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An X-autosome fusion chromosome of *Caenorhabditis elegans*

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Summary. The translocation *mnT12(IV;X)* is a fusion of holocentric chromosomes *IV* and *X*, the breakpoints occurring near the left end of *IV* and the right end of *X*. Animals homozygous for *mnT12* are viable and fertile; they contain five pairs of chromosomes rather than the normal set of six pairs. The *mnT12* chromosome is larger than all wild-type chromosomes and thus identifies linkage groups *IV* and *X* cytologically. Hermaphrodites heterozygous for *mnT12* show high frequency meiotic nondisjunction both between *mnT12* and the *X* chromosome, which results in a high incidence of male self progeny (27% compared to the wild-type incidence of 0.2%), and between *mnT12* and chromosome *IV*, which results in a high incidence of self progeny essentially trisomic for chromosome *IV* (karyotype *IV/mnT12/mnT12*). The viability of chromosome *IV* trisomics has been confirmed by constructing animals trisomic for only normal copies of chromosome *IV*; these animals are morphologically wild type. Meiotic chromosome disjunction in *mnT12* homozygotes appears to be normal, although the frequency of recombination between markers that are normally *X*-linked is significantly reduced. Males of genotype *IV/mnT12/0* are fertile. They can be thought of as having a neo-*X*(*mnT12*) neo-*Y*(*normal IV*) karyotype since it is possible to maintain a male-hermaphrodite stock of *C. elegans* consisting of such males and hermaphrodites carrying two neo-*X* chromosomes and no neo-*Y*; the organism is thus converted from an *XO:XX* type of sex determination to an *XY:XX* system.

Key words: Nematode – Nondisjunction – Translocation – Trisomy

Introduction

Chromosome fusions have been shown to be of major importance in the evolution of the karyotypes of many groups of organisms (White 1973). The most significant class of fusion, called centric fusion or Robertsonian translocation, involves the joining of two acrocentric chromosomes, each broken within or very near its centromere, to produce a metacentric fusion product. Centric fusions are generally thought to occur as a special case of reciprocal translocation, in which the short arms of the acrocentric chromosomes also fuse but are lost.

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In the small nematode *Caenorhabditis elegans*, the spindle microtubules at mitosis and meiosis have been shown to attach along the entire lengths of all six pairs of chromosomes (Albertson and Thomson 1982; D. Albertson, personal communication). *C. elegans* thus belongs to that group of organisms, which includes certain plants, protozoa, insects and other nematode species, that have holocentric or holokinetic chromosomes. One wonders whether any two holocentric chromosomes broken near their ends may form a stable fusion chromosome. It is worth noting that the classical localized centromere serves two meiotic functions: it provides sites for attachment of spindle fibers, and it plays a role in the orderly disjunction of the meiotic chromatids of a bivalent by keeping sister chromatids joined during meiosis I and by directing their splitting during meiosis II. Clearly a diffuse centromere cannot maintain attachment of sister chromatids all along the lengths of the chromosomes until meiosis II and still permit meiotic exchange. Indeed, it appears that one chromosome end (or possibly both) may play critical roles in the proper disjunction of holocentric chromosomes during meiosis (White 1973; and see Discussion). It is, therefore, possible that the meiotic behavior of a fusion chromosome generated from two holocentric chromosomes depends on which ends are joined.

In this paper we describe the behavior of the first known example of a *C. elegans* fusion chromosome. Our interest in this rearrangement is twofold: first, we hope to shed light on the types of chromosome rearrangement that holocentric chromosomes are capable of, about which little is known, and second, we hope to enhance the general genetic manipulability of the organism *C. elegans* because of its role as a model for developmental and behavioral genetics (for a recent review, see Sternberg and Horvitz 1984).

