Identification of *X* Chromosome Regions in *Caenorhabditis elegans* That Contain Sex-Determination Signal Elements

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ABSTRACT

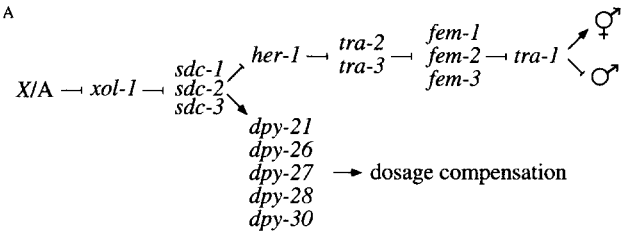
The primary sex-determination signal of *Caenorhabditis elegans* is the ratio of *X* chromosomes to sets of autosomes (*X*/A ratio). This signal coordinately controls both sex determination and *X* chromosome dosage compensation. To delineate regions of *X* that contain counted signal elements, we examined the effect on the *X*/A ratio of changing the dose of specific regions of *X*, using duplications in *X*O animals and deficiencies in *XX* animals. Based on the mutant phenotypes of genes that are controlled by the signal, we expected that increases (in males) or decreases (in hermaphrodites) in the dose of *X* chromosome elements could cause sex-specific lethality. We isolated duplications and deficiencies of specific *X* chromosome regions, using strategies that would permit their recovery regardless of whether they affect the signal. We identified a dose-sensitive region at the left end of *X* that contains *X* chromosome signal elements. *XX* hermaphrodites with only one dose of this region have sex determination and dosage compensation defects, and *X*O males with two doses are more severely affected and die. The hermaphrodite defects are suppressed by a downstream mutation that forces all animals into the *XX* mode of sex determination and dosage compensation. The male lethality is suppressed by mutations that force all animals into the *X*O mode of both processes. We were able to subdivide this region into three smaller regions, each of which contains at least one signal element. We propose that the *X* chromosome component of the sex-determination signal is the dose of a relatively small number of genes.

THE primary sex-determination signal in *Caenorhabditis elegans* is the ratio of *X* chromosomes to sets of autosomes (*X*/A ratio). Diploid animals with one *X* chromosome (*X*/A ratio of 0.5) are males, and those with two *X* chromosomes (*X*/A ratio of 1.0) are self-fertile hermaphrodites. Studies of triploid and tetraploid animals showed that an *X*/A ratio of 0.67 or lower specifies male development, while a ratio of 0.75 or higher specifies hermaphrodite development, indicating that a remarkably small difference in the relative *X* chromosome dose can determine sexual fate (MADL and HERMAN 1979). *C. elegans* sex determination provides an excellent opportunity to study how small differences in an initial signal are amplified to make the choice between two alternative developmental fates.

How might the *X* chromosome dose be measured? At one extreme, the dose of numerous elements along the entire *X* chromosome might contribute to the sex-determination signal if, for example, autosomal factors bind to dispersed sites along *X*. At the other extreme, the dose of a single *X*-linked gene might be all that is measured. To address this question, we varied the dose of specific *X* chromosome regions from one to two in *X*O animals using duplications, or from two to one in *XX* animals using deficiencies. If the dose of a large number of dispersed elements contributes to the *X*/A ratio, only large *X* chromosome duplications or deficiencies would affect the sex-determination signal. If only the dose of a single gene is measured then changing the dose of that gene, but not of other regions of *X*, should affect the signal. If there are a small number of *X*-linked elements, changing the dose of a few of these elements may be sufficient to affect the signal. Such a change could be achieved in *X*O animals, for example, by using multiple *X* chromosome duplications.

The phenotypes of mutations in the regulatory genes that are controlled by the signal tell us what phenotypes we can expect from changing the sex-determination signal itself. These genes direct not only sex determination, but also the essential process of *X* chromosome dosage compensation, a process that equalizes *X*-linked gene expression in *XX* and *X*O animals (DELONG *et al.* 1993; MILLER *et al.* 1988; NUSBAUM and MEYER 1989; VILLENEUVE and MEYER 1987) (Figure 1). *X*O-specific lethality and feminization are caused by mutations in the most upstream gene in the pathway, *xol-1*. *xol-1* is required in *X*O animals for proper male development; it achieves its role by negatively regulating the downstream *sdc* genes, which set the hermaphrodite mode of sex determination and dosage compensation. *xol-1* mutant *X*O animals die from inappropriately low *X* chromosome gene expression, because they adopt the *XX* mode of dosage compensation. In contrast, *XX*-specific lethality is caused by null mutations in *sdc-2* and *sdc-3*, which elevate *X* chromosome expression by failing to activate the downstream dosage compensation genes (*dpy-21*, *dpy-26*, *dpy-27*, *dpy-28* and *dpy-30*). These *dpy* genes equalize *X* chromosome expression by turning down tran-

A



B

	X/A	xol-1	sdc-1 sdc-2 sdc-3	Relative gene expression per X	Sex
XX	1.0	Low	High	0.5	Hermaphrodite
XO	0.5	High	Low	1.0	Male

FIGURE 1.—The primary sex-determination signal controls a hierarchy of genes that regulate sex determination and dosage compensation. (A) The sex determination and dosage compensation regulatory pathway. The primary signal and the master regulatory genes immediately downstream, *xol-1*, *sdc-1*, *sdc-2* and *sdc-3*, control both the sex determination and dosage compensation branches of this pathway. The regulatory genes that control somatic sex determination are shown on the upper branch, and the genes that implement dosage compensation are shown on the lower branch. The hierarchy for germline sex determination is not shown. Positive regulatory interactions are indicated by arrows, and negative regulatory interactions are indicated by bars. (B) The activity states of the master regulatory genes and the final outcome of the hierarchy in *XX* and *XO* animals. In *XX* animals, a high *X*/A ratio (1.0) represses *xol-1* activity and allows the *sdc* genes to be active and to positively regulate the *dpy* genes, which implement dosage compensation by reducing gene expression from both hermaphrodite *X* chromosomes. The *sdc* genes promote hermaphrodite sexual development by negatively regulating *her-1*, a gene required for male development. In *XO* animals, the low *X*/A ratio (0.5) activates *xol-1*. *xol-1* represses the *sdc* genes, resulting in a failure to activate the *dpy* genes and a failure to repress *her-1*. *X*-linked gene expression is not turned down, and the animal develops into a male (DELONG *et al.* 1993; HODGKIN 1980, 1983; HSU and MEYER 1994; KUWABARA and KIMBLE 1992; MEYER and CASSON 1986; MILLER *et al.* 1988; NUSBAUM and MEYER 1989; PLENEFISCH *et al.* 1989; VILLENEUVE and MEYER 1987).

scription of both hermaphrodite *X* chromosomes (HODGKIN 1983; HSU and MEYER 1994; MEYER and CASSON 1986; PLENEFISCH *et al.* 1989). The *sdc* mutations also cause masculinization of the dying *XX* animals. On the basis of these phenotypes, we expect that if we alter the sex-determination signal by changing the dose of signal elements, the resulting phenotype should be karyotype-specific lethality. *XO* animals with an *XX* dose of signal elements should turn off *xol-1* and die because the *sdc* genes become activated. *XX* animals with an *XO* dose of signal elements should turn on *xol-1* and die because the *sdc* genes are inappropriately repressed. Furthermore, the lethality caused by a 2*X* dose of signal elements in *XO* animals should be suppressed by *sdc* loss-of-function mutations, which force animals into the *XO* mode of dosage compensation regardless of karyotype. The lethality caused by a 1*X* dose of signal elements in *XX* animals should be suppressed by *xol-1* loss-of-function mutations, because *xol-1* mutant animals are forced into the *XX* mode of dosage compensation regardless of karyotype.

In *C. elegans*, duplications were available for most regions of the *X* chromosome (Figure 2), yet none of these duplications cause *XO*-specific lethality. The lack of lethality is not surprising since most of the duplications were initially recovered in viable *XO* males (HERMAN *et al.* 1976, 1979; HERMAN and KARI 1989). Many of the duplications that had no effect on *XO* males were subsequently shown to feminize 2*X*/3A animals, which are normally males–the larger the duplication, the greater the feminizing effect (MADL and HERMAN 1979; MENEELY 1994). These results were originally taken to indicate that the sex-determination signal is the cumulative dose of multiple dispersed weak signal elements, and that the duplications include elements sufficient to increase an *X*/A ratio of 0.67 (2*X*/3A), to the hermaphrodite level, but not sufficient to raise a ratio of 0.5 (1*X*/2A), to that threshold (MADL and HERMAN 1979). An alternative interpretation of these results, however, is that the duplications do not contain signal elements, but instead affect sex determination through a feedback mechanism, by which high levels of *X* chromosome gene expression caused by the duplication (MENEELY and NORDSTROM 1988) can feminize animals with an intermediate *X*/A ratio. Evidence for such a feedback mechanism comes from the observation that mutations in the dosage-compensation *dpy* genes, which cause increased *X*-linked gene expression, also feminize 2*X*/3A animals, but have no effect on the sexual phenotype of diploid animals (HODGKIN 1987; PLENEFISCH *et al.* 1989).

Some regions of *X* might not have been represented among the existing duplications and deficiencies because they harbor one or more signal elements whose dose strongly affects the sex-determination signal. With the knowledge that perturbations in the *X*/A ratio could cause sex-specific lethality, it was possible to devise strategies that would allow the recovery of duplications and deficiencies, regardless of whether they affected the signal. We reasoned that duplications of a region harboring signal elements could be isolated in either *XO* animals defective in an *sdc* gene or in wild-type *XX* animals. Deficiencies of such a region could be isolated in *xol-1* mutant *XX* animals.

In this study we isolated new duplications and deficiencies of specific *X* chromosome regions using these approaches. We found that a region near the left end of *X* strongly contributes to the *X*/A ratio. Duplications of this region cause *XO*-specific lethality that is suppressed by *sdc* mutations, while deficiencies of the same region cause heterozygous *XX* animals to have a weak Sdc phenotype that is suppressed by *xol-1* mutations. We were further able to subdivide this region into three smaller

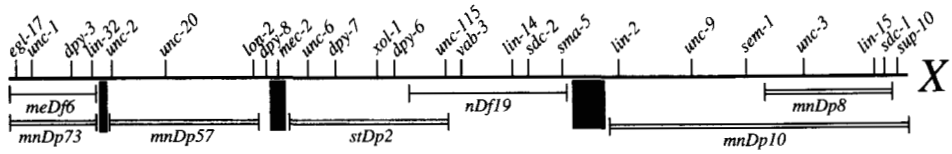


FIGURE 2.—Genetic map of the *C. elegans X* chromosome. The black boxes indicate regions that are not included in any previously characterized duplications or deficiencies. Representative duplications and deficiencies are shown for each region; duplications are shown as double lines, and deficiencies as single lines. Many other duplications and deficiencies of *X* have been characterized, particularly ones that map to either the left-most or right-most regions of the chromosome.

regions, each of which contributes to the signal. On the basis of these results, we predict that the *X* chromosome component of the *C. elegans* sex-determination signal is likely to be the dose of a relatively small number of genes.

MATERIALS AND METHODS

Strains and general methods: General methods used for maintaining *C. elegans* strains were as described by BRENNER (1974). N2 was the wild-type parent strain from which all the new strains described herein are derived. All experiments were performed at 20°. Mutagenesis with ethyl methanesulfonate (EMS) was according to BRENNER (1974), and mutagenesis by γ-irradiation was done by exposing L4 or young adult worms to approximately 5500 R, using a ^{137}Cs source. Abbreviations are as follows: *bli* (*bli*stered), *dpy* (*dumpy*), *egl* (*egg-laying defective*), *flr* (*fluoride resistant*), *flu* (abnormal gut *fluorescence*), *him* (*high incidence of males*), *lin* (*lineage*), *lon* (*long*), *Mec* (*mechanosensory defective*), *rol* (*roller*), *sdc* (*sex and dosage compensation*), *sem* (*sex-myoblast migration*), *sma* (*small*), *Tra* (sexually *transformed*), *unc* (*uncoordinated*), *xol* (*XO lethal*). The following mutations and chromosomal aberrations were used in this study:

Linkage group (LG) II: bli-2(e768).

LG III: dpy-27(y57) (PLENEFISCH *et al.* 1989); *dpy-28(y1).*

LG IV: him-8(e1489); yIs2 (Pxol-1::lacZ) (RHIND *et al.* 1995); *dpy-26(y65)* (PLENEFISCH *et al.* 1989); *bli-6(sc16); unc-22(e66).*

LG V: him-5(e1490); sdc-3(y128, y129) (DELONG *et al.* 1993); *unc-76(e911); dpy-21(e428); rol-9(sc148)* (LINK *et al.* 1992); *y249.*

LG X: egl-17(e1313, n1377) (M. J. STERN, personal communication); *unc-1(e538, e1598n1201); dpy-3(e27); lin-32(u282); unc-2(e55); unc-20(e112); unc-78(e1217); lon-2(e678); flu-2(e1003); xol-1(y9, y70)* (MILLER *et al.* 1988); *dpy-6(e14); vab-3(e648); lin-14(n179); sdc-2(y74, y93, y110* (NUSBAUM and MEYER 1989), *y202* (D. S. BERLIN and B. J. MEYER, unpublished), *y261* (C. C. AKERIB, unpublished); *sma-5(n678); lin-2(e1309); unc-9(e101); flr-1(ut11)* (KATSURA *et al.* 1994); *sem-1(n1382)* (M. J. STERN, personal communication); *unc-3(e151); sdc-1(n485)* (TRENT *et al.* 1983).

Duplications: mnDp8(X;I) (HERMAN *et al.* 1979); *mnDp57(X;I); mnDp66(X;I)* (HERMAN and KARI 1989); *yDp4(X;?); yDp5(X;?); yDp6(X;?); yDp7(X;?); yDp8(X;?); yDp9(X;?); yDp10(X;?); yDp11(X;IV); yDp12(X;f); yDp13(X;f); yDp14(X;I); yDp15(X;f); yDp16(X;f).*

Deficiencies: meDf5 X (VILLENEUVE 1994); *meDf6 X* (VILLENEUVE 1994); *yDf13 X; yDf14 X.*

Rearrangements: szT1 (X;I).

Extrachromosomal arrays: yEx68 [*sdc-2(+) rol-6(d)*] (D. BERLIN, unpublished), *yEx111* [*Pdpy-30::xol-1 unc-76(+)*] (RHIND *et al.* 1995), *yEx152* [*xol-1(+) unc-76(+)*] (J. B. KOPCZYNSKI, unpublished). Mutations not explicitly cited are described in HODGKIN *et al.* (1988), except for the new mutations described in this work.

