Classic Weinstein: Tetrad Analysis, Genetic Variation and Achiasmate Segregation in Drosophila and Humans

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ABSTRACT

A maximum-likelihood method for the estimation of tetrad frequencies from single-spore data is presented. The *m*ultilocus *e*xchange with *i*nterference and *v*iability (MEIV) model incorporates a clearly defined model of exchange, interference, and viability whose parameters define a multinomial distribution for single-spore data. Maximum-likelihood analysis of the MEIV model (MEIVLA) allows point estimation of tetrad frequencies and determination of confidence intervals. We employ MEIVLA to determine tetrad frequencies among 15 *X* chromosomes sampled at random from *Drosophila melanogaster* natural populations in Africa and North America. Significant variation in the frequency of nonexchange, or E_0 tetrads, is observed within both natural populations. Because most nondisjunction arises from E_0 tetrads, this observation is quite unexpected given both the prevalence and the deleterious consequences of nondisjunction in *D. melanogaster*. Use of MEIVLA is also demonstrated by reanalyzing a recently published human chromosome *21* dataset. Analysis of simulated datasets demonstrates that MEIVLA is superior to previous methods of tetrad frequency estimation and is particularly well suited to analyze samples where the E_0 tetrad frequency is low and sample sizes are small, conditions likely to be met in most samples from human populations. We discuss the implications of our analysis for determining whether an achiasmate system exists in humans to ensure the proper segregation of E_0 tetrads.

A classic, as we have all heard, is a work that is often referred to and never read.
Alexander Weinstein (1955)

IN 1936, Alexander Weinstein presented a mathematical method for inferring the frequency of tetrads with different numbers of exchanges in organisms where only one of the four products of meiosis (referred to as single-spore data) is recovered. His classic article contained the first theoretical model of crossing-over constructed on a four-strand basis and allowed him to infer two main conclusions (Weinstein 1936; see Weinstein 1958 for a review). First, he concluded that the vast majority of exchange at any single crossover event occurs between homologous chromatids, and few if any exchanges occur between sister chromatids. Second, he concluded that the choice of homologous chromatids that undergo exchange at different crossovers is random. Weinstein's results also suggest an important third conclusion, which he did not emphasize: a significant number of *X* chromosome tetrads in *Drosophila melanogaster* fail to undergo exchange during female meiosis. These tetrads are referred to as E_0 tetrads (see Figure 1).

The existence of E_0 tetrads presents something of a paradox, because the commonly accepted model suggests that at least a single exchange, or crossover, is necessary for proper segregation. Chiasmata, the cytological structures formed at the site of crossing-over, have long been thought necessary to ensure proper segregation (Darlington 1932; Nicklas 1977; reviewed in Hawley 1988). Two largely distinct research progams solved this paradox in *D. melanogaster*. The first involved chromosome mechanics and the direct demonstration that chromosomes that failed to cross over are still able to segregate with high efficiency (Bridges 1916; Sturtevant and Beadle 1936; Cooper 1945). Subsequent research led to the identification of a "backup" system, referred to as distributive pairing, that acts to ensure the segregation of nonexchange bivalents (Grell 1962, 1976). The fundamental inference leading to the identification of a backup system arose from the observation that the frequency of nondisjunction was significantly lower than that expected from a null model assuming random segregation of homologs in meiosis. The second strategy, involving screens for meiotic mutants, led to the identification of genetic loci, some of whose mutant phenotypes were defective in the segregation of nonexchange chromosomes (Sandler *et al.* 1968; Baker and Carpenter 1972; Carpenter 1973). Subsequent research along the first path led to the falsification of significant aspects of the distributive pairing model, and instead demonstrated that the "backup" system is composed of two genetically distinct pathways, now referred to as the homologous and heterologous achiasmate systems (Hawley *et al.* 1992; Haw-

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ley and Theurkauf 1993). Progress along the second line has led to the cloning, sequencing, and characterization of genetic loci defective in nonexchange chromosome segregation (Zhang and Hawley 1990; Zhang *et al.* 1990; Rasooly *et al.* 1991, 1994; Whyte *et al.* 1993; Afshar *et al.* 1995a,b; see Hawley *et al.* 1993 for review).

Accurate estimation of E_0 tetrad frequency is of interest for a number of reasons. Failure of the homologous achiasmate system in *D. melanogaster* is the most common cause of nondisjunction, with nearly 76% of *X* chromosome spontaneous nondisjunction events arising from E_0 tetrads (Koehler *et al.* 1996). Within *D. melanogaster*, E_0 tetrad frequency estimates for different chromosomes from several laboratory stocks demonstrate that acrocentric *X* chromosomes have a higher E_0 tetrad frequency than do metacentric autosomes (Weinstein 1936; Merriam and Frost 1964; Carpenter 1973; Charlesworth *et al.* 1985; Rutherford and Carpenter 1988; Hawley *et al.* 1992). No previous study has examined the patterns of variation in the frequency of E_0 tetrads among chromosomes sampled from natural populations. In a separate study, Zwick *et al.* (1999, this issue) report that *X* chromosomes from natural populations harbor high levels of genetic variation in rates of nondisjunction. They further identify two widespread intermediate frequency alleles at the *nod* locus, a chromokinesin required for proper segregation of achiasmate chromosomes, associated with increased nondisjunction. These observations make it of interest to determine if natural populations also contain high levels of variation in *X* chromosome E_0 tetrad frequency.

A number of recent studies report great similarity in the genetic events leading to spontaneous nondisjunction in Drosophila and humans (Koehler *et al.* 1996; Lamb *et al.* 1996, 1997a; Bugge *et al.* 1998; Robinson *et al.* 1998). This suggests the hypothesis that failure of an achiasmate system in humans might lead to nondisjunction, an event that occurs at an extraordinary frequency (Hassold *et al.* 1996). The existence of achiasmate systems in organisms other than *D. melanogaster* and the yeast *Saccharomyces cerevisiae* (Dawson *et al.* 1986; Guacci and Kaback 1991; Loidl *et al.* 1994) remains largely unknown. The observation of substantial E_0 tetrad frequencies in the human genome might suggest a requirement for an achiasmate system to ensure the proper segregation of nonexchange chromosomes. Much as was done in Drosophila, identifying an achiasmate system and the genetic loci that function in this pathway could be expected to make important contributions to understanding the causes of nondisjunction in humans.

Accurate estimation of tetrad frequencies with Weinstein's method requires a dense genetic map and large sample sizes. Until recently, such maps were rare. As a consequence, most quantitative analyses of meiotic crossing over have been focused on ordering markers into maps, accommodating missing data, and modeling interference. For example, mapping functions have been used to infer the genetic distance from the observed exchange fraction of widely spaced markers (Haldane 1919; Bailey 1961; Zhao and Speed 1996). Chiasma interference, first observed in *D. melanogaster*, has been modeled using a variety of approaches (Sturtevant 1915; Muller 1916; Morton and MacLean 1984; McPeek and Speed 1995; Zhao *et al.* 1995b). Statistical methods for analyzing ordered and unordered tetrads have also been published (Snow 1979; Zhao and Speed 1998a,b). However, the primary concerns of these studies have been the efficient construction of genetic maps, including accurate locus ordering and estimation of genetic distance. While tetrad estimation has long been possible in *D. melanogaster*, recent progress in the genomics of other model organisms and humans has resulted in both the dense genetic maps and the data from the progeny of a large number of meioses required for E_0 tetrad estimation.

Weinstein's method of tetrad frequency estimation has been successfully applied (Weinstein 1936; Merriam and Frost 1964; Koehler *et al.* 1996; Lamb *et al.* 1997b), but it suffers from a number of serious limitations. First, when sample sizes are limited, Weinstein's method frequently returns biologically meaningless negative tetrad frequency estimates, especially for the E_0 tetrad class. Application of a method that constrains tetrad frequency estimates within a biologically realistic range (*i.e.*, 0.0 to 1.0) may lead to a significantly better estimator of tetrad frequencies. A recently proposed alternative estimation procedure for Weinstein's model using the EM algorithm eliminates the possibility of negative tetrad frequency estimates (Bugge *et al.* 1998), but like Weinstein's method, suffers from other limitations. Weinstein's method ignores the sex of the progeny and the reciprocal marker arrangements of the individual chromatids recovered in an experimental cross. His method therefore cannot account for viability effects associated with the genetic markers or the sex of the progeny that might alter tetrad frequency estimates. Finally, we can find no published literature that has examined the effectiveness of Weinstein's estimator of tetrad frequencies with simulated datasets.

To overcome these limitations and improve on Weinstein's method of tetrad estimation, we first derive the *m*ultilocus *e*xchange with *i*nterference and *v*iability (MEIV) model. This model assumes that chromatid frequencies for single-spore data are multinominally distributed. The parameters of this distribution are derived from a plausible model of exchange, interference, and viability. We employ maximum-likelihood analysis of the MEIV model [MEIV *l*ikelihood *a*nalysis (MEIVLA)] to estimate tetrad frequencies for single-spore data. Second, we employ MEIVLA to estimate tetrad frequencies of a set of *X* chromosomes randomly sampled from *D. melanogaster* natural populations in North America and Africa. This is the first analysis of tetrad frequencies for

chromosomes randomly sampled from natural populations. We observed surprising levels of variation in the frequency of E_0 tetrads among *X* chromosomes from both natural populations. In the majority of cases, a model incorporating the viability effects of phenotypic markers fit significantly better than one lacking such effects. Third, we reanalyze a recently published human dataset (Lamb *et al.* 1997b) and show that MEIVLA is superior to Weinstein's (1936) method of tetrad frequency estimation. Finally, using parameters estimated from the Drosophila and human datasets, we employ simulations to investigate the efficiency of point estimation and the power of MEIVLA as compared to the Weinstein (1936) model. We particularly focus on the estimation of E_0 tetrad frequencies and the evidence for an achiasmate system in humans.