Materials and methods

All strains derive from *C. elegans* var. Bristol (Brenner 1974). The wild-type strain is designated N2. Genetic nomenclature follows Horvitz et al. (1979). Media and culture techniques were as described by Brenner (1974); all incubations were at 20° C. Mating and mapping methods were as described by Brenner (1974) and Herman (1978), using 35 mm diameter petri plates. Multiply mutant strains were constructed by standard methods (Brenner 1974). Figure 1 shows the relative map locations of all genes used in this work. Alleles used were the reference alleles listed by Swan-

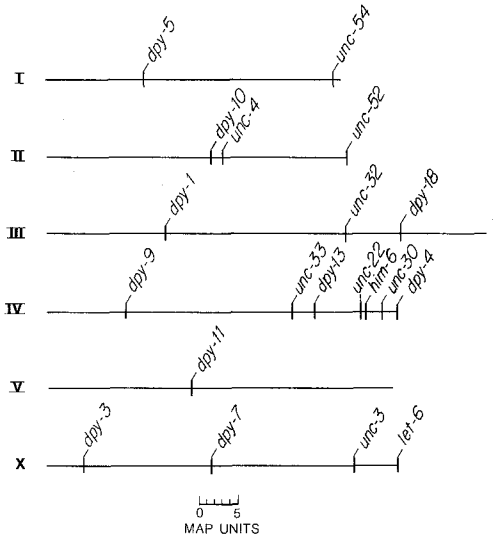

Fig. 1. A genetic map showing only loci used in this work

son et al. (1984); Fig. 1 was derived from their extensive genetic map except for the placement of *him-6*, which was positioned on the basis of the following three-factor crosses: 0 of 5 *dpy-13*$^+$ *unc-22* recombinant chromosomes derived from *dpy-13 unc-22/him-6* was *him-6*, and one of 10 *unc-22 dpy-4*$^+$ recombinant chromosomes derived from *unc-22 dpy-4/him-6* was *him-6*.

For recombination frequency measurements, parents were transferred daily, and complete broods were counted. Symmetric 95% confidence limits for frequencies of recombinant types were tabulated (Mainland et al. 1956) and used to calculate corresponding confidence limits for recombination frequencies (Brenner 1974). Measurements of egg-hatching frequencies were determined as previously described (Herman 1978).

The procedure for staining chromosomes with the fluorescent dye Hoeschst 33258 has been described (Herman et al. 1979).

The original *mnT12*-bearing animal was found among the progeny of an X-irradiated (7,500 r) parent in a screen for deficiencies (Sigurdson et al. 1984). It was first recognized that a genetic element, now called *mnT12*, conferred a high incidence of male self progeny when heterozygous but not when homozygous. Males carrying *mnT12* were crossed to *unc-3* hermaphrodites. The wild-type hermaphrodite progeny segregated many male offspring. Some of their hermaphrodite progeny gave no male progeny, but *mnT12*-bearing males could be recovered following mating with N2 males. This series of crosses was repeated three times before the experiments reported below were begun.

Results

The existence of a translocation involving linkage groups *IV* and *X* was apparent from the pseudolinkage observed between *dpy-9 IV* and *unc-3 X* in *mnT12* heterozygotes. Animals homozygous for *mnT12* were crossed to N2 males, and the male progeny were crossed to various double mutants, each carrying *unc-3* and an autosomal marker. The wild-type hermaphrodite cross progeny were picked, and their progeny were scored. All broods contained 25%–30% males, a trait referred to as Him, for high incidence of male self progeny; the wild-type incidence is about 0.2% (Hodgkin et al. 1979). The Him trait confirmed that each hermaphrodite had received *mnT12* from its male parent, since as shown below, hermaphrodites heterozygous for *mnT12* are Him. Among 666 adult self progeny of *dpy-9 IV/mn T12(IV;X)/unc-3 X* were the following phenotypes: 433 wild-type hermaphrodites, 122 wild-type males, 34 Dpy Unc hermaphrodites, 69 Dpy Unc males, 3 Dpy non-Unc hermaphrodites, 1 Dpy non-Unc male, 1 Unc non-Dpy hermaphrodite and 3 Unc non-Dpy males. Thus there is tight pseudolinkage of *dpy-9 IV* and *unc-3 X*. We also note that the recovery of *unc-3*$^+$ and *dpy-9*$^+$ in offspring of the *mnT12* heterozygote was favored over recovery of *unc-3* and *dpy-9*, respectively. For *unc-3 X* this is obvious for male offspring: the ratio of non-Unc to Unc plus Dpy Unc males was much greater than unity. For hermaphrodite offspring, the effect for each gene is apparent: the ratio of wild-type to mutant animals in each case was much greater than the 3:1 ratio expected for equal probabilities of recovery of the two alleles. In analogous crosses no pseudolinkage was apparent between *unc-3* and the following loci: *dpy-5 I, unc-54 I, dpy-10 II, unc-52 II, dpy-1 III, dpy-18 III, dpy-4 IV,* and *dpy-11 V*.

