Genetic Analysis of *X*-Chromosome Dosage Compensation in *Caenorhabditis elegans*

Philip M. Meneely[1] and William B. Wood[2]

Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309

Manuscript received February 17, 1987
Revised copy accepted May 30, 1987

ABSTRACT

We have shown that the phenotypes resulting from hypomorphic mutations (causing reduction but not complete loss of function) in two *X*-linked genes can be used as a genetic assay for *X*-chromosome dosage compensation in *Caenorhabditis elegans* between males (*XO*) and hermaphrodites (*XX*). In addition we show that recessive mutations in two autosomal genes, *dpy-21 V* and *dpy-26 IV*, suppress the phenotypes resulting from the *X*-linked hypomorphic mutations, but not the phenotypes resulting from comparable autosomal hypomorphic mutations. This result strongly suggests that the *dpy-21* and *dpy-26* mutations cause increased *X* expression, implying that the normal function of these genes may be to lower the expression of *X*-linked genes. Recessive mutations in two other *dpy* genes, *dpy-22 X* and *dpy-23 X*, increase the severity of phenotypes resulting from some *X*-linked hypomorphic mutations, although *dpy-23* may affect the phenotypes resulting from the autosomal hypomorphs as well. The mutations in all four of the *dpy* genes show their effects in both *XO* and *XX* animals, although to different degrees. Mutations in 18 other *dpy* genes do not show these effects.

DIFFERENTIAL gene expression during development commonly involves regulating a single gene or a set of developmentally or physiologically related genes. An exception in animals with sex chromosomes is the phenomenon of dosage compensation, in which the expression of most or all of the *X* chromosome is differentially regulated in the two sexes such that individuals with one *X* chromosome and individuals with two *X* chromosomes make equivalent amounts of *X*-linked gene products (MULLER, 1950).

Dosage compensation has been most extensively documented in Drosophila and mammals, which compensate by fundamentally different mechanisms. In Drosophila, both sexes are thought to express *X*-linked genes at the *2X* level: the single *X* chromosome in males appears to be hyperactivated with respect to the female *X* chromosomes and the autosomes (reviewed in BAKER and BELOTE, 1983). In mammals, both sexes express *X*-linked genes at the *1X* level: one of the two *X* chromosomes in females is inactivated (reviewed in GARTLER and RIGGS, 1983). These two mechanisms share the feature that compensation occurs in only one sex.

We are investigating the control of dosage compensation in the nematode *Caenorhabditis elegans*. There is clear and varied evidence that many *X*-linked genes in *C. elegans* are compensated. Most directly, MEYER and CASSON (1986) have reported molecular evidence for dosage compensation of transcript levels for three *X*-linked genes. DONAHUE, QUARANTILLO and WOOD (1987; cited in WOOD *et al.* 1985) have obtained similar results using a somewhat different experimental approach. This agrees with evidence from both enzyme assays and genetic studies. Two *X*-linked enzymes have been assayed: *ace-1* [J. DUCKETT and R. RUSSELL (cited by BULL 1983); R. RUSSELL, personal communication; P. M. MENEELY and K. NORDSTROM, unpublished data], which encodes one form of acetylcholinesterase, and *nuc-1 X* (W. B. WOOD, unpublished data) which encodes a DNA endonuclease (M. DEW and J. E. SULSTON, personal communication). In each case, similar levels of enzyme activity were found in males and hermaphrodites. However, the possibility of differential regulation in the two sexes due to physiological differences rather than dosage compensation makes these results difficult to interpret, especially for *ace-1* where a sexual difference is apparent. Indirect genetic evidence has been presented that the dosage of genes defined by *X*-linked lethals is compensated (MENEELY and HERMAN 1979), and arguments for dosage compensation were made based on initial descriptions of two of the unusual *dpy* genes discussed below (HODGKIN 1983a).

To investigate dosage compensation further in *C. elegans*, we have employed a genetic assay for the level of *X*-linked gene expression using hypomorphic mutations. MULLER (1950) defined a hypomorph as a mutant allele that causes partial loss of gene function, such that its phenotype appears to depend on the level of mutant gene product. He pointed out that such mutants could be employed as indicators of allele

[1] Present address: Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, Washington 98104.
[2] To whom correspondence should be addressed.

dosage or expression. We show here that *X*-linked hypomorphs result in similar phenotypes in *1X* and *2X* animals, indicating that these genes are dosage compensated. The genetic assay has the advantages that it can be used in direct screens for new mutations with abnormal levels of *X*-linked gene expression, and that it allows analysis of small numbers of mutant animals in strains that cannot be grown to large homogeneous populations.

We also present evidence supporting the involvement of at least two, and possibly four, previously identified genes in the dosage compensation mechanism. Mutations in these genes lead to a short morphology known as dumpy (Dpy). The phenotypes of most Dpy mutants are not affected by sex or the ratio of *X* chromosomes to sets of autosomes (*X/A* ratio). However, the genes *dpy-21 V*, *dpy-22 X*, *dpy-23 X* and *dpy-26 IV*, which we shall refer to subsequently as *X*-dependent *dpy* genes, are unusual in this regard. Their mutant phenotypes are postulated to result from inappropriate expression of the *X* chromosome, for three reasons (HODGKIN 1983a; MENEELY and WOOD 1984). First, phenotypes of mutations in these genes resemble *X* chromosome aneuploidy in being both Dpy and inviable. Second, unlike other *dpy* genes, their phenotypes are altered by changes in *X/A* ratio in the normal range between 0.5 and 1.0. Third, they either enhance (*dpy-21*, *dpy-26*) or suppress (*dpy-22* and perhaps *dpy-23*) the effects of increased *X* dosage in *X* chromosome aneuploids and segmental aneuploids (MENEELY and WOOD 1984; P. MENEELY and W. B. WOOD, unpublished data).

The phenotypes resulting from mutations in the two autosomal genes *dpy-21* and *dpy-26* are similar in that *2A;1X* animals are non-Dpy, *2A;2X* animals are Dpy, and *2A;3X* animals are inviable. Therefore, they resemble *dpy+ 2A;2X*, *2A;3X* and *2A;4X* animals, respectively. The *dpy-26(n199)* mutation also results in maternal-effect lethality of *2X* embryos and a Him (*H*igh *i*ncidence of *m*ales) phenotype reflecting an increased frequency of *X* chromosome nondisjunction. Heterozygous *n199/+* hermaphrodites produce one-quarter Dpy self-progeny. However, these *n199/n199* animals lay many eggs that do not hatch, and the majority of their viable offspring are non-Dpy *1X* males (HODGKIN 1983a). A homozygous *n199/n199 2X* strain cannot be propagated.

The phenotypes resulting from mutations in the two *X*-linked genes *dpy-22* and *dpy-23* are generally similar to each other but different from *dpy-21* and *dpy-26*. For *dpy-22* and *dpy-23* mutants, *2X* animals are sickly and Dpy (distinguishable from *dpy-21* and *dpy-26* and usually from each other), with small brood sizes of fewer than 100. *1X* males are inviable for *dpy-23* mutants and variably so for *dpy-22* mutants. Mated *dpy-23* hermaphrodites produce no male progeny. For mated *dpy-22* hermaphrodites, less than 50% of the male progeny (expected from observed numbers of cross-progeny hermaphrodites) survive to adulthood as small, Dpy, sterile animals. For *dpy-22*, HODGKIN and BRENNER (1977) showed that the phenotype depended on the number of *X* chromosomes and not on physiological sex. There has been no similar demonstration for *dpy-23*.

In this paper, we present evidence suggesting that the normal function of *dpy-21* and *dpy-26* is to decrease expression of *X*-linked genes. In contrast, the normal function of *dpy-22* and perhaps *dpy-23* may be to increase expression of *X*-linked genes, although the evidence is less clear. Effects of mutations in the *X*-dependent *dpy* genes are seen in both *1X* and *2X* animals, suggesting that *X* chromosome expression could be controlled by a balance of positive and negative regulation in both sexes.

Our experimental approach can be outlined as follows. First, mutations in *X*-linked and autosomal genes were demonstrated to be either hypomorphic or null, based on variation or lack of variation, respectively, of the resulting phenotypes with allele dosage in *2X* animals. We also demonstrated dosage compensation for one of the *X*-linked genes, *lin-15*, whose hypomorphic mutant phenotype was easily quantitated. Then, in order to assay for specific effects of the *dpy* genes on *X* chromosome expression, mutations in each of the four *X*-dependent *dpy* genes were tested in both *1X* and *2X* animals for suppression or enhancement of phenotypes resulting from the *X*-linked hypomorphs, in particular the quantifiable *lin-15* mutations. In general, results with the other *X*-linked hypomorphs support those obtained with *lin-15*, but are less quantitative, and therefore somewhat less reliable. As controls, similar tests were carried out in *2X* animals on phenotypes resulting from null mutations in the same *X*-linked genes and from autosomal hypomorphs.

The experiments, although straightforward in principal, are complicated by two factors associated with the use of mutations in genes controlling cell lineages (*lin* genes), which provided some of the most useful hypomorphic alleles. First, some of the Lin phenotypes used are sex-influenced, making it necessary to employ sex transformer mutations so that *2X* and *1X* animals of the same physiological sex can be compared. Second, some of the hypomorphic *lin* mutations are synergistic alleles, that is, animals must carry two mutations, one in each of two different *lin* genes, in order to exhibit the mutant phenotype (FERGUSON and HORVITZ 1987) (see RESULTS for further explanation). Although these complications made strain construction more tedious and interpretation of some results more difficult, they did not appreciably diminish the usefulness of the *lin* mutations. A preliminary

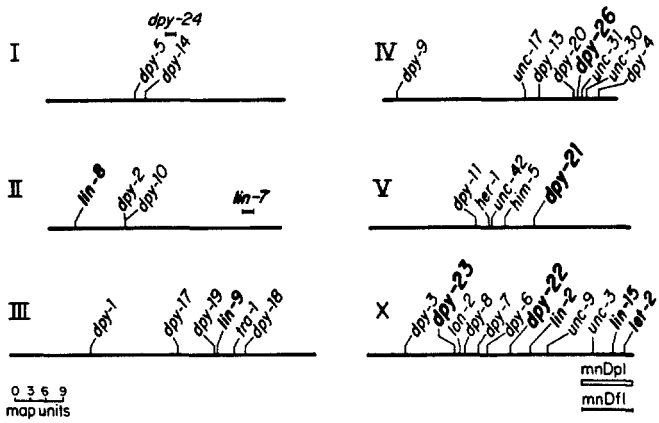

FIGURE 1.—Positions on the *C. elegans* genetic map of genes and rearrangements used in this study. Each of the six linkage groups is indicated by a *heavy horizontal line*, on which are shown positions of precisely mapped genes. *Horizontal bars* above these lines show intervals known to include map locations of other less precisely mapped genes, as indicated. *Double bars* and *single bars* below linkage groups indicate the extents of duplications and deficiencies, respectively. Genes indicated in small, **bold-faced** type are those for which hypomorphic alleles or putative hypomorphic alleles have been identified. Genes indicated in larger, ***bold-faced, italic*** type are the four *X*-dependent dpy genes.

account of some of these findings has appeared previously (WOOD *et al.* 1985).

MATERIALS AND METHODS

Genes, alleles, and strains of *C. elegans*

All strains are derived from N2, the Bristol strain of *C. elegans* (BRENNER 1974). The genes, alleles, and chromosomal rearrangements used are listed below by linkage group. Most have been described previously (MENEELY and HERMAN 1979, 1981; HODGKIN 1983a,b; MENEELY and WOOD 1984; FERGUSON and HORVITZ 1985); others are described in the text. Positions of these genes on the *C. elegans* genetic map, where known, are shown in Figure 1. Standard nomenclature for *C. elegans* genotypes and phenotypes is used, according to HORVITZ *et al.* (1979). Gene designations, based on mutant phenotypes are *dpy*, dumpy morphology; *her*, hermaphroditization (of *1X* animals); *him*, high incidence of males; *let*, lethal; *lin*, lineage defective; *lon*, long; *tra, transformer (of 2X* animals into males); *unc*, uncoordinated. The *lin* mutations used in this paper result in Multivulva (Muv), Vulvaless (Vul), and/or Egg-laying defective (Egl) phenotypes. Phenotypes are described subsequently in more detail where relevant.