MATERIALS AND METHODS

Drosophila lines: *D. 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 Lindsley and Zimm (1992). To minimize the effects of the autosomes on *X* chromosome exchange, an autosomal isogenic background was constructed by employing a stock whose genotype was *T(2;3) CyOTM6/+; mwh* ry^{506} 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* chromosome, the balancer *FM7a*, and an *X* chromosome containing the markers *y cv v f car*, were substituted into this common genetic background.

***D. melanogaster* experimental cross:** Experimental females were constructed by crossing *FM7/y cv v f car; b; ri; pol* virgin females to X_i/B^SY; *b; ri; pol* males in bottles. Virgin females whose genotype was X_i/*y cv v f car; b; ri; pol* were collected and aged for 2 days. An experimental cross consisted of crossing 30 males whose genotype was *y cv v f car/Y* to an equal number of experimental females in bottles containing fresh glucose media. 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 on days 11 through 18, after which the bottles were discarded. The raw count data for each chromosome line, separated by broods, is contained in appendix a. The North American bottles were uniformly more productive than the African bottles. It has previously been observed that female *D. melanogaster* from Zimbabwe, Africa exhibit premating isolation with males from other populations or laboratory stocks (Wu *et al.* 1995; Hollocher *et al.* 1997). In recent work on sperm displacement, it has been observed that in more than half of the matings of Zimbabwe females with Zimbabwe or non-Zimbabwe males, no sperm is transferred and these females often require multiple copulations to achieve insemination (J. Coyne, personal conversation). Similar processes affecting our extracted Zimbabwe *X* chromosomes stocks could explain the lower productivity of the African bottles.

Tetrad frequencies were calculated by MEIVLA as described in results. Two arrangements of the dataset were analyzed. First, tetrad frequencies were estimated from the total number of progeny for each *X* chromosome line in the study. Second, to examine the effect of brooding the parents in bottles, E_0 tetrad frequencies were estimated for the data from all three broods of a given *X* chromosome line. All tetrad frequency point estimates and confidence intervals were taken from one of three nested viability models. The model with the most parameters was the full viability model. This model assumes that each phenotypic marker has a different sex-specific viability effect. The single viability model assumes that each phenotypic marker has a specific viability effect that is identical in both males and females. The wild-type viability model assumes that the phenotypic markers have no effect on viability of the progeny in either sex. To determine the best-fitting model, we calculated the likelihood test statistic for each model and performed a likelihood-ratio test. We chose the full viability model as the best-fitting model if the *P* values from the likelihood-ratio test showed that the full model fit significantly better than the single and wild-type viability models. We chose the single viability model as the best-fitting model when it fit significantly better than the wild-type viability model and the full model did not provide a significantly better fit. We chose the wild-type viability model as the best-fitting model when neither of the two alternative models fit significantly better. We employed a *P* value of 0.05 as our significance threshold. appendix b contains the pertinent likelihood-ratio test results, the viability parameter point estimates, and their confidence intervals. All other statistical analyses were carried out with JMP 3.2.1 (SAS Institute).

Human data: Human data were obtained from Table 1 in Lamb *et al.* (1997b). Tetrad frequencies were calculated as described in results, using only the wild-type viability model.

RESULTS

Tetrad analysis model: The central problem of tetrad analysis is to employ the observed numbers or frequencies of chromatids to infer the unobservable frequencies of meiotic tetrads (Figure 1). We assume a known genetic map with $K + 1$ diallelic loci that divide an acrocentric chromosome into *K* regions. Alleles at each locus are labeled either + or −. For the *D. melanogaster* datasets, the − allele is assumed to be a visible mutant. Assume the regions between markers are small enough that there is at most a single exchange event within each region. Starting with a parent who is heterozygous at all loci, with one chromosome containing all + alleles and the other containing all − alleles, the basic experimental data consist of counting *N* individual chromatids, which are the products of *N* meioses. Chromatids are recovered in male or female progeny, contain either of two reciprocal marker arrangements, and can exhibit, or not exhibit, an exchange in any of the *K* regions. For a dataset with *K* regions, there are $4(2^K) = 2^{K+2}$ distinct observable exchange classes of chromatids.

Each distinct observable type of chromatid is designated by N_i^l with $(1 \le i \le 2^K)$ and $(1 \le l \le 4)$. The *i*'s partition the observable chromatids into 2^K exchange classes. We say two chromatids are in the same exchange

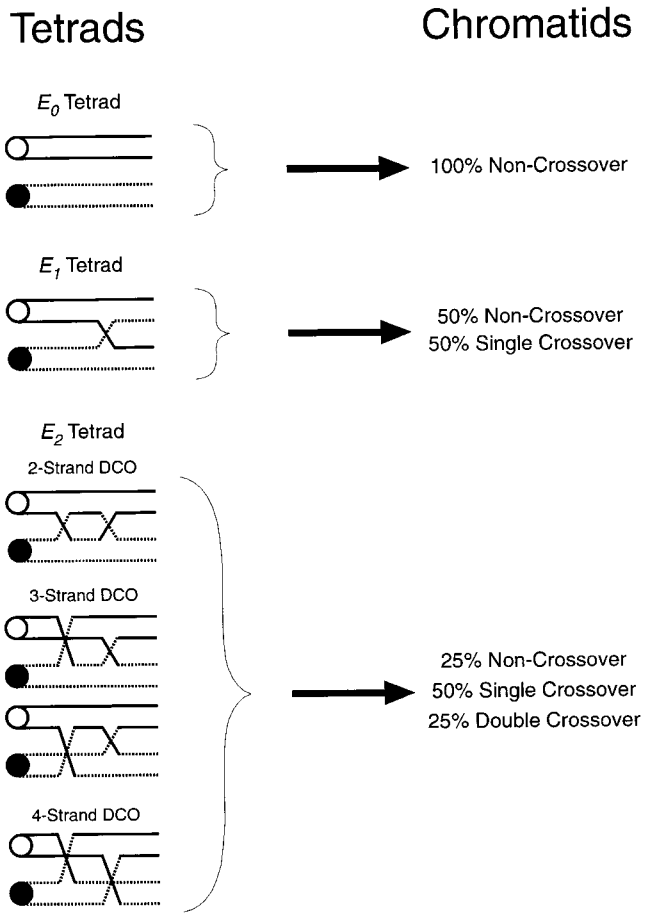

Figure 1.—Relationship between observed chromatids and the estimated tetrad frequencies in Drosophila and humans. Exchange tetrads of rank *n* produce chromatids with up to *n* exchanges in proportions first derived in Weinstein (1936). This derivation reflects the fundamental observation that *n* exchange tetrads produce chromatids that have fewer than *n* exchanges.

classes if they exhibit an identical pattern of exchange. Thus, for example, two chromatids are in the same exchange class if they both show exchanges in regions 1 and 3 but no others. The algorithm that relates a specific number *i* to a specific exchange class is unimportant. We require only that each unique exchange class be assigned to a unique *i*. The *l*'s divide each of the *i* exchange classes into four subclasses that account for the sex of the progeny and the reciprocal marker arrangement of the chromatid. Let N_i^1 be the observed number of chromatids of exchange class *i*, recovered in males with the − allele for marker 1, N_i^2 be the observed number of chromatids of exchange class *i*, recovered in females with the − allele for marker 1, N_i^3 be the observed number of chromatids of exchange class *i*, recovered in males with the + allele for marker 1, N_i^4 be the observed number of chromatids of exchange class *i*, recovered in females with the + allele for marker 1, where

$$N = \sum_{i=1}^{2^K} \sum_{l=1}^{4} N_i^l \tag{1}$$

reflects all observed chromatids. By assumption, the N_i^l's are multinomially distributed.

The following set notation for each exchange class conveniently indicates the exchange location(s). Let $I_{i,j}$ be an indicator variable that records whether exchange class *i* has an exchange in region *j*, where $1 \le j \le K$. Thus

$$I_{i,j} = \begin{cases} 1 & \text{if class } i \text{ has an exchange in region } j, \\ 0 & \text{otherwise.} \end{cases}$$

To maintain a complete list of the specific regions that have undergone exchange, for each exchange class *i*, create a set of integers S_i, such that $S_i = \{j \mid I_{i,j} = 1\}$. In other words, S_i is a list of those regions that are observed to have exchanges in class *i*. Let

$$|S_i| = \sum_{j=1}^{K} I_{i,j} \tag{2}$$

be the number of elements in set S_i. Thus $|S_i|$ reflects the number of exchanges in exchange class *i*. To derive the multinomial-likelihood expression, an explicit model incorporating exchange, crossover interference, and viability is required. Chromatid interference occurs when the chromatids that exchange at one site influence the choice of chromatids that undergo exchange at an adjoining site. Detection of significant chromatid interference is fairly rare in a variety of organisms (Weinstein 1936; Zhao *et al.* 1995a). We therefore assume no chromatid interference in our model.