Isolation and characterization of *yDp4*: We isolated *yDp4* from a screen in which N2 males irradiated with gamma rays were crossed with *flu-2 unc-9* hermaphrodites, and the cross progeny scored for the rare non-Unc males. The non-Unc males carry either an *X* chromosome duplication that includes *unc-9* or a dominant *unc-9* suppressor mutation, or they are patroclinous males. In screens of approximately 100,000 F_1 males, we isolated only two fertile non-Unc males that were not patroclinous. One of these males may carry a suppressor of *unc-9*, since the mutation, *y249 V*, can be made homozygous and does not complement any other *X*-linked gene tested. The other had a free duplication of *unc-9* and *unc-3*, but not *lin-2*, and was not maintained. We noticed, however, that most of the putative duplication-bearing males (*flu-2 unc-9*/O; *Dp*) that arose in this screen were infertile and slightly Dpy and appeared to be partially feminized. Many of these animals were picked to separate plates, and one of them gave rise to a few self progeny, indicating that this animal was intersexual. Its self progeny were Dpy non-Unc hermaphrodites, Unc hermaphrodites and males, and non-Unc pseudomales. The Dpy non-Unc hermaphrodites were presumed to be *flu-2 unc-9; Dp XX* animals, and were picked for further analysis. The Dpy phenotype was similar to that of diploid animals that have three *X* chromosomes (HODGKIN *et al.* 1979). Indeed, further analysis indicated that these animals are *XX* animals with a large duplication of the *X* chromosome, that we named *yDp4*. The self progeny of all these Dpy non-Unc hermaphrodites are Dpy non-Unc hermaphrodites, Unc hermaphrodites, and dead embryos, in a 2:1:1 ratio. This ratio of classes suggests that *yDp4* is stably attached to an autosome, and that the Dpy non-Unc hermaphrodites are *yDp4/+*, while the dead embryos are *yDp4/yDp4*. Because diploid animals with four *X* chromosomes are dead (HODGKIN *et al.* 1979), the death of *yDp4/yDp4* homozygotes could be due to their excess *X* chromosome dose. Rare non-Dpy non-Unc hermaphrodites arose from the self-progeny of this strain. These animals were inferred to be *yDp4/+ XO* hermaphrodites, because they invariably had a small brood with a high percentage of Unc male self-progeny. Preliminary mapping of *yDp4* showed that *yDp4* includes *flu-2* as well as *unc-9*, because the *yDp4/+; flu-2 unc-9* hermaphrodites have wild-type intestinal auto-fluorescence. *yDp4* also appears to include *lon-2*, since *yDp4/+; lon-2 unc-9* hermaphrodites are non-Lon. Further genetic mapping was hampered by the absence of fertile *yDp4* males.

Isolation, mapping and characterization of deletion derivatives of *yDp4*: We isolated deletion derivatives of *yDp4* that retain the *unc-9* portion of the duplication by gamma irradiating *yDp4/+; lon-2 unc-9 XX* hermaphrodites and screening for Lon non-Unc self-progeny. Many of these Lon non-Unc animals appeared to be *XO* hermaphrodites, since they had few progeny, most of which were Lon Unc males. These animals were inferred to be *yDp4(lon-2)/+; lon-2 unc-9*/O recombinants that had replaced the wild-type *lon-2* gene on

yDp4 with the mutant *lon-2* gene from the *X* chromosome. The remainder of the Lon non-Unc animals appeared to be *XX* animals with deletion derivatives of *yDp4*. To characterize these duplications, we assessed the fertility of duplication-bearing males, determined the genes included in the duplications and determined whether the duplications could be made homozygous in *XX* animals. *Dp/+; lon-2 unc-9/O* animals are fertile *XO* males for the duplication derivatives *yDp5*, *yDp6*, *yDp9*, *yDp11* and *yDp12* (see Figure 3). However, males carrying the largest duplications, *yDp5* and *yDp9*, are less successful at siring progeny than *yDp11* males, and *yDp11* males are less fertile than *yDp6* or *yDp12* males, which carry the smallest duplications.

Complementation tests with these duplications were done by crossing the *Dp/+; lon-2 unc-9/O* males with hermaphrodites that are homozygous for either *vab-3*, *lin-14*, *sma-5*, *flr-1* or *unc-3*, and assaying for wild-type male progeny. Because *lin-2* and *sem-1* have no male phenotype, complementation tests with *lin-2* and *sem-1* were done by crossing the *Dp/+; lon-2 unc-9/O* males with *lin-2 unc-9* or *unc-9 sem-1* hermaphrodites, and then back-crossing the non-Unc male F1 progeny with *lin-2 unc-9* or *unc-9 sem-1* hermaphrodites, respectively. If the duplication complements *lin-2* or *sem-1*, all the non-Unc hermaphrodite progeny of this cross should be non-Vul or non-Egl, respectively. *yDp5* and *yDp9* were tested for complementation of *sdc-2* by crossing *yDp5/+; lon-2 unc-9* or *yDp9/+; lon-2 unc-9* males with *szT1/xol-1(y70) sdc-2(y110) unc-9* hermaphrodites. The presence of wild-type male progeny indicated that these duplications do not include *sdc-2*. None of these duplications includes any gene tested to the right of *unc-9*, suggesting that the right endpoint of *yDp4* itself is just to the right of *unc-9*. *yDp5*, *yDp6*, *yDp7*, *yDp8*, *yDp9*, *yDp10* and *yDp11* could be made homozygous, suggesting that they are linked to an autosome. *yDp11* is attached to chromosome *IV*, since almost all of the Bli progeny of *yDp11/bli-6; lon-2 unc-9* hermaphrodites were also Unc, and almost all of the non-Unc progeny were also non-Bli. *yDp12* cannot be made homozygous and is probably free.

XO animals with *yDp7*, *yDp8* or *yDp10* are Dpy, infertile males. *yDp7*, *yDp8* and *yDp10* include *sdc-2* and *lin-14*, but not *xol-1*; for any of these duplications, putative *Dp/+; xol-1(y70) sdc-2(y110) unc-9* hermaphrodites were isolated that have no Unc hermaphrodite progeny, suggesting that the duplication complements the *sdc-2* mutation. When these hermaphrodites are crossed with wild-type males, the progeny include only Unc males but no Dpy non-Unc (*Dp/+*) males, suggesting that the duplication does not include *xol-1*. *yDp7*, *yDp8* and *yDp10* also include *lin-14(+)*, since *lin-14 (n179) sdc-2(y261)* hermaphrodites carrying smaller derivatives of these duplications are non-Lin-14, and give both Lin-14 and Dpy non-Lin-14 male progeny when crossed with wild-type males.

Comparison of the phenotype of *XX* animals homozygous for *yDp4* or its derivatives shows a strong correlation between the size of the duplication and the phenotype of the hermaphrodites. *XX* animals homozygous for *yDp4* are dead, while those homozygous for *yDp7*, *yDp8* or *yDp10* are Dpy and unhealthy, and animals homozygous for *yDp5*, *yDp6*, *yDp9*, *yDp11* or *yDp12* are wild type in phenotype. Therefore the lethality of *yDp4* is likely to be caused by an excessive *X* chromosome dose.

Isolation and mapping of duplications in the *unc-2* region of *X*: Duplications were isolated by crossing γ-irradiated N2 males with *unc-2 sdc-2(y74); yEx68* hermaphrodites and screening for non-Unc males. *yEx68* is an unstable extrachromosomal array carrying multiple copies of *sdc-2(+)* and *rol-6(d)* and was necessary for the viability of *sdc-2(y74) XX* animals. Rare non-Unc cross-progeny males may have a duplication of the *unc-2* region and be of genotype of *unc-2 sdc-2(y74)/O; Dp*. These males are crossed back with *unc-2 sdc-2(y74); yEx68* hermaphrodites. If they have a duplication that includes *unc-2*, many non-Unc male cross progeny should arise. Six free duplications of *unc-2*, including *yDp13(X;f)* and *yDp15(X;f)*, were isolated in this manner. They were isolated at a frequency of approximately 1/1000 mutagenized *X* chromosomes. All of these duplications behave genetically as free duplications, and for four of them, including *yDp13* and *yDp15*, an extra chromosomal element indicative of a free duplication was found when oocytes were stained with 4,6-diamidino-2-phenylindole (DAPI) (data not shown). To isolate duplications of *unc-2* that are missing the extreme left end of *X*, we used the same screen, except that we γ-irradiated *mnDp66; meDf5* males rather than N2 males. Therefore, any duplication of *unc-2* arising from a single break would have a left end at the *meDf5* endpoint. Four more duplications were isolated in this screen, including *yDp14(X;I)* and *yDp16(X;f)*, using the same strategy as with *yDp13* and *yDp15*.

Mapping and phenotypic analysis of *yDp13*, *yDp15*, *yDp14* and *yDp16*: To determine the phenotype of *XO* animals with these duplications in the absence of the *sdc-2* mutation, we crossed the *unc-2 sdc-2(y74); Dp* males with *unc-2* hermaphrodites. The resulting non-Unc males are *unc-2; Dp*, and their number was compared with the number of their *unc-2 sdc-2(y74)/unc-2 +; Dp* hermaphrodite siblings. Quantitative analysis of the viability of *Dp*-bearing *XO* animals was accomplished by crossing *unc-2; Dp* males with *unc-2 sdc-2(y93)* hermaphrodites, or *unc-2 sdc-2(y93); Dp* males with *unc-2* hermaphrodites, and comparing the number of non-Unc male and hermaphrodite cross progeny. We had previously tested whether *sdc-2(y93)* could suppress the *XO*-specific lethality caused by these duplications, because it is a weak allele of *sdc-2* that is not lethal to *XX* animals, and it could therefore facilitate analysis and maintenance of duplication-bearing *XX* and *XO* animals. *y93* fully suppresses the *XO*-specific lethality of *xol-1* null mutations (MILLER *et al.* 1988).

The viability of *yDp16 XO* animals was determined using an alternate method. We counted the total self-progeny broods of *him-8; dpy-3 unc-2; yDp16* hermaphrodites, and compared the percentage of wild-type male progeny to the percentage of male Dpy Unc progeny. Of 351 non-Dpy non-Unc animals, 15% were male; of 998 Dpy Unc animals, 42% were male. Since the number of *yDp16* males expected to be recovered in the absence of lethality was therefore 42% of the total duplication-bearing animals, we calculated that 24% of the expected *yDp16* males were recovered.

We mapped the extent of *yDp13*, *yDp14*, *yDp15* and *yDp16* by crossing duplication-bearing males with *unc-1 sdc-2(y93)* hermaphrodites, *dpy-3 sdc-2(y74); yEx68* hermaphrodites, *unc-20 sdc-2(y93)* hermaphrodites, *unc-78 sdc-2(y93)* hermaphrodites or *lon-2 sdc-2 (y93)* hermaphrodites, and assaying for wild-type male progeny. *yDp16* was not tested with *unc-78* or *lon-2*. *yDp13* and *yDp15* include *unc-78* but not *lon-2* and most likely extend to the left end of *X*, since they balance *meDf5* and *meDf6* homozygous animals. *yDp14* includes *unc-78* but not *lon-2*, and *yDp14* and *yDp16* most likely end at the *meDf5* breakpoint.

yDp14 was inferred to be attached to chromosome *I* because of its tight linkage to *mnDp66(X;I)*. When the rare *yDp14/mnDp66; unc-1 dpy-3* survivor males are crossed with *unc-1 dpy-3* hermaphrodites, only Unc (*yDp14/+*) or Dpy (*mnDp66/+*) cross progeny result ($n > 1000$), but when these males are crossed with *yDp14; unc-1 dpy-3* or *mnDp66; unc-1 dpy-3* hermaphrodites, numerous wild-type (*yDp14/mnDp66*) progeny result. These results suggest that the absence of progeny with both duplications in the first cross is

due to the segregation of these duplications away from each other.

A spontaneous derivative of *yDp14*, called *yDp14(y280)*, arose in the construction of the strain *yDp14/+; him-8; dpy-3 unc-2*. *yDp14(y280)*, like *yDp14*, includes *dpy-3*, *unc-2*, *unc-20* and *unc-78*, but it does not cause *X*O-specific lethality. *y280*, the mutation or deletion that suppresses the *X*O-specific lethality of *yDp14* in this strain, appears to be associated with the duplication itself, because the suppressor is dominant, and all the duplication-bearing F1 males are rescued when *yDp14(y280)/+* males are crossed with marked hermaphrodites.

Screen for *X*-linked or dominant suppressors of the *X*O-specific lethality caused by *mnDp66/yDp14*: *yDp14/+; him-8; unc-2* males were crossed with EMS-mutagenized *mnDp66; unc-1 dpy-3* hermaphrodites. Wild-type male progeny must be *mnDp66/yDp14*, and may have an *X*-linked or dominant suppressor of the *mnDp66/yDp14 X*O-specific lethality. They were individually crossed with *mnDp66; him-8; unc-1 dpy-3* hermaphrodites to obtain more males with the mutation and to maintain the strain. Suppressed males were tested for *X*-chromosome linkage by crossing them with *mnDp66; unc-1 dpy-3* and observing if they gave only wild-type hermaphrodite but no wild-type male cross progeny. A preliminary screen of 7000 F1 *mnDp66/yDp14 X*O animals yielded five *X*-linked suppressor mutations; four new mutations in *sdc-2*, and a small deficiency of the left end of *X*. Complementation tests with *sdc-2* were done by mating individual *mnDp66/yDp14; him-8; unc-1 dpy-3 sup* males with *unc-22; sdc-2(y202ts)* hermaphrodites, and assaying for the presence of male but not hermaphrodite cross progeny. The one suppressor that complemented *sdc-2* was mapped by crossing suppressed *mnDp66/yDp14* males with *egl-17(e1313) unc-1 unc-2* hermaphrodites and picking wild-type hermaphrodite cross progeny, which must be *mnDp66/+; him-8/+; sup unc-1 dpy-3 +/+ egl-17 unc-1 + unc-2*. These hermaphrodites had a dominant Him phenotype, suggesting that the suppressor is a deficiency of the left end of *X*. This deficiency, called *yDf13*, removes *egl-17*, since *yDp14/+; yDf13 unc-1 dpy-3/egl-17(n1377) dpy-3* hermaphrodites are Egl. The progeny of *mnDp66/yDp14; him-8; yDf13 unc-1 dpy-3* hermaphrodites include wild-type hermaphrodites and males as well as Dpy hermaphrodites and males, but no Unc (*yDp14; yDf13 unc-1 dpy-3*) animals, suggesting that *yDf13*, like *meDf5*, is homozygous lethal, but is balanced by *mnDp66*.

