

Genetic Variation in Rates of Nondisjunction: Association of Two Naturally Occurring Polymorphisms in the Chromokinesin *nod* With Increased Rates of Nondisjunction in *Drosophila melanogaster*

Michael E. Zwick, Jennifer L. Salstrom and Charles H. Langley

Center for Population Biology, University of California, Davis, California 95616

Manuscript received December 4, 1998

Accepted for publication April 9, 1999

ABSTRACT

Genetic variation in nondisjunction frequency among *X* chromosomes from two *Drosophila melanogaster* natural populations is examined in a sensitized assay. A high level of genetic variation is observed (a range of 0.006–0.241). Two naturally occurring variants at the *nod* locus, a chromokinesin required for proper achiasmate chromosome segregation, are significantly associated with an increased frequency of nondisjunction. Both of these polymorphisms are found at intermediate frequency in widely distributed natural populations. To account for these observations, we propose a general model incorporating unique opportunities for meiotic drive during female meiosis. The oötid competition model can account for both high mean rates of female-specific nondisjunction in *Drosophila* and humans as well as the standing genetic variation in this critical fitness character in natural populations.

IN the 83 years since Calvin Bridges (1916) employed nondisjunction to interpret the outcome of experiments that ultimately provided the foundation for the chromosome theory of inheritance, significant advances have been made in understanding the genetic events leading to the failure of chromosome segregation in *Drosophila melanogaster* (see Hawley *et al.* 1992 for a review). Recent studies suggest that the genetic events underlying nondisjunction appear remarkably similar in *Drosophila* and humans (Koehler *et al.* 1996a,b; Lamb *et al.* 1996; Bugge *et al.* 1998; Robinson *et al.* 1998). Spontaneous nondisjunction in both *Drosophila* and humans is characterized by three distinct properties: the vast majority of events occur in females at meiosis I, nondisjunctional chromosomes exhibit reduced levels and abnormal distributions of crossing-over, and rates of nondisjunction increase with maternal age (Penrose 1933; Merriam and Frost 1964; Tokunaga 1970a,b; Carpenter 1973; Warren *et al.* 1987; Sherman *et al.* 1991, 1994; Koehler *et al.* 1996a,b; Lamb *et al.* 1996, 1997; Bugge *et al.* 1998; Robinson *et al.* 1998). Despite progress in elucidating the proximate causes of nondisjunction, the evolutionary basis for the observed pattern and prevalence of spontaneous nondisjunction remains largely unknown.

Two aspects of spontaneous nondisjunction appear particularly paradoxical. First, why does spontaneous nondisjunction display a sex bias, with the vast majority of segregation errors occurring in females at meiosis

I? Second, what can account for the high frequency of spontaneous nondisjunction in both *Drosophila* (Bridges 1916; Merriam and Frost 1964; Koehler *et al.* 1996a) and human females (Hassold *et al.* 1996)? Two distinct game theory models constitute the only evolutionary analyses that attempt to explain the observed patterns of spontaneous nondisjunction (Axelrod and Hamilton 1981; Day and Taylor 1998). Close examination of both models leads to their rejection for a variety of reasons. First, Axelrod and Hamilton (1981) propose an unrealistic iterated prisoner dilemma (IPD) model that is rejected by a theoretical analysis in Day and Taylor (1998). But the Day and Taylor (1998) chromosome drive (CD) model also contains a critical flaw. Its one unique prediction, that all nondisjunction must result in trisomy (diplo exceptions) rather than nullosomies (nullo exceptions), is clearly not consistent with the observed data from either *Drosophila* or humans (Bridges 1916; Sturtevant and Beadle 1936; Merriam and Frost 1964; Koehler *et al.* 1996a; human data reviewed in Hassold *et al.* 1996). Thus neither evolutionary model provides an adequate explanation for the observed patterns of spontaneous nondisjunction.

One possible explanation for the observed sex bias in rates of nondisjunction is that natural populations do not harbor any genetic variation in rates of female-specific nondisjunction. As a consequence, natural selection could not act to increase the fidelity of chromosome transmission during female meiosis. Numerous previous studies have detected genetic variation for recombination rates in female meiosis (reviewed in Brooks 1988). Some *Drosophila* stocks harbor genetic variation in recombination rates as evidenced by their response to

Corresponding author: Michael E. Zwick, Department of Genetics, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106-4955. E-mail: mez4@po.cwru.edu

artificial selection (Roberts and Roberts 1921; Parsons 1958; Kidwell 1972a,b; Charlesworth and Charlesworth 1985a,b). *D. melanogaster* and *D. pseudoobscura* both exhibit variation in rates of recombination among stocks derived from natural populations (Levine and Levine 1954a,b; Lawrence 1958, 1963; Green 1959). Artificial selection to increase the rate of recombination on an autosome has been shown to concomitantly decrease rates of *X* chromosome nondisjunction (Charlesworth *et al.* 1985). And while variation in rates of nondisjunction has been detected in *Drosophila* laboratory stocks (Bridges 1916; Merriam and Frost 1964; Koehler *et al.* 1996a), with the exception of a single study designed to detect and isolate large-effect meiotic mutants from natural populations (Sandler *et al.* 1968), the existence of naturally occurring genetic variation in rates of spontaneous nondisjunction remains largely unknown.

Because spontaneous nondisjunction occurs at a frequency that makes it difficult to measure in a reasonably sized experiment, direct estimation of variation among a large number of different genotypes in their rates of nondisjunction is laborious. To overcome this impediment, we employ an alternative strategy to detect naturally occurring variation in rates of nondisjunction. The most frequent nondisjunction event in *D. melanogaster* involves the *X* chromosome resulting in diplo ($2X$) and nullo ($0X$) exceptions. Three-quarters of *X* chromosome nondisjunction arises from noncrossover chromosomes that normally segregate via the homologous achiasmate pathway (Hawley *et al.* 1992; Koehler *et al.* 1996a). Because most nondisjunction arises from the failure of the homologous achiasmate system, we devised a sensitized assay to measure transmission efficiency in this pathway among a set of *X* chromosomes sampled at random from natural populations in North America and Africa. Sensitized assays are exploited by many genetic enhancer/suppressor screens and also have been employed in evolutionary studies to detect activity differences among electrophoretic variants (Labate and Eanes 1992).

We report the results of a sensitized assay that demonstrates 10-fold differences among *X* chromosomes in their rates of nondisjunction in the homologous achiasmate system. We further identify two naturally occurring variants at the *nod* locus, a chromokinesin required for proper achiasmate chromosome segregation (Baker and Carpenter 1972; Carpenter 1973; Zhang and Hawley 1990; Zhang *et al.* 1990; Afshar *et al.* 1995a,b), that are significantly associated with an increased frequency of nondisjunction. Both polymorphisms are widely distributed and at intermediate frequency in natural populations. The observation of substantial genetic variation in rates of nondisjunction is consistent with the variation in E_0 tetrad frequency described in a companion article (Zwick *et al.* 1999, this issue). We propose a general model, the oötid competition (OC) model,

to account for the elevated mean rates of female-specific nondisjunction in both *Drosophila* and humans and for the large level of genetic variation in this critical fitness character within *D. melanogaster* natural populations.

