Gene Expression in *Caenorhabditis elegans*: Two Unusual Dumpy Genes

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Summary. The phenotypes caused by mutations in two autosomal genes of the nematode *Caenorhabditis elegans*, *dpy-21 V* and *dpy-26 IV*, are markedly affected by X chromosome dosage, independent of sexual phenotype. At high X chromosome to autosome ratio, in *2A;3X* animals, these dumpy mutations are lethal; at intermediate ratio, in *2A;2X* animals, they cause dumpiness or lethality; at low ratio, in *2A;1X* animals they cause neither dumpiness nor lethality. One gene, *dpy-26*, exhibits a strong maternal effect. Interactions between these genes and two major sex-determining genes *her-1 V* and *tra-1 III* have been examined. The dumpy mutations partly suppress the masculinization of *tra-1 2A;2X* animals and also increase the fertility of most *her-1 2A;1X* hermaphrodites. It is suggested that these dumpy genes are involved in X chromosome dosage compensation, and in some aspects of sexual differentiation. The *dpy-26* gene is compared with a similar *Drosophila* gene, *daughterless*.

Introduction

The nematode *Caenorhabditis elegans* occurs naturally in two sexes, which differ in the number of X chromosomes. Both sexes have five pairs of autosomes, but the self-fertilizing hermaphrodite has two X chromosomes (abbreviated *2A;2X* or *XX*) and the male has one X chromosome (*2A;1X* or *XO*). In many organisms, sex chromosome dosage differences of this kind are compensated, in order to achieve equal expression of sex-linked genes in the two sexes. Mammals carry out compensation by means of X chromosome inactivation, so that only one of the two X chromosomes in an *XX* female cell is expressed (Lyon 1961). The mechanism of compensation in *Drosophila* is quite different: levels of transcription on the X chromosomes are adjusted so that genes on the single X chromosome of male flies produce twice as much product as the corresponding genes on each of the two X chromosomes of female flies, thereby equalizing X chromosome expression in the two sexes (Stewart and Merriam 1980). Nevertheless, compensation is not a universal phenomenon. For example, it is believed that some animal groups, such as birds (Baverstock et al. 1982) and butterflies (Johnson and Turner 1979) do not compensate for sex chromosome dosage differences in the two sexes. Both of these groups have fairly high chromosome numbers (White 1973) so presumably sex-linked genes are relatively few in number and therefore dosage differences may be less deleterious than in other organisms.

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The X chromosome of *C. elegans* is relatively large, about one sixth of the genome. Of 321 genes listed by Riddle and Swanson (1982), 60 are sex linked (this count excludes *let* genes, which are a biased set). However, it is not known whether any or all of these sex-linked genes are dosage compensated. One possibility, X chromosome inactivation, can be excluded, because hermaphrodites heterozygous for any recessive sex-linked mutation and its wild-type allele are invariably wild-type. One can assume that some sex-linked genes are cell autonomous in their expression, and therefore heterozygotes of this type would be expected to show a partial or complete mutant phenotype if only one X chromosome were active in each cell. Since this is never observed, one can conclude that there is no X chromosome inactivation. On the other hand, compensation of the *Drosophila* type remains a strong possibility.

It is known that excessive imbalances in X chromosome to autosome ratio result in lethality. Previous work has shown that diploid zygotes with four X chromosomes (*2A;4X*) die before hatching (Hodgkin et al. 1979) and so also do triploid zygotes with one X chromosome (*3A;1X*: Madl and Herman 1979). However, wild-type *2A;1X* males are perfectly viable, and *2A;3X* hermaphrodites are viable and fertile, though clearly abnormal (Hodgkin et al. 1979). This viability could be explained as the result of compensation, or alternatively as the result of all sex-linked genes being relatively insensitive to dosage variation. *C. elegans* seems to be able to tolerate substantial but not extreme levels of aneuploidy, because deletions (e.g., *eDf2(III)*) or duplications (e.g., *eDp6(III;f)* or *sDp1(I;f)*) (Riddle and Swanson 1982) of perhaps 5% of the genome do not result in lethality. On the other hand, indirect evidence suggests that monosomy or trisomy for any of the five autosomes is lethal (Hodgkin et al. 1979). This implies that there is compensation of X chromosome expression, to permit viability of *2A;1X* and *2A;3X* animals. Studies of particular genes on the X chromosome also suggest that these genes are dosage compensated (Meneely and Herman 1979, 1981).

A related problem is the effect of X chromosome dosage on sexual phenotype. Nigon (1951), and Madl and Herman (1979) showed that a high X chromosome to autosome ratio (more than 0.75, as in a *4A;3X* tetraploid) results in hermaphrodite development, while a low ratio (less than 0.67) results in male development. However, many single gene

