THE THEORY OF MULTIPLE-STRAND CROSSING OVER[1]

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THE PROBLEM

ACCORDING to the theory of the chiasmatype as originally formulated by JANSSENS (1909), interchange between homologous chromosomes takes place when each is already split longitudinally, but at any level only two of the four strands exchange parts. It was pointed out by MULLER (1916) and BRIDGES (1916) that the theory could be demonstrated genetically if it could be shown that in eggs which have retained two maternal strands, one strand may be a crossover and the other a non-crossover, or both crossovers but not at the same level. Such individuals were obtained by MULLER and BRIDGES; and although in these cases there was, as MULLER pointed out, the possibility that the extra strands had arisen by non-disjunction prior to maturation, the correctness of JANSSENS's theory has since been demonstrated by the regular occurrence of such individuals in races with attached X's or high non-disjunction and in triploids (ANDERSON 1925b, BRIDGES AND ANDERSON 1925, L. V. MORGAN 1925, REDFIELD 1930, STURTEVANT 1931).

If crossing over occurred at a two-strand stage, each chromatid would be identical with one of the other chromatids of the tetrad and the complement of the remaining two; hence the enumeration of the strands and the determination of how they are combined in the tetrad would be a simple matter. In four-strand crossing over, the strands recovered are presumably still a random sample of all the strands; but there are two complications. (1) Each of the missing strands is not necessarily either identical with or the complement of the strand recovered. (2) There is the possibility that crossing over may have occurred between sister strands; and this would not be directly detectable.

A complete theory of crossing over must take into account the missing strands in each tetrad and the unrecognizable crossings over. At first

sight this may seem like a search for the substance of things hoped for, the evidence of things not seen; nevertheless the nature of the strands and tetrads can be deduced by calculation from the experimental data.

MATHEMATICAL METHOD

Definitions

Rank. The number of levels at which crossing over occurs in a strand or tetrad will be termed its rank. Non-crossovers are of rank 0, singles of rank 1, and so forth.
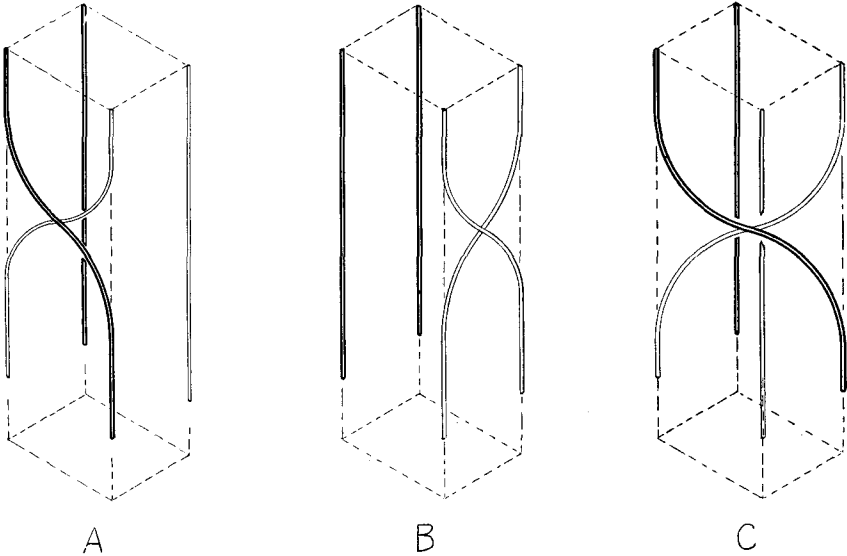

FIGURE 1. Types of single crossing over. A, lateral, involving homologous strands; B, lateral, involving sister strands; C, diagonal, involving homologous strands.

Types of single crossing over. A distinction must be made between homologous and sister-strand crossing over; also between lateral and diagonal crossing over if the strands of a tetrad are arranged along the edges of a quadrilateral prism (figure 1).

Types of multiple crossing over. In tetrads of rank 2, crossing over will be termed regressive, progressive, or digressive according to whether both or one or neither of the strands that cross over at the first level is involved in the crossing over at the second level (figure 2). A tetrad of higher rank than 2 may be mixed in type. In figure 3 are shown the various types of tetrads of ranks 2 and 3, but no distinction is made between lateral and diagonal crossing over.

A tetrad can give rise to strands of its own rank or of lower ranks but not to strands of higher rank. The rank of an emerging strand may however

be greater than the number of levels at which any one of the original strands that enter the tetrad crosses over (figure 3, tetrad G6).

Association of chromatids. Completely random association of chromatids in crossing over implies (1) that at any given level any two chromatids of a tetrad are equally likely to cross over (this may be termed random local association, or random occurrence of crossing over); (2) that the two chromatids which cross over at one level do not determine which shall cross over at other levels (random recurrence of crossing over).

A   B   C
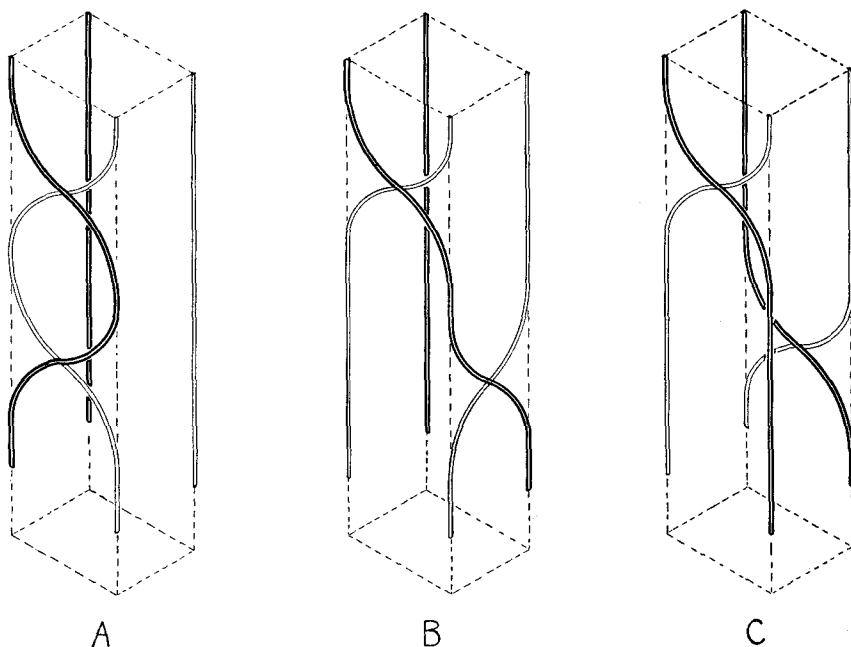
FIGURE 2. Types of double crossing over. A, regressive; B, progressive; C, digressive.

We shall first work out the theory of crossing over for completely random association; and then generalize it for cases where either occurrence, or recurrence, or both are not random.

Case 1. Recurrence random, chance of detecting crossing over constant.

A. Random occurrence (free sister-strand crossing over)

A tetrad of rank 0 can give rise only to non-crossover strands. In a tetrad of rank 1, half the strands will be crossovers and half non-crossovers, so that the chance of obtaining a strand of rank 1 will be 1/2, if elimination into polar bodies is a random matter. But not all the crossover strands will be recognizable as such: those resulting from crossing over between sister strands will remain unaltered. Since on a random basis one-third of the exchanges at a given level will be between sister
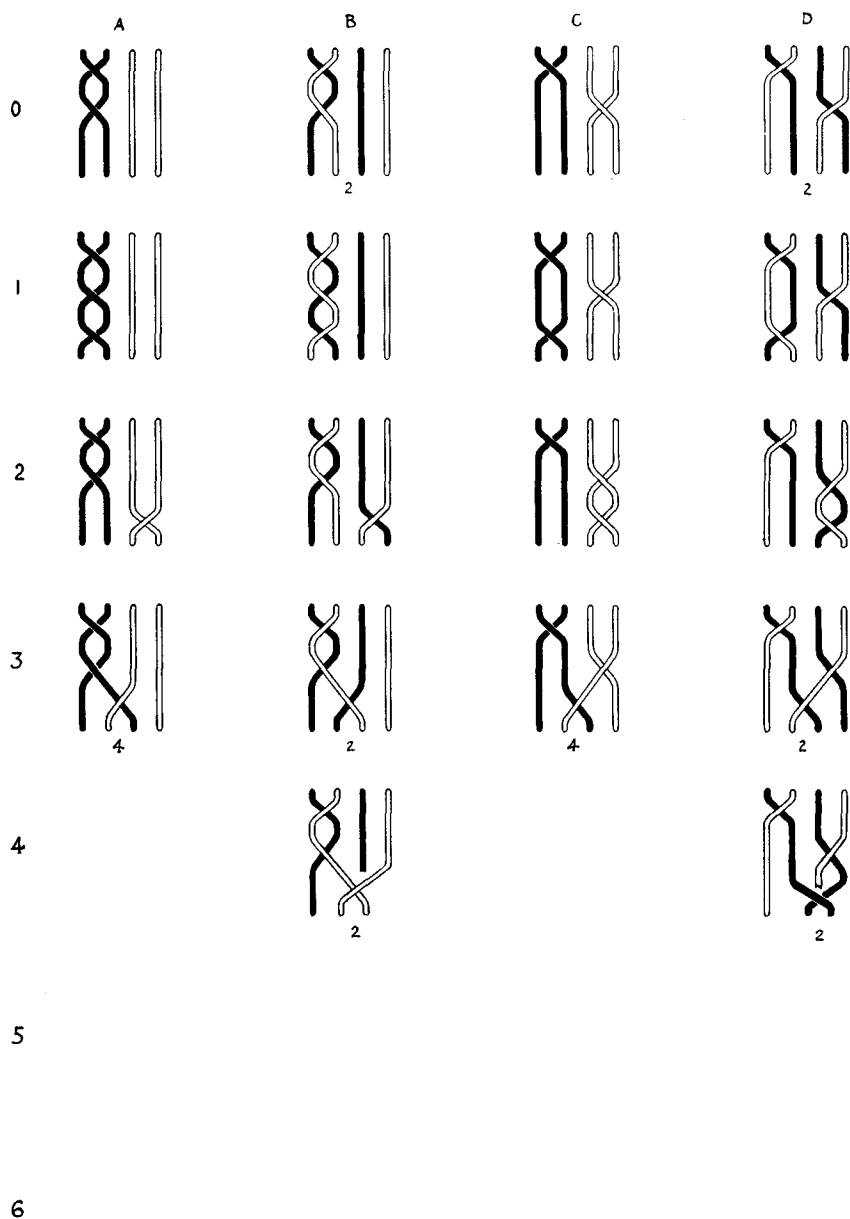
FIGURE 3. Types of double and triple crossing over. In the uppermost horizontal row (marked 0) are shown the types of tetrads of rank 2; the figure beneath each tetrad indicates its relative frequency. Beneath each tetrad of rank 2 are shown (rows 1–6) the tetrads that result when crossing over occurs in a third region. The numeral under each tetrad of rank 3 indicates its relative frequency among tetrads of rank 3 in the same vertical column; its frequency among all tetrads of rank 3 is the product of this number by the frequency of the tetrad of rank 2 from which it is derived. Where no number is indicated, 1 is understood. All frequencies in this figure are based on the assumption of random occurrence and recurrence.
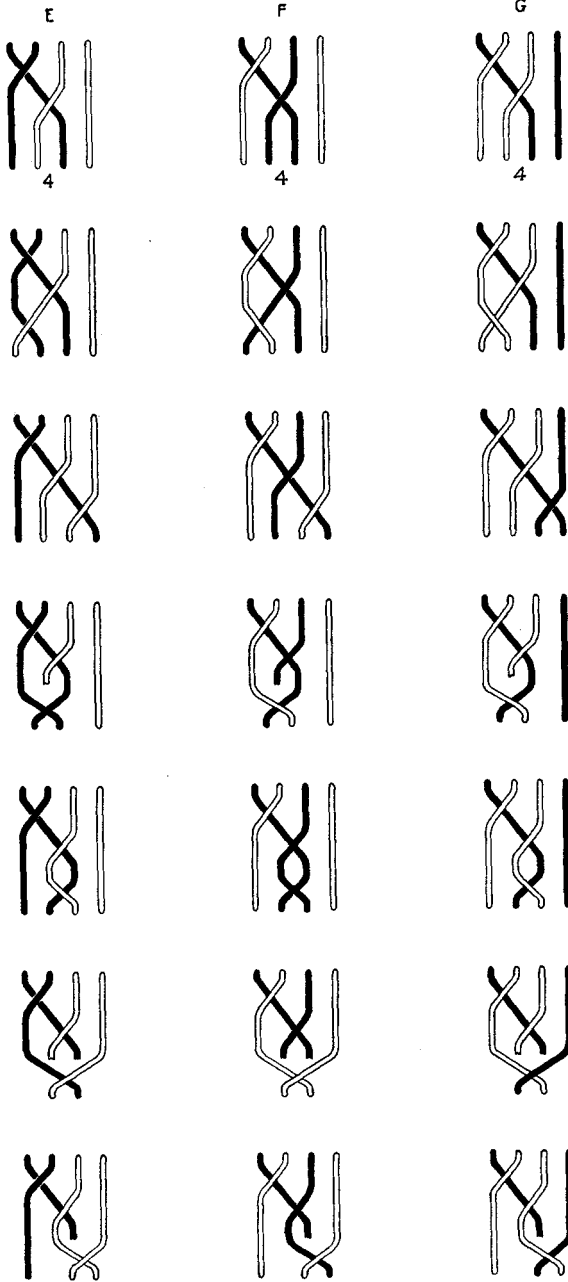
strands, only the other two-thirds will be recognizable. Hence the chance that a tetrad of rank 1 will give rise to a chromatid recognizable as a single crossover will be $1/2 \cdot 2/3 = 1/3$; that is, the observed frequency of crossing over in any region short enough to have only one crossing over at a time will be one-third of the true frequency.