MATERIALS AND METHODS

***Drosophila* lines:** *Drosophila melanogaster* isogenic *X* chromosome lines were sampled at random from natural populations in North America and Africa. North American lines were collected from Raleigh, North Carolina as described in Miyashita *et al.* (1993). African lines were collected from Zimbabwe as described in Begun and Aquadro (1993). All balancers and marker stocks are as described in Lindley and Zimm (1992). To minimize the effects of the autosomes on *X* chromosome nondisjunction, an autosomal isogenic background was constructed by employing a stock whose genotype was $T(2;3)CyOTM6/+; mwh ry^{206} e^1; spa^{pol}$ (hereafter, spa^{pol} will be referred to as *pol*). This allowed the simultaneous isolation of a single second (marked with *b*) and third chromosome (marked with *ri*) that were subsequently backcrossed and made homozygous. The resulting genotype of the common isogenic background was $b; ri; pol$. Each experimental X_i chromosome (where *i* refers to the *i*th *X* chromosome from either North America or Africa), the balancer *FM7a*, and an *X* chromosome containing the markers $y cv v f car$ were substituted into this common genetic background.

Experimental crosses: Experimental females were generated by crossing $FM7a, y sc^8 w^a v nod^d B/y^+ Y; pol$ males to $X_i; b; ri; pol$ virgin females. Virgin females whose genotype was $FM7a, y sc^8 w^a v nod^d B/X_i; b/+; ri/+; pol$ were aged in vials for 2 days. An experimental cross consisted of crossing $30 y v/y^+ Y; C(4)RM, ci ey^R$ males to an equal number of $FM7a, y sc^8 w^a v nod^d B/X_i; b/+; ri/+; spa^{pol}$ 2-day-old virgin females in bottles containing fresh glucose media (Figure 1). Each experimental cross was brooded, with the original parents transferred to new bottles on days 4 and 8. For any experimental cross, the first bottle was brood 1, the day 4 bottle was brood 2, and the day 8 bottle was brood 3. All experimental crosses were maintained in an incubator at 24° with a 12-hr dark/light cycle. For all broods within each experimental cross, all progeny were scored for their phenotypic markers (see Figure 1) on days 11 through 18, after which the bottles were discarded. *X*-chromosome nondisjunction was observed by the recovery of diplo *X* females ($FM7/X$) and nullo *X* males ($y v/O$). One-half of nondisjunctional classes are lethal (XXX and O/O), so the frequency of *X* chromosome nondisjunction was calculated as $(2 \times \text{Observed Diplo} + \text{Nullo Exceptions}) / (\text{Total Progeny} + \text{Diplo Exceptions} + \text{Nullo Exceptions})$. We also simultaneously measured rates of fourth chromosome nondisjunction by recovering $C(4)RM, ci ey^R$ nullo exceptions and *pol* diplo exceptions in both sexes. One-half of both the regular and nondisjunctional classes are *Minute* or lethal. Therefore, the rates of fourth chromosome nondisjunction are simply calculated as the $(\text{Observed 4th Exceptions}) / (\text{Total progeny})$. Fourth chromosomes do not undergo meiotic recombination and depend upon the homologous achiasmate system to ensure their proper segregation. Total sample sizes were 98,184 for North America and 103,921 for Africa.

The experimental cross was sensitized in three ways by the addition of the *FM7* balancer containing the *nod^d* null allele. First, *FM7* is a strong suppressor of exchange that forces all *X* chromosomes to segregate via the homologous achiasmate system (Hawley *et al.* 1992). Second, alleles at other meiotic loci can act as dominant enhancers of *nod^d* in *nod^d/+* heterozygotes (Knowles and Hawley 1991). Third, naturally

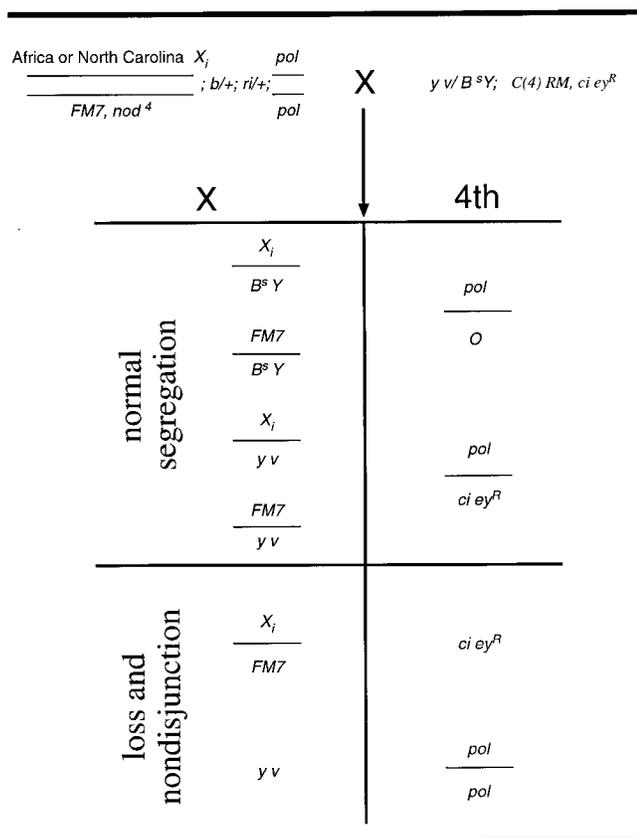


Figure 1.—Sensitized cross employed to assess variation in rates of X and 4 th nondisjunction. Each genotypic class results in a different phenotype, so it is possible to simultaneously score all progeny for their X and 4 th genotypes.

occurring DNA sequence variation in centromeric and extra-centromeric regions is expected to measurably influence the fidelity of chromosome transmission during female meiosis in $nod^+/+$ heterozygotes (Murphy and Karpen 1995). During the generation of experimental females, nondisjunction in males could lead to females whose genotype would be $FM7/X_1/y^+ Y$. Nondisjunction in these females will approach 60%, resulting in an excess of diplo X exceptions and $y^+ Y$ sons. Any lines that produced more than two $y v/y^+ Y$ males were discarded and assumed to have been contaminated during the generation of experimental females. There was no association between rates of nondisjunction measures and replicates of lines discarded due to $y^+ Y$ contamination ($P = 0.65$). All high nondisjunction rate lines showed an excess of nullo exceptions. To ensure that no unmarked Y chromosome was present, a subset of $y v/O$ males were tested for fertility by mating to multiple females in vials. No fertile males were detected (data not shown), suggesting that no unmarked Y chromosomes were present in any of our stocks.

Molecular analysis: Genomic DNA was prepared from ~ 100 flies from each isogenic X chromosome stock using an SDS lysis, organic extraction, ethanol precipitation protocol (Jowett 1986). PCR was conducted under standard conditions (50- μ l reaction volume: 2.5 μ l of two 10- μ m primers, 5 μ l of $10\times$ buffer with $MgCl_2$, 2 μ l of dNTP (0.2 mM each dNTP), 36 μ l distilled water, 1 μ l Taq polymerase, 1 μ l DNA sample). The following two unique primers from the nod locus genomic sequence were employed: 5'-GCGCTTATTTAATAGGTAGTCTAAG-3' and 5'-GCCACAACGTACGCTGCCAGCTG-3'.

DNA sequencing of PCR products was accomplished with internal primers (5'-GAAACGTGAAACCTGCGC-3' and 5'-CGGCGCAGCAGCGGCTGGC-3') in an ABI Prism 377 (Perkin Elmer, Norwalk, CT).

