

Chromosome-Wide Control of Meiotic Crossing over in *C. elegans*

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Summary

A central event in sexual reproduction is the reduction in chromosome number that occurs at the meiosis I division. Most eukaryotes rely on crossing over between homologs, and the resulting chiasmata, to direct meiosis I chromosome segregation, yet make very few crossovers per chromosome pair [1, 2]. This indicates that meiotic recombination must be tightly regulated to ensure that each chromosome pair enjoys the crossover necessary to ensure correct segregation. Here, we investigate control of meiotic crossing over in *Caenorhabditis elegans*, which averages only one crossover per chromosome pair per meiosis [3, 4], by constructing genetic maps of end-to-end fusions of whole chromosomes. Fusion of chromosomes removes the requirement for a crossover in each component chromosome segment and thereby reveals a propensity to restrict the number of crossovers such that pairs of fusion chromosomes composed of two or even three whole chromosomes enjoy but a single crossover in the majority of meioses. This regulation can operate over physical distances encompassing half the genome. The meiotic behavior of heterozygous fusion chromosomes further suggests that continuous meiotic chromosome axes, or structures that depend on properly assembled axes, may be important for crossover regulation.

Results

Eukaryotes that rely on crossovers to direct meiotic chromosome segregation have evolved alternative strategies to ensure that crossovers are formed between each chromosome pair during every meiosis. Some organisms (e.g., *Schizosaccharomyces pombe*) simply make large numbers of crossovers per chromosome pair, with means high enough to ensure that few chromosome pairs will fail to receive at least one crossover [5]. In such organisms, the number of crossovers per chromosome pair appears to be randomly distributed. Interestingly, however, most eukaryotes actually form very few crossovers per chromosome pair per meiosis — typically on the order of 1–3 per chromosome arm. This is true for diverse organisms with chromosome sizes that differ by 2–3 orders of magnitude (from 230 kb to 100 Mb). The nematode *Caenorhabditis elegans* represents an extreme example of this general case: despite a nearly 2-fold range in physical size between the smallest

(13.7 Mb) and largest (20.9 Mb) chromosomes, each of the six *C. elegans* chromosomes has a genetic length of roughly 50 cM, corresponding to a mean of one crossover per chromosome pair per meiosis. This is not simply an average of one, since chromosome pairs lacking chiasmata (the nonexchange, or E_0 class), which would be expected to occur at high frequency if crossovers were distributed randomly, are very rare (<1% of oocyte meioses) [6, 7]. Lack of E_0 meioses, together with 50 cM maps, implies a very narrow, nonrandom distribution of chiasma number/chromosome pair (very few E_0 , few $E > 1$). This nonrandom distribution indicates that crossing over must be tightly regulated to ensure that each pair enjoys the “obligate chiasma” required to direct orderly segregation [8].

To investigate the control of meiotic crossing over in *C. elegans*, we assessed the meiotic recombination behavior of end-to-end chromosome fusions (Figure 1). These complete chromosome fusions are stable in mitosis and meiosis ([9], see below). We first constructed genetic maps of two-chromosome fusions in the homozygous state by determining the frequencies of recombination between pairwise combinations of visible genetic markers distributed along their lengths (Figures 2A and 2B). The fusion chromosomes analyzed were *mnT12* and *eT6*, two *IV;X* fusions differing in the orientation of *X* [9, 10]. This analysis generated genetic maps of 48.8 cM for *mnT12* and 50.1 cM for *eT6* for the assayed portions of the fusion chromosomes. Since the markers used spanned 85%–90% of the total physical length of the chromosomes assayed, we estimate a total genetic length of 54 cM for *mnT12* and 59 cM for *eT6*; this estimate assumes that the frequency of recombination in the unassayed terminal portions of the chromosomes is similar to that of the chromosome as a whole. Remarkably, the map length of each of these two-chromosome fusions is nearly the same as those of each of the single constituent chromosomes in their unfused states. Further, segregation behavior and cytological analysis of fusion chromosomes indicated that E_0 meioses are very rare in these fusion chromosome homozygotes: *mnT12* and *eT6* homozygotes produced >99% viable progeny ($n = 797, 805$), indicating that segregation errors leading to aneuploidy are extremely infrequent. Moreover, no fusion chromosome pairs lacking chiasmata were observed in 170 and 145 late prophase nuclei scored in *mnT12* and *eT6* homozygotes, respectively; this finding indicates that the incidence of E_0 pairs is <1%. Together, these results indicate that the two fused chromosomes are now being perceived as a single chromosome “unit” by the organism: a segment of the genome that previously would have received two crossovers now typically receives one crossover. These results imply that meiotic crossovers in *C. elegans* are limited by a chromosome-wide interference mechanism that operates to discourage additional exchanges once a single initiated recombination event has been designated for the crossover pathway.

