Chiasma Interference as a Function of Genetic Distance

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ABSTRACT

For many organisms, meiotic double crossing over is less frequent than expected on the assumption that exchanges occur at random with respect to each other. This "interference," which can be almost total for nearby intervals, diminishes as the intervals in which the double crossovers are scored are moved farther apart. Most models for interference have assumed, at least implicitly, that the intensity of interference depends inversely on the physical distance separating the intervals. However, several observations suggest that interference depends on genetic distance (Morgans) rather than physical distance (base pairs or micrometers). Accordingly, we devise a model in which interference is related directly to genetic distance. Its central feature is that recombinational intermediates (C's) have two fates–they can be resolved with crossing over (Cx) or without (Co). We suppose that C's are distributed at random with respect to each other (no interference); interference results from constraints on the resolution of C's. The basic constraint is that each pair of neighboring Cx's must have between them a certain number of Co's. The required number of intervening Co's for a given organism or chromosome is estimated from the fraction of gene conversions that are unaccompanied by crossover of flanking markers. The predictions of the model are compared with data from Drosophila and Neurospora.

CROSSOVER interference was described by STURTEVANT (1915) and by MULLER (1916). They showed that an exchange in one interval on a Drosophila chromosome decreases the probability of exchange in a nearby interval. Subsequent genetic and cytological work revealed this phenomenon in many organisms and extended our understanding of it. A meiotic crossover discourages the presence of a second crossover in a distance-dependent manner. If a second crossover does occur, the choice of chromatids involved is typically at random, or nearly so.

Gene conversions, without regard to the presence of associated crossovers, are distributed (with respect to each other) without interference (MORTIMER and FOGEL 1974), but gene conversions with associated crossovers interfere with nearby exchanges (STADLER 1959; MORTIMER and FOGEL 1974) [but see KITANI (1978)].

Although interference can be widely demonstrated, it is limited to a different physical range in different organisms–interference extends over 10^4 kilobase pairs of DNA in Drosophila, while the effect is typically gone after only 10^3 kilobase pairs in Neurospora and 10^2 kilobase pairs in Saccharomyces. Our paper offers an explanation for this variability.

Models to explain interference can be divided into two classes. One class of models assumes that interference diminishes as a function of physical distance (base pairs or microns). For instance, FOX (1973) proposed a "chiasma determining mechanism" that travels along the bivalent at a constant rate and occasionally "fires" to determine chiasmata. After it has fired, the machine requires a certain amount of time to recharge, resulting in interference. KING and MORTIMER (1990) proposed that a crossover nucleates an inhibitory polymerization. Earlier models were based on hypothesized mechanical properties of chromosomes (*e.g.*, MULLER 1916).

Another class of models assumes that interference falls off as a function of genetic (*i.e.*, linkage map) distance (Morgans). To our knowledge, only one model falls in this category. MORTIMER and FOGEL (1974) [and see STAHL (1979)], analyzing their data from Saccharomyces, proposed that recombination intermediates (C) are distributed randomly with respect to each other. These intermediates are resolved in either of two ways, with or without crossing over (Cx's and Co's, respectively). Interference results from the "rule" that Cx's and Co's alternate along the length of the tetrad. We shall refer to such models, in which interference is an immediate function of genetic rather than physical distance, as "genetic models." By so doing, we do not mean to imply that interference is mediated by other than physical forces.

We are attracted to genetic models of interference primarily because organisms with low rates of recombination per kilobase pair (like *Drosophila melanogaster*) show interference over a longer physical dis-

tance than do organisms with high rates of recombination per kilobase pair (like *Saccharomyces cerevisiae*). Furthermore, the literature lends some direct support to genetic models of interference. Data from STADLER (1959) and from MORTIMER and FOGEL (1974) suggest that Co's promote the occurrence of nearby crossovers (Cx's). Among Co events detected at *cys* in a cross conducted at 18° in Neurospora, STADLER saw 41 nearby crossovers. By comparison, 28.2 were expected if crossovers were to have occurred at the rate observed when there was no conversion at *cys* ($0.1 > P > 0.05$). (A parallel cross at 25° showed no influence of the Co event.) Among detected Co events at *ARG4* in yeast, MORTIMER and FOGEL saw 33 crossovers in the interval centromere-*THR1*, which includes *ARG4*, when 23.3 were expected without interference. Among detected Co events at *HIS1*, 6 crossovers to one side and 97 to the other were found when 1.8 and 90.5, respectively, were expected without interference (MORTIMER and FOGEL 1974). Our inability to determine the precise origins of some of the numbers in the latter reference prevent us from estimating the significance of the observations in yeast.

In this paper, we describe a genetic model of interference and its mathematical formulation. We compare the predictions of the model with data from *Neurospora crassa* and *D. melanogaster*.

THE MATHEMATICAL MODEL

Symbols are defined in context and in Table 1.

The model presented here has the following features:

1. Recombination-initiation events occur at random with respect to each other. Among acts of meiosis, the numbers of such initiations (n) in any given interval are Poisson-distributed about the mean number (y).

2. Initiations lead to an intermediate structure, like a Holliday junction (HOLLIDAY 1964) or a region of double-chain gap repair flanked by Holliday junctions (SZOSTAK *et al.* 1983). An intermediate (C), were it positioned at a marked locus, would either result in conversion (SZOSTAK *et al.* 1983) or provide the opportunity for conversion via mismatch correction (HOLLIDAY 1964; MESELSON and RADDING 1975).

