

Estimating Meiotic Exchange Patterns From Recombination Data: An Application to Humans

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ABSTRACT

We present analytical methods to estimate the recombinational history of chromosomes in a human population. Our analysis, similar to those utilized in *Drosophila*, can be used to construct meiotic maps based upon crossover frequencies observed in family data. We apply this method of exchange estimation to a population of paternally and maternally inherited chromosomes 21. The patterns of chromosomal exchange estimated by this type of analysis are comparable to those obtained by the more technically difficult method of cytologically counting chiasmata among human male meiotic events (sperm). This type of analysis can be applied to both male and female meiosis, circumventing many technical problems inherent to cytological counting. Moreover, the distribution of exchange locations along a chromosome for each exchange type (*i.e.*, single, double, or triple exchanges) can be examined individually, an advantage compared to examination of genetic maps that only provide a summary of these distributions. We discuss how this analysis can be used to examine various assumptions concerning meiotic exchange in humans and investigate properties of the analysis that contribute to the accuracy of the results.

WEINSTEIN (1936) presented mathematical methods for inferring the frequency of tetrads of various ranks (*i.e.*, zero, single, multiple exchanges) in a population of *Drosophila* from the observed number of recombinants recovered from chromatids. He used this analysis to investigate questions about meiotic exchange, including the presence or absence of sister-chromatid exchange and the random assortment of strands into gametes. Subsequently, this method has been utilized to analyze both disjoined and nondisjoined chromosome populations from specific *Drosophila* matings (*e.g.*, CHARLES 1938; MERRIAM and FROST 1964; KOEHLER *et al.* 1996).

Our purpose is to investigate the usefulness of these methods for the analysis of human chromosomes. Examination of exchange distribution in humans is important to understand both the normal process of recombination as well as the abnormal processes that may lead to aneusomy. For example, it has long been assumed that in humans at least one exchange is necessary to ensure proper chromosome disjunction. In many organisms, achiasmate bivalents dissociate into univalents before metaphase I, leading to increased rates of meiosis I nondisjunction (reviewed in BAKER and HALL 1976). However, secondary segregation systems have been described for *Drosophila* (GRELL 1976; DERNBURG *et al.* 1996) that, to some extent, promote normal disjunction in the absence of exchange. It is not known if such an achiasmate backup system exists for humans. The identification of achiasmate bivalents

in the exchange pattern of a population of normally disjoined chromosomes may suggest the presence of such a system. On the other hand, the absence of achiasmate bivalents, while unable to rule out a secondary segregation system, would not support its existence.

In addition, this analysis allows exchange distributions to be estimated for both sexes. Genetic maps have shown that recombination differs significantly between males and females. In males, extensive exchange patterns have been cytologically determined using spermatocytes obtained from testicular biopsy (HULTEN 1974; LAURIE *et al.* 1981; LAURIE and HULTEN 1985; HULTEN *et al.* 1990). There are no comparable cytological observations on chiasma distributions in the human female, mainly due to technical problems in obtaining appropriately staged oocyte material for study. Our analysis circumvents these difficulties and, to the best of our knowledge, represents the first method for generating chiasma distributions for the human female.

We apply this method of exchange estimation to a population of maternally and paternally inherited chromosomes 21. Such methods provide estimates of chromosomal exchange comparable to those obtained from cytological observations and therefore are useful in studies of human chromosomal exchange. We discuss how this method can be used to examine assumptions concerning meiotic exchange and investigate factors that contribute to the accuracy of the results.

MATERIALS AND METHODS

Assumptions of exchange model: The observed frequencies, q , of each recombination type of interest (described

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below) were used to estimate frequencies, p , of each exchange type. Four major assumptions were made to estimate tetrad exchange based upon single chromatids: (1) no appreciable crossing over between sister chromatids, (2) the segregation of crossover strands into the gametes is a random event, (3) the viability of all possible crossover products is equal, and (4) there is no chromatid interference. This last assumption implies that (1) any two chromatids of a tetrad are equally likely to undergo exchange, and (2) the two chromatids that crossover at one location do not determine which chromatids undergo exchange at an adjacent location.