Beyond the overall 40%–50% reduction in the fre-

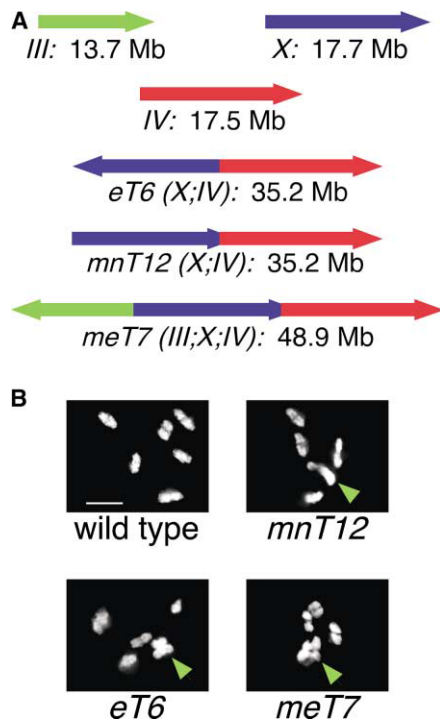


Figure 1. Wild-Type and Fusion Chromosomes Used in This Study
(A) Diagrammatic representation of unfused and fused chromosomes. The arrowheads signify the right ends of chromosomes. The exact structures of fusion points are unknown, but the viability of fusion chromosome homozygotes indicates that no essential sequences have been deleted.
(B) Late meiotic prophase karyotypes of fusion chromosome homozygotes. The wild-type nucleus has six pairs of DAPI-stained chromosomes attached by chiasmata. *mnT12*, *eT6*, and *meT7* nuclei have five, five, and four pairs, respectively; the fusion chromosome bivalent is indicated by the arrow. The scale bar represents 6 μ m.

quency of crossing over per unit of DNA, several additional features of the two-chromosome fusion maps were notable. Each of the *C. elegans* autosomes has a centrally located, gene-dense cluster in which recombination is greatly depressed compared with the chromosome average [11, 12]; during wild-type meiosis, the vast majority of autosomal crossovers occur in the regions flanking these clusters, within 25%–35% of chromosome length from a chromosome end. We found that the low frequency of recombination per unit of DNA characteristic of the chromosome IV cluster was retained in the fusion chromosomes, despite the region now being located proportionally closer to a chromosome end. This suggests that repression of recombination in the cluster may be conferred largely by local sequence intrinsic features. We also found evidence for chromosomal position effects influencing crossover frequency. Since the X chromosome portion of the fusion is inverted in *mnT12* relative to *eT6*, we could compare recombination in the *dpy-6 unc-3* interval (Figures 2A and 2B) in two different locations. The genetic length of this interval is significantly smaller in *mnT12*, where it is centrally located, than in *eT6*, where it is terminally located (8.1 cM versus 13.8 cM; $p < 0.01$). This suggests that the position along the chromosome can influence

the likelihood of crossing over and is consistent with an organismal preference for off-center exchanges.

We also determined the genetic map length of a three-chromosome fusion in the homozygous state (*meT7*: III; X; IV). *meT7* is 48.9 Mb long and comprises almost half of the genome on a single chromosome (Figure 1). We used a modified mapping procedure that permitted simultaneous scoring of four markers to assess recombination in three intervals in a single cross (Figure 2C). Whereas the mapping procedure used to generate the two-chromosome fusion maps was designed to sample the large numbers of meioses required for precision in measuring small intervals, the modified method used for *meT7* was designed to enable detection of possible exchanged chromosomes.

