TETRAD ANALYSIS

by

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INTRODUCTION

In a number of fungi, algae, Bryophytes, and some higher plants, it is possible to isolate and genetically analyze the four cells resulting from a single meiosis. The four meiotic products can be separated and grown into four gametophytes or gones (the term gone is used to designated a clone derived from a single meiotic product), or in the case of higher plants the pollen tetrads can be utilized to fertilize standard female parents. In this way the genotype of the four products of a single meiosis can be identified by suitable tests. This type of genetic analysis is called tetrad analysis in contradistinction to single strand analysis. Single strand analysis is typified by the familiar type of genetic studies done on *Drosophila* or maize, where single meiotic products (sperm or pollen) are selected at random from many meioses and analyzed for their genetic constitution by crossing to suitable tester strains.

A great deal more information about gene, chromosome, and chromatid behavior during meiosis can be inferred from tetrad analysis than from single strand analysis. One can obtain, for example, in a tetrad analysis a measurement of relative distance (genetic map distance) between a gene and it's centromere. One can also obtain an estimate of the frequency of double exchanges between two linked genes, whereas in a single strand analysis one needs three linked genes to detect double cross-overs. Tetrad analysis, furthermore, gives information as to which of the four chromatid strands are involved in the exchange events, and therefore information about the phenomenon of interference on the chromatid level.

Tetrads may be ordered (*Neurospora, Sordaria*) or unordered (yeast, *Chlamydomonas*). Although the techniques used to determine genetic map distance between two linked genes are the same in ordered and unordered tetrads, the determination of gene-centromere map distances is quite different in the two types of tetrads. Although tetrads are generally unordered, textbooks on genetics generally consider only ordered tetrads. In the analysis of unordered tetrads there are often special cases where the concepts of ordered tetrads are useful (see Lerche 1937, Mainx 1931, Starr 1954, Stein 1958).

Because of the above considerations, it seems wise to present the concepts and techniques of tetrad analysis to the general biologist who may be working with organisms in which tetrad analyses can be performed. The purpose of this paper

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is to bring together the techniques of ordered and unordered tetrads into a useful summary.

ESTIMATED EXCHANGE FREQUENCIES

In the analysis of tetrads, we base our gene-gene and gene-centromere map distances on estimated exchange frequencies. Exchange frequency is the frequency of tetrads of meiotic products in which a chromatid exchange has occurred in the gene-gene or gene-centromere region being investigated. It is, therefore:

$$\frac{\text{number of tetrads with an exchange in the region}}{\text{total number of tetrads}}$$

Or:

$$\frac{\text{number of meioses in which an exchange has occurred in the region}}{\text{total number of meioses}}$$

Exchange frequencies are commonly estimated for gene-centromere regions in unordered tetrads, and for gene-gene regions in both ordered and unordered tetrads from the frequency of tetratype tetrads (T) (see below). Gene-centromere exchange frequencies in ordered tetrads are estimated from the frequency of second-division segregation of alleles (see below).

TETRAD TYPES

In the analysis of tetrads, we are concerned with the relationship of pairs of genes (gene-gene relationships). For each gene-gene relationship within a single tetrad there is a *tetrad type*. There are just three kinds of tetrad types, namely: parental ditype (PD), non-parental ditype (NPD) and tetratype (T).

Consider the cross: $AB \times ab$. Tetrads containing two genetically different types of meiotic products, where two products are like one parent and two like the other (*AB, AB, ab, ab*) are designated parental ditype tetrads (PD). Tetrads containing two genetically different types of meiotic products, where neither type is like either parent (*Ab, Ab, aB, aB*) are designated non-parental ditype tetrads (NPD). Tetrads containing four genetically differing meiotic products, one like each parent, and two like neither parent (*AB, Ab, aB, ab*) are designated tetratype tetrads (T) (see figure 1).

Figure 1.

P_1:	*AB* × *ab*	
F_1:	*Aa Bb*	
f_1 tetrad types:	*AB AB ab ab*	parental ditype
	Ab Ab aB aB	non-parental ditype
	AB Ab aB ab	tetratype

A tetrad type designation can be given for each gene-gene relationship in each tetrad. The importance of these tetrad types was first recognized by Burgeff (1929), but it was not until later that the methods of analysis were developed (Mather & Beale 1942, Whitehouse 1942, Perkins 1949, Lindegren 1949, Whitehouse 1949, 1950).

THE ESTIMATION OF EXCHANGE FREQUENCES BETWEEN LINKED GENES

Assume in the cross of $AB \times ab$ (figure 1) that the two genes are linked. How would the three tetrad types (PD, NPD and T) be produced from a meiosis of a cell heterozygous for the two linked genes (*AB/ab*)?