If recurrence is random, the chances of detecting crossing over at different levels are independent. Hence the chance that a tetrad of rank 2 will result in an individual recognizable as a double crossover will be $1/3 \cdot 1/3 = 1/9$; the chance that a tetrad of rank 3 will result in an individual recognizable as a triple crossover will be $(1/3)^3 = 1/27$; and in general the chance that a tetrad of rank r will result in an individual recognizable as an r-ple crossover will be $(1/3)^r$.

The chance that a tetrad of rank 2 will result in an individual which is a recognizable crossover only in the first region is $1/3 \cdot 2/3 = 2/9$; and this is also the chance that the tetrad will give rise to an individual which is a recognizable crossover in the second region only. The chance that an individual will emerge which is a non-crossover or an apparent non-crossover will be $2/3 \cdot 2/3 = 4/9$.

Of strands derived from tetrads of rank 3, those that are recognizable as triple crossovers will be $(1/3)^3 = 1/27$; those that are recognizable as crossovers in the first two regions only will be $(1/3)^2 \, 2/3 = 2/27$, and this will also be the frequency of strands that are recognizable crossovers in regions 1 and 3 only, or in 2 and 3 only. The frequency of recognizable crossovers in region 1 only will be $1/3 \, (2/3)^2$; and this will also be the frequency of recognizable crossovers in region 2 only, or in region 3 only. Finally, the frequency of non-crossovers and apparent non-crossovers together will be $(2/3)^3 = 8/27$.

In general, if a tetrad is of rank r, the chance that it will give rise to a chromatid which is a recognizable crossover in some specified k of the r regions will be $(1/3)^k \, (2/3)^{r-k}$.

By this method we can deduce from a set of crossover data the frequencies of tetrads of different classes. In table 1, the experimental data in the first line are taken from a cross of BRIDGES (cited by WEINSTEIN 1918). Since each individual must have been derived from a tetrad of at least its own rank, the triples must be derived from tetrads of rank 3; for there were probably no tetrads of higher rank, because each of the three regions is too short to allow more than single crossing over within itself.

The one triple in the experimental data must represent 27 tetrads of rank 3. These tetrads must have produced in addition to the 1 triple, 2 individuals in each of the three rank-2 classes, 4 individuals in each class of rank 1, and 8 non-crossovers. If we subtract each of these from the total

in the corresponding class, the remainder represents those individuals in that class derived from tetrads of rank lower than 3.

We now turn to the 2, 3 class. The remainder in this class must be derived from tetrads that were crossovers in regions 2 and 3 only. From these tetrads emerged 41 individuals of class 2, 3; 82 that were crossovers in region 3 only, 82 that were crossovers in region 2 only, and 164 non-crossovers. In a similar way, we can calculate the individuals derived from the 1, 3 and the 1, 2 tetrads; and if we subtract all the individuals derived from tetrads of rank 2, the remainders represent the individuals derived from tetrads of lower ranks. The individuals in each crossover class of rank 1 constitute only 1/3 of those derived from the tetrads of the same class; the other 2/3 must have been non-crossovers and must be subtracted from the observed non-crossovers.

TABLE 1

Frequencies of tetrads calculated for random association including sister-strand crossing over.

REGIONS OF CROSSING OVER	0	1	2	3	12	13	23	123
Observed frequencies	9927	1949	1664	1651	88	207	43	1
Frequencies and distribution of tetrads of rank 3	8	4	4	4	2	2	2	*1*
Remainders	9919	1945	1660	1647	86	205	41	
Frequencies and distribution of tetrads of rank 2	164		82	82			*41*	
	820	410		410		*205*		
	344	172	172		*86*			
Remainders	8591	1363	1406	1155				
Frequencies and distribution of tetrads of rank 1	2310			*1155*				
	2812		*1406*					
	2726	*1363*						
Remainder	*743*							
Corrected frequencies of tetrads	743	4039	4218	3465	774	1845	369	27

Thus we arrive at the italicized figures along the diagonal, which give the number of individuals of each class derived from tetrads of the same class.

These frequencies however are not the frequencies of the tetrads of each class; for, as we have seen, in a tetrad of rank r, only $(1/3)^r$ of the emerging strands are of the same rank, r. Hence it is necessary to multiply the remainder in each single crossover class by 3, in each double crossover class by 9, in each triple crossover class by 27. The results, in the lowest horizontal line, give the true number of tetrads in each class on the assumption of random occurrence and recurrence with sister-strand crossing over.

B. No sister-strand crossing over

If we assume that there is no crossing over between sister strands but that otherwise association of strands is random, crossing over may occur in one of four ways at any level (ab, ab', a'b, a'b', where a is the sister strand of a' and b of b'). The chance that the strand recovered is a crossover is $1/2$, and this is also the chance of detecting the crossing over at that level in the tetrad, since every crossing over is recognizable once it is obtained.

Of the strands derived from a tetrad of rank r, those that are also of rank r will be $(1/2)^r$, those that are crossovers in any $(r-1)$ specified regions will be $(1/2)^{r-1} \cdot 1/2$, and so forth. In general the chance that a tetrad of rank r will give rise to a strand which is a crossover in some specified k of the r regions will be $(1/2)^k \, (1/2)^{r-k} = (1/2)^r$. That is, all the classes derived from tetrads of a given kind occur with equal frequency; since the frequency is independent of k, the number of regions in which the strands are crossovers.

This procedure is illustrated in table 2.

TABLE 2

Frequencies of tetrads calculated for random association without sister-strand crossing over.

REGIONS OF CROSSING OVER	0	1	2	3	12	13	23	123
Observed frequencies	9927	1949	1664	1651	88	207	43	1
Frequencies and distribution of tetrads of rank 3	1	1	1	1	1	1	1	*1*
Remainders	9926	1948	1663	1650	87	206	42	
Frequencies and distribution of tetrads of rank 2	42		42	42			*42*	
	206	206		206		*206*		
	87	87	87		*87*			
Remainders	9591	1655	1534	1402				
Frequencies and distribution of tetrads of rank 1	1402			*1402*				
	1534		*1534*					
	1655	*1655*						
Remainder	*5000*							
Corrected frequencies of tetrads	5000	3310	3068	2804	348	824	168	8

The remainder in each class will now have to be multiplied by 2^r, where r is the rank of the class, to give the number of tetrads of that class.

C. The general case

It is conceivable that sister chromatids cross over with a frequency which differs from what it would be on a random basis but is not 0. The chance of detecting a crossing over in a region would then be neither 1/3

nor $1/2$; let it be designated by p. Then if p has the same value in every region of the chromosome, the frequencies of tetrads can still be calculated by the method explained above.

A formula for this procedure can be derived as follows.

Let the chromosome be divided into regions so short that no crossing over of rank higher than 1 occurs within each. Let $a_0, a_1, a_2, \cdots a_n$ be the observed frequencies of individuals that are crossovers in $0, 1, 2, \cdots n$ regions. It should be noted that each subscript represents the cardinal number of crossings over, not the ordinal number of the region in which crossing over takes place. Let $x_0, x_1, x_2, \cdots x_n$ represent the number of tetrads that are crossovers in $0, 1, 2, \cdots n$ regions.

The chance that a tetrad of rank r will give rise to a strand which is a crossover in some specified k of these r regions is $p^k (1-p)^{r-k}$, or $p^k q^{r-k}$ where $q = 1-p$. The number of ways in which these k regions can be specified is $[r(r-1)(r-2) \cdots (r-k+1)]/(1 \cdot 2 \cdot 3 \cdot \cdots k)$. Hence the total chance of obtaining a crossover strand of rank k from a tetrad of rank r is the product of these two expressions.

We can now form the following equations.

$$a_0 = x_0 + qx_1 + q^2x_2 + q^3x_3 + q^4x_4 + q^5x_5 + \cdots + q^nx_n$$
$$a_1 = px_1 + 2pqx_2 + 3pq^2x_3 + 4pq^3x_4 + 5pq^4x_5 + \cdots + npq^{n-1}x_n$$
$$a_2 = p^2x_2 + 3p^2qx_3 + 6p^2q^2x_4 + 10p^2q^3x_5 + \cdots + C_2^np^2q^{n-2}x_n$$
$$a_3 = p^3x_3 + 4p^3qx_4 + 10p^3q^2x_5 + \cdots + C_3^np^3q^{n-3}x_n$$
$$a_4 = p^4x_4 + 5p^4qx_5 + \cdots + C_4^np^4q^{n-4}x_n$$
$$a_5 = p^5x_5 + \cdots + C_5^np^5q^{n-5}x_n$$
$$\cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots$$
$$a_n = p^nx_n$$

In these $(n+1)$ equations there are $(n+1)$ unknowns, the x's; for the a's are observed frequencies and p is determined by the assumptions as to the chance of detecting a crossing over within a region. Hence if the equations are solved simultaneously, the x's can be evaluated.

But the value of x_0 can be obtained without solving for the other x's. For if the equations are multiplied respectively by 1, $-q/p$, q^2/p^2, $-q^3/p^3$, $\cdots$ $(-q/p)^n$, and then added together, the coefficients of every x except x_0 will add up to 0. Hence

$$x_0 = a_0 - \frac{q}{p}a_1 + \left(\frac{q}{p}\right)^2 a_2 - \left(\frac{q}{p}\right)^3 a_3 + \cdots + \left(-\frac{q}{p}\right)^n a_n. \qquad (1)$$

In this procedure a_0 has been used to represent the frequency of the non-crossovers. But it may be used for the frequency of any class; for ex-

ample, the crossovers in region 1, or the crossovers in regions 1 and 3, provided that the classes which are not crossovers in these regions are excluded. Then in the data so selected, a_1, a_2, a_3, $\cdots$ a_n represent the frequencies of the classes that are crossovers in the same region or regions as the a_0 class and in 1, 2, 3, $\cdots$ n additional regions; and x_0, x_1, x_2, x_3, $\cdots$ x_n are the frequencies of the tetrads which are crossovers in the same regions as the a_0 class and in 0, 1, 2, 3, $\cdots$ n additional regions.

The solution for x_0 will now give the number of tetrads of any specified class that gave rise to strands of the same class. But (unless this class is the non-crossovers) x_0 is not the total number of tetrads of the class; for there must have been others that gave rise to strands of lower rank and these have been excluded from the calculations. Since the proportion of tetrads of rank r that give rise to strands of the same rank is p^r, it is necessary to multiply x_0 by $1/p^r$ to get the number of all the tetrads of the class in question. This frequency is therefore

$$X = \frac{1}{p^r} x_0 = \frac{1}{p^r}\left[a_0 - \frac{q}{p} a_1 + \left(\frac{q}{p}\right)^2 a_2 - \left(\frac{q}{p}\right)^3 a_3 + \cdots + \left(-\frac{q}{p}\right)^n a_n\right]$$

$$= \frac{1}{p^r}\left[a_0 - \left(\frac{1-p}{p}\right) a_1 + \left(\frac{1-p}{p}\right)^2 a_2\right.$$

$$\left. - \left(\frac{1-p}{p}\right)^3 a_3 + \cdots + \left(-\frac{1-p}{p}\right)^n a_n\right]. \tag{2}$$

where a_0 is the observed frequency of the class in question, and a_1, a_2, a_3, $\cdots$ a_n the observed frequencies of classes of additional rank 1, 2, 3, $\cdots$ n (WEINSTEIN 1928, 1932a).

For random occurrence and recurrence with sister strand crossing over, $p = 1/3$ and the formula becomes

$$X = 3^r x_0 = 3^r\left[a_0 - 2a_1 + 4a_2 - 8a_3 + \cdots + (-2)^n a_n\right]. \tag{2a}$$

If sister-strand crossing over is entirely excluded but occurrence and recurrence are otherwise random $p = 1/2$ and the formula becomes

$$X = 2^r x_0 = 2^r\left[a_0 - a_1 + a_2 - a_3 + \cdots + (-1)^n a_n\right]. \tag{2b}$$

Case 2. Recurrence random, chance of detecting crossing over variable.

In case 1, it was assumed that p, the chance of detecting crossing over, is the same for all regions. It is theoretically possible however that the chance is not invariant, for sister chromatids might cross over in some regions and not in others, or more frequently in some regions than in others. Such differences might be caused by local conditions like proximity to the

end of the chromosome or to the spindle fibre or to inert regions or perhaps to particular genes.

In such cases we may designate the chance of detecting crossing over in regions 1, 2, 3, $\cdots$ n as p_1, p_2, p_3, $\cdots$ p_n. Let $q_1 = 1 - p_1$, $q_2 = 1 - p_2$, $q_3 = 1 - p_3$, $\cdots$ $q_n = 1 - p_n$.

If recurrence is random, the chance that a tetrad of rank r will give rise to a strand which is a crossover in some specified k of the r regions is the product of the p's for the k regions and the q's for the remaining regions. Thus the chance that a tetrad which is a crossover in regions 1, 2, and 3 will give rise to a strand which is a crossover only in regions 1 and 3 is $p_1 p_3 q_2$.

The frequencies of tetrads can now be calculated as in table 3.

TABLE 3

Frequencies of tetrads calculated for random association except that sister strands cross over only in region 1.