Incorporating exchange: Assuming that all regions are small enough such that there is never more than one exchange per region, then in the absence of interference, the probability of an exchange event in region *j* is R_j. Let Z_i be the probability that any meiosis has exchanges only in the regions of S_i. In the absence of interference, Z_i would equal U_i, where U_i is given by

$$U_i = \prod_{j=1}^{K} [I_{i,j}R_j + (1 - I_{i,j})(1 - R_j)]. \tag{3}$$

Incorporating crossover interference: To model crossover interference, first note that if there is more than one exchange in class *i* ($|S_i| > 1$), then interference will reduce Z_i from U_i and increase the frequency of classes with fewer exchanges. Let $1 - P_i$ be the proportion by which the *i*th exchange class is decreased due to interference, with $0 \le P_i \le 1$. For $P_i = 1$, no interference is acting, while $P_i = 0$ indicates complete suppression of the *i*th exchange class. For a model with *K* regions, there are $2^K - K - 1$ P_i terms that may differ from one. For a specific example, suppose class *i* consists of a triple crossover event in regions one, two, and three. Given that interference occurs (*i.e.*, $P_i < 1$), by assumption, the proportion of triple exchanges will decrease while the number of double exchanges increases.

There are three different possible classes of double exchanges that increase in frequency for a given triple exchange. By assumption, the probabilities of these events are

$$\text{Double in regions 1 and 2} = \frac{R_1R_2}{R_1R_2 + R_1R_3 + R_2R_3}$$

$$\text{Double in regions 1 and 3} = \frac{R_1R_3}{R_1R_2 + R_1R_3 + R_2R_3}$$

$$\text{Double in regions 2 and 3} = \frac{R_2R_3}{R_1R_2 + R_1R_3 + R_2R_3}.$$

In general, multiple crossovers in other regions can be resolved in a similar fashion. To do this, let

$$M_i = \prod_{j \in S_i} R_j, \tag{4}$$

$$Y_i = U_i + \sum_{\substack{S_i \subset S_j, \\ |S_j| - |S_i| = 1}} \frac{M_i(1 - P_j)Y_j}{\sum_{\substack{S_k \subset S_j, \\ |S_j| - |S_k| = 1}} M_k}. \tag{5}$$

Note that Y_i is given in terms of Y_j, where $S_i \subset S_j$ and $|S_j| - |S_i| = 1$. Therefore, one must first calculate Y for the class with exchanges in all regions, then for all classes with exchange in all but one region, and so forth, down to the class with no exchanges. Z_i, the probability that a meiosis has exchanges only in regions S_i, is given by

$$Z_i = P_iY_i. \tag{6}$$

Incorporating viability effects of markers: The viability effects of markers in progeny of both sexes will act to decrease the recovery of certain chromatids relative to others. To incorporate viability, we assume that the + allele for each marker has no effect on fitness (*i.e.*, has fitness = 1). Assume that the − allele at marker i has fitness effect v_i^{m} in males and v_i^{f} in females and that these fitness effects are not influenced by culture conditions. We assume a multiplicative model of epistasis. Let

$$W_{i,j} = \begin{cases} 1 & \text{if } \sum_{l=1}^{j} I_{i,j} \text{ is odd,} \\ 0 & \text{if } \sum_{l=1}^{j} I_{i,l} \text{ is even,} \end{cases}$$

be the indicator that the allele at the $j + 1$ locus differs from the allele at the first locus of exchange class i. ($W_{i,j}$ is 0 if locus 1 and $j + 1$ are both + or are both −, and equal to 1 otherwise.) Let

$$G_i^1 = \frac{v_1^{\mathrm{m}}Z_i}{4} \prod_{j=2}^{K+1} (1 - W_{i,j})v_j^{\mathrm{m}} + W_{i,j}, \tag{7}$$

$$G_i^2 = \frac{v_1^{\mathrm{f}}Z_i}{4} \prod_{j=2}^{K+1} (1 - W_{i,j})v_j^{\mathrm{f}} + W_{i,j}, \tag{8}$$

$$G_i^3 = \frac{Z_i}{4} \prod_{j=2}^{K+1} W_{i,j}v_j^{\mathrm{m}} + 1 - W_{i,j}, \tag{9}$$

$$G_i^4 = \frac{Z_i}{4} \prod_{j=2}^{K+1} W_{i,j}v_j^{\mathrm{f}} + 1 - W_{i,j}, \tag{10}$$

Thus G_i^l is proportional to the frequency of chromatids after accounting for marker genotype and sex of the progeny. Letting

$$T = \sum_{i=1}^{2^K} \sum_{l=1}^{4} G_i^l \tag{11}$$

be the sum of these proportions, we normalize by

$$E_i^l = \frac{G_i^l}{T} \tag{12}$$

to obtain the final estimate of chromatid frequencies.

Final tetrad frequency estimation: In organisms where all four products of a single meiosis can be recovered, the N_i^l's are multinomially distributed with means NE_i^l. But with single-spore data, only one of the four products of a single meiosis is recovered. Chromatids derived from any particular exchange are recovered with probability 1/2 because exchange is assumed not to occur between sister chromatids (Weinstein 1936). Therefore, our N_i^l are multinomially distributed with means NF_i^l, where

$$F_i^l = \sum_{S_i \subseteq S_j} E_j^l \left(\frac{1}{2}\right)^{|S_j|}, \tag{13}$$

The overall likelihood, L, of the observations is

$$L = \binom{N}{N_1^1, N_1^2, \ldots, N_{2K}^3, N_{2K}^4} \prod_{i=1}^{2^K} \prod_{l=1}^{4} (F_i^l)^{N_i^l}. \tag{14}$$

Our approach to solving this likelihood expression is to numerically find the values of our parameters (K exchange frequencies, R's; $2^K - K - 1$ P's; and $2K + 2$ v's, for a total of $2^K + 2K + 1$) that maximize (14). This was accomplished by minimizing $-\log(L)$ using the "Powell" algorithm (Press *et al.* 1992). Once the parameters associated with the maximum have been found, tetrad frequency E_i can be found by noting

$$E_i = \sum_{|S_j|=1}^{4} E_i^l, \quad 0 \le i \le K. \tag{15}$$

Confidence intervals for all the tetrad frequency point estimates were determined by using the Powell algorithm to search the surrounding likelihood surface. For a calculated maximum likelihood, L_{m}, the range of parameter values that gave an L such that $\log(L_{\mathrm{m}}) - \log(L) < 2$ are considered within the 95% confidence interval. The minimum and maximum tetrad frequencies implied by parameters in the 95% confidence interval are considered the confidence limits. This procedure does not guarantee that all tetrad frequencies between the minimum and maximum will be within the 95% confidence interval. However, for a sufficiently smooth likelihood surface, all intermediate values will be contained.

Tetrad frequency estimation in Drosophila: Tetrad frequency estimates calculated from the best-supported viability model for six African *X* chromosomes and nine North American *X* chromosomes are contained in Tables 1 and 2, respectively (see appendices for raw data, viability parameter estimates, and their confidence intervals). For three of six African *X* chromosomes and nine of nine North Carolina *X* chromosomes, a model incorporating viability was the best-fitting model. Despite the observed viability effects of the morphological mutants employed in this study, their effect on estimates of E_0 tetrad frequency appears quite small.

The mean estimated E_0 tetrad frequencies for the African (0.118) and the North American (0.105) populations are not significantly different ($P = 0.23$). The *X* chromosome samples, however, exhibit significant variation within both natural populations. This is most clearly seen by the nonoverlapping E_0 tetrad frequency point estimates and their confidence intervals in Figure 2. Because *X* chromosomes from both populations were substituted into a common isogenic background, the source of this variation should reside on the individual *X* chromosomes.

The E_0 tetrad frequency estimates for each brood were also calculated from the best-supported viability model for the *X* chromosomes from both natural populations. The mean E_0 tetrad frequency estimates for the three broods are significantly different (Figure 3; $P = 0.02$). Comparing each pair of means, corrected for multiple comparisons by the Tukey-Kramer HSD, shows that first and third broods are the only two that are significantly different ($P < 0.05$).

Tetrad frequency estimation in humans: We determined the raw count data from the human dataset in Lamb *et al.* (1997b) and reanalyzed their data (Table 3). We conclude that the maximum-likelihood estimate for the frequency of E_0 tetrads in human females is 1.5%. Our E_0 tetrad frequency estimate is nearly identical to that in Lamb *et al.* (1997b) for this single dataset. The E_1 and E_2 tetrad frequency point estimates differ. For all tetrad frequency estimates, however, MEIVLA returns confidence intervals that are significantly smaller than those in Lamb *et al.* (1997b). Furthermore, the confidence intervals for the E_0 tetrad frequency point estimate do not include biologically meaningless negative tetrad frequency estimates.

Simulation results: To investigate the efficiency of the MEIVLA point estimation procedures and the accuracy of confidence intervals for various sample sizes, we simulate datasets using parameters estimated from the *D. melanogaster* NC14X line and the human dataset. For the Drosophila NC14X parameter set, we generated 500 simulated datasets for each of six different sample sizes. For the human parameter set, we generated 500 simulated datasets for each of nine different sample sizes.

Using parameters determined from the *D. melanogaster* NC14X line, the majority of E_0 tetrad frequency esti-

TABLE 1

Tetrad frequency estimates from the best-fitting viability model for African *X* chromosomes

Line	Model	$\hat{E}_0$	(C.I.)	$\hat{E}_1$	(C.I.)	$\hat{E}_2$	(C.I.)	$\hat{E}_3$	(C.I.)
5EX	Wild type	0.120	(0.084, 0.156)	0.713	(0.709, 0.717)	0.167	(0.144, 0.182)	0	(0.0, 0.001)
15EX	Wild type	0.142	(0.102, 0.185)	0.734	(0.660, 0.801)	0.124	(0.106, 0.145)	0	(0.0, 0.002)
31EX	Single	0.085	(0.067, 0.101)	0.734	(0.732, 0.737)	0.181	(0.169, 0.189)	0	(0.0, 0.0)
32EX	Single	0.195	(0.169, 0.220)	0.612	(0.596, 0.648)	0.181	(0.159, 0.202)	0.012	(0.001, 0.014)
34EX	Wild type	0.119	(0.097, 0.139)	0.738	(0.702, 0.764)	0.141	(0.129, 0.153)	1.7×10^{-3}	$(1.5 \times 10^{-3}, 2.1 \times 10^{-3})$
36EX	Single	0.175	(0.159, 0.191)	0.679	(0.650, 0.701)	0.146	(0.137, 0.155)	0	(0.0, 0.0)

E_3 tetrad frequency estimates of 0 for lines 5EX, 15EX, 31EX, and 36EX reflect the fact that no triple-crossover chromatids were recovered.

