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Primary sex determination in the nematode *C. elegans*

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Summary

Most nematodes have XO male/XX female sex determination. *C. elegans* is anomalous, having XX hermaphrodites rather than females. The hermaphrodite condition appears to result from the modification of a basic male/female sex-determination system, which permits both spermatogenesis and oogenesis to occur within a female soma. This modification is achieved by a germ-line-specific control acting at one step in a cascade of autosomal regulatory genes, which respond to X-chromosome dosage and direct male, female, or hermaphrodite development. Mutations of one of these genes can be used to construct artificial strains with ZZ male/WZ female sex determination.

Primary sex determination normally depends on the ratio of X chromosomes to autosomes, as in *Drosophila*, and there appear to be multiple sites on the X chromosome that contribute to this ratio. Also, as in *Drosophila*, X-chromosome expression is compensated to equalize gene activity in XX and XO animals. Interactions between dosage compensation and sex determination are described and discussed.

Key words: nematode, *C. elegans*, sex determination, dosage compensation, *Drosophila*, hermaphrodite.

Introduction

The nematode *Caenorhabditis elegans* has a sexual system that at first sight appears to be profoundly different from the sexual systems found in higher vertebrates. First, the two sexes normally encountered are hermaphrodite and male (Fig. 1), rather than the more usual female and male, and the animal can reproduce both by self-fertilization and by cross-fertilization (Fig. 2). Second, the primary sex-determining signal is not a dominant sex chromosome like the Y chromosome in mammals, or the W chromosome in birds. Instead, sex is determined by X-chromosome dosage, so that diploid animals with two X chromosomes (XX) are hermaphrodite and those with one X chromosome (XO) are males. Males can arise spontaneously by X-chromosome loss in a self-fertilizing hermaphrodite population, clearly demonstrating that there is no Y chromosome in this system. Sex determination in *C. elegans* has much more in common with sex determination in *Drosophila* than it has with mammalian mechanisms; nevertheless, there are some underlying similarities between all three systems, and it has been possible to modify the *C. elegans* system in a variety of ways so as to make it resemble the *Drosophila* system or the vertebrate system more closely.

An X-chromosome dosage mechanism could rely either on a chromosome-counting mechanism, in which the absolute number of chromosomes is important, or on a ratio mechanism, in which the relative number of X chromosomes to autosomes is the determinative variable. The first kind of system is found in species with haploid male/diploid female sex determination, such as Hymenoptera; the second kind is the system found in *Drosophila*. Classical work by Nigon (1951) showed that tetraploid *C. elegans* carrying three or four X chromosomes (4A;3X or 4A;4X) were hermaphrodite, but those with two X chromosomes (4A;2X) were male. This shows that it is the X/A ratio that determines sex, not the absolute number of X chromosomes. Madl & Herman (1979) confirmed Nigon's findings and demonstrated that 3A;2X triploids were male, in contrast to the 4A;3X tetraploid hermaphrodites. The ratio-measuring mechanism is therefore capable of distinguishing between X/A ratios as similar as 0·67 and 0·75. They also showed that there must be at least three different sites on the X chromosome that were involved in computing the ratio, by observing the effect of small X-chromosome duplications on the

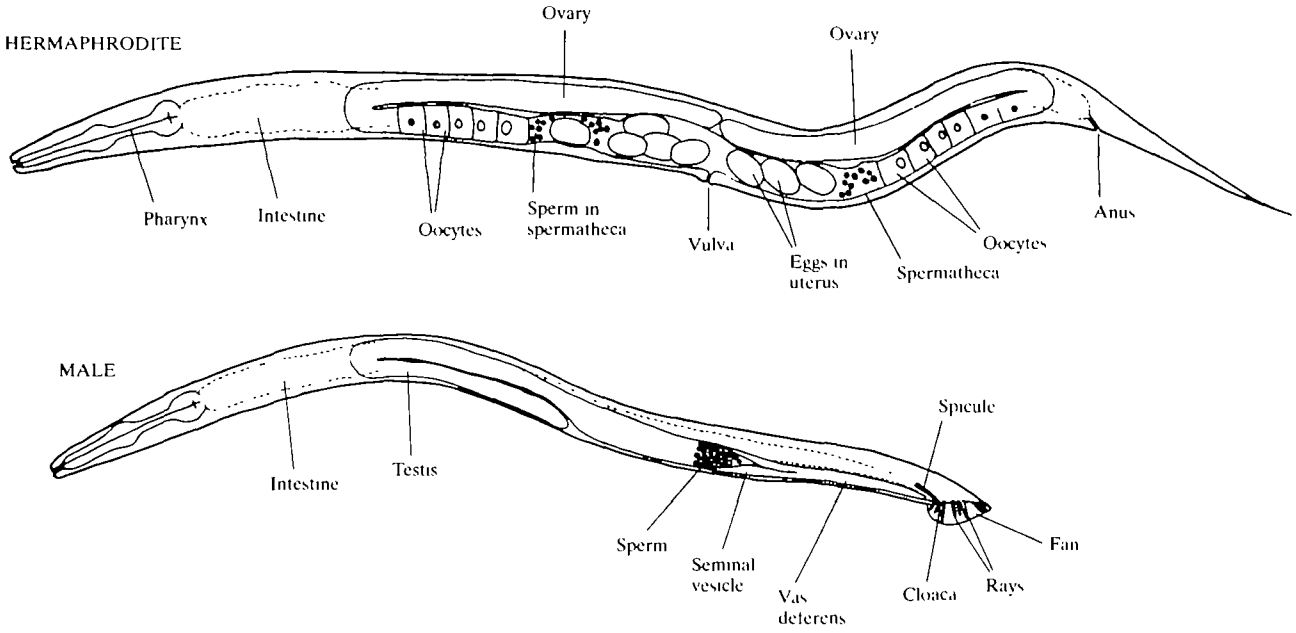


Fig. 1. Schematic diagrams of hermaphrodite and male, indicating major differences between the sexes.

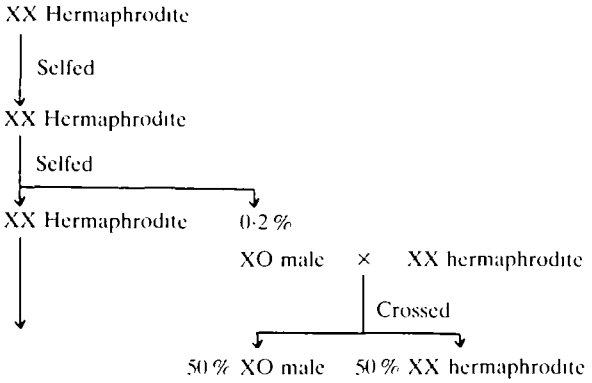


Fig. 2. Sexual system of *C. elegans*. Hermaphrodites normally reproduce by self-fertilization, but if mated with a male, reproduce by cross-fertilization, producing both male and hermaphrodite progeny.

sex of 3A;2X animals. This work is further discussed below.