Despite consisting of three whole chromosomes and comprising almost half of the genome, the measured genetic length of *meT7* was only 54 cM. As the markers used span 88% of the chromosome, some chromosomes likely had crossovers in the unassayed region. By assuming that the frequency of crossing over in the terminal regions was similar to that of the chromosome as a whole, we estimate a total map length of 61 cM for *meT7*. This map length, coupled with a low incidence of E_0 meioses (assayed cytologically) in *meT7* homozygotes (1/189), implies that in the majority of meioses, the three-chromosome fusion receives only a single crossover over a physical distance that would normally accommodate three crossovers. Moreover, the distribution of exchanges along the chromosomes in the 22 meiotic products with two crossovers differed significantly from that expected if crossovers in the different intervals were occurring independently. Double-crossover products involving exchanges in the two terminal intervals (interval 1 and interval 3, Figure 2C) were over-represented ($p = 0.0020$), whereas those involving exchanges in the adjacent intervals 1 (the smallest interval) and 2 were underrepresented ($p = 0.0021$). The nonindependent behavior of the different intervals implies that when two exchanges do occur, they are still governed by an interference mechanism that acts along the length of *meT7* to discourage nearby double exchanges, resulting in a wide spacing between coincident crossovers. Taken together, these results indicate that the chromosome-wide interference mechanism that limits meiotic crossovers is capable of acting over half the genome.

Having demonstrated the existence of a robust, chromosome-wide interference mechanism that limits the number of crossovers along a chromosome pair in *C. elegans*, we sought to further investigate the nature of the functional unit upon which this mechanism operates. Specifically, we examined meiosis in animals heterozygous for fused and unfused chromosomes.

We assessed chiasma formation, a cytological read-out of meiotic crossing over, in hermaphrodites heterozygous for *mnT12* and one copy each of unfused IV and X (Figure 3A). During early and mid-prophase, both the IV and X segments pair as efficiently in *mnT12* heterozygotes as in wild-type worms (data not shown). By late prophase (diakinesis), chromosomes have become highly condensed, and homologous chromosome pairs that have enjoyed crossovers are held together by chias-

mata [13]. Thus, six DAPI-stained bodies, corresponding to six pairs of attached homologs (bivalents), are detected at diakinesis in wild-type oocytes (Figure 1B). Further, five bivalents are seen in *mnT12* and *eT6* homozygotes, and four are seen in *meT7* homozygotes. In *mnT12* heterozygotes, diakinesis nuclei with five DAPI-stained bodies (indicating that crossing over had occurred between *mnT12* and both *X* and *IV*) comprised 55% of the total, while nuclei with six DAPI-stained bodies (presumably reflecting meioses with crossovers between either *X* and *mnT12* or *IV* and *mnT12*, but not both) comprised 45% of the total. These data suggest that, when heterozygous, *mnT12* and its cognate unfused partners enjoy two (or more) crossovers in roughly half of meioses and may undergo only a single crossover in the remaining half of meioses. These data can be used to estimate a minimum genetic map length of about 78 cM for heterozygous *mnT12*, indicating that heterozygosity for *mnT12* results in an increased frequency of crossovers relative to *mnT12* homozygotes.

In addition, we measured crossing over between SNP markers located near opposite ends of chromosome *IV* in males heterozygous for *mnT12* and one copy of unfused *IV* (Figure 3B). The frequency of recombination in these heterozygotes (40%, 35/87) was not significantly different from that in control males (49%, 34/69); these data suggest that the partnerless *X* portion of *mnT12* does not reduce the overall frequency of crossing over on the *IV* portion.