mutations have been identified that override the sex-determining effect of this ratio, resulting in *XX* males (*tra* mutants: Hodgkin and Brenner 1977) or *XO* hermaphrodites (*her* mutants: Hodgkin 1980) or *XO* females, i.e. spermless hermaphrodites (*fem* mutants: Edgar et al. in preparation; Doniach and Hodgkin in preparation). These mutants define at least six autosomal genes (*tra-1*, *tra-2*, *tra-3*, *her-1*, *fem-1*, *fem-2*). The epistatic interactions between the mutations have led to the construction of a model in which these genes act sequentially (Hodgkin 1980; Doniach and Hodgkin in preparation). It is proposed that the activity of one of these genes, *her-1*, is controlled by the X: autosome ratio, and *her-1* activity in turn controls the 'downstream' *fem* and *tra* genes. However, nothing is known about the mechanism whereby a high X:autosome ratio switches *her-1* 'off', and a low ratio switches *her-1* 'on'. This mechanism must be capable of fine discrimination, because ratios as similar as 0.67 and 0.75 result in opposite effects. In *Drosophila*, Cline (1979) has shown that the mechanisms for dosage compensation and for sex determination are partly overlapping, because the gene *Sxl* is involved in both processes. Certain other *Drosophila* genes are involved only in one process or the other, e.g., *mle* mutations affect dosage compensation but not sex determination (Belote and Lucchesi 1980), and *tra* mutations affect sex determination but not dosage compensation (Baker and Ridge 1980). So far, no gene directly comparable to *Sxl* has been discovered in *C. elegans*. It will be illuminating to learn how much similarity there is between the genetic strategies employed by *C. elegans* and *Drosophila*, two organisms that are phylogenetically remote from each other.

An obvious approach to these problems is to investigate genes with differential expression in individuals with different X chromosome dosage. Many mutations of this type have been identified in *Drosophila* (Belote and Lucchesi 1980) and some of these are known to exert their effects by interfering with the process of dosage compensation. One might expect analogous mutations to be discovered in *C. elegans*. Partly for this reason, the male (*XO*) and hermaphrodite (*XX*) phenotypes have been compared for mutations in a large number of genes (Hodgkin 1983a). In most cases phenotypes in the two sexes are similar, implying no differential expression. Those cases showing significant differences can be divided into four categories. (1) sex determining genes: *her-1* mutations have no effect on *XX* animals, and *tra* mutations have no effect on *XO* animals. (2) sexual differentiation genes, which affect structures found only in one sex (e.g., *lin-2*, which affects vulval differentiation, and *mab-9*, which affects male copulatory organs). (3) a few behavioral mutants, which exhibit weaker phenotypes in the male probably as a result of the greater mobility of male animals (e.g., *mec* mutants, *unc-31*, *unc-45*) (4) four 'chauvinistic dumpy' genes: *dpy-21 V*, *dpy-22 X*, *dpy-23 X*, and *dpy-26 IV*.

One can distinguish between the effects of sexual differentiation and the effects of X chromosome dosage by means of the sex determination genes *her-1*, and *tra-1*, which permit the construction of *XO* hermaphrodites and *XX* males, respectively. By applying this criterion, one can eliminate categories two and three, in which the differential expression is a secondary consequence of sexual differentiation. However, the four dumpy genes appear to be directly affected by X chromosome dosage, independent of sexual phenotype (Hodgkin and Brenner 1977, and this paper). Mutations in the two sex-linked genes *dpy-22* and *dpy-23* result in a variable small scrawny phenotype in *XX* animals and a more severe, sometimes lethal, phenotype in *XO* animals. They are difficult to study because their phenotypes are so variable. Opposite behaviour is found for the two autosomal genes, *dpy-21* and *dpy-26*: *XX* animals are more severely affected than *XO* animals. The present paper reports some additional data on these two genes and describes their interaction with the sex determining genes. One gene, *dpy-26*, has properties strongly reminiscent of the *Drosophila* gene *daughterless* (*da*), which affects dosage compensation and also interacts with *Sxl* (Cline 1979, 1981).

Materials and Methods

Methods of culture, genetics and nomenclature were essentially as described previously (Brenner 1974; Hodgkin, Horvitz and Brenner 1979; Horvitz et al. 1979).

All experiments were carried out at 20–22° C.

Strains

For clarity, the phenotypes of the various sex-determination mutants and dumpy mutants discussed in this paper are summarized in Table 1. The particular mutations and other markers used were:

LGIII	*sup-5(e1464)*, *tra-1(e1099)* (recessive allele), *tra-1(e1575)* (dominant allele)
LGIV	*dpy-13(e184)*, *fem-1(hc17,e1927)*, *him-8(e1489,g203)*, *dpy-20(e1282)*, *him-3(e1256)*, *him-6(e1423)*, *him-12(g47)*, *unc-22(e66)*, *dpy-26(n198,n199)*, *unc-31(e169)*, *unc-30(e191)*, *dpy-4(e1166)*, *sDf2*
LGV	*dpy-11(e224)*, *her-1(e1518, e1519, e1520, e1561, e1564, e1574)*, *him-5(e1490)*, *unc-76(e911)*, *dpy-21(e428, e459)*, *unc-51(e369)*
LGX	*dpy-3(e27)*, *dpy-8(e130)*, *dpy-23(e840)*, *dpy-7(e88)*, *unc-18(e81)*, *dpy-6(e14)*, *dpy-22(e652)*, *unc-7(e5)*

Most of these genes and alleles are listed by Riddle and Swanson (1982), and stocks are available from the Caenorhabditis Genetics Center. The gene *isx-1*, originally defined by the temperature-sensitive mutation *hc17* (Nelson et al. 1978) has been renamed *fem-1* because stronger alleles such as *e1927* cause complete feminization of *XO* and *XX* animals (Doniach and Hodgkin in preparation). The dominant mutation *e1575*, originally assigned to *her-2*, is now known to be a *tra-1* allele (Hodgkin 1980, 1983b). The mutants *him-12(g47)* and *him-8(g203)* were provided by

Table 1. Mutant phenotypes

Genotype	*XX* Phenotype	*XO* Phenotype
Wild type	hermaphrodite	male
her-1	hermaphrodite	hermaphrodite
tra-1(rec)	male	male
tra-1(dom)/+	female	female
fem-1	female	female
dpy-21	dumpy	non-dumpy
dpy-26	dumpy/lethal	non-dumpy

Dr. R. Cassada; the two *dpy-26* alleles were provided by N. Tsung and H.R. Horvitz; the deficiency *sDf2* (Moerman and Baillie 1981) was provided by T. Rogalski.