For the geographic survey, PCR products were digested for 1 hr at 65 $^\circ$ with *TaqI* restriction endonuclease. The resulting fragments were run on a 1.5% agarose gel, stained with ethidium bromide, photographed, and scored. Alleles with the nod^+ GenBank reference sequence contain a *TaqI* site at nucleotide position 4515, while those with the nod^P -like haplotype lack this *TaqI* site. As a consequence, a *TaqI* restriction digest of a nod^+ haplotype results in five fragments (sizes: 289, 266, 150, 78, 40, 28 bp), while a similar digest of a nod^P haplotype results in five fragments (sizes: 344, 289, 150, 40, 28 bp). The two haplotypes are easily differentiated by the presence of the 344-bp or 266-bp fragments.

Statistical analysis: To test for possible associations between genetic markers and frequency of nondisjunction, we employed a permutation testing approach (Churchill and Doerge 1994; Long *et al.* 1998). Mean frequencies of nondisjunction for each line were \log_{10} transformed to make the variance independent of the mean. To test for differences between the means of the two populations or among genetic variants within a single population, the test statistic

$$t = \frac{\mu_1 - \mu_2}{\sqrt{s_1^2 + s_2^2}} \quad (1)$$

was employed, where μ_1 and μ_2 are the means of two partitions of the dataset and s_1^2 and s_2^2 are the sample variances for the same partition. The permutation test consists of random resampling without replacement of the observed mean log-transformed frequencies to generate two new subsamples identical in size to the original sample, calculating the test statistic, and storing the value. This operation was repeated 100,000 times to determine the distribution of the test statistic. Significance was assessed by determining the proportion of simulated test statistics exceeding the observed test statistic using both tails of the simulated distribution. To test for different interactions between the nod^+ and nod^P haplotypes in distinct geographic regions, the test statistic

$$t = \frac{((\mu_{A1} - \mu_{A2}) - (\mu_{N1} - \mu_{N2}))}{\sqrt{s_{A1}^2 + s_{A2}^2 + s_{N1}^2 + s_{N2}^2}} \quad (2)$$

was employed, where μ_{A1} and μ_{A2} are the means of the two partitions in the African population, μ_{N1} and μ_{N2} are the means of the same two partitions in the North American population, and s_{A1}^2 , s_{A2}^2 , s_{N1}^2 , s_{N2}^2 are the sample variances of the partitions within each geographic location. This analysis was conducted in a similar permutation test framework. All statistical analyses were also performed on the raw and arc-sin-transformed frequencies, giving similar results. ANOVA to test for differences in brood means and nonparametric Mann-Whitney U -tests were conducted with JMP 3.2.1 (SAS Institute).

RESULTS

Genetic variation in *D. melanogaster* nondisjunction rates: A surprisingly high level of genetic variation in rates of nondisjunction is observed among randomly sampled X chromosomes from North America and Africa (Figure 2). With average sample sizes of ~ 3400 flies per X chromosome, the largest standard error of the mean frequency of nondisjunction for any X chromosome is 5.9×10^{-3} . No effect of brood on the mean frequency of nondisjunction is detected ($P = 0.38$). The

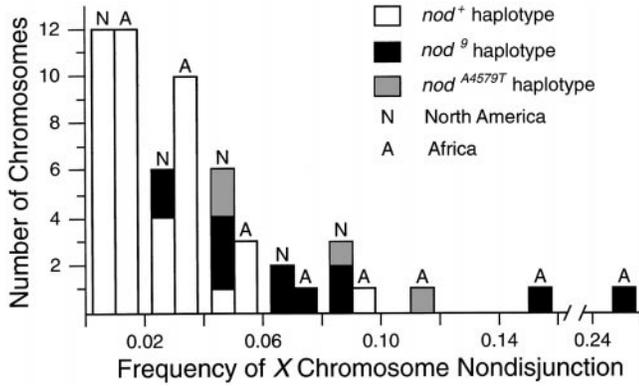


Figure 2.—Histogram showing number of chromosomes with different frequencies of nondisjunction in our sensitized assay. The randomly sampled African (A) and North American (N) chromosomes are identified by an A and N, respectively, above each column. The number of chromosomes containing the *nod*⁺ (white), *nod*^g (black), or *nod*^{A4579T} (gray) haplotypes and their observed frequency of nondisjunction are shown.

variation that we observe cannot be attributed to zygotic viability for two reasons. First, the null exception class is genotypically identical in the assays of all X chromosome lines. Second, if zygotic viability were significantly influencing our results, we would not expect the high correlation between rates of X and 4 nondisjunction that we observe (Figure 3). Because our experimental design consisted of substituting each of the X_i chromosomes into a common autosomal isogenic background, the genetic causes of the variation we observe must reside on the individual X chromosomes.

Identification and characterization of a candidate locus: Naturally occurring variants at X-linked loci that function in meiosis are a plausible source of genetic variation in our experiment. One such candidate is the

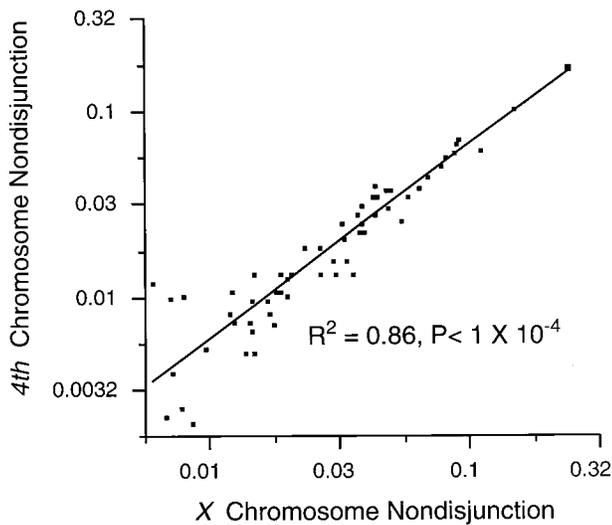


Figure 3.—Graph showing the correlation of log-transformed mean frequencies of X and 4th nondisjunction ($R^2 = 0.86$; $P < 1 \times 10^{-4}$).

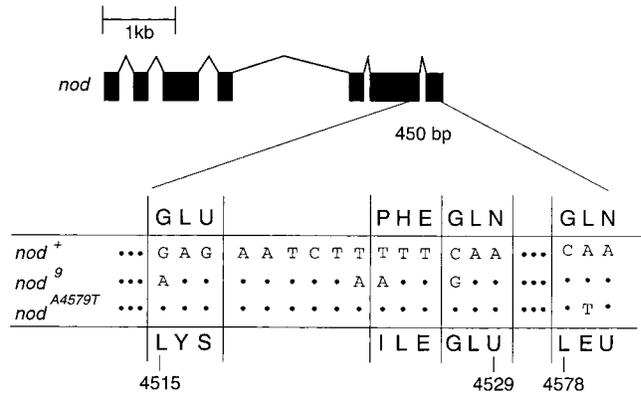


Figure 4.—The *nod* transcript and the location of polymorphic regions are shown. The *nod*^g haplotype apparently arose from a gene conversion event from either of two downstream (non-*nod*) exons, which share 95% sequence identity with the 3' portion of the *nod* transcript (Rasooly *et al.* 1994). DNA sequence of *nod*⁺ and *nod*^g haplotypes shows the location of four nucleotide differences, three of which result in amino acid polymorphism. The upper amino acids are coded for by the *nod*⁺ reference sequence. The first three lower amino acids are coded for by the *nod*^g sequence. The last lower amino acid is coded for by the *nod*^{A4579T} sequence. The *nod*^{A4579T} sequence differs from *nod*⁺ at nucleotide site 4579 and is found on three chromosomes in the North American sample and a single chromosome from the African sample. Other rare and silent nucleotide variants within the 450 bp sequenced are not shown in the figure.