LG I: *dpy-5(e61), dpy-14(e188), dpy-24(s71), lin(n833).*

LG II: *lin-8(n111), dpy-2(e8), dpy-10(e128), lin-7(e1413, n106).*

LG III: *dpy-1(e1), dpy-17(e164), dpy-19(e1259), lin-9-(n112), tra-1(e1099), dpy-18(e364).*

LG IV: *dpy-9(e12), unc-17(e245), dpy-13(e184), dpy-20-(e1282), dpy-26(n199), unc-31(e169), unc-30(e191), dpy-4-(1166).*

LG V: *dpy-11(e224), her-1(e1520), unc-42(e270), him-5 (e1467,e1490), dpy-21(e428,e459).*

LG X: *dpy-3(e27,e182), dpy-23(e840), lon-2(e678), dpy-8(e130), dpy-7(e88), dpy-6(e14), dpy-22(e652), lin-2-(e1309,n768), unc-9(e101) unc-3(e151), lin-15(n309,n374, n765,n767), let-2(mn114,mm153).* [The *dpy-3(e27)* mutation maps very close to and fails to complement *e182*, indicating that the latter, previously designated as the only *dpy-12* allele (BRENNER 1974), is in fact an allele of *dpy-3*.] The deficiencies *nDf19* and *mnDf1* include the *dpy-22* locus and the *unc3–let-2* interval, respectively. *mnDp1* is a duplication, linked to chromosome *V*, that includes the region of *X* deleted by *mnDf1*. *mnDp1/+;mmDf1* animals are viable. Unless otherwise indicated, mutant strains were obtained from the Caenorhabditis Genetics Center or from the Boulder collection. *dpy-21(e459)* was obtained from R. HORVITZ; *dpy-26(n199)* was obtained from J. HODGKIN as a balanced heterozygote with *unc-17(e245)*.

General techniques

Culturing, handling and genetic manipulation of *C. elegans* were carried out as described by BRENNER (1974).

Scoring of phenotypes resulting from hypomorphic alleles

***lin* mutants:** Phenotypes of the *lin* mutants have been described by FERGUSON and HORVITZ (1985) and will be summarized only briefly here. Mutations in *lin-2 X* and *lin-7 II* result in an egg-laying-defective (Egl) phenotype and in addition, for stronger alleles, a vulvaless (Vul) phenotype, both of which can be scored with the dissecting microscope. No attempt was made to distinguish these phenotypes in our experiments; animals showing either the Vul or Egl phenotype or both were scored as Lin in determinations of penetrance. Mutations in *lin-15 X* and combinations of mutations in *lin 8 II* and *lin-9 III* result in multiple pseudovulvae (Muv phenotype), visible in the dissecting microscope as protrusions on the ventral side of the animal (see Figure 2). *lin-15* animals, even if non-Muv often show a protrusion at the vulva. Therefore, only animals exhibiting two or more protrusions (i.e. at least one pseudovulva) were scored as Muv.

Severities of Lin and Muv phenotypes, which can vary with mutant allele and temperature, were compared based on penetrance (percent of mutant animals exhibiting the phenotype), determined by counting all of a particular class of progeny (for example all the male progeny) from single hermaphrodites. Uncertainties (95% confidence intervals) were determined using the normal deviate (z equivalent to χ^2), according to the formula $1.96\ (p*q/N)^{1/2}$, where p and q are the fraction of mutant and nonmutant animals, respectively. Expressivity of the Muv phenotype (size and number of pseudovulvae) also varies with *lin-15* allele and temperature (FERGUSON and HORVITZ 1985). Penetrance was used in these experiments to compare apparent levels of expression, because presence or absence of pseudovulvae was easier to determine and was less affected by morphological changes resulting from other mutations in the strains (*e.g.*, *dpy*) than were number and size of pseudovulvae.

***let* mutants:** *let-2* mutations cause early lethality or sterility (MENEELY and HERMAN 1981). Stages of arrest were judged from size and appearance of arrested larvae as observed under the dissecting microscope. Mutants carrying strong alleles die as embryos; mutants carrying weak alleles survive to become fourth stage larvae (L4) or sterile adults, which rarely lay eggs with an egg shell. Severities of Let phenotypes were compared based on expressivity, determined by observing the latest stage to which mutant animals survived and, for surviving adult hermaphrodites, measuring fertility.

Construction of multiply mutant strains

General constructions: Most strains were constructed by standard crosses and maintained as homozygous stocks. Sterile or inviable homozygotes were generated as segregants

from appropriate heterozygotes, as indicated in the table legends. Strains carrying *let-2* were maintained as marked homozygotes balanced by one copy of the duplication *mnDp1* (see Figure 1); for example, *mnDp1/+;unc-3 let-2*. For allele dosage experiments with mutations in *lin-15* and *let-2* (see Figure 1), deficiency heterozygotes were constructed by mating *mnDp1/+;mnDf1/0* males with hermaphrodites carrying the mutation and a marker allele not deleted by *mnDf1*; for example, *dpy-7 lin-15* hermaphrodites were mated, and the non-Dpy Lin hermaphrodite progeny, of genotype *dpy-7 lin-15/mnDf1*, were examined.

Generation of *3X* hermaphrodites and *1X* males from him strains carrying X-linked mutations: The recessive him mutations *him-5(e1467)* and *him-5(e1490)* cause increases in the frequency of *X* chromosome nondisjunction, leading to generation in hermaphrodites of both nullo-*X* and diplo-*X* gametes, which result in production of *1X* male and *3X* hermaphrodite self progeny, respectively (HODGKIN, HORVITZ and BRENNER 1979). *3X him-5* animals are recognized by their dumpy (Dpy) phenotype and rare male self progeny. *him-5(e1467)* hermaphrodites produce about 20% *1X* and 3% *3X* self progeny; *him-5(e1490)* hermaphrodites produce about 30% *1X* and 7% *3X* self progeny. In tests for allele dosage effects on *X*-linked mutations, *1X* and *3X* animals were obtained as male and Dpy hermaphrodite self progeny, respectively, of hermaphrodites homozygous for the hypomorphic allele and a *him-5* mutation. *1X* males were also obtained from crosses of wild-type males with hermaphrodites homozygous for an *X*-linked mutation. *3X* hermaphrodites carrying *let-2* mutations were obtained as Dpy Unc hermaphrodite progeny of *mnDp1 him-5(e1467)/ him-5(e1467);unc-3(e151) let-2* hermaphrodites.

Generation of *1X* hermaphrodites and *2X* males using sex reversal mutations: *1X lin-15* hermaphrodites were produced using the recessive sex reversal mutation *her-1(e1520)*, which transforms *1X* animals into fertile hermaphrodites (HODGKIN 1980). Males of genotype *her-1 him-5(e1490)/+ him-5(e1490);lin-15/0* were mated to hermaphrodites of genotype *her-1 unc-42(e270);lon-2(e678)lin-15*. [For *lin-15(n767)*, both strains were also homozygous for the autosomal mutation *n833*; see following section.] Cross progeny were recognized as non-Unc animals. The *lon-2* mutation is an *X*-linked recessive used to identify *1X* progeny by their long (Lon) phenotype; *lon-2* did not affect the penetrance of the *lin-15* Muv phenotype. From this cross, half the *1X* progeny are expected to be of genotype *her-1 unc-42/him-5;lon-2 lin-15/0*, which are Lon males. The other half of the *1X* progeny will be *her-1 unc-42/her-1 him-5;lon-2 lin-15/0*, which are Lon hermaphrodites homozygous for *her-1*. The penetrance of the *lin-15* Muv phenotype in *1X* animals was determined as the frequency of Lon Muv hermaphrodites among total Lon hermaphrodites. To determine the effect of *dpy-21* mutations on the penetrance of the *lin-15* Muv phenotype in *1X* hermaphrodites, the non-Dpy (*1X*) self progeny of *her-1 him-5(e1490) dpy-21;lin-15* animals were scored.

2X lin-15 males were produced using the recessive sex reversal mutation *tra-1(e1099)*, which transforms *2X* animals into fertile pseudomales (HODGKIN and BRENNER 1977). A strain with which to examine the penetrance of *lin(n833);lin-15(n767)* in *tra-1(e1099)* 2X pseudomales was constructed as follows. *tra-1* is closely linked to *dpy-18 III* (Figure 1); *lin(n833)* has been assigned to *LGI* (FERGUSON and HORVITZ 1987). *tra-1 2X* pseudomales were mated to *lin(n833);dpy-18;lin-15(n767)* hermaphrodites. Cross-progeny hermaphrodites of genotype *lin(n833)/+;dpy-18/tra-1; lin-15/+* were then allowed to self-fertilize, and Muv non-Dpy progeny were picked and maintained as a balanced heterozygous strain of genotype *lin(n833);dpy-18/tra-1;lin-15*. The penetrance of the *lin-15* Muv phenotype was measured in the *2X* pseudomale self progeny of these animals.

Construction of strains carrying *dpy-26* mutations: *dpy-26(n199) 2X* animals have few viable *2X* progeny and cannot be maintained as homozygotes (HODGKIN 1983a), as mentioned in the introduction. *2X* strains carrying this mutation were kept as balanced heterozygotes, for example *dpy-26/unc-17;lin-2*, *dpy-26/unc-30;lin-15(n765)*, or *lin-(n833);dpy-26/unc-31;lin-15(n767)*. Dpy progeny of these animals were scored to test the effects of *dpy-26* on penetrance of the Lin phenotypes. Therefore, we are testing the maternally rescued F_1 Dpy-26 phenotype, which may be weaker than if we had scored the few viable hermaphrodite F_2 progeny of these animals. When we looked among the viable F_2 progeny, the effect on the Lin-15(*n765*) phenotype was no stronger than among the F_1 animals, but only about 50 worms were examined. The three *unc* markers in trans to *dpy-26* were used interchangeably with no obvious differences in the results. To test *let-2* alleles, strains of genotype *dpy-26/unc-31;mnDp1/+;unc-3(e151) let-2* were constructed, and the viability and fertility of the Dpy Unc progeny were scored.

Because *2X dpy-26* homozygotes produce non-*Dpy 1X* animals among their few progeny (HODGKIN 1983a), it was possible to test the *dpy-26* mutation on *lin-15 1X* animals by scoring the male self-progeny of *dpy-26;lin-15* hermaphrodites. For some of these tests, *dpy-26;lin-15* males were also generated by mating *dpy-26* males to *dpy-26;lin-15* hermaphrodites. To test *dpy-26* effects in *lin-15 1X* hermaphrodites, strains of genotype *dpy-26;her-1-(e1520);lin-15(n309)*, *dpy-26;her-1;lin-15(n765)*, and *lin-(n833);dpy-26;her-1;lin-15(n767)* were constructed. From each of these strains a single *2X* Dpy hermaphrodite was picked and its *2X* Dpy and *1X* non-Dpy progeny were scored. For the latter (*n767*) strain, some experiments were done using *1X* hermaphrodites as the parents and examining their *2X* Dpy and *1X* non-Dpy progeny.

Construction of strains carrying *dpy-22* and *dpy-23* mutations: Because *dpy-22* and *dpy-23 1X* males are generally inviable and are infertile when they survive, *dpy-22* and *dpy-23* were used as the hermaphrodites in crosses for strain construction. For analysis of *dpy-22* effects in *1X* animals, a mutation in *him-5* was included because *him-5; dpy-22/0* males appear to survive at a somewhat higher frequency than *dpy-22/0* males generated by mating. This effect was not seen for *dpy-23* males. Many strains homozygous for *dpy-22* or *dpy-23* and another mutation were sickly and had low viability, especially at 25°. Therefore, in several of the tests for effects of *dpy-22* and *dpy-23* on phenotypes resulting from other hypomorphic alleles, the desired homozygotes were obtained as segregants from strains heterozygous for the *dpy-22* or *dpy-23* mutation, as indicated in the table legends. The unexpectedly low viability of some double mutant strains may mean that *dpy-22* and especially *dpy-23* have non-specific effects.

Identification of hypomorphic and null mutations in X-linked genes

***lin-2*:** Presumed null and hypomorphic alleles have been described by FERGUSON and HORVITZ (1985), and were further characterized here. The allele resulting in the highest penetrance of the Vul defect, *e1309* (89 ± 6%), was assumed to be a null allele, and the allele with the lowest penetrance, *n768* (38 ± 5%) was assumed to be a hypomorphic allele for these experiments. Consistent with this assumption, penetrance of the Lin phenotype in het-

eroallelic *n768/e1309* individuals was similar (92 ± 5%) to that seen in *e1309* homozygotes (see Tables 4 and 9).