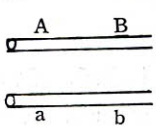
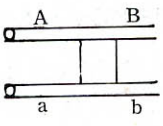
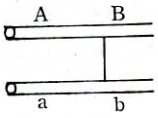
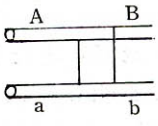
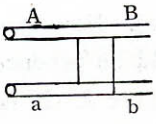
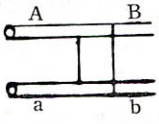
We must first make three assumptions, all of which are likely or at least reasonable: 1) Centromeres segregate at the first division of meiosis (prereduction of the centromeres). 2) All chromatid exchanges occur at the four strand stage of meiosis. 3) There are no sister-strand exchanges (there are no exchanges between chromatids attached to the same centromere). Having made these assumptions, we can conclude that the three tetrad types would be produced for a linked gene-gene relationship by the events diagrammed in figure 2. If no exchanges occurred between the genes *a* and *b*, then both genes would segregate at first division of meiois. That is, the large *A* alleles would segregate from the small *a* alleles, and the large *B* alleles from the small *b* alleles at first meiotic divison. Since large *A* and large *B* are attached to the same centromere, they would move to the same pole at first division. After the second meiotic division the four products would be *AB*, *AB*, *ab*, and *ab;* or a PD tetrad. A single exchange between non-sister chromatids and in the region between genes *a* and *b* would lead to first division segregation of *a* and second division segregation of *b*, and produce a tetratype tetrad. In figure 2 only one of four possible single exchanges has been diagrammed. If we label the chromatids from top to bottom as 1, 2 3, and 4; then the four possible non-sister chromatid single exchanges will be: 1 with 3, 1 with 4, 2 with 3, and 2 with 4. All four of these single exchanges will produce tetratype tetrads.

Two-exchange tetrads are of three types: 2-strand doubles, 3-strand doubles, and 4-strand doubles. In figure 2 one exchange in these two exchange tetrads has been kept constant (the exchange to the left), always involving chromatids 2 and 3. The second exchange has involved 2 and 3 (2-strand double exchange), 1 and 3 and 2 and 4 (3-strand double exchanges), and 1 and 4 (4-strand double exchange). One can draw 12 more possible two-exchange tetrads for a total of 16, by varying the exchange to the left. These 16 possible two-exchange tetrads, however, can all be classified as 2-strand, 3-strand or 4-strand double exchanges. The ratio of these three types of double exchanges, if chromatid involvement is random (no chromatid interference), will be one 2-strand to two 3-strand to one 4-strand double exchange.

Figure 2 can be summarized as follows:

Figure 2.

Relationship Between Exchanges and Tetrad Types in a Cross involving the Linked Genes $AB \times ab$

Multiplicity of exchanges			Genotypes of f_1 products	Tetrad Type
No exchanges	Single exchanges	Two exchanges		
			*AB* *AB* *ab* *ab*	PD
		 	*AB* *Ab* *aB* *ab*	T
			*Ab* *Ab* *aB* *aB*	NPD

No exchange = PD

Single exchange = T

Double exchange	frequency
a) 2-strand = PD	¼
b) 3-strand = T	½
c) 4-strand = NPD	¼

It is important to note that 4-strand double exchanges produce a unique tetrad type (NPD). This is a tetrad type which cannot be produced in a linked gene-gene relationship by either non-exchange or single-exchange events. The frequency of non-parental ditype tetrads, therefore, is a measure of the frequency of multiple exchanges. If we assume that double exchanges are the highest multiple exchanges occurring, then the total frequency of double exchanges can be estimated by multiplying the non-parental ditypes by four. (Perkins 1949).

Genes which are relatively closely linked will produce primarily non-exchange tetrads (PD). Single exchange tetrads (T) will occur with a lower frequeny (depending on the degree of linkage). Double exchange tetrads (detected by NPD)

will be rare and will vary with the degree of chiasma interference.

The frequency of parental ditype tetrads is a measure of the frequency of non-exchange meioses. However, some of these PD tetrads occur not as a result of non-exchange events, but as a result of 2-strand double exchange events. Although we have no way of directly measuring 2-strand double exchanges, we can estimate the frequency of 4-strand double exchanges from the frequency of NPD tetetrads. Since 2-strand doubles and 4-strand doubles occur with equal frequency (each ¼ of total double exchanges), we can use the NPD tetrad frequency as an estimate of the frequency of occurrence of 2-strand double exchange. Thus: frequency of non exchanges = frequency of PD − frequency of NPD. Using brackets to designate frequency:

[non-exchange tetrads] = [PD] − [NPD] *Equation 1.*

We can obtain the frequency of single exchange tetrads from the frequency of tetratype tetrads, when that frequency is corrected for those tetratypes which result not trom single exchanges but from 3-strand double exchanges. Since 3-strand doubles occur with twice the frequency of 4-strand doubles:

[single exchange tetrads] = [T] − 2 [NPD] *Equation 2.*

It has previously been pointed out that:

[double exchange tetrads] = 4 [NPD] *Equation 3.*

We are now ready to derive a formula for the estimation of *exchange frequency* from the observed tetrad type frequencies. The exchange frequency is the average number of exchanges per total number of tetrads (meioses). It is directly equivalent to chiasma frequency (assuming that each chiasma represents an exchange). Exchange frequency is given by the following formula:

$$\text{Exchange frequency} = \frac{\text{single exchanges} + 2 \times \text{double exchanges}}{\text{total tetrads}}$$

Note that the double exchanges are multiplied by two, since each double exchange tetrad represents two separate exchange events. The above formula can be expressed entirely in frequencies:

$$\text{Exchange frequency} = \frac{[\text{single exchanges}] + 2 \times [\text{double exchanges}]}{[\text{non-exchanges}] + [\text{single exchanges}] + [\text{double exchanges}]}$$

By substitution from *equations 1, 2,* and *3:*

$$\text{Estimated exchange frequency} = \frac{[T] - 2 \times [NPD] + 2 \times 4 \times [NPD]}{[PD] - [NPD] + [T] - 2 \times [NPD] + 4 \times [NPD]}$$

By reduction:

$$\text{Estimated exchange frequency} = \frac{[T] + 6 \times [NPD]}{[PD] = [NPD[+ [T]}$$

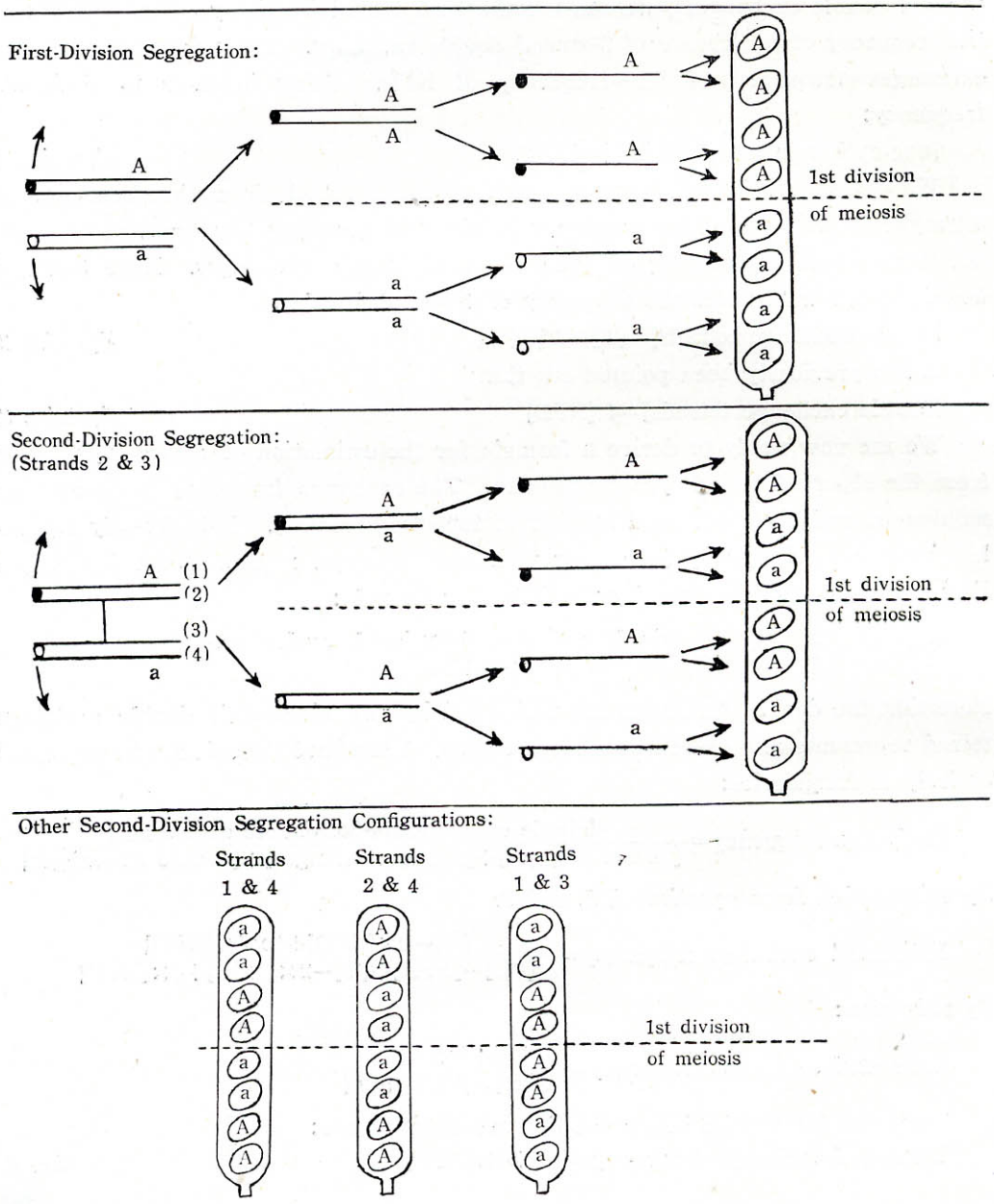
Since the frequencies below the line are equal to one:

Estimated exchange frequency = [T] + 6 × [NPD] *Equation 4.*

(Perkins 1949)

Although *equation 4* is logically correct if one makes the given assumptions, it is not generally applied (see Barratt, Garnjobst, Perkins & Newmeyer 1954; Ebersold, Levine, Levine & Olmstead 1962) because of the large sampling error which may be involved in multiplying the NPD tetrads by six. It should be emphasized that

Figure 3.
First-and Second-Divison Segregation in the Ordered Tetrads of *Neurospora crassa*



equation 4 assumes no chromatid interference (it assumes that the NPD tetrads will be ¼ of the total number of double exchanges).

In general practice, the tetratype frequency (T) is utilized in computing gene-gene map distances, and the NPD tetrads are ignored in this calculation (see below).

THE ESTIMATION OF GENE-CENTROMERE EXCHANGE FREQUENCIES IN ORDERED TETRADS

In *Neurospora crassa*, the eight ascospores in a single ascus are the result of the two meiotic divisions plus a postreductional mitosis of each meiotic product. The importance of the *Neurospora* ascus is that the two daughter nuclei of the first division of meiosis, as well as their respective daughter nuclei remain (with rare exceptions) in opposite ends of the ascus. Thus, a line drawn through the center of the ascus (transversely) separates those centromeres, chromatids, and genes which separated at first division of meiosis (see figure 3).

Since it is highly likely that centromeres are prereduced (the two homologous centromeres from the two parents segregate at first division), the only way the two alleles of a gene could segregate at the second division of meiosis would be to become attached to a different centromere by a chromatid exchange. The frequency of second-division segregation of alleles is, therefore, a measure of the frequency of exchange between a gene and it's centromere. It is an estimated exchange frequency.