Genetic Mapping

The positions of the two dumpy genes discussed in this paper were obtained as follows. For *dpy-21*, 2-factor cross data: segregation from *unc-76 dpy-21(e428)/+ +* hermaphrodites gave 1446 wild, 80 Dpy, 419 Unc & Unc Dpy (ratio wild: dumpy gives linkage $p = 8.2\%$). Segregation from *dpy-21(e428) unc-51/+ +* gave 658 wild, 59 Dpy, 57 Unc, 171 Dpy Unc ($p = 13.2\%$). 3-factor cross data: 6/6 Dpy recombinants from *+unc-76 dpy-21/him-5 + +* were *+ unc-76 dpy-21/him-5 + dpy-21*. 9/9 Dpy recombinants from *+dpy-21 unc-51/him-5 + +* were *+ dpy-21 unc-51/+ dpy-21+*.

For *dpy-26*, 2-factor cross data: segregation from *dpy-20 dpy-26(n199)/ + +* hermaphrodites gave 1193 wild, 8 Dpy-20, 9 Dpy-26, 389 Dpy-20 Dpy-26 (these three dumpy phenotypes are distinguishable; $p = 1.1\%$). 3-factor cross data: from *dpy-20 + dpy-26/+ unc-22 +*, 5/6 Dpy-20 and 1/3 Dpy-26 recombinants carried *unc-22*. From *dpy-20 + unc-31/+ dpy-26 +*, 10/15 Dpy-20 and 1/6 Unc-31 recombinants carried *dpy-26*. Also, *+/sDf2* hermaphrodites crossed with *dpy-26* males yielded Dpy-26 progeny, indicating that *sDf2* includes *dpy-26*.

Complementation Tests. Tests were carried out to confirm non-allelism with linked *dpy* genes. Thus, *dpy-21(e428)* complements *dpy-11(e224)* and *sma-1(e30)*. *dpy-22* and *dpy-23* complement *dpy-3*, *dpy-6*, *dpy-7*, *dpy-8*, and also complement each other. *dpy-26(n199)* complements *dpy-13*, *dpy-20*, and *dpy-4*. The *dpy-26* mutants also have a Him phenotype (i.e., a high frequency of male self-progeny), so complementation tests with linked *him* genes were carried out: *dpy-26* complements *him-3*, *him-6*, *him-8*, and *him-12*.

Suppression. Double mutants of *e428*, *e459*, *n198* and *n199* with the amber suppressor *sup-5(e1464)* (Wills et al. 1983) were constructed; none of the dumpy mutations was suppressed.

Results

A. Phenotypes of dpy-21 V

Two independent ethyl methane sulfonate (EMS) induced isolates of *dpy-21* found by S. Brenner, *e428* and *e459*, are both recessive mutations that exhibit dumpy expression in *XX* hermaphrodites and non-dumpy expression in *XO* males. The *XO* males of *e428* and *e459* are morphologically indistinguishable from wild type *XO* males, and in fertility measurements (Hodgkin 1983a) *e428* males scored 88%, *e459* males scored 87%, relative to 100% for wild type males. Thus, these *dpy-21 XO* males are essentially wild type.

The lack of expression of *dpy-21* in *XO* animals has been confirmed by constructing *her-1 XO* hermaphrodite mutants (Hodgkin 1980). As previously reported, the morphologies of *her-1(e1518)XO* and *her-1(e1518) dpy-21(e428)XO* are identical and the fertilities are similar (average self progeny broods 60 zygotes for *dpy-21(+)*, 112 for *dpy-21(−)*). Furthermore, *XO* animals can also be transformed into females (i.e., spermless hermaphrodites) by the rare dominant *tra-1* allele *e1575*, which has effects opposite to those of recessive masculinizing alleles such as *e1099* (Hodgkin 1980, 1983b), or alternatively by the recessive mutation *fem-1(e1927)* (Doniach and Hodgkin in preparation). *XO dpy-21* females of these two genotypes have been constructed, and in both cases the *XO* females are non-dumpy.

The expression of *dpy-21* in masculinized *XX* animals has also been investigated. As previously reported, (Hodgkin and Brenner 1977), *dpy-21 XX* phenotypic males are dumpy in all three *tra* mutant genotypes (*tra-1*, *tra-2*, *tra-3*). Although *tra-1(e1099) XX* individuals are invariably completely male in non-gonadal phenotype, and frequently male also in gonadal and germ-line phenotype, the *tra-1; dpy-21 XX* males are sterile and clearly abnormal in male tail anatomy (Fig. 2e). This partial suppression of the Tra phenotype is unlikely to be a consequence of dumpiness, because other *tra-1 dpy XX* males appear identical to *dpy XO* males (e.g. *dpy-20*, Fig. 2c). These abnormal phenotypes are similar to those produced by *dpy-26* (see below), and also to those observed in *tra-1 XXX* individuals (Fig. 2d).