TABLE 2

Tetrad frequency estimates from the best-fitting viability model for North American *X* chromosomes

Line	Model	$\hat{E}_0$	(C.I.)	$\hat{E}_1$	(C.I.)	$\hat{E}_2$	(C.I.)	$\hat{E}_3$	(C.I.)
NC6X	Single	0.073	(0.062, 0.083)	0.671	(0.668, 0.674)	0.254	(0.247, 0.261)	2.0×10^{-3}	$(1.8 \times 10^{-3}, 2.2 \times 10^{-3})$
NC7X	Single	0.091	(0.075, 0.104)	0.720	(0.719, 0.722)	0.188	(0.176, 0.194)	1.9×10^{-3}	$(1.7 \times 10^{-3}, 2.2 \times 10^{-3})$
NC11X	Single	0.108	(0.090, 0.124)	0.710	(0.708, 0.713)	0.179	(0.167, 0.195)	2.5×10^{-3}	$(2.2 \times 10^{-3}, 2.8 \times 10^{-3})$
NC12X	Full	0.141	(0.128, 0.152)	0.694	(0.693, 0.695)	0.165	(0.157, 0.173)	6.1×10^{-4}	$(5.6 \times 10^{-4}, 6.7 \times 10^{-4})$
NC14X	Single	0.104	(0.090, 0.118)	0.762	(0.745, 0.779)	0.133	(0.126, 0.141)	7.5×10^{-4}	$(6.7 \times 10^{-4}, 8.3 \times 10^{-4})$
NC19X	Single	0.135	(0.117, 0.151)	0.742	(0.712, 0.764)	0.123	(0.115, 0.132)	0	(0.0, 0.0)
NC20X	Single	0.126	(0.110, 0.139)	0.714	(0.713, 0.715)	0.159	(0.149, 0.168)	1.7×10^{-3}	$(1.6 \times 10^{-3}, 2.0 \times 10^{-3})$
NC29X	Single	0.118	(0.104, 0.130)	0.734	(0.716, 0.752)	0.148	(0.141, 0.156)	0	(0.0, 0.0)
NC50X	Single	0.088	(0.074, 0.099)	0.761	(0.745, 0.777)	0.150	(0.143, 0.158)	5.8×10^{-4}	$(5.3 \times 10^{-4}, 6.4 \times 10^{-4})$

E_3 tetrad frequency estimates of 0 for lines NC19X and NC29X reflect the fact that no triple-crossover chromatids were recovered.

mates calculated by MEIVLA are closer to the true value (0.104, Table 2) than those of Weinstein (1936) for all sample sizes (Table 4). Employing the human dataset parameters, an even greater majority of E_0 tetrad frequency estimates calculated by MEIVLA are closer to the true value (0.0145, Table 5) than those of Weinstein (1936). This advantage is particularly evident for small sample sizes. Both methods converge to similar point estimates as sample size increases, but even for large samples sizes, MEIVLA consistently outperforms the model of Weinstein (1936).

We draw two conclusions from analysis of the human data in Lamb *et al.* (1997b) and the results of our simulation studies. First, the maximum-likelihood estimate for the frequency of chromosome *21* E_0 tetrads in human female meiosis is ~1.5%. Second, on the basis of our simulation results, we conclude that with sample sizes of 276, it is not possible to exclude either that there are no chromosome *21* E_0 tetrads or the alternative, that E_0 tetrad frequencies are similar to those observed for the *X* chromosome in Drosophila. Our simulation analyses employing estimated human parameters (Table 5) suggest that if chromosome *21* E_0 tetrad frequency were as low as 1.5%, with moderate sample sizes (1000–5000 meioses), it should be possible to determine an upper bound that would exclude the E_0 tetrad frequencies seen for the Drosophila *X* chromosome. To obtain a chromosome *21* E_0 estimate that excludes zero at the lower bound, significantly larger sample sizes of 20,000–30,000 meioses are required. Our simulations with the Drosophila parameters also point out the inadequacy of current human sample sizes, because with a Drosophila sample size of 276, we cannot exclude the lower bound of zero in an organism where E_0 tetrad frequency is ~10% (see sample size 276 in Table 4).

DISCUSSION

We present MEIVLA, a method of tetrad frequency estimation that significantly improves upon those originally derived by Weinstein (1936) and extended in Lamb *et al.* (1997b): First, the MEIV model incorporates a clearly defined model of exchange, interference, and viability whose parameters define a multinomial distribution for single-spore data. The derivation of the MEIV model ensures that biologically meaningless results such as negative tetrad frequency estimates are not produced by MEIVLA of the MEIV model. Second, the MEIV model allows the determination of the magnitude of marker viability effects, permitting their incorporation into MEIVLA. Previous methods of tetrad frequency estimation have not incorporated viability in their estimation procedures. Third, simple methods that explore the likelihood surface surrounding its maximum allow the direct determination of confidence intervals. Finally, MEIVLA point estimates and confidence intervals are consistently superior to previous methods. This advantage is most evident in situations where the E_0 tetrad

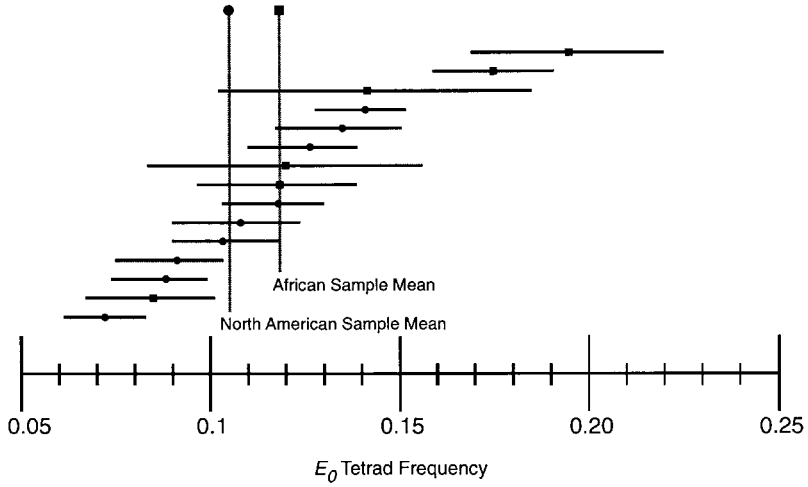

Figure 2.—E_0 tetrad frequency point estimates and their confidence intervals as calculated for *X* chromosomes from North American (circle) and African (square) natural populations. Note that most of the variation is found among chromosomes within populations—the population means (vertical lines with either a circle or a square) were not significantly different ($P = 0.23$).

frequency is low and sample sizes are small. Because both of these conditions are met in most samples from human populations, MEIVLA is ideally suited for the analysis of human datasets.

One potential criticism of our exchange model is that we only allow zero or one exchange between markers. However, we do not believe that this is a significant problem for two reasons. First, our method is conditioned upon known, dense genetic maps—such as those found in model organisms and that are increasingly available in nonmodel organisms. Given a sufficiently dense map, it is possible to choose markers so that this assumption is met. Second, the examination of genetic maps from model organisms supports the view that meiosis is regulated in such a manner as to favor a single exchange per chromosome arm. This is evidenced by the excess of single-crossover (E_1) tetrads (Tables 1 and 2; Lamb *et al.* 1997b; see review in Hawley 1988). Furthermore, when double crossovers do occur during meiosis, interference acts to space the chiasmata, making it very unlikely that a meiotic double-exchange event would occur even between moderately spaced markers. Analysis of our Drosophila data rejects a MEIV model lacking interference (data not shown). Exchange events of a nonmeiotic origin might occur, leading to apparent closely spaced double crossovers (Suzuki *et al.* 1966), but these events are expected to be rare. Our model is specifically designed to analyze data from acrocentric chromosomes and assumes that the fitness effects of morphological markers are not influenced by culture conditions. A more general model that relaxes these assumptions, allowing the analysis of metacentric chromosomes while incorporating fitness variation between cultures, is in development.