We also measured crossing over between SNP markers located near opposite ends of both chromosomes *III* and *IV* in males heterozygous for *meT7* and one copy each of unfused *III* and *IV* (Figure 3C). In these animals, unfused *III* and *IV* can pair and recombine with *meT7*, while the *X* portion of *meT7* remains partnerless. Unfused *III* and *IV* segregate efficiently from *meT7* in these animals (see Experimental Procedures), suggesting that chiasmata may form on both the *III* and *IV* segments in each meiosis. Recombination data support this conclusion: recombination frequencies for the chromosome *III* interval (48%) and the chromosome *IV* interval (41%) in the *meT7* heterozygote males were statistically indistinguishable from those in control males (46% and 49%, respectively), consistent with each of the autosomal portions of the fusion enjoying a crossover in each meiosis. In addition, the frequency of chromosomes with crossovers on both the *III* and *IV* portions (20%) was not different from that expected if crossovers on *III* and *IV* occurred independently, suggesting a lack of interference across the partnerless *X* portion of the fusion.

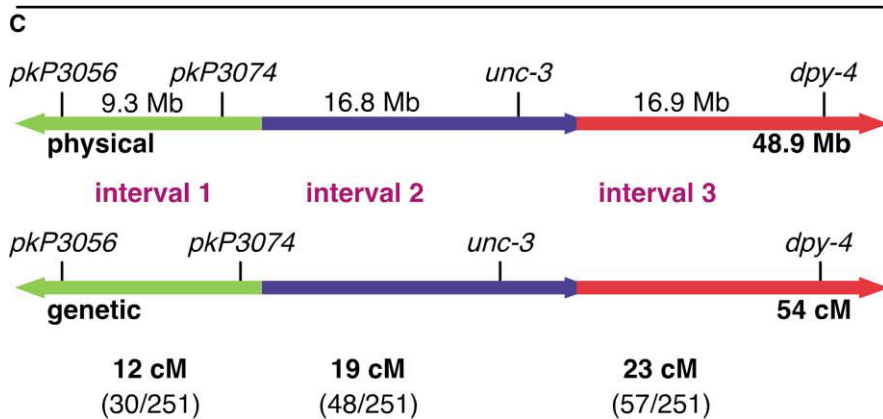
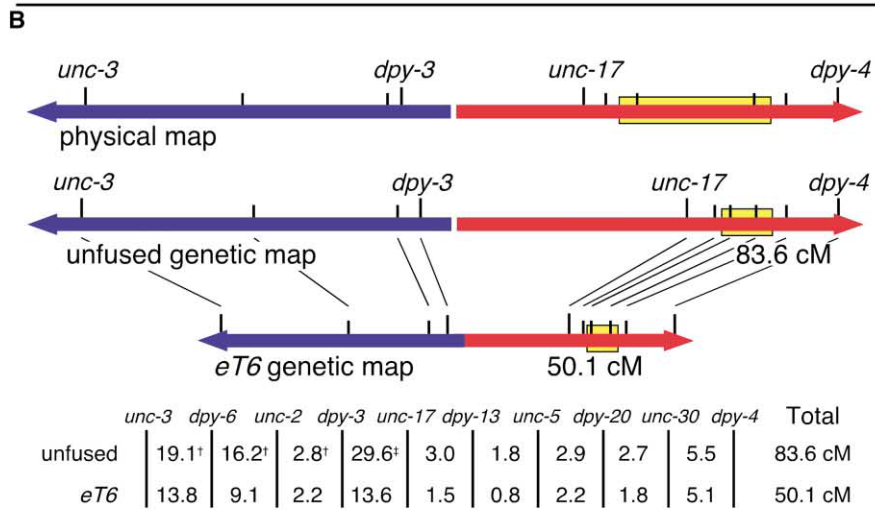
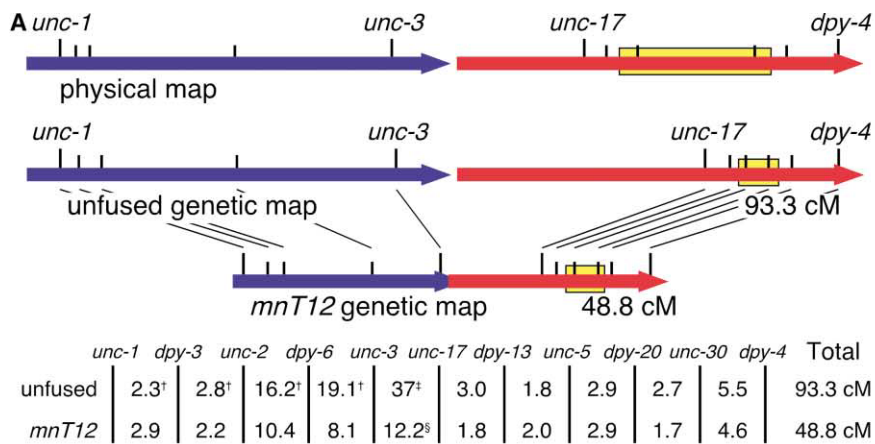
Discussion