The Him-conferring property was strongly correlated with heterozygosity for *mnT12* among the hermaphrodite self progeny of *dpy-9/mnT12/unc-3* hermaphrodites: 0 of 24 Dpy Unc progeny and 92 of 131 fertile wild-type progeny were Him (13 of 144 wild-type hermaphrodites picked were sterile or semi-sterile and hence could not be classified). Among the 92 wild-type Him hermaphrodites, 75 gave progeny ratios like that of their parents; at least most of the other 17, which segregated only wild-type progeny, we show below had the genotype *dpy-9 IV/mnT12/mnT12*. Most of the 39 non-Him wild-type hermaphrodite progeny of *dpy-9/mnT12/unc-3* hermaphrodites were homozygous for *mnT12:* 33 segregated no Dpy, Unc or Dpy Unc offspring; four of these were crossed to N2 males, and their male progeny were shown to produce *mnT12*-bearing sperm in crosses with *dpy-9; unc-3* hermaphrodites.

Mapping mnT12 breakpoints

We have detected considerable meiotic exchange both between *mnT12* and the *X* chromosome and between *mnT12* and chromosome *IV* in *IV/mnT12/X* hermaphrodites, where *IV* and *X* represent wild-type chromosomes *IV* and *X*, respectively. The male self progeny of *mnT12/dpy-3 unc-3* animals included 153 wild type, 100 Dpy Unc, 47 Dpy non-Unc and 37 Unc non-Dpy males (as already noted, recovery of *unc-3*$^+$ *mnT12* was favored over recovery of the *unc-3* chromosome). These frequencies correspond to a distance of 25 map units, compared to the wild-type *dpy-3* to *unc-3* distance of 42 map units (see below). We also counted male self progeny of *dpy-9 unc-33/mnT12* hermaphrodites; by concentrating on males we avoided the possible confusion between Dpy recombinants and *3X* hermaphrodites, which are shorter than wild type animals (Hodgkin et al. 1979). We counted 396 wild-type, 207 Dpy Unc, 62 Dpy non-Unc and 38 Unc non-Dpy males. It is impossible to derive a proper recombination frequency from these data because of the extensive nondisjunction that is occurring (see below), but the observed frequency of recombinant types is roughly two-thirds that normally found for the *dpy-9* to *unc-33* interval. Because of the considerable recombination

that occurs in *mnT12* heterozygotes between *mnT12* and the normal chromosome *IV* and between *mnT12* and the normal *X*, pairs of markers representing other regions of LG*IV* and *X* would not show as tight pseudolinkage as *dpy-9 IV* and *unc-3 X*; thus the translocation breakpoints must be in the regions of these markers. The following crosses show that the Him-conferring element also maps near these markers. Thirteen of the 13 viable Unc recombinant progeny descended from *dpy-7 mnT12/unc-3 let-6* hermaphrodites were Him, which indicates that the Him-conferring element maps very near or to the right of *let-6*, the right-most gene known on the *X* map (Meneely and Herman 1981). Similarly, 7 of 7 Unc recombinants segregating from *dpy-9 unc-33 IV/mnT12/+X* were Him, which indicates that the Him-conferring element also maps near or to the left of *dpy-9*, in the vicinity of the left end of linkage group (LG)*IV*. We conclude that *mnT12* when heterozygous is itself responsible for the Him trait and that the breakpoints of the translocation are near the right tip of *X* and in the general region of the left end of *IV*.

Genetics and cytology of mnT12 homozygotes

Translocation breakpoints near the ends of *IV* and *X* suggested that *mnT12* may be a fusion of *IV* and *X* rather than consisting of two elements. This idea was confirmed by Albertson (1984), who prepared spreads of metaphase chromosomes fluorescently stained with Hoechst 33258 from homozygous *mnT12* embryos. The karyotype of these animals consists of 10 chromosomes instead of the usual 12, with two chromosomes roughly twice as large as the others, which are all about the same size as the twelve wild-type chromosomes. Albertson (1984 and personal communication) has made extensive use of *mnT12* homozygotes in her work on in situ DNA hybridization of mitotic chromosomes and has not detected any small chromosomal elements in addition to the basic set of 10. We have looked at oocytes at diakinesis in homozygous *mnT12* hermaphrodites and have seen five bivalents instead of the usual six, with one bivalent much larger than the others (Fig. 2a, b); we have been unable to detect any additional elements. We consider it extremely unlikely that *mnT12* homozygotes contain a second element too small to be detected cytologically (see Discussion) and therefore conclude that *mnT12* is in fact a fusion chromosome consisting of both *IV* and *X*. Assuming that the tips of *IV* and *X* have been lost, the losses must not have been extensive because *mnT12* homozygotes are viable and fertile, although the animals are slightly smaller and more slowly growing than N2 hermaphrodites. Brood sizes are quite variable, but many broods include more than 200 animals.