***lin-15*:** Alleles at this locus were used extensively in these experiments, and further characterization was carried out to supplement the results of FERGUSON and HORVITZ (1985). The strongest known allele is *n309*; however, this allele is probably not a null for all aspects of the *lin-15* phenotype as shown by FERGUSON and HORVITZ (1985) and below. The weaker alleles *n765* and *n767* are temperature sensitive, resulting in penetrance of the Muv phenotype that is complete at 25°, reduced at 20°, and further reduced at 16°. For the purposes of this study, these alleles were characterized further as follows. For *lin-15(n309)* and *lin-15(n765)*, the character of the allele was determined by mating *mnDp1/+;mnDf1/0* males to *dpy-7(e88) unc-3 lin-15* hermaphrodites. *mnDf1* deletes *unc-3* and *lin-15* but not *dpy-7* (Figure 1). The penetrance of the Muv phenotype in the non-Dpy Unc progeny from this cross, of genotype *dpy-7 unc-3 lin-15/mnDf1*, was compared to the penetrance of the Muv phenotype of the corresponding *lin-15* homozygotes, grown at the appropriate growth temperature for at least one generation prior to testing. Many of the *lin-15/mnDf1* animals did not mature to adults, supporting the view that neither allele is truly null for all aspects of the *lin-15* phenotype. For the Muv phenotype among surviving animals, however, *n309* behaves as a null allele, and is designated as null or "operational null" throughout the remainder of this paper. In contrast, the Muv phenotype resulting from the *n765* allele behaves as a hypomorphic characteristic (see RESULTS, Table 2). To be sure that *dpy-7* did not contribute to the phenotype, the cross was also done using *dpy-18(e364);unc-3 lin-15* hermaphrodites. No differences were seen, and the results were pooled.

An additional complication in using *lin-15* mutants is that many alleles are synergistic mutations; that is, a mutation in another gene is also needed to produce a synthetic Muv phenotype (FERGUSON and HORVITZ 1987). The synergistic mutations can be divided into two classes, named A and B. Only strains homozygous for a mutation in each class have a Muv phenotype. The prototype mutations defining classes A and B are *lin-8(n111)* and *lin-9(n112)*, respectively (see Table 1). The only synergistic allele tested with *mnDf1* was *n767*, which is a class A allele; strains carrying this allele were also homozygous for *lin(n833)*, an autosomal class B mutation. Throughout this paper, strains homozygous for *lin-15(n767)* always are also homozygous for *lin(n833)*. To test the phenotype of *n767/mnDf1* animals, *lin(n833);him-5(e1490);lin-15(n767)/0* males were mated to *mnDp1/+;mnDf1* hermaphrodites. The cross-progeny males, of genotype *lin(n833)/+;mnDp1/him-5;mnDf1/0* were mated to *lin(n833);unc-9(e101) lin-15(n767)* hermaphrodites. The Muv non-Unc hermaphrodite progeny of this cross were then grown at the appropriate temperature. These animals have the genotype *lin(n833);unc-9 lin-15(n767)/mnDf1*. They are known to lack the duplication because *mnDp1* carries *lin-15+*, so that duplication-bearing animals would be non-Muv. The progeny of the Muv non-Unc hermaphrodites will include Muv non-Unc animals hemizygous for *lin(n767)* (*i.e.*, *n767/mnDf1*) like the parent, and Muv Unc animals homozygous for the *unc-9 lin-15(n767)* chromosome. Virtually no recombinant *unc-9+ lin-15(n767)* chromosomes are expected since *mnDf1* severely reduces or eliminates recombination in *unc-9 unc-3/mnDf1* animals, although *mnDf1* does not delete *unc-9* (P. MENEELY, unpublished data). The penetrance of the *lin-15* Muv phenotype was determined by scoring non-Unc animals. As shown in Table 2, the synergistic allele *n833;767* behaves as a hypomorph. The synergistic allele *lin-8(n111);lin-15(n374)* also behaves as a hypomorph, based on alleviation of the resulting Muv phenotype by increased *X* dosage (Table 3).

***let-2*:** MENEELY and HERMAN (1981) described null and hypomorphic mutations among *X*-linked lethals and steriles. The *let-2* mutations include hypomorphic and null alleles with conveniently distinguishable phenotypes. The hypomorphic allele *mn114* results in late larval lethality or adult sterility, whereas the null allele *mn153* results in embryonic lethality. The hypomorphic character of *let-2(mn114)* was determined in dosage experiments using *X*-linked deficiencies (MENEELY and HERMAN 1981). To retest these alleles, males of genotype *mnDp1/+;unc-3(e151) let-2/0* were mated to *mnDp1/+;mnDf1* hermaphrodites. The presence of non-Unc male progeny indicated success of the cross, and each cross was done three or more times because *mnDf1* is not efficiently transmitted via ova (MENEELY and HERMAN 1979). For the *let-2(mn114)* allele, no Unc progeny were observed, indicating that *mn114/mnDf1* heterozygotes die as embryos or young larvae. *mn114/mn153* animals are similarly inviable (see Table 9).

Identification of hypomorphic and null mutations in autosomal genes

***lin-7 II*:** FERGUSON and HORVITZ (1985) concluded that *e1413* is a null allele (resulting Vul phenotype more than 90% penetrant) whereas *n106* is hypomorphic (resulting Vul phenotype about 50% penetrant).

***lin- 8 II* and *lin-9 III*:** Synthetic mutants homozygous for both a *lin-8* and a *lin-9* mutation show a Muv phenotype; either mutation alone results in a non-mutant phenotype. FERGUSON and HORVITZ (1985) have suggested that *lin-8(n111)* is hypomorphic based on dosage experiments using a deficiency that deletes *lin-8*; animals heterozygous for *lin-8(n111)* and the deficiency and homozygous for *lin-9(n112)* showed increased sterility and sickness compared to *lin-8(n111);lin-9(n112)*. The *lin-9(n112)* was concluded to be hypomorphic based on comparison with a stronger *lin-9* allele, which results in a sterile phenotype.

RESULTS

Identification of hypomorphic alleles of *X*-linked and autosomal genes: By definition, for a hypomorphic mutant allele *m*, a heterozygote carrying *m* in trans with either a deficiency of the locus (*m/Df*) or a null allele of the same gene (*m/null*) is more severely mutant than the homozygote *m/m*, which in turn may be more severely mutant than a duplication or aneuploid strain carrying three copies of the mutant allele (*m/m/m*). In contrast, null alleles, which result in complete loss of function, do not show dependence of phenotype on dose (MULLER 1950). In general, these criteria were applied in identifying hypomorphic and null alleles of three *X*-linked and three autosomal genes.

The identifications of these alleles, as well as their resulting phenotypes are summarized in Table 1. Designations in the table are based on published results of others as well as our observations, details of which are described in MATERIALS AND METHODS. Some of the supporting data, where relevant, are included in subsequent tables. The *lin-15* hypo-

TABLE 1

Phenotypes resulting from null and hypomorphic alleles in several X-linked and autosomal genes

Gene	Null allele: resulting phenotype[a]	Hypomorphic allele[b]: resulting phenotype[a]
X-linked genes		
let-2 V	*mn153*: embryonic lethal	*mn114*: sterile adult
lin-2 X	*e1309*: 89% Lin	*n768*: 38% Lin
lin-15 X	*n309*[c]: Muv	*n765*: Muv, *ts* *n374*: synergistic Muv[d] *n767*: synergistic Muv, *ts*[e]
Autosomal genes		
lin-7 II	*e1413*: 95% Lin	*n106*: 50% Lin
lin-8 II	None: sterile?[f]	*n111*: synergistic Muv
lin-9 III	None: sterile?[f]	*n112*: synergistic Muv

[a] For more detailed descriptions, see MATERIALS AND METHODS.
[b] Criteria by which these alleles were concluded to be hypomorphic are described in MATERIALS AND METHODS.
[c] This allele is used throughout these experiments as an operational null allele with regard to the resulting Muv phenotype; however, it probably does not result in complete loss of all *lin-15* functions (see MATERIALS AND METHODS and text).
[d] A class-B *lin-15* allele (see text).
[e] A class-A *lin-15* allele (see text).
[f] No null allele identified; FERGUSON and HORVITZ (1985) have obtained evidence that the null phenotype is sterile (see text).

morphic alleles *n374* and *n767* are synergistic alleles: as explained in the introduction, their phenotypes depend upon a second mutation in an autosomal *lin* gene, as well as the X-linked *lin-15* mutation (see MATERIALS AND METHODS). Synergistic mutations causing the Muv phenotype fall into two classes, termed A and B (FERGUSON and HORVITZ 1987). In order for the Muv phenotype to be expressed, a strain must be homozygous for both a class A and a class B mutation. *lin-15* is unusual among genes with synergistic Muv mutations in that some alleles are of class A (*e.g.*, *n767*) and others are of class B (*e.g.*, *n374*).

Demonstration of X-chromosome dosage compensation: To test for dosage compensation, the severities of phenotypes resulting from hypomorphic alleles of X-linked genes were compared in *1X* and *2X* animals. Assuming that severity of such a phenotype reflects the level of expression of the mutant gene, then *1X* animals should show a phenotype similar to that of *2X* animals if there is dosage compensation, and a more severe phenotype if there is not. In general, phenotypes resulting from the X-linked hypomorphs tested appeared to be the same in *1X* and *2X* animals. For example, homozygous *let-2(mn114)/let-2(mn114)* hermaphrodites grow to be sterile adults, whereas hemizygous *let-2(mn114)/mnDf1* hermaphrodites, with only one copy of the hypomorphic allele, die as embryos or L1 larvae (MENEELY and HERMAN 1981; MATERIALS AND METHODS), showing that the lethal phenotype resulting from *mn114* is dose-dependent in *2X* animals. However, hemizygous *let-2(mn114)/0* males grow to adulthood, despite having only one copy of the hypomorphic allele, indicating compensation for the difference in dosage of this gene between *1X* and *2X* animals.

More detailed experiments were carried out with *lin-15* hypomorphic alleles, which result in temperature-sensitive phenotypes that provide more quantitative measures of expression (Table 2). Mutations in *lin-15* result in multiple pseudovulvae (Muv phenotype; see MATERIALS AND METHODS), visible as a series of protrusions along the ventral side of the animal (Figure 2). Penetrance of the Muv phenotype is often incomplete (<100%) and shows different reproducible values characteristic of different alleles and temperatures. Table 2 shows the evidence that penetrance of the Muv phenotype resulting from either of the two hypomorphic alleles *n765* or *n767* is dose-dependent in hermaphrodites (columns 1, 2, and 3), whereas the Muv phenotype resulting from *n309*, used as the control null allele in these experiments, is not dose-dependent (see MATERIALS AND METHODS).

A complication with comparing penetrance in *1X* male and *2X* hermaphrodites is that the Muv phenotypes resulting from several *lin-15* alleles are sex-influenced. For example, the phenotype resulting from the hypomorphic allele *n767* is almost completely sex-limited to hermaphrodites at 16° and 20°; likewise the phenotype resulting from *lin-15(n765)* is less penetrant in males than in hermaphrodites at 20° (Table 2). Therefore, comparisons were made between *1X* and *2X* animals of the same sex, making use of appropriate sex-reversal mutations as described in MATERIALS AND METHODS. *1X* hermaphrodites homozygous for *her-1(e1520)* and *2X* hermaphrodites showed similar penetrance of the Muv phenotypes resulting from two different hypomorphic *lin-15* alleles, *n765* and *n767*. Although the *1X* hermaphrodites (column 5) have only one copy of the mutant allele, their phenotypic response clearly resembles that of *2X* hermaphrodites with two copies (homozygotes; column 2), rather than that of *2X* hermaphrodites with one copy (heterozygous for the hypomorphic allele and a null allele; column 1). Control experiments showed that the *her-1* mutation has no significant effect on penetrance of the Muv phenotype in *2X* animals: *her-1;lin-15(n765)* and *lin(n833);her-1;lin-15(n767) 2X* hermaphrodites gave penetrance values of 10% (N = 168) and 26% (N = 242), respectively.