***D. melanogaster* natural populations harbor a significant level of variation among *X* chromosomes in their E_0 tetrad frequency:** This study is the first to examine the E_0 tetrad frequency of *X* chromosomes sampled from natural populations. Previous studies, concerning a small number of laboratory stocks, provided estimates of E_0 tetrad frequency that largely agree with those presented in this study (Weinstein 1936; Merriam and

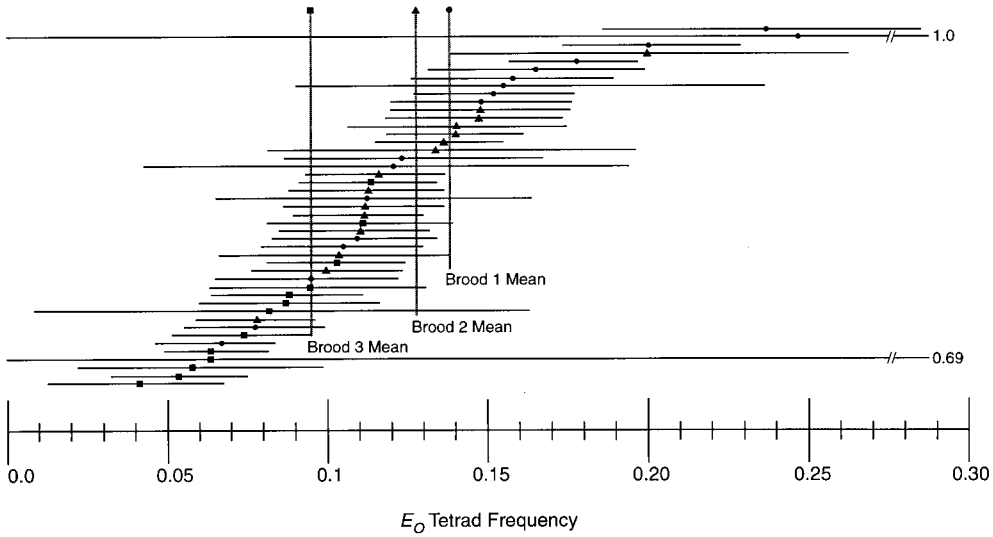

Figure 3.—E_0 tetrad frequency point estimates and their confidence intervals as calculated for each of three broods for all *X* chromosomes from North America and Africa. Brood 1 (circle), brood 2 (triangle), and brood 3 (square) are presented along with the mean E_0 tetrad frequency for each brood (vertical lines marked with either a circle, triangle, or square). The means of broods 1 and 3 were found to be statistically significantly different ($P < 0.05$) after correcting for multiple tests.

TABLE 3

Estimated tetrad frequencies for human chromosome *21* during female meiosis

Lamb *et al.* (1997b)		MEIVLA	
Estimate	95% C.I.	Estimate	95% C.I.
$\hat{E}_0 = 0.015$	(−0.106, 0.135)	$\hat{E}_0 = 0.015$	(0.000, 0.083)
$\hat{E}_1 = 0.580$	(0.363, 0.797)	$\hat{E}_1 = 0.520$	(0.507, 0.639)
$\hat{E}_2 = 0.406$	(0.285, 0.526)	$\hat{E}_2 = 0.466$	(0.339, 0.485)

Frost 1964; Carpenter 1973; Charlesworth *et al.* 1985; Rutherford and Carpenter 1988; Hawley *et al.* 1992; Koehler *et al.* 1996). However, none of these studies were able to address the patterns of variation in natural populations. It is quite striking that both the African and North American samples contained *X* chromosomes that significantly differed in their E_0 tetrad frequency. Given the observation that the majority of spontaneous nondisjunction arises from nonexchange (E_0) tetrads (Koehler *et al.* 1996) and the uniformly deleterious consequences of nondisjunction, it is quite surprising that natural populations harbor this variation.

One might expect that natural selection would act to decrease the frequency of E_0 tetrads because of the deleterious consequences of aneuploidy arising from nondisjunction. In a separate companion study of the patterns of genetic variation underlying nondisjunction in female meiosis, Zwick *et al.* (1999, this issue) demonstrate that *X* chromosomes from the same two natural populations harbor high levels of genetic variation in rates of nondisjunction in a sensitized assay of E_0 tetrads. They furthermore identify two widespread intermediate frequency alleles at the *nod* locus, a chromokinesin required for the proper segregation of achiasmate chromosomes, which are significantly associated with an increased frequency of nondisjunction. To account for the high levels of genetic variation observed in female meiosis, Zwick *et al.* (1999, this issue) present an evolutionary model, referred to as the oötid competition model, that can account for high rates of genetic variation in the efficiency of chromosome segregation during female meiosis.

Although the effects on E_0 tetrad frequency estimation were not large, incorporation of a multiplicative viability model significantly improved the fit of the MEIV model in a majority of cases. Incorporation of a specific viability model can improve tetrad frequency estimation when the viability effects are large. Our data further show a significant decrease in mean E_0 tetrad frequency for the third brood as compared to the first brood. This pattern largely agrees with those in other studies that have shown increased exchange as female Drosophila age. The pattern of variation in exchange in relation to maternal age, however, is not straightforward and exhibits substantial variation in different experiments (see Ashburner 1989 for a review). One important point to consider in the observation of elevated exchange in older female Drosophila is the possible nonmeiotic origin of exchange. Mitotic crossing over, perhaps associated with transposable element activity, in meiotic stem cells can result in an apparent increase in observed meiotic exchange. Such nonmeiotic exchange is expected to increase with maternal age. Simple "single-spore" tetrad estimation cannot distinguish between these sources of exchange and normal meiotic crossing-over.

We disagree with the conclusions in Lamb *et al.* (1997b) that analysis of the human dataset supports the concept of obligate exchange and fails to provide evidence for a secondary segregation system in human females: First, the concept of obligate exchange either

TABLE 4

Mean E_0 tetrad frequency estimates from 500 simulated datasets using *D. melanogaster* (NC14X) parameter values ($E_0 = 0.104$)

	Weinstein (1936)		MEIVLA		Proportion of estimates closer than Weinstein (1936)
Sample size	Mean E_0 estimate	Lower/upper percentiles	Mean E_0 estimate	Lower/upper percentiles	
276	0.1039	(−0.014, 0.225)	0.1000	(0.000, 0.217)	0.588
500	0.1020	(0.004, 0.196)	0.0990	(0.004, 0.184)	0.574
1,000	0.1018	(0.040, 0.164)	0.1008	(0.040, 0.161)	0.574
5,000	0.1033	(0.077, 0.130)	0.1033	(0.077, 0.130)	0.516
10,000	0.1040	(0.086, 0.123)	0.1046	(0.086, 0.123)	0.512
20,000	0.1040	(0.088, 0.119)	0.1040	(0.088, 0.119)	0.562

TABLE 5

Mean E_0 tetrad frequency estimates and confidence intervals determined from 500 simulated datasets (E_0 = 0.0145)

	Weinstein (1936)		MEIVLA		Proportion of estimates closer than Weinstein (1936)
Sample size	Mean E_0 estimate	Lower/upper percentiles	Mean E_0 estimate	Lower/upper percentiles	
276	0.0143	(−0.115, 0.130)	0.0133	(0.000, 0.059)	0.868
500	0.0154	(−0.076, 0.104)	0.0124	(0.000, 0.059)	0.812
1,000	0.0150	(−0.048, 0.078)	0.0130	(0.000, 0.046)	0.786
5,000	0.0145	(−0.014, 0.042)	0.0131	(0.000, 0.034)	0.738
10,000	0.0150	(−0.007, 0.034)	0.0142	(0.000, 0.029)	0.714
20,000	0.0144	(0.0001, 0.0028)	0.0141	(0.0001, 0.026)	0.612
30,000	0.0149	(0.004, 0.026)	0.0147	(0.004, 0.025)	0.570
40,000	0.0145	(0.004, 0.025)	0.0144	(0.004, 0.024)	0.536
50,000	0.0145	(0.006, 0.023)	0.0145	(0.006, 0.023)	0.546

requires that all tetrads undergo exchange or that homologous chromosomes in tetrads that fail to exchange segregate at random. Our maximum-likelihood estimate for the frequency of chromosome *21* E_0 tetrads in human females is 1.5%, nearly identical to that observed in Lamb *et al.* (1997b). Because of the limited sample size upon which this estimate is based, the confidence intervals determined from the MEIV model range from 0.0 to 8.3%. Nevertheless, the maximum-likelihood estimate is not 0, and it seems clear that the proper interpretation of both analyses is that they are consistent with a low frequency of chromosome *21* E_0 tetrads. Therefore, because this analysis and Lamb *et al.* (1997b) suggest that the E_0 frequency of chromosome *21* is not 0, to conclude that the model of obligate exchange is correct for humans, one must demonstrate that chromatids in E_0 tetrads segregate at random. This has not been done, nor can it be done with the data contained in Lamb *et al.* (1997b). The analysis of Bugge *et al.* (1998) suffers from a similar set of problems. Thus, the conclusion that the concept of obligate exchange is supported is not warranted.

For quite different reasons, the analysis of the Lamb *et al.* (1997b) dataset cannot speak to the alternative hypothesis of the existence of a secondary segregation system in humans. In Drosophila, the vast majority of nondisjunction arises from the *X* chromosome. Thus, to a good approximation, the genome-wide E_0 tetrad frequency, which we refer to as ${}^{G}E_0$, is simply equal to the *X* chromosome E_0 tetrad frequency. In humans, ${}^{G}E_0$ must reflect the probability that one or more chromosome pairs may fail to undergo exchange during female meiosis. Because the human karyotype has 23 pairs of chromosomes, tetrad frequency analysis of a single chromosome cannot be used to estimate ${}^{G}E_0$.

One possible hypothesis is that the Drosophila and human ${}^{G}E_0$ tetrad frequencies might both be ~10%. If this were the case, the E_0 tetrad frequency for any individual chromosome in humans would be expected to be lower than that observed for the Drosophila *X* chromosome. To determine the ${}^{G}E_0$ tetrad frequency for the human genome, the best experimental design would require the simultaneous analysis of many, if not all, human chromosomes. Simultaneous analysis is required because exchange patterns of different chromosomes may not be independent. One example of such interactions is the interchromosomal effect (Sturtevant 1919; reviewed in Lucchesi 1975), the observation that inversions suppressing exchange on one chromosome act to increase exchange on other chromosomes. Studies of single human chromosomes cannot account for this possible source of variation. The analysis in Bugge *et al.* (1998), which employs a hypothesis-testing approach in calculating simultaneous tetrad frequencies for multiple chromosomes, cannot detect interactions between chromosomes. Furthermore, our simulation studies (Tables 4 and 5) make it quite clear that sample sizes of ~80 meioses analyzed by Bugge *et al.* (1998) are far too small to allow any conclusion. However, given the appropriate data, an achiasmate system could be detected in humans much as was done in Drosophila if the observed level of female-specific nondisjunction arising from nonexchange tetrads was significantly lower than that predicted by a null model assuming random segregation of all chromosomes in ${}^{G}E_0$ tetrads.