Even if there were considerable meiotic nondisjunction of *mnT12* chromosomes in homozygous *mnT12* hermaphrodites, the animals would not be expected to be Him because it would be impossible for the male to have a single copy of the *X* chromosomal information without simultaneously carrying only a single copy of LG*IV*, which is probably lethal (Hodgkin et al. 1979). It seems likely based on the following evidence, however, that there is little meiotic nondisjunction of *mnT12* in homozygotes. First, the measured egg-hatching frequency was high: 94% (822/875) of eggs hatched to give viable progeny. Second, we have screened for nondisjunction as follows: *him-6* males were crossed to *unc-33 mnT12/unc-33 mnT12; dpy-11 V* hermaphrodites,

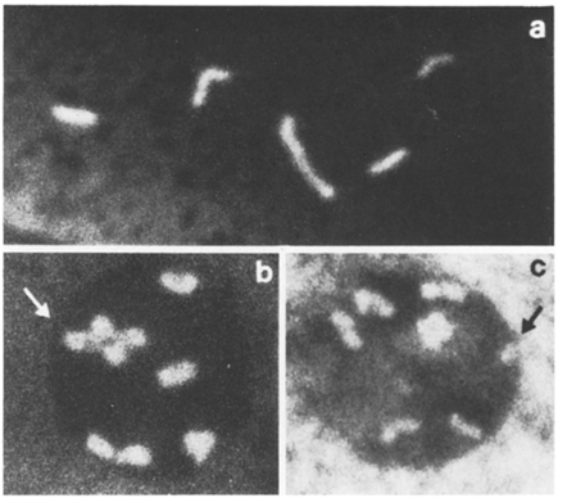

Fig. 2a–c. Fluorescence microscopy of oocytes stained with Hoechst 33258. Magnification: 2,200×. **a** *mnT12* homozygote with one large and four small bivalents. **b** *mnT12* homozygote with one large (note *arrow*) and four small bivalents. The chromosomes are more condensed than in **a**; bivalents characteristically assume the cross-shaped appearance apparent here for *mnT12* in mid-diakinesis (Nigon and Brun 1955). **c** Trisomic for linkage group *IV*, showing six bivalents and one univalent (note *arrow*)

and the non-Dpy progeny were screened for Unc animals, which could result from the fusion of nullo-*IV* nullo-*X* sperm with diplo-*mnT12* ova. The *him-6* mutation increases meiotic nondisjunction of the *X* chromosome in hermaphrodites (which results in a high incidence of male self-progeny) and the autosomes in both sexes (Hodgkin et al. 1979). No Unc animals were found among 2,000 non-Dpy progeny. In a control cross between *him-6* males and *dpy-5 I; him-6 unc-33 IV* hermaphrodites, 6 non-Dpy Unc-33 progeny (all male) were found among 1,048 non-Dpy animals. This result is consistent with the expected frequency of nullo-*IV* nullo-*X* sperm in *him-6* males of about 4–10% (Hodgkin et al. 1979). We conclude that the incidence of diplo-*mnT12* ova is probably less than one or two percent.

We have investigated recombination frequencies in *mnT12* homozygotes. The results, given in Table 1, show that the frequencies of exchange between homologous fusion chromosomes in the *dpy-3* to *unc-3* interval, which corresponds to most of the normal *X* chromosome, is less than half the wild-type frequency. The frequency of recombination between *unc-33 IV* and *dpy-4 IV* is only slightly if at all reduced in *mnT12* homozygotes (we have not measured recombination frequencies in *mnT12* homozygotes over *LGIV* segments to the left of *unc-33*). The frequency of exchange in the *dpy-1* to *unc-32* interval of LG*III* is little affected by the presence of *mnT12*.