REGIONS OF CROSSING OVER	0	1	2	3	12	13	23	123
Observed frequencies	9927	1949	1664	1651	88	207	43	1
Frequencies and distribution of tetrads of rank 3	2	1	2	2	1	1	2	*1*
Remainders	9925	1948	1662	1649	87	206	41	
Frequencies and distribution of tetrads of rank 2	41		41	41				*41*
	412	206		412		206		
	174	87	174		87			
Remainders	9298	1655	1447	1196				
Frequencies and distribution of tetrads of rank 1	1196				*1196*			
	1447		*1447*					
	3310	*1655*						
Remainder	*3345*							
Corrected frequencies of tetrads	3345	4965	2894	2392	522	1236	164	12

A formula for this procedure can also be deduced.

Let the observed frequency of the non-crossovers be represented by b_0; of the singles by b_1, b_2, b_3, $\cdots$ b_n; of the doubles by b_{12}, b_{13}, $\cdots$ b_{23}, b_{24}, $\cdots$ b_{34}, $\cdots$ $b_{(n-1)n}$; and similarly for the remaining classes. Each subscript now represents not, as before, the cardinal number of crossings over, but the ordinal numbers of the regions in which crossing over occurs. The frequencies of tetrads of various classes can be denoted by y with corresponding subscripts; thus y_0 is the frequency of non-crossover tetrads, y_1 of tetrads that are crossovers in region 1 only, and so forth.

We can now form a set of equations analogous to those in case 1. For

the sake of simplicity the equations will be given for only three regions; but the method is applicable to any number of regions.

$$b_0 = y_0 + q_1 y_1 + q_2 y_2 + q_3 y_3 + q_1 q_2 y_{12} + q_1 q_3 y_{13} + q_2 q_3 y_{23} + q_1 q_2 q_3 y_{123}$$

$$b_1 = \quad\quad p_1 y_1 \quad\quad\quad\quad\quad + p_1 q_2 y_{12} + p_1 q_3 y_{13} \quad\quad\quad + p_1 q_2 q_3 y_{123}$$

$$b_2 = \quad\quad\quad p_2 y_2 \quad\quad + p_2 q_1 y_{12} \quad\quad\quad\quad + p_2 q_3 y_{23} + p_2 q_1 q_3 y_{123}$$

$$b_3 = \quad\quad\quad\quad p_3 y_3 \quad\quad\quad + p_3 q_1 y_{13} + p_3 q_2 y_{23} + p_3 q_1 q_2 y_{123}$$

$$b_{12} = \quad\quad\quad\quad\quad\quad p_1 p_2 y_{12} \quad\quad\quad\quad\quad + p_1 p_2 q_3 y_{123}$$

$$b_{13} = \quad\quad\quad\quad\quad\quad\quad\quad p_1 p_3 y_{13} \quad\quad\quad + p_1 p_3 q_2 y_{123}$$

$$b_{23} = \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad p_2 p_3 y_{23} + p_2 p_3 q_1 y_{123}$$

$$b_{123} = \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad p_1 p_2 p_3 y_{123}$$

These equations can now be solved for the y's. But again it is possible to obtain the value of y_0 without solving for the other y's; for if the equations are multiplied respectively by

$$1, \ -\frac{q_1}{p_1}, \ -\frac{q_2}{p_2}, \ -\frac{q_3}{p_3}, \ \frac{q_1 q_2}{p_1 p_2}, \ \frac{q_1 q_3}{p_1 p_3}, \ \frac{q_2 q_3}{p_2 p_3}, \ -\frac{q_1 q_2 q_3}{p_1 p_2 p_3},$$

and then added together, the coefficients of the y's in every column, except y_0 will add up to 0. Hence

$$y_0 = b_0 - \left(\frac{q_1}{p_1} b_1 + \frac{q_2}{p_2} b_2 + \frac{q_3}{p_3} b_3 \right) + \left(\frac{q_1 q_2}{p_1 p_2} b_{12} + \frac{q_1 q_3}{p_1 p_3} b_{13} + \frac{q_2 q_3}{p_2 p_3} b_{23} \right) \quad (3)$$

$$- \frac{q_1 q_2 q_3}{p_1 p_2 p_3} b_{123}.$$

If the b_0 class is of rank 0, then y_0 is the number of non-crossover tetrads. But as in case 1, the method can be applied to part of the data: then b_0 is the frequency of a crossover class and y_0 is the number of tetrads of this class that gave rise to chromatids of the same class. Hence y_0 must be divided by the product of the p's for the regions in which the class is a crossover. These regions are not any of those numbered from 1 to 3, for the b_0 or y_0 class is not a crossover in 1 or 2 or 3. Hence the regions in which the class is a crossover can be numbered separately from 1 to r, where r is the rank of the class. The chance of detecting crossing over in each of these r regions may be denoted by p' with the proper subscript; and the total number of tetrads of the class now becomes

$$X = \frac{1}{p_1' p_2' p_3' \cdots p_r'} y_0. \quad (4)$$

If the equations had included n regions, the general formula for the number of tetrads of any class would be

$$X = \frac{1}{p_1' p_2' p_3' \cdots p_r'} \left[b_0 - \left(\frac{q_1}{p_1} b_1 + \frac{q_2}{p_2} b_2 + \cdots + \frac{q_n}{p_n} b_n \right) \right.$$

$$+ \left(\frac{q_1 q_2}{p_1 p_2} b_{12} + \frac{q_1 q_3}{p_1 p_3} b_{13} + \cdots + \frac{q_2 q_3}{p_2 p_3} b_{23} + \cdots + \frac{q_{(n-1)} q_n}{p_{(n-1)} p_n} b_{(n-1)n} \right)$$

$$\left. + \cdots + (-1)^n \frac{q_1 q_2 q_3 \cdots q_n}{p_1 p_2 p_3 \cdots p_n} b_{123 \cdots n} \right]. \tag{5}$$

Case 3. Recurrence not random, chance of detecting crossing over constant.

The frequencies of regressive, progressive, and digressive crossing over are not necessarily determined by chance alone: it is conceivable that they may vary with the nature of the crossings over (whether homologous or sister strand, lateral or diagonal), with their distance apart, and with the particular regions involved. They might also depend on other crossings over: their number, their distance away, the regions in which they occur, and their nature (including now not only whether they are homologous or sister-strand, and lateral or diagonal, but also whether they are regressive, progressive, or digressive with respect to the crossings over under consideration).

In making a table like tables 1 and 2, we may therefore subdivide each class of tetrad into its different types (such as are shown in figure 3) and distribute separately the chromatids emerging from each type.

If when we are considering tetrads of a given class we add together all the emerging strands that are crossovers in some specified k of the r regions, the proportion of such strands is no longer $p^r q^{r-k}$. The ratio of the actual proportion to the proportion expected on random recurrence may be designated by t; its value will in the most general case vary with the regions of crossing over in the tetrad and in the emerging chromatid; these regions may therefore be indicated by numerical subscripts, positive if the crossing over of the tetrad appears in the chromatid, negative if it does not. For example, the chance that a tetrad which is a crossover in regions 1, 2, and 3 will give rise to a chromatid which is a crossover in regions 1 and 3 only may be written as $t_{1-2+3} p^2 q$.

In a table like table 1 the proper value of t will enter into the frequency of each class if recurrence is not random; for example, the distribution of tetrads of rank 3 will be as follows:

Regions of crossing over	0	1	2	3	12	13	23	123
Frequencies and distribution of tetrads of rank 3		$8t_{-1-2-3}$	$4t_{1-2-3}$	$4t_{-1+2-3}$	$4t_{-1-2+3}$	$2t_{1+2-3}$	$2t_{1-2+3}$	$2t_{-1+2+3}$	t_{1+2+3}

The x_0 values (the figures on the diagonal) for classes of rank 2 or more must be divided not merely by p^r but by the product of p^r and the ap-

propriate t: the remainder in the 123 column must be divided by $t_{1+2+3}p^3$, that in the 23 column by $t_{2+3}p^2$, and so on.

The t's will also enter into the equations corresponding to those on page 163; this will be considered in connection with the next case. The general formula derived from the equations will now be more complicated; but if ranks above 2 are absent, the frequency of tetrads of rank 0 is

$$y_0 = a_0 - \frac{q}{p}\,a_1 + \frac{t_{1-2}+t_{-1+2}-t_{-1-2}}{t_{1+2}}\,\frac{q^2}{p^2}\,a_2. \tag{6}$$

Case 4. Recurrence not random, chance of detecting crossing over variable.

The same considerations apply to this case as to case 3 and the t factors enter into it in the same way. Thus the chance that a tetrad of rank 3 will give rise to a strand which is a recognizable crossover in regions 1 and 3 only will be $t_{1-2+3}p_1p_3q_2$.

With non-random recurrence, the distribution of tetrads of rank 3 in table 3 would be as follows:

Regions of crossing over	0	1	2	3	12	13	23	123
Frequencies and distribution of tetrads of rank 3	$2t_{-1-2-3}$	t_{1-2-3}	$2t_{-1+2-3}$	$2t_{-1-2+3}$	t_{1+2-3}	t_{1-2+3}	$2t_{-1+2+3}$	t_{1+2+3}

and each x_0 value must be divided by the product of $p_1'p_2' \cdots p_r'$ and the appropriate t.

The equations on page 166 will now become as follows.

$$\begin{aligned}
b_0 &= y_0 + q_1y_1 + q_2y_2 + q_3y_3 + t_{1-2}q_1q_2y_{12} + t_{1-3}q_1q_3y_{13} + t_{-2-3}q_2q_3y_{23} + t_{1-2-3}q_1q_2q_3y_{123}\\
b_1 &= p_1y_1 + t_{1-2}p_1q_2y_{12} + t_{1-3}p_1q_3y_{13} + t_{1-2-3}p_1q_2q_3y_{123}\\
b_2 &= p_2y_2 + t_{-1+2}p_2q_1y_{12} + t_{2-3}p_2q_3y_{23} + t_{-1+2-3}p_2q_1q_3y_{123}\\
b_3 &= p_3y_3 + t_{-1+3}p_3q_1y_{13} + t_{-2+3}p_3q_2y_{23} + t_{-1-2+3}p_3q_1q_2y_{123}\\
b_{12} &= t_{1+2}p_1p_2y_{12} + t_{1+2-3}p_1p_2q_3y_{123}\\
b_{13} &= t_{1+3}p_1p_3y_{13} + t_{1-2+3}p_1p_3q_2y_{123}\\
b_{23} &= t_{2+3}p_2p_3y_{23} + t_{-1+2+3}p_2p_3q_1y_{123}\\
b_{123} &= t_{1+2+3}p_1p_2p_3y_{123}
\end{aligned}$$

The formula for y_0 is now still more complicated; but if crossovers of ranks above 2 are absent or neglected,

$$\begin{aligned}
y_0 = b_0 &- \left(\frac{q_1}{p_1}\,b_1 + \frac{q_2}{p_2}\,b_2 + \cdots + \frac{q_n}{p_n}\,b_n\right)\\
&+ \frac{t_{1-2}+t_{-1+2}-t_{-1-2}}{t_{1+2}}\,\frac{q_1q_2}{p_1p_2}\,b_{12} + \frac{t_{1-3}+t_{-1+3}-t_{-1-3}}{t_{1+3}}\,\frac{q_1q_3}{p_1p_3}\,b_{13} \tag{7}\\
&+ \cdots + \frac{t_{(n-1)-n}+t_{-(n-1)+n}-t_{-(n-1)-n}}{t_{(n-1)+n}}\,\frac{q_{(n-1)}q_n}{p_{(n-1)}p_n}\,b_{(n-1)n}.
\end{aligned}$$

In this formula, as in the equations from which it is derived, y_0 and b_0

represent respectively the number of tetrads and of chromatids of rank 0. If b_0 is used for a class of higher rank, the equations must be modified by omitting every term in which the y is not a crossover in at least the same regions as the b_0 class. The y's can then be renumbered so that the class of lowest rank is y_0, and a general formula can be derived as in cases 1 and 2. This, however, is complicated; and the frequencies of tetrads of classes ranking above 0 can be obtained by solving for the y's in the equations as they stand.

The frequency of chromatids

The observed frequency of any class includes of course only the chromatids that are homologous-strand crossovers in the specified regions. The true frequency of chromatids of the class would include sister-strand crossovers as well.

The relations between tetrad frequencies and true frequencies of chromatids are given by the sets of equations on pages 163, 166, and 168, and by equations 1–7 if the x's and y's retain their original meanings, but in the definitions of the a's, b's, p's, and t's the true frequencies of chromatids are substituted for the observed frequencies. The value of p will now vary from 1/2 when at any level of crossing over only two strands are involved to 1 when four strands are always involved in pairs.

The sets of equations on pages 163, 166, and 168, and equations 1–7 also express the relations between the observed and the true frequencies of chromatids if the a's and b's denote observed frequencies, the x's and y's the true frequencies of chromatids (not of tetrads), if each p denotes what proportion of exchanges are between homologous strands, and if the t's are modified accordingly (WEINSTEIN 1928, 1932a).

Each p as originally defined is of course the product of the p's of the two preceding paragraphs.

ORDINARY DIPLOIDS

The results of applying the multiple-strand method to ordinary diploids are illustrated in tables 4 and 8.

Table 4 is based on a cross involving almost the entire length of the X chromosome of *Drosophila melanogaster (sc ec cv ct v g f)*. The experimental data (column A) comprise 28239 individuals, including 24034 from BRIDGES and OLBRYCHT (1926), 2047 from ANDERSON (1925a, table IV), and 2158 from an experiment (hitherto unpublished) by the writer. In the other columns are given tetrad frequencies calculated on the assumption of random recurrence for various values of p. The column headed $p = 1/2$ is based on the assumption that sister chromatids do not cross over (formula 2b); that headed $p = 1/3$ on the assumption that they cross over as freely

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TABLE 4

Crossing over involving sc ec cv ct v g f (28239 individuals).