To describe how such an analysis would be performed, we assume a very simple model with *n* independent chromosomes, each with a probability ε of forming an E_0 tetrad (${}^{G}E_0 \approx n\varepsilon$), then

$$D = \sum_{i=0}^{n} \binom{n}{i} \varepsilon^i (1 - \varepsilon)^{n-i} (\rho^i), \tag{16}$$

where *D* is the expected frequency of normal disjunction and nondisjunction arising from tetrads with at least one exchange in females. Therefore, $1 - D$ is the expected frequency of female-specific nondisjunction

TABLE 6

Expected frequency of nondisjunction in the absence of an achiasmate system (ρ = 0.5, see text)

ε (E_0 tetrad freqency for each chromosome)	*N* (number of chromosomes that can undergo nondisjunction)		
	5	10	23
0.005	0.012	0.025	*0.056*
0.01	0.025	*0.049*	*0.109*
0.02	*0.049*	*0.096*	*0.206*
0.03	*0.073*	*0.140*	*0.294*
0.04	*0.096*	*0.183*	*0.372*
0.05	*0.119*	*0.224*	*0.441*
0.06	*0.141*	*0.263*	*0.504*
0.07	*0.163*	*0.300*	*0.559*
0.08	*0.184*	*0.335*	*0.609*
0.09	*0.206*	*0.369*	*0.653*
0.10	*0.226*	*0.401*	*0.692*

TABLE 7

Expected frequency of nondisjunction in the absence of an achiasmate system (ρ = 0.25, see text)

ε (E_0 tetrad frequency for each chromosome)	*N* (number of chromosomes that can undergro nondisjunction)		
	5	10	23
0.005	0.019	0.037	*0.083*
0.01	0.037	*0.073*	*0.159*
0.02	*0.073*	*0.140*	*0.294*
0.03	*0.108*	*0.204*	*0.408*
0.04	*0.141*	*0.263*	*0.504*
0.05	*0.174*	*0.318*	*0.585*
0.06	*0.206*	*0.369*	*0.653*
0.07	*0.236*	*0.417*	*0.711*
0.08	*0.266*	*0.461*	*0.759*
0.09	*0.295*	*0.503*	*0.800*
0.10	*0.323*	*0.541*	*0.834*

arising from E_0 tetrads. For a single E_0 tetrad, ρ is the expected frequency of normal segregants. This equation reduces to

$$D = (1 + \varepsilon(\rho - 1))^n. \quad (17)$$

Because of the great variation among chromosomes in their frequency of nondisjunction, to simplify this analysis, we chose three different numbers of chromosomes. The sample size of five was chosen to reflect those chromosomes (*15*, *16*, *18*, *21*, and *22*) whose frequencies of nondisjunction are the best characterized (Lamb *et al.* 1996, 1997a; Bugge *et al.* 1998; Robinson *et al.* 1998; reviewed in Hassold *et al.* 1996). A sample size of 10 was chosen to encompass nondisjunction arising from the 10 smallest human chromosomes (*13–22*). A sample size of 23 was chosen to reflect the assumption that each pair of human chromosomes is equally likely to undergo nondisjunction. For ease of analysis, ε and ρ are assumed to be identical for each set (*5*, *10*, and *23*) of chromosomes. Violation of this assumption, as evidenced by variation among chromosomes in their frequency of nondisjunction (reviewed in Hassold *et al.* 1996) or the nonindependence of recombination among different chromosomes, could be incorporated into future, more complicated models.

If we first assume that the actual genomic rate of female-specific nondisjunction is 0.2 (see review in Hassold *et al.* 1996) and that only 20% of this total arises from nonexchange tetrads, then 4% of all meioses would be expected to generate female-specific nondisjunction arising from E_0 tetrads. Table 6 contains the expected frequency of female-specific nondisjunction ($1 - D$) for a small set of representative parameters with ρ = 0.5. In Drosophila, it has long been recognized that nullo exceptions are more frequent than diplo exceptions and that this can cause the rate of nondisjunction to be >0.5 in the absence of an achiasmate system. To reflect these observations, Table 7 contains the expected frequency of female-specific nondisjunction ($1 - D$) for the same small set of representative parameters with ρ = 0.25. The values in italic type in Tables 6 and 7 represent expected frequencies of nondisjunction originating from nonexchange tetrads in the absence of an achiasmate system that are greater than our assumed frequency of female-specific nondisjunction arising from E_0 tetrads. Thus the parameter sets that lead to levels of nondisjunction >4% represent values necessary to reject the null model of random segregation and thereby would cause one to conclude that an achiasmate system exists in humans.

Thus, much as in the case of Drosophila, there are two alternative strategies one could employ to detect achiasmate systems. The first method requires estimation of ${}^{G}E_0$ and an estimate of the female-specific rate of nondisjunction. Large datasets would be required, but in principle, if an achiasmate system exists in a specific organism, it should be possible to eliminate a null model assuming no achiasmate system. Datasets are rapidly becoming available in a number of other model and nonmodel organisms, which should allow detection of putative achiasmate systems. A second strategy, which is more direct but more difficult to carry out, would aim to characterize genetic loci whose null phenotypes specifically affect the segregation of achiasmate chromosomes. The identification and characterization of mammalian homologs of Drosophila genes that function in achiasmate segregation (*i.e.*, Toaki *et al.* 1996) will likely lead to the direct genetic identification and characterization of an achiasmate system, if such a system exists.

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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.

Ashburner, M., 1989 *Drosophila*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Bailey, N. T. J., 1961 *Introduction to the Mathematical Theory of Genetic Linkage*. Oxford University Press, London.

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.

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., 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.

Cooper, K. W., 1945 Normal segregation without chiasmata in female *Drosophila melanogaster*. Genetics **30:** 472–484.

Darlington, C. D., 1932 *Recent Advances in Cytology*. The Blakiston Co., Philadelphia.

Dawson, D. S., A. W. Murray and J. W. Szostak, 1986 An alternative pathway for meiotic chromosome segregation in yeast. Science **234:** 713–717.

Grell, R. F., 1962 A new hypothesis on the nature and sequence of meiotic events in the female of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **48:** 1737–1754.

Grell, R. F., 1976 Distributive pairing, pp. 435–486 in *Genetics and Biology of Drosophila*, edited by E. Novitski and M. Ashburner. Academic Press, New York.

Guacci, V., and D. B. Kaback, 1991 Distributive disjunction of authentic chromosomes in Saccharomyces cerevisiae. Genetics **127:** 475–488.

Haldane, J. B. S., 1919 The combination of linkage values, and the calculation of distances between the loci of linked factors. J. Genet. **8:** 299–309.

Hassold, T., M. Abruzzo, K. Adkins, D. Griffin, M. Merrill *et al.*, 1996 Human aneuploidy: incidence, origin, and etiology. Environ. Mol. Mutagen. **28:** 167–175.

Hawley, R. S., 1988 Exchange and chromosome segregation in eukaryotes, pp. 497–525 in *Genetic Recombination*, edited by R. Kucherlapati and G. Smith. American Society for Microbiology, Washington, DC.

Hawley, R. S., and W. E. Theurkauf, 1993 Requiem for distributive segregation: achiasmate segregation in Drosophila females. Trends Genet. **9:** 310–317.

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.

Hawley, R. S., K. S. McKim and T. Arbel, 1993 Meiotic segregation in *Drosophila melanogaster* females: molecules, mechanisms, and myths. Annu. Rev. Genet. **27:** 281–317.

Hollocher, H., C. T. Ting, M. L. Wu and C. I. Wu, 1997 Incipient speciation by sexual isolation in Drosophila melanogaster: extensive genetic divergence without reinforcement. Genetics **147:** 1191–1201.

Koehler, K. E., C. L. Boulton, H. E. Collins, R. L. French, K. C. Herman *et al.*, 1996 Spontaneous X chromosome MI and MII nondisjunction events in *Drosophila melanogaster* oocytes have different recombinational histories. Nat. Genet. **14:** 406–414.

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.*, 1997a Characterization of susceptible chiasma configurations that increase the risk for maternal nondisjunction of chromosome 21. Hum. Mol. Genet. **6:** 1391–1399.

Lamb, N. E., E. Feingold and S. L. Sherman, 1997b Estimating meiotic exchange patterns from recombination data: an application to humans. Genetics **146:** 1011–1017.

Lindsley, D. L., and G. C. Zimm, 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego.

Loidl, J., H. Scherthan and D. B. Kaback, 1994 Physical association between nonhomologous chromosomes precedes distributive disjunction in yeast. Proc. Natl. Acad. Sci. USA **91:** 331–334.

Lucchesi, J. C., 1975 Interchromosomal effects, pp. 313–329 in *The Genetics and Biology of Drosophila*, edited by M. Ashburner and E. Novitski. Academic Press, London.

McPeek, M. S., and T. P. Speed, 1995 Modeling interference in genetic recombination. Genetics **139:** 1031–1044.

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. T., M. Aguade and C. H. Langley, 1993 Linkage disequilibrium in the *white* locus region of *Drosophila melanogaster*. Genet. Res. **62:** 101–109.

Morton, N. E., and C. J. MacLean, 1984 Multilocus recombination frequencies. Genet. Res. **44:** 99–108.

Muller, H. J., 1916 The mechanism of crossing over. Am. Nat. **50:** 193–221, 284–305, 350–366, 421–434.