Estimation of exchange frequencies: As previously mentioned, this work was initiated to investigate the usefulness of applying WEINSTEIN's 1936 tetrad analysis to populations of human chromosomes. As done by WEINSTEIN, we assigned a probability distribution to the outcomes of each meiotic exchange event. For example, tetrads that lack exchanges yield only noncrossover chromatids. Single exchange tetrads yield single crossover strands $1/2$ of the time and noncrossover strands the other $1/2$ of the time. For a double exchange tetrad, the chance of obtaining a double crossover chromatid is $1/4$, the chance of obtaining a single crossover chromatid is $1/2$, and the chance of observing a noncrossover chromatid is $1/4$. In general, the chance of observing k crossovers in a chromatid obtained from a tetrad with n exchanges is $q = (1/2)^n \binom{n}{k}$. This calculation can take into account the locations of the exchanges in the following manner. The chromosome is divided into several intervals, and it is assumed that there is no more than one exchange per interval. The intervals need not be the same length; they need only to be short enough in genetic distance to justify the assumption of no more than one exchange. An exchange in any given interval has a 50% chance of being detected, independently of events in the other intervals. Thus, if there are n exchanges, each in a different interval, there are 2^n types of chromatids that can be observed, each with equal probability. Specifically, there are $\binom{n}{0} = 1$ type of noncrossover chromatid, $\binom{n}{1} = n$ types of single crossover chromatids, . . . , and $\binom{n}{n} = 1$ type of n -crossover chromatid. For example, a triple exchange in regions 1, 5, and 8 can give rise to $2^3 = 8$ different products of meiosis, determined by which two of the four strands are involved in each exchange. The eight products are as follows: a triple crossover in regions 1, 5, and 8; a double crossover in regions 1 and 5, regions 5 and 8, or regions 1 and 8; a single crossover in region 1, region 5, or region 8; and a strand with no observable crossovers. Each of these resulting products occurs with a frequency of $1/8$.

Let q_i be the frequency in the population of chromatids of crossover type i . Then \hat{q}_i , the observed frequency in the sample, is the maximum likelihood estimate of q_i . The distributions described above give equations relating the observed recombinant frequencies (\hat{q}_i) and the estimated exchange frequencies (\hat{p}_i). The equations (from the law of total probability) are $\hat{q}_i = \sum \hat{p}_j$ and $P(\text{recombination type } i | \text{exchange type } j)$. The maximum likelihood estimates, \hat{p}_i , of the p 's can be obtained by solving these equations, using the invariance property of maximum likelihood estimation. The following example illustrates this procedure. Suppose we divide the chromosome into just two intervals and observe $q_{0,0}$ = frequency of nonrecombinants, $q_{1,0}$ = frequency of chromatids with crossovers in the first interval but not the second, $q_{0,1}$ = frequency of chromatids with crossovers in the second interval but not the first, and $q_{1,1}$ = frequency of double recombinants. The equations relating p 's and q 's are as follows:

$$\begin{aligned} q_{0,0} &= p_{0,0} + 1/2 p_{1,0} + 1/2 p_{0,1} + 1/4 p_{1,1} \\ q_{1,0} &= 1/2 p_{1,0} + 1/4 p_{1,1} \\ q_{0,1} &= 1/2 p_{0,1} + 1/4 p_{1,1} \\ q_{1,1} &= 1/4 p_{1,1} \end{aligned}$$

Solving these gives estimates of the frequencies of each exchange type:

$$\begin{aligned} \hat{p}_{0,0} &= \hat{q}_{0,0} - \hat{q}_{1,0} - \hat{q}_{0,1} + \hat{q}_{1,1} \\ \hat{p}_{1,0} &= 2(\hat{q}_{1,0} - \hat{q}_{1,1}) \\ \hat{p}_{0,1} &= 2(\hat{q}_{0,1} - \hat{q}_{1,1}) \\ \hat{p}_{1,1} &= 4\hat{q}_{1,1} \end{aligned}$$

In general, if all exchange types are allowed and d intervals are used, there will be $2d$ equations to solve. These are simple sets of linear equations that can easily be solved by standard software. However, in practice it is unnecessary to include all $2d$ types. If a maximum of m crossovers has been observed in the data, it is only necessary to use the equations involving that number or fewer, giving a total number of equations of $\binom{d}{0} + \binom{d}{1} + \dots + \binom{d}{m}$. This reduction in the number of equations, in combination with the many symmetries, allows the equations to be solved by hand for most cases.