3. An intermediate is resolved so as to give crossing over (Cx) or not to give crossing over (Co) of markers that flank the intermediate. For simplicity, we ignore the possibility of conversion of these markers. Thus, the validity of our expressions is limited to distances that are long compared to the length of conversion tracts.

4. Recombination occurs in the four-strand stage of meiosis and proceeds without chromatid interference. Therefore, any deviation of the coefficient of coincidence (S) from unity is a consequence of chiasma interference.

TABLE 1

Basic symbols employed

Symbol	Definition
C	An event in a tetrad (a recombinational intermediate) that can result in conversion (without regard to accompanying crossover)
Co	C that is resolved without crossover
Cx	C that is resolved with crossover
d	Empirically determined map distance (in Morgans) between markers
m	Fixed number of Co's between neighboring Cx's
n	Actual number of C's in a tetrad in a given interval
p	In models with variable numbers of Co's between neighboring Cx's, p is the probability for each additional Co (after the fixed number, m)
q	Observed fraction of gene conversions accompanied by crossover of flanking markers
R	Frequency of recombinant haplonts
r	Fraction of C's that are Cx estimated by correcting data on the assumption that Cx's and Co's are independent
ρ	Fraction of C's that are Cx estimated by correcting data on the assumption of a fixed number of Co's between neighboring Cx's
S_3	Inclusive coefficient of coincidence for adjacent intervals
S_4	Inclusive coefficient of coincidence for separated intervals
X	Linkage map distance (in Morgans) ≡ (1/2)(mean number of Cx's per tetrad in a given interval)
y	Mean number of C's per tetrad in a given interval

5. Chiasma interference is a result of nonindependence in the resolution of intermediates. In particular, resolution of an intermediate as a Cx makes it impossible for the nearest neighbor intermediates to be resolved as Cx–they will be resolved as Co. This feature of our model was suggested by MORTIMER and FOGEL (1974) and makes the mechanism of interference directly dependent on genetic linkage distance rather than on the physical distance between marked intervals. In our model, m Co's follow each Cx.

Interference is conventionally measured as a "coefficient of coincidence" (MULLER 1916). For each value of m, we present two expressions for the coefficient of coincidence, which have been widely employed (BRIDGES 1915; MULLER 1916; WEINSTEIN 1918; STEVENS 1936). For each definition, S is (frequency of double recombinants predicted by the model)/(frequency of double recombinants predicted in the absence of interference). Both coefficients are "inclusive" in that they ignore exchanges in other intervals. The two definitions differ in the double recombinant frequency that is measured.

i. One definition for the coefficient of coincidence is for a four-factor cross in which recombination is scored in two marked intervals (1–2 and 3–4) and in which the distance (X) between the two marked intervals is varied:

TABLE 2

Deriving R for $m = 1$

n	P_n	Probability of Cx	Frequency of recombination given Cx
1	ye^{-y}	1/2	1/2
>1	$1 - e^{-y} - ye^{-y}$	1	1/2

1 2 3 4

We call this coefficient of coincidence S_4. For mathematical convenience, we take the recombination frequencies R_{12} and R_{34} to be equal and small, and we examine the variation of S_4 with variation in the map distance (X, in Morgans) of the interval 2–3.

ii. A second definition of S, frequently used, is based on a three-factor cross in which the lengths (X) of the two marked intervals are varied. We call this coefficient of coincidence S_3.

1 2 3

For convenience, we take $R_{12} = R_{23}$ and write mapping functions $R \equiv R(X)$ for $R_{12} = R_{23}$ and for R_{13}. The dependence of S_3 on R can be determined from the relationship that defines R:

$$R_{13} = R_{12} + R_{23} - 2R_{12\&23}^{\text{double}}$$

Then, from the definition

$$S_3 = R_{12\&23}^{\text{double}}/(R_{12}R_{23}),$$

we can compute S_3 from any mapping function as

$$S_3 = \frac{R_{12} - \frac{1}{2}Rei_{13}}{R_{12}^2} = \frac{R(X) - \frac{1}{2}R(2X)}{[R(X)]^2}.$$

With HALDANE's (1919) mapping function, $R = (\frac{1}{2})(1 - e^{-2X})$ (which lacks interference), $S_3 = 1$ and $S_4 = 1$.

In general, when $R(X) \to 0.5$, $R(2X) \to 0.5$ and $S_3 \to 1$. S_4, on the other hand, can, in principle, deviate from unity when $R(X) \to 0.5$.

Alternating CxCo ($m = 1$): For this case, C events are alternately Co and Cx along the length of a chromosome. This was briefly explored previously (STAHL 1979).

The mapping function: In the absence of chromatid interference, tetrads enjoying one or more crossovers in a marked interval produce 50% recombinant haploid products on the average (*i.e.*, in the presumed absence of chromatid interference, parental ditype tetrads = nonparental ditype tetrads). Thus, the general form for a mapping function is $R = (\frac{1}{2})$(Probability $\geq$ 1 Cx).

If one C event occurs, the probability of Cx is $\frac{1}{2}$. If more than one C event occurs, the probability of one or more Cx is unity. In Table 2 y is the mean number of C's in a given interval. With the aid of Table 2, the mapping function in terms of a Poisson distribution of C's with mean y can then be written as

$$R = \tfrac{1}{2}(1 - e^{-y} - \tfrac{1}{2}ye^{-y}).$$

y is equal to $4X$, because half the C's are Cx's and events per chromatid are half of those per bivalent. Thus:

$$R = \tfrac{1}{2}[1 - (1 + 2X)e^{-4X}]. \qquad (1)$$

This mapping function is graphed in Figure 1 along with others developed here and with HALDANE's (1919) function for no interference. S_3 is graphed in Figure 2.