The *dpy-21* mutation appears to be lethal to *2A;3X* individuals. This was inferred by means of the *him-8* mutation *e1489*. Mutant *him-8* hermaphrodites exhibit high levels of X chromosome nondisjunction in gametogenesis (Hodgkin et al. 1979) such that self progeny consist of about 34% *XO*, 56% *XX*, 8% *XXX*, 3% inviable zygotes (probably mostly *2A;OO* zygotes). These counts for *XXX* and *OO* zygotes are somewhat higher than those previously reported; similar counts were also obtained with another *him-8* allele, *g203*. As can be seen from Table 2, both *dpy-21* and *him-8* mutants segregate a few inviable zygotes, but the double mutant *him-8; dpy-21* segregates inviable zygotes at an enhanced frequency (14.7% cf. 1.9% and 2.5%). Furthermore, 102/102 hermaphrodites from a *him-8;dpy-21* strain all segregated over 25% self progeny males, so all must have been *2A;2X* not *2A;3X*, though at least 14 should have been *2A;3X* (which would have segregated less than 12% males). Thus, it is likely that the *dpy-21 XXX* genotype is not viable. Other dumpy genes (*dpy-4*, *dpy-11*, *dpy-20*) are viable in a *2A;3X* karyotype.

Reversion experiments on *dpy-21* have been carried out, mainly with the object of obtaining *XO* hermaphrodite mutants (Hodgkin 1980). However, *XX* non-dumpy revertants have also been obtained. Most of these carry second site *lon* mutations, which partly suppress the dumpy phenotype. One significent revertant was obtained from a *dpy-21; unc-7* mutagenized strain. A large slow-growing non-dumpy hermaphrodite was picked, which segregated both dumpy and non-dumpy hermaphrodite progeny, and many non-dumpy males (although the parent strain was non-Him). In subsequent generations the dumpy hermaphrodites bred true, producing dumpy hermaphrodite self-progeny, while the non-dumpy hermaphrodites behaved like the worm first isolated. When crossed with *lon-2 XO* males, both the dumpy and the non-dumpy hermaphrodites produced non-Unc non-Lon male progeny. The interpretation is that the original worm was a *4A;3X* tetraploid, which segregated *4A;2X* (male), *4A;3X* (non-dumpy hermaphrodite), and *4A;4X* (true-breeding dumpy hermaphrodite progeny). Chromosomes of these worms were examined using Hoechst 33258 staining and many polyploid meiotic figures (Madl and Herman 1979) were indeed observed. It follows that *dpy-21*

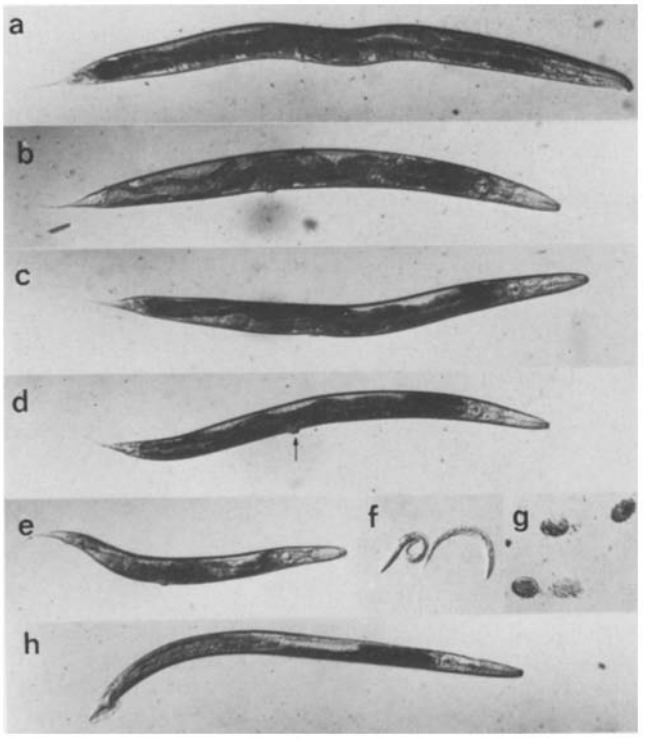

Fig. 1a–h. Light micrographs of young adult nematodes, Nomarski optics. Magnification: 75×. **a** Wild type *2A;2X*. **b** Wild type *2A;3X*. **c** *dpy-21 2A;2X*. **d** *dpy-26 2A;2X* (from *dpy-26/+* parent). The enlarged vulva (arrowed) is characteristic. **e–h** self progeny of *dpy-26* hermaphrodites: **e** Viable *2A;2X* escaper (note severe dumpy phenotype) (1% of brood). **f** Arrested larvae (76%). **g** unhatched eggs (19%). **h** Non-dumpy *2A;1X* male (4%)

expression, like sexual phenotype, is governed by the X chromosome to autosome ratio, but the threshold for *dpy-21* expression is higher than for hermaphrodite sex determination. That is, at an X:A ratio of 0.75, hermaphrodite development ensues but *dpy-21* is not expressed.

One unusual feature of this revertant strain was that some of the non-dumpy animals were intersexual in phenotype. Madl and Herman (1979) did not observe any intersexual worms in their *4A;3X* populations, so this may be an effect due to *dpy-21*. It was impossible to breed from the intersexes, so it is not known whether these worms were *4A;2X* or *4A;3X* or mosaic in karyotype.