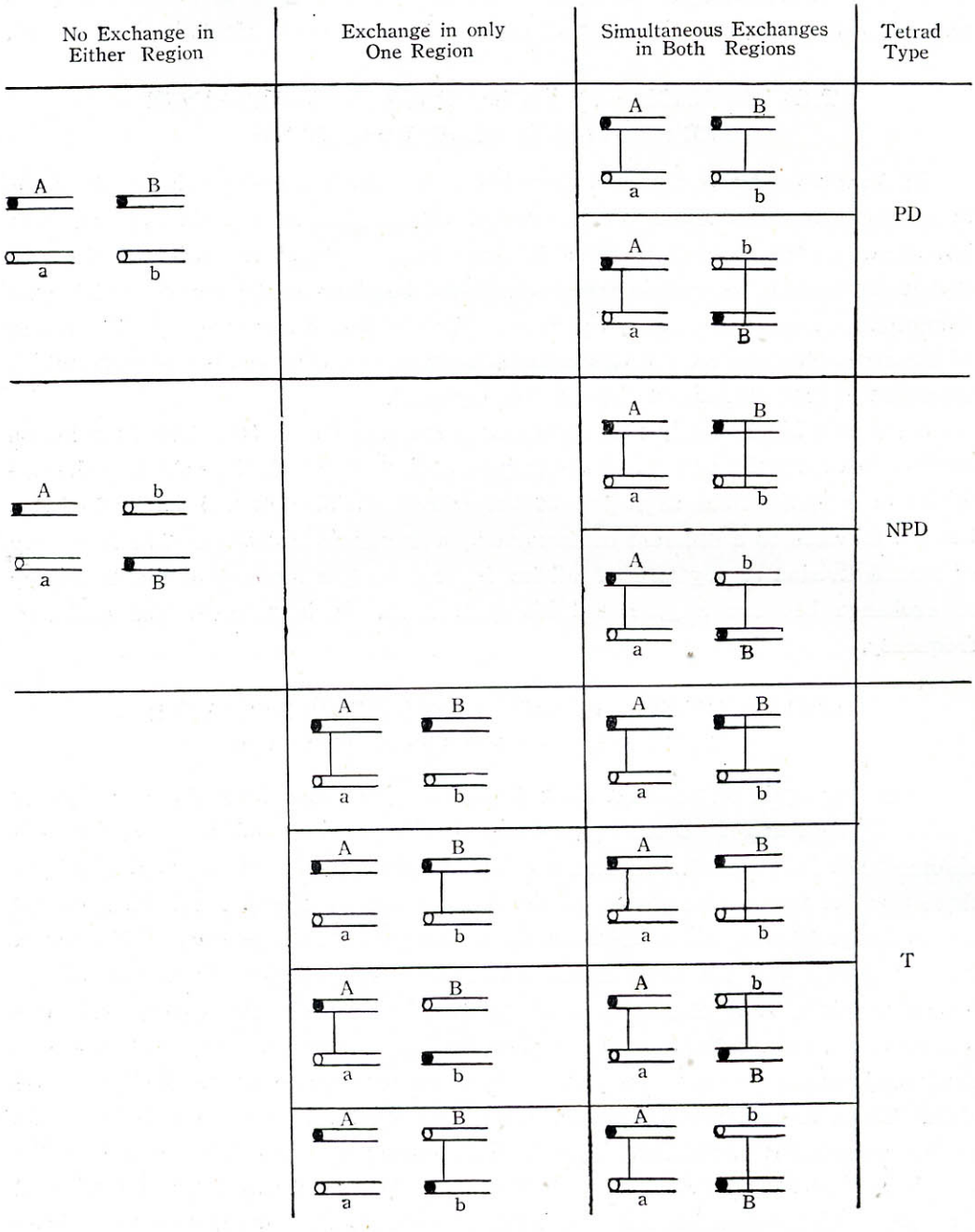
THE ESTIMATION OF GENE-CENTROMERE EXCHANGE FREQUENCIES IN UNORDERED TETRADS

Returning again to the cross $AB \times ab$ (figure 1), consider how the three tetrad types (PD, NPD and T) could be produced should genes *a* and *b* be on different chromosomes (non-linked). In figure 4 the simplest modes of production of the three types of tetrads are given. In the first column of figure 4 are diagrammed non-exchange tetrads, where there are no exchanges between gene *a* and it's centromere or gene *b* and it's centromere. There are two possible orientations of the metaphase plate. They may be in a *cis* position with both centromeres from one parent on one side of the metaphase plate, or in a *trans* position, with the two non-homologous centromeres from one parent on opposite sides of the metaphase plate. These two orientations should occur with equal frequency and lead to the random assortment of non-linked genes. The first arrangement (*cis*) leads to a PD tetrad, and the second (*trans*) to a NPD tetrad. Non-linked genes, therefore, should show an equal frequency of PD and NPD tetrads. It is, indeed, deviations from this equality which are evidence for linkage in tetrad analysis (see below).

An exchange between either gene and it's centromere will lead to a tetratype tetrad (column 2 of figure 4). Thus, the frequency of T tetrads is a

Figure 4.

The Simplest Modes of Production of the Three Tetrad Types (PD, NPD & T)
When Genes are on Different Chromosomes (Not-Linked)



measure of the frequency of exchanges in the two regions between the genes and their respective centromeres. Simultaneous exchanges in both regions are diagrammed in column 3 of figure 4. Half of these simultaneous exchanges will produce

tetratype tetrads, the other half producing ditypes (¼ PD: ¼ NPD).

Let: p=tetratype frequency

x=frequency of exchanges between a and it's centromere

y=frequency of exchanges between b and it's centromere

If we ignore the simultaneous occurrence of exchanges in both regions (consider only non-exchange tetrads and exchanges involving one region), then the following relationship exists:

$p=x+y$

Simultaneous exchanges in both regions should occur at a frequency which is the product of their independent frequencies (xy). Since simultaneous exchanges do not necessarily lead to tetratype tetrads, we must correct the above equation as follows:

$p=(x-xy)+(y-xy)$

Since half of the simultaneous exchanges *do* produce tetratype tetrads (see figure 4, we can correct the above equation by adding back this portion of the simultaneous exchanges:

$p=(x-xy)+(y-xy)+1/2xy$

By reduction:

$p=x+y-3/2xy$ *Equation 5.*

(Perkins 1949)

Equation 5 gives the relationship between two unknown quantities (x and y) which we would like to calculate, and one known quantity (p) which we can observe in any cross involving segregation of two non-linked genes. An equation with two unknowns and one known cannot, obviously, be solved.