Nicklas, R. B., 1977 Chromosome distribution: experiments on cell hybrids and *in vitro*. Philos. Trans. R. Soc. London B Biol. Sci. **277:** 267–276.

Press, W. H., S. A. Teukolsky, W. T. Vetterling and B. P. Flannery, 1992 *Numerical Recipes in C*. Cambridge University Press, Cambridge, United Kingdom.

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.

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.

Rutherford, S. L., and A. T. C. Carpenter, 1988 The effect of sequence homozygosity on the frequency of *X*-chromosomal exchange in *Drosophila melanogaster* females. Genetics **120:** 725–732.

Sandler, L., D. L. Lindsley, B. Nicoletti and G. Trippa, 1968 Mutants affecting meiosis in natural populations of Drosophila melanogaster. Genetics **60:** 525–558.

Snow, R., 1979 Maximum likelihood estimation of linkage and interference from tetrad data. Genetics **92:** 231–245.

Sturtevant, A. H., 1915 The behavior of chromosomes as studied through linkage. Z. Indukt. Abstammungs. Vererbugsl. **13:** 234–287.

Sturtevant, A. H., 1919 Contributions to the genetics of Drosophila melanogaster. III. Inherited linkage variations in the second chromosome. Carnegie Inst. Wash. Publ. **278:** 305–341.

Sturtevant, A. H., and G. W. Beadle, 1936 The relations of inver-

sions in the *X* chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21:** 554–604.

Suzuki, D. T., D. Baillie and D. Parry, 1966 The origin of multiple crossover chromoatids in short genetic intervals in *Drosophila melanogaster*. Genetics **54:** 1359–1370.

Toaki, N., A. Fujimoto-Nishiyama, Y. Toyoshima, S. Yonemura, S. Tsukita *et al.*, 1996 Kid, a novel kinesin-like DNA binding protein, is localized to chromosomes and the mitotic spindle. EMBO J. **15:** 457–467.

Weinstein, A., 1936 The theory of multiple-strand crossing over. Genetics **21:** 155–199.

Weinstein, A., 1955 Unraveling the chromosomes. J. Cell. Comp. Physiol. **45:** 249–269.

Weinstein, A., 1958 The geometry and mechanics of crossing over. Cold Spring Harbor Symposia Quant. Biol. **XXIII:** 177–196.

Whyte, W. L., H. Irick, T. Arbel, G. Yasuda, R. L. French *et al.*, 1993 The genetic analysis of achiasmate segregation in *Drosophila melanogaster*. III. The wild-type product of the Axs gene is required for the meiotic segregation of achiasmate homologs. Genetics **134:** 825–835.

Wu, C. I., H. Hollocher, D. J. Begun, C. F. Aquadro, Y. Xu *et al.*, 1995 Sexual isolation in Drosophila melanogaster: a possible case of incipient speciation. Proc. Natl. Acad. Sci. USA **92:** 2519–2523.

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.

Zhao, H., and T. P. Speed, 1996 On genetic map functions. Genetics **142:** 1369–1377.

Zhao, H., and T. P. Speed, 1998a Statistical analysis of half-tetrads. Genetics **150:** 473–485.

Zhao, H., and T. P. Speed, 1998b Statistical Analysis of Ordered Tetrads. Genetics **150:** 459–472.

Zhao, H., M. S. McPeek and T. P. Speed, 1995a Statistical analysis of chromatid interference. Genetics **139:** 1057–1065.

Zhao, H., T. P. Speed and M. S. McPeek, 1995b Statistical analysis of crossover interference using the chi-square model. Genetics **139:** 1045–1056.

Zwick, M. E., J. L. Salstrom and C. H. Langley, 1999 Genetic variation rates of nondisjunction: association of two naturally occurring polymorphisms in the chromokinesin *nod* with increased rates of nondisjunction in *Drosophila melanogaster*. Genetics **152:** 1605–1614.

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APPENDIX A: *Drosophila melanogaster* RAW DATA

	North American X Chromosomes																		African X Chromosomes																	
	NC6X			NC7X			NC11X			NC12X			NC14X			NC19X			NC20X			NC29X			NC50X			5EX			15EX			31EX			32EX			34EX			36EX		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
y cv v f car , male	399	463	447	267	439	284	178	370	225	504	592	515	392	469	412	277	392	252	356	439	318	394	576	421	519	604	498	34	87	45	58	60	24	85	369	235	47	116	58	239	206	132	322	374	273
y cv v f car , female	390	485	426	237	421	289	153	344	241	522	602	609	494	468	435	242	427	252	356	401	348	407	568	514	535	552	495	43	82	29	67	74	21	93	374	255	43	104	64	230	214	100	339	343	319
+++++ , male	514	550	512	258	493	335	171	425	237	537	595	567	475	502	459	307	482	271	399	462	350	445	627	566	570	697	650	43	85	49	76	65	30	98	352	272	105	173	106	230	254	144	352	335	317
+++++ , female	541	601	462	255	495	333	183	447	279	640	659	580	496	489	464	267	440	270	461	518	405	461	711	549	611	694	563	46	85	51	75	75	20	112	435	283	87	141	115	271	238	148	390	412	351
y ++++ , male	107	131	119	57	82	83	33	63	41	75	84	98	88	81	83	47	80	49	69	72	60	64	94	95	93	111	121	5	15	10	12	10	5	7	72	57	7	17	25	35	43	22	39	65	63
y ++++ , female	106	133	124	55	88	76	33	82	55	82	122	121	77	91	94	50	72	62	51	97	54	67	86	113	133	122	136	8	13	9	7	7	6	23	81	68	6	23	11	31	31	28	46	55	57
+ cv v f car , male	105	102	98	37	95	68	32	75	38	81	88	94	84	84	81	47	68	57	55	73	53	71	89	82	116	113	120	7	10	7	12	7	4	11	81	53	9	29	19	31	37	36	59	51	70
+ cv v f car , female	94	125	124	61	89	67	35	63	54	75	103	90	74	86	103	52	83	47	60	77	57	69	81	90	123	121	126	8	11	7	11	13	7	24	91	48	7	20	9	27	36	28	53	57	56
y cv +++ , male	164	198	193	85	154	125	44	119	77	113	150	178	155	138	157	73	118	90	113	142	107	144	202	174	183	231	194	18	27	16	21	16	8	30	117	95	16	43	26	70	78	47	85	87	79
y cv +++ , female	184	196	181	101	154	122	47	119	89	132	176	172	160	175	154	67	132	95	122	136	119	136	200	188	207	202	216	18	31	14	19	21	9	37	135	116	14	41	18	69	81	46	90	95	101
++ v f car , male	176	191	181	89	159	114	43	123	89	150	178	173	150	146	138	71	140	92	137	149	128	148	181	174	215	217	188	17	26	19	37	10	14	33	151	127	16	39	21	72	83	48	88	103	80
++ v f car , female	168	192	175	78	185	137	52	139	65	137	174	189	138	138	137	81	123	94	118	166	135	156	213	183	209	222	211	11	34	20	16	12	7	38	132	101	18	52	20	68	85	53	73	108	98
y cv v ++ , male	116	141	112	85	110	79	53	119	69	133	157	149	139	165	116	66	96	74	96	124	84	131	178	136	184	141	132	4	17	20	21	19	3	28	83	71	13	26	10	68	56	35	92	84	68
y cv v ++ , female	161	141	131	73	115	88	46	111	82	155	204	185	120	146	138	80	112	58	94	104	96	121	169	152	157	156	145	10	23	8	25	16	10	29	95	68	15	36	11	58	69	49	82	79	76
+++ f car , male	141	161	140	76	133	85	44	118	88	186	201	167	144	116	110	83	127	75	101	145	109	128	168	148	159	178	151	8	17	14	18	21	4	22	102	85	16	40	11	61	85	39	89	96	74
+++ f car , female	145	152	139	73	110	107	61	124	78	193	191	177	122	134	146	73	115	89	126	148	110	128	181	157	151	208	163	9	24	8	10	21	8	33	121	95	15	36	23	66	63	48	88	100	98
y cv v f + , male	27	34	27	25	34	22	10	32	22	59	37	33	28	36	15	9	29	10	18	27	29	35	33	34	32	37	24	4	4	4	5	3	2	2	22	10	3	7	1	15	16	4	28	29	17
y cv v f + , female	43	33	26	13	39	22	14	28	15	39	46	39	38	25	45	13	22	13	27	30	11	33	32	34	32	31	44	2	7	0	5	9	1	6	16	11	5	11	5	18	12	13	23	37	19
++ + f car , male	39	50	31	17	30	28	14	29	19	40	53	37	35	36	30	16	22	12	27	38	30	20	24	24	30	43	32	0	4	5	1	2	1	5	23	17	3	10	3	20	12	2	29	29	23
++ + f car , female	38	35	25	16	21	21	13	39	29	36	43	51	35	34	37	17	16	10	31	46	19	29	42	24	45	48	30	2	3	2	2	4	1	5	42	13	0	9	5	20	21	7	24	23	24
y + v f car , male	7	11	3	2	0	1	1	2	2	4	0	2	5	1	1	2	5	1	0	1	1	3	1	1	1	1	1	0	1	0	1	2	0	1	2	4	0	1	0	0	0	0	1	1	1
y + v f car , female	7	4	7	3	1	1	0	1	2	0	6	3	1	0	2	3	0	1	3	2	1	0	1	3	1	5	5	0	0	0	0	0	0	1	2	4	0	1	0	0	0	1	1	2	2
+ cv +++ , male	6	4	2	3	3	1	0	1	2	0	4	0	2	0	0	0	3	2	2	0	2	1	2	0	7	4	4	1	2	0	0	0	0	1	5	1	0	2	0	0	0	1	2	1	0
+ cv +++ , female	3	4	0	4	3	1	3	2	2	3	1	2	0	3	5	0	5	2	4	0	0	2	5	6	6	2	7	0	1	0	0	2	0	0	4	2	0	2	1	0	0	2	1	2	1
y ++ f car , male	30	21	28	8	15	16	3	17	7	16	17	16	11	12	8	7	3	19	15	16	6	12	11	19	16	17	14	0	3	1	0	2	2	7	13	9	3	3	2	2	6	6	9	10	14
y ++ f car , female	19	20	32	7	9	15	6	11	9	16	20	18	7	8	10	5	8	9	13	13	10	9	15	12	14	20	15	0	4	5	1	2	1	2	13	10	2	4	3	2	9	3	4	7	7
+ cv v ++ , male	18	33	28	7	16	11	10	10	7	15	14	14	9	9	7	6	3	4	7	14	7	11	10	15	13	15	11	2	1	0	3	1	1	3	7	8	2	9	2	5	4	1	10	4	11
+ cv v ++ , female	29	30	32	6	12	20	8	14	10	14	14	23	13	13	13	8	10	5	11	11	8	12	14	17	16	12	14	1	1	0	2	0	1	2	10	19	2	8	3	4	6	5	7	6	5
y + v f car , male	11	9	11	2	5	5	1	7	4	7	8	9	5	1	5	4	3	2	3	5	7	6	2	1	5	5	4	1	2	0	0	1	0	0	1	9	1	2	0	2	1	3	6	5	3
y + v f car , female	5	8	9	5	8	4	3	1	4	6	10	6	6	3	7	1	2	0	3	4	5	5	4	4	4	6	3	0	2	0	1	6	3	0	1	1	1	2	0	1	1	1	2	3	4
+ cv v f + , male	7	6	8	2	3	4	1	2	5	4	2	2	3	5	1	0	1	0	5	6	3	3	2	8	6	2	8	1	2	0	0	3	5	0	1	1	0	2	1	1	1	1	6	2	2
+ cv v f + , female	8	6	11	3	5	6	1	8	2	5	7	6	3	5	5	0	3	2	5	5	5	0	6	3	7	5	8	0	2	0	0	2	3	1	1	0	0	1	1	1	1	1	1	3	2
y cv + f car , male	10	21	14	2	9	11	4	8	4	8	9	12	5	7	4	3	11	4	11	12	14	8	18	15	2	2	0	0	1	2	2	14	9	2	2	5	3	4	5	5	8	8	9	9	8
y cv + f car , female	21	19	19	2	8	6	1	10	13	9	10	7	7	15	8	7	3	4	8	10	6	8	9	15	14	8	8	0	1	0	1	8	7	0	3	3	3	6	8	4	9	9	8	7	6
++ v v ++ , male	19	11	15	10	12	9	1	9	1	8	11	8	4	2	11	4	10	6	6	8	8	10	12	11	10	7	11	4	1	3	0	1	2	1	11	5	1	3	1	7	5	4	3	5	4
++ v v ++ , female	14	17	14	8	18	14	2	10	8	18	14	11	6	11	6	2	4	7	7	9	5	7	13	21	19	15	11	2	1	0	1	0	1	1	9	9	1	4	1	4	4	5	5	6	5
y cv ++ car , male	3	9	7	2	5	5	1	2	2	5	9	8	4	3	4	1	2	3	5	5	5	4	6	6	6	8	7	1	0	0	1	0	1	2	1	2	1	3	2	5	1	2	3	3	1
y cv ++ car , female	8	10	10	6	3	4	4	6	8	8	8	7	3	4	3	0	12	4	3	6	5	3	6	3	4	6	5	1	2	1	0	1	0	1	3	2	1	4	1	1	1	2	5	5	6
++ v f + , male	12	6	8	8	6	4	3	6	4	8	9	9	3	5	5	3	1	1	9	9	6	5	9	10	10	10	5	0	1	1	1	0	0	0	3	1	0	3	1	1	3	2	7	4	2
++ v f + , female	9	14	8	7	2	4	4	2	3	3	6	7	12	10	5	3	2	3	4	7	4	5	7	7	7	9	5	1	0	1	0	0	0	1	6	3	0	3	2	2	1	1	3	5	4
y cv v + car , male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
y cv v + car , female	0	2	0	0	0	1	0	0	2	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0
+ + v f + , male	1	1	4	1	3	0	0	1	1	4	3	0	1	0	0	0	2	0	3	1	2	1	1	0	3	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	1	1	0	2	0
+ + v f + , female	2	0	1	0	1	1	0	1	1	4	2	1	2	1	0	0	0	0	3	0	1	0	2	0	4	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
y + v ++ , male	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
y + v ++ , female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
+ cv + f car , male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
+ cv + f car , female	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
y + v + car , male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
y + v + car , female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
+ cv v f + , male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
+ cv v f + , female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
++ v v car , male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
++ v v car , female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
y + v v car , male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
y + v v car , female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
+ cv + f + , male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
+ cv + f + , female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX B: Viability parameter values and *P* values for best-supported viability model