nod locus, a chromokinesin required for proper achiasmate chromosome segregation (Baker and Carpenter 1972; Carpenter 1973; Zhang and Hawley 1990; Zhang *et al.* 1990; Rasooly *et al.* 1991; Afshar *et al.* 1995a,b). A candidate allele, *nod*^g, was found in a laboratory stock (Rasooly *et al.* 1994) employed in one of the first large EMS screens for meiotic mutants (Baker and Carpenter 1972). This screen was highly successful in that it recovered an extraordinary number of newly induced meiotic mutants, some of which were quite weak and were not recovered in later screens. Subsequent analysis of a laboratory stock containing a descendant copy of the X chromosome mutagenized by Baker and Carpenter (1972) yielded elevated rates of female-specific nondisjunction (Rasooly *et al.* 1994). Rasooly *et al.* (1994) identified a variant nucleotide sequence in the 3' DNA binding domain (*nod*^g) that they characterized as a “weak *nod* mutation.” They suggested that the recovery of weak meiotic mutants reported in Baker and Carpenter (1972) is best explained if one assumes that the original EMS screen was carried out in a “sensitized” background caused by the presence of the *nod*^g allele.

The *nod*⁺ GenBank reference sequence and the *nod*^g haplotype differ at four nucleotide sites, three of which result in amino acid changes (Figure 4). A DNA sequence survey of North American and African X chromosomes in our sample demonstrates that the *nod* locus

		Location		
		Africa	North America	
Genotype	<i>nod</i> ⁺	0.030 (27)	0.022 (20)	0.027
	<i>nod</i> ^β	0.128 (3)	0.069 (9)	0.081
		0.044	0.035	

Figure 5.—The mean rates of nondisjunction for each genotype/location combination. The genotype column labels refer to the *nod*⁺ and *nod*^β DNA sequences shown in Figure 2. Marginal column and row frequencies are located outside the box. Numbers in parentheses are the numbers of *X* chromosomes in each category.

is highly polymorphic for two haplotypes in the small region that distinguishes *nod*⁺ from *nod*^β (Figures 4 and 5). Alleles with the typical *nod*⁺ sequence contain a *TaqI* site at nucleotide position 4515, while those with the *nod*^β-like haplotype lack this *TaqI* site (*TaqI*⁻, Figure 4). A PCR-based RFLP screen shows that the *TaqI*⁻ haplotype is also found at intermediate frequency in populations from Barcelona, Spain (0.44, *N* = 27) and Davis, California (0.48, *N* = 54). The frequency of the *TaqI*⁻ haplotype is higher in the three temperate populations than in the tropical African population. A second candidate allele, *nod*^{A4579T}, is found on three chromosomes from the North American sample and a single chromosome from the African sample. This haplotype was determined by DNA sequencing to result in a distinct amino acid change in the putative *nod* coding region (Figure 4).

Association of polymorphic sites at the *nod* locus with an increased frequency of nondisjunction: We employed a permutation-based statistical design (Churchill and Doerge 1994; Long *et al.* 1998) to determine that the mean frequency of *X* chromosome nondisjunction does not significantly differ between our samples from North America and Africa ($P < 0.85$). Chromosomes containing the *nod*^β sequence (Figures 2 and 4) have significantly higher mean frequencies of nondisjunction in both the African ($P < 0.006$) and the North American ($P < 10^{-4}$) samples (Figures 2 and 5). A nonparametric Mann-Whitney *U*-test confirms this association (Africa, $P < 0.008$; N. America, $P < 4 \times 10^{-4}$). A test of the interaction between geographic location and the *nod*^β haplotype was not significant ($P < 0.13$).

The *nod*^{A4579T} variant is marginally associated with an increased frequency of nondisjunction in the North Carolina sample (permutation, $P < 0.08$; Mann-Whitney *U*-test, $P < 0.07$). Because the *nod*^{A4579T} variant is found only once in the African sample, it is not possible to carry out the permutation test while the nonparametric Mann-Whitney *U*-test is not significant (Mann-Whitney *U*-test, $P = 0.15$). Because the mean frequency of nondisjunction did not differ between the samples from the two natural populations, it seemed reasonable to perform the permutation test on the total dataset to test the association of the *nod*^{A4579T} polymorphism with nondisjunction frequency. The permutation test ($P = 0.02$) and a nonparametric Mann-Whitney *U*-test ($P < 0.02$) both showed that the *nod*^{A4579T} polymorphism is significantly associated with an increased frequency of nondisjunction (Figure 2). Further evidence for the association of both *nod* haplotypes with elevated rates of nondisjunction comes from the DNA sequencing of two independently derived *nod* alleles from *X* chromosome laboratory stocks that exhibit an elevated frequency of nondisjunction in an unsensitized background (R. S. Hawley, personal communication). One *X* chromosome stock's sequence is identical to that observed for the *nod*^β haplotype while the other contains the *nod*^{A4579T} haplotype. These observations, in conjunction with those in Rasooly *et al.* (1994), lead us to surmise that the variation we detected in our sensitized assay reliably reflects variation present in natural populations. However, the absolute magnitude of nondisjunction attributable to specific *nod* haplotypes or rates of spontaneous *X* chromosome nondisjunction cannot be determined by our assay.

We propose the OC model to account for both the high rates of female-specific nondisjunction and the high levels of genetic variation we observe (Figure 6): We first note a fundamental difference between the mechanisms of chromosome segregation in males and females. Male meiosis can be referred to as symmetric meiosis to reflect the fact that during a normal meiotic division, each of the four products of meiosis, referred to as spermatids, will eventually be found in a functional, similarly sized sperm. In contrast, the pattern of chromosome segregation and cell division during female meiosis is fundamentally asymmetric. Asymmetric meiosis occurs when only one of the four products of meiosis, referred to as oötid, is included in the pronucleus of an oocyte. In *Drosophila*, for example, the interior-, and usually posterior-, most oötid is included in the pronucleus (King 1970). As a consequence of this asymmetry, female meiosis is uniquely at risk of meiotic drive (Sandler and Novitski 1957), resulting from competition among oötid for inclusion into the pronucleus. Because the orientation of the female spindle is fixed, resulting in the pronucleus being situated at a specific location in the oocyte, any chromosomal element that