Analysis of animals homozygous for *X*-chromosome duplications: No male progeny arose from the strains *yDp14; him-8; unc-2* and *yDp14(y280); him-8; dpy-3 unc-2*. These hermaphrodites were inferred to have the *him-8* mutation because they arose as the self-progeny of *yDp14/+; him-8; unc-2* or *yDp14(y280)/+; him-8; dpy-3 unc-2* mothers. All the progeny of these mothers that were heterozygous for the duplication continued to give rise to males, while all the progeny that were homozygous for the duplication (characterized by the absence of Unc or Dpy Unc progeny) failed to give rise to males.

Effect of sex-determination and dosage-compensation mutations on duplication-bearing XO animals: The ability of *yEx111* or *yEx152* to suppress the lethality of duplication-bearing *X*O animals was assayed by crossing N2, *unc-76; yEx111* or *unc-76; yEx152* males with *unc-2; Dp* hermaphrodites, and examining the cross progeny for phenotypically wild-type males. Suppression by *sdc-1* was assayed by crossing duplication-bearing males with *unc-2 sdc-1* hermaphrodites, and examining the phenotype of the non-Unc males. The Unc non-Sdc hermaphrodite progeny of this cross (*unc-2 sdc-1/ unc-2 +*) were crossed with *unc-2; Dp* males to determine whether *sdc-1* must be homozygous in the mother to rescue the duplication-bearing male progeny. Suppression by *sdc-3(y129)* was assayed by crossing *yDp14/+; sdc-3(y129); unc-2* males with *sdc-3(y129); unc-2* hermaphrodites, and observing numerous wild-type male progeny. The phenotype of duplication-bearing XO animals in dosage compensation *dpy* mutant backgrounds was determined using the same scheme for all the combinations tested. For instance, to build *yDp14/+; dpy-28; unc-2*, we crossed *yDp14/+; unc-2* males with *dpy-28; unc-2* hermaphrodites. The non-Unc males (*yDp14/+; dpy-28/+; unc-2*/O) were crossed back with *dpy-28; unc-2* hermaphrodites. Individual non-Unc males that were either *yDp14/+; dpy-28; unc-2*/O or *yDp14/+; dpy-28/+; unc-2*/O were separately crossed back with *dpy-28; unc-2* hermaphrodites. Males that gave no Unc non-Dpy hermaphrodite progeny in this cross were inferred to be homozygous for the *dpy-28* mutation. All the non-Dpy non-Unc progeny from such a cross are *yDp14/+ X*O animals, and we counted the number of these animals that were hermaphrodites, intersexes, and males, based on their dissecting-microscope phenotype.

Isolation, mapping and characterization of deficiencies of the left end of *X*: Since the phenotype of *meDf6/+ XX* animals suggests that *meDf6* does not remove all the signal elements in this region of *X*, we mapped the right endpoint of *meDf6* with respect to *lin-32* to further localize the sex-determination signal element(s) in the *dpy-3* to *unc-2* interval. *yDp14/+; lin-32* males were crossed with *meDf6/dpy-3 unc-2* hermaphrodites. One fourth of the *XX* cross progeny should be *meDf6/lin-32* hermaphrodites, and they should have a Lin-32 phenotype if *meDf6* deletes *lin-32*. Indeed, many of the cross-progeny hermaphrodites had a posterior Mec phenotype, were somewhat Egl, and appeared indistinguishable from *lin-32(u282)* homozygotes. All the progeny of these hermaphrodites were Mec and some were Lin-32 males, further verifying that the F1 animals were *lin-32/meDf6*, and that *meDf6* deletes *lin-32*.

Larger deficiencies of the left end of *X* were isolated in an *unc-2* non-complementation screen. *xol-1(y9); yEx152 X*O males were mutagenized with gamma rays and crossed with *unc-2 xol-1(y9) dpy-6* hermaphrodites. Unc non-Dpy hermaphrodites were picked to test if they carried a deficiency of *unc-2*. Two of these Unc non-Dpy hermaphrodites had deficiencies of the entire left end of *X*, based on the following criteria. All their Unc non-Dpy progeny segregated Unc Dpy hermaphrodites and dead eggs, indicating a homozygous-lethal phenotype. Both deficiencies failed to complement *egl-17, dpy-3* and *unc-20*. In addition, *Df xol-1/++* animals were Him, a characteristic phenotype caused by deficiencies of the left end of *X* (Villeneuve 1994). *yDf14* is one of the two deficiencies that arose in this screen. In order to study the deficiencies in a *xol-1(+)* background, we needed to isolate recombinants that had lost the *xol-1(y9)* mutation from the deficiency chromosome. We did not know what phenotype to expect of these recombinants, so we instead isolated new deficiencies of *unc-2* in a *xol-1/+* background, taking advantage of our knowledge that such deficiency heterozygotes should be viable. Deficiencies isolated on a *xol-1(+)* chromosome can then easily be tested for the phenotypes they cause in a *xol-1(+)* background.

To obtain the new deficiencies, we used the same screen as above, except we crossed mutagenized N2 males with the *unc-2 xol-1 dpy-6* hermaphrodites. Four Unc non-Dpy hermaphrodites were isolated that have a deficiency of the left end of *X*. All four cause a slightly Dpy and Egl phenotype in *XX* heterozygotes. We tested their phenotype in a *xol-1(+)* background by crossing *lon-2* males with the *Df ++/unc-2 xol-1 dpy-6* hermaphrodites, and we found that many of the progeny

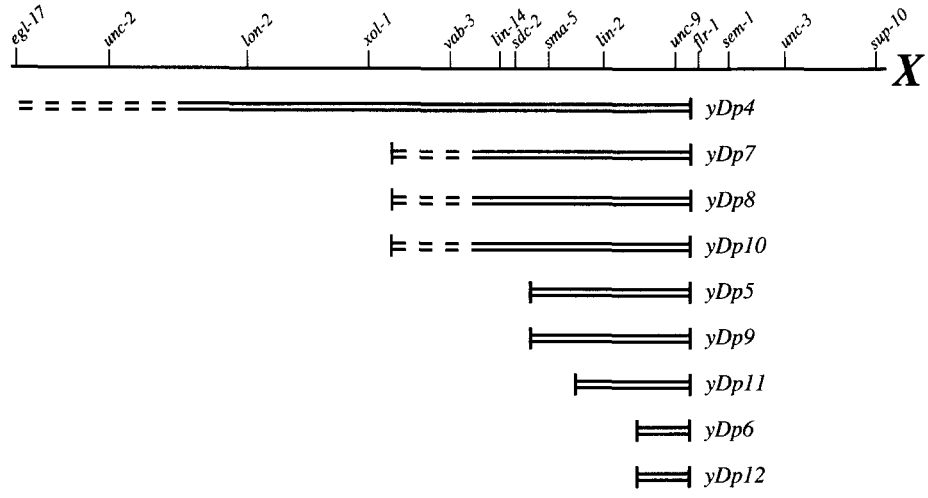


FIGURE 3.—Genetic map of the *X* chromosome indicating the extent of *yDp4* and its derivatives. The genes used to map these duplications, *lon-2, xol-1, vab-3, lin-14, sdc-2, sma-5, lin-2, unc-9, flr-1, sem-1* and *unc-3* are shown. Additional genes are indicated for easier comparison to the maps in Figures 2 and 4. The known extent of the duplications is indicated by solid double lines. The dotted double lines indicate possible further extension of a duplication, and the vertical bars indicate their furthest possible extent. All these duplications except *yDp12* are attached to an autosome. *yDp11* was mapped to chromosome *IV*; the autosomal linkage group of the other duplications was not determined.

of this cross were Dpy or Dpy Tra hermaphrodites. These animals were selfed individually, and all were found to be *Df+/+ lon-2*. In contrast, all the wild-type progeny of this cross were *unc-2 + xol-1 dpy-6/+ lon-2 + +*. We progeny-tested 16 wild-type animals from each cross and in each case all 16 animals lacked the deficiency. The phenotype of the *Df +/+ lon-2* hermaphrodites was variable; some animals were Dpy Tra and infertile, and the majority were Dpy and Egl. Progeny counts on three of these deficiencies in the *Df+/+ lon-2* background showed that the *Df/+* animals were almost twice as abundant as their *lon-2* siblings, suggesting that there was little, if any, dominant lethality. These three deficiencies appear to extend to the left end of *X* on the basis of their failure to complement *unc-1*.

The phenotype of the new deficiencies in a *xol-1(+)* background suggests that the phenotype of the *yDf14/+* recombinants may also be Dpy or Dpy Tra. Indeed, such animals arose among the self progeny of *yDf14 + xol-1/+ lon-2 +* hermaphrodites. We selfed several such Dpy animals individually and demonstrated in the following series of experiments that they carried a *yDf14 +* recombinant chromosome. We isolated Dpy Unc (*yDf14/unc-2*) hermaphrodites among the progeny of *mnDp57/+; unc-2* males crossed with the putative *yDf14 +/+ lon-2* hermaphrodites. These Dpy Unc animals had Dpy Unc, Dpy Unc Tra, and Unc hermaphrodite progeny, as well as Unc male progeny. The presence of male progeny provided an independent proof that the deficiency was still present. The Dpy Unc hermaphrodites were crossed with *lon-2 xol-1(y9); yEx152* males, and slightly Dpy non-Unc (*yDf14 ++/+ lon-2 xol-1*) progeny arose. These hermaphrodites had the same phenotype as the *yDf14 + xol-1/+ lon-2 +* hermaphrodites. We further confirmed that the *yDf14* chromosome carried a wild-type copy of *xol-1* because these *yDf14 ++/+ lon-2 xol-1* animals initially segregated no males, but a few wild-type and Lon male recombinants arose while maintaining this strain. In the absence of recombination, all the *X*O animals should be dead, because a *xol-1* mutation is in *trans* to the deficiency.

All the deficiencies isolated appear to extend farther rightward than *yDp13* or *yDp15*. We crossed *unc-1; yDp13* or *unc-1; yDp15* males with *Df/unc-1* or *Df/unc-2* hermaphrodites, and expected that wild-type male progeny would arise if the *Df; Dp* males were viable. In every cross there were numerous cross progeny, none of which were wild-type males. Since *meDf6; yDp13* and *meDf6; yDp15* males appear wild type, the most likely explanation for the absence of wild-type males with these larger deficiencies is that the deficiencies extend farther to the right than the duplications, so that the duplications cannot balance deficiency homozygous or hemizygous animals.

Assaying the effect of duplications on expression of a *xol-1* reporter gene: The strains *yDp14; him-8 yIs2; unc-2* and *mnDp66; him-8 yIs2; unc-1* were stained for β-galactosidase activity concurrently with the control strains *unc-24 yIs2* and *him-8 yIs2*. To ensure statistical significance, total animals from at least two 5-cm plates with worms at high density but not starved, were stained for each genotype tested. We modified FIRE *et al.*'s (1990) protocol, in that we incubated worms in approximately 1 mg/ml levamisole in M9 for 30 min prior to drying them. This treatment causes hermaphrodites to contract and thereby release their embryos. Worms were stained for approximately 20 hr using an X-gal concentration of 0.012%. These experiments were repeated several times with different concentrations of X-gal and time of staining. The *yDp14*-containing strain consistently resembled the *him-8* control strain, while the *mnDp66*-containing strain consistently stained less than the *him-8* control strain.

RESULTS

Duplications of *unc-9* confer no sex-specific phenotypes: Our goal was to identify regions of *X* that contain strong components of the sex-determination signal, using the criterion that duplications of these regions should be deleterious to *X*O males. We initially isolated duplications of the *unc-9* region because this region had not been well characterized with regard to duplications or deficiencies. Using HERMAN *et al.*'s (1976) screen for *X* chromosome duplications in *X*O animals, we isolated the attached duplication, *yDp4*, as a *yDp4/+; unc-9/*O *X*O animal that was a non-Unc, slightly Dpy, self-fertile intersex (see MATERIALS AND METHODS). We subsequently determined that *yDp4/+ XX* animals are Dpy hermaphrodites. The Dpy phenotype is most likely a consequence of the large size of *yDp4* (Figure 3), which causes the *XX yDp4/+* animals to resemble *3X/2A* animals. Although *yDp4* is too large to be informative about the *unc-9* region because it encompasses almost the entire *X* chromosome, it proved useful for isolating smaller duplications of this region as deletion derivatives in *XX* animals.

TABLE 1

yDp5* and *yDp9* do not affect the viability of *XO* animals, either alone or in combination with *mnDp57

Parental genotypes		No. of cross progeny			Male viability	
Male	Hermaphrodite	*Dp/+ XX*[a]	*Dp/+ XO*[b]	*mnDp57/+; Dp/+ XO*[b]	Percent viable *Dp/+ XO*[c]	Percent viable *mnDp57/+; Dp/+ XO*[d]
N2	*yDp5; lon-2 unc-9*	795	804		101	
N2	*yDp9; lon-2 unc-9*	605	605		100	
mnDp57/+; unc-2	*yDp5; lon-2 unc-9*	563	545	289	97	112
mnDp57/+; unc-2	*yDp9; lon-2 unc-9*	565	553	280	98	102

[a] All *XX* cross progeny are *yDp5/+* or *yDp9/+*, and are non-Lon non-Unc hermaphrodites (*lon-2 unc-9/++* or *+ lon-2 unc-9/unc-2 ++*). They are distinguishable from *XX* self progeny, which are Lon non-Unc hermaphrodites.

[b] All *XO* cross progeny are *yDp5/+* or *yDp9/+* and *lon-2 unc-9/O*. They are Lon non-Unc males in the absence of *mnDp57*, or non-Lon non-Unc males in the presence of *mnDp57/+*. The numbers in the *Dp/+* column indicate total males with *yDp5* or *yDp9*, and the numbers in the *mnDp57/+; Dp/+* column indicate only those males with both duplications.

[c] Calculated as (total males)/(cross hermaphrodites) × 100.

[d] Calculated as (non-Lon males)/(Lon males) × 100.

These derivatives allowed us to assess whether the *unc-9* region harbors sex-determination signal elements by assaying whether these smaller duplications cause lethality of *XO* animals.

From *yDp4/+; lon-2 unc-9 XX* hermaphrodites, we isolated deletion derivatives of *yDp4* that still include *unc-9(+)* and have new left breakpoints between *lon-2* and *unc-9* (see MATERIALS AND METHODS and Figure 3). With the exception of *yDp12 (X;f)*, each of the duplications is attached to an autosome and can be made homozygous. For five of these duplications, *yDp5, yDp6, yDp9, yDp11* and *yDp12* (Figure 3), the duplication-bearing *XO* animals are fertile males. To determine the effect of duplicating this region in *XO* animals, we took the two largest duplications, *yDp5* and *yDp9*, and compared the viability of *yDp5/+* and *yDp9/+ XO* males with that of their duplication-bearing hermaphrodite siblings. The results of this comparison show that there is no karyotype-specific effect of these duplications on viability (Table 1). The observation that *yDp5/+* and *yDp9/+ XO* animals are fully viable suggests that the *sma-5* to *unc-9* interval does not include strong components of the sex-determination signal.