Statistical methods: WEINSTEIN's method of estimating exchange frequencies, described above, is most appropriate when sample sizes are large. With human data, sample sizes will generally be smaller, and his estimation method can yield estimates that are less than zero or greater than one. Maximum likelihood estimates of the exchange class frequencies that are constrained to the $[0,1]$ interval can be obtained instead by the EM algorithm. However, we recommend that both constrained and unconstrained estimates be calculated in most cases. Reliance on the constrained estimates alone can disguise large sampling errors or deviations from model assumptions. A further issue is that the values one gets for the constrained estimates depend on what one specifies as the maximum possible number of crossovers. In this article, we present only unconstrained estimates, as we are interested in demonstrating clearly the amount of random error involved.

Given the small sample sizes available for human data, it is important to quantify the accuracy of the exchange frequency estimates. To our knowledge, there is very little literature on this topic. WEINSTEIN (1936) suggested a very approximate method, based on the delta method, of calculating standard errors for the exchange class frequencies. CHARLES (1957) attempted to refine this, but his method is applicable only to certain problems, and, in addition, is not adequately theoretically justified. We assessed the accuracy of our estimates by calculating exact standard errors based on the fact that the estimates are functions of multinomial random variables. An example of this type of calculation is given in the APPENDIX. (This is similar in spirit to the method proposed by WEINSTEIN, but is exact rather than approximate.) We then constructed approximate confidence intervals assuming asymptotic normality of the estimates. Our confidence intervals are crude; they are neither simultaneous nor independent, and they are symmetrical and thus include negative numbers. In theory it is possible to refine them. A perhaps more promising approach for the future is to compute bootstrap or permutation standard errors, though there may be some technical or theoretical questions about the correct way to do so.

Methods for testing hypotheses with this type of data are also an open question. One might, for example, wish to test the null hypothesis that the frequency of the achiasmate class is zero. Permutation or bootstrap methods are probably most appropriate to this type of test. In some cases it might also make sense to calculate a likelihood ratio and determine its null hypothesis distribution by simulation. (Standard asymptotic methods for likelihoods are not applicable here because of the small sample sizes and the constraints on the estimates). We did not perform any hypothesis tests on our ex-

change frequencies. We were in fact interested in whether the achiasmate class has frequency zero, but looking at that question by hypothesis testing methods would be an exercise in trying to prove the null hypothesis, so we considered an estimation framework (confidence intervals) to be more appropriate.

The standard errors we calculated for the HULTEN *et al.* (1990) data are straightforward, since the frequencies were directly observed in that case. Those calculations do assume that the observations are independent and identically distributed. This is not quite the case because the data were based on multiple sperm counts from a few men. However, the distributions in each man appear similar, so this approximation is probably reasonable.

Data analysis: Genotype data was obtained from the CEPH dataset (WWW address: <http://www.cephb.fr/HomePage.html>). Extensive chromosome 21 marker data were available for 38 families, with an average of 8.02 individuals per family. The recombination status across each chromosome arm was determined with the CHROMPIC option of the mapping program CRIMAP (LANDER *et al.* 1987), assuming the most likely phase. We did not use families whose most likely phase was <0.8 . For our analysis, the chromosome was separated into roughly equal physical intervals. The arms of the chromosome were initially partitioned into tenths, similar to the intervals used in the chiasma counts of HULTEN *et al.* (1990). This, however, resulted in very small (<4 Mb) intervals, many of which were uninformative as they contained few genetic markers. Subsequently we collapsed the number of intervals to five in both our data set and that of HULTEN. An integrated genetic and physical map that provided the estimated physical location (in Mb) for each genetic marker was used to determine which genetic markers lay within each physical interval (LAWRENCE *et al.* 1993). Interval genetic lengths were kept relatively small (30 cM or less) to reduce the possibility of double exchanges occurring within an interval. Although the longest interval was 30 cM, the average genetic length of a chromosome interval was 11.9 cM for males and 17.4 cM for females. According to the Kosambi mapping function, the probabilities of a double exchange in intervals of this length are 0.0011 and 0.0035, respectively.