A solution to the distances x and y, however, is possible if we have one more independent gene segregating in the cross (if the cross is $ABC\times abc$) (Perkins 1949). Now we can observe tetratype frequencies for three gene-gene relationships, namely: the tetratype frequency between genes a and b (p), between b and c (q), and between a and c (r). If we designate exchange frequency between c and it's centromere by z, then:

$p=x+y-3/2xy$

$q=y+z-3/2yz$

$r=x+z-3/2xz$

Where: p=tetratype frequency between genes a and b

q=tetratype frequency between genes b and c

r=tetratype frequency between genes a and c

And: x=exchange frequency between gene a and it's centromere

y=exchange frequency between gene b and it's centromere

z=exchange frequency between gene c and it's centromere

We now have three observed (known) values (p, q, and r), and three unknown

values (x, y, and z), and three simultaneous equations. It is possible to solve for exchange frequencies between the three genes and their respective centromeres (Perkins 1949). The solutions of the three simultaneous equations are given by Whitehouse (1950):

$$x=2/3(1\pm\sqrt{\frac{4-6p-6r+9pr}{4-6q}}) \qquad \textit{Equation 6.}$$

$$y=2/3(1\pm\sqrt{\frac{4-6p-6q+9pq}{4-6r}}) \qquad \textit{Equation 7.}$$

$$z=2/3(1\pm\sqrt{\frac{4-6q-6r+9qr}{4-6p}}) \qquad \textit{Equation 8.}$$

Once one has established an exchange frequency between a particular gene and it's centromere, one can utilize this gene to establish exchange frequencies between other independent genes and their respective centromeres. This can be done by a rearrangement of *equation 5* (see Gowans 1960):

$$x=\frac{2(p-y)}{2-3y} \qquad \textit{Equation 9.}$$

Where: x=unknown exchange frequency between gene d and it's centromere
y=known exchange frequency between gene e and it's centromere
p=tetratype frequency between unmapped gene d and mapped gene e

COMPARISON OF MAPPING FUNCTIONS IN TETRAD ANALYSIS AND SINGLE STRAND ANALYSIS

The exchange frequency between two linked genes or between a gene and it's centromere is an important relationship to measure, since it is a measure of the relative distance of the region being considered. It is assumed that the exchange frequency for any region is constant (for constant conditions). Since we cannot directly observe the number of exchanges in a whole series of tetrads, we utilize the tetrad types or second-division segregation frequencies to make an estimate of the exchange frequency (estimated exchange frequency).

In classical genetic organisms such as maize and *Drospophila*, the frequency of crossing-over is estimated from the frequency of observable recombinations between two linked genes. *Crossing-over frequency** is the frequency of chromatids or strands which have been involved in a cross over:

$$\text{Crossing-over frequency}=\frac{\text{cross-over strands}}{\text{total strands}}$$

* "Cross-over frequency" is often used interchangeably in the literature with recombination frequency. In order to separate the physcial event from the method of measurement of that event, we will refer to the actual frequency of cross-overs as the cross*ing*-over frequency, and the observed measure of these events as the recombination frequency (synonym: cross.over frequency) (Perkins 1955)-

The crossing-over frequency, like the exchange frequency, is assumed to be constant between any two genes. Crossing-over frequency is estimated from the recombination frequency.

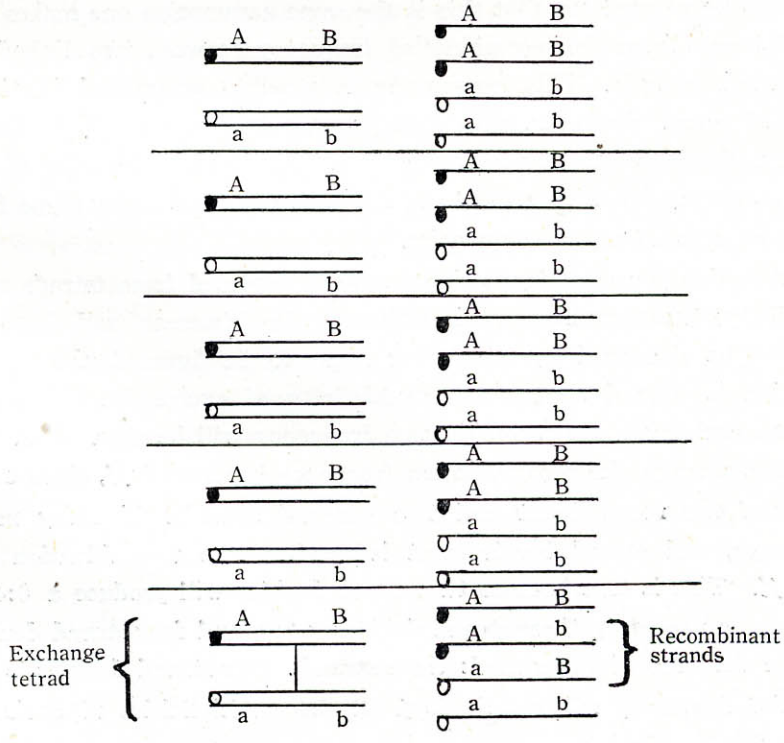
Now consider the relationship between crossing-over frequency and exchange frequency. Consider a case where five tetrads are analyzed, one of which has a single exchange (figure 5). Obviously the exchange frequency will be one in five (1/5). Suppose, however, a single strand analysis were done on the meiotic products from these same five tetrads. Each tetrad produces four strands for a total of twenty. Only two of these strands would be recombinant for the two linked genes, for a recombination frequency of 2/20 or 1/10. It is obvious, therefore, that:

Exchange frequency/2=crossing-over frequency

Also, for this simple case where only non-exchange and single-exchange events are involved, and where the same strands are being used for the tetrad analysis and the single strand analysis, the following equality will hold:

Figure 5.

Relationship Between Exchange Frequency and Recombination Frequency in a Simple Case



$$\text{Exchange frequency} = \frac{\text{number of exchanges}}{\text{number of tetrads}} = \frac{1}{5}$$

$$\text{Recombination frequency} = \frac{\text{number of recombinant strands}}{\text{total number of strands}} = \frac{2}{20} = \frac{1}{10}$$

Extimated exchange frequency/2=recombination frequency

Since the concept of genetic map distance was developed in higher organisms utilizing single-strand analyses, map units are based on an estimate of crossingover frequency (recombination frequency or derivatives therefrom). Map distance is generally defined as the percent recombination. If we utilize tetratype frequency or second division segregation frequency in determining exchange frequency, then the general equation for converting estimated exchange frequency (tetratype frequency or second-division segregation frequency) to map units is:

$$\text{map units} = \frac{\text{estimated exchange frequency}}{2} \times 100 \qquad \textit{Equation 10.}$$

Where: estimated exchange frequency=[T] OR [2nd division segregation]

Equation 10 is the formula generally utilized to determine map distance between two linked genes or between a gene and it's centromere in a tetrad analysis (see Barratt, Garnjobst, Perkins and Newmeyer 1954 for a discussion). It should be pointed out that in utilizing this equation one is assuming complete interference (one is assuming that all tetrads are either non-exchange or single-exchange tetrads). It should also be pointed out that this is the same assumption one makes in computing map distances from the recombination frequency between two linked genes in a single-strand analysis. This assumption is possibly warranted in the case of closely linked genes. However, with distant genes it seems probable that double- and higher multiple-exchanges are taking place. Increased multiplicity of exchanges (increases above single-exchanges) has a quite different effect on tetratype frequencies than it has on recombination frequencies.

The proportion of recombinant single strands expected from tetrads of any rank (or multiplicity) whatever except 0 is uniformly ½ (Emerson and Rhoades 1933). This can best be visualized by consulting figure 6. In figure 6, the frequency of recombination is plotted against the multiplicity of exchanges. If none of the meioses have exchanges, the recombination frequency will be zero. If every meiosis has a single exchange, the recombination frequency becomes 50%, since each tetrad will consist of two recombinant and two parental strands. If every meiosis has two exchanges, and strand involvement is random, the recombination frequency remains 50%. This is true because two-strand doubles will produce a 0:4 ratio of recombinants to parentals, three-strand doubles a 2:2, and four-strand doubles a 4:0 ratio. Since the ratio of two-strand: three-strand: four-strand doubles is 1:2:1, the recombination frequency will remain 50%. Higher multiplicities of exchanges will not change this equilibrium.

The proportion of each successive rank or multiplicity of exchange per meiosis to be tetratypes or to show second-division segregation is given by the following formula:

Figure 6.