1X males were also compared with *2X* males homozygous for *tra-1(e1099)*. Among *2X* males of genotype *lin(n833);tra-1;lin-15(n767)*, 5% (N = 171) were mutant at 20°. This penetrance is not significantly

TABLE 2

Gene dosage effects on penetrance of the Muv phenotype resulting from three alleles of *lin-15 X*[a]

Allele	Temperature	(1) *m*/null[b]	(2) *m/m*	(3) *m/m/m*	(4) *m*/0 ♂	(5) *m*/0 ♀
n309[d]	16°	100 (55)	100 (243)	100 (22)	100 (46)	100 (81)
n765[e]	16°	98 (179)	9 (402)	1 (137)	0 (81)	14 (108)
	20°	100 (83)	100 (364)	65 ± 13 (49)	65 ± 10 (96)	99 (79)
	25°	100 (90)	100 (164)	98 (47)	100 (93)	100 (130)
n833;n767[e,f]	16°	95 (637)	30 (389)	7 (66)	0 (350)	21 (103)
	20°	100 (208)	100 (1046)	47 (138)	1 (226)	96 (110)
	25°	100 (418)	100 (1089)	96 (132)	24 (248)	99 (67)

[a] Penetrance values are expressed as percent of animals with multiple pseudovulvae (Muv). Uncertainties (95% confidence limits) are ≤ ± 8% unless indicated otherwise. After each value is shown in parentheses the total number of animals examined.

[b] *m* represents the indicated *lin-15* allele. The *m*/null strains were of genotype *lin-15 mnDf1*.

[c] Homozygous for *her-1(e1520)*.

[d] Operational null allele (see MATERIALS AND METHODS and text). Penetrance of the Muv phenotype at 20° and 25° was 100% for all *n309* genotypes.

[e] Hypomorphic allele (see MATERIALS AND METHODS).

[f] Synergistic allele (see MATERIALS AND METHODS and text).

different from that seen in *1X lin(n833);lin-15(n767)* males (Table 2, column 4). *1X tra-1* males were not tested.

In summary, these results demonstrate that *lin-15* is dosage compensated, because *1X* and *2X* hermaphrodites exhibit the same penetrance of the Muv phenotype resulting from two different hypomorphic *lin-15* alleles. They also suggest that *her-1* and *tra-1* mutations, which alter sexual phenotype, do not affect dosage compensation.

Compensation of the *lin-2* gene, whose mutant phenotype is also sex-limited to hermaphrodites, was not tested.

***dpy-21* and *dpy-26* effects in *2X* animals:** The mutant phenotypes of two autosomal *X*-dependent dpy genes, *dpy-21* and *dpy-26* have been postulated to result from inappropriately high levels of *X*-chromosome expression, as reviewed in the introduction. For *dpy-21*, this has been confirmed by demonstration of elevated transcript levels for several *X*-linked genes in *dpy-21* mutants (MEYER and CASSON 1986; DONAHUE, QUARANTILLO and WOOD 1987); consistent with these results, the following experiments demonstrate that *dpy-21* and *dpy-26* mutations suppress phenotypes resulting from *X*-linked hypomorphic alleles, whereas these *dpy* mutations do not suppress similar phenotypes resulting either from null alleles of the same *X*-linked genes or from autosomal hypomorphic alleles.

Quantitative experiments were carried out using several alleles of *lin-15* and observing effects on penetrance of the resulting Muv phenotype. The results are presented in Table 3. Either an increase in allele dosage in the form of a third *X* chromosome or the presence of the *dpy-21(e428)* or *dpy-26(n199)* mutation substantially decreases the severity of phenotypes resulting from the hypomorphs *lin-15(n765)*, *lin-15(767)* and *lin-15(n374)* (particularly apparent at 16° and 20°), but does not affect the phenotype resulting from the operational null mutation (see MATERIALS AND METHODS) *lin-15(n309)*. The presence of a third *X* chromosome decreases severity of phenotype less strongly than does either of the two *X*-dependent *dpy* mutations, which show similar levels of suppression. A second allele of *dpy-21, e459*, tested with *lin-15(n765)* and *lin-15(n767)*, gave levels of suppression indistinguishable from those observed with *dpy-21(e428)* (data not shown).

The synergistic Muv mutations tested included an allele of each class: *n767* is of class A and *n374* is of class B. For both alleles, the penetrance of the resulting phenotype is about equally decreased by increased *X* dosage or by either of the two *X*-dependent *dpy* mutations. Two other class B *lin-15* alleles, *n743* and *n744*, were also suppressed by *dpy-21(e428)* (data not shown). Because these *lin-15* alleles are synergistic mutants, it could be argued that suppressor effects in these strains are not on expression of the *X*-linked *lin-15* allele, but rather on expression of the autosomal mutation or on the combination of *X*-linked and autosomal mutations. However, the suppression seen with the non-synergistic allele *lin-15(n765)* argue against this interpretation, as does the observation presented below that *dpy-21* and *dpy-26* mutations appear to suppress only *X*-linked and not autosomal hypomorphs.

Less quantitative analysis of *dpy-21* and *dpy-26* effects on two other *X*-linked hypomorphs and the corresponding null mutants is summarized and compared with the effects of increased *X* dosage in Table 4. In one case, presence of a third *X* chromosome or either of the two *X*-dependent *dpy* mutations decreased the severity of the phenotype resulting from the hypomorphic allele tested and did not affect the

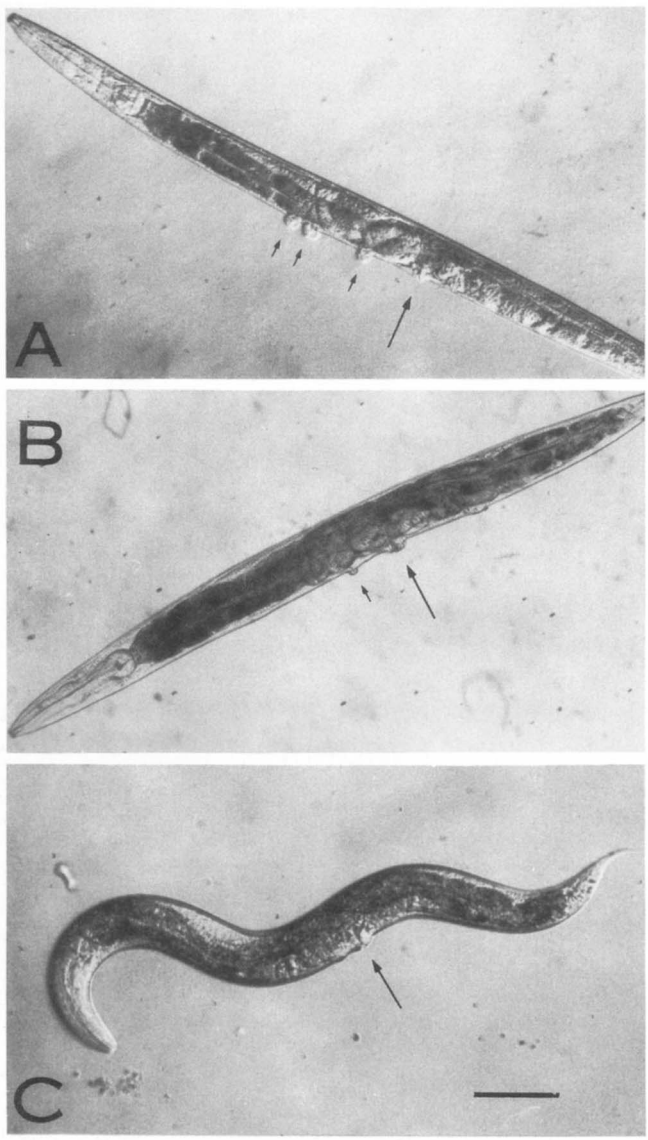

FIGURE 2.—Brightfield photomicrographs of hermaphrodites homozygous for the synergistic hypomorphic allele *lin(n833);lin-15(n767)*, which causes production of multiple pseudovulvae (Muv phenotype). (A) A *2X* hermaphrodite, with small arrows indicating the pseudovulvae and a large arrow indicating the vulva. (B) A *3X* hermaphrodite of genotype *lin(n833);him-5(e1490);lin-15(n767)*, identified by its Dpy phenotype; despite the reduction in both number and size of the pseudovulvae, this animal would be scored as mutant. (C) A *dpy-21 2X* hermaphrodite of genotype *lin(n833); dpy-21(e428);lin-15(n767)*; this animal lacks pseudovulvae. Animals were reared at 20°. For construction of these strains, see MATERIALS AND METHODS. Photographed with a Zeiss Universal microscope; scale bar indicates 100 μm.

phenotype resulting from the null allele tested. Suppression of *let-2(mn114)* was seen as alleviation of sterility, with production of a few viable progeny embryos. In the other case, no suppression was apparent with the assays employed. Penetrance of the Lin phenotype resulting from *lin-2(n768)* was not decreased either by presence of a third *X* chromosome or by either of the *X*-dependent *dpy* mutations. We do not know if *lin-2* is subject to dosage compensation.

In order to determine whether the suppressor effects observed with *dpy-21* and *dpy-26* mutations are specific for *X*-linked hypomorphs, tests for suppression were carried out with autosomal hypomorphic mutations that result in a Muv phenotype similar to that resulting from *lin-15* mutations or a Lin phenotype similar to that resulting from *lin-2* mutations. Effects of *dpy-21(e428)* and *dpy-26(n199)* on penetrance of the Lin phenotype resulting from the hypomorphic allele *lin-7(n106) II* and on penetrance of the Muv phenotype resulting from the synergistic hypomorphic allele *lin-8(n111) II;lin-9(n112) III* are shown in Table 5. Neither of the two *X*-dependent *dpy* mutations appeared to affect penetrance of either phenotype. The results suggest that in general, *dpy-21* and *dpy-26* mutations do not suppress autosomal hypomorphs. Interpretation of these experiments is subject to the caveat that although the phenotypes resulting from the *lin-7* and *lin-8;lin-9* hypomorphic alleles are known to be enhanced in heterozygotes with a deficiency or null allele, that is, by removal of one copy of the mutant gene (MATERIALS AND METHODS), they are not known to be decreased in severity by addition of an extra copy of the mutant gene.

***dpy-21* and *dpy-26* effects in *1X* animals:** Both *dpy-21* and *dpy-26* males are non-Dpy, fertile animals that are essentially wild type in phenotype, although *dpy-26* males (and *dpy-26;her-1 1X* hermaphrodites) are sometimes less fertile than the corresponding *dpy-26+* strains (HODGKIN 1983a). These observations suggest that *dpy-21* and *dpy-26* might be almost or completely inactive in *1X* animals (HODGKIN 1983a). This possibility was tested by examining the effects of *dpy-21* and *dpy-26* mutations on *lin-15 1X* males and *her-1;lin-15 1X* hermaphrodites, with the results shown in Table 6. For *lin-15(n765)* animals at both 20° and 25°, for *lin-15(n767)* animals at 25°, and for animals carrying the operational null allele (see MATERIALS AND METHODS) *lin-15(n309)* at all growth temperatures, a substantial fraction of the males show the Muv phenotype (Table 6A, column 1). For both *n765* and *n767*, but not for *n309*, this phenotype is significantly suppressed by *dpy-21(e428)* (Table 6A, column 2). The effect of *dpy-26(n199)* is less clear (column 3); it appears to suppress the Muv phenotype significantly in *lin-15(n765)* animals, but not in *lin-15(n767)* animals.