B. Phenotypes of dpy-26 IV

The two *dpy-26* mutations *n198* and *n199* were isolated by N. Tsung after EMS mutagenesis. They have identical properties, as does the heterozygote *n198/n199*. Both are recessive, and the phenotype of *n199/sDf2* is similar to that of *n199*, so it is likely that *n199* results in complete or almost complete loss of *dpy-26* gene function. The gene exhibits a strong maternal rescue effect: homozygous *dpy-26* hermaphrodite progeny from heterozygous (*dpy-26/+*) hermaphrodite parents are only slightly dumpy (Fig. 1d) and entirely viable (Table 2, row 8). However, these homozygotes produce a self-progeny brood consisting predominantly of unhatched eggs and arrested larvae. One or two hermaphrodites in each brood reach adulthood, but these are slow growing and much more dumpy in phenotype than their parents, and they produce almost no viable progeny (Table 2, rows 2 and 3). Consequently *dpy-26* mutants cannot be maintained as homozygous self-fertilizing stocks. In addition to these inviable hermaphrodite self-progeny, each brood contains 1 to 18 non-dumpy *XO* males, which are almost wild type in phenotype. Thus, *dpy-26* appears to have a lethal maternal effect on *XX* progeny, but no effect on *XO* progeny. In addition, these mutations have a Him (high incidence of males) property, indicating loss of X chromosomes in hermaphrodite gametogenesis.

Maternal effect lethal mutations in *C. elegans* have been classified into 6 groups by Wood et al. (1980). By their criteria, *dpy-26(n199)* is an MNZ mutant, which means that expression of a wild type allele in either the mother or the zygote, but not in the father, is sufficient to rescue the lethal mutant phenotype. Expression of the wild type allele in the mother permits full viability of *dpy-26* offspring. Expression of the wild type allele in the zygote also permits full viability, because entirely wild type hermaphrodite

Table 2. Self progeny broods of *2A;2X* hermaphrodites

Genotype	Number of broods counted	Average brood					Percent males	Percent inviable
		Wild type hermaphrodite	Dumpy hermaphrodite	Wild type male	Arrested larvae	Unhatched eggs		
Wild type	12	327	0	1	ND	2	0.2	0.7
dpy-21	6	0	187	0	ND	3	0.1	1.9
dpy-26(z)[a]	6	0	2	7	117	30	4.7	94.3
dpy-26(m)[b]	9	0	1	2	24	16	4.2	93.8
dpy-26; dpy-21	10	0	1	2	19	6	6.2	90.1
him-8	7	156	21[c]	96	ND	7	34.3	2.5
him-8;dpy-21	6	0	105	78	ND	31	36.4	14.7
dpy-26/+[d]	6	229	75	0	2	2	0.0	1.3
him-8 dpy-26/him-8+	6	133	63	86	5	19	28.2	7.8

ND = not determined. In these instances arrested larvae accounted for less than 1% of the brood.
[a] Daughters of *dpy-26/+* mothers.
[b] Daughters of *dpy-26* mothers, i.e. no maternal rescue.
[c] These dumpy animals are *2A;3X* individuals.
[d] Progeny of *dpy-26* mothers crossed with wild-type males

progeny are obtained by crossing *dpy-26* hermaphrodites with wild type males. Crossing *dpy-26* hermaphrodites with *dpy-26/+* males gives wild type *dpy-26/+ XX* progeny and inviable *dpy-26/dpy-26 XX* progeny indistinguishable from the self progeny, so there is no rescue due to expression of *dpy-26(+)* in the male parent.

The combination of *dpy-26* and a *2A;3X* karyotype appears to be lethal, as with *dpy-21*. Because of the maternal lethal effects of *dpy-26*, it is not easy to distinguish *XX* and *XXX* self progeny of *dpy-26* homozygotes. All of the hermaphrodite escapers in self-progeny broods appear to be *XX* rather than *XXX*, because they produce male self progeny and very few *XXX* cross progeny when mated with wild type males. With regard to zygotic lethality, it was found that *him-8 dpy-26/him-8* + self progeny broods exhibit an enhanced inviable zygote frequency (7.8%) relative to *him-8* (2.5%) or *dpy-26/+* (1.3%). This suggests that there is a lethal zygotic interaction between *dpy-26* and a *2A;3X* karyotype, as with *dpy-21*.

These lethal interactions prompted the construction of a *dpy-26; dpy-21* double mutant. If the two genes affect independent processes, then a synergistic interaction between them might be observed. Surprisingly, the double mutant not only is viable but is able to survive as a fertile stock, unlike *dpy-26* alone, although it grows exceedingly slowly (Table 2, row 5). Males segregated by this stock are fertile, but less fecund than either mutant alone (21% cf. 54% and 88%). In this respect there is some synergy between the two mutants.

The effects of other abnormal karyotypes such as *4A;3X*, on the expression of *dpy-26* have not been investigated.

Both *dpy-26* alleles, and the heterozygotes *n198/n199*, and *n199/sDf2*, have a Him phenotype, such that about 4% of the self progeny zygotes are *XO* rather than *XX*. It is conceivable that this phenotype results from a closely linked *him* mutation, independent of the *dpy-26* locus, but this is unlikely for several reasons. First, in all crosses and mapping experiments the two phenotypes (Him and Dpy) have cosegregated. Second, both alleles show the same two phenotypes. It is unlikely that two independent isolates should be identical double mutants.