A chromosome was included in the analysis when at least one marker in each interval was informative. This approach yielded 262 male and 276 female meiotic events. Each interval was examined for the presence or absence of a recombinant event. If a recombinant event was observed, the interval was noted, for example, "1 3 5" indicates a chromatid with recombinant events in the first, third, and fifth intervals. If a recombination event occurred at the junction of two intervals, the recombination was recorded as occurring in both intervals, each with a probability of occurrence of $1/2$. For a chromosome arm divided into 10 roughly equal intervals, 1024 exchange types are possible. However, if only five intervals are considered, this number is reduced to a more manageable 32. Given these data, the frequency of each class of recombinants was determined (Table 1). Once recombination frequencies were known, chromosomal exchange distributions were estimated using the maximum likelihood method described above.

RESULTS

Male exchange events: We initially analyzed 262 paternally inherited chromosomes 21 to study the events of male meiosis. Male meiotic tetrads have previously been extensively characterized by cytological visualization of chromosome 21 chiasma in human spermatocytes (HULTEN *et al.* 1990). These spermatocytes, obtained from testicular biopsy, were subjected to sequential staining to visualize the chromosome tetrads. Tetrads were then projected at ~ 2500 times magnification and the chiasma positions measured. We compared our estimated exchange results with these cytological data to test the accuracy of the analysis. On average, 1.03 [95% confidence interval (CI) = 0.90, 1.16] exchanges per chromosome 21 bivalent were estimated by our method. This value is similar to that obtained by HULTEN *et al.* (1.09, 95% CI = 0.91, 1.27). Additionally, the estimated frequencies of zero, one, and two exchange bivalents mirrored the values observed cytologically (see Table 2).

TABLE 1
Observed recombinant frequency for each recombination class for paternally and maternally inherited chromosomes 21

Recombination type ^a	Male meioses (<i>n</i> = 262)	Female meioses (<i>n</i> = 276)
No recombination	0.492	0.406
Single recombinant	0.500	0.493
Interval location		
1	0.042	0.138
2	0.050	0.087
3	0.051	0.081
4	0.076	0.047
5	0.281	0.140
Double recombinant	0.008	0.101
Interval location		
1 and 3	—	0.019
1 and 4	—	0.013
1 and 5	0.004	0.045
2 and 5	—	0.020
3 and 5	0.004	0.004

^a Number and interval for each recombinant.

Figure 1 shows the percentage of all chiasmata occurring in each interval of the chromosome for both our estimates and the data of HULTEN *et al.* (1990). The overall chiasma distributions obtained by the two methods are comparable. Both show that more than half of the total exchange for these chromosomes occurs in the telomeric region, as previously suggested by the genetic map. A chi-square analysis to compare these two distributions was performed. It showed no statistically significant difference between the two data sets ($\chi^2 = 7.61$; d.f. = 4; $P = 0.11$). (This test was performed as a comparison of our recombination data to HULTEN's exchange data, as the proportions in each interval are the same whether one counts recombinants or exchanges.)

Figure 1 shows the distribution of all chiasmata from both single and double exchange tetrads. Distributions similar to those in Figure 1 can also be computed for single or double exchange tetrads only, as shown in Table 3. However, for the male chromosome 21 analysis

TABLE 2
Estimated exchange frequencies and cytologically observed exchange frequencies for chromosome 21

Exchange type	Male meioses				Female meioses	
	This analysis (n = 262)		HULTEN <i>et al.</i> (1990) (n = 131)		This analysis (n = 276)	
	Estimated exchange	95% CI	Observed exchange	95% CI	Estimated exchange	95% CI
Achiasmate	-0.001	-0.123, 0.121	0.000		0.015	-0.106, 0.135
Single exchange	0.970	0.841, 1.099	0.909	0.860, 0.958	0.580	0.363, 0.797
Double exchange	0.030	-0.013, 0.073	0.091	0.042, 0.140	0.406	0.285, 0.526

this provides little additional information, since nearly all bivalents have only a single exchange. This application is more informative for the female chromosome 21 exchange distribution, as discussed below.