To examine the effects of these mutations on *lin-15(n767) 1X* hermaphrodites, the strains *lin(n833); dpy-26(n199);her-1(e1520);lin-15(n767)* and *lin(n-833);her-1 him-5(e1490) dpy-21(e428);lin-15(n767)* were constructed, and their non-Dpy progeny were scored for the Muv phenotype. For *him-5 dpy-21* strains, these non-Dpy animals are *1X* individuals that

TABLE 3

Effects of increased *X* dose and mutations in genes *dpy-21* and *dpy-26* on penetrance of the Muv phenotypes resulting from four alleles of *lin-15* in hermaphrodites[a]

lin-15 allele	Temperature	(1) *dpy+*	(2) *dpy+ 3X*	(3) *dpy-21 2X*	(4) *dpy-26 2X*
n309	16°	100 (243)	100 (22)	100 (54)	100 (143)
n765	16°	9 (402)	1 (137)	3 (112)	1 (120)
	20°	100 (364)	65 ± 13 (49)	49 (240)	46 (174)
	25°	100 (164)	98 (47)	100 (184)	100 (45)
n833;n767[c]	16°	30 (389)	7 (66)	6 (414)	10 (473)
	20°	100 (1046)	47 (138)	17 (608)	19 (402)
	25°	100 (1089)	96 (132)	96 (611)	97 (271)
n111;n374[b]	16°	5 (226)	0 (26)	2 (44)	9 ± 12 (23)
	20°	96 (481)	46 ± 13 (54)	25 ± 9 (97)	17 ± 11 (47)
	25°	100 (108)	100 (16)	100 (18)	95 (57)

[a] *X*-dependent *dpy* mutations used were *dpy-21(e428)* and *dpy-26(n199)*. Penetrance values are expressed as percent of animals with multiple pseudovulvae (Muv). Uncertainties (95% confidence limits) were ≤ ± 8% unless indicated otherwise. After each value is shown in parentheses the total number of animals examined.

[b] Operational null allele (see MATERIALS AND METHODS and text). Penetrance of the Muv phenotype at 20° and 25° was 100% for all *n309* genotypes.

[c] *n767* is a class-A and *n374* is a class-B synergistic allele of *lin-15* (see MATERIALS AND METHODS and text).

TABLE 4

Effects of increased *X* dose and mutations in *dpy-21* and *dpy-26* on phenotypes resulting from null and hypomorphic alleles three *X*-linked genes in hermaphrodites[a]

Gene (allele)	*2X*	*3X*	*dpy-21 2X*	*dpy-26 2X*
let-2(mn153)[b]	Embryonic lethal	Embryonic lethal	Embryonic lethal	—[d]
let-2(mn114)[c,e]	Sterile adult	Slightly fertile	Slightly fertile	Slightly fertile
lin-2(e1309)[b,f]	89% ± 6% Lin[b]	90% ± 6% Lin	91% ± 6% Lin	90% ± 6% Lin
lin-2(n768)[c]	36% ± 5% Lin	50% ± 14% Lin	41% ± 5% Lin	40% ± 8% Lin

[a] See MATERIALS AND METHODS for more complete descriptions of phenotypes. The *X*-dependent *dpy* mutations used were *dpy-21(e428)* and *dpy-26(n199)*.

[b] Null allele (see MATERIALS AND METHODS).

[c] Hypomorphic allele (see MATERIALS AND METHODS).

[d] Not determined.

[e] Self-fertility assay with *let-2(mn114)* hermaphrodites gave the following results. *2X* animals, generated as Unc segregants of *mnDf1/+; unc-3(e151) let-2(mn114)*: 10 hermaphrodites laid an average of 7 embryos each, none of which were viable. *3X* animals, generated as Dpy Unc segregants of *mnDp1 him-5(e1467)/him-5(e1467);unc-3(e157)let-2(mn114)* (see MATERIALS AND METHODS): 10 hermaphrodites produced an average 9 viable progeny each, which survived to adulthood. *dpy-21 2X* animals: 13 animals produced an average of 19 viable progeny each, which survived to adulthood. A *dpy-21;unc-3(e151) let-2(mn114)* strain was maintained for three generations before drying out, whereas an *unc-3(e151)let-2(mn114)* strain could not be maintained. A *dpy-26;let-2(mn114)* animal was also constructed (see MATERIALS AND METHODS) and found to produce viable progeny. However, this strain could not be maintained beyond the first generation, as expected because of the *dpy-26* mutation.

[f] For determining penetrance in *lin-2(e1309)* strains, between 100 and 110 animals of each genotype were examined. For *lin-2(n768)* strains, numbers of animals examined were as follows: *2X*, 418; *3X*, 52; *dpy-21 2X*, 351; *dpy-26 2X*, 156.

are produced at a frequency of about 30% as a consequence of nondisjunction resulting from the *him-5(e1490)* mutation (HODGKIN, HORVITZ and BRENNER 1979); they are transformed into hermaphrodites by the *her-1* mutation (HODGKIN 1980). For *dpy-26* strains, no *him* mutation is needed to produce *1X* animals, because the *n199* mutation itself causes a high frequency of nondisjunction (HODGKIN 1983a). The results tabulated in Table 6B (column 2) show that a *dpy-21* mutation substantially suppresses the Muv phenotype resulting from the hypomorphic mutation *lin-15(n767)* in *1X* hermaphrodites. Again the *dpy-26* mutation suppressed the Muv phenotype only slightly in these animals.

Also examined in these experiments was the effect of maternal *X*-chromosome number on *dpy-26* suppression of *lin-15(n767)* (data not shown). There was no significant difference in the effect of *dpy-26* on the *lin-15(n767) 1X* hermaphrodite progeny of *1X vs. 2X* hermaphrodites.

However, suppression of *lin-15* hypomorphs by both *dpy-21* and *dpy-26* mutations is less pronounced in *1X* hermaphrodites than in *2X* hermaphrodites, as seen by comparison of results from Tables 3 and 6B. This difference is not due to the *her-1* mutation, as shown in Table 7. Suppression is significantly weaker in *lin(n833);her-1;lin-15(n767) 1X* hermaphrodites

TABLE 5

Effects of *X*-dependent *dpy* mutations on penetrance of mutant phenotypes resulting from autosomal hypomorphic alleles in *2X* hermaphrodites[a]

Genes (alleles)	*dpy+*	*dpy-21*	*dpy-26*	*dpy-22*	*dpy-23*
lin-7(n106)[b]	49 ± 9 (116)	49 ± 9 (114)	41 ± 16 (37)	56 ± 8 (156)	50 ± 20 (24)
lin-8(n111);lin-9(n112)[c]	100 (71)	100 (155)	100[d] (108)	92 (164)	100[e] (132)

[a] *X*-dependent *dpy* mutations used were *dpy-21(e428)*, *dpy-26(n199)*, *dpy-22(e652)*, and *dpy-23(e840)*. Penetrance values are expressed as per cent of animals with the appropriate mutant phenotype. Uncertainties shown are 95% confidence limits. After each value is shown in parentheses the total number of animals examined.

[b] Hypomorphic allele resulting in a vulval-defective (Lin) phenotype; see Table 1 and MATERIALS AND METHODS for further descriptions of phenotypes and strain constrictions.

[c] Synergistic hypomorphic allele resulting in a multiple pseudovulvae (Muv) phenotype; see MATERIALS AND METHODS.

[d] The *lin-8;lin-9dyp-26* animals were Dpy progeny of *lin-8;lin-9;dpy-26/unc-31*.

[e] The *lin-8;lin-9;dpy-23* animals were Dpy progeny of *lin-8;lin-9;dpy-23/+*.

TABLE 6

Effects of *dpy-21* and *dpy-26* mutations on penetrance of the Muv phenotype in *lin-15 1X* males and *her-1;lin-15 1X* hermaphrodites[a]

lin-15 allele	Temperature	(1) *dpy+*	(2) *dpy-21*	(3) *dpy-26*
A. *lin-15 1X* males				
n309[b]	16°	100 (46)	100 (64)	100 (33)
n765	16°	0 (81)	0 (121)	0 (109)
	20°	65 (96)	25 (148)	25 (120)
	25°	100 (93)	100 (69)	56 (34)
n833;n767[c]	16°	0 (350)	0 (63)	0 (65)
	20°	1 (226)	0 (371)	0 (193)
	25°	24 (248)	11 (229)	21 (132)
B. *her-1;lin-15 1X* hermaphrodites				
n833;n767[c,d]	16°	21 (103)	7 (236)	27 (45)
	20°	96 (110)	31 (235)	81 (124)
	25°	99 (67)	83 (152)	90 (11)

[a] *X*-dependent dpy mutations used were *dpy-21(e428)* and *dpy-26(n199)*. Penetrance values are expressed as percent of animals with multiple pseudo-vulvae (Muv). Uncertainties (95% confidence limits) were ≤ ± 8%. After each value is shown in parentheses the total number of animals examined.

[b] Operational null allele (see MATERIALS AND METHODS and text). Penetrance of the Muv phenotype at 20° and 25° was 100% for all *n309* genotypes.

[c] Synergistic allele (see MATERIALS AND METHODS and text).

[d] Progeny of *2X* hermaphrodites.

TABLE 7

Comparison of *dpy-21* and *dpy-26* effects on penetrance of the Muv phenotype in *lin(n833);her-1;lin-15(n767) 1X* and *2X* hermaphrodites and *lin(n833);lin-15(n767) 2X* hermaphrodites at 20°[a]

her-1 genotype and karyotype	(1) *dpy+*	(2) *dpy-21*	(3) *dpy-26*
her-1 1X	96 ± 4 (110)	31 ± 6 (235)	85 ± 4[b] (260)
her-1 2X	100 (434)	12 ± 4 (350)	26 ± 3 (217)
her-1+ 2X	100 (1046)	17 ± 3 (608)	19 ± 4 (402)

[a] *X*-dependent *dpy* mutations used were *dpy-21(e428)* and *dpy-26(n199)*. Penetrance values are expressed as percent of animals with multiple pseudovulvae. Uncertainties are shown as 95% confidence limits. After each value is shown in parentheses the total number of animals examined.

[b] Combined results from *1X* and *2X* mothers.

than in either *lin(n833);her-1;lin-15(n767) 2X* or *lin(n833);her-1+;lin-15(n767) 2X* hermaphrodites. The difference is striking for the *dpy-26* mutation, which appears to show little suppression of *n767* in *1X* animals but does suppress *lin-15(n765)* (Table 6). It is less striking for the *dpy-21* mutation, for which a significant difference in expression can be seen only at 20°. The findings that suppression is stronger in *2X* than in *1X* animals are consistent with the observation that the Dpy morphology of *dpy-21* and *dpy-26* mutants is limited to *2X* animals.

In summary, the *dpy-21* and *dpy-26* mutations suppress *X*-linked hypomorphs strongly and to approximately equal extents in *2X* animals. In *1X* animals these mutations suppress less strongly and to different extents: mutations in *dpy-21* show consistent intermediate levels of expression, whereas a *dpy-26* mutation shows inconsistent effects, suppressing one of the *X*-linked hypomorphs tested equally as strongly as the *dpy-21* mutations and the other hypomorphs much less strongly. Differences in the *dpy-21* and *dpy-26* effects could result from a difference in the time of expression of these genes and from a maternal effect: *n765* shows a strong maternal effect, whereas *n767* does not; the suppression of *n765* could result from a *dpy-26* effect in the *2X* parent.

***dpy-22* and *dpy-23* effects in *2X* animals:** The phenotypes of *dpy-22* and *dpy-23* mutants do not correlate with that of any known *X*-chromosome aneuploid. However, the low viability of *dpy-22* males and the inviability of *dpy-23* males (HODGKIN 1983a) suggested that both the *dpy-22* and *dpy-23* mutations affect *X* expression, resulting in inviability of *1X* animals and a sickly, Dpy phenotype in *2X* animals. A persistant difficulty in working with *dpy-22* and *dpy-23* is that the animals are very sickly, and the phenotypes quite variable. The effects of *dpy-22* and *dpy-23* mutations on *X*-chromosome expression in *2X* animals were tested as before by building multiply

TABLE 8

Effects of *dpy-22* and *dpy-23* mutations on penetrance of the Muv phenotype resulting from three alleles of *lin-15* in 2X hermaphrodites[a]

lin-15 allele	Temperature	(1) *dpy+*	(2) *dpy-22*	(3) *dpy-23*
n309[b]	16°	100 (243)	100 (206)	100 (174)
n765	16°	9 (402)	74 (277)	52 (132)
	20°	100 (364)	100 (559)	100 (163)
n833;n767[c]	16°	30 (389)	97 (110)	66 (193)
	20°	100 (1046)	100 (237)	100 (168)

[a] X-dependent *dpy* mutations used were *dpy-22(e652)* and *dpy-23(e840)*. Penetrance values are expressed as percent of animals with multiple pseudovulvae (Muv). Uncertainties (95% confidence limits) were ≤ ± 7%. After each value is shown in parentheses the number of animals examined. All three alleles show 100% penetrance at 25°C. See text for further explanation.