For the other three duplication derivatives, *yDp7, yDp8* and *yDp10*, both *XX* and *XO* animals with one copy of the duplication are slightly Dpy, and males are rarely, if ever fertile. The phenotypes caused by *yDp7, yDp8* and *yDp10* suggest that these duplications include a gene or genes with deleterious effects on both *XX* and *XO* animals at increased dose. The duplications do not appear to affect *XO* animals specifically, as might be expected if they act solely by increasing the dose of sex-determination signal elements. While these three duplications include *sdc-2*, duplication of *sdc-2* is unlikely to be solely responsible for the Dpy phenotype, since *XX* animals with extrachromosomal arrays of *sdc-2* are wild type (D. S. BERLIN, unpublished).

Our analysis of the *unc-9* region therefore fails to show that its dose has any importance for the sex-determination signal. However, we have not excluded the possibility that the dose of the *unc-9* region contributes weakly to the signal. One test of a possible weak contribution is to examine *XO* animals with *yDp5* or *yDp9* and additional *X*-chromosome duplications for any apparent feminization or death. We observed no difference in the viability or phenotype of *XO* animals with *mnDp57* and *yDp5* or *yDp9*, compared to *XO* animals with *yDp5* or *yDp9* alone (Table 1), suggesting that these combinations of duplications do not strongly affect the sex-determination signal. One experiment, however, does suggest that this region may contribute weakly to the signal. *yDp9* causes additional *XO*-specific lethality in combination with a newly isolated duplication, *yDp14*, as we will describe.

An approach to isolate duplications that may affect the sex-determination signal in XO animals: We devised a method of isolating *X* chromosome duplications in *XO* animals regardless of their potential effect on the *X*/A ratio. We modified HERMAN *et al.*'s (1976) method of isolating duplications in males by using an *sdc-2* mutant background, in which all animals are locked into the *XO* fate of sex determination and dosage compensation, regardless of karyotype. Just as *xol-1* mutant *XO* animals are rescued by loss-of-function mutations in an *sdc* gene, mutations that increase the *X*/A ratio of an *XO* animal should also be suppressed by loss of downstream *sdc* activity. This scheme allows us to isolate duplications of targeted regions of *X* that may or may not affect the sex-determination signal in males. If strong signal elements exist in the targeted region, duplications of the region should kill *XO* males in an *sdc(+)* background.

We initially chose the left end of the *X* chromosome for this duplication screen. Our selection of this region was motivated by an observation made by J. HODGKIN and D. ALBERTSON (unpublished results) that a new *XO*-specific lethal mutation they had identified was associated with a large inverted duplication of the left end of *X*. The *XO*-specific lethal phenotype could be due to an effect of the duplication itself on the sex-determination signal. Alternatively, the phenotype could be due to a

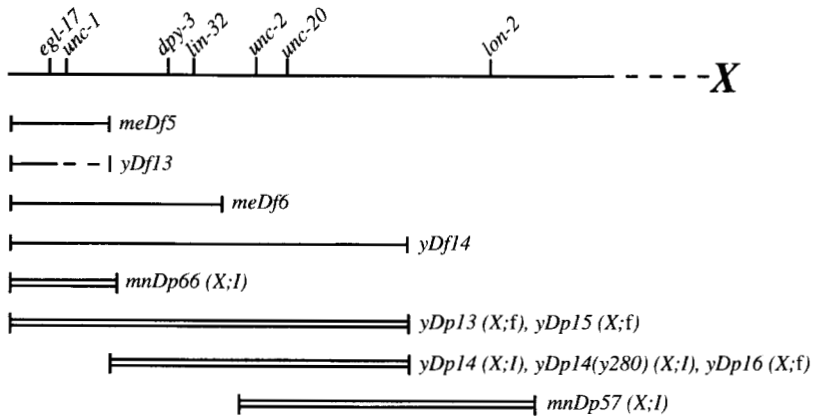


FIGURE 4.—Genetic map of the left end of *X*, indicating the extent of duplications and deficiencies analyzed herein. The remainder of the chromosome is indicated by the dotted line. Duplications are shown as double lines, and deficiencies as single lines. Because *yDf13* was induced on an *unc-1 dpy-3* chromosome, we could not determine if *yDf13* includes *unc-1*, so a dotted line was used to indicate its possible extent. For each duplication or deficiency, vertical bars indicate their furthest possible extent.

mutation that is linked to the duplication chromosome. To test whether this region does include sex-determination signal elements that cause *X*O-specific lethality in two doses, we screened for duplications of this region and then tested which, if any, of these duplications cause *X*O-specific lethality in the absence of the suppressor mutation.

Duplications of the left end of *X* cause *X*O-specific lethality: We isolated six duplications that include the *unc-2* gene (see Figure 4) in *sdc-2* mutant males, all of which cause *X*O-specific lethality in an *sdc-2*(+) background (see MATERIALS AND METHODS). We examined the effect of two of these duplications, *yDp13 (X;f)* and *yDp15 (X;f)* (Figure 4) on the viability of *X*O animals by comparing the recovery and phenotype of duplication-bearing *X*O animals with or without an *sdc-2* partial loss-of-function mutation. The data presented in Table 2 indicate that *yDp13* or *yDp15* *X*O animals in an *unc-2 sdc-2* strain are completely wild type. However, only 6% of the expected *yDp13* and 10% of the expected *yDp15* *X*O males are recovered in an *unc-2 sdc-2*(+) background, and they are small, thin, slow growing, but fertile. The missing *yDp13* and *yDp15* *X*O animals are most likely dead, rather than transformed into hermaphrodites, since we saw some arrested or dead L1 larvae among the cross progeny of animals with these duplications. The most compelling evidence suggesting that duplications of this region kill rather than transform *X*O animals comes from a quantitative analysis of *yDp14*, an attached duplication of part of this region described later in this paper. The fact that all six duplications of *unc-2* cause an *X*O-specific lethal phenotype suggests that *X*O-specific lethality is an inherent consequence of duplicating this region of the *X* chromosome. Furthermore, the clear rescue of the duplication-bearing *X*O animals by an *sdc-2* mutation indicates that these duplications affect the sex-determination decision of *X*O animals and act upstream of *sdc-2*. These properties suggest that this region includes one or more sex-determination signal elements, which when duplicated in *X*O animals, cause a shift in their sex-determination signal toward the *XX* setting.

TABLE 2

Duplications of the left end of *X* cause *X*O-specific lethality

Genotype of *X*O animals	Percent males recovered[a]	*n*[b]
unc-2; yDp13	14[c]	98
unc-2 sdc-2(y93); yDp13	234[d,e]	77
unc-2; yDp15	10[c]	78
unc-2 sdc-2(y93); yDp15	98[d]	85
yDp14/+; unc-2	38[f]	814
yDp14/+; unc-2 sdc-2(y93)	111[d]	913

[a] Percent males recovered was calculated as the (number of *Dp* males)/(number of *Dp* hermaphrodite siblings) × 100, using the progeny of the following crosses: To determine the relative recovery of *unc-2; Dp* males, *unc-2 sdc-2(y93); Dp* males were crossed with *unc-2* hermaphrodites. To determine the relative recovery of *unc-2 sdc-2(y93); Dp* males, *unc-2; Dp* males were crossed with *unc-2 sdc-2(y93)* hermaphrodites. All non-Unc progeny were counted.

[b] *n* is the total number of duplication-bearing males and hermaphrodites counted.

[c] These males are small, thin, slow growing, but fertile.

[d] These males are completely wild type.

[e] Approximately 2.3 times as many *unc-2 sdc-2(y93); yDp13* males were recovered compared to *unc-2 sdc-2(y93)/unc-2 +; yDp13* hermaphrodites, suggesting that *yDp13*, like many other free duplications, segregates away from the single *X* chromosome in male meioses and is therefore more frequently present in nullo-*X* sperm (DELONG *et al.* 1987; HERMAN *et al.* 1979; MCKIM and ROSE 1990). If we apply this distortion factor of 2.3 to the *yDp13* males recovered in the *unc-2* background, we calculate that the relative viability of *yDp13* males in this *sdc-2*(+) background is only 6% (instead of 14%) that of the *yDp13* hermaphrodites.

[f] These males are variable, ranging from wild type to small, thin, slow-growing, but fertile.

At least two signal elements contribute to the *X*O-specific lethal phenotype of *yDp13* or *yDp15*: All six of the duplications of *unc-2* that were isolated are easily lost through recombination with the *X* chromosome. They proved to be impossible to maintain in *XX* animals that have an intact *X* chromosome. This type of behavior was previously seen with other duplications that include the left end of *X* (HERMAN and KARI 1989), and is probably due to the presence of the meiotic chromosome pairing center at the left end of *X* (VILLENEUVE 1994). To study this region further, we wanted to isolate new duplications that are missing the pairing center but that still may affect the *X*/A ratio. Numerous duplications and deficiencies of the extreme left end of *X* had been isolated

TABLE 3

yDp14/+ XO animals are dead, rather than transformed into hermaphrodites

Parental genotypes		No. of progeny[a]			
Male	Hermaphrodite	*yDp14/+*[b] *XX*	*yDp14/+*[b] *XO*	*+/+*[b] *XX*	*+/+*[b] *XO*
yDp14/+; unc-2 sdc-2(y93)	*dpy-3 unc-2*	694 (94%)	109 (15%)	736 (100%)	654 (89%)

[a] Total cross progeny were counted; in parentheses are shown the percentage of each class relative to the number of *XX* cross progeny without *yDp14*. All four classes should be equally represented.

[b] *yDp14/+* or *+/+* refer only to the presence or absence of *yDp14*. Complete genotypes are *yDp14/+; + unc-2 sdc-2/dpy-3 unc-2 + XX* or *yDp14/+; dpy-3 unc-2/O XO* or *+ unc-2 sdc-2/dpy-3 unc-2 + XX* or *dpy-3 unc-2/O XO*. *yDp14/+ XX* cross progeny are non-Unc non-Dpy hermaphrodites. *yDp14/+ XO* cross progeny are non-Unc non-Dpy males. *+/+ XX* cross progeny are Unc non-Dpy hermaphrodites. *+/+ XO* cross progeny are Unc Dpy males. Self progeny are Unc Dpy hermaphrodites and were not counted.

previously (HERMAN and KARI 1989; VILLENEUVE 1994) and none of them had any apparent effect on the sex-determination signal. Therefore, we reasoned that the increased dose of a more internal region, near *unc-2*, was likely to be primarily responsible for the strong effects of our duplications, and we isolated new duplications of *unc-2* that were missing the left end of *X*.

To isolate such duplications, we used the same screen but started with a deficiency-*X* chromosome (*meDf5*, Figure 4) (MATERIALS AND METHODS). Both duplications that were characterized from this screen, the attached duplication *yDp14(X;I)* and the free duplication *yDp16*, can be maintained in *XX* animals without noticeable loss of the duplication by recombination. Results shown in Table 2 indicate that *yDp14* causes *XO*-specific lethality. Only 38% of the expected number of *yDp14/+ XO* animals are recovered in an *unc-2 sdc-2(+)* background. This recovery is in the same range as that of *yDp16 XO* animals (24% recovery in a *him-8; dpy-3 unc-2* background, see MATERIALS AND METHODS). Both *yDp14/+* and *yDp16 XO* animals are variable in phenotype, ranging from nearly wild type to small, thin, slow growing, but fertile males. *yDp14/+ XO* animals are completely rescued by an *sdc-2* mutation (Table 2), suggesting that the phenotype of *yDp14/+ XO* animals is due to a defect early in the sex-determination pathway. *yDp16 XO* animals also appear wild type in the *sdc-2* mutant background.

We established that the inability to recover the expected number of males with *yDp14* is due to *XO*-specific lethality, rather than to transformation of *XO* animals into hermaphrodites. In the cross shown in Table 3, we expected equal numbers of cross progeny with one copy of *yDp14* and with no copies of *yDp14*. We observed only 15% of the expected number of males with *yDp14*, but the number of hermaphrodites with *yDp14* was equal to the number of cross-progeny hermaphrodites without *yDp14*. Therefore, the missing *yDp14/+ XO* animals must be dead rather than sexually transformed. Most *yDp14/+ XO* animals arrest as L1 larvae. The *XO* phenotype of *yDp14/+* is similar to, but less severe than that of *xol-1*. 100% of *xol-1* mutant *XO* animals are dead and arrest variably as embryos or L1 larvae (MILLER *et al.* 1988).

As the previous sets of experiments demonstrate, the percent viability of *XO* animals with these duplications varies among different strain backgrounds. Only 15% of *yDp14/+* males were recovered in the *dpy-3 unc-2* background (Table 3), compared to 38% in the *unc-2* background (Table 2). We observed similar variability in numerous other experiments with all these duplications; for any of them, the duplication-bearing males were more viable in an *unc-2* background than in a *dpy-3* or *unc-1* background. Therefore, whenever possible, we compared the viability of *XO* animals with different duplications in the same strain background.

What accounts for the difference in viability between *yDp14/+ XO* animals and *yDp13* or *yDp15 XO* animals? The most obvious difference between these duplications is that *yDp14* is missing the left end of *X*. If duplication of the very end of *X* is responsible for this phenotypic difference, we should be able to reconstruct the phenotype of *yDp13* or *yDp15* by examining animals with one copy of *yDp14* and a second duplication that includes only the left end of *X*. We tested the combination of *yDp14* and *mnDp66*, because these two duplications together include approximately the same region of *X* as *yDp13* or *yDp15* (see Figure 4). *mnDp66* and *yDp14* do indeed cause synergistic *XO*-specific lethality. Only 1.7% of *mnDp66/yDp14* males are viable, compared to 12% of *yDp14/+* males and 100% of *mnDp66/+* males in the same strain background (Table 4). The sevenfold lower viability of *mnDp66/yDp14* males compared to *yDp14/+* males in the same strain background is similar in magnitude to the sixfold lower viability of *unc-2; yDp13 XO* animals compared to *yDp14/+; unc-2 XO* animals (Table 2). Therefore, the *XO*-specific lethality caused by the combination of *mnDp66* and *yDp14* is similar in severity to that caused by *yDp13* or *yDp15*.