S in a four-factor cross: To get a double crossover in 1–2 and 3–4, two conditions must be met in 2–3. (i) The outermost events in the 2–3 interval must be Co's. Thus, there must be an odd number of Poisson-distributed C events in 2–3:

$$P_{\text{odd}} = \tfrac{1}{2}(1 - e^{-2y})$$

(HALDANE 1919). (ii) Given an odd number of events,

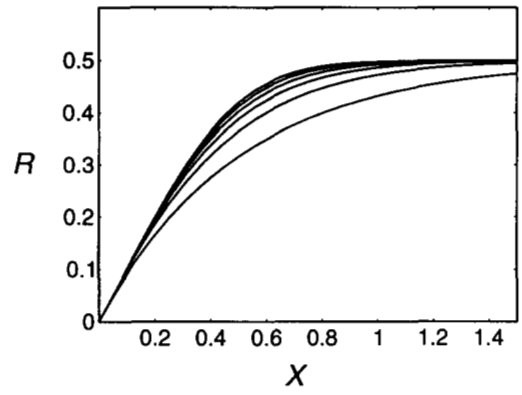

FIGURE 1.—Mapping functions for the Cx(Co)^m model. R, the recombination frequency is graphed *vs.* X, the map distance in Morgans. From right to left are HALDANE's function ($m = 0$) and functions for $m = 1$ to $m = 5$ (Equations 1, 3, 5, 7); m is the fixed number of Co's between neighboring Cx's.

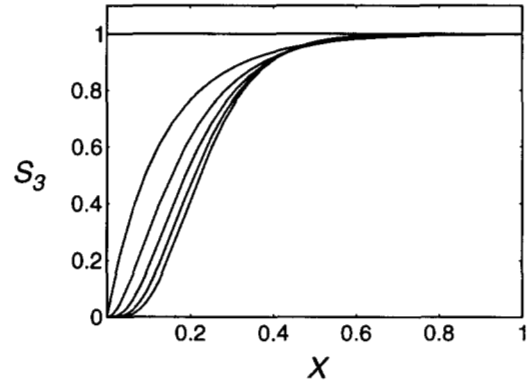

FIGURE 2.—S_3 as a function of X for the Cx(Co)^m model for $m =$ 1 to 5 (left to right). X is the map length of interval 1–2 or 2–3, which equals the map distance from the midpoint of interval 1–2 to that of interval 2–3. For HALDANE's function ($m = 0$), $S_3 = 1$ at all values of X.

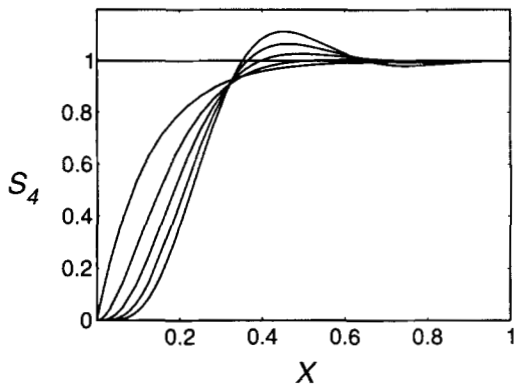

FIGURE 3.—S_4 as a function of X for $m = 1$ to 5 (left to right; Equations 2, 4, 6, 8). In the absence of interference, $S_4 = 1$ at all values of X.

TABLE 3

Deriving R for $m = 2$

n	P_n	Probability of Cx	Frequency of recombination given Cx
1	ye^{-y}	1/3	1/2
2	$(y^2/2)e^{-y}$	2/3	1/2
>2	$1 - (1 + y + y^2/2)e^{-y}$	1	1/2

the probability that both terminal events are Co (rather than Cx) is $\frac{1}{2}$, so that $(\frac{1}{4})(1 - e^{-2y})$ is the probability that events in 2–3 permit a double exchange in 1–2 and 3–4. Among bivalents in which this requirement is met, the probability of recombination in 1–2 (or 3–4) is $2R_{12}$, because any C event in either one of those short intervals must be a Cx, whereas in an unselected population only half the time will a C event in 1–2 or 3–4 be a Cx. Thus, the probability of double crossover in 1–2 and 3–4 is

$$\tfrac{1}{2} P_{\text{odd}}(2R_{12})^2 = R_{12}^2(1 - e^{-2y}).$$

Then the ratio of doubles predicted by the model (with $m = 1$) to that predicted in the absence of interference is

$$S_4 = 1 - e^{-2y}.$$

Again, the scaling factor to convert y (the mean number of Poisson-distributed initiation events) to X (Morgans) is 4, so that

$$S_4 = 1 - e^{-8X}. \tag{2}$$

S_4 as a function of X is graphed in Figure 3.

Cx's alternate with runs of two Co events ($m = 2$): This case was partially developed previously (STAHL 1979).

The mapping function: Adapting the procedure described for $m = 1$ (above) gives Table 3. For $m = 2$, $y = 6X$, so

$$R = \tfrac{1}{2}[1 - (1 + 4X + 6X^2)e^{-6X}]. \tag{3}$$

This function differs in the coefficient of the X^2 term from that presented by STAHL (1979) because of a typographical error in that reference. The function is graphed in Figure 1. The corresponding S_3 as a function of X is graphed in Figure 2.