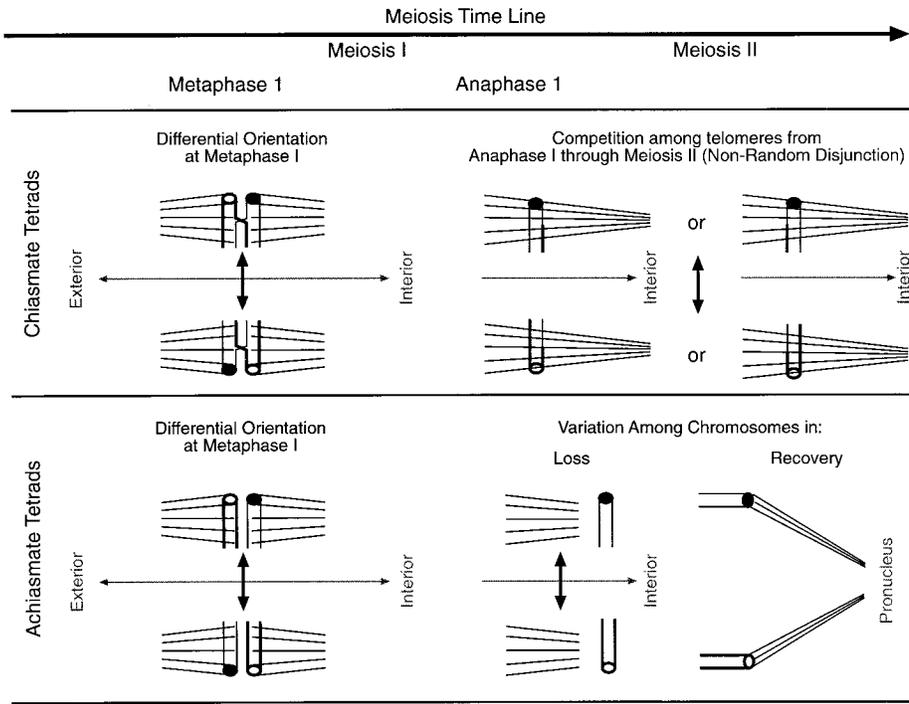


Figure 6.—Possible mechanisms of oötid competition for chiasmate and achiasmate tetrads during female meiosis are shown. Centromeres of both chiasmate and achiasmate oötid are expected to competitively orient during meiosis I. During anaphase I through meiosis II, telomeres or other chromosomal elements on the distal arms of chiasmate oötid are expected to compete for orientation toward the pronucleus, resulting in nonrandom disjunction. Polymorphic alleles of loci involved in the proper segregation of oötid during canonical female meiosis, in addition to variation at centromeres, telomeres, or other chromosomal elements, are likely to provide multiple opportunities for competitive interactions among achiasmate and terminal exchange oötid during the processes of loss from the main spindle and recovery to the pronucleus. These competitive interactions are expected to alter the probability of an oötid's inclusion in the pronucleus and lead to variation in rates of nondisjunction.

increases its frequency of inclusion into the pronucleus will be strongly selected.

The OC model consists of three main features. First, during asymmetric meiosis, natural selection will favor selfish chromosomal elements that can successfully compete with other such elements for inclusion into the pronucleus (*i.e.*, exhibit meiotic drive). Centromeres and telomeres are two types of chromosomal elements that are expected to compete during asymmetric meiosis. Second, single-locus population genetic theory predicts that chromosomal elements that exhibit only meiotic drive will rapidly go to fixation. Chromosomal elements are expected to be polymorphic when a balance between meiotic drive and a deleterious viability or fertility effect exists in a population (Sandler and Novitski 1957; Hiraizumi *et al.* 1960). We propose that the high levels of genetic variation that we observe are best explained if female-specific nondisjunction acts as a deleterious effect countering meiotic drive of chromosomal elements during asymmetric meiosis. High rates of nondisjunction are female-specific because no opportunity for a similar competition among chromosomal elements exists in symmetric meiosis or during mitosis. Multiple genetic elements and their modifiers may interact, resulting in complex dynamics (Feldman and Otto 1991). Third, the maternal-age effect in rates of nondisjunction arises as a consequence of strong early-acting natural selection favoring alleles with later-acting deleterious phenotypes (Williams 1957). Because there is no opportunity for spermatid competition in males, a paternal-age effect is expected to be weaker. We envision

three plausible genetic mechanisms of oötid competition during asymmetric meiosis (Figure 6).

1. *Competitive orientation of homologous centromeres at meiosis I:* Any centromere that preferentially orients in the direction of the pronucleus, thereby avoiding inclusion in the polar bodies, will be strongly selected. This competition can occur between chiasmate and achiasmate bivalents only during metaphase I of meiosis when centromeres are heterozygous (Figure 6). We suggest two mechanisms leading to nondisjunction as a pleiotropic consequence of this competition. First, both centromeres might simply orient and move toward the pronucleus and be unable to exclude their competitor, resulting in a diplo exception. Second, a "strong" centromere finding itself oriented in the "wrong" direction is expected to reorient and attempt to exclude its homolog from the pronucleus. Depending upon the characteristics of the two competing centromeres, we would occasionally expect such competition to result in diplo exceptions.

Our model predicts that natural populations harbor centromeres that vary in their abilities to competitively pair, orient, and move toward the pronucleus. Classic *Drosophila* experiments suggest centromere strength variation among laboratory stocks does exist (Novitski 1952, 1955). Recent analyses of minichromosomes in *Drosophila* have demonstrated that variation in the length of centromeric sequences, extracentromeric sequences, and *nod* dos-

age can significantly influence the fidelity of chromosome transmission during female meiosis (Murphy and Karpen 1995; Karpen *et al.* 1996). Centromeric satellite sequences, thought to be involved in centromere function, often show substantial variation between populations and species. While much of this variation is thought to be neutral (Charlesworth *et al.* 1994), we expect that the evolutionary dynamics of sequences that influence chromosome transmission may be strongly influenced by natural selection.

2. *Competition among heterozygous telomeres or distal arms of chiasmate bivalents from anaphase I through meiosis II:* Entry into anaphase I requires the release of sister chromatid cohesion distal to the chiasmata, allowing telomeres to act independently during the subsequent movement of chromosomes. Telomeres or other distally located chromosomal elements that can alter the orientation of the chromatids, perhaps through differential binding of the NOD protein, may be preferentially transmitted. Nonrandom disjunction, the classic genetic observation of differential recovery of structurally different chromatids, suggests that such a mechanism is plausible (Novitski 1951; Novitski and Sandler 1956; Mark and Zimmering 1977). We do not expect nondisjunction to arise from this phase of oötid competition. Nevertheless, because telomeres and centromeres are not likely to be in linkage disequilibrium, their competition and dynamics are predicted to be only loosely coupled. This may preclude the fixation of a single optimum set of chromosomal elements and act to maintain genetic variation.
3. *Competition among oötid during the loss and recovery of achiasmate and terminal-exchange bivalents during anaphase I:* Cytological observations of *D. melanogaster* demonstrate that centromeres begin to move chromosomes toward the poles during metaphase I, prior to completion of the acentriolar spindle and metaphase I arrest (Theurkauf and Hawley 1992; Dernburg *et al.* 1996). Achiasmate chromosomes and chiasmate chromosomes with distal exchanges have been observed to occasionally fall off the spindle and less frequently, recover from this loss. Cytological observations have demonstrated that single chromosomes are able to nucleate microtubules and form spindles that orient toward the pronucleus (Theurkauf and Hawley 1992). Nullo exceptions occur when chromosomes fall off the spindle and fail to recover. Diplo exceptions are produced in situations where both homologs are lost and subsequently recover to the region of the pronucleus (Theurkauf and Hawley 1992). Loss of oötid from the main spindle and their recovery to the pronucleus probably involve gene products of loci that function to ensure the proper segregation of oötid during canonical female meiosis. Polymorphisms at these loci, in addition to variation among centromeres,

telomeres, or other chromosomal elements, are expected to provide multiple opportunities for competitive interactions between oötid during the processes of loss from the main spindle and recovery to the pronucleus. These competitive interactions may act to alter the probability of an oötid's inclusion in the pronucleus. Mechanisms of competition among achiasmate or terminal exchange oötid are liable to be especially sensitive to the dosage of NOD protein.

DISCUSSION

Ever since Bridges (1916) utilized nondisjunction to fix a cornerstone in the chromosome theory of heredity, great progress has been made in understanding the proximate mechanisms that lead to nondisjunction. Nevertheless, prior to this study, the patterns of any genetic variation underlying nondisjunction rates in natural populations remained largely unknown. Our study was designed to detect variation in rates of nondisjunction in the homologous achiasmate pathway among *D. melanogaster* X chromosomes sampled at random from natural populations in Africa and North America.