REGIONS OF CROSSING OVER	OBSERVED CHROMATID FREQUENCIES* p=2/3	TETRAD FREQUENCIES CALCULATED FOR RANDOM RECURRENCE							
		p=2/3	p=1/2	p=1/3 IN REGIONS INDICATED, 1/2 IN OTHER REGIONS					p=1/3
				1	12	123	1234	12345	
	A	B†	C	D	E	F	G	H	I
0	12776	6716	1709	837	−459	−2007	−4392	−5305	−5316
1	1407	1696	1744	2616	2601	2577	2220	1707	1203
2	2018	2483	2602	2592	3888	3864	3492	2724	1836
3	1976	2649	3130	3112	3096	4644	4527	4116	3546
4	3378	4515	5328	5094	4848	4770	7155	6861	6102
5	2356	2951	3180	2812	2296	2022	1826	2739	2610
6	2067	2284	1998	1626	1016	616	108	22	33
12	9	15	20	30	45	36	45	45	45
13	16	27	36	54	48	72	90	63	54
14	142	291	468	702	702	714	1071	981	846
15	198	430	736	1104	1104	1086	1026	1539	1530
16	206	440	744	1116	1110	1104	1014	1008	1512
23	11	21	36	32	48	72	81	81	99
24	136	291	492	492	738	744	1116	1098	1008
25	261	584	1032	1032	1548	1548	1536	2304	2295
26	318	701	1224	1220	1830	1842	1782	1776	2664
34	42	88	148	152	156	234	351	351	306
35	148	324	560	548	548	822	822	1233	1188
36	212	463	800	792	800	1200	1170	1140	1710
45	123	262	440	400	392	392	588	882	873
46	315	674	1136	1076	1036	1016	1524	1518	2277
56	59	124	204	200	196	176	172	258	387
123	3	7	8	12	18	27			−27
124	1	2				−18	−27	−27	−27
126	2	5	8	12	18				
134		−2	−8	−12	−24	−36	−54	−54	−54
135	3	10	24	36	36	54	54	81	81
136	3	8	16	24	12	18	18	18	27
145	10	34	80	120	120	120	180	270	270
146	15	51	120	180	180	180	270	270	405
156	1	3	8	12	12	12	12	18	27
234	1	2		−8	−12	−18	−27	−27	−27
236		−2	−8	−16	−24	−36	−36	−36	−54
245	2	7	16	16	24	24	36	54	54
246	10	34	80	80	120	120	180	180	270
256	1	3	8	8	12	12	12	18	27
346	5	17	40	40	40	60	90	90	135
356	5	17	40	40	40	60	60	90	135
456	1	3	8	8	8	8	12	18	27
1234	1	5	16	24	36	54	81	81	81
1236	1	5	16	24	36	54	54	54	81

† In the p=2/3 column, the only one in which fractional values occur, they are given to the nearest unit. The values in the p=2/3 column also represent the true frequencies of chromatids when p=1/3.

* From BRIDGES and OLBRYCHT 1926, ANDERSON 1925a, and WEINSTEIN.

as other chromatids (formula 2a). In the latter column the frequency of the non-crossover class is negative; and since this is impossible, it follows that there cannot be random association of chromatids with free crossing over between sister strands.

The results are of course subject to errors of sampling as well as errors due to differential viability and other causes. These errors may become exaggerated in the course of the calculations because the observed frequencies of the crossovers of higher rank are small and are multiplied by relatively large factors, so that slight differences may be magnified. For this reason the negative frequencies among the crossovers (they are all among the triples) are obviously not significant, for they would become 0 or positive with small changes in the observed numbers of triples or quadruples, or in some cases if the quadruples were derived from tetrads of rank 5.

The errors of sampling may be calculated by the formula for the standard error of a function of several variables, which may be written

$$E = \sqrt{\left(\frac{\partial F}{\partial v_0}\right)^2 e_0{}^2 + \left(\frac{\partial F}{\partial v_1}\right)^2 e_1{}^2 + \left(\frac{\partial F}{\partial v_2}\right)^2 e_2{}^2 + \cdots + \left(\frac{\partial F}{\partial v_n}\right)^2 e_n{}^2}$$

where F is a function of the v's, E its standard error, and the e's are the standard errors of the respective v's. This formula holds for all cases where the distribution of errors is Gaussian (SCARBOROUGH 1930, pp. 337–338). For F we may substitute the tetrad frequency X; and for the v's we may substitute the a's of formula 2 or 2a or 2b, or the b's of formula 5.

The frequency of the non-crossovers in the $p = 1/3$ column is approximately 20 times its standard error, which is 268. The result cannot therefore be due to errors of sampling. Nor can it be due to differential viability, for viability was good in the experiments on which the calculations are based.

We may conclude that sister strands do not cross over as freely as homologous strands if the association of chromatids in crossing over is otherwise random. It does not follow however that they do not cross over at all: they might cross over only in some regions, or throughout the chromosome but to a smaller extent than homologous strands.

To test the first of these alternatives, tetrad frequencies were calculated by means of formula 5 for $p = 1/3$ (free sister-strand crossing over) in some regions and $p = 1/2$ (no sister-strand crossing over) in others. The results are given in columns D–H of table 4. As sister-strand crossing over is restricted to a shorter and shorter region at the left of the X chromosome, the negative frequencies approach 0 and finally become positive. The negative frequencies are from about 10 to about 20 times their standard errors, except -459, which is about 2.5 times its standard error. Thus it is shown

that free crossing over between sister chromatids, if it occurs at all, must be limited to a short region. This is not necessarily at the left end of the X, for the results are similar if we postulate sister-strand crossing over in other regions.

There remains the possibility that recurrence is not random. This can be tested by seeing whether the relative frequencies of tetrads can be altered without changing the frequencies of strands as given by experiment.

TABLE 5

Frequencies of tetrads of ranks 0, 1, and 2 and of strands derived from them, on the assumption of random association without sister-strand crossing over $(p=1/2)$.

RANK AND TYPE OF TETRAD	FREQUENCY OF TETRAD	FREQUENCIES OF STRANDS		
		NON-CROSSOVERS	SINGLES	DOUBLES
0	1709	1709		
1	17982	$1/2=8991$	$1/2=8991$	
2				
$1/4=$ regressives	2019	$1/2=1009.5$		$1/2=1009.5$
$1/2=$ progressives	4038	$1/4=1009.5$	$1/2=2019$	$1/4=1009.5$
$1/4=$ digressives	2019		2019	

Table 5 gives the frequencies of tetrads of ranks 0, 1, and 2 and of strands derived from them on the assumption of random association without sister-strand crossing over. The following equations indicate what combinations of tetrads are equivalent with respect to the strands derived from them:

$$1 \text{ non-crossover } +1 \text{ digressive} = 2 \text{ singles.} \tag{8}$$
$$1 \text{ regressive } +1 \text{ digressive} = 2 \text{ progressives.} \tag{9}$$
$$1 \text{ non-crossover } +2 \text{ progressives} = 1 \text{ regressive } +2 \text{ singles.} \tag{10}$$

Still other substitutions are possible if ranks above 2 are included; for example,

$$1 \text{ single } +1 \text{ regressive } = 1 \text{ non-crossover } +1 \text{ digressive}$$
$$\text{(or regressive-digressive) of rank 3.} \tag{11}$$

By a rank-3 digressive is meant a tetrad like D1 in figure 3; by a rank-3 regressive-digressive is meant one like D2 or B2.

In making these substitutions, it is necessary to consider the regions of crossing over, so that the proper classes and frequencies of strands may result; also in order to avoid digressives with two crossings over in the same region, unless it is desired to test the possibility of such digressives. The substitutions are also limited by the frequencies of tetrads to be replaced; hence it follows that deviations from random recurrence will, if too great, lead to negative frequencies of tetrads. Nevertheless considerable deviations are possible without changes in the frequencies of strands;

but coincidence of tetrads will be altered, as will also the frequencies of progeny of attached X's. These results will be considered below.

Equations 8 and 11 hold when sister strands cross over freely; the other equations now assume the following forms:

$$\text{1 regressive } +3 \text{ digressives} = 4 \text{ progressives.} \tag{12}$$

$$\text{3 non-crossovers } +4 \text{ progressives} = 1 \text{ regressive } +6 \text{ singles} \tag{13}$$

If sister strands cross over freely, then as can be seen from table 6, random recurrence results in an excess of tetrads of ranks higher than 0 and of non-crossover strands derived from them, so that a negative frequency of tetrads of rank 0 must be postulated to bring the total number of tetrads and of non-crossover strands down to the observed figure. The negative frequency will disappear if non-random recurrence can reduce by 5316 the number of those tetrads of ranks higher than 0 that give rise to non-crossover strands. The simplest way to do this is to replace 10632 tetrads of rank 1 by their equivalent 5316 non-crossover tetrads and 5316 digressives, in accordance with equation 8.

TABLE 6

Frequencies of tetrads of ranks 0, 1, and 2 and of strands derived from them on the assumption of random association including sister-strand crossing over ($p = 1/3$).

RANK AND TYPE OF TETRAD	FREQUENCY OF TETRAD	FREQUENCIES OF STRANDS		
		NON-CROSSOVERS	SINGLES	DOUBLES
0	-5316	-5316		
1	15330	$2/3 = 10220$	$1/3 = 5110$	
2				
$1/6 =$ regressive	2799	$2/3 = 1866$		$1/3 = 933$
$2/3 =$ progressive	11196	$5/12 = 4665$	$1/2 = 5598$	$1/12 = 933$
$1/6 =$ digressive	2799	$1/3 = 933$	$2/3 = 1866$	

TABLE 7

Frequencies of tetrads of ranks 0, 1, and 2 and of strands derived from them on the assumption of non-random association with sister strand crossing over ($p = 1/3$). (For explanation see text.)

RANK AND TYPE OF TETRAD	FREQUENCY OF TETRAD	FREQUENCIES OF STRANDS		
		NON-CROSSOVERS	SINGLES	DOUBLES
0	0	0		
1	4698	3132	1566	
2				
regressive	2799	1866		933
progressive	11196	4665	5598	933
digressive	8115	2705	5410	

The tetrad and strand frequencies as revised by this method are given in Table 7. They may be modified by other substitutions.

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TABLE 8

Crossing over involving sc ec cv ct v s f car bb (16136 individuals).

REGIONS OF CROSSING OVER	OBSERVED CHROMATIN FREQUENCIES*	p=2/3	p=1/2	TETRAD FREQUENCIES CALCULATED FOR RANDOM RECURRENCE p=1/3 in regions indicated, 1/2 in other regions							p=1/3
				1	12	123	1234	12345	123456	1234567	
	A	B†	C	D	E	F	G	H	I	J	K
0	6607	3300	904	697	175	−301	−1161	−1547	−1683	−1462	−1339
1	506	530	414	621	618	609	501	357	18	−120	−204
2	1049	1167	1046	1044	1566	1566	1497	1266	624	327	189
3	855	996	960	952	952	1428	1371	1230	717	483	399
4	1499	1817	1876	1804	1758	1720	2580	2466	1914	1494	1305
5	937	1143	1196	1096	942	848	772	1158	1080	981	903
6	1647	1867	1710	1480	1036	692	324	272	408	363	333
7	683	661	420	320	100	−66	−346	−412	−442	−663	−651
8	379	350	202	140	24	−42	−180	−234	−254	−246	−369
12	3	4	4	6	9	0	0	0	−9	−9	−9
13	6	11	16	24	18	27	27	18	18	18	18
14	41	87	144	216	216	216	324	315	306	288	279
15	55	118	200	300	300	294	288	432	432	414	405
16	118	262	460	690	684	684	678	678	1017	1017	1008
17	54	117	200	300	300	300	288	276	276	414	414
18	34	73	124	186	186	186	180	174	168	168	252
23	3	4	4	0	0	0	0	0	−9	−9	−9
24	38	69	92	92	138	138	207	207	171	126	72
25	85	182	308	308	462	462	462	693	666	630	621
26	237	517	892	888	1332	1326	1302	1284	1926	1908	1881
27	123	262	440	440	660	660	630	606	594	891	873
28	70	144	232	232	348	348	312	306	288	276	414
34	22	46	76	76	76	114	171	171	171	153	144
35	55	116	192	188	188	282	282	423	414	396	369
36	177	394	692	692	688	1032	1032	1026	1539	1530	1521
37	88	192	332	332	332	498	486	474	468	702	702
38	38	80	132	132	132	198	192	174	168	168	252
45	41	90	156	152	152	152	228	342	342	342	333
46	198	435	756	752	736	736	1104	1104	1656	1656	1620
47	159	346	596	588	568	560	840	840	840	1260	1251
48	91	189	308	304	280	276	414	408	384	378	567
56	35	73	120	120	108	104	104	156	234	234	225
57	49	101	164	156	140	132	132	198	198	297	297
58	40	82	132	128	124	112	108	162	156	156	234
67	21	44	72	72	64	60	60	60	90	135	135
68	30	56	80	76	64	60	44	40	60	60	90
78	2	1	−4	−4	−12	−12	−16	−16	−16	−24	−36

* From BRIDGES. † See footnote to table 4.

TABLE 8 (*Continued*)

Crossing over involving sc ec cv ct v s f car bb (16136 individuals).