TABLE 4

Deriving S_4 for $m = 2$

n	P_n	Condition	Frequency
2	$y^2e^{-y}/2!$	CoCo	1/3
5	$y^5e^{-y}/5!$	CoCoCxCoCo	1/3
8	$y^8e^{-y}/8!$	CoCoCxCoCoCxCoCo	1/3
and so on			

S in a four-factor cross: In order to qualify for double crossover in the 1–2 and 3–4 intervals, the conditions with probabilities shown in Table 4 must be met for the interval 2–3.

When conditions in 2–3 permit a crossover in 1–2 or 3–4, that crossover will have a probability proportional to $3R_{12}$, while the probability of that crossover in unselected bivalents will be proportional to R_{12}. Thus, the probability of a double crossover among bivalents that permit a double crossover is $(3R_{12})^2$.

Because the scaling factor to convert y to X is 6, we write:

$$\begin{aligned} S_4 &= 3e^{-6X}\left[\frac{(6X)^2}{2!} + \frac{(6X)^5}{5!} + \frac{(6X)^8}{8!} + \ldots\right] \\ &= 1 - 2e^{-9X}\cos\left(\frac{\pi}{3} - 3\sqrt{3}X\right). \end{aligned} \tag{4}$$

S_4 as a function of X is graphed in Figure 3.

Cx's alternate with runs of three Co's ($m = 3$): *The mapping function*: The method used for $m = 2$ leads to the following mapping function for $m = 3$:

$$R = \frac{1}{2}\left[1 - \left(1 + 6X + 16X^2 + \frac{64}{3}X^3\right)e^{-8X}\right]. \tag{5}$$

This function is graphed in Figure 1. The corresponding S_3 as a function of X is graphed in Figure 2.

S in a four-factor cross: The method used for the CxCoCo case leads to the following expression for S_4:

$$\begin{aligned} S_4 &= 4e^{-8X}\left[\frac{(8X)^3}{3!} + \frac{(8X)^7}{7!} + \frac{(8X)^{11}}{11!} + \ldots\right] \\ &= 1 - e^{-16X} - 2e^{-8X}\sin(8X). \end{aligned} \tag{6}$$

S_4 as a function of X is graphed in Figure 3.

The general Cx(Co)^m model: The mapping function, R, and the four-factor coefficient of coincidence, S_4, for the general Cx(Co)^m model can be derived by induction from the foregoing cases, in which m, the number of Co's intervening between Cx's, equals 1, 2 and 3, respectively.

$$R = \frac{1}{2}\left[1 - e^{-y}\sum_{i=0}^{m}\frac{y^i}{i!}\left(1 - \frac{i}{m+1}\right)\right] \tag{7}$$

and

$$S_4 = (m + 1)e^{-y} \sum_{i=0}^{\infty} \frac{y^{m+(m+1)i}}{[m + (m + 1)i]!}. \quad (8)$$

Use the change of scale $y = 2(m + 1)X$ to get R and S_4 in terms of X. Equation 7 is equivalent to Equation 30 of COBBS (1978).

Although we failed to find closed-form solutions in terms of elementary functions for these series, they are useful for calculation and graphics. R for $m = 4$ and 5, respectively, is graphed in Figure 1. S_3 for these m values is graphed in Figure 2. S_4 is graphed in Figure 3.

Model in which Cx events alternate with runs of Co of variable length: The foregoing deterministic model can be generalized by requiring that at least m Co's follow each Cx, but that more Co's can occur, with probability p for each additional Co. For the generalized Cx(Co)^m model, the mean run-length of Co's is then $m + p + p^2 + \ldots = m + p/(1 - p)$. When $p/(1 - p) \gg m$, the distribution of Cx's in any interval becomes nearly Poisson, and the model approaches HALDANE's (no interference). Thus, when we add such a random number of Co's to the deterministic model, we expect the mapping and interference functions to interpolate between those for the Cx(Co)^m model and HALDANE's as p approaches 1. In APPENDIX 1, we consider explicitly the simplest cases, where $m = 0$ or 1.

RESULTS AND DISCUSSION

Some previous models for interference (*e.g.*, KING and MORTIMER 1990) are measuring models; they assume, at least implicitly, that interference is a function of physical distance. The model by KING and MORTIMER supposes that a substance polymerizes bidirectionally from a crossover. If the polymer reaches a crossover intermediate, that intermediate is negated. This model was fitted to data by adjusting the rates of polymerization. Thus, the model relates S to a physical event that needs have no relationship to linkage map distance. Drosophila has far fewer conversions and crossovers per unit physical distance than does *Saccharomyces*, presumably because it has a lower density of recombination initiation sites and/or because the sites are less active on the average. At the same time, interference extends over far greater (physical) distances in Drosophila than it does in Saccharomyces. To fit data as different as those from flies and yeast, the parameters of the physical models must be adjusted (arbitrarily) to suit the differing relationships between genetic and physical distances. For instance, were the physical parameters that characterize yeast interference applied directly to Drosophila, interference would vanish within a small fraction of the length of a chromosome arm when, in fact, it does not. Whereas the physical models *measure* distance and adjust the probability of a second crossover accordingly, our models *count* Co's and, thus, relate S directly to genetic distance. By its nature our model accounts for the major difference between Drosophila and Saccharomyces.