Female exchange events: Because of technical difficulties, it has been impossible to directly observe female exchange events as HULTEN *et al.* (1990) did with male events. In an attempt to understand the behavior of female exchanges, we applied the exchange analysis to a population of 276 maternally inherited chromosomes 21. As expected, we found that female chromosomes undergo more exchange than their male counterparts, 1.39 exchanges per bivalent compared to 1.03 exchanges, respectively (Table 2). Nearly 41% of the maternally inherited chromosomes developed from a double exchange bivalent, 13 times as many as estimated for males. The overall distribution of chiasmata also differed between the sexes, as expected (Figure 1). In female meiosis, chromosome 21 exchanges were estimated to occur near both the centromere and telomere with equal frequency (~30%), unlike the telomeric emphasis of male meiotic events. From the overall distribution, we cannot determine if this attraction for the chromosomal ends is primarily a result of the placement of single exchanges, double exchanges, or both events. Examining single and double exchanges sepa-

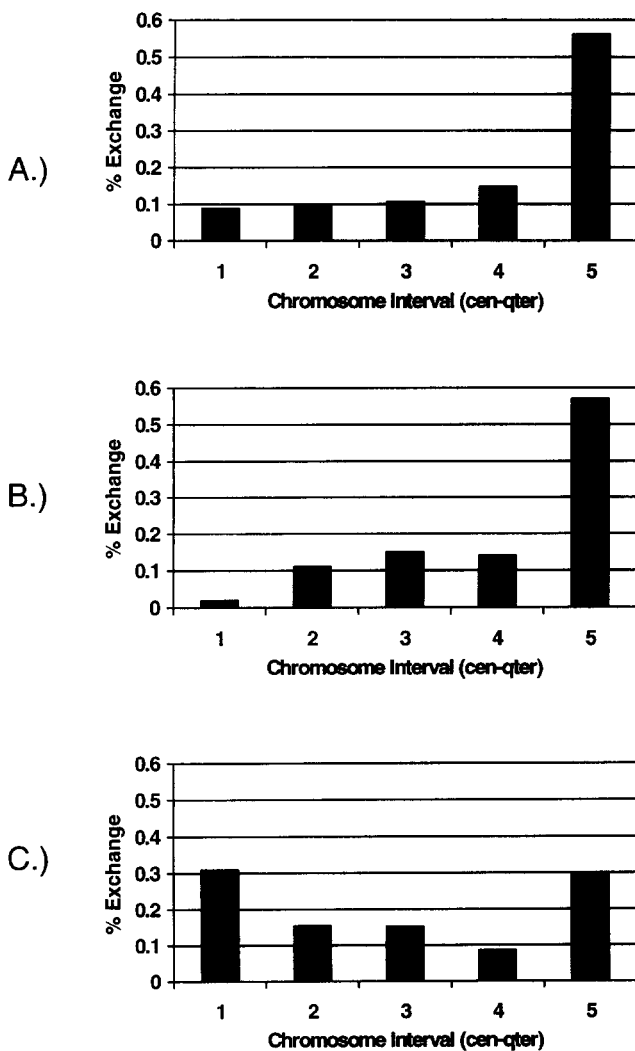


FIGURE 1.—Comparison of chromosome 21 meiotic exchange. Percentage of exchanges in each interval based on estimations from recombination data for paternally inherited chromosomes (A), observed cytological counts in spermatocytes (HULTEN *et al.* 1990) (B), estimations from recombination data for maternally inherited chromosomes (C).

TABLE 3

Estimated exchange frequency for each exchange class for paternally and maternally inherited chromosomes 21

Exchange type ^a	Male meioses (n = 262)	Female meioses (n = 276)
Achiasmate	-0.001	0.015
Single exchange		
Interval location		
1	0.076	0.112
2	0.099	0.134
3	0.095	0.116
4	0.153	0.069
5	0.547	0.141
Double exchange		
Interval location		
1 and 3	0	0.080
1 and 4	0	0.051
1 and 5	0.015	0.181
2 and 5	0	0.080
3 and 5	0.015	0.014

^a Number and interval for each exchange.