Meiotic crossovers and chiasmata are nonrandomly distributed in many organisms, a phenomenon that has been appreciated for decades. One manifestation of nonrandomness is the relative rarity of E_0 chromosome pairs, reflecting the formation of an “obligate chiasma” [8, 14]. A second manifestation of nonrandomness is crossover or chiasma interference, the tendency of a (nascent) crossover event in one region of a chromosome to discourage the formation of other crossovers

nearby. Crossover interference was first observed in *Drosophila* nearly a century ago by Muller [15], and subsequent observations have revealed similar effects in most eukaryotes, including *C. elegans* (e.g., [16–20]). This study demonstrates that during *C. elegans* meiosis, fusion of two or three whole chromosomes apparently removes the requirement for placing a chiasma in each of the constituent chromosome segments. We found that most fusion chromosome pairs in homozygous worms undergo only a single crossover event over a length of chromosome that would normally enjoy two or three crossovers, indicating that the cell perceives the fusions as single chromosomes and is no longer obliged to form two or three crossovers. Moreover, multiple crossovers are actively discouraged: although it is clear that these chromosomes can readily and reproducibly accommodate two or three crossovers in the unfused state, such multiple events are infrequent on the fusion chromosomes. The fact that removing the obligation for multiple crossovers is accompanied by such a marked reduction in the incidence of multiple crossovers indicates that the fusion chromosomes are being treated as single chromosome units with regard to both obligate chiasma and crossover interference and may reflect an underlying mechanistic linkage between these two facets of crossover control.

Is there an evolutionary advantage imparted by crossover interference per se? It is possible that widely spaced crossovers might confer a favorable orientation of bivalents on the meiosis I spindle that imparts a selective advantage. Alternatively, the tendency for wide spacing between coincident crossovers may instead be a secondary consequence of the chromosome properties that ensure obligate exchange.

What is the functional unit upon which crossover control mechanisms operate — that is, what qualifies to be recognized as a “chromosome”? Since analogous phenomena operate in diverse organisms over physical distances that differ by several orders of magnitude, it is unlikely that the unit corresponds to the DNA molecule per se. Instead, our observations provide support for the idea that the unit upon which chromosome-wide crossover control mechanisms operate may correspond to a region capable of continuous homologous synapsis — that is, a region capable of assembling the meiosis-specific synaptonemal complex (SC) structure connecting the axes of aligned homologous chromosome segments [8, 21]. The fact that hermaphrodites heterozygous for *mnT12* enjoy chiasmata in both segments in some meioses and in only one segment in others suggests that the presence of an axial discontinuity on only one partner “chromosome” can partially, but not completely, disrupt the ability to communicate the presence of a (nascent) crossover and/or to discourage others in response. Further, in *meT7* heterozygous males, where homology between the fusion chromosome and its unfused counterparts is interrupted by a partnerless *X* chromosome segment, the two chromosome segments with homologous partners apparently each enjoy a chiasma in every meiosis; this suggests that each of these segments may be treated separately as a “chromosome” unit by the crossover control system. Absence of interference between crossovers on the *III* and *IV*