The model presented here has one adjustable parameter, the fractions of C's that are Cx's. If we make the usual (though not universal) assumption that conversions accompanied by crossing over have the same properties as those that are not, we can estimate m (the number of Co's between adjacent Cx's) from the observed fraction of conversions accompanied by crossing over. For example, in *D. melanogaster* conversions are associated with crossovers 22% of the time (HILLIKER and CHOVNICK 1981) or "less than 20% of the time" (HILLIKER, CLARK and CHOVNICK 1991), which implies that $m = 4$ or 5. In *N. crassa*, the fraction of C's that are Cx's is about 0.3, leading to the estimate $m = 2$ (see APPENDIX 2). *S. cerevisiae* was initially reported as showing a 50% association of crossing over with conversion (HURST, FOGEL and MORTIMER 1972), consistent with $m = 1$, the model proposed by MORTIMER and FOGEL (1974). Later (FOGEL, MORTIMER and LUSNAK 1983), it was concluded that a better estimate of the fraction of C's that are Cx's is 0.35, consistent with $m = 2$. Our model predicts how S_4 rises with X for different values of m (Figure 3).

We choose to examine the relationship of S_4 to X because it is more revealing than the three-factor method. The three-factor method allows one to relate recombination frequency (R), the coefficient of coincidence (S_3) and the map distance (X) without any assumptions, and it has been utilized in the construction of empirical mapping functions (*e.g.*, AMATI and MESELSON 1964). However, the three-factor definition has the shortcoming that, as a simple consequence of the algebra of the definition, $S_3 \to 1$ when $R \to 0.5$. Thus, any interesting long-range interactions between crossovers, like the negative interference ($S_4 > 1$) seen when $m > 1$ (see below), are obscured.

In our model, S_4 rises as X^m for small X, where m is the number of intervening Co's. (Adding variable numbers of Co's to the runs of fixed length gives S_4 curves that fall between the deterministic ones and HALDANE's. See APPENDIX 1.) Thus our model with $m > 1$ predicts that interference is essentially complete until some threshold X value is reached, whereas when $m = 1$ there is an immediate rise in S_4. WEINSTEIN (1959) summarized observations in Drosophila with the statement that ". . .exchanges have not been detected closer together than 5–15 units [0.05–0.15 Morgan], the precise length differing in different chromosomes and in different parts of the same chromosome." The data of BRIDGES and CURRY (1935),

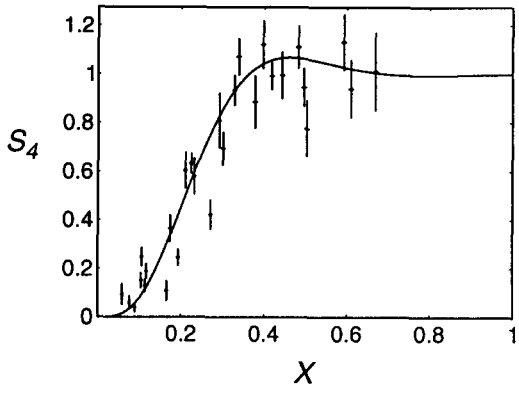

FIGURE 4.—Drosophila S_4 data (BRIDGES and CURRY 1935) compared to the model with $m = 4$ (Equation 8). X values plotted here are measured by the sum of the X values in all of the subintervals between the midpoint of the 1–2 interval and the midpoint of the 3–4 interval. In our models, R for the outside intervals (1–2 and 3–4) is assumed to be negligible. In experimental data, however, these R values are apt to be of appreciable length compared to the interval 2–3. This makes the "S_4" values tend toward S_3 values. Thus, any tendency of Drosophila S_4 values to exceed unity at $X \cong 0.5$ would be partially obscured. Lines emanating from data points denote one standard error and were computed from formulas in MULLER and JACOBS-MULLER (1925) and STEVENS (1936).

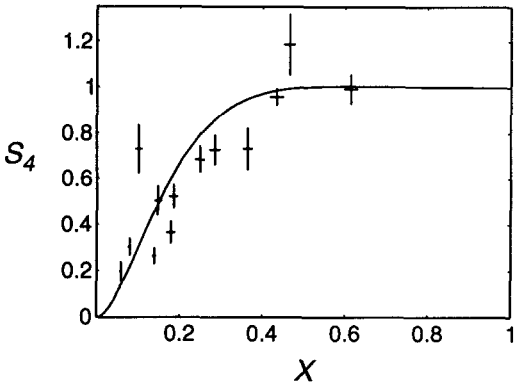

FIGURE 5.—Neurospora S_4 data, plotted as in Figure 7, compared to the model with $m = 2$ (Equation 4). Data points are from pooled data in PERKINS (1962), except for the three points on the lower left, which are from pooled data in STRICKLAND (1961). Map distances between adjacent markers were computed as $X = (\frac{1}{2})(T + 6NPD)$, where T and NPD are the proportions of tetratype and nonparental ditype tetrads, respectively (PERKINS 1949). Approximate standard errors were computed from formulas in MULLER and JACOBS-MULLER (1925) and STEVENS (1936), modified to account for NPD's. The very few conversion tetrads that were exchanged for flanking markers were omitted from the calculations. The estimate of $m = 2$ was selected on the basis of a literature survey and analysis conducted by DAVID PERKINS plus additional considerations (see APPENDIX 2).

shown in Figure 4, clearly manifest such an initial lag. Data from Neurospora, shown in Figure 5, indicate a smaller lag.