Breakage of the fusion chromosome

There is a mechanism by which homozygous *mnT12* hermaphrodites would be expected to produce male self progeny: if during gametogenesis the fusion chromosome were to break near its fusion site and if a gamete that contained only the mostly-*IV* piece were then produced, that gamete would be essentially nullo-*X* and would be expected, upon fusion with a haplo-*mnT12* gamete, to lead to male development. We presume that this is the mechanism by which rare males occur spontaneously (5/16,450) among the self

Table 1. Recombination in *mnT12* homozygotes

Genotype	Self progeny[a]				Recombination frequency[b]
	WT	D	U	DU	
dpy-3 unc-3X/+ +	1,084	315	326	226	0.38–0.45
dpy-3 unc-3 mnT12/mnT12	757	74	87	213	0.13–0.18
unc-33 dpy-4 IV/+ +	919	91	79	216	0.12–0.16
mnT12 unc-33 dpy-4/mnT12	3,771	290	306	1,060	0.11–0.13
dpy-1 unc-32 III/+ +	1,045	153	136	287	0.17–0.22
dpy-1 unc-32/+ +; mnT12/mnT12	2,290	265	333	633	0.17–0.20

[a] Abbreviations: WT, wild type; D, dumpy; U, uncoordinated; DU, dumpy uncoordinated
[b] 95% confidence limits

progeny of *mnT12* homozygotes. The incidence of such males was greatly enhanced by exposure to gamma rays: 7 males were found among 1,530 progeny of *mnT12* homozygotes exposed as young adults to 7,200 r. Several free duplications have been identified following exposure of *C. elegans* to ionizing radiation (Herman et al. 1976, 1979, 1982; Albertson and Thomson 1982; Rose et al. 1984; Rosenbluth et al. 1985).

Heterozygous mnT12 males

When homozygous *mnT12* hermaphrodites are crossed with N2 males, the male progeny have the genotype *IV/mnT12/0*. When these males are crossed with *dpy-9; unc-3* hermaphrodites having a normal chromosome complement, the hermaphrodite cross progeny carry *mnT12* and are wild type, and the male progeny do not carry *mnT12* and are Unc non-Dpy. We measured the ratio of hermaphrodites to males in such crosses and found it to be close to 1:1 (986 hermaphrodites and 958 males). Heterozygous males were also crossed with *dpy-5 I; him-6 unc-33 IV* hermaphrodites, and the non-Dpy progeny were screened for Unc animals, which would result from the union of nullo-*IV* nullo-*mnT12* sperm and diplo-*IV* ova. Diplo-*IV* ova are expected at a frequency of about 2–3% owing to the presence of *him-6* (Hodgkin et al. 1979). Among 2,105 progeny, two exceptional animals were found; this corresponds to a frequency of nullo-*IV* nullo-*mnT12* sperm produced by *mnT12* heterozygous males of roughly 4%.

Making chromosome IV a sex chromosome

When heterozygous *mnT12* males are crossed to homozygous *mnT12* hermaphrodites, the resulting progeny include *mnT12/mnT12* hermaphrodites and *mnT12/IV* males, which can be picked and mated to each other. We repeated such matings for six generations and showed that the resulting stock retained *mnT12*. We note that in a line reproducing in this way, *mnT12* can be thought of as a neo-*X* chromosome and chromosome *IV* is behaving as a neo-*Y* chromosome: hermaphrodites have two neo-*X* chromosomes and males have one neo-*X* and one neo-*Y*. The organism is thus converted from an *XO:XX* type of sex determination to an *XY:XX* system.

Meiotic chromosome nondisjunction in heterozygous mnT12 hermaphrodites

It is clear simply from their strong Him phenotype that *IV/mnT12/X* animals exhibit meiotic nondisjunction of *mnT12* and *X* because nullo-*mnT12* nullo-*X* gametes must be produced to generate the male self-progeny. We next show that these animals also exhibit nondisjunction of *mnT12* and chromosome *IV*.