	A	B	C	D	E	F	G	H	I	J	K
123	1	3	8	12	18	27	27	27	27	27	27
126	1	3	8	12	18	18	18	18	27	27	27
135	1	3	8	12	12	18	18	27	27	27	27
145	1	3	8	12	12	12	18	27	27	27	27
146	1	3	8	12	12	12	18	18	27	27	27
147	2	7	16	24	24	24	36	36	36	54	54
148	1	3	8	12	12	12	18	18	18	18	27
157	2	7	16	24	24	24	24	36	36	54	54
158	1	3	8	12	12	12	12	18	18	18	27
168	1	3	8	12	12	12	12	12	18	18	27
236	1	3	8	8	12	18	18	18	27	27	27
246	4	13	32	32	48	48	72	72	108	108	108
247	5	17	40	40	60	60	90	90	90	135	135
248	6	20	48	48	72	72	108	108	108	108	162
256	3	10	24	24	36	36	36	54	81	81	81
257	4	13	32	32	48	48	48	72	72	108	108
258	1	3	8	8	12	12	12	18	18	18	27
267	2	7	16	16	24	24	24	24	36	54	54
268	3	10	24	24	36	36	36	36	54	54	81
278	2	7	16	16	24	24	24	24	24	36	54
347	2	7	16	16	16	24	36	36	36	54	54
348	1	3	8	8	8	12	18	18	18	18	27
356	1	3	8	8	8	12	12	18	27	27	27
357	2	7	16	16	16	24	24	36	36	54	54
358	3	10	24	24	24	36	36	54	54	54	81
367	1	3	8	8	8	12	12	12	18	27	27
368	1	3	8	8	8	12	12	12	18	18	27
458	1	3	8	8	8	8	12	18	18	18	27
468	4	13	32	32	32	32	48	48	72	72	108
478	1	3	8	8	8	8	12	12	12	18	27
568	1	3	8	8	8	8	8	12	18	18	27

In the above analysis it has been assumed that while the relative frequencies of tetrads may differ, within each type of tetrad the frequencies of strands that are recognizable as crossovers in any specified regions are the same as when recurrence is random. This is necessarily true when sister strands do not cross over, but otherwise need not be true for all tetrads. Hence substitutions differing from those given above might be possible.

The results of non-random recurrence can also be tested by means of the formulas for cases 3 and 4. For the frequency of tetrads of rank 0, formula 6 will be sufficient, at any rate for purposes of illustration.

An increase of regressives, or of progressives at the expense of digressives, will decrease t_{1-2} and t_{-1+2} and will increase t_{1+2} and t_{-1-2}. This will lessen the value of the term in a_2 and consequently of y_0; hence there will be even more negative tetrads if sister strands cross over. An increase of digressives, or of progressives at the expense of regressives, will increase t_{1-2} and t_{-1+2} and will decrease t_{1+2} and t_{-1-2}. This will increase the value of the term in a_2 and if the increase is sufficient the negative frequency of non-crossover tetrads will disappear.

Other classes can be evaluated in the same way; and the method is essentially equivalent to the analysis already given. For some purposes it may be preferable, since it can be used more systematically; for example, the t's may be made to vary according to some rule, such as that $t_{1+2+3} = t_{1+2}t_{2+3}$, and the correctness of the rule can thus be tested.

The analysis of the Xple data is supported by the results of applying the multiple-strand theory to a cross involving the genes *sc ec cv ct v s f car bb*, which cover practically the entire crossover map of the X chromosome (table 8). The observed frequencies (column A) are from an experiment made by DR. C. B. BRIDGES, who kindly placed them at my disposal before they were published (MORGAN, BRIDGES, and SCHULTZ 1935).

Negative values appear in the non-crossover tetrads as well as some of those of rank 1 when sister strands are allowed to cross over beyond the first two regions.

Here the negative values cannot be eliminated so simply as in the X-ple cross.

The analysis is further supported by the application of the theory to other data, both sex-linked and autosomal, in *Drosophila melanogaster* and to sex-linked data in *D. virilis*. These results will be published elsewhere.

The maximum amount of recombination between two linked genes

In most organisms the amount of recombination between two linked genes approaches 50 percent as an upper limit as the intermediate distance lengthens. This relation is a corollary of equation 2b, which can be written

$$(a_0 + a_2 + a_4 + \cdots) - (a_1 + a_3 + a_5 + \cdots) = 2^r X.$$

If the assumptions on which the equation is based are correct, then $2^r X$ cannot be negative. Hence $(a_0 + a_2 + a_4 + \cdots) \geqq (a_1 + a_3 + a_5 + \cdots)$; that is, the apparent non-crossovers (classes of 0 and even rank) will equal or exceed the apparent crossovers (classes of odd rank).

This relation can be deduced directly from the set of equations on page 163; as in fact it has been deduced by EMERSON and RHOADES (1933) from the table of BELLING (1931) which corresponds to a special case of these equations. In each vertical column of the equations the coefficients of the

x's are the successive terms of the expansion $(p+q)^r$, where $q = 1-p$. If $p = 1/2$, the sum of the alternate terms is $1/2$; and in any column (except the x_0 column) the strands with no crossings over or with an even number will constitute half the total. These strands together with those in the x_0 column make up the apparent non-crossovers; hence the observed recombination frequency will be less than 50 percent. As the chromosome lengthens genetically, the x_0 class will decrease and approach 0 and the proportion of recombination between the end genes will approach 50 percent as an upper limit.

A more general relation can be obtained if the equations are multiplied respectively by $1, -1, 1, \cdots (-1)^n$ and added together. The result is the equation

$$(a_0+a_2+a_4+ \cdots) - (a_1+a_3+a_5+ \cdots)$$
$$= x_0+(q-p)x_1+(q-p)^2x_2+ \cdots +(q-p)^nx_n. \qquad (14)$$

If $p = 1/2$, $q - p = 0$, and we have the case just discussed.

If $p < 1/2$, the right-hand side of the equation will remain greater than 0, and the recombination frequency between the end genes will remain less than 50 percent as n increases.

If $p > 1/2$, the terms in $(q-p)$ will be positive if the exponent is even, negative if it is odd. Hence their sum will be increased by the terms with even exponents, decreased by those with odd exponents. The precise nature of the result will therefore depend on the number of x's and their relative sizes; that is, on the length of the chromosome and the coincidence. This situation would result if more than two chromatids crossed over at one level or within a region.

The relations just deduced are only part of still more general relations. For in the equations on page 163, a_0 may represent the frequency of any class, not necessarily the non-crossovers. It follows that if $p = 1/2$, the sum of the crossovers in any specified region or regions and in $0, 2, 4, \cdots$ additional regions will exceed the sum of the crossovers in the specified region or regions and in $1, 3, 5, \cdots$ additional regions. This relation holds for *Drosophila melanogaster* and also for *D. virilis* (WEINSTEIN, unpublished data involving the X chromosome from sepia to rugose, a distance of about 100 units.

The minimum distance within which double crossing over occurs

An upper limit can be set to the possible frequency of crossing over between sister strands by the length of the shortest distance between adjacent levels of crossing over in a chromatid. In the X chromosome of *D. melanogaster*, this is about 14 units. Since the frequency of tetrad crossing over in such a region cannot exceed 1.00, the chance of detecting a

crossing over, being the ratio of the observed to the actual frequency, cannot be less than 0.14. But the chance of detecting a crossing over in a tetrad is composed of two factors: the chance of recovering a crossover chromosome and the chance of recognizing it when recovered. The first factor is 1/2 (provided that only two strands cross over within the region); hence the second is not less than 0.28. That is, crossover strands can be recognized in at least 0.28 of cases, and sister strands cannot cross over in more than 0.72 of cases.

It might be possible to draw a further conclusion in some cases. If every tetrad were a crossover in the region, the amount of crossing over in the region could not exceed 33 1/3 percent if sister strands crossed over and 50 percent if they did not. Thus single crossing over exceeding 33 1/3 percent within a region where no double crossing over occurred might be an indication that there was no crossing over between sister strands.

COINCIDENCE AND INTERFERENCE

The coincidence of two regions may be expressed as

$$\frac{D/N}{A/N \ \ B/N} = \frac{DN}{AB},$$

where N is the total number of individuals, A the number of crossovers in the first region, B in the second region, and D in both regions simultaneously. The regions whose coincidence is being measured will be referred to as nodal regions; the points of crossing over as nodes. The distance between the nodes will be termed the internode, and the distance between the nodal regions will be termed the intermediate region.

Coincidence may be of several types. In the type originally defined, A, B, and D include all individuals that are crossovers in the nodal regions, regardless of whether they are also crossovers in other regions. They may be termed inclusive totals, and coincidence so calculated inclusive coincidence. Inclusive coincidence, which is a measure of interference, increases as the internode lengthens (MULLER 1916).

If crossovers in the intermediate region are excluded from D, the resultant coincidence may be termed select; it measures the frequencies of internodes of different lengths (WEINSTEIN 1918).

If crossovers in the intermediate region are excluded from A, B, D, and N, the resultant coincidence has been termed partial (MULLER 1925). The concept of partial coincidence may be extended by excluding regions other than the intermediate ones; in the extreme case all regions may be excluded except those whose coincidence is being measured. This extreme type may be termed exclusive coincidence.

All the above types of coincidence as ordinarily measured are based on the observed frequencies of chromatids. They may therefore be termed chromatid or strand coincidence. Coincidence might also be based on true chromatid frequencies, or on tetrad frequencies. Tetrad coincidence might be based merely on the levels of crossing over, regardless of which strands are involved; or it might be calculated separately for regressive, progressive, and digressive crossing over; and further distinctions are possible according to whether the exchanges are between lateral or diagonal, homologous or sister strands.

Inclusive coincidence

If recurrence is random, inclusive coincidence for tetrads or for the true frequencies of strands is equal to the strand coincidence as ordinarily calculated. For if X_A, X_B and X_D denote respectively the inclusive frequencies of tetrads that are crossovers in the first nodal region, in the second nodal region, and in both nodal regions, then strand coincidence whether based on observed or true values is $p_A \, p_B \, X_D \, N/p_A \, X_A \, p_B \, X_B = X_D \, N/X_A \, X_B$, which is the tetrad coincidence. This invariance holds regardless of whether or not sister strands cross over, and regardless of whether the p's are the same or different in different regions.

But if recurrence is not random, strand coincidence is

$$t_{A+B} \, p_A \, p_B \, X_D \, N/p_A \, X_A \, p_B \, X_B;$$

hence

$$\text{inclusive tetrad coincidence} = \frac{\text{inclusive chromatid coincidence}}{t_{A+B}} \qquad (15)$$

If random recurrence is altered by an increase of progressives at the expense of digressives, or by an increase of regressives, $t_{A+B} > 1$, and tetrad coincidence will be less than the observed value. If random recurrence is altered by an increase of progressives at the expense of regressives, or by an increase of digressives, $t_{A+B} < 1$, and tetrad coincidence will be greater than the observed value.

Only those deviations from random recurrence need be considered that yield the observed frequencies of chromatids: for example, those indicated in equations 8–13 and in table 7. These deviations will not alter the frequency of recognizable crossing over in any one region, and hence will not alter the denominator of the coincidence fraction. Their effect on coincidence will result from an alteration of the number of double crossover tetrads and hence of the numerator of the fraction.

The substitutions indicated in equations 9, 11, and 12 would not alter the total number of tetrads that are crossovers in both regions involved and hence would not affect the coincidence. This applies to equation 11

only if the rank-2 regressive involves both nodal regions. But a change would result from the substitutions indicated in the other equations. Since, on random recurrence without sister-strand crossing over, the progressives constitute one-half of rank-2 tetrads, the regressives and digressives one quarter each, the substitutions in equation 10 might increase or decrease tetrad coincidence by as much as 25 percent. A similar decrease might result from the elimination of digressives indicated in equation 8. The reverse substitution in this equation would increase tetrad coincidence by an amount dependent on the number of singles available. Thus the coincidence of regions 1 and 6, which is $912 \cdot 28239/4036 \cdot 6442 = 1.0$, could be increased to 2.9 if all the 1744 singles in region 1 and an equal number in region 6 were replaced by 1744 digressives and 1744 non-crossovers. Similarly, the coincidence of regions 2 and 6 could be increased from 1.0 to 2.5 if the 1998 singles in region 6 and an equal number in region 2 were replaced by digressives and non-crossovers.

Not all the coincidence values could be altered simultaneously. Thus if all the 1998 singles in region 6 were used to raise the 2, 6 coincidence, there would be none left to raise the coincidence of 1 and 6. Again, only 1709 digressives could be added or eliminated by equation 8 since there are only 1709 non-crossovers. If the changes are distributed among the various classes, the increase or decrease in any one class would be much less than indicated above.

Similar procedures with similar results apply if there is crossing over between sister strands. The distribution of tetrads in table 7 would increase coincidence since it adds 5316 tetrads to rank 2. The increase would be negligible for all values involving region 6, for the additional doubles are at the expense of singles, of which there are only 33 in region 6 (table 4, column I); but other values might be considerably affected. The increases could be minimized if the additional tetrads were distributed among all classes; but they could not be counteracted by substitutions since there are no non-crossovers at whose expense these could be made.

It is perhaps unlikely that inclusive tetrad coincidence differs greatly from the observed value, for the following reasons: (1) Great deviations are brought about only by restricting substitutions of tetrads to one or a few classes, with the result that two adjacent regions have very different coincidences with the same region. (2) An increase of coincidence to more than 1 would imply that crossing over in one region is helped by that in another. (3) A decrease of inclusive coincidence for widely separated regions to less than 1 is not consistent with the mutual independence that might be expected from such regions. But we do not know how widely separated two regions must be to achieve independence; and in our present state of knowledge none of these reasons is conclusive.

Select coincidence

If X_d, X_{d+1}, X_{d+2}, $\cdots$ X_{d+n} are the frequencies of tetrads that are cross-overs in both nodal regions and also at 0, 1, 2, $\cdots$ n levels in the intermediate region, then if recurrence is random and p constant,

$$\text{select chromatid coincidence} = \frac{p^2[X_d+qX_{d+1}+q^2X_{d+2}+ \cdots +q^nX_{d+n}]N}{p \, X_A \quad p \, X_B}$$

$$= \text{select tetrad coincidence} + \frac{[qX_{d+1}+q^2X_{d+2}+ \cdots +q^nX_{d+n}]N}{X_A \, X_B} \qquad (16)$$

Hence select coincidence for tetrads is less than the true value for chromatids, which in turn is less than the observed value since q for the true value is less than for the observed value. These relations hold if the p's differ, but not necessarily if recurrence is not random.