Double Crossovers:

intervals 1 and 2: 0/251
 intervals 1 and 3: 14/251
 intervals 2 and 3: 8/251

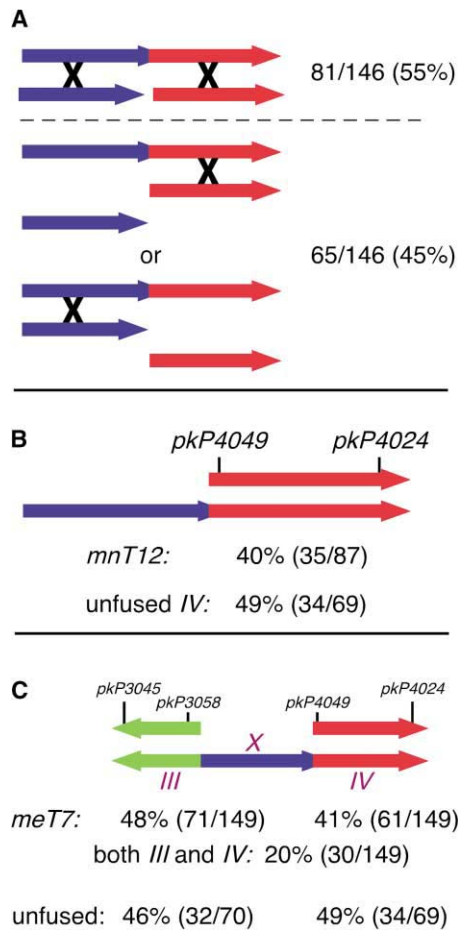


Figure 3. Meiotic Behavior of Fusion Chromosome Heterozygotes
(A) Chiasma formation in *mnT12* heterozygous hermaphrodites (X; *mnT12*; IV). The upper portion of the diagram represents oocyte nuclei containing a trivalent in which both unfused X and IV were connected to *mnT12* by chiasmata. The lower portion of the diagram represents nuclei containing an asymmetric bivalent (with either X or IV connected to *mnT12* by a chiasma) and a univalent.
(B and C) Crossing over in *mnT12* and *meT7* heterozygous males. The diagrams depict the relevant portions of karyotypes of fusion chromosome heterozygote males and the positions of SNP markers typed. Fractions of total meiotic products assayed that were recombinant in a given interval are indicated below the diagrams for both fusion heterozygotes and controls.

segments in *meT7* heterozygous males might be interpreted as evidence that mature SC structure (which does not form on the X in males; K.J.H., unpublished

data) is required to communicate the status of recombination events. However, this result is also consistent with models wherein crossover regulation is conferred by properties inherent to the meiotic chromosome axis per se and does not require SC polymerization [22], since the X chromosome is known to be heterochromatic during male meiosis [23, 24] and its axis appears to differ in organization from those of the euchromatic autosomes (K.J.H., unpublished data). Indeed, preliminary results from our lab indicate a role for properly constructed axes (and/or SC structures that depend on proper axis morphogenesis) in conferring crossover interference in *C. elegans* (Nabeshima and K.J.H., unpublished data).

The idea that meiotic chromosome axes or SC might represent relevant functional units for crossover regulation is consistent with several independent correlative observations. For example, organisms such as *S. pombe* that lack continuous axial structures and SCs also lack crossover interference [5, 25, 26]. Further, mean chiasma frequencies are highly correlated with mean SC lengths in males of *Locusta migratoria* [27], and recent studies in humans and mice have shown that the number of MLH1 foci (a cytological marker for meiotic crossovers) in individual meiocytes covaries with SC length [28–30].

Several previous studies have provided evidence that the frequency of crossovers enjoyed by a given chromosome segment can vary depending on chromosomal context [31–36]. For example, studies by Kaback et al. of chromosome bisections and translocations in *Saccharomyces cerevisiae* demonstrated an inverse correlation between crossover frequency in a particular DNA segment and the size of the chromosome on which that segment resides. Further, Rose and coworkers showed that when crossovers are restricted to a limited region of a chromosome pair in *C. elegans* (by heterozygosity for chromosome rearrangements), the organism compensates by increasing the frequency of recombination in the crossover-competent region. Our analysis of whole-chromosome fusions complements and extends these previous findings in several ways. By removing the necessity for a crossover within segments as large as whole chromosomes, we have revealed a strong proclivity of *C. elegans* to limit the number of crossovers to one per chromosome pair. Moreover, our results demonstrate that crossover control mechanisms can operate across fusion chromosomes much larger than any chromosome normally present in the wild-type organism. Together, these findings indicate a remarkable ca-

Figure 2. Physical and Genetic Maps of Fusion Chromosomes

Positions of markers on the physical maps are from Wormbase [4]; physical positions of uncloned loci (*dpy-6* and *dpy-4*) are estimates based on known correlations between physical and genetic maps.