Consider the wild-type Him hermaphrodite self-progeny of *dpy-9/mnT12/unc-3* hermaphrodites. About 20% (32/167) of these animals segregated only non-Dpy non-Unc self progeny. We shall demonstrate that animals showing this segregation pattern have the genotype *dpy-9/mnT12/mnT12*. About 81% (457/600) of the progeny of these animals were hermaphrodites, the remaining 19% being male; 36% (63/175) of the hermaphrodites were non-Him. The presence of *mnT12* in the non-Him hermaphrodite progeny was determined as follows: descendants of 25 non-Him animals were mated with N2 males, male progeny were mated with *dpy-9; unc-3* hermaphrodites, and wild-type hermaphrodite progeny were picked and scored for pseudolinkage of *dpy-9* and *unc-3* and for the Him trait. All 25 of the original non-Him animals scored were thus judged to have been homozygous for *mnT12*. We next show that the male progeny of the putative *dpy-9/mnT12/mnT12* animals had the genotype *dpy-9/mnT12/0*. First, we mated male self progeny of 14 independently-derived putative *dpy-9/mnT12/mnT12* animals with *dpy-9; unc-3* hermaphrodites and picked wild-type hermaphrodite progeny. The presence of *mnT12* was then ascertained by checking for both the Him trait and pseudolinkage of *dpy-9* and *unc-3*. In all 14 cases, the males were shown to carry *mnT12*. Second, we mated male self progeny from 15 independently-derived putative *dpy-9/mnT12/mnT12* hermaphrodites with *unc-4 II; dpy-9 IV* hermaphrodites. In all 15 cases, the resulting cross progeny (non-Unc) males were all Dpy, hence the male parents contained a *dpy-9* chromosome *IV*. Because the putative *dpy-9/mnT12/mnT12* animals segregated *dpy-9/mnT12/0* males, they must have carried a *dpy-9* chromosome *IV* and at least one *mnT12* fusion chromosome. We consider two possible reasons why these animals did not segregate any Dpy (or Dpy Unc) offspring: first they had the genotype we have been putatively ascribing to them, in which case there would be no normal *X* chromosome to segregate with the *dpy-9* chromosome, or second, they carried an unfused but recessive lethal *X* chromosome (genotype *dpy-9/mnT12/X*); breakage of *mnT12*, for example, might have produced an unfused but deficient *X* chromosome. We distinguished between these possibilities as follows. Animals were generated having the following putative genotype: *unc-4 II; dpy-9 IV/mnT12/mnT12;* these animals were Him but segregated no Dpy (Unc-4) self-progeny. Individual animals were mated with *dpy-9/+* males. The

(Unc-4) self progeny were screened to confirm that each hermaphrodite parent was Him. All Dpy non-Unc-4 progeny (25/25) were male; this result is consistent with our first explanation but not the second. We thus conclude that animals of genotype *IV/mnT12/mnT12* are generated as self progeny of *IV/mnT12/X* parents. This indicates that chromosomes *IV* and *mnT12* (as well as *X* and *mnT12*) are undergoing meiotic nondisjunction in the *IV/mnT12/X* hermaphrodites. The hatching frequency of eggs laid by *IV/mnT12/X* hermaphrodites was only about 55% (1267/2323); we expect that at least some of the unhatched eggs were essentially monosomic for LG*IV* (and others were nullo-*X*) owing to meiotic nondisjunction.

Trisomy for chromosome IV

The *dpy-9 IV/mnT12/mnT12* animals described in the previous section are essentially trisomic for LG*IV*. In this section we prove that normal chromosome *IV* trisomics can be constructed and maintained.

Males of genotype *unc-30 dpy-4 IV/++* were crossed with *him-6 unc-22 dpy-4 IV/him-6 unc-22 unc-30 IV* hermaphrodites. The role of *him-6* was to increase the frequency of diplo-*IV* ova (Hodgkin et al. 1979). The three markers *unc-22*, *unc-30* and *dpy-4* are all situated in a 4 map unit segment and thus tend to balance each other. Among 123 fertile wild-type progeny of the cross, two were trisomic for LG*IV*, as judged by progeny testing. These animals were non-Him and had the following genotype (omitting *him-6*): *unc-30 dpy-4/unc-22 dpy-4/unc-22 unc-30*. We maintained a trisomic line by simply picking wild-type progeny and checking for the appearance of appropriate offspring. The trisomics segregated principally seven phenotypic classes of self progeny. These classes included (with their frequencies in parentheses; 707 total animals counted); wild type (0.25), Unc-22(0.22), Unc-30(0.15), Dpy(0.20), Dpy Unc-22(0.08), Unc-22 Unc-30(0.05) and Dpy Unc-30(0.05). We have confirmed the presence of an extra chromosome by cytological inspection of oocytes, which generally showed six bivalents and one univalent (Fig. 2c). The trisomic hermaphrodites have small broods (about 150 animals per brood) but seem largely indistinguishable morphologically from wild-type diploids under the dissecting microscope.