Some crosses have been carried out to investigate the Him property, along the lines of those described for other *him* mutations (Hodgkin et al. 1979), using the sex-linked marker *unc-7*. Crosses of *dpy-26; unc-7* hermaphrodites (daughters of *dpy-26/+ ;unc-7* hermaphrodites) with wild-type males yielded a total of 402 wild hermaphrodites: 15 wild males: 5 Unc hermaphrodites: 414 Unc males. The patroclinous wild males indicate that nullo-X ova are being produced at a frequency of 3.5%, which is comparable to the self-progeny male frequency of 3.7% for unmated *dpy-26; unc-7* hermaphrodites (eight complete broods yielded 57 males out of 1554 zygotes). Therefore, X chromosome loss may be confined to the egg line. The matroclinous Unc hermaphrodite progeny indicate that diplo-X ova are also being produced, but at a lower frequency (as in other *him* mutants). The production of these diplo-X ova is significant because it suggests that X chromosome loss is occurring by meiotic nondisjunction, rather than pre-meiotically. Otherwise, it might have been possible to explain the Him phenotype as a consequence of the *XX* lethality. For example, if the lethal effects of *dpy-26* were already manifested in the mitotic germ line, so that rare *XO* nuclei proliferated at the expense of *XX* nuclei, then a Him phenotype would result. However, if this were the case, one would not expect to detect diplo-X gametes, nor would one expect diplo-X gametes if loss occurred during early embryogenesis. Another test of pre-meiotic loss was made by constructing *dpy-26; unc-18/+* hermaphrodites, and scoring male self-progeny. If the males arose from rare pre-meiotic events, followed by proliferation, then one would expect to see 'jackpots' of all Unc or all wild males. In fact, only 3 out of 30 broods scored were unmixed, and all consisted of 2 or fewer males. Thus, there is no obvious causal connection between the maternal lethal phenotype and the meiotic phenotype.

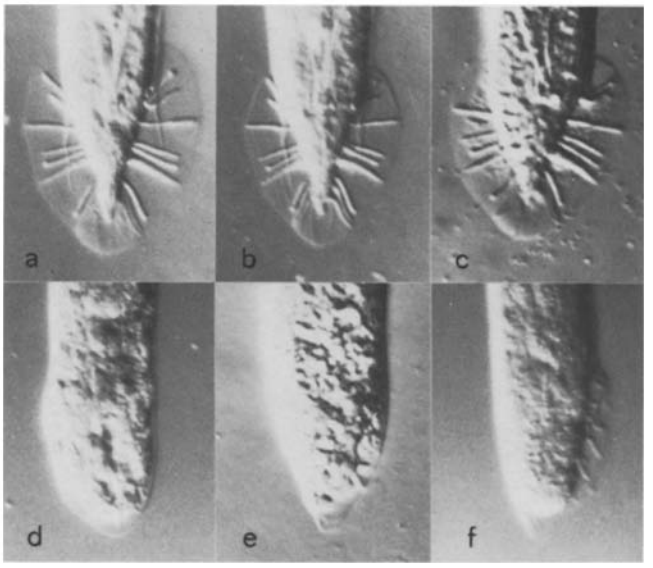

Fig. 2a–f. Light micrographs of adult male tails, dorsal view, Nomarski optics. Magnification: 350×.
a Wild type *2A;1X*. **b** *tra-1(e1099) 2A;2X*. **c** *tra-1; dpy-20 2A;2X*. **d** *tra-1 2A;3X*. **e** *tra-1; dpy-21 2A;2X*. **f** *tra-1; dpy-26 2A;2X*

No meiotic abnormalities are observed in *dpy-26 XO* males, which sire equal numbers of *XX* and *XO* progeny. No inviable zygotes are sired, indicating that autosomal behaviour is unaffected (Hodgkin et al. 1979). Fertility is lower than in wild type or *dpy-21 XO* males, (54% for *n199*, 45% for *n198*) indicating that these mutations have some effect on XO individuals.

Interactions of *dpy-26* with sex determining genes followed approximately the same pattern as with *dpy-21*. Animals of genotype *tra-1;dpy-26 XX* are incompletely masculinized (Fig. 2f), even if their mothers carried *dpy-26(+)*. The phenotype is still predominantly male, however. These transformed *XX* individuals are no less dumpy than their *XX* hermaphrodite siblings.

Conversely, transformed *dpy-26;her-1 XO* hermaphrodites are invariably non-dumpy. The double mutant *dpy-26; her-1 XO* strains grow almost exclusively as non-dumpy *XO* hermaphrodites. Two male/female (gonochoristic) non-dumpy *XO* strains were also constructed, one consisting of *tra-1(e1575)/+ ;dpy-26 XO* females and *dpy-26 XO* males, the other consisting of *fem-1(e1927) dpy-26 XO* females and *fem-1 dpy-26/+ dpy-26 XO* males. Again, all *dpy-26 XO* animals were non-dumpy and viable, confirming that the dumpy and lethal phenotypes are not expressed in *XO* individuals.