In Figure 4 we present the test of our model. Estimates of S_4 from Drosophila are compared to our model with $m = 4$. In Figure 5, Neurospora data are compared to our model with $m = 2$. It is important to note that these m values were not selected to give a best fit to these data relating S_4 to X but from the entirely independent experiments, cited above, that measure the fraction of conversions that is accompanied by crossing over.

In attempts to describe multilocus recombination data in Drosophila, previous authors (COBBS 1978; M. S. MCPEEK, unpublished manuscript) have fitted to data a model assuming a Gamma distribution of map distance between adjacent chiasmata. Our formulas relating R and S_4 to map distance are consistent with this description, and our general formula for R (Equation 7) was previously derived by COBBS (1978). However, the previous works did not specify a mechanism that would generate such a relationship. Our formulas, on the other hand, are derived from a particular biological model of interference. The Gamma distribution of map distance between adjacent chiasmata is a result of our model, rather than assumption. In the Introduction, we noted the data that led us to our model. One of these data was the apparent increase of crossovers (Cx's) unassociated with but in the vicinity of a simple conversion (a Co). A cogent test of our model (in progress) is an extension of that observation. Our model can be tested by a five-factor cross. Tetrads will be examined for Co at the central marker. Crossing over will be scored for the two outside intervals. The model predicts that coincidence (S_4) for these intervals will be higher in the presence of the Co than it will be in tetrads that have no detected events in the interval between the flanking intervals. Such a result tests the model by demonstrating that the mechanism that causes interference *counts* Co events. Economy of hypothesis would suggest that no other aspect of distance, regardless of how distance is measured, has an influence on interference.

For both the Drosophila and Neurospora data used here, the fraction of exchanges accompanied by conversion of one of the markers is negligible, and our models apply. The ability of our model to describe the dependence of interference on linkage map distance is gratifying. We have been unable to test our model against yeast data because a substantial fraction of all recombinants arising at interesting values of X involve conversion of one or the other of the markers. This is an expected property of any creature in which the density of C's per unit physical distance is high (and conversion tracts are of normal length).

In discussing the relationship of S_4 to X we have focused on the model in which a fixed number of Co's follows the obligatory Co that comes after Cx. If the estimate of $\frac{1}{5}$ for the fraction of C's that are Cx's is accurate for *D. melanogaster*, the close fit of our model with $m = 4$ to the data in Figure 4 would rule out an alternative model with a variable number of Co's (unless p is near zero).

Our model allows a formal rule for ensuring that

few chromosomes go without a chiasma even when the mean number of chiasmata (*i.e.*, Cx's) per bivalent is small and may have to be kept so to avoid excessive entanglements (MERRIAM and FROST 1964). One need only suppose that the initial decision regarding the fate of C events is made by the (random?) selection of one such event to be a Cx. Other decisions are then made such that the appropriate pattern of Cx's and Co's is achieved. The fraction of chromosomes receiving no chiasmata will, then, be the (small) fraction of cells totally lacking in the (Poisson-distributed) C events. If this speculation is correct, estimates of the fraction of C's that are Cx's do not lead in a simple way to the value of m. However, if the number of C's per chromosome is greater than $m + 1$, the simple estimate of m will be reasonably good.

For *D. melanogaster* autosomes, on the other hand, we speculate that the pattern of Cx's and Co's is laid down from the centromere toward the telomere starting with a run of four Co's [and see COBBS (1978)]. This would explain both the absence of interference across the centromere and the inhibition of crossing over near the centromere (BEADLE 1932). This speculation predicts that there is no inhibition of gene conversion near the centromere. Consistent with this, GREEN (1975) suggested that conversions occur near the centromere with normal frequency in Drosophila. One consequence of this speculation is that the genetically (and physically) short chromosome *IV* in Drosophila should usually fail to receive any chiasma, in keeping with observation. The efficient distributive pairing system of Drosophila (GRELL 1964) prevents the nondisjunction that might accompany such a situation in some other organisms.

If Drosophila does follow the rules of our model modified as described above, our equation for S_4 *vs.* X cannot describe the Drosophila data precisely. However, it is reasonably accurate beyond the first Cx, where S_4 is necessarily measured, as will be detailed in a subsequent publication.

CARPENTER (1987) has speculated that early (ellipsoidal) recombination nodules seen in Drosophila signal either C or Co events. Late (spherical) nodules, on the other hand, clearly signal Cx events [for a review see CARPENTER (1987)]. CARPENTER's speculation combined with our model as applied to Drosophila predicts that the ratio of early to late nodules will be relatively high near autosomal centromeres, as was observed (CARPENTER 1979). If our speculation regarding a string of Co events near Drosophila centromeres is correct, the estimate of 0.2 for the fraction of C's that are Cx's, obtained from HILLIKER and CHOVNICK (1981) and HILLIKER, CLARK and CHOVNICK (1991), is slightly compromised. Our speculation predicts that the fraction of C's that are Cx's depends on linkage map position. The dependency would be strong for markers close to the centromere. However, the estimate of 0.2 is based on studies of the *rosy* locus, which is far (0.52 Morgan) from the centromere of chromosome *III*.

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Appendix 1
Cx's Followed by Variable Numbers of Co's

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In the case of $m = 0$ (no obligatory Co following a Cx), Cx's and Co's have independent Poisson distributions, and the model is obviously equivalent to HALDANE's regardless of p. (In this case, p is the probability that any single event will be a Co.)