[b] Operational null allele (see MATERIALS AND METHODS and text). Penetrance of the Muv phenotype at 20° was 100% for all *n309* genotypes.

[c] Synergistic allele (see MATERIALS AND METHODS and text).

mutant strains carrying one of the two X-dependent *dpy* mutations and X-linked hypomorphic mutation. In general, both *dpy-22* and *dpy-23* mutations enhance the phenotypes resulting from X-linked hypomorphs.

Two *lin-15* hypomorphs, *n765* and *n767*, show striking increases in penetrance of the mutant Muv phenotype at 16° as a result of a *dpy-22* or *dpy-23* mutation (Table 8). For *n765*, both of the increased values are still somewhat lower than the penetrance of 98% seen in the hemizygous strain *lin-15(n765)/mnDf1* (Table 2). For the synergistic hypomorph *lin(n833);lin-15(n767)*, the increased penetrance resulting from the *dpy-22* mutation is about the same as the value of 95% seen in the hemizygous strain *lin(n833);lin-15(n767)/mnDf1* (Table 2); the increase in penetrance resulting from the *dpy-23* mutation is somewhat less.

Enhancement of the operational null allele (see MATERIALS AND METHODS) *lin-15(n309)* could not be tested using the resulting Muv phenotype, because *lin-15(n309)* strains already show 100% penetrance. The *dpy-22 lin-15(n309)* and *dpy-23 lin-15(n309)* strains grew very poorly. Their low viability could be interpreted as enhancement of *lin-15(n309)*, since the true null phenotype of *lin-15* may be inviability (FERGUSON and HORVITZ 1985; see MATERIALS AND METHODS). However, it could also be due to sickness of these strains resulting from the presence of two generally deleterious mutations.

Tests for *dpy-22* and *dpy-23* effects with two other hypomorphic alleles are summarized in Table 9, which compares the effects of the two X-dependent dpy mutations with the effect of decreased dosage of the hypomorphic allele when heteroallelic with the corresponding null allele. Clear effects of *dpy-22* and *dpy-23* mutations were seen in tests with a *lin-2* hypomorphic allele. As shown in Table 9, both X-dependent dpy mutations enhance the penetrance of the Lin phenotype resulting from *lin-2(n768)*, though not as strongly as does the twofold decrease in dosage of the hypomorphic allele in a heteroallelic *lin-2(n768)/lin-2(e1309)* strain. Both the doubly mutant strains were quite sick, and the *dpy-23 lin-2(n768)* strain could not be maintained for more than a few generations in culture. For *let-2(mn114)*, enhancement was seen as an earlier effective lethal phase: *dpy-22 let-2(mn114)* homozygotes die at an early larval stage, either in the egg or at L1, similarly to *let-2(mn114)/let-2(mn153)* animals but unlike *let-2(mn114)/let-2(mn114)* homozygotes, which develop into sterile adults. However, *dpy-23 let-2(mn114)* homozygotes also survive to become sterile adults, indicating that the *dpy-23* mutation does not appreciably enhance *let-2(mn114)*.

It is important to stress that *dpy-22* and *dpy-23 2X* animals are themselves slow-growing and sluggish, and have low brood sizes. It is possible that the results with *let-2* could be due to a non-specific effect of having several deleterious mutations in the same strain. The effects seen with *lin-15* and *lin-2* hypomorphs, however, may represent specific enhancement of the resulting phenotypes.

In order to determine whether the enhancement effects observed with *dpy-22* and *dpy-23* mutations are restricted to X-linked hypomorphs, tests were also carried out with autosomal hypomorphs. Effects of *dpy-22(e652)* and *dpy-23(e840)* on penetrance of the Lin phenotype resulting from the hypomorphic allele *lin-7(n106) II* and on penetrance of the Muv phenotype resulting from the synergistic hypomorphic allele *lin-8(n111) II;lin-9(n112) III* are shown in Table 5. Neither of the two X-dependent *dpy* mutations appeared to affect penetrance of the Lin phenotype resulting from *lin-7(n106)*. Because the penetrance of the Muv phenotype resulting from the synergistic allele *lin-8(n111);lin-9(n112)* is already 100%, enhancement of this phenotype would not have been observable. However, since FERGUSON and HORVITZ (1985) found that the phenotype resulting from reduced dose of *lin-8(n111)* or a stronger allele of *lin-9* is sterility, reduced fertility should serve as an indication of enhancement of the hypomorphic synergistic allele. The *dpy-22(e652)* mutations did not significantly affect fertility of *lin-8(n111);lin-9(n112)* (data not shown). The results with *dpy-23* were difficult to interpret. A *lin-8;lin-9; dpy-23* strain is virtually inviable. The results in Table 5 are based on the *dpy-23* progeny of *lin-8;lin-9;dpy-23/+* animals; all were Muv, but also almost completely sterile, and the strain could not be propagated. It is possible that *dpy-23* specifically enhances

TABLE 9

Summary of effects of allele dosage and *dpy-22* or *dpy-23* mutations on phenotypes resulting from two *X*-linked hypomorphic alleles in *2X* hermaphrodites[a]

Allele	$\frac{m}{m}$	$\frac{m^b}{\text{null}}$	$\frac{dpy\text{-}22\ m}{dpy\text{-}22\ m}$	$\frac{dpy\text{-}23\ m}{dpy\text{-}23\ m}$
let-2(mn114)[c]	Sterile adult	L1 lethal	L1 lethal	Sterile adult
lin-2(n768)	38% ± 5% Lin[d]	92% ± 5% Lin	67% ± 7% Lin	65% ± 13% Lin

[a] *X*-dependent dpy mutations used were *dpy-22(e652)* and *dpy-23(e840)*. *m* in headings represents *let-2* or *lin-2* hypomorphic allele.

[b] Heteroallelic strains tested were *let-2(mn114)/let-2(mn153)*, and *lin-2(n768)/lin-2(e1309)*. The *let-2* strain was obtained by crossing *mnDp1/+;unc-3 let-2(mn114)* males with *mnDp1/+;unc-3 let-2(mn153)* hermaphrodites. No Unc progeny larvae were observed, although a few Unc L1's might have been missed; we conclude that the resulting phenotype is either embryonic or L1 lethality.

[c] *let-2* homozygotes were obtained as Unc segregants of *mnDp1/+;unc-3(e151) let-2(mn114)*. *let-2 dpy-22* and *let-2 dpy-23* homozygotes were obtained as Dpy Unc segregants of the corresponding *mnDp1/+;dpy unc-3 let-2* strains.

[d] Lin: vulvaless or egg-laying defective (see MATERIALS AND METHODS); percentages given indicate penetrance of the Lin phenotype with an uncertainty representing the 95% confidence interval. The numbers of animals scored as Lin among total animals examined in the four columns were 159 of 418, 108 of 117, 109 of 163, 32 of 49, respectively.

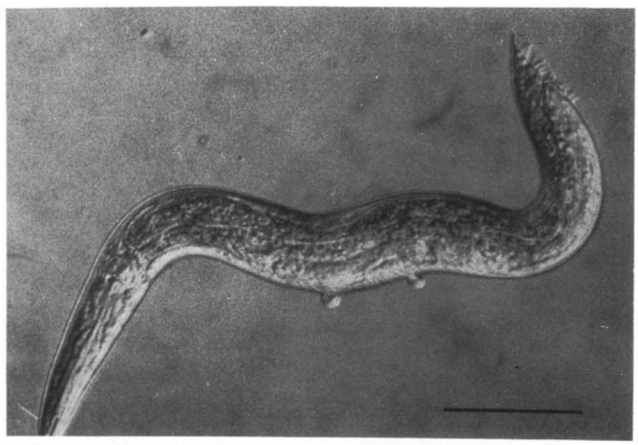

FIGURE 3.—Brightfield photomicrograph of a surviving *dpy-22* male carrying a hypomorphic allele of *lin-15*, showing male tail morphology and multiple ventral protrusions. The animal, of genotype *lin(n833);him-5(e1490);dpy-22(e652) lin-15(n767)/0* was reared at 20° and photographed with a Zeiss Universal microscope. Scale bar indicates 100 μm.

these autosomal hypomorphs, causing sterility. However, since many other *dpy-23* strains are sickly and sterile, this effect could also be nonspecific.

***dpy-22* and *dpy-23* effects in *1X* animals:** The effects of *dpy-22* and *dpy-23* mutations on phenotypes resulting from *X*-linked hypomorphic alleles in *1X* animals were difficult to test, because in general *dpy-22* and *dpy-23 1X* animals are inviable. Nevertheless, some evidence for enhancement of such a phenotype by *dpy-22* was obtained using *lin-15* alleles. The null allele *lin-15(n309)* results in males with multiple ventral protrusions that resemble pseudovulvae, whereas the hypomorphic allele *n767* causes this phenotype in males only rarely at 20° and almost never at 16° (Table 6A). However, the surviving male progeny of *lin(n833);him-5;dpy-22 lin-15(n767)* hermaphrodites invariably have multiple protrusions (Figure 3) regardless of growth temperature. In one brood, reared at 16°, 12 of 12 surviving males showed the Muv phenotype, as did more than 100 males observed in the course of maintaining stocks of this strain at 16° or 20° over a 2-year period. Similar results were obtained with a *him-5;dpy-22 lin-15(n765)* strain; although *him-5;lin-15(n765)/0* males are not mutant at 16°, almost all *him-5;dpy-22 lin-15(n765)/0* males show the Muv phenotype. In a *him-5;dpy-22 lin-15(n309)* strain, no viable males were observed. Thus, the *dpy-22* mutation enhances the phenotype of a *lin-15* hypomorph in *1X* males, presumably by decreasing the level of *lin-15* expression. The effect of *dpy-23* could not be tested by this method, since *dpy-23* males from a similar strain did not survive.

The lethality in *1X* animals caused by these mutations as well as the striking enhancement by a *dpy-22* mutation of the Muv phenotype resulting from a *lin-15* hypomorphic allele in *1X* males suggests that the effects of *dpy-22* and *dpy-23* mutations are stronger in *1X* than in *2X* animals. The effects of *dpy-22* mutation may be explained by decreases in *X*-chromosome expression, more pronounced in *1X* than in *2X* animals. For *dpy-23*, the evidence is less clear. Conclusions regarding both these genes must be qualified by the fact that each is presently defined by only a single mutant allele, and that the resulting mutant phenotypes are somewhat variable.

Effect of other *dpy* genes: To test for possible effects of Dpy morphology in general, or for effects of mutations in other *dpy* genes on phenotypes resulting from an *X*-linked hypomorphic allele, appropriate mutant strains were constructed and characterized phenotypically. The tests were conducted with *lin-15(n767)*, a hypomorph showing strong suppression by *dpy-21* and *dpy-26* mutations (Table 3) and enhancement by *dpy-22* and *dpy-23* mutations (Table 8). Strains of genotype *lin(n833);dpy;lin-15(n767)* were constructed for alleles of all *dpy* genes available from the Caenorhabditis Genetics Center, except for *dpy-25*, which we found to be sterile and nearly inviable in this combination. These strains were grown at 20°, where suppression could be seen

TABLE 10

Effect of *dpy* gene mutations on penetrance of the Muv phenotype resulting from a hypomorphic allele of *lin-15* at two temperatures[a]

Gene (allele)	16°	20°
dpy+	30 (389)	100 (1046)
dpy-1(e1)	34 (179)	100 (191)
dpy-2(e8)	31 (160)	99 (135)
dpy-3(e27)	33 (214)	99 (198)
dpy-3(e182)[b]	28 (120)	98 (190)
dpy-4(e1166)	30 (157)	100 (270)
dpy-5(e61)	29 (154)	100 (247)
dpy-6(e14)	37 (200)	99 (141)
dpy-7(e88)	29 (180)	99 (115)
dpy-8(e130)	31 (283)	100 (108)
dpy-9(e12)	31 (139)	100 (189)
dpy-10(e128)	30 (153)	100 (146)
dpy-11(e224)	26 (168)	100 (136)
dpy-13(e184)	31 (165)	99 (113)
dpy-14(e188)	27 (103)	100 (102)
dpy-17(e164)	30 (103)	98 (127)
dpy-18(e364)	31 (166)	100 (300)
dpy-19(e1259)[c]	30 (188)	98 (312)
dpy-20(e1281)[c]	26 (257)	100 (107)
dpy-21(e428)	6 (414)	17 (608)
dpy-22(e652)	97 (110)	100 (237)
dpy-23(e840)	66 (193)	100 (168)
dpy-24(s71)	25 (143)	100 (118)
dpy-26(n199)[d]	10 (473)	19 (402)

[a] All strains were homozygous for the *lin(n833)* and *lin-15(n767)* mutations as well as the indicated *dpy* allele. Penetrance values are expressed as percent of animals with multiple pseudovulvae (Muv). Uncertainties (95% confidence limits) were ≤ ± 9%. After each value is shown in parentheses the total number of animals examined.