The synergistic *XO*-specific lethality caused by *mnDp66* and *yDp14* can be explained by two different models. In the first model, *mnDp66* and *yDp14* both include sex-determination signal elements, and their synergistic lethal effect is due to the increased dose of

TABLE 4

***yDp14* and *mnDp66* cause synergistic XO-specific lethality**

Parental genotypes		Percent viability of males			
Male	Hermaphrodite	*yDp14/+* XO[a]	*mnDp66/+* XO[b]	*mnDp66/yDp14* XO[c]	*n*[d]
N2	*yDp14; unc-1 dpy-3*[e]	12			1099
yDp14/+; dpy-3 sdc-2(y74)	*mnDp66; unc-1 dpy-3*[e]		102	1.7	1847

[a] Percent viability of *yDp14/+* males was calculated as (total Unc non-Dpy (*yDp14/+; unc-1 dpy-3*) males)/(total non-Unc non-Dpy (*yDp14/+; unc-1 dpy-3/++*) hermaphrodites) × 100.

[b] Percent viability of *mnDp66/+* males was calculated as the (total Dpy (*mnDp66/+; unc-1 dpy-3*) males)/[total non-Dpy hermaphrodites (*yDp14/mnDp66; unc-1 dpy-3 +/+ dpy-3 sdc-2*)] × 100.

[c] Percent viability of *mnDp66/yDp14* males was calculated as the (total non-Unc non-Dpy (*mnDp66/yDp14; unc-1 dpy-3*) males)/[total non-Dpy hermaphrodites (*yDp14/mnDp66; unc-1 dpy-3 +/+ dpy-3 sdc-2*)] × 100.

[d] *n* for the first cross is the total live cross progeny. *n* for the second cross is the total live cross progeny except the *mnDp66/+* hermaphrodites, because this class was indistinguishable from the self progeny.

[e] *unc-1(e1598n1201)*.

both sets of elements. However, the results shown in Table 4 demonstrate that *X*O animals with one copy of *mnDp66* are fully viable. Therefore, it was possible that *mnDp66* contributes non-specifically to the lethality of *yDp14/+* *X*O animals, and includes no sex-determination signal elements itself. If the increased lethality of *mnDp66/yDp14* *X*O animals compared to *yDp14/+* *X*O animals is due only to an effect on the sex-determination signal, and not to non-specific effects, we expect that this lethality should be suppressed by an *sdc-2* mutation. Indeed, we found that *mnDp66/yDp14; sdc-2* *X*O animals are wild-type males. In fact, we isolated four new *sdc-2* mutations in a pilot screen for *X*-linked suppressors of the *X*O-specific lethality of *mnDp66/yDp14* (see MATERIALS AND METHODS). These results suggest that the increased dose of more than one signal element contributes to the *X*O-specific lethality caused by the large duplications *yDp13* and *yDp15*.

***yDp13* and *yDp15* XO males are partially rescued by the small *X* chromosome deficiency *meDf5*:** To confirm that more than one signal element contributes to the *X*O-specific lethality of *yDp13* and *yDp15*, we assayed whether *meDf5* could partially rescue the *X*O-specific lethality caused by *yDp13* or *yDp15* (see Figure 4), by analyzing the self progeny of *meDf5; yDp13* and *meDf5; yDp15* hermaphrodites. Hermaphrodites homozygous for *meDf5* have a strong Him phenotype; approximately 32% of the self-progeny of *mnDp66 (X;I); meDf5* hermaphrodites are males (VILLENEUVE 1994). Since we do not expect the percentage of males among live progeny in these strains to be altered by a free duplication compared to an attached duplication, we could determine the viability of *meDf5; yDp13* or *meDf5; yDp15* males by comparing the percentage of males in the self-progeny broods of *meDf5; yDp13* and *meDf5; yDp15* with that of *mnDp66; meDf5* hermaphrodites. The number of *meDf5; yDp13* males corresponds to 38% of the expected number, and that of *meDf5; yDp15* males corresponds to 29% of the expected number of males compared to *mnDp66; meDf5* (Table 5). For both *yDp13* and *yDp15*, these males are variable in phenotype, with some appearing close to wild type in size and growth rate. Thus, *meDf5; yDp13* and *meDf5; yDp15* *X*O males are similar to *yDp14/+; unc-2* males in viability and phenotype, as expected from the fact that the major difference in the extent of these duplications is the presence or absence of the *meDf5* region. A further confirmation that the dose of the *meDf5* region partially contributes to the *X*O-specific lethal effect of these duplications is our isolation of a new deficiency of this region, *yDf13* (see Figure 4), as a suppressor of the *X*O-specific lethality caused by *mnDp66/yDp14* (see MATERIALS AND METHODS). These results confirm that the dose of the extreme left end of *X*, defined by *meDf5* or *mnDp66*, contributes to the *X*/A ratio.

TABLE 5

meDf5* and *meDf6* suppress the XO-specific lethality caused by *yDp13* and *yDp15

Genotype	Percent males	*n*[a]	Percent viability of males[b]
mnDp66; meDf5[c]	32	7289	100
meDf5; yDp13	15	400	38
meDf5; yDp15	12	294	29
meDf6; yDp13[d]	27	622	79
meDf6; yDp15[d]	23	287	65

[a] *n* is the number of live progeny counted. For *yDp13* and *yDp15* strains, only approximately 50% of the progeny were viable, due to loss of the duplication. Total self-progeny broods were scored. Percent males is the percentage of total live self progeny that were male.

[b] Male viability was calculated as the ratio of the number of males observed to the number of males expected if the strain behaves like *mnDp66; meDf5*.

[c] Data from VILLENEUVE (1994).

[d] In this experiment, we could not compare the percentage of self-progeny males with that of a *meDf6* homozygous reference strain, because *meDf6* is homozygous lethal and no attached duplications are known that balance this deficiency. Because the percent self-progeny males of *meDf6/+* is similar to that of *meDf5/+* (VILLENEUVE 1994), we calculated male viability using the *mnDp66; meDf5* strain as a reference.

***yDp14* may include more than one sex-determination signal element:** To begin to delineate the region(s) within *yDp14* whose dose contributes to the *X*O-specific

lethality, we determined whether *meDf6*, which partially overlaps in extent with the region covered by *yDp14* (see Figure 4), can suppress the *X*O-specific lethality of *yDp13* or *yDp15* more strongly than *meDf5*. If the dose of the region between the right endpoints of *meDf5* and *meDf6* contributes to the *X*O-specific lethal effect of these duplications, the phenotype of the *meDf6; Dp* XO animals should be less severe than that of the *meDf5; Dp* *X*O animals. We examined the self-progeny broods of *meDf6; yDp13* and *meDf6; yDp15* hermaphrodites, and found that for *meDf6; yDp13*, 27% of the live self progeny are male, while for *meDf6; yDp15*, 23% of the live self progeny are male (Table 5). Moreover, most of these males appear completely wild type. *meDf6* appears to be an even better suppressor of the *X*O-specific lethality caused by these duplications than *meDf5*, because *meDf5; yDp13* hermaphrodites produced only 15% males, and *meDf5; yDp15* hermaphrodites produced only 12% males (Table 5). Furthermore, in contrast to the *meDf6; Dp* males, which appear wild type, the *meDf5; Dp* males were more variable in phenotype, with far fewer males appearing wild type and most males appearing small, thin, and slow growing. This result suggests that the region between the *meDf5* and *meDf6* endpoints contributes to the effect of *yDp13* and *yDp15* on males, and is also likely to contribute to the lethal effect of *yDp14/+* on males. However, *meDf6* does not appear to delete all the sex-determination signal elements in the *yDp14* region, because some *meDf6; yDp13* and *meDf6; yDp15* males are most likely inviable (Table 5). In addition, *meDf6* has no obvious dominant effects on hermaphrodites. *meDf6/+ XX* hermaphrodites are fully viable and wild type in phenotype (data not shown), while *Df/+* hermaphrodites with larger deficiencies of the left end of *X*, described in a later section, have sex determination and dosage compensation mutant phenotypes. Therefore at least one, but probably not all the signal elements in *yDp14* map within the region deleted by *meDf6*.

Synergistic *X*O-specific lethality caused by combinations of duplications: Our discovery that the *mnDp66* region contributes to the sex-determination signal, even though *mnDp66/+* *X*O animals are wild type, suggests that duplications of other regions of *X* may also affect the sex-determination signal. Like *mnDp66*, their effect may only be seen in combination with *yDp14* or another duplication that affects the *X*/A ratio. In order to initiate a survey of the *X* chromosome, we tested two duplications of different regions of *X* for synergistic lethality with *yDp14*, *mnDp8*, which maps to the right side of *X* (Figure 2), and *yDp9*, which maps to the central part of *X* (Figure 3). There was no difference in the phenotype or viability of *yDp14/mnDp8* males compared to *yDp14/+* males (Table 6). With *yDp9*, however, we found that *X*O animals with both *yDp14* and *yDp9* are twofold less viable than *yDp14/+* *X*O animals in the same strain background (Table 6, and data not shown). These results show that *mnDp8* has no apparent effect on the sex-determination signal, and *yDp9* has at most a small effect; we have not yet ruled out that the increased lethality of *yDp14/+; yDp9/+* *X*O animals may be nonspecific.

It was important to determine if *mnDp57* contains signal elements because *mnDp57* and *yDp14* overlap extensively (see Figure 4); if *mnDp57* does not contain signal elements, the region of *yDp14* that must include such elements is more precisely defined. There are no appropriate markers we could use to identify animals with both *mnDp57* and *yDp14*. We therefore tested whether *mnDp66* and *mnDp57* cause synergistic *X*O-specific lethality, and found that *X*O animals with both *mnDp57* and *mnDp66* are completely wild type (Table 6). By comparison with the strong synergistic lethal effect of *mnDp66* and *yDp14* in the same strain background (Table 6), it seems unlikely that *mnDp57* contains any of the signal elements present in *yDp14*. Therefore, all the signal elements within *yDp14* probably map to the left of *unc-2* (Figure 4).

Two duplications together may contain signal elements sufficient to kill *X*O animals, even though neither one of them individually causes any *X*O-specific lethality. We observed this phenomenon in our analysis of a spontaneous derivative of *yDp14*, named *yDp14(y280)* (see MATERIALS AND METHODS). *yDp14(y280)* causes no *X*O-specific lethality in one copy (Table 6), suggesting that *yDp14(y280)* is missing one or more signal elements present in *yDp14*. *yDp14(y280)*, however, does cause synergistic *X*O-specific lethality in combination with *mnDp66*. Only 8% of the expected number of *X*O animals with both *mnDp66* and *yDp14(y280)* survived (Table 6). In contrast, just as *mnDp8/yDp14* males are as viable as *yDp14/+* males, *mnDp8/yDp14(y280)* *X*O animals are fully viable, wild-type males (Table 6). The fact that two duplications like *mnDp66* and *yDp14(y280)* cause *X*O-specific lethality in combination, although neither duplication confers any *X*O-specific lethality individually, provides further evidence that the sex-determination signal is made up of multiple elements, and that changing the dose of a single element from one to two in *X*O animals may be insufficient to alter the signal itself.

Multiple copies of a single region cause increased *X*O-specific lethality: The above analysis indicates that the phenotype caused by combining duplications of different regions of the left end of *X* in *X*O animals is much stronger than the phenotype caused by duplicating each smaller region individually. We wondered whether having multiple copies of a single smaller region could substitute for simultaneous duplication of two of these regions in increasing the perceived *X*/A ratio of *X*O animals. Since *yDp14*, *yDp14(y280)* and *mnDp66* can all

TABLE 6

Effects of pairwise combinations of duplications

Duplications paternal/maternal[a]	Percent viability of males with both duplications	Percent viability of males with only maternal duplication	Percent viability of males with only paternal duplication	*n*
yDp14/mnDp8[b]	48			1322
yDp14/yDp9[c]	12	101		1494
mnDp57/+[d]			113	1131
mnDp57/mnDp66[e]	95			997
yDp14/mnDp66[f]	1.4	83		669
yDp14(y280)/+[g]			97	698
yDp14(y280)/mnDp66[h]	8			1535
yDp14(y280)/mnDp8[i]	90			2053

[a] The progeny of crosses were counted in which the male parents were heterozygous for the paternal duplication, and the hermaphrodite parents were homozygous for the maternal duplication. For the two crosses shown with a single duplication, the + indicates no maternal duplication.

[b] *yDp14/+; him-8; unc-2* males were crossed with *mnDp8; dpy-3 unc-3* hermaphrodites. Percent viability of males with both duplications was calculated as (total males with both duplications)/(one-half the total cross-progeny hermaphrodites) × 100. *n* is the total cross-progeny males with both duplications and total cross-progeny hermaphrodites.

[c] *yDp14/+; unc-2* males were crossed with *yDp9; dpy-3 unc-9* hermaphrodites. Percent viability of males with both duplications was calculated as (total males with both duplications)/(half the total cross-progeny hermaphrodites) × 100. Percent viability of males with only the maternal duplication was calculated as (total males with only the maternal duplication)/(half the total cross-progeny hermaphrodites) × 100. *n* is the total cross progeny.

[d] *mnDp57/+; unc-2* males were crossed with *unc-2* hermaphrodites. Percent viability of males with the duplication was calculated as (total non-Unc males)/(total non-Unc hermaphrodites) × 100. *n* is the total duplication-bearing cross progeny.

[e] *mnDp57/+; unc-2* males were crossed with *mnDp66; egl-17(e1313) unc-1(e1598 n1201) unc-2* hermaphrodites. Percent viability of males with both duplications was calculated as (total males with both duplications)/(total hermaphrodites with both duplications) × 100. *n* is the total cross progeny with both duplications.

[f] *yDp14/+; unc-2* males were crossed with *mnDp66; egl-17(e1313) unc-1(e1598 n1201) unc-2* hermaphrodites. Percent viability of males with both duplications was calculated as (total males with both duplications)/(total hermaphrodites with both duplications) × 100. Percent viability of males with only the maternal duplication was calculated as (total males with only the maternal duplication)/(total hermaphrodites with both duplications) × 100. *n* is the total cross-progeny hermaphrodites with both duplications and total cross-progeny males.

[g] *yDp14(y280)/+; him-8; dpy-3 unc-2* males were crossed with *unc-2* hermaphrodites. Percent viability of males with the duplication was calculated as (total non-Unc males)/(total non-Unc hermaphrodites) × 100. *n* is the total duplication-bearing cross progeny.

[h] *yDp14(y280)/+; him-8; dpy-3 unc-2* males were crossed with *mnDp66; unc-1(e1598 n1201) dpy-3* hermaphrodites. Percent viability of males with both duplications was calculated as (total males with both duplications)/(total hermaphrodites with both duplications) × 100. *n* is the total cross progeny with both duplications.