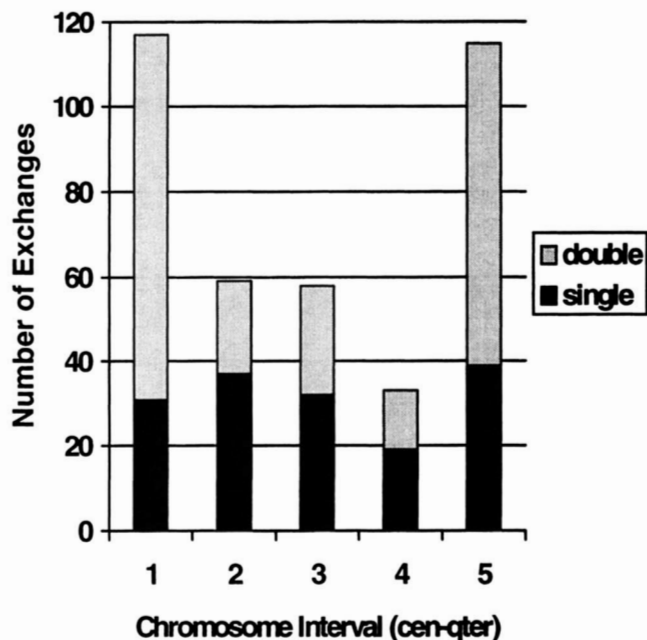


FIGURE 2.—Chromosome 21 estimated female meiotic exchange. The estimated number of meiotic exchanges that involved single (darkly shaded regions) or double (lightly shaded regions) exchange configurations.

rately, however, provides insight into this issue (Figure 2 and Table 3). According to the analysis, single exchange events occur across the chromosome with a relatively equal frequency. The high frequency of centromeric and telomeric exchanges observed in the overall distribution is due to the prevalence of double exchange bivalents with one exchange near the centromere and the other along the most distal region of the chromosome. In other words, the most common double exchange configuration places the two exchanges as far apart as possible as expected based on the observations of positive interference. This demonstrates the additional information this type of analysis is capable of providing. Such a result could not have been inferred from the examination of a genetic map for chromosome 21.

The “0,” or nonexchange, class: It has long been assumed that at least one exchange (the so-called “obligate exchange”) is necessary to ensure proper chromosome disjunction. If this is true, the exchange frequency for the achiasmate class should be zero for a normally disjoining population. On the other hand, backup segregation systems exist in some organisms [such as *Drosophila* (GRELL 1976; DERNBURG *et al.* 1996)] to ensure proper disjunction, even in the absence of meiotic exchange. If such a secondary system were present in humans, achiasmate bivalents might then be observed. Both the male and female chromosome 21 analyses estimated zero or near zero frequencies for the achiasmate class, supporting the concept of obligate exchange and failing to provide evidence for a secondary segregation system in humans.

TABLE 4

Estimated exchange frequencies, standard errors and confidence intervals for chromosome 1

Exchange type	Exchange	Male meioses	
		SE	95% CI
Achiasmate	-0.068	0.106	-0.280, 0.145
Single exchange	0.290	0.479	-0.668, 1.247
Double exchange	-0.273	1.012	-2.296, 1.750
Triple exchange	0.669	1.270	-3.210, 1.871
Quadruple exchange	0.0000	0.983	-1.966, 1.966
Quintuple exchange	0.358	0.357	-3.554, 1.072

Accuracy of assumptions and estimations: The results of our analysis suggest that WEINSTEIN’s mathematical methods for estimating meiotic exchange patterns from genetic recombination maps can be extended to human chromosomes. The similarity between the exchange results estimated by our methods and those observed cytologically support not only our analysis, but the basic assumptions used to generate genetic maps.