As the intermediate region lengthens, there will ultimately be a decrease in the frequency of tetrads that are not crossovers in it, and consequently a decrease of select tetrad coincidence. Select coincidence for strands will also ultimately decrease if recurrence is random, and even if it is not random except on rather special assumptions.

Partial coincidence

Partial coincidence for tetrads will in general differ from the observed value; for when tetrad frequencies are replaced by the corresponding strand frequencies, the changes in the numerator and denominator do not necessarily compensate for each other. The same is true when non-intermediate regions or all non-modal regions are excluded.

A detailed discussion of coincidence in *D. melanogaster* and *D. virilis* will be published separately.

NON-DISJUNCTION AND ATTACHED X'S

The multiple-strand method can be applied to cases of non-disjunction and attached X's if allowance is made for the fact that two chromatids are recovered instead of one and that they are not necessarily a random pair. The situation is clearest in attached X's, where the genes in the attached strands tend to remain together, the tendency being absolute at the point of attachment and decreasing distally because of crossing over with strands of the other attached pair.

It will be best therefore to begin at the point of attachment; and if we do this, the strands resulting from tetrads of any given rank can be deduced as follows.

Crossing over between the spindle fibre and the first heterozygous pair

of genes might occur in three ways: (1) between strands attached to each other (these are homologous); (2) between sister strands; and (3) between a strand and its homologue in the other attached pair. Types 1 and 2 will leave each attached pair heterozygous; type 3 will result in homozygosis in each pair. If any two chromatids are equally likely to cross over, the three types will occur with equal frequency, and homozygosis will be produced in 1/3 of the cases.

If the first crossing over leaves each attached pair of chromatids heterozygous, then crossing over in the next region can occur in the same three ways, with the same results. If the first crossing over has produced homozygosis, the next crossing over can occur in two ways: (1) between strands attached to each other (these are now sister strands), (2) between strands of different attached pairs (these are now all homologous). If it is a matter of chance which strands cross over, type 1 will occur in 1/3 of cases, type 2 in 2/3.

This procedure can be continued for the entire length of the chromosome. It may be represented by the following diagram, in which a represents homozygosis for one strand, b for the other, and H heterozygosis.

The chance that a tetrad of any rank will give rise to a particular type of offspring can be obtained by multiplying the appropriate fractions. For example, among offspring derived from tetrads of rank 3, there will be $(2/3)^3$ that are heterozygous throughout and $(1/6)^3$ that are homozygous for strand a throughout.

If crossing over does not occur between sister strands, but association of strands is otherwise random, the procedure must be modified to conform with the following diagram:

TABLE 9*

Calculated and observed frequencies of daughters of attached-X females
(Observed frequencies from Sturtevant 1931)

I $\dfrac{+}{sc\ ec\ cv\ ct\ v\ g\ f}$

	Obs.	Calc.
+..........	614	613
sc..........	27	19
sc ec........	36	29
sc ec cv......	18	27
sc ec cv ct....	47	45
sc ec cv ct v..	16	25
sc ec cv ct v g..	9	15
sc ec cv ct v g f.	5	9
es..........	1	0.2
ec cv........	2	0.4
ec cv ct......	4	4
ec cv ct v......	5	6
ec cv ct v g....	9	6
ec cv ct v g f...	3	6
cv..........	2	0.4
cv ct........	7	4
cv ct v........	12	8
cv ct v g......	10	9
cv ct v g f.....	3	6
ct..........	4	1
ct v..........	4	5
ct v g........	6	6
ct v g f........	5	6
v..........	6	3
v g..........	8	10
v g f..........	9	7
g..........	1	2
g f..........	4	4
f..........	1	1
sc v g........	2	0.2
Total.......	880	

II $\dfrac{ec\ cv\ ct\ v\ g\ f}{sc}$

	Obs.	Calc.
+..........	557	578
sc..........	198	210
ec..........	34	36
ec cv........	29	35
ec cv ct......	54	61
ec cv ct v......	32	38
ec cv ct v g....	24	26
ec cv ct v g f...	6	19
cv ct........	9	5
cv ct v........	19	10
cv ct v g......	7	12
cv ct v g f.....	8	8
ct..........	0	1.6
ct v..........	15	6
ct v g........	10	8
ct v g f........	7	8
v..........	9	4
v g..........	18	12
v g f..........	21	9
g..........	3	2
g f..........	17	4
f..........	2	1.1
ec v g........	1	0.2
ec cv g f......	2	0.1
ec cv ct g f....	2	0.5
ec cv ct f......	1	0.2
sc v g........	1	0.6
sc v g f......	3	0.3
sc g..........	1	0.2
sc g f........	4	0.7
sc f..........	1	0.2
sc ct v g f......	1	0
Total.......	1096	

III $\dfrac{g\ f}{sc\ ec\ cv\ ct\ v}$

	Obs.	Calc.
+..........	515	534
sc..........	16	19
sc ec........	20	28
sc ec cv......	22	27
sc ec cv ct.....	28	44
sc ec cv ct v....	39	48
g..........	56	49
g f..........	38	32
ec cv........	2	0.4
ec cv ct......	7	4
ec cv ct v......	28	17
cv..........	1	0.4
cv ct........	5	4
cv ct v........	28	23
ct..........	2	1.3
ct v..........	30	17
v..........	27	20
f..........	2	1.1
sc v........	2	0.5
sc ec v........	1	0.2
sc g..........	1	0.3
sc ec g f......	3	0.2
Total.......	873	

IV $\dfrac{ec\ cv\qquad g\ f}{sc\qquad ct\ v}$

	Obs.	Calc.
+..........	308	318
sc..........	74	61
sc ct........	39	37
sc ct v........	36	40
ec..........	28	46
ec cv........	83	65
ec cv g......	9	18
ec cv g f.....	5	13
ct..........	17	8
ct v..........	37	48
cv..........	6	10
cv g..........	7	8
cv g f........	4	5
g..........	21	15
g f..........	17	14
v..........	25	16
f..........	2	0.7
ec g..........	1	0.1
ec g f........	1	0.2
ec cv f........	1	0.1
sc g..........	1	0.5
sc g f........	1	0.5
sc f..........	1	0.1
sc ct g........	1	0.0
Total......	725	

V $\dfrac{ct\ v\qquad f}{sc\ ec\ cv\qquad g}$

	Obs.	Calc.
+..........	349	362
sc..........	22	16
sc ec........	15	23
sc ec cv......	56	80
sc ec cv g......	11	21
ct..........	46	44
ct v..........	68	66
ct v f........	15	23
ec cv..........	17	8
ec cv g......	10	10
cv..........	14	10
cv g..........	15	13
v..........	15	11
v f..........	7	6
g..........	44	29
f..........	14	4
sc g..........	3	0.5
sc ec g........	2	0.3
sc ct v........	1	0.1
sc v f........	1	0.2
sc f..........	1	0.1
sc ec v f......	1	0.1
sc ec f........	1	0.2
Total.......	728	

VI $\dfrac{ec\qquad v\qquad f}{sc\qquad cv\ ct\qquad g}$

	Obs.	Calc.
+..........	386	397
sc..........	62	49
sc cv........	20	28
sc cv ct......	37	72
sc cv ct g......	9	26
ec..........	102	109
ec v..........	43	54
ec v f........	13	16
cv..........	2	0.8
cv ct........	32	22
cv ct g........	28	29
ct..........	14	6
ct g..........	12	13
v..........	61	43
v f..........	24	20
g..........	39	23
f..........	11	4
cv g..........	1	0
sc g..........	5	1
sc v..........	6	0.6
sc v f........	4	0.3
sc f..........	1	0.3
ec g..........	4	1
Total.......	916	

VII $\dfrac{ec\quad ct\ v\qquad f}{sc\quad cv\qquad g}$

	Obs.	Calc.
+..........	352	398
sc..........	69	49
sc cv........	90	100
sc cv g........	12	26
ec..........	65	58
ec ct........	48	50
ec ct v........	54	53
ec ct v f........	4	16
cv..........	31	23
cv g..........	34	29
ct..........	14	6
ct v..........	44	29
ct v f........	15	13
v f..........	5	7
g..........	47	36
f..........	3	4
sc g..........	2	1
ec v..........	3	0.3
ec ct f........	1	0.5
sc ct v........	1	0.0
sc v f........	2	0.3
sc f..........	1	0.3
ec g..........	2	0.6
ec ct g........	1	0.4
ec cv..........	1	0
Total........	916	

VIII $\dfrac{cv\qquad g}{sc\ ec\quad ct\ v\qquad f}$

	Obs.	Calc.
+..........	413	433
sc..........	35	20
sc ec........	59	59
sc ec ct......	38	47
sc ec ct v......	21	42
sc ec ct v f...	2	9
cv..........	121	125
cv g..........	40	55
ec..........	8	0.6
ec ct..........	7	4
ec ct v........	17	12
ec ct v f......	3	6
ct..........	10	6
ct v..........	41	30
ct v f........	21	13
g..........	43	37
v..........	17	14
v f..........	19	7
f..........	4	5
sc v f........	1	0.2
sc ec v......	1	0.4
sc cv g........	1	0.1
sc g..........	1	0.6
sc ec g......	3	0.6
sc ec ct g.....	1	0.3
ec g..........	1	0.0
Total.......	928	

* This table includes all calculated frequencies of 1 or more; and, where the observed frequency is not 0, every calculated frequency of less than 1. Calculated figures are given to the nearest unit; except those of 1 or less, which are given to the nearest 0.1.

In either of the above cases, if recurrence is not random, the frequency of each class obtained from a tetrad of a given kind will not be the simple product of the fractions in the table, but this product multiplied by a factor which, in the most general case, will differ according to the rank of the tetrad, the regions involved, and the nature of the crossings over: whether homologous or sister-strand; lateral or diagonal; regressive, progressive, or digressive.

Thus if the frequencies of tetrads are known, the frequencies of genotypes and phenotypes among the offspring can be calculated. In table 9 are given the frequencies of phenotypes expected among offspring of attached-X females heterozygous for *sc ec cv ct v g f*, on the assumption that sister strands do not cross over but association of chromatids is otherwise random. The tetrad frequencies in the column headed $p = 1/2$ in table 4 have been used as a basis; but since they do not include crossing over between the spindle fibre and forked, a correction has been applied by taking into account what proportion of each class must also have been crossovers to the right of forked. This correction is based on a cross involving the loci *y bi cv ct v g B bb*, the unpublished data of which were kindly placed at my disposal by DR. C. B. BRIDGES in 1932. The crossover values in this cross agree closely with those in the X-ple cross (MORGAN, BRIDGES and SCHULTZ 1933).

The calculations have been made for the eight types of heterozygous mothers that gave the greatest number of offspring in the experiments of STURTEVANT (1931), and STURTEVANT's actual counts are included for comparison. The frequencies are on the whole in agreement. The discrepancies are probably due at least in part to the small counts and to differential viability; there may also have been differences in proportions of crossing over between the attached-X stock and those on which the calculated values are based.

A comparison can also be made between calculated and observed frequencies of offspring heterozygous for all the genes involved. The calculated value is 39.9 on the basis of table 4 (corrected) and 40.9 on the basis of table 8. The value observed by STURTEVANT (1931) is 34.5; and from the data given by BEADLE and EMERSON (1935, table 2) it appears that among 1478 offspring whose genetic constitutions were tested, 668 were heterozygous for all genes from scute to forked inclusive, this being a proportion of 45.2. The calculated values are almost precisely half way between the observed values. It should be noted also that STURTEVANT's figure is based on tests of only 383 wild-type daughters; and that the proportions of crossing over in the attached-X stock used by BEADLE and EMERSON were somewhat different from those of the stocks on which the calculated values are based.

The proportion of completely heterozygous individuals in attached X's, is equal to $x_0+qx_1+q^2x_2+q^3x_3+ \cdots +q^nx_n$ provided that at any level of crossing over only two strands are involved; hence it is precisely equal to the proportion of non-crossovers in the same region among offspring of females with unattached X's. The variations in the frequencies of x's and p's compensate for one another, and the result is therefore independent of the value of p. Nevertheless a figure obtained on the assumption of crossing over between sister strands would not be significant despite its agreement with observation; for the tetrad frequencies on which it would be based are incorrect, involving as they do negative values. The agreement would therefore be purely formal since it would be due to compensating errors.

The proportion of homozygosis for each gene can also be calculated. This is done by adding the proportions of the appropriate classes in the diagrams on page 182. If $p = 1/2$, the sum is

$$\tfrac{1}{4}x_1+\tfrac{1}{8}x_2+\tfrac{3}{16}x_3+\tfrac{5}{32}x_4+ \cdots$$

where the x's are the frequencies of tetrads that are crossovers at 1, 2, 3, 4, $\cdots$ levels between the point of attachment and the gene in question. Values have been calculated on the basis of the $p = 1/2$ columns of table 4 (corrected for crossing over between f and bb) and of table 8. These, together with observed values, are given in table 10 and illustrated in figure 4.

The solid curves in figure 4 illustrate homozygosis plotted against actual map distance in each cross. The two lines coincide almost completely from the spindle fibre to about v; beyond v the curve based on table 4 rises above that based on table 8.

The total map distance is almost exactly the same in the two crosses; but corresponding regions do not always have the same length. Hence, while the ends of the curves lie in the same perpendicular, the other corresponding loci do not. In order to facilitate the comparison of corresponding genes, the curves have been redrawn so that the abscissa of each gene is the average of the values in the two crosses. The results are the dash line for the data of table 4 and the dot-and-dash line for those of table 8.