Genetic variation in rates of nondisjunction: We present three main conclusions and an evolutionary model to account for our experimental results. First, *D. melanogaster* natural populations harbor surprisingly high levels of genetic variation in rates of nondisjunction. Second, a significant proportion of this variation is attributable to two haplotypes at the *nod* locus, a gene required for achiasmate chromosome segregation during female meiosis. This is the first demonstration in any organism that naturally occurring alleles at a meiotic candidate locus are associated with an increased frequency of nondisjunction. Third, the *nod*⁹ and *nod*^{A4579T} haplotypes are observed to be geographically widespread and at intermediate frequency in natural populations. Finally, we present the OC model to account for both the high rates of female-specific nondisjunction and the high levels of genetic variation we observe.

What can explain the high frequency of the *nod*⁹ haplotype in natural *Drosophila* populations? The *nod* locus codes for an N-terminal kinesin motor domain and a C-terminal cargo-binding domain. The genetic variants associated with an elevated frequency of nondisjunction that we identify are located in the cargo-binding domain—the portion of the NOD protein that binds to chromosomes. However, the specific variants we identify are not located within the region shown to bind DNA *in vitro* (Afshar *et al.* 1995a,b). Because polymorphic markers >2 kb apart in regions of normal recombination are usually not in linkage disequilibrium (Miyashita and Langley 1988; Miyashita *et al.* 1993), we consider it highly likely that variants within the *nod* locus, which is located in a region of normal recombination, cause the associations we observe. We predict that the variants that we have identified will alter the pheno-

typic characteristics of the NOD protein. Similar to the pattern that we observe in *D. melanogaster*, preliminary analysis of an ongoing DNA sequence survey of the *nod* locus from three sibling species (*D. simulans*, *D. mauritiana*, and *D. sechellia*) suggests excess replacement polymorphism in the cargo-binding domain of the *nod* locus (data not shown). The central chromosomal location of *nod* means that linkage disequilibria with variant centromeres or telomeres are unlikely. Thus the *nod*^p haplotype either must itself exhibit meiotic drive, perhaps through haplotype-specific binding of the NOD protein at the *nod* locus, or act as an “unlinked modifier” of other meiotic drive elements.

OC model: Given the prevalence of female-specific nondisjunction and its uniformly deleterious consequences, one might anticipate that simple directional natural selection would act to reduce rates of female-specific spontaneous nondisjunction. Yet in spite of ample variation described in this study and in a companion article (Zwick *et al.* 1999, this issue), natural selection seems unable to act on this variation in the manner predicted by a simple directional selection model. We propose the OC model to account for elevated rates of female-specific nondisjunction and the high levels of genetic variation we observe.

While portions of the OC model are admittedly speculative, it is based upon observations of chromosome behavior in a variety of experiments conducted with *D. melanogaster* laboratory stocks. We predict that the types of chromosome transmission behavior observed in laboratory experiments will also be found among chromosomes in natural populations. Transmission variation in female meiosis among different centromeres, telomeres, or other genetic elements in natural populations remains largely unknown. However, such variation is difficult to characterize unless one performs an experiment specifically designed to detect it. Any such experiment must account for viability differences among genotypes, because viability and transmission are confounded in the recovery of different progeny genotypes. Most previous evolutionary studies assume that all variation in the recovery of progeny genotypes is attributed to viability differences, but when an experiment designed to detect transmission variation has been carried out, it has often been detected (Hiraizumi and Gerstenberg 1981; Curtisinger 1984). Our results suggest that evolutionary significant transmission variation in female meiosis might be quite prevalent in natural populations and should be detected in appropriate laboratory experiments.

While the OC model appears superficially similar to previous CD models in that it assumes that nondisjunction arises as a consequence of meiotic drive, it differs in two important respects. First, the OC model does not simply assume that whole chromosomes exhibit meiotic drive as does the CD model. Instead, the OC model predicts that drive occurs due to competition among

chromosomal elements (perhaps centromeres and telomeres) that attempt to bias their transmission. Models that assume whole-chromosome drive are unlikely to incorporate the full dynamics expected in female meiosis. Second, the OC model explains the high levels of genetic variation we observe, while the CD model does not address this issue. Future models of nondisjunction incorporating observed proximate mechanisms, as opposed to idealized game theory models, are more likely to reflect the expected complex dynamics of meiotic drive during female meiosis.

Alternative models to account for the maintenance of polymorphism at the *nod* locus are possible. One model could assume a balance between the meiotic effects on nondisjunction that we observe with an effect on mitotic cell division during development. Although the peak level of expression of the *nod* locus is during female meiosis, it is expressed at different times during development in mitotically dividing cells (Zhang *et al.* 1990). Furthermore, a single gain-of-function allele, *nod*^{PTW}, has a cold lethal mitotic phenotype (Rasooly *et al.* 1991), leading to the suggestion that *nod* may have a redundant mitotic phenotype that is not detected in the null mutant. However, because *nod*'s meiotic and putative mitotic functions occur at widely spaced times during development, we consider such an explanation unlikely. If such a trade-off between the meiotic and mitotic functions of the *nod* locus in fact existed, we would expect that natural selection would quickly select for modifiers leading to elimination of any such trade-off. Given the absence of an obvious mitotic phenotype in the *nod* null mutant and the pattern of expression of the *nod* locus, we consider it more likely that oötid competition during female meiosis accounts for the observed polymorphism. A second alternative model, incorporating chromosome transmission variation during male meiosis, was not addressed by our study. Centromeres, telomeres, or other chromosomal elements might alter transmission in males and females in different ways. Variation in these chromosomal elements might act to maintain variation at the *nod* locus. Characterization of the male-specific transmission of our *X* chromosomes would determine if such a model is plausible.

Inversion polymorphism and temperature: Levels of inversion polymorphism and temperature are two likely interacting selective agents. Common cosmopolitan inversions [*i.e.*, *In(2L)t*, *In(2R)NS*, *In(3L)P*, and *In(3R)P*] in *D. melanogaster* natural populations are observed to have a three- to fivefold higher mean frequency at tropical as opposed to temperate latitudes (Table 9 in Lemeunier and Aulard 1992). Low temperature has been shown to cause a large increase in nondisjunction in females (Tokunaga 1970a,b). Because we substituted all the *X* chromosomes into an isogenic autosomal background and conducted our experimental crosses at a single temperature, our experiment cannot assess the

role of heterozygous autosomal inversions or temperature on rates of *X* chromosome nondisjunction. It has long been known that heterozygous autosomal inversions in a variety of laboratory stocks will act to increase rates of *X* chromosome nondisjunction (Morgan and Sturtevant 1944; Roberts 1962). Selection for increased *nod* function should be strong in populations with high levels of inversion polymorphism because of the greater frequency of achiasmate chromosomes. The impact of polymorphic inversions could explain the lower frequency of the *nod^P* haplotype in the African sample. Temperature may also act directly on the *nod^P* haplotype, causing it to function more efficiently at lower temperatures. We are currently conducting an experiment to determine if rates of nondisjunction for *X* chromosomes with different *nod* haplotypes interact with temperature. In addition, further sampling of natural populations to characterize a possible cline at the *nod* locus is warranted.