Hermaphrodites are modified females

Increasing the X/A ratio, as in a 2A;3X animal (ratio 1·5) does not cause feminization: such animals are viable self-fertile hermaphrodites, like 2A;2X animals (Hodgkin, Horvitz & Brenner, 1979). However, both evolutionary considerations and genetic analysis indicate that the *C. elegans* sexual system is a modified male/female system, and that the hermaphrodite is in essence a female that has the ability to make both sperm and eggs. The number of species in the phylum Nematoda is unknown, but probably well over 10^5; most of these have an XX female/XO male system (White, 1973). Several species within the genus *Caenorhabditis* itself have XX females and XO males (e.g. *C. remanei*: Sudhaus, 1974), so it is reasonable to suspect that hermaphroditism is a secondary specialization. This would be of obvious value to a fast-growing ubiquitous soil organism like *C. elegans*, permitting more efficient exploitation of transient habitats.

Genetic analysis has supported the view that the hermaphrodite is a modified female and provided a preliminary explanation of how spermatogenesis can be allowed to proceed in a female body. The starting point of this analysis has been the isolation and analysis of (by now) hundreds of mutations that alter the normal process of sex determination, causing XX animals to develop into males or females (rather than the normal hermaphrodite), or causing XO animals to develop into hermaphrodites or females (rather than the normal males). Most of these mutations fall into seven autosomal complementation groups, each of which we have studied in some detail. Table 1 summarizes the phenotypes of some of these mutants and the locations of the genes are shown in Fig. 3. The epistatic interactions between these genes have been extensively investigated and have been interpreted in terms of the basic model for sex determination shown in Fig. 4. The autosomal genes appear to be organized in a cascade of at least four regulatory steps: the X/A ratio controls activity of *her-1*, *her-1* activity controls *tra-2*, and so on. As a result, the regulatory genes can take on one of two activity states, depending on the X/A ratio. For most of the genes, putative null mutations are available: these have little or no effect on sexual phenotype in one

Table 1. *Phenotypes of some sex-determination mutants*

	XX phenotype	XO phenotype
Wild type	hermaphrodite	male
her-1(0)	hermaphrodite	hermaphrodite
her-1(gf)	masculinized hermaphrodite	male
tra-2(0)	incomplete male	male
tra-2(gf)	female	feminized male
fem-3(0)	female	female
fem-3(gf)	germline masculinized	male
tra-1(0)	low fertility male	low fertility male
tra-1(gf)	female	female

In this table, and in the text, '*(0)*' refers to putative null (or close to null) alleles, which are usually recessive, and '*(gf)*' refers to gain-of-function alleles, which are usually dominant. Gene abbreviation are *fem*: feminization, *her*: hermaphroditization, *tra*: transformer Summarized from Hodgkin (1980, 1986, 1987), Doniach (1986), Trent, Tsung & Horvitz (1983), Barton, Schedl & Kimble (1987).

chromosomal sex, but cause a transformation of the other sex. Therefore, it is possible that this gene activity is either low or absent during normal development of the unaffected sex. For four of the genes, both loss-of-function (putative null) mutations and gain-of-function mutations have been isolated. The loss-of-function mutations cause transformation in one direction and the gain-of-function mutations cause transformation in the other. An example is shown in Fig. 5: a *her-1(0)* (null mutation, loss-of-function) mutation has no effect on XX animals (normal hermaphrodites), but transforms XO animals into hermaphrodites (Hodgkin, 1980). Conversely, a rare *her-1(gf)* (gain-of-function) mutation has no effect on XO animals (normal males), but partly masculinizes XX animals (Trent, Tsung & Horvitz, 1983). This mutation is also weakly dominant, as expected for a gain-of-function allele, whereas the *her-1(0)* mutation is recessive. The existence of two classes of mutation causing opposite transformations provides strong evidence for a model in which these genes play key roles in normal sex determination: the loss-of-function mutations show that *her-1* activity is *necessary* for XO male sex determination, and the *her-1(gf)* mutation shows that *her-1* activity is *sufficient* for at least part of male sex determination. However, the effect of both kinds of mutation can be completely suppressed by appropriate mutations of *tra-2*: *her-1(0);tra-2(0)* animals are masculinized, and *her-1(gf);tra-2(gf)* animals are feminized (Hodgkin, 1980; Doniach, 1986). Therefore *her-1* must act by regulating, either directly or indirectly, the *tra-2* gene or gene product. Similar arguments have led to the proposed cascade in its present form.

At step one, the *her-1* gene appears to control a choice between male and hermaphrodite development, but at step three, the *fem* genes control a choice between male and *female* development. Both XX and XO *fem* mutants develop into fertile females, in which the early gametes normally destined for spermatogenesis develop into oocytes instead. This suggested that spermatogenesis in the hermaphrodite was achieved by a germ-line-specific modification of the normal regulatory cascade, acting at a step prior to the *fem* gene control, most probably *tra-2*. Support for this idea comes from the properties of certain dominant *tra-2* mutations (*tra-2(gf)*) (Doniach, 1986; Hodgkin, 1986), which eliminate spermatogenesis from XX animals but not from XO animals. Homozygous *tra-2(gf)* stocks therefore grow as XX female/XO male cross-fertilizing populations. The interpretation is that these *tra-2(gf)* mutations render *tra-2* insensitive to the germ-line-specific modulation that normally occurs in the XX animal, but does not affect the control exerted by *her-1* action in the XO animal; consequently *tra-2(gf)* XO animals are fertile males. If this interpretation is correct, one might expect to find a gene activity that would be responsible for germ-line modulation, and a plausible candidate has been identified in the gene *fog-2 V* (*fog* standing for 'feminization of germ line': Schedl & Kimble, 1987). Recessive (probably loss-of-function) mutations of *fog-2* have the same effect as *tra-2(gf)* mutations, causing feminization of XX but not of XO. It appears that *fog-2* is active in both XX and XO animals (though irrelevant in the latter), but that it acts only during early germ-line development, resulting in the initial phase of hermaphrodite spermatogenesis. During later development, *fog-2* does not or cannot act, so the hermaphrodite germ line switches to sustained oogenesis.