	North American *X* Chromosomes																													
	NC6X		NC7X		NC11X		NC19X		NC20X		NC29X		NC50X		NC12X										NC14X					
Best Supported Viability Model	Single		Single		Single		Single		Single		Single		Single		Full										Full					
P value (Single vs. No Viability)	1.0×10^{-6}		6.03×10^{-4}		1.4×10^{-3}		2.9×10^{-3}		1.0×10^{-6}		1.0×10^{-6}		1.0×10^{-6}		3.7×10^{-4}										0.02					
P value (Full vs. No Viability)	1.0×10^{-6}		6.3×10^{-3}		1.3×10^{-2}		0.01		1.0×10^{-6}		1.0×10^{-6}		1.0×10^{-6}		1.1×10^{-5}										2.4×10^{-3}					
P value (Full vs. Single)	0.07		0.7		0.74		0.5		0.8		0.11		0.8		2.5×10^{-3}										0.02					
y viability (C.I.)	0.96	(0.89, 1.03)	0.97	(0.89, 1.06)	0.92	(0.83, 1.02)	0.96	(0.89, 1.03)	0.96	(0.89, 1.03)	0.96	(0.89, 1.03)	0.96	(0.89, 1.03)	0.96	(0.86, 1.07)	(Male)	1.06	(0.95, 1.18)	(Female)	0.98	(0.87, 1.09)	(Male)	0.98	(0.87, 1.07)	(Female)				
cv viability (C.I.)	0.96	(0.88, 1.04)	0.95	(0.86, 1.05)	1.01	(0.89, 1.14)	0.96	(0.88, 1.04)	0.96	(0.88, 1.04)	0.96	(0.88, 1.04)	0.96	(0.88, 1.04)	0.94	(0.83, 1.06)	(Male)	0.91	(0.80, 1.02)	(Female)	0.99	(0.86, 1.13)	(Male)	1.10	(0.97, 1.25)	(Female)				
v viability (C.I.)	0.93	(0.87, 1.02)	0.99	(0.90, 1.08)	0.96	(0.87, 1.07)	0.93	(0.87, 1.02)	0.93	(0.87, 1.02)	0.93	(0.87, 1.02)	0.93	(0.87, 1.02)	0.93	(0.83, 1.03)	(Male)	1.03	(0.93, 1.14)	(Female)	1.01	(0.90, 1.13)	(Male)	0.93	(0.83, 1.05)	(Female)				
f viability (C.I.)	1.04	(0.87, 1.12)	1.09	(0.95, 1.26)	0.98	(0.84, 1.14)	1.04	(0.87, 1.12)	1.04	(0.87, 1.12)	1.04	(0.87, 1.12)	1.04	(0.87, 1.12)	1.09	(0.92, 1.27)	(Male)	0.97	(0.82, 1.14)	(Female)	0.87	(0.72, 1.06)	(Male)	1.08	(0.89, 1.29)	(Female)				
car viability (C.I.)	0.92	(0.86, 1.08)	0.88	(0.78, 1.01)	1.00	(0.87, 1.15)	0.92	(0.86, 1.08)	0.92	(0.86, 1.08)	0.92	(0.86, 1.08)	0.92	(0.86, 1.08)	0.99	(0.86, 1.14)	(Male)	1.02	(0.89, 1.18)	(Female)	1.05	(0.89, 1.23)	(Male)	0.91	(0.77, 1.08)	(Female)				

	African *X* Chromosomes													
	5EX 1		15EX 2		31EX 3		32EX 1		34EX 2		36EX 3			
Best Supported Viability Model	No Viability		No Viability		Single		Single		No Viability		Single			
P value (Single vs. No Viability)	0.06		0.7		1.0×10^{-4}		1.0×10^{-6}		0.06		0.02			
P value (Full vs. No Viability)	0.17		0.19		1.5×10^{-4}		1.0×10^{-6}		0.35		0.11			
P value (Full vs. Single)	0.62		0.06		0.12		0.38		0.996		0.82			
y viability (C.I.)	-		-		0.95	(0.86, 1.05)	0.76	(0.64, 0.91)	-		0.96	(0.87, 1.05)		
cv viability (C.I.)	-		-		0.95	(0.86, 1.07)	1.03	(0.83, 1.27)	-		1.00	(0.86, 1.13)		
v viability (C.I.)	-		-		0.94	(0.85, 1.05)	0.87	(0.72, 1.05)	-		0.92	(0.83, 1.03)		
f viability (C.I.)	-		-		0.94	(0.78, 1.13)	1.04	(0.76, 1.41)	-		1.07	(0.92, 1.24)		
car viability (C.I.)	-		-		1.12	(0.94, 1.34)	0.86	(0.65, 1.13)	-		0.98	(0.86, 1.12)		