In conclusion, our results suggest that natural populations harbor a great deal of genetic variation in rates of nondisjunction during female meiosis and that a significant proportion of this variation may consist of intermediate frequency alleles at meiotic candidate loci. If, as we have argued, similar evolutionary forces operate during female meiosis in humans (perhaps most dioecious organisms), intermediate-frequency alleles predisposing human females to increased risk of nondisjunction can also be expected to be found at candidate loci or to be detected in genome-wide scans for association (Risch and Merikangas 1996).

We thank Michael Turelli, John Gillespie, Mark Grote, and two anonymous reviewers for their discussion and assistance in improving the manuscript. We also thank R. Scott Hawley for providing thoughtful answers to our many questions concerning chromosome segregation during female meiosis. Fellowship and research support was provided to M.E.Z. by a National Science Foundation Pre-Doctoral Fellowship, a National Science Foundation Dissertation Improvement Grant DEB 96-23970, the Center for Population Biology at UC Davis, and the Daphne and Ted Pengelley Research Award. This research was also partially funded by a National Science Foundation Grant DEB 95-09548 to C.H.L.

LITERATURE CITED

- Afshar, K., N. R. Barton, R. S. Hawley and L. S. Goldstein, 1995a DNA binding and meiotic chromosomal localization of the *Drosophila nod* kinesin-like protein. *Cell* **81**: 129-138.
- Afshar, K., J. Scholey and R. S. Hawley, 1995b Identification of the chromosome localization domain of the *Drosophila nod* kinesin-like protein. *J. Cell Biol.* **131**: 833-843.
- Axelrod, R., and W. D. Hamilton, 1981 The evolution of cooperation. *Science* **211**: 1390-1396.
- Baker, B. S., and A. T. C. Carpenter, 1972 Genetic analysis of sex chromosomal meiotic mutants in *Drosophila melanogaster*. *Genetics* **71**: 256-286.
- Begun, D. J., and C. F. Aquadro, 1993 African and North American populations of *Drosophila melanogaster* are very different at the DNA level. *Nature* **365**: 548-550.
- Bridges, C. B., 1916 Non-disjunction as proof of the chromosome theory of heredity. *Genetics* **1**: 1-52, 107-163.
- Brooks, L. D., 1988 The evolution of recombination rates, pp. 87-105 in *The Evolution of Sex*, edited by R. E. Michod and B. R. Levin. Sinauer Associates, Sunderland, MA.
- Bugge, M., A. Collins, M. B. Petersen, J. Fisher, C. Brandt *et al.*, 1998 Non-disjunction of chromosome 18. *Hum. Mol. Genet.* **7**: 661-669.
- Carpenter, A. T., 1973 A meiotic mutant defective in distributive disjunction in *Drosophila melanogaster*. *Genetics* **73**: 393-428.
- Charlesworth, B., and D. Charlesworth, 1985a Genetic variation in recombination in *Drosophila*. I. Responses to selection and preliminary genetic analysis. *Heredity* **54**: 71-83.
- Charlesworth, B., and D. Charlesworth, 1985b Genetic variation in recombination in *Drosophila*. II. Genetic analysis of a high recombination stock. *Heredity* **54**: 85-98.
- Charlesworth, B., I. Mori and D. Charlesworth, 1985 Genetic variation in recombination in *Drosophila*. III. Regional effects on crossing over and effects on non-disjunction. *Heredity* **55**: 209-221.
- Charlesworth, B., P. Sniegowski and W. Stephan, 1994 The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature* **371**: 215-220.
- Churchill, G. A., and R. W. Doerge, 1994 Empirical threshold values for quantitative trait mapping. *Genetics* **138**: 963-971.
- Curtisinger, J. W., 1984 Components of selection in *X* chromosome lines of *Drosophila melanogaster*: sex ratio modification by meiotic drive and viability selection. *Genetics* **108**: 941-952.
- Day, T., and P. D. Taylor, 1998 Chromosomal drive and the evolution of meiotic nondisjunction and trisomy in humans. *Proc. Natl. Acad. Sci. USA* **95**: 2361-2365.
- Dernburg, A. F., J. W. Sedat and R. S. Hawley, 1996 Direct evidence of a role for heterochromatin in meiotic chromosome segregation. *Cell* **86**: 135-146.
- Feldman, M. W., and S. P. Otto, 1991 A comparative approach to the population-genetics theory of segregation distortion. *Am. Nat.* **137**: 443-456.
- Green, M. M., 1959 Effects of different wild-type isoalleles on crossing-over in *Drosophila melanogaster*. *Nature* **184**: 294.
- Hassold, T., M. Abruazzo, K. Adkins, D. Griffin, M. Merrill *et al.*, 1996 Human aneuploidy: incidence, origin, and etiology. *Environ. Mol. Mutagen.* **28**: 167-175.
- Hawley, R. S., H. Irick, A. E. Zitron, D. A. Haddox, A. Lohe *et al.*, 1992 There are two mechanisms of achiasmate segregation in *Drosophila* females, one of which requires heterochromatic homology. *Dev. Genet.* **13**: 440-467.
- Hiraizumi, Y., and M. V. Gerstenberg, 1981 Gametic frequency of second chromosomes of the T-007 type in a natural population of *Drosophila melanogaster* in Texas. *Genetics* **98**: 303-316.
- Hiraizumi, Y., L. Sandler and J. F. Crow, 1960 Meiotic drive in natural populations of *Drosophila melanogaster*. III. Populational implications of the segregation-distorter locus. *Evolution* **14**: 433-444.
- Jowett, T., 1986 Preparation of nucleic acids, pp. 275-286 in *Drosophila: A Practical Approach*, edited by D. B. Roberts. IRL Press, Oxford.
- Karpen, G. H., M. H. Le and H. Le, 1996 Centric heterochromatin and the efficiency of achiasmate disjunction in *Drosophila* female meiosis. *Science* **273**: 118-122.
- Kidwell, M. G., 1972a Genetic change of recombination value in *Drosophila melanogaster*. II. Simulated natural selection. *Genetics* **70**: 433-443.
- Kidwell, M. G., 1972b Genetic change of recombination value in *Drosophila melanogaster*. I. Artificial selection for high and low recombination and some properties of recombination-modifying genes. *Genetics* **70**: 419-432.
- King, R. C., 1970 *Ovarian Development in Drosophila melanogaster*. Academic Press, New York.
- Knowles, B. A., and R. S. Hawley, 1991 Genetic analysis of microtubule motor proteins in *Drosophila*: a mutation at the *ncd* locus is a dominant enhancer of *nod*. *Proc. Natl. Acad. Sci. USA* **88**: 7165-7169.
- Koehler, K. E., C. L. Boulton, H. E. Collins, R. L. French, K. C. Herman *et al.*, 1996a Spontaneous *X* chromosome MI and MII nondisjunction events in *Drosophila melanogaster* oocytes have different recombinational histories. *Nat. Genet.* **14**: 406-414.
- Koehler, K. E., R. S. Hawley, S. Sherman and T. Hassold, 1996b Recombination and nondisjunction in humans and flies. *Hum. Mol. Genet.* **5 Spec. No.**: 1495-1504.