This model explains how hermaphrodite spermatogenesis is achieved, and also provides a possible justification for the rather elaborate cascade of gene interactions. That is, the cascade may be necessary in order to permit germline modulation, and that a simpler system would permit development of only males and females. Molecular probes for several of the genes in the cascade are becoming available, so it may be possible to determine how much of this regulatory cascade is operative in related nematode species with male/female systems, the presumed ancestral arrangement.

Alternative sexual systems

The *fog-2* and *tra-2(gf)* mutations demonstrate how the sexual system can be modified in one step from a male/hermaphrodite system to a male/female system. Such an artificial strain has XX female/XO male

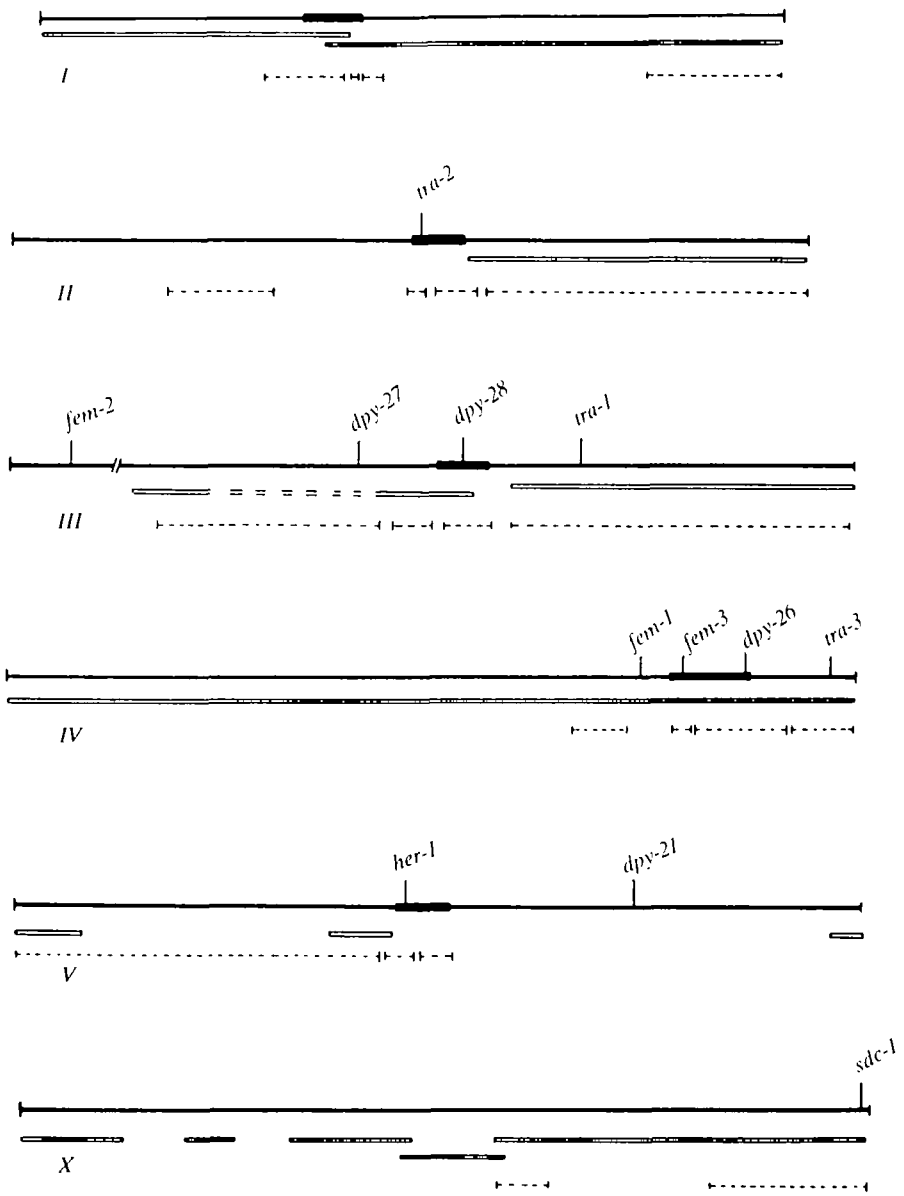


Fig. 3. Simplified genetic map of *C. elegans*, showing locations of genes affecting sex determination and genes affecting dosage compensation. Also shown are the approximate extents of some of the duplications (hollow bars) and deficiencies (dotted lines) that have been obtained. The thick solid bars on each of the five autosomes indicates clusters, in which most of the autosomal genes are concentrated. Each chromosome is about 50 map units (50 % recombination) in length, apart from *LGIII* which is slightly larger. For more details, see Swanson, Edgley & Riddle (1984).

sex determination, like most nematodes or many other animals – for example, the Drosophila species *Drosophila annulimana*.

It is also possible to provide *C. elegans* with a Y chromosome, as shown by Sigurdson *et al.* (1986). The X-autosome translocation *mnT12* attaches the whole of *LGIV* to the X chromosome. This translocation is homozygous viable, so it is possible to construct a strain with only four pairs of autosomes, plus a neo-X (*mnT12*) and a neo-Y (the original *LGIV*). In this strain, hermaphrodites are XX and males are XY. If such a strain were made homozygous for a *fog-2* mutation, the result would be an XX female/XY male strain, essentially like *Drosophila melanogaster* (in which a Y chromosome is present, but does not determine sex).