Discussion

We have two reasons for believing that *mnT12* is a single genetic element consisting of a fusion of all the essential genes of chromosomes *IV* and *X*. First, no additional elements are observable cytologically, either by us or by Albertson (1984 and personal communication); even the smallest chromosome fragments or free duplications, initially identified genetically as the duplications of single loci, have been readily recognized cytologically (Herman et al. 1976, 1979), probably because fragments below a minimal size are too unstable to be maintained (Albertson and Thomson 1982). Our second reason concerns the likely meiotic instability of a minute chromosomal element. First, it seems unlikely that minute elements would pair and disjoin regularly. In the one case in which animals with two copies of a free duplication could be reliably identified (Herman et al. 1979), nullo-duplication self progeny were frequent, indicating that meiotic stability was not conferred by having potential meiotic pairing partners. Furthermore, the small chromosomal elements that have been studied tend to be lost pre-meiotically during gametogenesis, particularly in the ovum line (Herman et al. 1976, 1979). It therefore seems likely that if the viability of *mnT12* homozygotes depended on the presence of a minute second element (either one or two copies per cell), then a considerable fraction of the eggs laid by such animals would be deficient for the second element; but 94% of the eggs laid by *mnT12* homozygotes hatched to give viable offspring. We conclude that *mnT12* is a simple fusion of chromosomes *IV* and *X*, with both breaks presumably occurring near enough to the tips of the chromosomes such that no essential genes have been deleted.

Our conclusion that considerable meiotic nondisjunction between *mnT12* and *IV* occurs in *mnT12* heterozygotes was based on the demonstration that *IV/mnT12/mnT12* animals are produced as self progeny of *mnT12* heterozygotes. Although we did not demonstrate their presence, we expect that two other genotypes trisomic for LG*IV* were also produced by *mnT12* and *IV*: *IV/IV/mnT12* males and *IV/IV/mnT12/X* hermaphrodites; if the latter are Him, they would have been classified as *mnT12* heterozygotes. Zygotes monosomic for *IV* were probably also produced, but we would expect them to be inviable (Hodgkin et al. 1979). Similarly we expect that high frequency nondisjunction between *mnT12* and *X* in *mnT12* heterozygotes would have generated the following triplo-X hermaphrodite self progeny: *mnT12/mnT12/X* and *IV/mnT12/X/X*. Triplo-*X* animals are shorter than wild type (Hodgkin et al. 1979); some of the self progeny of *mnT12* heterozygotes that we classified as wild type were in fact short (but were not Dpy) and may have been triplo-*X*, but we did not attempt to prove that they were. Animals of genotype *mnT12/mnT12/X* would have been classified as *mnT12* homozygotes because they would not be capable of generating male self progeny. If *IV/mnT12/X/X* animals are Him, they would have been classified as *mnT12* heterozygotes. About 3% ($6/39 \times 433/666$) of the self-progeny of *dpy-9/mnT12/unc-3* animals were non-Him and segregated both wild-type and Dpy Unc self progeny; we suspect that these animals may have been either *IV/IV/mnT12/X* or *IV/mnT12/X/X* or both.

We do not know whether the nondisjunction observed between *mnT12* and *X* and between *mnT12* and *IV* in *mnT12* heterozygotes is the result of reduced pairing between the fusion chromosome and its normal homologues or is due to difficulties in a subsequent stage of the segregation process. We have looked at oocytes of *mnT12* heterozygotes by light microscopy; they generally showed the fusion chromosome and its two wild-type homologues together, suggesting that *mnT12* generally pairs with both *IV* and *X*, but the evidence for that view is weak. It might be interesting to see reconstructions of the synaptonemal complexes formed by *mnT12* heterozygotes (Goldstein and Slaton 1982; von Wettstein et al. 1984). If *mnT12* invariably pairs with both *IV* and *X* to produce a trivalent, and if the trivalent is maintained until anaphase I, then by analogy with the behavior of monocentric chromosomes in translocation heterozygotes, the observed meiotic nondisjunctions might be the simple consequence of different possible orientations of the trivalent on the metaphase plate. Segregation from the "alternate" orientation of a translocation heterozygote with monocentric chromosomes produces euploid gametes,

but segregation from "adjacent-1" and "adjacent-2" orientations generates aneuploid gametes. Unfortunately, conclusions about the orientations of *C. elegans* chromosomes at metaphase I are made very difficult by their extremely condensed state at that stage (Nigon and Brun 1955); we thus do not know in what respects, if any, the meiotic orientations and segregations of the holocentric *C. elegans* chromosomes might be analogous to the behavior of monocentric chromosomes.