- Labate, J., and W. F. Eanes, 1992 Direct measurement of in vivo flux differences between electrophoretic variants of G6PD from *Drosophila melanogaster*. *Genetics* **132**: 783-787.
- Lamb, N. E., S. B. Freeman, A. Savage-Austin, D. Pettay, L. Taft *et al.*, 1996 Susceptible chiasmate configurations of chromosome 21 predispose to non-disjunction in both maternal meiosis I and meiosis II. *Nat. Genet.* **14**: 400-405.
- Lamb, N. E., E. Feingold, A. Savage, D. Avramopoulos, S. Freeman *et al.*, 1997 Characterization of susceptible chiasma configurations that increase the risk for maternal nondisjunction of chromosome 21. *Hum. Mol. Genet.* **6**: 1391-1399.
- Lawrence, M. J., 1958 Genotypic control of crossing-over on the first chromosome of *Drosophila melanogaster*. *Nature* **182**: 889-890.
- Lawrence, M. J., 1963 The control of crossing-over in the X-chromosome of *Drosophila melanogaster*. *Heredity* **18**: 27-46.
- Lemeunier, F., and S. Aulard, 1992 Inversion polymorphism in *Drosophila Melanogaster*, pp. 339-405 in *Drosophila Inversion Polymorphism*, edited by C. B. Krimbas and J. R. Powell. CRC Press, Boca Raton, FL.
- Levine, R. P., and E. E. Levine, 1954a The genotypic control of crossing over in *Drosophila pseudoobscura*. *Genetics* **39**: 677-691.
- Levine, R. P., and E. E. Levine, 1954b Variable crossing over arising in different strains of *Drosophila pseudoobscura*. *Genetics* **40**: 399-405.
- Lindsley, D. L., and G. C. Zimm, 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego.
- Long, A. D., R. F. Lyman, C. H. Langley and T. F. C. Mackay, 1998 Two sites in the *Delta* gene region contribute to naturally occurring variation in bristle number in *Drosophila melanogaster*. *Genetics* **149**: 999-1017.
- Mark, H. F., and S. Zimmering, 1977 Centromeric effect on the degree of nonrandom disjunction in the female *Drosophila melanogaster*. *Genetics* **86**: 121-132.
- Merriam, J. R., and J. N. Frost, 1964 Exchange and nondisjunction of the X-chromosomes in female *Drosophila melanogaster*. *Genetics* **49**: 109-122.
- Miyashita, N., and C. H. Langley, 1988 Molecular and phenotypic variation of the white locus region in *Drosophila melanogaster*. *Genetics* **120**: 199-212.
- Miyashita, N. T., M. Agueade and C. H. Langley, 1993 Linkage disequilibrium in the *white* locus region of *Drosophila melanogaster*. *Genet. Res.* **62**: 101-109.
- Morgan, T. H., and A. H. Sturtevant, 1944 Maintenance of a *Drosophila* stock center, in connection with investigations on the constitution of germinal material in relation to heredity. *Carnegie Inst. Wash. Year Book* **43**: 164-165.
- Murphy, T. D., and G. H. Karpen, 1995 Interactions between the *nod+* kinesin-like gene and extracentromeric sequences are required for transmission of a *Drosophila* minichromosome. *Cell* **81**: 139-148.
- Novitski, E., 1951 Non-random disjunction in *Drosophila*. *Genetics* **36**: 267-280.
- Novitski, E., 1952 The genetic consequences of anaphase bridge formation in *Drosophila*. *Genetics* **37**: 270-287.
- Novitski, E., 1955 Genetic measures of centromere activity in *Drosophila melanogaster*. *J. Cell. Comp. Physiol.* **45**: 151-169.
- Novitski, E., and L. Sandler, 1956 Further notes on the nature of nonrandom disjunction in *Drosophila melanogaster*. *Genetics* **41**: 194-206.
- Parsons, P. A., 1958 Selection for increased recombination in *Drosophila melanogaster*. *Am. Nat.* **92**: 255-256.
- Penrose, L. S., 1933 The relative effects of paternal and maternal age in mongolism. *J. Genet.* **27**: 219-224.
- Rasooly, R. S., C. M. New, P. Zhang, R. S. Hawley and B. S. Baker, 1991 The *lethal(1)TW-6cs* mutation of *Drosophila melanogaster* is a dominant antimorphic allele of *nod* and is associated with a single base change in the putative ATP-binding domain. *Genetics* **129**: 409-422.
- Rasooly, R. S., P. Zhang, A. K. Tibolla and R. S. Hawley, 1994 A structure-function analysis of NOD, a kinesin-like protein from *Drosophila melanogaster*. *Mol. Gen. Genet.* **242**: 145-151.
- Risch, N., and K. Merikangas, 1996 The future of genetic studies of complex human diseases. *Science* **273**: 1516-1517.
- Roberts, P., 1962 Interchromosomal effect and the relation between crossing-over and nondisjunction. *Genetics* **47**: 1691-1709.
- Roberts, F. A., and E. Roberts, 1921 Studies on crossing over I. The effect of selection on crossover values. *J. Exp. Zool.* **32**: 333-354.
- Robinson, W. P., B. D. Kuchinka, F. Bernasconi, M. B. Petersen, A. Schulze *et al.*, 1998 Maternal meiosis I non-disjunction of chromosome 15: dependence of the maternal age effect on level of recombination. *Hum. Mol. Genet.* **7**: 1011-1019.
- Sandler, L., and E. Novitski, 1957 Meiotic drive as an evolutionary force. *Am. Nat.* **41**: 105-110.
- Sandler, L., D. L. Lindsley, B. Nicoletti and G. Trippa, 1968 Mutants affecting meiosis in natural populations of *Drosophila melanogaster*. *Genetics* **60**: 525-558.
- Sherman, S. L., N. Takaesu, S. B. Freeman, M. Grantham, C. Phillips *et al.*, 1991 Trisomy 21: association between reduced recombination and nondisjunction [see comments]. *Am. J. Hum. Genet.* **49**: 608-620.
- Sherman, S. L., M. B. Petersen, S. B. Freeman, J. Hersey, D. Pettay *et al.*, 1994 Non-disjunction of chromosome 21 in maternal meiosis I: evidence for a maternal age-dependent mechanism involving reduced recombination. *Hum. Mol. Genet.* **3**: 1529-1535.
- Sturtevant, A. H., and G. W. Beadle, 1936 The relations of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. *Genetics* **21**: 554-604.
- Theurkauf, W. E., and R. S. Hawley, 1992 Meiotic spindle assembly in *Drosophila* females: behavior of nonexchange chromosomes and the effects of mutations in the *nod* kinesin-like protein. *J. Cell Biol.* **116**: 1167-1180.
- Tokunaga, C., 1970a Aspects of low-temperature-induced meiotic nondisjunction in *Drosophila* females. *Genetics* **66**: 653-661.
- Tokunaga, C., 1970b The effects of low temperature and aging on nondisjunction in *Drosophila*. *Genetics* **65**: 75-94.
- Warren, A. C., A. Chakravarti, C. Wong, S. A. Slaugenhaupt, S. L. Halloran *et al.*, 1987 Evidence for reduced recombination on the nondisjoined chromosomes 21 in Down syndrome. *Science* **237**: 652-654.
- Williams, G. C., 1957 Pleiotropy, natural selection, and the evolution of senescence. *Evolution* **11**: 398-411.
- Zhang, P., and R. S. Hawley, 1990 The genetic analysis of distributive segregation in *Drosophila melanogaster*. II. Further genetic analysis of the *nod* locus. *Genetics* **125**: 115-127.
- Zhang, P., B. A. Knowles, L. S. Goldstein and R. S. Hawley, 1990 A kinesin-like protein required for distributive chromosome segregation in *Drosophila*. *Cell* **62**: 1053-1062.
- Zwick, M. E., D. J. Cutler and C. H. Langley, 1999 Classic Weinstein: tetrad analysis, genetic variation and achiasmate segregation in *Drosophila* and humans. *Genetics* **52**: 1615-1629.

Communicating editor: R. S. Hawley