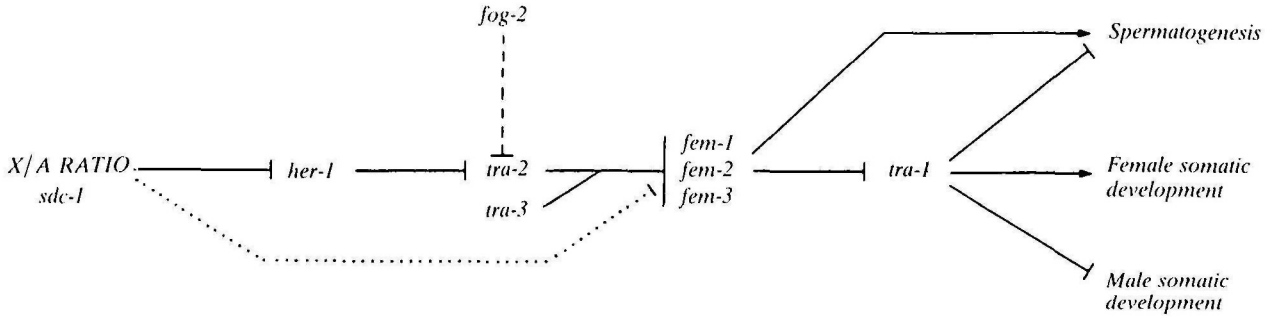
By using different mutations, it is possible to achieve a more extreme alteration. As indicated in Table 1, complete sexual transformation in either direction can be achieved by appropriate mutations of the last gene in the cascade, *tra-1*. For *tra-1(0)* alleles, both XX and XO develop into males, and for *tra-1(gf)*, both XX and XO develop into females. Because *tra-1(gf)* is dominant to *tra-1(0)*, heterozygous *tra-1(gf)/tra-1(0)* animals are also female. They can be cross-fertilized by *tra-1(0)* males to yield equal numbers of *tra-1(gf)/tra-1(0)* females, and *tra-1(0)* males. This establishes a stable male/female population which can be grown indefinitely, and in which sex is determined by a dominant W system (Hodgkin, 1983*b*). The autosome bearing *tra-1(gf)* is in essence a W chromosome, and the autosome bearing *tra-1(0)* is a Z chromosome, so we have a WZ/ZZ system, as in birds and many reptiles. In these strains, the X chromosome dosage does not affect sex. Instead, primary sex determination is

achieved by the presence or absence of a single active gene, which appears to be generally the case in mammals. Thus, sexual systems that appear to be extremely different on the surface may have significant underlying similarities.

The detailed interactions underlying this cascade and the subsequent events required for correct sexual development of many different tissues, are being actively investigated at both genetic and molecular levels in several different laboratories. It is reasonable to hope that a molecular definition of some of the postulated regulatory interactions will soon be possible. Also, molecular analysis should reveal whether there are significant homologies between sex determination in nematodes and sex determination in other organisms.

Dosage compensation

Sex determination in *C. elegans* depends on a chromosomal dosage mechanism, so it has been important to establish whether there is dosage compensation for the different numbers of X chromosomes in the two sexes and, if so, how dosage compensation and sex determination are integrated. In mammals, dosage compensation occurs by X-chromosome inactivation,



Low (XO)	soma:	HIGH	LOW	HIGH	LOW	Male soma
	germ:	HIGH	LOW	HIGH	LOW	Sperm
HIGH (XX)	soma:	LOW	HIGH	LOW	HIGH	Female soma
	early germ:	LOW	LOW	HIGH	LOW	Sperm
	late germ:	LOW	HIGH	LOW	HIGH	Oocytes

Fig. 4. A regulatory cascade controlling sexual phenotype. The top part of the figure indicates the postulated regulatory interactions (pointed arrows mean positive regulation, blunt arrows mean negative regulation). The dotted line signifies a minor interaction and the dashed line indicates a germ-line-specific control (see text). The lower part of the figure sets out the proposed activity states for the various genes in the two sexes.

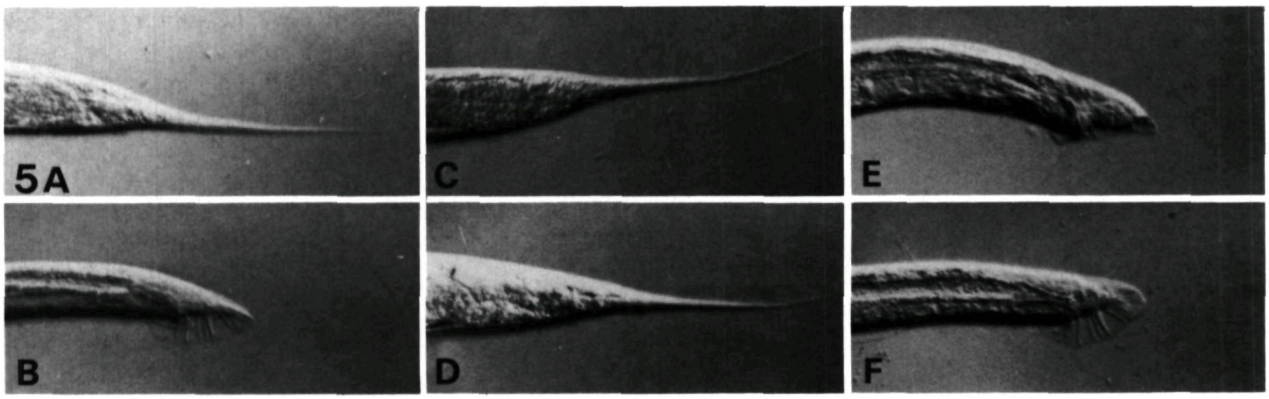


Fig. 5. Tail phenotypes of wild type and *her-1* mutants. (A) wild type (*WT*) XX; (B) wild type (*WT*) XO; (C) *her-1(O)* XX; (D) *her-1(O)* XO; (E) *her-1(gf)* XX; (F) *her-1(gf)* XO. The allele used for C and D was *e1518*, and the allele for E and F was *n695*.

a process wholly independent from sex determination. For example, the single X is never inactivated in XO female mammals, whereas only one X is active in XXY male mammals. In *Drosophila*, dosage compensation is achieved by increasing transcription from the single X chromosome of XY flies, to match the total output of the two X chromosomes of XX flies. Failure to compensate correctly is lethal. Both sex and compensation are controlled by a single 'master control gene', *Sxl* (*Sex-lethal*), which has been the subject of a long and fascinating series of studies by Cline (1983, 1984, 1986). His experiments demonstrate that in XX flies, *Sxl* adopts a state which dictates both female development and low X chromosome transcription; in XY flies, *Sxl* adopts a state dictating male development and high X transcription. As in *C. elegans*, the primary signal is the X/A ratio, which in some manner not yet understood (see Nothiger, this volume) sets the state of *Sxl*; this state is thereafter propagated by an autoregulatory mechanism, independent of X/A ratio (Cline, 1984). The arrangement in *Drosophila* therefore neatly solves a problem that would otherwise arise: compensation is necessary for viability, but perfect compensation removes the difference in X-chromosome dosage that is required for sex determination. There are, of course, other possible solutions to this problem: for example, the genes involved in determining X/A ratio might be specifically uncompensated, so that the perceived X/A ratio would not be affected by compensation.