As noted in the introduction, a diffuse centromere cannot keep sister chromatids joined all along the chromosome lengths until meiosis II and still permit crossing over. Earlier work with dominant *X* chromosome nondisjunction mutants of *C. elegans* led to the proposal that the left end of the *X* chromosome plays a critical structural role in the segregation of *X* chromosomes during meiosis in *XX* animals, perhaps through maintaining end-to-end attachment of sister chromatids until the appropriate time for disjunction (Herman et al. 1982). The proposal was based primarily on the properties of two mutations. One mutation mapped to the left end of the *X* chromosome, promoted equational nondisjunction of itself but not its wild-type allele in heterozygotes and also promoted *X* chromosome nondisjunction in homozygotes; it was suggested that the mutation disrupted the normal segregational function of the left end of the *X* chromosome. The other mutation was a half translocation, called *mnT10(X)*, which had a small region of the left end of linkage group *V* substituted for the left tip of *X*; this element showed meiotic nondisjunction when either heterozygous (with a normal *X* chromosome) or homozygous; the segregational defect was attributed to the absence of the left tip of the *X*. The left end of the *X* chromosome is intact as an end of *mnT12* and may be responsible for the regular disjunction of the fusion chromosome in *mnT12* homozygotes. The fact that animals of genotype *IV/mnT12/mnT12* segregate males at high frequency (19% of self progeny) indicates that the disjunction of the two fusion chromosomes is disrupted by the presence of a normal *IV*, however.

The *mnT12* chromosome has proved useful in work directed at localizing genes by *in situ* hybridization of cloned probes (Albertson 1984). Because the wild-type chromosomes are largely indistinguishable cytologically, the large size of the *mnT12* chromosome served to distinguish linkage groups *IV* and *X* from the other four linkage groups. Homozygous *mnT12* animals may also prove useful in an assay for chromosome breakage, as we have illustrated; only through chromosome breakage and loss of the bulk of the *X* portion of one copy of *mnT12* (or mutation conferring a male phenotype of *XX* animals) should *mnT12* homozygotes yield male self progeny, which are easy to identify. We have not investigated the positions of these breaks in *mnT12*. They might occur at sites somewhat removed from the original fusion site, in which case a broken chromosome could carry either a duplication of the right end of X or a deficiency of the left end of LG*IV*.

The existence of viable triplo-*X* animals has been known for some time (Hodgkin et al. 1979), but we have here reported the first example of a viable autosomal trisomic. Because triplo-*X* animals are shorter than diploid hermaphrodites, it was a little surprising that triplo-*IV* animals have essentially wild-type morphology and movement. We now see no reason why other autosomal trisomics may not also prove to be viable and fertile.

Numerous instances of evolutionary reversions from an *XO:XX* sex chromosome system to an *XY:XX* system have been identified, particularly in grasshoppers (White 1973). These reversions have usually come about through the centric fusion of an acrocentric autosome and an acrocentric *X* chromosome to produce a neo-*X* chromosome. Once unfused *X* chromosomes have been eliminated from the population, the original acrocentric autosome is confined to the male line and constitutes a neo-*Y* chromosome. We have shown that *mnT12* and autosome *IV* are capable of playing the roles of neo-*X* and neo-*Y* chromosomes, respectively, in *C. elegans*, thereby converting the sex determination system from *XO* to *XY*. The *XO* system has an obvious advantage for *C. elegans* in that males can arise spontaneously as self progeny of hermaphrodites through meiotic nondisjunction; with an *XY* system, males can only arise as progeny of male-by-hermaphrodite crosses. A possible advantage of a *Y* chromosome is in providing a pairing partner for the *X* in males. A special mechanism is required for handling the meiotic segregation of the single *X* chromosomes in *XO* males of *C. elegans* (Hodgkin et al. 1979); this mechanism is inoperative or ineffective in both gamete lines of *XO* hermaphrodites (made hermaphrodite by *her-1* mutation), in which the unpaired *X* chromosome tends to be lost during meiosis (Hodgkin 1980).

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