In the case of $m = 1$, where a Cx is certainly followed by a single Co and by additional Co's each with probability p, the mapping and interference functions are as follows.

The mapping function: Let p_x = the probability of Cx (given C) = $(1 - p)/(2 - p)$, and p_o = probability of Co (given C) = $1/(2 - p)$.
Thus,

$$y = \frac{2}{p_x} X = \frac{2(2 - p)}{1 - p} X. \qquad (9a)$$

The probability of at least one crossover in a tetrad

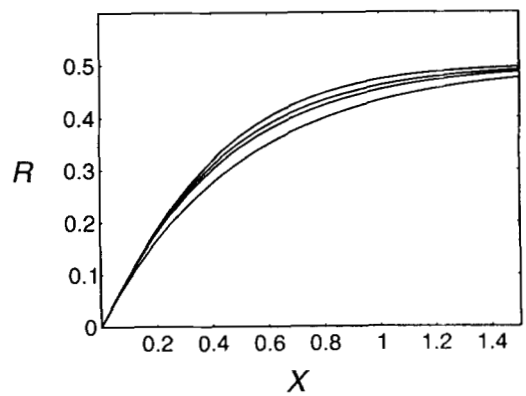

FIGURE 6.—Mapping function for the model in which Cx is invariably followed by a single Co and then by variable numbers of additional Co's, each with probability p (Equation 9a). From left to right, $p = 0$ (CxCo), $p = \frac{2}{3}$, $p = \frac{4}{5}$, $p = \infty$ (HALDANE).

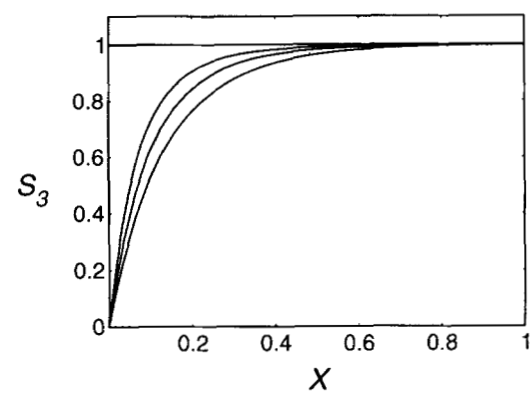

FIGURE 7.—S_3 as a function of X for the model in which Cx is invariably followed by a single Co and then by variable numbers of additional Co's, each with probability p. From right to left, $p = 0$ (CxCo), $p = \frac{2}{3}$, $p = \frac{4}{5}$, $p = \infty$ (HALDANE).

with n C's equals 1 minus the probability of n Co's in a row. In such a tetrad, the probability of n consecutive Co's is 1 for $n = 0$ and $[1/(2-p)]p^{n-1}$ for $n \geq 1$. Then

$$R = \frac{1}{2}\left[1 - e^{-y}\left(1 + \sum_{n=1}^{\infty} \frac{y^n}{n!}\frac{p^{n-1}}{2-p}\right)\right] = \frac{1}{2}\left[1 - e^{-y}\left(1 + \frac{e^{py}-1}{p(2-p)}\right)\right]. \quad (9b)$$

When p approaches zero, Equation 9b approaches tl mapping function for alternating CoCx ($m = 1$). Whe p approaches 1, using Equation 9a it can be shown that Equation 9b is asymptotically equivalent to HALDANE's mapping function. Thus, as Co runs of variable length becomes longer, they result in less chiasma interference. R as a function of X for several values of p is graphed in Figure 6. Corresponding graphs for S_3 are in Figure 7.

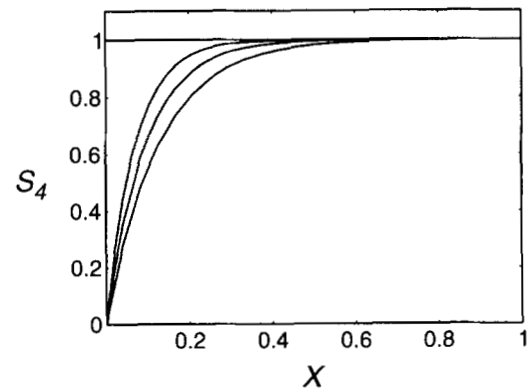

FIGURE 8.—S_4 as a function of X for the model in which Cx is invariably followed by a single Co and then by variable numbers of additional Co's, each with probability p (Equation 10). From right to left, $p = 0$ (CxCo), $p = \frac{2}{3}$, $p = \frac{4}{5}$, $p = \infty$ (HALDANE).