The standard errors calculated for chromosome 21 are relatively large, but not so large as to make the study uninteresting. The basic observations of the analysis still stand. For larger chromosomes, however, the standard errors become much larger because the number of exchange types increases. Subsequently, the sample size required for analysis increases. For example, chromosome 1 is ~263 Mb long, five times the size of chromosome 21, and makes up ~8.2% of the total human genome. Extensive genotype data from the CEPH dataset for chromosome 1 was available on only eight families, resulting in a sample size of ~90 chromosomes. Exchange values and their resulting confidence intervals were calculated from the recombination data obtained from this group (Table 4). The confidence intervals are enormous, due both to the small sample size and six classes of exchange types, making it difficult, if not impossible, to accurately identify underlying patterns of meiotic exchange for this chromosome. In the first application of this analysis (WEINSTEIN 1936) large standard errors were not present, due to the large sample sizes used. For example, the estimated proportion of single exchange events derived from the 28,239 *Drosophila* sampled is $q_1 = 0.467$, SE = 0.008. In comparison, the estimated proportion of single exchange events derived from the chromosome 1 sample of 90 is $q_1 = 0.290$, SE = 0.479, a 60-fold increase.

DISCUSSION

We have extended WEINSTEIN’s methods to estimate chiasma distributions based upon observed recombinant events recovered from chromatids and examined its application to the study of human meiosis. The observed crossover frequencies for this analysis can be

calculated from chromosomes of either maternal or paternal origin. Based upon a study of chromosome 21, we have demonstrated that this analysis can be extended to humans. The resulting pattern of chromosomal exchange is comparable to that obtained by the more technically difficult method of cytologically counting chiasmata. Human genetic maps can, then, be successfully transformed into chiasma distributions

There are several strengths of this type of analysis. Most notably, exchange distributions can be estimated for both sexes. To the best of our knowledge, this represents the first method for generating such distributions for the human female. The exchange distributions obtained for female chromosome 21 are notably different from those of the male, as suggested by the variations between their genetic maps. Another strength is the ability of the overall exchange distribution to be separated into its individual parts. A benefit of this method is the ability to separate higher order exchanges from single exchanges and examine each distribution individually. Although genetic maps represent locations and clustering of exchanges, they cannot identify the underlying causes of the observed pattern as they are a composite of exchanges due to single, multiple, or achiasmate tetrads. Our analysis separates the overall distribution and allows the contribution from each exchange type to be individually observed.

The sample size of meiotic events appears to be a critical factor in the analysis, especially when examining large chromosomes. As shown by the results from the chromosome 1 population (Table 4), the sample must be large enough to achieve a reasonable representation for each recombination class. The presence of higher-order tetrad exchanges, such as quadruple and quintuple exchanges, will otherwise skew the exchange results. In addition, the number of genetic markers used on the population must also be of appropriate size to ensure that all regions of the chromosome are represented.

These methods can additionally be extended to examine populations arising from abnormal meiotic events, such as nondisjunction. In these cases, two of the four tetrad strands are available for study. Accordingly, the equations that transform the recombination frequencies to exchange values must be modified. These modifications will differ for each exchange class depending upon the origin of the nondisjunction error. Errors arising at meiosis I produce progeny with chromatids from each of the homologues while errors of meiosis II origin yield progeny with both sister chromatids from the same homologue. It is a relatively simple procedure to modify these equations to take this into account. We are currently employing these techniques to examine the estimated exchange patterns for a population of over 300 individuals with chromosome 21 nondisjunction. Such an analysis should yield new insight

into the types and patterns of meiotic exchange found both in normal and abnormal meiotic events.