Both genetic and molecular investigations have shown that *C. elegans* does indeed compensate for the difference in X-chromosome dosage between XX and XO animals (Wood, Meneely, Schedin & Donahue, 1985; Meyer & Casson, 1986). Most (but not all) sex-linked genes are expressed at similar levels in both XX and XO animals, and both X chromosomes in an XX hermaphrodite are active, so a mechanism of the *Drosophila* type is likely. Compensation appears to occur at the mRNA level: Meyer and Casson (1986) found that transcript levels for three different sex-linked genes were at equal levels in XX and XO animals (comparing either wild-type hermaphrodites and males, or *tra-1* XX males and wild-type XO males). A fourth sex-linked gene examined in this study was found to be uncompensated; also, three sex-linked amber suppressors appear to be uncompensated, because suppression is higher in homozygous XX animals than in hemizygous XO animals (Hodgkin, 1985 and unpublished data).

Several autosomal genes have been identified that are involved in the process of dosage compensation (Table 2). Mutations of these genes, for example *dpy-21* and *dpy-26*, have little or no effect on XO animals, but cause either dumpiness or lethality to XX animals (Hodgkin, 1983c). It was proposed that these effects arise from increased levels of X-chromosome expression, and this has been shown to be the case for *dpy-21* (Meyer & Casson, 1986; Meneely & Wood, 1987). Two other mutations with effects similar to *dpy-26* have been identified subsequently: *rh18* (isolated by E. Hedgecock) and *yl* (isolated by J. Plenefisch & B. J. Meyer). The *rh18* mutation was found to complement *dpy-26*, and maps between the *LGIII* markers *unc-93* and *dpy-17* (J. H., unpublished data); it is therefore assigned to a new gene, *dpy-27*. The *yl* mutation also maps to *LGIII*, but to a different location, and *yl* complements *rh18*, so *yl* defines a fourth gene in this class, *dpy-28* (L. DeLong, J. Plenefisch & B. J. Meyer, personal communication). Both *dpy-27* and *dpy-28* mutations cause elevated levels of transcripts from sex-linked genes, like *dpy-21* (Meyer & Casson, 1986).

Table 2. *Mutations affecting dosage compensation*

Gene	XX phenotype	XO phenotype
(A) 'Chauvinist dumpies' (autosomal)		
dpy-21(e428)	dumpy hermaphrodite	wild-type male
dpy-26(n199)	inviable or very dumpy hermaphrodite	wild-type male
dpy-27(rh18)	..	..
dpy-28(yl)	..	..
(B) *Sdc* (Sex and dosage compensation) (sex-linked)		
sdc-1(n485)	masculinized dumpy hermaphrodite	wild-type male

Summarized from Hodgkin (1983c), Meyer & Casson (1986), Villeneuve & Meyer (1987), and unpublished data from E. Hedgecock, J. Hodgkin, J. Plenefisch, L. Delong and B. J. Meyer.

These 'chauvinist dumpy' genes are significantly different from dosage compensation genes identified in *Drosophila* (reviewed by Baker & Belote, 1983). The fly genes *mle*, *msl-1*, *msl-2* and *msl-3* are required for *increasing* X-chromosome transcription in flies with only one X chromosome, so mutations of these loci do not affect female flies (XX) but are lethal to male flies (XY); hence the gene names *maleless* (*mle*) and *male-specific lethal* (*msl*). In contrast, the nematode genes *dpy-21*, *dpy-26* etc. appear to be required for *decreasing* X-chromosome transcription, since mutations are deleterious to XX rather than XO. Also, three of the genes exhibit a strong maternal rescue effect: *dpy-26* XX progeny derived from *dpy-26*/+ mothers show little abnormality, but those derived from *dpy-26* homozygous mothers usually die as embryos or young larvae. The *dpy-27* and *dpy-28* mutations have a similar, but somewhat weaker phenotype (J. H., unpublished data; L. DeLong & B. J. Meyer, unpublished data). Mutations of *dpy-21*, in contrast, do not normally show any maternal effect.

Some possible candidates for genes with the opposite functional role have been identified, but have been harder to analyse than the XX-deleterious class. Mutations of two sex-linked genes, *dpy-22* and *dpy-23* cause abnormal phenotypes in both XX and XO, but the XO phenotype is much worse (Hodgkin & Brenner, 1977). Some evidence for decreased X-chromosome expression in *dpy-22* and *dpy-23* mutants has been obtained (Meneely & Wood, 1987). Another sex-linked mutation, *y9*, has a more clean-cut effect, leading to lethality in XO hemizygotes but not in XX homozygotes; it was isolated as a suppressor of XX lethality in *dpy-28* mutants, so there is good reason to believe that it is involved in dosage compensation (L. Miller & B. J. Meyer, personal communication).

A possible link between compensation and sex determination

The mutations described in the previous section do not have any conspicuous effect on sex determination, although they do affect dosage compensation. A sex-linked locus with significant effects on both processes has recently been characterized, *sdc-1* (formerly *egl-16* (Trent *et al.* 1983); *sdc-1* stands for 'sex and dosage compensation': Villeneuve & Meyer, 1987). Two mutations of *sdc-1* have been studied, which result in partial masculinization and also in elevated X-chromosome expression and transcript levels. The masculinization effects, but not the altered dosage compensation, can be suppressed by *her-1(0)* mutations, indicating that *sdc-1* acts at an early step in the sex-determination pathway, and also acts on dosage compensation. The phenotypes are stronger in *sdc-1/mnDf1* XX heterozygotes, which indicates that these mutations do not completely eliminate *sdc-1* function. Also, both *sdc-1* alleles show a maternal effect: little masculinization is seen in *sdc-1* daughters of *sdc-1*/+ mothers.

There is a formal similarity between *sdc-1* and the *Drosophila* gene *Sxl*: both are sex-linked loci which appear to be involved at an early step in sex determination and dosage compensation. For both genes, loss-of-function mutations lead to masculinization and elevated X-chromosome transcription in XX animals, but have no obvious effect on XO (or XY) animals. However, as discussed by Villeneuve & Meyer (1987) there are other possible roles for this gene: it could be one of the elements measured in the assessment of the X/A ratio. It cannot be the only such element, because a deficiency for the *sdc-1* region has no dominant effect on sexual phenotype in XX animals (normal hermaphrodites), and a duplication for *sdc-1* has also no effect on XO animals (normal males). Nevertheless, it could be one of a number of such elements. More detailed analysis of this locus will be necessary to define its role. A different locus on the X chromosome, with properties related to those of *sdc-1*, has been identified by Wood *et al.* (1987).