***S* in a four-factor cross:** S_4 can be computed as the probability that the initial and final events in the middle interval (2–3) are both Co, multiplied by the probability of Cx in both end intervals (1–2 and 3–4) given the initial and final Co's in the middle interval. The unconditional probability that the first event in the interval is Co is p_o. The probability that $n - 1$ remaining events terminate in Co is given by the total probability of all possible combinations of single Co's and CxCo pairs,

$$P_{n^{Co-Co}} = \sum_{i=0}^{[(n-1)/2]} \binom{n-1-i}{i} (1-p)^i p^{n-1-2i},$$

in which $[(n-1)/2]$ is the integer part of $(n-1)/2$; *e.g.*, $[3/2] = 1$. Thus, the probability of initial and final Co's in the middle interval is

$$P_{\text{Co—Co}} = p_o e^{-y} \sum_{n=1}^{\infty} \frac{y^n}{n!} P_{n^{Co-Co}}.$$

Given initial and final Co's in the middle interval, there is a probability $1 - p$ that an event flanking the middle interval is Cx. When events in the middle interval permit a crossover in the end intervals, the probability of a crossover in one of the end intervals is $(R_{12})/p_x$, whereas the probability of a crossover in the unselected bivalents is R_{12}. Hence,

$$S_4 = [(1-p)/i_X]^2 P_{\text{Co—Co}} = (2-p)p^{-1}e^{-y} \sum_{n=1}^{\infty} \frac{(py)^n}{n!} \sum_{i=0}^{[(n-1)/2]} \binom{n-1-i}{i} \left(\frac{1-p}{p^2}\right)^i$$

When $p \to 1$, $P_{n\text{Co—Co}} \to 1$, and $S_4 \cong 1 - e^{-y} \cong 1 - e^{-2X/(1-p)}$. From this it can be seen that when $p \to 1$, $S_4 \to 1$, as in HALDANE's model (see Figure 8).

Appendix 2

Estimates of the Proportion of Recombination Intermediates That Are Resolved With Crossing Over in *Neurospora crassa*

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Crossing over between markers is increased among tetrads of chromatids where conversion has occurred at a locus between them. Flanker exchanges in 445 of 907 conversion yeast tetrads (HURST, FOGEL and MORTIMER 1972) led to the generalization, rather widely held, that the probability of crossing over among convertants is 1/2. But the observed 50% was shown to be fortuitous, resulting partly from crossing over in the intervals between the middle locus and the flanking markers (STADLER 1973; FOGEL *et al.* 1979); such crossing over is expected because Co's do not positively interfere with crossing over.

If we assume that Co and Cx events are independent of each other, correction can be made for crossing over outside the converted locus (WHITEHOUSE and HASTINGS 1965). Assuming $d \ll 1$ so that $d \approx R$ (the recombination rate between the flanking markers), the fraction of C's that are Cx is $r = (q - d)/(1 - d)$, where r = corrected fraction of crossovers among conversions, q = observed fraction, and d = empirically determined map units (in Morgans) between the flankers (PERKINS 1979; FOGEL *et al.* 1979).

(A minor source of error is recombination events that terminate reciprocally between marked sites within the middle gene. Such recombinants are obligatorily flanker-crossovers but not mutant-site conversions, and so should be excluded. This error can be corrected with tetrad data, but not with the Neurospora data presented here, all of which were collected by random spore analysis.)

When corrected as described above, conversion-associated crossing over is found to be below 50% for all published tetrad data and most chromatid data (D. D. PERKINS, unpublished results).

In Table 5, published data from Neurospora are presented and are corrected by the procedure established above. The data corrected in the manner described (r) imply that 0.33 ± 0.01 of conversions enjoy an associated crossover (*i.e.*, the fraction of C's that are Cx = 0.33).

The correction described is not fully appropriate for this paper, whose basic assumption is that Co and Cx events are *not* independent of each other. A more accurate estimator, ρ, of the fraction of C's that are

TABLE 5

Fraction of selected intragenic recombinant chromatids (Neurospora ascospores) that is crossed over for flanking markers

Locus	Fraction recombinant for flankers				Reference
	Observed		Corrected		
	q	d	r	ρ	
met-2	0.47	0.10	0.41	0.37	MURRAY (1963)
am	0.32	0.08	0.26	0.24	SMYTH (1973)
his-1	0.45	0.10	0.39	0.35	THOMAS and CATCHESIDE (1969)
ad-3A	0.32	0.06	0.28	0.26	DE SERRES (1960)
pan-2	0.50	0.12	0.43	0.38	CASE and GILES (1959)
cys-2	0.51	0.16	0.42	0.35	STADLER and TOWE (1963)
met-7	0.22	0.04	0.19	0.18	MURRAY (1969)
pdx	0.38	0.06	0.34	0.32	MITCHELL (1956)
mtr	0.26	0.03	0.24	0.23	STADLER and KARIYA (1969)
Mean	0.38		0.33	0.30	
SE	±0.01		±0.01	±0.01	

Cx recognizes that a Co increases the likelihood of a Cx in the (short) interval defined by the flankers. Since the fraction of C's that are Cx (ρ) equals $1/(m + 1)$, the factor by which a Co increases this likelihood is

$$\frac{m + 1}{m} = \frac{1}{1 - \rho}.$$

Then we can write

$$q = \rho + (1 - \rho)(\text{prob. of Cx between flankers, given Co})$$

$$= \rho + (1 - \rho)\left(\frac{1}{1 - \rho}\right)d = \rho + d.$$

Thus,

$$\rho = q - d.$$

Values for ρ are given in Table 5. The average ρ value is 0.30 ± 0.01, suggesting that the fraction of C's that are Cx = $\frac{1}{3}$ (*i.e.*, $m = 2$).

Some fraction of the conversions apparently unaccompanied by crossing over were likely to have been Cx events that, despite chiasma interference, enjoyed a second exchange between the flankers. Since our correction procedures have ignored that possibility, the ρ values are apt to be slight underestimates of the fraction of C's that are Cx. To minimize this effect, we have included in Table 5 only those studies for which $d < 0.2$, so that $d \approx R$ and double recombinants will be of little consequence.