(A and B) *mnT12* and *eT6*. Genetic maps of *mnT12* and *eT6* constructed by measuring recombination frequencies between pairs of visible markers. The ticks on the maps correspond to the map positions of markers indicated in the tables at the bottom of the panel; the tables present genetic map distances in cM between each adjacent pair of markers. The distances between the markers are to scale; yellow boxes delineate the approximate extent of the chromosome IV gene cluster. †, data from [6]. ‡, estimated distance from published map; see the Experimental Procedures. §, calculated from *unc-3-dpy-13* (14 cM) and *unc-17-dpy-13* (1.8 cM) genetic distances.

(C) *meT7*. Genetic map distances were determined by using a procedure that assessed recombination simultaneously in the three indicated intervals; *pkP3056* and *pkP3074* are SNP markers. For each interval, the genetic map length in cM is indicated; [number of crossovers in the interval]/[total meiotic products assayed] is given in parentheses. The types and numbers of double crossover products are also indicated; no triple crossover products were found.

capacity of eukaryotic organisms to recognize new combinations as a “chromosome” and to modulate meiotic recombination accordingly. This has important implications for chromosome evolution, as it suggests that a means to rapidly stabilize a new karyotype may be an inherent feature of the meiotic program. Given an opportunity for inbreeding, such a capacity could promote rapid reproductive success of individuals homozygous for new karyotypes while at the same time imposing reduced reproductive fitness on heterozygotes, providing a powerful driving force for reproductive isolation.

Experimental Procedures

Except where noted, the strains and markers used were derived from the standard *C. elegans* strain Bristol N2.

Fusion Chromosomes

C. elegans chromosomes are holokinetic [37, 38], so end-to-end chromosome fusions are stable in mitosis and meiosis. Since meiotic nondisjunction leads to the production of inviable aneuploid embryos, success of meiotic segregation in fusion homozygotes was assessed by determining the frequency of inviable embryos produced. Wild-type hermaphrodites produce >99% viable progeny, indicating accurate chromosome segregation during meiosis. *mnT12* and *eT6* homozygotes also produce >99% viable progeny (*mnT12*: 790/797 embryos hatched; *eT6*: 803/805 embryos hatched). *meT7* homozygotes produce 92% viable progeny (326/355 embryos hatched), indicating successful chromosome segregation in most meioses. As E_3 meioses are rare in *meT7* homozygotes (<1%), the inviability of 8% of embryos is not caused by failure to form chiasmata. Rather, we speculate that the large size of the condensed fusion bivalent may sometimes exceed the capacity of the very short oocyte meiotic spindle [38].

The success of meiotic segregation in *meT7* heterozygous males was assessed by crossing them with *dpy-4 spe-8* hermaphrodites (impaired for self-sperm activation). 98.5% of embryos laid were viable (1024/1040), and 51.0% of viable outcross progeny were male (522/1024), indicating that aneuploid gametes were very infrequent.

Construction of *mnT12* and *eT6* Genetic Maps

Pairs of recessive visible markers were crossed onto the fusion chromosome, and doubly marked fusion hermaphrodites were crossed with males bearing the unmarked fusion to generate marker heterozygotes. Progeny of heterozygous hermaphrodites were scored for parental and recombinant phenotypes; for each marker pair, all self-progeny from at least 5 heterozygotes were scored (a minimum of 1100 progeny, except for the *unc-5 dpy-20* interval on *eT6* where 948 progeny were scored). Map distances (p) were calculated from the fraction of recombinant progeny (R) by using the standard equation $p = 1 - (1 - 2R)^{1/2}$ [11]. Statistical analyses were performed by using Fisher's exact test. For unfused chromosomes, the *unc-3-unc-17* and *dpy-3-unc-17* genetic distances were estimated by summing estimated genetic distances from *unc-3* to X_R (or *dpy-3* to X_L) and IV_L to *unc-17*. For *mnT12*, the genetic distance from *unc-3* to *unc-17* was estimated by subtracting the *unc-17-dpy-13* genetic distance from the *unc-3-dpy-13* genetic distance.