[i] *yDp14(y280)/+; him-8; dpy-3 unc-2* males were crossed with *mnDp8; dpy-3 unc-3* hermaphrodites. Percent viability of males with both duplications was calculated as (total males with both duplications)/(total hermaphrodites with both duplications) × 100. *n* is the total cross progeny with both duplications.

be made homozygous in hermaphrodites without causing obvious deleterious effects, we could study the phenotype of XO progeny that have two copies of these duplications, that therefore have three doses of the duplicated region.

yDp14; him-8 and *yDp14(y280); him-8* hermaphrodites should produce approximately 37% XO self progeny because of the *him-8* mutation (HODGKIN *et al.* 1979). We found, however, that for both of these homozygous duplications, no male progeny arose (Table 7), suggesting that these duplications are completely lethal to XO animals in two copies (see MATERIALS AND METHODS). We confirmed the complete lethality of the *yDp14/yDp14* XO animals by crossing *yDp14/+* males with *yDp14; bli-2; unc-1 dpy-3* hermaphrodites. If *yDp14/yDp14* XO animals are hermaphrodites, half of the XO cross progeny should be Unc non-Bli non-Dpy hermaphrodites (*yDp14; bli-2/+; unc-1 dpy-3*/O). No such hermaphrodites were found among more than 100 cross progeny, indicating that *yDp14/yDp14* XO animals are dead, rather than transformed into hermaphrodites.

All the *yDp14/yDp14* XO animals could be dead because of a further increase in their perceived X/A ratio

TABLE 7

Two copies of *yDp14* or *mnDp66* cause increased XO-specific lethality

Genotype	Percent males	*n*[a]	Percent viability of males[b]
him-8[c]	37		100
yDp14; him-8; unc-2	0	>1000	0
yDp14(y280); him-8; dpy-3 unc-2	0	>1000	0
mnDp66; him-8; unc-1 dpy-3	24	3111	54
mnDp57; him-8; unc-2	35	413	92

[a] Entire self-progeny broods of each genotype were counted; *n* is the total number of live animals.

[b] The percent viability of males was calculated as the ratio of the number of males observed to the number of males expected if the strains behave like the *him-8* control.

[c] Data from HODGKIN *et al.* (1979).

caused by elevating the dose of the *yDp14* region from the normal single dose to three doses. However, because *yDp14* is a large duplication, it was possible that the XO animals are dead because they have too high a dose of nonspecific *X* chromosome sequences, and that the effect of *yDp14/yDp14* on the sex-determination signal is

TABLE 8

An *sdc-2* mutation fully rescues the *XO*-specific lethality caused by two copies of *yDp14*

Parental genotypes		Number of cross progeny[a]		Percent viability of males[b]
Males	Hermaphrodites	Males	Hermaphrodites	
yDp14/+; unc-2	*yDp14; rol-9; unc-2 sdc-2(y93)*	284	299	95

[a] Total non-Rol progeny were counted. Half the cross progeny are *yDp14/+*, and half are *yDp14/yDp14*; these two genotypes are indistinguishable.

[b] Percent viability of males was calculated as the (total number of male)/(total number of hermaphrodite cross progeny) × 100. Because one-half of the cross progeny are *yDp14/yDp14*, the 95% recovery of the expected total males indicates that at least 90% of the *yDp14/yDp14* males must be rescued by *sdc-2(y93)*.

not any stronger than that of *yDp14/+*. If the death is due only to the effect of *yDp14* on the sex-determination signal, these *XO* animals should be completely rescued by an *sdc* loss-of-function mutation. However if the death is due to a combination of specific and nonspecific effects, the increased *XO*-specific lethality may be only partially suppressed or not suppressed at all by an *sdc* mutation. We found that the strain *yDp14; him-8; unc-2 sdc-2(y93)* produces numerous wild-type males, indicating that a *yDp14; him-8* strain does produce numerous *XO* embryos and that an *sdc-2* mutation at least partially suppresses the lethality of *yDp14/yDp14* males. We subsequently determined that an *sdc-2* mutation completely suppresses the lethality caused by two copies of *yDp14*, using the cross shown in Table 8. At least 90% of *yDp14; unc-2 sdc-2(y93) XO* males were viable, by comparison with their *yDp14; unc-2 sdc-2(y93)/unc-2 + XX* siblings. Therefore, the increased *XO*-specific lethality of *yDp14/yDp14* compared to *yDp14/+* is due to an increased dose of sex-determination signal elements.

We also determined that *mnDp66* can cause *XO*-specific lethality at a higher copy number. We found that *mnDp66; him-8; unc-1 dpy-3* hermaphrodites produce only 24% male self progeny (Table 7), compared to 37% males expected from *him-8* hermaphrodites (HODGKIN *et al.* 1979), and the *mnDp66/mnDp66* males are small, thin, and slower growing than normal males. While the previous experiments with *mnDp66/+* males showed that this region in two doses causes no *XO*-specific lethality (Table 4), these results suggest that three doses of the *mnDp66* region are deleterious to *XO* animals, but are not as deleterious as three doses of the elements included in *yDp14* and *yDp14(y280)*.

The lethality of *mnDp66* homozygous males is likely to be due to an effect on the sex-determination signal. The pattern of lethality and the phenotype of animals is much different from that seen with *mnDp57/mnDp57* animals, for example. *mnDp57/+* does not appear to have any effect on the sex-determination signal (Table 6), and there is little change in the sex ratio of *mnDp57; him-8; unc-2* progeny compared to *him-8* alone (Table 7). *mnDp57* is, however, deleterious to both *XX* and *XO* animals when homozygous. *mnDp57/mnDp57 XX* animals are slightly Unc hermaphrodites, and have a small brood size of only approximately 70 progeny; many of the males are Dpy, suggesting they may be sick because they have three copies of this large region of *X*. The Dpy phenotype of many of the *mnDp57/mnDp57 XO* animals is remarkably distinct from the small, thin phenotype of *XO* animals homozygous for *mnDp66*, which is typical of males with *yDp13*, *yDp15* or *mnDp66/yDp14*.

Our analysis of combinations of duplications with each other and with deficiencies suggests that the left end of *X* includes multiple signal elements. The fact that neither *meDf6; yDp13*, *meDf6; yDp15*, nor *yDp14(y280)/+*, cause a strong *XO* phenotype is most likely explained by a requirement to duplicate more than one signal element in order to strongly affect the sex-determination signal of *XO* animals. These results suggest that the dose of at least two signal elements in the *yDp14* region contribute to the *XO*-specific lethality caused by *yDp14/+*. Together with the phenotypes of *meDf5; yDp13*, *meDf5; yDp15*, and *yDp14/mnDp66* animals, these results further indicate that the increased dose of at least three signal elements contributes to the

TABLE 9

XO-specific lethality caused by duplications is suppressed by mutations in the sex-determination and dosage compensation regulatory genes

Suppressor	Duplications			
	yDp14 / +	*yDp13*	*yDp15*	*yDp14* / *mnDp66*
yEx111 (*Pdpy-30:: xol-1*)	Yes[a]	Yes	Yes	Yes
yEx152 (high copy *xol-1*)	Yes	ND	ND	Yes
sdc-2(y74)[b]	Yes	Yes	Yes	Yes
sdc-2(y93)[c]	Yes	Yes	Yes	ND
sdc-1(n485)	Yes[d]	ND	ND	ND
sdc-3(y129)	Yes	ND	ND	ND

[a] Yes indicates that abundant duplication-bearing males with a wild-type phenotype were observed in the presence of the suppressor array (in the case of *yEx111* or *yEx152*), or in a mutant homozygote (in the case of the *sdc-1*, *sdc-2* or *sdc-3* mutations). ND indicates not done.

[b] *sdc-2(y74)* is a null allele of *sdc-2* (NUSBAUM and MEYER 1989).

[c] *sdc-2(y93)* is a weak allele of *sdc-2* that causes no *XX*-specific lethality, but it completely rescues the lethality of *xol-1(y9) XO* animals (MILLER *et al.* 1988; NUSBAUM and MEYER 1989).

[d] *sdc-1(n485)* rescues only if the mother is homozygous for the *sdc-1* mutation.

TABLE 10

Mutations in the dosage-compensation *dpy* genes feminize duplication-bearing *XO* animals

Genotype of *XO* animals	Phenotype of *XO* animals[a]			
	Percent male	Percent intersex	Percent hermaphrodite	n[b]
dpy-28(y1); xol-1(y9)[c]			100	305
dpy-28(y1); yDp13[d]	26	13	61	53
yDp14/+; dpy-28(y1)[d]	65	15	20	1218
yDp14(y280)/+; dpy-28(y1)[d]	49	20	31	586
sdc-3(y128); xol-1(y9)[e]			100	
yDp14/+; sdc-3(y128)[d]	76	6	17	190
dpy-26(y65); xol-1(y70)[c]	18	7	75	307
yDp14/+; dpy-26(y65)[d]	77	14	9	108
dpy-27(y57); xol-1(y9)[f]	<1		>99	386
dpy-27(y57); yDp13[d]	58	8	34	153
yDp14/+; dpy-27(y57)[d]	80	7	13	209
dpy-21(e428); xol-1(y9)[c]	44	17	39	688
yDp14/+; dpy-21(e428)[d]	99		1	468

[a] *XO* animals were examined with a dissecting-microscope. Animals classified as hermaphrodites were all Egl, but self-fertile, and had a normal or nearly normal tail. Animals classified as intersex had a hermaphrodite vulva, were often self fertile, but had a male-like tail. Most animals classified as male were completely wild-type fertile males, but a few were abnormal, and may have been partially feminized.

[b] n is the number of *XO* animals scored.

[c] Data from MILLER *et al.* (1988).

[d] Non-Dpy non-Unc progeny from *dpy; unc-2; Dp XO* males crossed with *dpy; unc-2 XX* hermaphrodites were examined.

[e] *sdc-3(y128)* affects dosage compensation but not sex determination. Data from DELONG *et al.* (1993).

[f] Non-Dpy progeny from *dpy-27; xol-1 XO* males crossed with *dpy-27; xol-1 XX* hermaphrodites were examined.

XO-specific lethality caused by *yDp13* and *yDp15*. In addition, the finding that each of the duplications, *yDp14*, *yDp14(y280)*, and *mnDp66*, can cause increased *XO*-specific lethality when present in two extra copies compared to one extra copy, suggests that multiple copies of just the set of signal elements within each one of these duplications are sufficient to increase the *X*/A ratio of *XO* animals.

XO-specific lethal duplications act upstream of the known genes in the sex-determination regulatory pathway: If these duplications indeed affect the sex-determination signal as this analysis suggests, they should act upstream of *xol-1*. The model in Figure 1 predicts that duplications which increase the *X*/A ratio and cause *XO* animals to adopt the *XX* fate should act by reducing *xol-1* activity toward the level in *XX* animals. We expect that such animals should be rescued if their *xol-1* activity is elevated to the male level, regardless of karyotype. Such deregulated high *xol-1* activity has been observed with extrachromosomal arrays that either have *xol-1* driven by the constitutively acting *dpy-30* promoter (*yEx111*) or simply have *xol-1* in high copy (*yEx152*). Both types of arrays cause *XX*-specific lethality, indicating that *xol-1* is improperly activated in these *XX* animals (RHIND *et al.* 1995). In side-by-side comparisons of males from crosses with or without the *yEx111* array, we only observed duplication-bearing males with a wild-type phenotype in crosses with *yEx111*. *yEx111* rescues *XO* animals with *yDp13*, *yDp14*, *yDp15* or *mnDp66/yDp14* (Table 9). In addition, the *yEx152* array, with the wild-type *xol-1* gene in high copy, also rescues duplication-bearing *XO* animals. These results strongly suggest that the duplications act upstream of *xol-1*, as expected if they affect the sex-determination signal.

Just as *sdc-2* mutations rescue the *XO*-specific lethality of these duplications, we expected that loss-of-function mutations in the *sdc-1* or *sdc-3* genes would also suppress this lethality. We found that *yDp14/+ XO* males have a completely wild-type phenotype in either of these *sdc* mutant strains (Table 9). An *sdc-1* mutation, however, rescued the *yDp14/+* males only if the mother was homozygous for this mutation. This maternal requirement for rescue was previously observed for the rescue of *xol-1 XO* animals by *sdc-1* mutations (MILLER *et al.* 1988), and it is consistent with the partial maternal rescue of the sex-determination and dosage compensation defects of *sdc-1* mutant *XX* animals (VILLENEUVE and MEYER 1987).

Mutations in the dosage-compensation *dpy* genes also suppress the *XO*-specific lethality caused by the duplications, as expected, since these mutations block the *XX* mode of dosage compensation. The *XO* duplication-bearing animals with these mutations are variably feminized, reminiscent of the phenotype of *xol-1 XO* animals that are rescued by dosage compensation *dpy* mutations (Table 10) (MILLER *et al.* 1988). The duplication-bearing *XO* animals are less feminized than *xol-1* mutant animals that have been rescued by the same *dpy* mutation. We see a general correlation between the percentage of feminized *dpy XO* animals and the extent of *XO*-specific lethality in the absence of the suppressor. *xol-1* causes complete *XO*-specific lethality, and all the *dpy-28; xol-1 XO* animals are hermaphrodite. *yDp13* causes less lethality, and 74% of the *dpy-28; yDp13 XO*

animals are hermaphrodite or intersex. *yDp14* causes even less lethality, and only 35% of the *yDp14; dpy-28* animals are hermaphrodite or intersex. *dpy-28* *X*O animals with one copy of *yDp14(y280)*, however, are quite feminized; 51% are hermaphrodite or intersex even though this duplication causes no *X*O-specific lethality, indicating that there is no absolute correlation between lethality and feminization. This feminization of some of the duplication-bearing *X*O animals in a *dpy* background indicates that these duplications of the left end of *X* affect the sex determination decision as well as the dosage compensation decision of *X*O animals, consistent with their proposed effect on the *X*/A ratio.

Deficiencies of the left end of *X* cause an Sdc phenotype: We expected that just as duplications of the left end of *X* cause an *X*O-specific lethal phenotype by increasing the sex-determination signal from the *X*O toward the *XX* setting, deficiencies of this same region should cause a dominant lethal phenotype in *XX* animals, because the *Df/+ XX* animals should have an *X*O dose of these signal elements and should adopt the *X*O mode of dosage compensation. We further expected that a loss-of-function mutation in *xol-1* should suppress the sex determination and dosage compensation (Sdc) mutant phenotype of *XX* deficiency heterozygotes, because a *xol-1* mutant animal is locked into the *XX* state, regardless of karyotype. To test these expectations, we isolated deficiencies of the left end of *X* in a *xol-1* mutant background, and then assayed the phenotype of deficiency heterozygotes in a *xol-1*(+) background.