Table 3. Self fertility of *XO* hermaphrodites

Genotype	Number of broods counted	Average brood	
		Viable zygotes	Total zygotes
A. her-1(e1518)			
her-1 XO	32	29	60
her-1 dpy-21 XO	12	50	112
dpy-26; her-1 XO	6	3	46
dpy-26; her-1 dpy-21 XO	6	24	51
B. her-1(e1520)			
her-1 XO	15	3	28
her-1 dpy-21 XO	8	22	46
dpy-26; her-1 XO	6	46	113
dpy-26; her-1 dpy-21 XO	6	40	98

An anomaly was found in examining the effect of *dpy-26* on the fertility of *her-1 XO* hermaphrodites, in that different *her-1* alleles exhibited different behavior (Table 3). For the allele *her-1(e1518)*, *dpy-26(n199)* caused a striking decrease in the production of viable progeny, so that the *n199;e1518* double mutant strain is almost inviable. Fertility is restored by constructing the *n199;e1518 dpy-21(e428)* triple mutant. Opposite behavior was found for the allele *her-1(e1520)*: *e1520 XO* hermaphrodites produce few viable self progeny, but *n199;e1520 XO* hermaphrodites produce many. Double mutants of *dpy-26(n199)* with four other *her-1* alleles were also constructed, and all behaved like *e1520* rather than *e1518 (e1519, e1564, e1574* at 20° C, and the temperature-sensitive allele *e1561* at 25°). Thus, the *her-1* allele *e1518*, previously used as the reference allele for this gene, is exceptional in its properties. These observations also suggest that the genes *her-1*, *dpy-21*, and *dpy-26* interact in some way.

Discussion

The genes *dpy-21* and *dpy-26* are unusual in that they are located on the autosomes (*LGV* and *LGIV*), yet they have phenotypic consequences that are dependent on X chromosome dosage. It is proposed that these genes are involved in an X chromosome dosage compensation mechanism, so that mutations in *dpy-21* or *dpy-26* cause increased X chromosome gene expression in *XX* animals while having little effect on X chromosome expression in *XO* animals. The reasons for suggesting this are firstly that rather similar weak dumpy phenotypes are expressed by *2A;3X* aneuploids; by *dpy-21 XX* mutants; and by *dpy-26* (zygotic) *XX* mutants. Secondly, all three of these genotypes have a similar effect in partly suppressing the masculinizing effect of *tra-1* mutations, while other *dpy* mutations do not have this effect. Thirdly, both *dpy-21* and *dpy-26* exhibit a zygotic lethal interaction with a *2A;3X* karyotype. This lethality can be interpreted as the result of X chromosome overactivity: since it is known that a *2A;4X* karyotype is lethal, it is plausible that increasing X chromosome expression from a *2A;3X* karyotype would also be lethal. In addition, *dpy-26* shows a maternal lethal effect on *2A;2X* progeny, though the time of lethal arrest is variable.

The results of this paper do not directly address the question of whether the various phenotypes of *dpy-21* and *dpy-26* (dumpiness, *tra* suppression, lethality etc.) result from interaction with a single X chromosome locus or with many. If only one locus were involved one might expect easily to find a dominant sex-linked suppressor of *dpy-21*. No such suppressor has been found, so it is probable that more than one locus is involved. It should also be possible to answer this question by using X chromosome duplications and deficiencies, of which a large number are now available. Meneely and Wood (1983) have carried out such an analysis of *dpy-21* and conclude that these phenotypes are affected by multiple X chromosome loci.

One can speculate that these two genes, *dpy-21* and *dpy-26*, are part of a general dosage compensation mechanism. The obvious suggestion is that the wild type function of these genes is to prevent hyper-expression of X chromosomes. In an *XO* animal, some mechanism would block this function, permitting the single X to be expressed at a high level. The mutations in these genes are recessive, indicating partial or complete loss of function; therefore, they should affect *XX* individuals more than *XO* individuals, as observed. The absence of any lethal synergy between *dpy-21* and *dpy-26* suggests that they affect the same process, rather than independent processes. However, the two genes show strikingly different maternal effects. One type (*dpy-21*) shows no maternal effect and no lethal phenotype; the other (*dpy-26*) has very weak dumpy zygotic expression and a lethal maternal phenotype. Perhaps the two genes function at different times: *dpy-26* ensuring correct dosage compensation during early development and *dpy-21* becoming important later. It would not be surprising if an early failure of dosage compensation had lethal consequences while a later failure was less serious. Furthermore, if correct early dosage compensation is vital, then a strong maternal effect might be expected.

In this context it is worth comparing the *C. elegans* gene *dpy-26* with the *Drosophila* gene *daughterless (da)*. Both genes are autosomal, and show a lethal maternal effect on *XX* progeny, while having weaker zygotic expression. Animals with one X chromosome (*XO* or *XY*) are much less severely affected. Despite interactions with sex determining genes (*Sxl* in the case of *da*, *tra-1* and *her-1* in the case of *dpy-26*), the mutations by themselves do not cause sexual transformation. These similarities are striking, and suggest that some of the underlying phenomena are the same in *C. elegans* and *Drosophila*. However, there are significant differences: *da* acts as a zygotic lethal on both *XX* and *XY* flies at high temperature (Cline 1981), while *dpy-26* is probably not a zygotic lethal. Also, zygotic expression of *dpy-26(+)* is sufficient to rescue *XX* progeny from *dpy-26(−)* hermaphrodite parents (by mating with wild type males) but XX progeny cannot be rescued from *da* female flies by zygotic da^+ expression. These differences might be explained if *da* were a more extreme mutant than *dpy-26*. A more mysterious difference is the meiotic phenotype of *dpy-26*.