X/A ratio: the numerator

The remainder of this paper will be devoted to analysis of the natural primary sex-determining signal, the X/A ratio. This is a problem which may have wider biological significance than is at first apparent, because it involves the translation of a quantitative signal into a qualitative choice. Most biological switch mechanisms that we understand at present operate on qualitative distinctions, as in the presence or absence of the mammalian Y determinant. However, there are also cases where small quantitative differences appear to trigger very different consequences. Nervous systems obviously have the computational ability to make such distinctions, but quantitative discrimination is a much harder task at the level of a single cell or part of a cell. It is widely believed that some kind of morphogen gradient is responsible for many events in pattern formation; the correct interpretation and response to such a gradient is likely to require accurate measurements by the cells in the morphogenetic field. No convincing example of a natural morphogen gradient in a multicellular organism has yet been put forward, so this is a highly speculative area of developmental biology. In the case of X/A ratio assessment in *Drosophila* and *Caenorhabditis*, we have examples of small differences in ratio leading to entirely different consequences. In these systems, it may be possible to define the signals precisely and determine how they are measured. It is also at this point that the sex-determination systems of *C. elegans* and *Drosophila* seem most similar, and one can hope that insights gained from these two organisms will be mutually illuminating, even if they shed little light on mammalian sex determination.

The original analysis of X/A ratio by Madl & Herman (1979) and subsequent unpublished work by R. K. Herman (personal communication), suggested that there were at least four sites on the right half of the X chromosome that contributed to the 'numerator' of the X/A ratio. They examined the effect of partial X duplications on the sexual phenotype of triploid 3A;2X animals, which are normally male. A small duplication (*mnDp8*: see Fig. 6) had no obvious effect on 3A;2X animals, but larger duplications did have an effect: 3A;2X;*mnDp9* animals were usually intersexual and 3A;2X;*mnDp10* animals were hermaphrodite. This showed that there must be at least two sites, one included in *mnDp10* but not *mnDp9*,

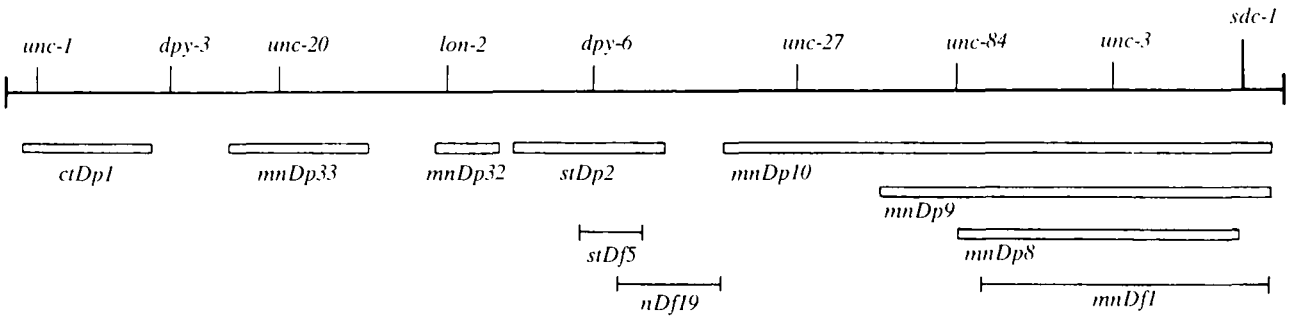


Fig. 6. Duplications and deficiencies of the X chromosome. See Swanson *et al.* (1984) for a more detailed map.

and another within *mnDp9* but not *mnDp8*. Furthermore, since diploid 2A;XO;*mnDp10* animals are male, there must be at least one more site not included in *mnDp10*. In these experiments, 3A;2X animals were generated by crossing tetraploid hermaphrodites (4A;4X) with diploid males carrying the duplication; in the reverse cross, mating diploid 2A;2X;*mnDp8* hermaphrodites with 4A;2X tetraploid males, several of the progeny were hermaphrodite or intersex. This suggests that there is a fourth site within *mnDp8* and also that there can be a maternal effect on the measurement of X/A ratio. Note that the gene *sdc-1* lies in this region, so it would be of interest to repeat these experiments using *sdc-1;mnDp* mothers.

Subsequent to this work a number of additional X chromosome duplications have become available, with extents approximately as shown in Fig. 6. These have not yet been investigated for their effect on 3A;2X animals. None of them appears to cause any hermaphroditization in 2A;XO;*Dp* males.

X/A ratio: the denominator

So far, it has been possible to infer rather little about the nature of the autosomal part of the X/A ratio. If there were a single site responsible for this 'denominator signal', it might be expected to show dosage effects in aneuploid animals: a deficiency for such a site might cause a shift towards hermaphrodite development in *Df*/+;XO animals, and a duplication might cause masculinization of +/+;*Dp*;XX animals. A large number of autosomal duplications and deficiencies are now available in *C. elegans*, with approximate extents shown in Fig. 3. About 80% of the map is covered by duplications and about 50% by deficiencies. None of these have been shown to affect sexual phenotype in diploid animals. Possibly, they might have effects in animals with intermediate X/A ratios, such as 3A;2X males or 4A;3X hermaphrodites, but such an analysis would be hard to carry out, and has not yet been attempted. It is difficult to make a large quantitative shift in the X/A ratio by means of autosomal alterations.

The possibility that the first gene in the sex determination pathway, *her-1*, is involved directly in measuring X/A ratio can be considered, but is unlikely for several reasons. A simple model might be that the *her-1* locus produces a fixed amount of some product which is titrated by X-chromosome sites or products. At high X dosage, the *her-1* product is completely titrated out and therefore inactive; at low X dosage only partial titration is achieved, so some active *her-1* product remains and is able to put the pathway into the male state. However, with such a model one would expect to see dosage effects for *her-1*, and these are not usually observed. Both *her-1*/+ and *mDf1*/+ XO animals are normal fertile males (Hodgkin, 1980 and unpublished data; *mDf1* is a deficiency that fails to complement *her-1* and flanking markers). Doniach (1986) did observe an effect on sexual phenotype in *tra-2(gf)*/+;*her-1*/+ XO animals, which are more feminized than the corresponding *tra-2(gf)*/+;+/+ XO animals, but this is the only dominant effect so far observed.

One can sustain the *her-1* titration model by proposing that the *her-1* gene is able to autocompensate in some way, so that equal amounts of product are made both by *her-1*/+ and by wild-type animals. Alternatively, it could be that none of the *her-1* loss-of-function alleles is completely null for *her-1* product. There is some heterogeneity amongst these alleles (Hodgkin, 1980, 1983*c*), suggesting that at least some are not null: for example, *her-1(e1520)* XO animals are less fertile than *her-1(e1518)* XO animals. A deficiency for the region, *mDf1*, has become available (D. L. Riddle, unpublished data) and has been used in complementation tests with these two alleles. Both *e1518/mDf1* XO and *e1520/mDf1* XO animals are hermaphrodites, with similar very low fertility. This test therefore does not show which of the two alleles is closer to a null, because it is possible that dominant effects due to *mDf1* are causing a general reduction in fertility. C. Trent & W. B. Wood (personal communication) have carried out tests with weak alleles of *her-1* which suggest that *e1520* does

have residual *her-1* activity. Nevertheless, it is still possible that some of the other recessive *her-1* alleles are null alleles.