[b] *e182*, previously described as the only allele defining the *dpy-12* gene (BRENNER 1974) was shown by mapping and complementation tests to be an allele of *dpy-3* (see MATERIALS AND METHODS).

[c] Animals are non-Dpy at 16°.

[d] Animals examined were the Dpy progeny of *lin(n833);dpy-26(n199)/unc-30(e191);lin-15(n767)*.

as a decrease in penetrance of the Muv phenotype, and at 16°, where enhancement could be seen as an increase in penetrance. The results are presented in Table 10. For comparison, the penetrance for *lin(n833);lin(n767)* in *dpy+*, *dpy-21*, *dpy-22*, *dpy-23* and *dpy-26* backgrounds is also shown. Only for strains carrying one of the four *X*-dependent dpy mutations is the penetrance significantly different from that seen in the *dpy+* strain. We conclude that the enhancement and suppression effects seen with *dpy-21*, *dpy-22*, *dpy-23* and *dpy-26* mutations are not a general property of mutations resulting in the Dpy phenotype, but rather are unusual to mutations in the four *X*-dependent *dpy* genes among those tested.

DISCUSSION

For this study we identified hypomorphic mutant alleles of several *X*-linked and autosomal genes, by the criterion that the resulting phenotypes vary with allele dosage. We could therefore use severity of these phenotypes as rough assays for levels of mutant gene expression. We have exploited such assays to investigate two questions: first, whether *C. elegans* globally regulates expression of *X*-linked genes to compensate for the difference in *X*-chromosome dosage between *1X* and *2X* animals; and second, whether expression of *X*-linked genes is specifically affected by mutations in the four unusual *X*-dependent dpy genes *dpy-21*, *dpy-22*, *dpy-23* and *dpy-26*. We also hoped to define a genetic assay for *X*-chromosome expression that would allow us to find and characterize new dosage compensation mutations.

Our results can be summarized briefly as follows. First, the phenotypes resulting from hypomorphic alleles of at least two *X*-linked genes are similar in *1X* and *2X* animals. Second, mutations in the *X*-dependent *dpy* genes *dpy-21* and *dpy-26* suppress the phenotypes resulting from *X*-linked hypomorphic alleles, but do not suppress either those resulting from null alleles in the same genes or similar phenotypes resulting from autosomal hypomorphic alleles. Suppression of *X*-linked hypomorphs by *dpy-21* and *dpy-26* mutations is seen in both *1X* and *2X* animals, but is more pronounced in *2X* animals, particularly for the *dpy-26* mutation.

A mutation in another *X*-dependent *dpy* gene, *dpy-22*, appears to have the opposite effect, enhancing the phenotypes resulting from three *X*-linked hypomorphic alleles, but not those resulting from three autosomal hypomorphic alleles. The effect of the *dpy-22* mutation is also seen in both *1X* and *2X* animals, but is probably more pronounced in *1X* animals. A mutation in a fourth *X*-dependent dpy gene, the *X*-linked gene *dpy-23*, enhances hypomorphs in two *X*-linked genes and does not affect one autosomal hypomorph; however, its role remains unclear because one *X*-linked hypomorph is unaffected, and at least one autosomal hypomorph may be affected. Mutations in 18 other *dpy* genes, which do not result in *X*-dependent phenotypes, show no significant suppression or enhancement of the phenotype resulting from an *X*-linked hypomorphic allele.

X-chromosome dosage compensation in *C. elegans*: The similarity of phenotypes resulting from hypomorphic alleles of the *X*-linked genes *let-2* and *lin-15* in *1X* and *2X* animals is consistent with other indications that many genes on the *X* chromosome are regulated to compensate for the difference in *X* dosage. The most direct evidence for this conclusion has come from observations on levels of transcription from cloned *X*-linked sequences (MEYER and CASSON 1986; DONAHUE, QUARANTILLO and WOOD 1987). Additional support comes from assays of enzymes coded by *X*-linked genes in males and hermaphrodites, as summarized in the introduction, as well as

from an earlier observation of MENEELY and HERMAN (1981) that *let-36(mn140)*, another *X*-linked hypomorph, results in sterile adults for both homozygous males and homozygous hermaphrodites, yet causes arrest before adulthood in hemizygous *let-36(mn140)/Df* hermaphrodites.

X-chromosome dosage compensation is a sex-related characteristic, because males are normally *1X* and hermaphrodite *2X*. However, our findings with sex transformer mutants, that phenotypes resulting from hypomorphic alleles of *X*-linked genes are similar in *1X* and *2X* animals of the same sex, indicate that dosage compensation in *C. elegans* can occur independently of sexual phenotype. When *1X* animals are transformed into hermaphrodites, they retain the *1X* level of expression normally characteristic of males. Similar results have been obtained with *tra-1 2X* pseudomales in *C. elegans* by MEYER and CASSON (1986) and in Drosophila by SMITH and LUCCHESI (1969).

Effects of *dpy-21* and *dpy-26* mutations on *X* expression: The Dpy phenotypes of *dpy-21* and *dpy-26* mutant *2X* animals are similar to that of *2A;3X* aneuploids, which by our hypomorph assay have higher than normal *X* expression. Like the presence of a third *X* chromosome in *2A:3X* animals, recessive mutations in *dpy-21* and *dpy-26* specifically suppress the phenotypes of *X*-linked hypomorphs, as if the *dpy* mutations lead to an increase in *X* expression. These findings suggest that the *dpy-21* and *dpy-26* gene products might function normally as negative regulators of *X* expression in the dosage compensation process. Before the implications of these suggestions are examined further, however, several aspects of the experiments require further comment.

Some *X*-linked hypomorphs show more suppression and enhancement effects than others. For example, the phenotype resulting from *lin-2(n768)* is not noticeably suppressed by mutations in either *dpy-21* or *dpy-26*. Such results could be due to the nature of the mutant phenotype and how markedly it changes over the range of expression affected by a particular *X*-dependent dpy mutant. In support of this explanation, *lin-2(n768)* is not suppressed in *2A; 3X* animals either. Our conclusion that *dpy-21* and *dpy-26* mutations do not affect autosomal hypomorphs must be qualified in view of this realization: conceivably, some of the autosomal hypomorphic alleles chosen for our tests were affected by mutations in *X*-dependent *dpy* genes, but not enough to show clear phenotypic changes. Checking the possibility that suppression could have been seen by increasing the dose of the mutant allele is less straightforward with the autosomal than with the *X*-linked hypomorphs and was not done in these experiments. The possibility that the hypomorphs we tested are not good indicators can be evaluated only by testing additional autosomal hypomorphs. Meanwhile, however, findings cited in the introduction that *dpy-21* mutations cause increases in the levels of several *X*-linked-gene products normalized to autosomal controls supports the view that effects of *dpy-21* on autosomal gene expression are small relative to its effects on *X*-linked-gene expression (MEYER and CASSON 1986; DONAHUE, QUARANTILLO and WOOD, 1987; P. MENEELY and K. NORDSTROM, unpublished data).

An alternative explanation for the lack of suppression effects on some *X*-linked hypomorphs is that some *X*-linked genes may be compensated or regulated either not at all, or not by these particular *X*-dependent *dpy* genes. The tRNA gene *sup-7 X* is apparently not dosage compensated (HODGKIN 1985). Transcript levels of the functionally unidentified gene *uxt-2 X* appear similar to *sup-7* in this respect (MEYER and CASSON 1986). *X*-linked genes whose expression is sex-limited, such as vitellogenin genes (KIMBLE and SHARROCK 1983; W. B. WOOD *et al.* 1985) may be uncompensated in *C. elegans*, as they are in Drosophila (QTA *et al.* 1981). Consistent with this possibility is our finding that expression of the sex-limited *lin-2* mutant phenotype is not affected by *dpy-21* and *dpy-26* mutations; we do not know whether *lin-2* is dosage compensated. It seems clear, however, that *dpy-21* and *dpy-26* affect expression of many genes on the *X*-chromosome. The hypomorphs shown to be affected represent genes with differing physiological functions: *let-2* mutations may affect basement membranes (J. KRAMER, J. PRIESS and D. HIRSH, personal communication), and *lin-15* mutants have a hypodermal defect (E. FERGUSON, unpublished results). A hypomorphic *lin-14 X* mutation is apparently suppressed by *dpy-21* mutations, but again, the assay involved scoring ectodermal cells (P. MENEELY, unpublished results; L. DELONG and B. MEYER, personal communication). *dpy-21* also affects the expression of *X*-linked genes for myosin (*myo-2*) (MEYER and CASSON 1986; DONAHUE, QUARANTILLO and WOOD 1987) actin (*act-4*) (DONAHUE, QUARANTILLO and WOOD 1987), and an acetylcholinesterase (*ace-1*) (P. MENEELY and K. NORDSTROM, unpublished data).

Since these studies were initiated, two additional genes have been discovered that share properties with *dpy-21* and *dpy-26* and are also likely candidates for involvement in the dosage compensation mechanism. These genes are *dpy-27 III* (J. HODGKIN and E. HEDGECOCK, personal communication) and *dpy-28 III* (MEYER and CASSON 1986). Mutations in these genes result in phenotypes similar to those caused by *dpy-26* mutations. Mutations in *dpy-27* suppress *lin-15(n765)* in *2X* animals (P. MENEELY, unpublished

results), and mutations in both *dpy-27* and *dpy-28* increase the levels of three *X*-linked transcripts in *2X* animals (MEYER and CASSON 1986).

The *dpy-21(e428)* and *dpy-21(e459)* mutations are recessive, and therefore probably result in loss or reduction of gene product function, but the *dpy-21* null phenotype has not been established. The *dpy-26(n199)* mutation is recessive, and behaves like a null allele in dosage tests reported by HODGKIN (1983a). The increases in *X* expression seen as a result of these mutations therefore suggest that the products of these genes normally function to decrease *X* expression.

To bring about *X*-chromosome dosage compensation, negative regulators of *X* expression might be expected to act only in *2X* animals. HODGKIN (1983a) showed that *dpy-26* and, in some strains, *dpy-21* mutations affect the fertility of *1X* animals. We have shown *dpy-21* and *dpy-26* effects on *X*-linked hypomorphs in both *1X* and *2X* animals. However, in the assays using *lin-15* hypomorphs, the effects observed were more pronounced in *2X* than in *1X* animals, particularly for *dpy-26*, which suppresses appreciably only one of the hypomorphs tested in *1X* animals. MEYER and CASSON (1986) have suggested that neither *dpy-27* nor *dpy-28* affects *1X* animals.

In summary, therefore, our results are consistent with the possibility that *dpy-21* and *dpy-26* gene products are negative regulators of *X* expression in the dosage compensation process, acting preferentially in *2X* animals to help lower *X* expression to about the same level as in *1X* animals. The differences in maternal effects seen with these two genes suggests that the *dpy-26* product could be maternally produced for early embryonic function, while the *dpy-21* product could function during later development. Additional discussion of *dpy-21* and *dpy-26* functions has been presented elsewhere (WOOD *et al.* 1987).

Effects of *dpy-22* and *dpy-23* mutations on *X* expression: In contrast to mutations in the autosomal genes *dpy-21* and *dpy-26*, mutations in the *X*-linked genes *dpy-22* and *dpy-23* may enhance the phenotypes resulting from *X*-linked hypomorphs in *2X* animals, as if causing a decrease in *X* expression. However, the effects of *dpy-22* and *dpy-23* mutations are more difficult to interpret, for at least three reasons.

First, exceptions were noted; for example, the phenotype resulting from *let-2(mn114)* is not noticeably enhanced by the *dpy-23* mutation, although it is enhanced by the twofold decrease in dose in a *let-2(mn114)/mnDf1* strain. Such exceptions, as discussed above, could result from insensitivity of the hypomorphic mutant phenotype to levels of expression in the range affected by the *dpy-23* mutation, or from actual differences in the control of different *X*-linked genes.