Two deficiencies that remove the left end of *X* were isolated using an *unc-2* non-complementation screen in a *xol-1* null mutant background (MATERIALS AND METHODS), and both cause a dominant Sdc phenotype that is rescued by a *xol-1* null mutation. Figure 5 shows the phenotype of *XX* animals heterozygous for one of these deficiencies, *yDf14*, in three strain backgrounds: homozygous *xol-1* null, *xol-1* null/*xol-1*(+), and homozygous *xol-1*(+). The *yDf14/+ XX* animals in a homozygous *xol-1* mutant background appear wild type. In a *xol-1/+* background they have a slightly Dpy, slightly Egl phenotype, but are completely viable (Table 11). In a homozygous *xol-1*(+) background, the *yDf14/+* animals are Dpy, some of them are masculinized, but most are viable (Table 11). This phenotype is similar to that of a weak *sdc* mutation (VILLENEUVE and MEYER 1987). Because the *xol-1* mutation suppresses the phenotype of *yDf14/+* animals, this phenotype must be due to a sex-determination and dosage compensation defect, and the deficiencies must act upstream of *xol-1*, consistent with our expectation that these deficiencies should lower the sex-determination signal of *XX* animals.

The fact that the deficiency heterozygotes in a *xol-1* null/+ background are not completely suppressed indicates that *xol-1*(+), even in a single copy, can be deleterious to *XX* animals when activated. Previous experiments demonstrated that extrachromosomal arrays with *xol-1* in high copy, or arrays with *xol-1* expressed from the constitutive *dpy-30* promoter, cause *XX*-specific lethality (RHIND *et al.* 1995). It was not clear from those experiments whether higher *xol-1* activity than that normally found in *X*O animals is required to kill *XX* animals. In the experiments shown here, *xol-1* can only be expressed from the endogenous gene, which is present in two copies in the *yDf14/+* animals, and in one copy (as in wild-type *X*O animals) in the *yDf14 xol-1(y9)/++* animals. The phenotypes of these animals indicate that their level of *xol-1* activity, which cannot be any higher than that of wild-type *X*O animals, is at least deleterious, if not lethal, to *XX* animals.

Why are the phenotypes of the deficiency heterozygotes not more severe? One possibility that could account for the fact that these deficiencies do not cause dominant *XX*-specific lethality is that these deficiencies are so large that the "*XX*" animal now has an interme-

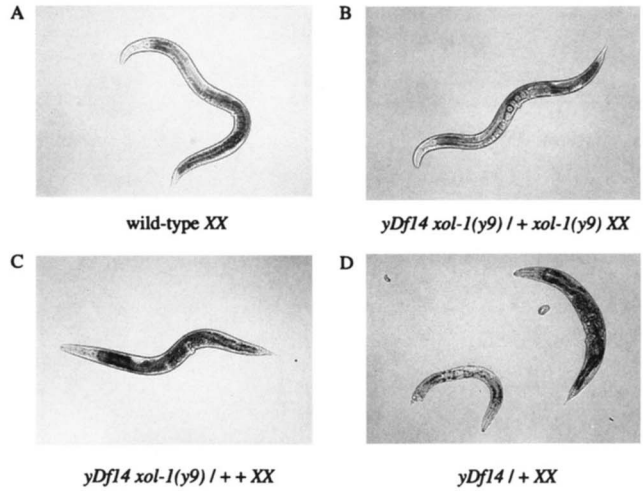


FIGURE 5.—Phenotype of *yDf14/+* XX animals. All panels are bright-field photomicrographs at the same magnification and enlargement. Animals were picked at the fourth larval (L4) stage and photographed 1 day later to ensure that young adult animals were photographed for each genotype. (A) Wild-type *XX* animal, shown for comparison. (B) *yDf14 + xol-1(y9)/+ lon-2 xol-1(y9) XX* animal. Animals of this genotype are wild type in length (compare to panel A) showing no evidence of a dosage-compensation defect. Comparison with panel D indicates that the deleterious phenotype of *yDF14/+* animals is suppressed by *xol-1(y9)*. (C) *yDf14 + xol-1(y9)/+ lon-2 + XX* animal. Animals of this genotype are slightly Dpy, suggesting they have a partial dosage compensation defect. Their phenotype is intermediate between that of *yDf14/+* animals in a homozygous *xol-1(y9)* background (shown in B) and *yDf14/+* animals in a homozygous *xol-1* (+) background (shown in D), suggesting that they are partially suppressed by *xol-1(y9)/+*. (D) *yDf14 +/+ lon-2 XX* animals. Animals of this genotype are variable in phenotype. Approximately 80% are Dpy and Egl, like the animal shown in the top right. This animal also has a bivulva phenotype typical of *yDf14/+* and other dosage-compensation defective *XX* animals (PLENEFISCH *et al.* 1989). Approximately 20% are Dpy, sick and variably masculinized or Tra, like the animal shown in the lower left.

TABLE 11

Viability and phenotype of *yDf14/+ XX* animals

Genotype	Percent viability of *yDf14/+*[a]	Phenotype of *yDf14/+ XX* animals[b]	*n*
yDf14 + xol-1/+ lon-2 +	104	100% slightly Dpy	898[c]
yDf14 +/+ lon-2	83	83% Dpy, Egl 17% Dpy, Tra	200[d]

[a] Percent viability was calculated as the (number of *Df/+* animals)/(twice the number of Lon hermaphrodites) × 100.

[b] See Figure 5 for photos of representative animals of these genotypes.

[c] *n* is the total number of self progeny of *yDf14 + xol-1/+ lon-2 +* hermaphrodites; 36% were slightly Dpy and Egl (*yDf14 + xol-1/+ lon-2 +*) hermaphrodites, 17% were Lon hermaphrodites, 5% were Lon males, 0.8% were Dpy or Dpy Tra hermaphrodites (presumably some were *XXX* animals and some were *yDf14 +/+ lon-2* recombinants), and 40% were dead embryos. We expected equal numbers of dead embryos and Lon animals, since the corresponding *yDf14/yDf14* or *yDf14/O* animals should be dead. The extra lethality in this strain cannot be specific to *yDf14* heterozygotes, because we recovered these animals in the expected number compared to *lon-2* homozygotes.

[d] Only live progeny were counted. Total live progeny included 125 Dpy animals, 75 Lon hermaphrodites, and 15 Lon males. Of 114 Dpy animals examined, 94 were Dpy, Egl hermaphrodites and 20 were masculinized and sick.

diate dose of *X* chromosomes and is therefore more tolerant of dosage compensation upsets. While all the deficiencies we isolated appear to be larger than *yDp13* and *yDp15* (see MATERIALS AND METHODS), a smaller deficiency causing a similar phenotype was isolated in a screen for suppressors of the *X*O-specific lethality caused by *mnDp66/yDp14* (I. CARMI, personal communication). Since *mnDp66/yDp14; Df/O* males are viable, this deficiency must not extend beyond the right endpoint of *yDp14*. The phenotype of *XX* animals heterozygous for this deficiency is similar to that of *yDf14/+* animals, suggesting that the higher viability of the *Df/+ XX* animals compared to the duplication-bearing *X*O animals is unlikely to be due simply to a larger size of the deficiencies compared to the duplications. An alternative possibility is that the increase in *X*-linked gene expression in these *XX* animals that arises from their lower dose of signal elements may not be as deleterious to them as the corresponding decrease in *X*-linked expression of *X*O animals caused by a higher dose of the same elements is to males. In any case, these results clearly demonstrate that changing the dose of the left end of the *X* chromosome, either from one dose to two in males, or from two doses to one in hermaphrodites, causes the reciprocal type of sex determination and dosage compensation mutant phenotypes that are expected if the dose of this region contributes strongly to the *X* chromosome component of the *X*/A ratio.

***mnDp66* affects expression of a *xol-1::lacZ* reporter gene:** To begin to determine how the different *X* chromosome elements of the *X*/A ratio affect the activity of downstream regulatory genes, we assessed the effect of *X*-chromosome duplications on expression of *xol-1* using a *xol-1* reporter gene, *yIs2 (Pxol-1::lacZ)*. *yIs2* is an integrated array containing multiple copies of a reporter gene in which the *xol-1* upstream regulatory region and part of the *xol-1* structural gene are fused to *lacZ*. We chose to examine *xol-1* expression using this reporter construct because *xol-1* is the most upstream gene in the pathway and because *xol-1* expression, and *lacZ* expression from *yIs2*, is sex-specifically regulated (RHIND *et al.* 1995). The self-progeny embryos of *yIs2* mothers, which are all *XX*, have only low β-galactosidase activity as observed by staining with X-gal (see Figure 6D). In contrast, *him-8 yIs2* mothers, which have 40% *X*O progeny, produce numerous embryos with high β-galactosidase activity, suggesting that *X*O embryos, but not *XX* embryos, express high levels of β-galactosidase from this reporter construct (see Figure 6C).

We expected that duplications that affect the sex-determination signal of *X*O animals might act by decreasing *xol-1* expression in *X*O embryos. To test this hypothesis, we compared the self-progeny embryos of *yDp14*; *him-8 yIs2* or *mnDp66*; *him-8 yIs2* hermaphrodites with those of *him-8 yIs2* and *yIs2* hermaphrodites by staining them for β-galactosidase activity (Figure 6). All the experimental embryos should be homozygous for either *yDp14*, which causes complete *X*O-specific lethality (Table 7), or *mnDp66*, which causes 70% *X*O-specific lethality in the *yIs2* background (Table 12). We found that the *yDp14; him-8 yIs2* strain appeared indistinguishable from the *him-8 yIs2* control strain in both the number of embryos staining and the intensity of staining, suggesting that *yDp14* has no effect on *lacZ* expression from this reporter gene. We repeated this experiment at least ten times, examining a total of thousands of embryos of both the *yDp14; him-8 yIs2* and the *him-8 yIs2* genotypes, and never saw any noticeable difference in staining between these strains. On the basis of this result, however, we cannot exclude the possibility that *yDp14* might regulate the endogenous *xol-1* gene. In marked contrast, far fewer embryos stained in the *mnDp66; him-8 yIs2* strain compared to the *him-8 yIs2* control strain, and those that did stain appeared to stain less strongly. We examined at least one thousand embryos in four separate experiments, and consistently observed approximately 5–10 fold less staining in the *mnDp66; him-8 yIs2* strain compared to the *him-8 yIs2* strain. This result suggests that *mnDp66* affects expression of β-galactosidase from the *xol-1::lacZ* reporter

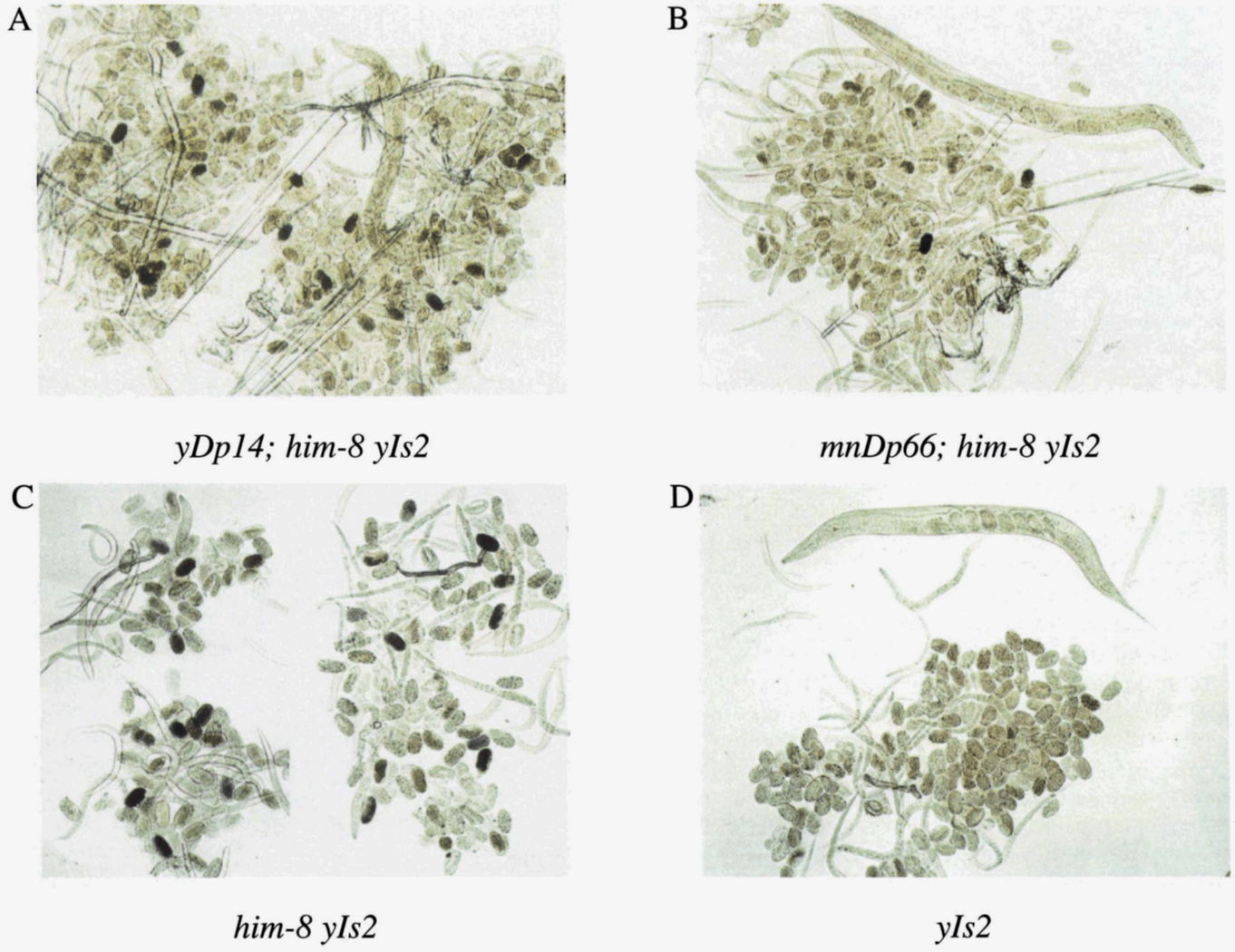


FIGURE 6.—Effect of signal element duplications on expression of β-galactosidase from the integrated *xol-1* reporter gene, *yIs2*. *X*O embryos from the 28-cell stage through gastrulation normally express high levels of β-galactosidase from *yIs2*, whereas *XX* embryos express very low levels (RHIND *et al.* 1995). Animals of each genotype were stained with X-gal (0.012%) for 20 hr at room temperature. Bright-field photomicrographs of representative groups of embryos are shown. Thousands of embryos of each genotype were examined. (A) *yDp14; him-8 yIs2; unc-2*. Within the cluster of embryos shown, several embryos stain the dark blue color indicative of high level β-galactosidase expression found in the *X*O control embryos (*him-8 yIs2*) shown in panel C (B) *mnDp66; him-8 yIs2; unc-1*. A cluster of embryos is shown with only two embryos staining blue, one of them very weakly. Nearly all of the *mnDp66* embryos examined exhibited no apparent β-galactosidase activity, indicating the *xol-1* reporter is turned down in response to the increased *X*/A ratio. (C) *him-8 yIs2*, the positive control for staining, shows many *X*O embryos that stain strongly. This panel is a composite of three separately photographed groups of embryos, assembled to show a number of embryos comparable to the number in the other samples. (D) *unc-24 yIs2*, the negative control for staining shows the very low background of β-galactosidase activity typical of *XX* embryos. Only one darkly staining embryo (not shown) was observed among hundreds of embryos; it was probably a rare *X*O embryo that arose from spontaneous *X* chromosome non-disjunction, which occurs at a frequency of 1/500 progeny.

gene, apparently reducing its expression in *X*O embryos. *mnDp66* is therefore likely to also reduce expression of the endogenous *xol-1* gene in *X*O embryos, consistent with our previous analysis that *mnDp66* includes sex-determination signal elements and that this duplication increases the perceived *X*/A ratio of *X*O animals.