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APPENDIX

Example standard error calculation: For the example given in MATERIALS AND METHODS of a chromosome divided into two intervals, we gave the following estimates of exchange probabilities:

$$\hat{p}_{1,1} = 4\hat{q}_{1,1}$$

$$\hat{p}_{0,1} = 2(\hat{q}_{0,1} - \hat{q}_{1,1})$$

$$\hat{p}_{1,0} = 2(\hat{q}_{1,0} - \hat{q}_{1,1})$$

$$\hat{p}_{0,0} = \hat{q}_{0,0} - \hat{q}_{1,0} - \hat{q}_{0,1} + \hat{q}_{0,1}$$

Let $n_{0,0}$, $n_{1,0}$, $n_{0,1}$, $n_{1,1}$ be the number of chromosomes observed of each exchange type, so that $\hat{q}_i = n_i/n$, where $n = n_{0,0} + n_{1,0} + n_{0,1} + n_{1,1}$. The standard errors of these estimates are calculated as follows, using standard properties of the multinomial distribution.

$$SE(\hat{p}_{1,1}) = \sqrt{\text{var}(\hat{p}_{1,1})} = 4\sqrt{\text{var}(\hat{q}_{1,1})} \approx 4\sqrt{\frac{\hat{q}_{1,1}(1 - \hat{q}_{1,1})}{n}}$$

$$\begin{aligned} SE(\hat{p}_{0,1}) &= \sqrt{\text{var}(\hat{p}_{0,1})} \\ &= 2\sqrt{\text{var}(\hat{q}_{0,1}) + \text{var}(\hat{q}_{1,1}) + 2 \text{cov}(\hat{q}_{0,1}, \hat{q}_{1,1})} \\ &\approx 2\sqrt{\frac{\hat{q}_{0,1}(1 - \hat{q}_{0,1})}{n} + \frac{\hat{q}_{1,1}(1 - \hat{q}_{1,1})}{n} + \frac{2(\hat{q}_{0,1}\hat{q}_{1,1})}{n}} \end{aligned}$$

$$\begin{aligned} SE(\hat{p}_{1,0}) &= \sqrt{\text{var}(\hat{p}_{1,0})} \\ &= 2\sqrt{\text{var}(\hat{q}_{1,0}) + \text{var}(\hat{q}_{1,1}) + 2 \text{cov}(\hat{q}_{1,0}, \hat{q}_{1,1})} \\ &\approx 2\sqrt{\frac{\hat{q}_{1,0}(1 - \hat{q}_{1,0})}{n} + \frac{\hat{q}_{1,1}(1 - \hat{q}_{1,1})}{n} + \frac{2(\hat{q}_{1,0}\hat{q}_{1,1})}{n}} \end{aligned}$$

$$\begin{aligned} SE(\hat{p}_{0,0}) &= \sqrt{\text{var}(\hat{p}_{0,0})} \\ &= \sqrt{\text{var}(\hat{q}_{0,0}) + \text{var}(\hat{q}_{1,0}) + \text{var}(\hat{q}_{0,1}) + \text{var}(\hat{q}_{1,1}) \\ &\quad + 2 \text{cov}(\hat{q}_{0,0}, \hat{q}_{1,0}) + 2 \text{cov}(\hat{q}_{0,0}, \hat{q}_{0,1}) \\ &\quad + 2 \text{cov}(\hat{q}_{0,0}, \hat{q}_{1,1}) + 2 \text{cov}(\hat{q}_{1,0}, \hat{q}_{0,1}) \\ &\quad + 2 \text{cov}(\hat{q}_{1,0}, \hat{q}_{1,1}) + 2 \text{cov}(\hat{q}_{0,1}, \hat{q}_{1,1})} \end{aligned}$$

$$\approx \sqrt{\frac{\hat{q}_{0,0}(1 - \hat{q}_{0,0})}{n} + \frac{\hat{q}_{1,0}(1 - \hat{q}_{1,0})}{n} + \frac{\hat{q}_{0,1}(1 - \hat{q}_{0,1})}{n} + \frac{\hat{q}_{1,1}(1 - \hat{q}_{1,1})}{n} + \frac{2(\hat{q}_{0,0}\hat{q}_{1,0})}{n} + \frac{2(\hat{q}_{0,0}\hat{q}_{0,1})}{n} - \frac{2(\hat{q}_{0,0}\hat{q}_{1,1})}{n} - \frac{2(\hat{q}_{1,0}\hat{q}_{0,1})}{n} + \frac{2(\hat{q}_{1,0}\hat{q}_{1,1})}{n} + \frac{2(\hat{q}_{0,1}\hat{q}_{1,1})}{n}}$$