The theoretical and observed results are of the same general type, rising from nearly 0 at the proximal end to above 16 2/3 (the value expected on pure chance) at the distal end. The greatest discrepancies are the low values observed by EMERSON and BEADLE in the region from car to ct; these are undoubtedly due to the fact that there was less crossing over in the attached X stock used by EMERSON and BEADLE than in the stocks of tables 4 and 8. Differences in crossing over and in coincidence may also account in part for the discrepancies in ct and more distal genes; but differ-

ential viability is suggested by the low value of *cv* as compared with *ct* and of *sc* as compared with *y* in some of the data.

The theoretical curve based on table 4 and on the *y bi cv ct v g B bb* cross was exhibited at the Sixth International Congress of Genetics and before the Genetics Society of America in 1932. Homozygosis when sister strands do not cross over was independently calculated by SAX (1932) and
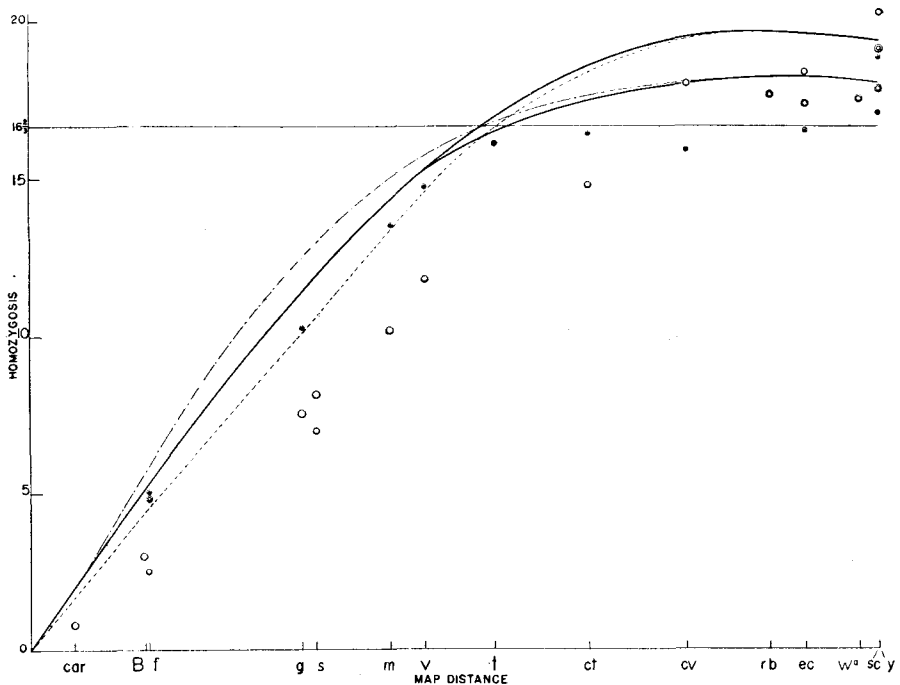
FIGURE 4. Proportions of homozygosis for recessive genes in offspring of heterozygous attached-X females. The abscissa indicates distance from the spindle fibre. The curves are drawn through the theoretical values given in table 10. The observed values in table 10 are also indicated; those from ANDERSON, L. V. MORGAN, and STURTEVANT by dots enclosed in circles; those from RHOADES by concentric circles; those from EMERSON and BEADLE by simple circles.

subsequently by BELLING (1933), KIKKAWA (1933)[2] and MATHER (1935); the data on which these calculations were based did not include the entire length of the X chromosome. BEADLE and EMERSON (1935) have obtained a similar curve from an analysis of crossing over in the attached X's themselves.

[2] KIKKAWA's previous (1932) method was, as he himself pointed out, incorrect because the highest frequencies of homozygosis yielded by it were distinctly less than 16 2/3 percent. KIKKAWA in 1932 stated that the writer's theory as embodied in formula 2 of the present paper would not allow for *aaa/aba* individuals. This objection is answered by the analysis given above, which shows that such individuals are expected and with a frequency corresponding to the one observed experimentally. KIKKAWA's objection was due to a misinterpretation of the writer's theory; special cases of the theory have since been used by KIKKAWA himself.

The agreement of a theory with observation is of course not significant unless other theories agree less closely. It is therefore necessary to consider how the curve would be modified if the assumptions on which it was calculated were altered.

For regions near the point of attachment the curve is a straight line, the homozygosis being half the map distance from the spindle fibre. This

TABLE 10*

Percentages of homozygosis in daughters of attached-X females.

GENE	CALCULATED		OBSERVED			
	TABLE 4 p=1/2 CORRECTED FOR CROSSING OVER BETWEEN f AND bb	TABLE 8 p=1/2	ANDERSON 1925 L. V. MORGAN 1925 STURTEVANT 1931 SUMMARIZED BY STURTEVANT	RHOADES 1931	EMERSON 1933	BEADLE 1935
car		2.2				0.8
B			4.1		3.0	
f	4.6	5.9	5.1	4.9		2.5
g	10.1		10.3		7.5	
s		13.0			8.1	7.0
m			13.5		10.2	
v	14.6	15.8	14.8		11.9	11.8
lz			14.9			
t			16.1			
ct	18.4	17.6	16.4			14.8
cv	19.5	18.1	15.9			18.0
rb				17.6		
ec	19.6	18.3	16.6	17.3		18.4
w or w^a			16.5 (w)	17.5 (w^a)		
sc	19.4	18.0	17.1	17.9		20.3
y			19.0	19.2		

* This table includes all the data hitherto published. The proportions headed RHOADES (1931), and EMERSON and BEADLE (1933), have been calculated by the writer from the total counts of these workers. The homozygosis values calculated by RHOADES differ slightly from those recorded here because he excluded some of his data.

In the experiment of EMERSON and BEADLE (1933) the value marked B is the average for Bar and its normal allelomorph.

would be expected on random association without sister strand crossing over; because homozygosis for a given gene is 1/4 of the frequency of tetrads of rank 1, while the map distance is 1/2, and there are few if any tetrads of higher rank. If sister strands cross over freely, homozygosis is 1/6 and map distance 1/3 of rank-1 tetrads; so that the agreement is equally good and this part of the curve cannot be used as evidence for or against sister-strand crossing over. But the distal part of the curve, as has been pointed out by the writer (WEINSTEIN 1932c) supplies the necessary evidence; for if sister strands crossed over, the proportion of homozygosis (as can be seen from the first diagram on page 182) would be

$$\tfrac{1}{6}x_1 + \tfrac{1}{6}x_2 + \tfrac{1}{6}x_3 + \cdots + \tfrac{1}{6}x_n$$

so that even if all tetrads were crossovers, the amount of homozygosis would not exceed one-sixth. The fact that both calculated and experimental curves rise above this level shows that crossing over does not occur freely between sister strands.

The curve also proves that only two of the four strands cross over at any level. For if all four always crossed over, no homozygosis would be produced at all; and if all four sometimes crossed over, the homozygosis in the proximal part of the curve would not be half the map distance but less. This is true regardless of whether crossing over occurs between sister strands.

The curve can also be used to test whether recurrence is random. That the proportion of homozygosis depends on the nature of recurrence can be seen from the fact that in tetrads of rank 2 homozygosis of genes distal to both exchanges results not from regressives or digressives but only from some of the progressives. If there is no crossing over between sister strands, homozygosis is produced only by those progressives in which the proximal crossing over is lateral (the distal crossing over must then be lateral also); this type is illustrated in figure 2B. The terms lateral and diagonal are applied according to the relative positions of the strands in the region of crossing over, and do not necessarily describe their relation at the proximal end.

If recurrence is random, these progressives constitute one-half of all progressives or one quarter of all rank-2 tetrads.[3] That is, the same proportion of rank-2 progressives as of single crossover tetrads produce homozygosis; while regressives, digressives, and non-crossovers do not produce it at all.

The substitutions indicated in equations 8–13 and table 7 can be tested with respect to their effect on homozygosis for scute with the aid of the following table.

TABLE 11

Tetrad frequencies for the X chromosome (random recurrence, no sister-strand crossing over)

RANK	0	1	2 REGRESSIVE	2 PROGRESSIVE	2 DIGRESSIVE	3	4
Table 4 (corrected to include crossing over between *f* and *bb*)	557	15678	2755	5510	2755	888	96
Table 8	904	7824	1730	3460	1730	488	

Equation 8. The replacement of non-crossovers and digressives by singles would increase homozygosis. In the corrected Xple figures, since

[3] All these proportions must be divided by 2 when homozygosis for the recessive gene only is considered.

there are only 557 non-crossover tetrads there could be not more than 1114 additional singles, of which 278.5 would produce homozygosis for scute; this would raise the proportion from 19.4 to 20.4—not so good an agreement with the observed values for *sc* and *y*.

The reverse substitution (non-crossovers and digressives for singles) could have a much greater effect. If all of the 15678 singles were replaced, homozygosis for *scute* would fall to 5.5, an impossibly low figure. A reduction of homozygosis to 16.7 would require the replacement of 1/5 of the singles; this is highly improbable, since most of the observed values for *sc* and *y* differ significantly from 16.7.

On the basis of table 8, the highest value would be 20.8, the lowest 5.9; and a decrease to 16.7 would involve replacing 1/9 of the singles.

Equation 9. The progressives could be doubled or eliminated; and homozygosis would fall as low as 14.6 or rise as high as 24.2 on the basis of table 4 (corrected). The corresponding values on the basis of table 8 would be 12.6 and 23.4. Hence only a small part of the substitutions would be possible.

Equation 10. This would not alter homozygosis, since the effectiveness of a progressive is equivalent to that of a single.

Equation 11. Homozygosis would remain unaltered because a non-progressive tetrad of rank 3 has the same effect as a single. This follows from the fact that in the rank-3 tetrad two of the crossings over are regressive with respect to each other and neutralize each other; the third crossing over effects homozygosis if it is diagonal but not if it is lateral.

If sister strands cross over freely, homozygosis results from 1/3 of the tetrads of rank 1 and 1/2 of the progressives of rank 2, but not from regressives or digressives of rank 2 or from non-crossovers (see footnote 3). Since homozygosis calculated on free sister-strand crossing over and random recurrence is too low, only those deviations from random recurrence are possible that raise the calculated proportion to the observed level.

The method by which the negative frequency of non-crossovers was eliminated (table 7) consisted in decreasing the number of tetrads of rank 1 by 10632 and increasing the digressives by 5316. This would decrease the proportion of homozygosis for distal genes and is therefore impossible.

Of the substitutions indicated in equations 8, 11, 12, and 13 the only ones that would increase homozygosis are the replacement of the left-hand by the right-hand side of equations 8 and 12. But singles cannot be increased by the substitutions indicated in equation 8, since there are no non-crossovers at whose expense this could be accomplished; hence the only substitution that remains is of progressives for regressives and digressives (equation 12). To test this completely the figures in table 7

do not suffice, since they include only the region from *forked* to *scute;* but they can be considered as roughly applicable to the region from the point of attachment to *crossveinless* or *echinus*, which is about as long as that from *forked* to *scute*. On this basis there might be as many as 10820 additional progressives; and the proportion of homozygosis due to singles and progressives might be increased to as much as $1/28239$ $(1/6\ 4698+1/4\ 22016) = 22.2$—too high a figure, so that only part of the substitution is possible.

<div align="center">POLYPLOIDS</div>

<div align="center">*Triploids*</div>

In triploids there is evidence that only two of the six strands cross over at any level (BRIDGES and ANDERSON 1925, REDFIELD 1930). Hence among the strands emerging from a hexad which is a crossover at a given level, the proportion that are recognizable crossovers at that level is the product of $1/3$ by the chance of recognizing a crossover strand once it is recovered. This chance depends on two factors: (1) whether all three chromosomes are distinguishable from each other or two are indistinguishable, and (2) whether crossing over takes place between sister strands. The results for four possible cases are as follows:

	CROSSING OVER BETWEEN SISTER STRANDS AS FREQUENT AS ON CHANCE	NO CROSSING OVER BETWEEN SISTER STRANDS
All 3 chromosomes distinguishable	$1/3(4/5) = 4/15$	$1/3$
2 chromosomes indistinguishable	$1/3(8/15) = 8/45$	$1/3(8/12) = 2/9$

These fractions represent the chances of detecting a crossing over at one level if one chromatid is recovered ("regular" offspring). If two chromatids are recovered ("exceptional" offspring), then p for hexads is the product of the chance that *at least* one is a crossover by the chance of recognizing a crossover strand once it is obtained provided that the two chromatids are recovered at random.

BRIDGES and ANDERSON (1925) showed that a chromosome may cross over with different chromosomes at different levels; and they concluded that synapsis involves all three chromosomes equally throughout their length. On this theory recurrence would be random for all six chromatids, except that sister-strand crossing over may be excluded. But when hexad frequencies are calculated on this basis, negative values are encountered (WEINSTEIN 1932c).

In table 12 are given the hexad frequencies calculated for the cross of REDFIELD (1930, table 1, broods 1 and 2 combined), which covers about half of the third chromosome. Here all three chromosomes are marked;

hence formula 2 can be applied with $p = 4/15$ and $p = 1/3$. When $p = 4/15$, the largest negative value (-135) is 1.6 times its standard error. When $p = 1/3$, the negative value (-30) is 0.6 times its standard error. The results are not conclusive; still they suggest that with longer stretches of chromosome significant negative frequencies would appear.