The partial suppression of *tra-1(−)* mutants by *dpy-21*, *dpy-26*, or a *2A;3X* karyotype deserves comment. In normal *XX* diploids, absence of *tra-1* product results in complete masculinization of all non-gonadal characters, while gonadal characters are less completely masculinized. However, in the *XXX* and dumpy *XX* individuals both gonadal and non-gonadal characters are incompletely masculinized. Thus, it seems that an overactive set of X chromosomes is able to exert an influence which is independent of the

action of the *tra-1* gene. Therefore *tra-1* activity is not the only way in which X chromosome dosage can affect sexual phenotype, although it may be the major way. Certainly *tra-1* activity is the most important factor in the wild type (Hodgkin 1983b), and it may be that the abnormal phenotype of these *tra-1(−)* dumpy incomplete males is merely a consequence of the generally abnormal physiology of these mutants. Alternatively, this phenotype may reflect an influence which is important in the wild type, and which is responsible for the variable gonadal phenotype of *tra-1(−) XX* males. For example, one can speculate that some sex-linked genes are more important in hermaphrodite development than in male development. These might have evolved to become less sensitive to the putative dosage compensation mechanism, so that they would then exert a feminizing influence proportional to the number of X chromosomes. Another possibility is that there are minor sex-determining genes (either autosomal or sex-linked) that act downstream from *tra-1*, yet can be affected directly by the X to autosome ratio.

The observations described in this paper raise several questions which can only be answered by further investigation. Several tentative conclusions can nevertheless be drawn. Firstly, the available evidence suggests that the sex-linked genes of *C. elegans* are dosage compensated. Secondly, there appear to be at least two autosomal genes involved in this process, one of which may be the nematode analogue of the *Drosophila* gene *daughterless*. Thirdly, sexual phenotype in *C. elegans* can be affected by X chromosome dosage, even in the absence of the major sex-determining gene *tra-1*.

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References

Baker BS, Ridge KA (1980) Sex and the single cell. I On the action of major loci affecting sex determination in *Drosophila melanogaster*. Genetics 94:383–423

Baverstock PR, Adams M, Polkinghorne RW, Gelder M (1982) A sex-linked enzyme in birds – Z chromosome conservation but no dosage compensation. Nature 296:763–766

Belote JM, Lucchesi JC (1980) Male specific lethal mutations of *Drosophila melanogaster*. Genetics 96:165–186

Brenner S (1974) The genetics of *Caenorhabditis elegans*. Genetics 77:71–94

Cline TW (1979) A male-specific lethal mutation in *Drosophila melanogaster* that transforms sex. Develop Biol 72:266–275

Cline TW (1981) Maternal and zygotic sex-specific gene interactions in *Drosophila melanogaster*. Genetics 96:903–926

Hodgkin J (1980) More sex-determination mutants of *Caenorhabditis elegans*. Genetics 96:649–664

Hodgkin J (1983a) Male phenotypes and mating efficiency in *Caenorhabditis elegans*. Genetics 103:43–64

Hodgkin J (1983b) Two types of sex determination in a nematode. Nature 304:267–268

Hodgkin JA, Brenner S (1977) Mutations causing transformation of sexual phenotype in the nematode *Caenorhabditis elegans*. Genetics 86:275–287

Hodgkin J, Horvitz HR, Brenner S (1979) Nondisjunction mutants of the nematode *Caenorhabditis elegans*. Genetics 91:67–94

Horvitz HR, Brenner S, Hodgkin J, Herman RK (1979) A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol Gen Genet 175:129–133

Johnson MS, Turner JRG (1979) Absence of dosage compensation for a sex-linked gene in butterflies (Heliconius). Heredity 43:71–77

Lyon MF (1961) Gene action in the X chromosome of the mouse (*Mus musculus* L.). Nature 190:372–373

Madl JE, Herman RK (1979) Polyploids and sex determination in *Caenorhabditis elegans*. Genetics 93:393–402

Meneely PM, Herman RK (1979) Lethals, steriles, and deficiencies in a region of the X chromosome of *Caenorhabditis elegans*. Genetics 92:99–115

Meneely PM, Herman RK (1981) Suppression and function of X-linked lethal and sterile mutations in *Caenorhabditis elegans*. Genetics 97:65–84

Meneely PM, Wood WB (1983) An autosomal gene that affects both X-chromosome expression and sex determination in *Caenorhabditis elegans*. Genetics in press

Moerman DG, Baillie DL (1981) Formaldehyde mutagenesis in the nematode *Caenorhabditis elegans*. Mut Res 80:273–279

Nelson GA, Lew KK, Ward S (1978) Intersex, a temperature-sensitive mutant of the nematode *Caenorhabditis elegans*. Dev Biol 66:386–409

Nigon V (1951) Polyploidie experimentale chez un Nematode libre, *Rhabditis elegans* Maupas. Bull biol Fr Belg 85:187–225

Riddle DL, Swanson MM (1982) The nematode *Caenorhabditis elegans*. Genetic Maps 2:244–258

Stewart B, Merriam J (1980) Dosage compensation. In: Ashburner M, Wright TRF (eds) The Genetics and Biology of Drosophila. Vol 2d, Academic Press, New York, 107–140

Wills N, Gesteland RF, Karn J, Barnett L, Bolten S, Waterston RH (1983) The genes *sup-7 X* and *sup-5 III* suppress amber nonsense mutations via altered transfer RNA. Cell 33:575–583

White MJD (1973) Animal Cytology and Evolution. Cambridge University Press

Wood WB, Hecht R, Carr S, Vanderslice R, Wolf N, Hirsh D (1980) Parental effects and phenotypic characterization of mutations that affect early development in *Caenorhabditis elegans*. Develop Biol 74:446–469

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