Expression of the gain-of-function *her-1* mutation, *n695* (Trent *et al.* 1983) can be affected by X-chromosome dose or activity, as expected if *her-1* were a denominator element. For example, the phenotypes of 2A;2X and 2A;3X animals were compared, by examining the progeny of a 2A;3X hermaphrodite of genotype *him-5(e1467) her-1(n695)*. 16/28 XX animals showed some masculinization (as in Fig. 5E), but only 1/13 XXX animals. Also, a strain of genotype *her-1(n695) dpy-21(e428)* XX was constructed and found to show almost no masculinization (2/55 animals examined had a slightly masculinized tail). However, it may be that these effects are indirect or insignificant, given that *n695* is an incompletely penetrant mutation; its expression may therefore be very sensitive to genetic background.

The fact that *sdc-1* appears to act upstream of *her-1* and to control both sex and dosage compensation might be taken as evidence against a role for *her-1* as denominator, but the argument is not compelling. First, the function of *sdc-1* is not certain at this point, being based on only two alleles. Second, there is no *a priori* reason why sex and dosage compensation should be regulated together; it could be that *sdc-1* is a numerator element for both processes, and *her-1* is the denominator for sex alone, there being some other denominator for dosage compensation. Another (less likely) possibility is that *her-1* does have a function in dosage compensation control, but that none of the *her-1* mutations so far obtained affects this function.

The *her-1* gene cannot be the only gene responding to X/A ratio, because there is a 'minor pathway' (indicated by the dotted line in Fig. 4), whereby X/A ratio is able to influence sexual phenotype independent of *her-1* and *tra-2* (see Hodgkin, 1980). It is still not clear what gene activities are involved in this minor pathway or whether it plays any role during the normal process of sex determination.

Wood *et al.* (1987) have proposed that the autosomal 'chauvinist dumpy' genes constitute the denominator elements. This proposal is based in part on an analogy with the *Drosophila* gene *da* (*daughterless*), which is believed to act in setting the state of *Sxl*, and therefore to have some kind of denominator function. Like *da*, the *C. elegans* genes *dpy-26*, *dpy-27* and *dpy-28* have an extreme maternal effect, being lethal or almost lethal to XX progeny but not to XO progeny. However, unlike *da*, these genes do not show a strict maternal effect, because mating *dpy-26* mothers with wild-type males yields perfectly normal viable *dpy-26*/+ XX progeny. No dosage effects have been demonstrated for the three loci *dpy-26*, *dpy-27*, and *dpy-28*, for which both duplications and deficiencies are available. Moreover, in diploid animals the mutations of these genes do not cause any noticeable feminization of XO animals: a double mutant *dpy-26(n199); dpy-21(e428)* XO is still a fertile male (Hodgkin, 1983*b*).

Meneely & Wood (1984) reported that one allele of *dpy-21*, *ct16*, did cause feminization of diploid XO animals, leading to an abnormal male phenotype. This effect, however, is due to a second mutation. A strain BW139, genotype supposedly *him-5(e1467) dpy-21(ct16) V* was obtained from P. Meneely and found to have a predominantly non-dumpy hermaphrodite phenotype, but to segregate males with a Mab (male abnormal) phenotype. A few dumpy hermaphrodites were also segregated; these appeared to be viable 2A;3X animals, as expected for a strain carrying the meiotic non-disjunction mutation *him-5(e1467)* (Hodgkin *et al.* 1979). When BW139 hermaphrodites were mated with *dpy-21(e428)* XO males, all cross progeny were completely wild type. The strain was also mated with wild-type males and the segregation of the *mab* mutation followed: it was unlinked to *him-5* and was eventually mapped to *LGII* by M. Shen (personal communication). He has found that it fails to complement the known *mab* mutation *mab-3(e1240)* II, and therefore is assigned to this gene, being given a new allele designation *e2093* (to avoid confusion). The '*ct16*' Mab phenotype illustrated by Meneely & Wood (1984) is strikingly similar to that of *mab-3(e1240)* (illustrated by Hodgkin, 1983*a*; Hodgkin, Doniach & Shen, 1985), so there is no doubt that the abnormal male phenotype described by these workers is due to *mab-3*, not *dpy-21*.

An effect of dosage compensation on sex determination

Meneely & Wood (1984) also described a different feminizing effect of *dpy-21*: in diploid XO animals with large X duplications, (such as *mnDp10*), *dpy-21* mutations caused a shift in phenotype from male to intersex. Related experiments are reported here which demonstrate a more extreme effect with both *dpy-21* and *dpy-27* (which has stronger effects than *dpy-21*).

In these experiments (Table 3), 3A;2X progeny are generated by mating 2A;2X hermaphrodites with 4A;2X males. Normally (cross 6), these animals are all male; the occasional hermaphrodites are probably 3A;3X animals, resulting from imperfect disjunction in the tetraploid male parent (i.e. a few sperm are probably 2A;2X rather than 2A:1X). However, if the diploid mother carries either *dpy-21* or *dpy-27*, a

Table 3. *Effects of dosage compensation mutations on sexual phenotype*

	Maternal genotype		Paternal genotype		Total progeny	Inviable progeny	Percent of viable progeny				
							WH	WI	WM	DH	UM
1.	+	*2A*	+	*2A*	259	3	48	0	0	0	52
2.	*dpy-21*	*2A*	+	*2A*	138	1	48	0	0	0	52
3.	*dpy-21*	*2A*	*dpy-21*	*2A*	182	10	0	0	0	43	57
4	*dpy-27*	*2A*	+	*2A*	287	8	47	0	1	0	52
5	*dpy-27*	*2A*	*dpy-27*	*2A*	276	115	0	0	0	11	89
6	+	*2A*	+	*4A*	115	1	4	1	95	0	0
7	+	*2A*	*dpy-21*	*4A*	436	14	10	10	78	0	2
8.	+	*2A*	*dpy-27*	*4A*	199	8	10	7	80	0	3
9.	*dpy-21*	*2A*	+	*4A*	236	16	45	9	45	1	0
10	*dpy-21*	*2A*	*dpy-21*	*4A*	241	29	33	18	43	4	2
11	*dpy-21*	*2A*	*dpy-27*	*4A*	110	4	50	12	38	0	0
12.	*dpy-27*	*2A*	+	*4A*	270	19	48	23	29	0	0
13.	*dpy-27*	*2A*	*dpy-21*	*4A*	141	10	52	25	20	0	3
14.	*dpy-27*	*2A*	*dpy-27*	*4A*	352	27	92	3	3	1	1