TABLE 12

Crossing over in triploids of constitution	$+$	D	ma	Sb	$+$
	se	Mh	$+$	$+$	H
	h	$+$	cu	bx	e^s

REGIONS OF CROSSING OVER	OBSERVED FREQUENCIES REDFIELD 1930 n = 1030	TETRAD FREQUENCIES CALCULATED FOR RANDOM RECURRENCE		
		$p = 1/2$	$p = 1/3$	$p = 4/15$
0	565	272	127	101
1	87	100	63	17
2	171	226	183	97
3	70	56	-30	-135
4	47	48	21	-7
12	22	76	144	193
13	11	36	63	77
14	8	20	18	-4
23	27	104	225	341
24	12	40	72	91
34	6	20	36	46
123	1	8	27	53
124	2	16	54	105
134	1	8	27	53

Negative values are in fact obtained for the cross covering almost the entire length of the third chromosome given in REDFIELD's table 5. Here only one of the chromosomes is marked, hence $p = 2/9$. And a further modification of procedure is necessitated by the fact that since there are two strands of one kind and one of the other, the chances of recognizing crossing over at different levels are not independent, even though recurrence be random. It becomes necessary therefore to use equations like those in case 3. The analysis need not be given in detail because a similar one has been made by other workers (MATHER, 1933, 1935; KIKKAWA, 1934; see KIKKAWA for the equations). These negative frequencies are not in themselves significant; but they point in the same direction as those in the previous cross.

These results suggest that, for considerable distances, only two of the

chromosomes conjugate while the third goes unmated to either pole. On this assumption, the unmated chromosome region can be neglected; for the conjugating chromosomes form what is essentially a tetrad, and the chromosome recovered in regular offspring is always one of those that conjugate. Hence the data in table 12 can be treated as in diploids; that is, formula 2 can be applied with $p = 1/3$ if sister strands cross over and $p = 1/2$ if they do not. When $p = 1/3$, as we have seen, some negative values are encountered, though their significance is doubtful; when $p = 1/2$, however, all frequencies are positive and support the theory on which they are based.

If the conclusions suggested by these results are correct, we should expect that when two strands are recovered (exceptional offspring), usually not more than one strand will be a crossover. This is borne out by the results with the X chromosome: from BRIDGES and ANDERSON (1925 table 5) it appears that a crossover chromatid is associated with a non-crossover 82 times out of 97, and with a crossover only 15 times. The tendency is complete at the spindle fibre and decreases for more distal regions because of crossing over between them and the spindle fibre. In the autosomes there is a similar relation between association of chromosomes and distance from the spindle fibre (REDFIELD 1930). As has been pointed out by MULLER (unpublished, cited by WEINSTEIN 1932c), these facts support the view that only two of the chromosomes conjugate while the third remains unmated, although there may be some change of partners.

Similar conclusions concerning conjugation in triploids have been reached by MATHER (1933, 1935); and on cytological grounds by BELLING (1921), MULLER (1922) and DARLINGTON (1932). RHOADES (1933) and KIKKAWA (1934) have presented somewhat different interpretations. The questions raised by these workers cannot be discussed here but will be treated elsewhere.

Frequency of crossing over in diploids and triploids

In comparing the amount of crossing over in diploids and triploids, it is necessary to distinguish between crossing over per strand and crossing over per tetrad or hexad (WEINSTEIN 1932a).

If in any given region crossing over is constant per chromatid, then if chromatids are recovered at random there would be no change in crossing over per chromatid in triploids if sister strands do not cross over, but an increase if they do. For in the latter case one third of the crossings over in a tetrad would be undetectable, whereas in the hexad the proportion would be only $3/15 = 1/5$. This was pointed out by BRIDGES and ANDERSON (1925), who suggested that thus it might be possible to discover whether or not crossing over occurs between sister strands.

There is however another possibility which they did not consider; namely, that the amount of crossing over might be constant not per strand but per group of chromatids (tetrad or hexad). In this case the amount per chromatid would be decreased, since there would be the same number of crossings over but more strands. The ratio of recognizable cross-over strands in triploids to that in diploids would then be $4/15 \div 1/3 = 4/5$ if sister strands cross over, and $1/3 \div 1/2 = 2/3$ if they do not; provided of course that strands are recovered at random. Here again it might be possible to discover whether sister strands cross over.

In BRIDGES and ANDERSON's data for the X chromosome, the amount of crossing over per strand recovered was half as great in triploids as in diploids (except for the leftmost region of the X, which showed an increase). If strands were recovered at random, this would agree better with the sup-position that crossing over is the same for hexad as for tetrad and that sister strands do not cross over. But the decrease is too great and suggests that non-crossover strands are recovered more often than on chance. If two strands are recovered and one is usually a non-crossover, the amount of crossing over per recovered strand in triploids would be approx-imately halved, as it is; hence the figures support the theory that only two strands usually associate in synapsis while the third goes unmated to one pole. We may still conclude that crossing over per hexad is the same as per tetrad; but no conclusion can be drawn directly as to crossing over between sister strands, for the result would be the same whether this occurs or not, since the hexad acts essentially as a tetrad.

At the left end of the X, where there must be a greater tendency for strands to be recovered at random (because of crossing over between this region and the spindle fibre), the ratio might be expected to approach $2/3$ if sister strands do not cross over; instead it rises to 2. This increase re-mains unexplained.

In REDFIELD's data, since only those individuals were selected that did not receive the unmated chromosome, the triploid-diploid ratio per strand might be expected to decrease from 1 at the spindle fibre to about $2/3$ for distal regions, where strands are recovered more nearly at random. The latter figure is realized near the ends of the chromosome; and there is a rise toward the center. Near the spindle fibre however the ratio increases to 3 or 4; and this increase is not accounted for.

MULLER (unpublished, see WEINSTEIN 1932c) and RHOADES (1933) have reached similar conclusions, though RHOADES's interpretation of synapsis in triploids differs from that given above.

Higher polyploids

The multiple-strand theory can be applied to higher polyploids; it is

merely necessary to take into account which chromosomes are distinguishable and how they undergo synapsis. This may result in complicated situations; but where (as in some tetraploids) synapsis is in pairs, the treatment is not essentially different from that of diploids.

SUMMARY, WITH SOME CONSIDERATION OF THE MECHANISM OF CROSSING OVER

A mathematical method is described which makes it possible to calculate from the observed frequencies in a crossover experiment (1) the frequency of undetected crossing over including that between sister strands, which cannot be recognized directly, and (2) how the individual chromatids are associated in tetrads. The method can be applied to ordinary diploids, to cases of non-disjunction and attached chromosomes, and to polyploids. The calculated results differ according to the assumptions made as to crossing over at any given level and the mutual relations of crossings over at different levels; and since some of the results are inconsistent with the experimental data or (as in the case of negative frequencies) meaningless, the assumptions on which they rest can be ruled out and our knowledge of the mechanism of crossing over thus becomes more precise.

The experimental results when subjected to this mathematical treatment lead to the following conclusions regarding the mechanism of crossing over:

(1) There is no crossing over between sister chromatids.

(2) At any level only two of the four chromatids may cross over.

(3) Otherwise it is a matter of chance which chromatids cross over at any level.

(4) The chromatids that cross over at one level do not determine which ones cross over at other levels.

(5) This mechanism implies that for inclusive coincidence the true value for strands or tetrads is identical with the observed value regardless of whether sister strands cross over or not; for select coincidence the tetrad value is less than the observed value where the two differ; and for partial coincidence the tetrad and the observed values are in general not identical, though they may be very similar. If recurrence is not random, all types of tetrad coincidence will in general differ from the observed values.

Coincidence for chromatids remains identical with the observed values if sister strands do not cross over.

All these propositions and others are summed up in formula 2 for $p = 1/2$; that is, that the frequency of any class of tetrads is given by

$$X = 2^r [a_0 - a_1 + a_2 - a_3 + \cdots + (-1)^n a_n].$$

(6) In triploids the same mechanism holds; but the evidence indicates

that two of the chromosomes undergo synapsis to the exclusion of the third, over considerable distances, though there is some change of partners; but recurrence is probably random among the strands of the conjugating chromosomes.

Deviations from the mechanism described above lead to results that are inconsistent or incorrect:

(1) Crossing over between sister chromatids would involve negative frequencies of tetrads, would not allow the proportion of homozygosis for any gene to exceed 16 2/3 percent in cases of attached X's and non-disjunction and would result in other discrepancies. Evidence in the same direction is available in the work of EMERSON and BEADLE (1933) and BEADLE and EMERSON (1935) on attached X's; and of L. V. MORGAN (1933) on the ring-shaped X; and in the non-occurrence of sister-strand crossing over in Bar either in its ordinary locus (STURTEVANT 1925) or when translocated to the left end of the chromosome (MULLER and WEINSTEIN 1932 and unpublished data).

(2) Crossing over between more than two chromatids at a given level would diminish or eliminate homozygosis in offspring of attached X's. This conclusion is supported by the non-occurrence of identical crossovers in offspring of attached X's (ANDERSON 1925b) and of triploids (BRIDGES and ANDERSON 1925).

(3) Random occurrence of crossing over is shown most simply in the equality of lateral and diagonal crossing over near the spindle fibre (or a 2:1 ratio if sister strands cross over); and this involves random occurrence in other regions to give the observed results.

(4) Deviations from random recurrence would, if too great, lead to negative frequencies, and would modify the proportions of homozygosis in attached X's from the observed values.

The above results would follow the modification of one condition at a time. Modification of two or more conditions simultaneously might increase discrepancies; for example, the crossing over of all four chromatids at the same level would produce some of the same effects as an increase of digressive crossing over. On the other hand, some modifications would compensate for one another: an increase in digressive crossing over will counteract some effects of increased regressive crossing over, and it may also eliminate negative frequencies produced by crossing over between sister strands.

The conclusions have been stated in terms implying that at the time when crossing over occurs each chromosome is split into two separate strands or rows of genes. This assumption is not required by the mathematical analysis, and it leads to some difficulties which, together with the modifications they suggest, will be briefly considered.

(1) Crossing over between diagonal chromatids might prevent interchange between two others at the same or neighboring levels by keeping them apart; but it is not obvious why a lateral crossing over should have the same effect. This suggests that when crossing over occurs sister chromatids are not completely independent of each other.

(2) It is difficult to see why two completely independent sister chromatids should not cross over with each other, particularly since a sister chromatid might change positions with a homologous one by crossing over with it. The evidence however does not actually show that sister strands do not cross over: such crossings over are not directly detectable since they result in no recombination of characters. Our criterion of their occurrence is really whether crossing over between homologous strands at the same or neighboring levels is prevented, and the evidence shows that it is not. This could be explained if the sister genes are not arranged in two distinct rows but the genes at one level are oriented at random with respect to the genes at other levels; or to put it somewhat differently, there would be one row of double genes instead of two rows of single genes, and there would be no sister strand crossing over because there would be no sister strands (WEINSTEIN 1932c).

It is not necessary however to assume that orientation of genes at one level is random with respect to all other levels; but only that it is random with respect to the next level at which crossing over occurs—a considerable distance, being in the X chromosome of Drosophila some 14 units or about one-fifth of its genetic length. It would be sufficient if within this distance orientation of genes were random on two sides of one interlocus; or if genes did tend to form two strings, but the tendency of two genes to remain in the same string decreased with distance between them and finally disappeared when the distance became long enough for a second crossing over to occur. This might help to explain also why there is crossing over at all.

(3) It is difficult to understand why two chromatids that are sufficiently closely associated to exchange parts are no more likely to associate together at the next crossing over than either is to associate with a third. If at levels sufficiently far apart the orientation of sister genes is random, this difficulty disappears.

Thus all three difficulties suggest that the two chromatids of a chromosome are not entirely separate strands, and two of the difficulties can be explained if the orientation of sister genes at one level is random with respect to the sister genes of the same chromosome at other levels at which crossing over occurs.

The limitation of crossing over at a given level to two chromatids requires however further explanation. If both sister genes are already formed at the time that crossing over occurs, it must for some reason be difficult

or impossible for both to become detached from their neighbors in the chromosome. This might be due to the persistence of a material connection between successive genes, or of some physical force.

It is possible however that both sister genes are not already formed before crossing over takes place; particularly if the new genes are formed not by division of the old ones but by being catalyzed by them. The limitation of crossing over could then be explained if the old genes remain linked at the points of crossing over and only the new genes can become attached to new genes of the other chromosome, more or less as BELLING (1933) postulated. This might be brought about by the formation of the new genes on different sides of the old genes at different levels. There would still however have to be emerging chromatids made up of old and new genes of the same chromosome, to account for progressive crossing over; and this would come about if as the chromatids separate, old and new genes move at random at different levels of crossing over, as has been suggested above.

HISTORICAL NOTE

The part of the present investigation dealing with random recurrence and constant p was carried out in 1928. An abstract published in that year (WEINSTEIN 1928) included a statement of the problem, the general formula numbered 2, the invariance of coincidence under certain conditions, and the applicability of the method to attached X's and polyploids. The conclusion was drawn that association of strands in crossing over can not be entirely a matter of chance; this was based on the application of the formula for various values of p, including 1/3 (free sister-strand crossing over) and 1/2 (no sister-strand crossing over). The crossover frequencies worked out by BELLING (1931) and SAX (1932) correspond to the case p = 1/2 if different classes of the same rank are not separated.

The theory was generalized by WEINSTEIN (1930); and the complete theory was presented in a paper and exhibits at the Sixth International Congress of Genetics (WEINSTEIN 1932a, 1932b). The exhibits included the originals of all the figures in the present paper, but the additional data based on table 8 have since been added to figure 4. The work on attached X's and triploids was reported in the same year (WEINSTEIN 1932c).

The derivation of formulas by sets of equations, as given in the present paper, was included in the writer's report before the Congress of Genetics in 1932. Special cases of such sets of equations have since been used by MATHER (1933, 1935) and KIKKAWA (1934).

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