Second, an inherent ambiguity in using phenotypes resulting from hypomorphic mutations to indicate effects of other mutations on level of expression of the hypomorphic allele is that the phenotype of the double mutant may be non-specifically influenced by both mutations in ways that are difficult to interpret. This ambiguity clouds our interpretation of *dpy-23* effects in particular, somewhat less so with *dpy-22*. Both mutations alone result in sickly animals, and many doubly and multiply mutant strains carrying these mutations are nearly inviable. For example, *lin-7;dpy-23* and *dpy-23 lin-2* strains were virtually sterile regardless of the allele of *lin-7* or *lin-2* used, despite the fact that neither *lin-7* nor *lin-2* null phenotypes are thought to involve sterility or inviability (FERGUSON and HORVITZ 1985). On the other hand, the null phenotypes for both *lin-8* and *lin-9* are thought to be inviability and sterility, and *lin-8(n111);lin-9(n112);dpy-23* is sterile and nearly inviable. Therefore, we cannot rule out the possibility that the *dpy-23* mutation enhances these, and perhaps other autosomal hypomorphs.

Third, in addition to the ambiguity in some of the observed effects of *dpy-22* and *dpy-23* mutations, the nature of these mutations themselves is unclear. Both genes are defined so far only by the alleles used here, *dpy-22(e652)* and *dpy-23(e840)*. Both mutations have a variable phenotype, and *e840* was induced by *X*-rays. Therefore, although both are recessive, the suggestion that they result in loss or reduction of gene product function can be made with less confidence. Neither is known to be a null allele; moreover, *dpy-22(e652)* may be a hypomorph, based on a preliminary finding that *dpy-22(e652)/nDf19* animals die as embryos (W. B. WOOD, unpublished data). With these reservations, our results are consistent with the possibility that the *dpy-22* and *dpy-23* gene products could normally function to increase *X* expression.

Again, if these gene products were postulated to play a role in dosage compensation, they would be expected to show more activity in *1X* than in *2X* animals. The evidence presented here and previously, though not compelling, is consistent with this expectation. The *dpy-22* and *dpy-23* mutations are lethal in *1X*, but not in *2X* animals. The *dpy-22* mutation causes strong enhancement of the phenotype resulting from *lin-15 X* hypomorphic alleles in *1X* males. However, although our results are consistent with some role for *dpy-22* and perhaps *dpy-23* in regulation of *X* expression, further study of these genes will be required before conclusions can be drawn regarding their involvement in the dosage compensation mechanism.

Relationship of the *X*-dependent *dpy* genes to sex

determination: Sex determination in *C. elegans* is controlled by a set of interacting autosomal genes, the *her*, *fem* and *tra* genes (HODGKIN and BRENNER 1977; HODGKIN 1980; KIMBLE, EDGAR and HIRSH 1984; DONIACH and HODGKIN 1984). The first gene in the regulatory pathway, *her-1*, appears to be controlled somehow by the *X/A* ratio. We previously presented evidence that *dpy-21* affects sex determination as well as *X*-chromosome expression (MENEELY and WOOD 1984), based on three lines of evidence: (1) *dpy-21* mutations enhance the hermaphroditizing effects of large *X* duplications in *1X* animals, resulting in intersexual phenotypes; (2) *dpy-21* mutations interact with mutations in the sex determining genes *tra-1* and *her-1*; and (3) *ct16*, which we found to be a weak allele of *dpy-21*, causes what appeared to be an abnormal tail phenotype in *1X* males, but does not make these animals Dpy. The first two of these effects could result from increased *X* expression, which could affect other genes controlling sexual dimorphism or perhaps cause a mistakenly high assessment of the *X/A* ratio. Similar effects on *tra-1* and *her-1* mutants result from the abnormally high *X* dose in *2A;3X* aneuploids (HODGKIN 1983a; C. TRENT, personal communication).

The effect of *ct16* cannot be explained this way. An alternative explanation for the abnormal tails of (*ct16*) mutant males is that these animals carried a second mutation affecting male tail morphology. Backcrosses with linked markers failed to separate two mutations (MENEELY and WOOD 1984); however, a strain heterozygous for *ct16* and the presumed null allele *dpy-21(e428)* produced fertile males (P. M. MENEELY, unpublished results). Even more confusing, many "*ct16*" strains are no longer Dpy, although that was the phenotype used for mapping and complementation testing. M. SHEN and J. HODGKIN (personal communication) have shown that an isolate of *ct16* carries a mutation in the unlinked gene *mab-3 II*, but no *dpy-21* mutation. Other strains carrying *ct16* have also given variable results. The nature of the original *ct16* remains unclear. Therefore, there is at present no evidence for a direct effect on sex determination of *dpy-21* or any of the other *X*-dependent *dpy* genes apart from effects on the level of *X* expression.

However, the observation that *dpy-21* mutations, and in more recent experiments, *dpy-26* mutations as well, enhance the hermaphroditizing effect of *X* duplications on sex determination (MENEELY and WOOD 1984 and unpublished results) suggests that the products of *X*-dependent *dpy* genes could act as components of the denominator of the *X/A* ratio, perhaps by negatively regulating the level of expression of certain early acting *X*-linked genes that in turn could control sex determination through *her-1*, and subsequently control dosage compensation in later development as well by regulation of the *X*-dependent *dpy* genes (WOOD *et al.* 1987). One such early acting *X*-linked gene could be *sdc-1 X* and shown by VILLENEUVE and MEYER (1987) to be involved in both sex determination and dosage compensation.

In summary, we have shown that two autosomal genes appear to exert negative control and that one and possibly two *X*-linked genes may exert positive control of *X*-chromosome expression in *C. elegans*. The evidence for positive control is much weaker. These genes appear to be active, although with different levels of effect, in both males and hermaphrodites, suggesting that dosage compensation may be achieved by an appropriate balance between activation and repression of *X*-chromosome expression in each of the two sexes. Searches for other dosage compensation mutants are in progress, and more genes will undoubtedly turn out to be involved (C. TRENT, J. MANSER and S. BURGESS, personal communications; B. MEYER, personal communication). The genetic assay we describe here will be useful in finding and characterizing new mutations. Identification of additional genes and additional alleles of the genes described here should help to clarify the mechanism by which *X*-chromosome expression is regulated, as well as the relationship between *X*-chromosome dose, dosage compensation, and sex determination in *C. elegans*.

This research was supported by a grant to W.B.W. from the National Institutes of Health (HD-11762), by postdoctoral fellowships to P.M.M. from the American Cancer Society (PF-1840) and the National Institutes of Health (GM-07775), and by research grants to P.M.M. from the National Science Foundation (DCB-8417215) and Basil O'Connor Starter Research Grant Program (5-525) of the March of Dimes. We are particularly grateful to E. FERGUSON, for introducing us to many of the alleles, for providing information on their genetics and the resulting phenotypes prior to publication as well as furnishing us with the appropriate mutant strains, and for his detailed criticisms and suggestions during preparation of the manuscript. We also thank him for the initial observation that a *dpy-21* mutation suppressed *lin-15(n767)*. We thank R. HERMAN, J. HODGKIN, R. HORVITZ, C. TRENT and R. WATERSTON for providing mutant strains. Other strains were obtained from the Caenorhabditis Genetics Center, which is supported by a contract between the National Institutes of Health and the Curators of the University of Missouri. We are also grateful to J. HODGKIN, B. MEYER and R. RUSSELL for communication of unpublished results, to R. HORVITZ and C. TRENT for much helpful discussion, to C. TRENT, P. MAINS and B. MCCOUBREY for their constructive comments on the manuscript, and to J. WILSON for its preparation through many revisions.

LITERATURE CITED

BAKER, B. S. and J. M. BELOTE, 1983 Sex determination and dosage compensation in *Drosophila melanogaster*. Annu. Rev. Genet. **17:** 345–393.

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

BULL, J. J., 1983 *The Evolution of Sex-determining Mechanisms*. Benjamin-Cummings, Menlo Park, Calif.

DONAHUE, L., B. QUARANTILLO and W. B. WOOD, 1987 Molecular analysis of *X*-chromosome dosage compensation in *C. elegans*. Proc. Natl. Acad. Sci. USA. In press.

DONIACH, T. and J. HODGKIN, 1984 A sex-determining gene, *fem-1*, required for both male and hermaphrodite development in *Caenorhabditis elegans*. Dev. Biol. **106:** 223–235.

FERGUSON, E. L. and H. R. HORVITZ, 1985 Identification and characterization of 22 genes that affect the vulval cell lineages of the nematode *Caenorhabditis elegans*. Genetics **110:** 17–72.

FERGUSON, E. L. and H. R. HORVITZ, 1987 Synthetic multivulva mutants of *Caenorhabditis elegans*. Genetics. In press.

GARTLER, S. M. and A. D. RIGGS, 1983 Mammalian *X*-chromosome inactivation. Annu. Rev. Genet. **17:** 155–190.

HODGKIN, J., 1980 More sex-determination mutants of *Caenorhabditis elegans*. Genetics **96:** 649–664.

HODGKIN, J., 1983a *X* chromosome dosage and gene expression in *C. elegans*: two unusual dumpy genes. Mol. Gen. Genet. **192:** 452–458.

HODGKIN, J., 1983b Male phenotypes and mating efficiency in *Caenorhabditis elegans*. Genetics **103:** 43–64.

HODGKIN, J., 1985 Novel nematode amber suppressors. Genetics **111:** 287–310.

HODGKIN, J. and S. BRENNER, 1977 Mutations causing transformation of sexual phenotype in the nematode *Caenorhabditis elegans*. Genetics **86:** 275–287.

HODGKIN, J., H. R. HORVITZ and S. BRENNER, 1979 Non-disjunction mutants of the nematode *Caenorhabditis elegans*. Genetics **91:** 67–94.

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

KIMBLE, J. and W. J. SHARROCK, 1983 Tissue-specific synthesis of yolk proteins in *Caenorhabditis elegans*. Dev. Biol. **105:** 234–239.

KIMBLE, J., L. EDGAR and D. HIRSH, 1984 Specification of male development in *Caenorhabditis elegans*: the *fem* genes. Dev. Biol. **105:** 234–239.

MENEELY, P. M. and R. K. HERMAN, 1979 Lethals, steriles and deficiencies on a region of the *X* chromosome of *Caenorhabditis elegans*. Genetics **92:** 99–115.

MENEELY, P. M. and R. K. HERMAN, 1981 Suppression and function of *X*-linked lethal and sterile mutations in *Caenorhabditis elegans*. Genetics **97:** 65–84.

MENEELY, P. M. and W. B. WOOD, 1984 An autosomal gene that affects *X* chromosome expression and sex determination in *Caenorhabditis elegans*. Genetics **106:** 29–44.

MEYER, B. and L. CASSON, 1986 *Caenorhabditis elegans* compensates for the difference in *X* chromosome dosage between sexes by regulating transcript levels. Cell **47:** 871–881.

MULLER, H. J., 1950 Evidence of the precision of genetic adaptation. Harvey Lect. **43:** 165–229.

OTA, T., A. FUKUNAGA, J. KAWABE and K. OISHI, 1981 Interactions between sex-transformation mutants of *Drosophila melanogaster*. I. Hemolymph vitellogenins and gonad morphology. Genetics **99:** 429–441.

SMITH, P. D. and J. C. LUCCHESI, 1969 The role of sexuality in dosage compensation in *Drosophila*. *Genetics* **61:** 607–618.

VILLENEUVE, A. and B. MEYER, 1987 *sdc-16*: a link between sex determination and dosage compensation in *C. elegans*. Cell **48:** 25–37.

WOOD, W. B., P. MENEELY, P. SCHEDIN and L. DONAHUE, 1985 Aspects of dosage compensation and sex determination in *Caenorhabditis elegans*. Cold Spring Harbor Symp. Quant. Biol. **50:** 575–583.

WOOD, W. B., C. TRENT, P. MENEELY, J. MANSER and S. BURGESS, 1987 Control of *X*-chromosome expression and sex determination in embryos of *Caenorhabditis elegans*. pp. 191–199. In: *Genetic Regulation of Development*, Edited by W. LOOMIS. Alan R. Liss, Inc., New York.

Communicating editor: R. K. HERMAN