In all of these crosses, the maternal parent was homozygous for the recessive mutations *fem-1(hc17) IV* and *unc-7(e5) X*, and the paternal parent carried neither of these mutations The sex-linked *unc* (uncoordinated) marker allows distinction between animals carrying one and two X chromosomes, because animals carrying only one (matroclinous) X chromosome will be uncoordinated, but *unc*/+ or *unc*/+/+ animals move well. The *fem-1* mutation was used to ensure that only cross progeny were examined all maternal parents were raised at 25°C during early larval life, and then shifted to 20°C for crossing. This treatment converts XX animals from self-fertile hermaphrodites into females, as a result of the temperature sensitive *hc17* mutation (Nelson, Lew & Ward, 1978; Doniach & Hodgkin, 1984) Consequently, all progeny, both viable and inviable, must be cross-progeny. 'Inviable progeny' refers to the number of unhatched eggs or arrested young larvae. Percentages in the last five columns are calculated relative to total viable progeny. Abbreviations in these columns are, WH, wild-type hermaphrodite; WI, wild-type intersex, WM, wild-type male; DH, dumpy hermaphrodite, UM, uncoordinated male. Wild type in this context means expressing neither a dumpy nor an uncoordinated phenotype.

Tetraploid 4A;2X males were generated by selfing 4A;3X hermaphrodites. Conveniently, the mutations *dpy-21(e428)* and *dpy-27(rh18)* can be maintained in stocks of homozygous *dpy* 4A;3X tetraploids, which are non-dumpy viable hermaphrodites. These segregate dumpy or inviable 4A;4X hermaphrodites, non-dumpy 4A;2X males, and more 4A;3X hermaphrodites.

considerable shift is seen towards either an intersexual or a hermaphrodite phenotype (crosses 9–14). If both mother and father carry *dpy-27*, most of the progeny are hermaphrodite (cross 14).

Given the information that these *dpy-21* and *dpy-27* mutations cause elevated levels of most X-chromosome transcripts (Meyer & Casson, 1986), the obvious interpretation is that the 'perceived' X/A ratio has been shifted, leading to the change in sexual phenotype. In a diploid XO animal, the shift is insufficient to reach the critical level, but in a 3A;2X animal, a much smaller shift is required, so sexual phenotype is switched. If this interpretation is correct, then it follows that X-chromosome *transcripts* (rather than sites) are being measured in the computation of X/A ratio and that there can be a significant maternal contribution to this computation (because there is asymmetry between crosses 7 and 9, and 8 and 12).

Various other conclusions can be inferred from the data in Table 3. For example, it appears that X-chromosome disjunction is slightly impaired in tetraploid *dpy-21* and *dpy-27* males. A meiotic effect has already been seen for *dpy-26* (Hodgkin, 1983c) but not hitherto for *dpy-21* or *dpy-27*. As a result of this, some 3A;1X male animals are generated in crosses 7, 8 etc.; they are recognizable because they express the matroclinous *unc-7* marker. Such animals had previously been thought to be inviable, (Madl & Herman, 1979), but at least some appear to survive, although they are thin, small and pale. A cross to confirm this was carried out, which generated non-Unc 3A;1X males (Table 4). Fewer than the expected number were found, and none was able to mate successfully (unlike 3A;2X males), confirming this conclusion. The presence of a *dpy-27* or a *dpy-21* mutation did not seem to improve the phenotype (Table 3, cross 10 and cross 14), suggesting that the compensation machinery is only able to work within certain limits (2A;3X animals are also abnormal and 2A;4X animals die as embryos: Hodgkin *et al.* 1979).

It is possible that the autosomes also fail to disjoin perfectly in tetraploid males, so that some of the resulting 3A;2X animals have either two or four copies of a particular autosome, rather than three. This could account for some of the variability in these crosses – for example, the occasional hermaphrodites and intersexes generated in the control cross (Table 3,

Table 4. *Generation of 3A;1X triploid males*

Male parent	Inviable progeny	Viable progeny counts			
		WH	WM	UH	UM
1. Diploid: 2A;1X	73	0	54	73	72
2. Tetraploid: 4A;2X	12	0	37	12	105

Phenotype abbreviations as in Table 3; also UH, uncoordinated hermaphrodites. For both crosses, maternal parents were of genotype *fem-1(hc17) him-8(e1489) IV; unc-1(e1598) X*, and were raised at 25°C as larvae, to convert them into functional females (see Table 3). The *unc-1* mutation is dominant (Park & Horvitz, 1986) and the *him* mutation leads to a high frequency of nullo-X oocytes (Hodgkin *et al.* 1979). Consequently, patroclinous non-Unc males are produced in cross 1, implying a nullo-X oocyte frequency of $2\times54/272 = 40\,\%$. The patroclinous non-Unc male progeny in cross 2 should be 3A;IX. Fewer than expected are produced (37 observed, 66 (i.e. 166×0·4) predicted).

cross 6). Conceivably one could identify which autosomes are important in computing X/A ratio, by repeating these crosses with animals carrying autosomal markers.

Many, and perhaps all, of the 'intersexes' generated in these crosses are 'mosaic' intersexes, containing a variety of mixtures of male and female parts. They have not been examined in detail, but it seems likely that this could result from independent assessment of X/A ratio in different tissues. When the signal is ambiguous (around 0·7), different tissues might make different decisions, resulting in a mosaic intersex, as in triploid *Drosophila* (Cline, 1983).

The main conclusion from these experiments is that the dosage compensation genes can affect assessment of the X/A ratio, so the X-chromosome part of the ratio is not uncompensated. This returns us to the problem mentioned above: compensation will tend to abolish the primary sex determining signal, so the system should not work, unless some other process is involved. At present, a likely possibility is that *C. elegans* uses the same solution as *Drosophila*. If the *sdc* gene(s) are the nematode analogues of *Sxl*, then one would predict that they, too, should exhibit autoregulation, and become insensitive to X/A ratio once their initial state has been set. Given the present tools available for molecular and genetic analysis of *C. elegans*, experiments to test these ideas should soon be possible.

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