We also observed that *yIs2(Pxol-1::lacZ)* and *mnDp66* together cause synergistic *X*O-specific lethality. *yIs2* alone causes no *X*O-specific lethality, but only 35% of *mnDp66; him-8 yIs2; unc-1* males are viable compared to 54% of *mnDp66; him-8; unc-1 dpy-3* males (Table 12). One possible explanation for the increased lethality is that *yIs2* could compete with the endogenous *xol-1* gene for an activator that binds to the *xol-1* upstream regulatory region. The effect of *mnDp66* on *lacZ* expression from *yIs2* suggests that increasing the dose of the *mnDp66* region represses *xol-1* expression. These two effects together, the proposed competition of *yIs2* with *xol-1* for an activator, and *xol-1* repression by

TABLE 12

mnDp66 and *yIs2(Pxol-1::lacZ)* cause synergistic XO-specific lethality

Genotype	Percent males	n^a	Percent viability of males[b]
mnDp66; him-8; unc-1 dpy-3	24	3111	54
mnDp66; him-8 yIs2(Pxol-1::lacZ); unc-1	17	1795	35
him-8 yIs2(Pxol-1::lacZ); unc-2	41	2723	118

[a] Entire self-progeny broods of each genotype were counted; *n* is the total number of live animals.
[b] The percent viability of males was calculated as the ratio of the number of males observed to the number of males expected if the strains behave like the *him-8* control strain shown in Table 7.

mnDp66, may cause a strong enough decrease in *xol-1* expression to kill most *mnDp66; yIs2* males.

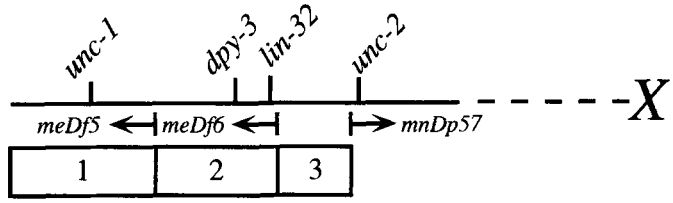
DISCUSSION

Sex-determination signal elements are located near the left end of X: Our results demonstrate that the left end of *X*, between the left telomere and the *unc-2* gene, includes signal elements whose dose is measured for the *X*/A ratio. Almost all *XO* animals with an extra dose of this entire region are dead. Within this region, the dose of three smaller regions, each of which contains one or more signal elements, contributes to the *XO*-specific lethality as shown in Figure 7. A summary of the data establishing the location of each region is provided in the Figure 7 legend. Increasing the dose of any of these regions individually from an *XO* to an *XX* dose has little or no effect on males. One extra dose of region 1 causes no *XO*-specific lethality, while one extra dose of region 3 appears to cause approximately 25% *XO*-specific lethality. The live *XO* animals with one extra dose of either of these regions are wild-type males. Further increases in the dose of one or more of these regions cause more extensive *XO*-specific lethality. 50% of *XO* animals with two extra doses of region 1 are dead, and 75% of *XO* animals with one extra dose of both regions 2 and 3 are dead. The *XO* animals that survive are variable in phenotype; some appear wild type, while others are small, thin, slow growing, fertile males, which we will refer to as midget males. At least 90% of *XO* animals with one extra dose of all three regions are dead; the few that escape lethality are all midget males. Finally, no *XO* animals with two extra doses of both regions 2 and 3 survive. The regions may not make equivalent contributions to the signal; for example, region 3 may contain either more or stronger elements than region 1, based on the relative lethality caused by two doses of these regions in *XO* animals.

This study provides the first observation of the phenotypes of diploid *XO* animals with increased doses of signal elements. It suggests that higher doses of signal elements cause both increased *XO*-specific lethality and an increase in the proportion of midget males among the *XO* animals that escape lethality. The midget male phenotype of the *XO* animals that escape lethality is likely to be due to an inappropriately low level of *X*-linked gene expression, resulting from the shift of these animals toward the *XX* mode of dosage compensation. Further analysis of how the sex-determination signal regulates downstream genes will be necessary to understand the phenotype of the midget males, particularly, why they survive, and why they are not sexually transformed.

We expected that *XX* animals with a single dose of the signal elements in this entire region would exhibit an equally severe phenotype as that of *XO* animals with two doses. Surprisingly, these *XX* animals have only a weak Sdc phenotype–they are only dumpy and partially masculinized, rather than dead. Moreover, *XX* animals with a single dose of regions 1 and 2 shown in Figure 7 exhibit no mutant phenotypes. The cause of this lack of reciprocity is not clear, nor is it clear why the *XX* animals with one dose of this region exhibit a sex determination defect as well as a dosage compensation defect, while *XO* animals with two doses of this region only exhibit a dosage compensation defect. With regard to the high viability of hermaphrodites, it may be that they can better tolerate increases in *X*-linked gene expression than males can tolerate decreases in *X*-linked gene expression. The opposite situation is true in Drosophila; females appear to be more sensitive to a lowered dose of signal elements than are males to an increased dose of the same elements (CLINE 1988).

The region near the left end of *X* satisfies all the requirements for a region that contains dose-dependent sex-determination signal elements. The karyotype-specific effects of changing the dose of this region are suppressed by all appropriate mutations in the downstream genes controlled by the *X*/A ratio. *XO* animals with a duplication of the region are rescued by constitutive *xol-1* expression and by loss-of-function *sdc* mutations and dosage-compensation *dpy* mutations, indicating that this lethality is caused by *XO* animals adopting the *XX* mode of dosage compensation, and that the duplications act upstream of *xol-1*. The dominant Sdc phenotype caused by deficiencies of this region is suppressed by a *xol-1* null mutation, indicating that this phenotype also results from a defect in the sex-determination regulatory pathway upstream of *xol-1*. This Sdc phenotype is partially suppressed simply by loss of one copy of *xol-1*(+), providing the first evidence that a small, twofold, difference in the level of *xol-1* can cause significant dosage-compensation defects in *XX* animals.



Region Duplicated or Deleted	Partial Genotypes	*XO*-specific Lethality caused by Duplications	*XX* Phenotype of *Df*/+ Animals
1	*mnDp66*/+	none	
	meDf5/+		WT
2	none		
3	*meDf6; yDp13*	≈ 25% lethality	
	meDf6; yDp15		
1+1	*mnDp66/mnDp66*	≈ 50% lethality	
1+2	*meDf6*/+		WT
2+3	*yDp14*/+	≈ 75% lethality	
	yDp16		
	meDf5; yDp13		
	meDf5; yDp15		
1+2+3	*yDp13*	≈ 90% lethality	
	yDp15		
	mnDp66/yDp14		
	yDf14/+		Sdc
2+3+2+3	*yDp14/yDp14*	100% lethality	

FIGURE 7.—Three regions near the left end of *X* include sex-determination signal elements. Region 1 is the region included in *mnDp66* and *meDf5*. Region 2 is the region included in *meDf6* that is not also included in *meDf5*. Region 3 is the region to the right of *meDf6* and the left of *unc-2*. Delineation of the regions harboring signal elements is based on the following results: for region 1, the combination of *mnDp66* and *yDp14* causes the same degree of *XO*-specific lethality as *yDp13* or *yDp15*, while *yDp14* alone causes less lethality, and *meDf5* partially rescues *yDp13* or *yDp15* males; for region 2, *meDf6* rescues *yDp13* males more completely than *meDf5* does; for region 3, *meDf6; yDp13* and *meDf6; yDp15* males are not completely viable, and *meDf6*/+ XX animals are wild type, but *yDf14*/+ *XX* animals have an Sdc phenotype, suggesting that signal elements map to the right of *meDf6*. The signal elements removed by *yDf14* and the other deficiencies of this region are likely to be the same ones as those included in the duplications, with no elements removed by the deficiencies that are not included in the duplications. This conclusion is based on the finding that an *X* chromosome deficiency that does not extend rightward beyond *yDp14*, and thus cannot include additional signal elements, causes the same dominant Dpy, Egl and incomplete Tra *XX* phenotype as *yDf14*. This deficiency was isolated as a suppressor of the *XO*-specific lethality caused by *mnDp66/yDp14* (I. CARMI, personal communication), and the hemizygous *mnDp66/yDp14; Df*/O males are wild type. Region 3 most likely does not extend as far as *unc-2* because *mnDp57* includes *unc-2* but has no apparent effect on the sex-determination signal. The chart below the map summarizes the degree of *XO*-specific lethality caused by duplicating these regions and the *XX* phenotype caused by reducing the dose of these regions. The chart also shows the combinations of different duplications and deficiencies that cause these phenotypes. The percent lethality indicated is only a rough estimate, since the effects of duplications in different strain backgrounds are combined in this summary. No genotype we tested duplicates or deletes only region 2. Combinations of regions that could not be tested with the duplications and deficiencies described herein are not listed.

Additional signal elements elsewhere on X: Complete lethality of *XO* animals, similar to that caused by *xol-1* mutations, can be achieved by increasing the dose of signal elements in the left end of *X*, as demonstrated by the phenotype of animals with two extra doses of the *yDp14* region. Yet, one extra dose of the signal elements in *yDp13* and *yDp15*, the largest duplications of the left end of *X*, neither kills all *XO* animals nor feminizes all the *XO* animals that are rescued by dosage compensation *dpy* mutations. With respect to the *X*/A ratio, these

results indicate that a hermaphrodite dose of just this region of *X* is not quite equivalent to two complete *X* chromosomes, and additional signal elements must exist elsewhere on *X*. The region between *sma-5* and *unc-9* may contain a weak signal element, since a duplication of that region, *yDp9*, causes increased lethality of *yDp14*/+ *X*O animals. However, we have not yet proven that the increased lethality of *X*O animals caused by this combination can be suppressed by an *sdc* mutation and must therefore be caused by an increase in the perceived *X*/A ratio.

In contrast, other duplications, *mnDp8* and *mnDp57*, do not appear to include sex-determination signal elements, since neither of these duplications causes increased *X*O-specific lethality in combination with *yDp14* or *mnDp66*. However, both *mnDp8* and *mnDp57*, like most other previously isolated *X* chromosome duplications, feminize 2*X*/3A males (Meneely 1994), suggesting that the feminization of 2*X*/3A males by *X* chromosome duplications is not likely to be caused by increasing the dose of signal elements (see also Meneely 1994).

This study provides useful tools to allow the identification of specific *X* chromosome signal elements. If strong signal elements exist within *yDp13* or elsewhere, mutations in individual elements may arise as suppressors of the *X*O-specific lethality caused by this duplication. If the effect of *yDp13* is due to the cumulative dose of many weaker elements, only deficiencies of this region that remove more than one element may be recovered in a suppressor screen. Regardless of the location or strength of individual elements, we can at least identify the regions that must contain such elements by determining whether *X* chromosome duplications cause synergistic *X*O-specific lethality with *yDp14* or *mnDp66*. If the duplicated regions harbor signal elements, the synergistic lethality should be suppressed by downstream *sdc* and dosage compensation *dpy* mutations.

Regulation of *xol-1* by the *X*/A ratio: It has recently been shown that the *X*/A ratio directs the choice of sexual fate by regulating the level of *xol-1* transcripts. High *xol-1* expression during gastrulation promotes male development, while low *xol-1* expression at that time permits hermaphrodite development. High *xol-1* expression kills *XX* animals by forcing them to adopt the *X*O mode of dosage compensation. Together these results indicate that *xol-1* acts as an early developmental switch to set the choice of sexual fate, and it may be the direct target of the *X*/A ratio (Rhind *et al.* 1995). One prediction of this model is that elevating the dose of signal elements in an *X*O animal should reduce the expression of *xol-1*. Indeed, the increase in dose of signal elements in *X*O animals homozygous for *mnDp66* correlates with a lower expression level of the *xol-1::lacZ* reporter gene compared to that in wild-type *X*O animals. These results suggest that a high dose of the signal element(s) within *mnDp66* directly or indirectly represses the endogenous *xol-1* gene. Surprisingly, there appeared to be no effect of increasing the dose of signal elements in *yDp14* on the expression of this reporter gene, despite the complete *X*O-specific lethality caused by two copies of *yDp14*. Additional experiments are necessary to determine if the elements in this region also affect *xol-1* expression. It is possible that these elements regulate *xol-1* in a manner not reflected by the reporter gene, despite the fact that this reporter gene is sex-specifically regulated.

In addition to *X*-linked sex-determination signal elements, other genes are likely to regulate the level of *xol-1* expression. These could be maternally supplied activators or repressors of *xol-1* expression as well as dose-sensitive autosomal factors, as in Drosophila (Cline 1993). Mutations in genes that negatively regulate *xol-1*, as well as mutations in the signal elements themselves could be isolated in screens for suppressors of the *X*O-specific lethal phenotype caused by duplication of signal elements. Mutations in *xol-1* itself and in genes that positively regulate *xol-1* would be isolated in screens for suppressors of the Sdc phenotype of *XX* animals heterozygous for deficiencies that remove signal elements. Both suppressor screens promise to yield further insight into the early steps of the sex-determination regulatory pathway.

Our analysis provides a preliminary indication that the *X*/A ratio in *C. elegans* may be assessed in a manner similar to that in Drosophila. Although we have not yet identified individual signal elements, the fact that the dose of the left end of *X* has a strong effect on the perceived *X*/A ratio in diploids, while that of other regions of *X* has no such effect, suggests that the dose of only a few genes may comprise the *X* chromosome component of the *X*/A ratio in *C. elegans*, as in Drosophila. The Drosophila *X* chromosome signal elements are dose-sensitive transcriptional activators of the feminizing switch gene *Sxl*, the primary target of the sex-determination signal (Cline 1993). In *C. elegans*, the primary target of the signal may be *xol-1*, whose expression is also sex-specifically regulated. We predict that the *C. elegans* signal elements are genes that directly regulate *xol-1* expression.

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