Construction of the *meT7* Genetic Map

meT7 was mapped by using a combination of recessive visible markers (*unc-3 X* and *dpy-4 IV*) and SNP markers. A version of *meT7* was constructed wherein the majority of chromosome *III* sequences (originally derived from N2) were replaced by sequences from strain CB4856 (which has many mapped DNA sequence differences from N2). In contrast to the standard mapping procedure (used for *mnT12* and *eT6*) that assesses recombination in both spermatocyte and oocyte meioses, the procedure used for *meT7* assessed recombination only in oocyte meiosis. Hermaphrodites homozygous for *meT7* and heterozygous for all four markers were crossed with *dpy-4 IV* males of normal karyotype, and male progeny were scored for all four markers. As males carry a single copy of the *X* chromosome,

the allele present at *unc-3 X* could be scored directly. As all male progeny receive *dpy-4 IV* from their father, those that also receive *dpy-4* from the hermaphrodite will be *Dpy*. Since the male parent contributes only N2-derived alleles for the SNP markers, the presence or absence of the CB4856-derived alleles indicates which alleles were contributed by the oocyte. Since each progeny male contains a single product of an oocyte meiosis, and since all four markers can be scored for each product, this method allows detection of multiply exchanged chromosomes. For each pair of intervals, Fisher's exact test was used to calculate the probability that crossovers in the two intervals occurred independently of each other; in addition, the signs of departures from independence were inferred by comparing the observed incidence of doubles to the expected incidence determined by multiplying the individual frequencies. The fit of the overall distribution of crossovers in our data set to a model assuming independent behavior of all intervals was assessed by using a Pearson's χ -square test to evaluate all classes simultaneously; this yielded a p value of 0.0002 (for 4 degrees of freedom).

Recombination Frequencies in Males

mnT12 or *meT7* homozygous hermaphrodites were mated to CB4856 males to derive male progeny with one copy of the fusion chromosome and one copy each of the CB4856-derived autosomes. These males were mated to *unc-3* (N2) hermaphrodites, and non-*Unc* hermaphrodite progeny (which will have inherited the fusion chromosome from their fathers) were collected and scored for the indicated SNP markers. *pkP4049* and *pkP4024* are 14.7 Mb apart (91% of the physical length of *IV*). *pkP3045* and *pkP3075* are 11.4 Mb apart (84% of the physical length of *III*).

Cytological Assessment of Diakinesis Chromosomes

For Figure 1, oocyte nuclei were prepared and imaged as in [39]. For quantitation, adult hermaphrodites (24 hr post-L4 stage) were fixed and stained with DAPI as in [6], and late diakinesis oocyte nuclei were scored for number and appearance of DAPI-stained bodies. This analysis assumes that attachment at diakinesis reflects the presence of a chiasma and underlying crossover and that crossovers reliably result in detectable chiasmata.

Supplemental Data

Supplemental Data including additional details regarding markers and strain constructions are available at <http://www.current-biology.com/cgi/content/full/13/18/1641/DC1/>.

Acknowledgments

We thank the *Caenorhabditis* Genetics Center and Shawn Ahmed for strains, F. Stahl, K. McKim, N. Hunter, H. Scherthan, and members of the Villeneuve laboratory for comments on the manuscript, members of the Villeneuve lab for technical assistance, and A. Owen for advice regarding statistical analysis. This work was supported by an American Cancer Society postdoctoral fellowship (PF-99-139-01-DDC) to K.J.H. and by a Searle Scholars Award and the Esther Ehrman Lazard Faculty Scholar Award to A.M.V.

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