Relationship of Recombination Frequency to Multiplicity of Exchanges

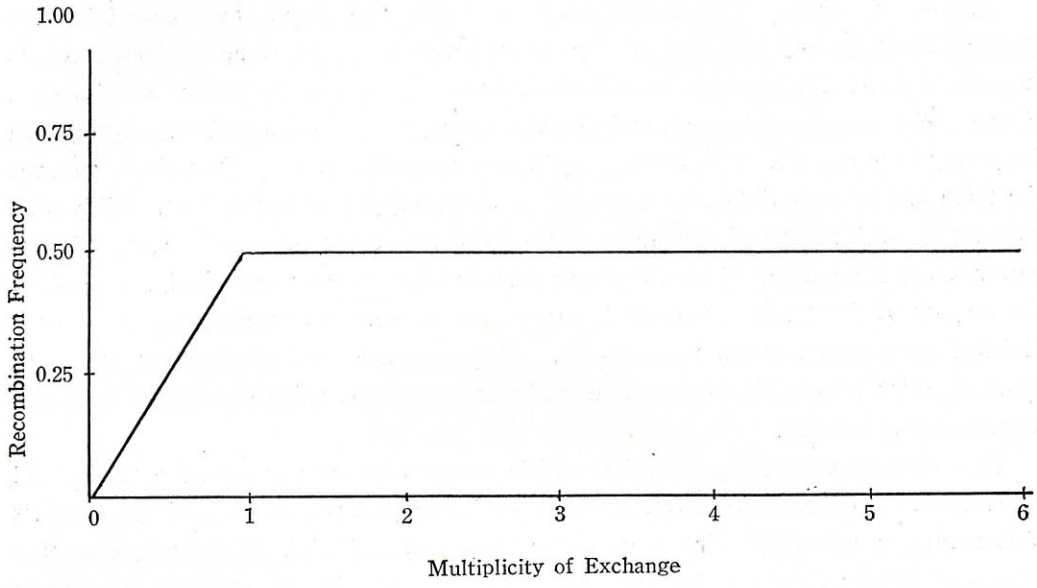
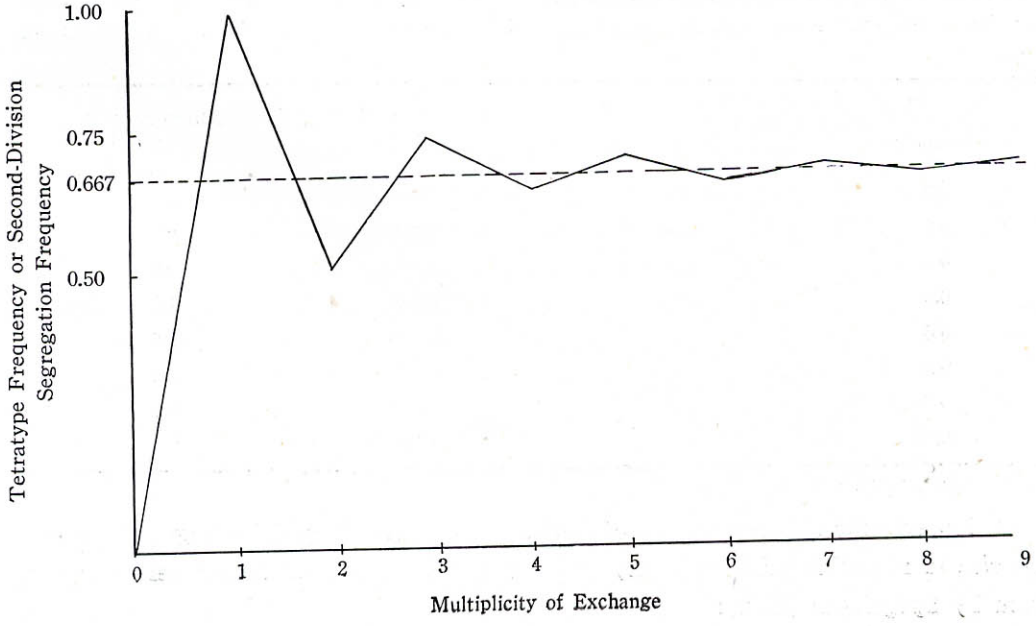


Figure 7.

The Relationship of Tetratype Frequency or Second Division Segregation Frequency to Multiplicity of Exchanges



[T] or [2nd division seg'n.]=2/3 [1−(−½)^r]

Where: r=rank or multiplicity of exchange (Mather 1935)

Figure 7 shows the relationship between tetratype or second-division segregation frequency and multiplicity of exchanges. Again, if no exchange occurs in each meiosis, the tetratype or second-division segregation frequency will be zero. If one exchange occurs in each meiosis, the tetratype or second-division segregation frequency becomes 1.0. The addition of a second exchange to each meiosis reduces the tetratype or second-division segregation frequency by one-half (0.5). With each increase in multiplicity of exchanges half of the tetratypes or second-division segregation types are converted to ditypes or first-division types respectively, and all of the ditypes of first-divison segregation types are converted to tetratypes or second-division segregation types respectively. This produces the fluctuating curve in figure 7 which eventually reaches an equilibrium at 0.667 tetratype or second-divivison segregation frequency.

It is obvious that for a situation which assumes no interference, the theoretical upper limit of recombination frequency is 50%, and that the theoretical upper limit of tetratype or second-division segregation frequency is 66.7%. Thus, map distances calculated from recombination frequencies in a single strand analysis, and from one half the tetratype or second-division segregation frequency in a tetrad analysis are not completely comparable, especially when the map distances are large. The differences involved for this situation of complete interference are summarized in figure 8.

Figure 8*

The Comparison of Recombinant Map Distances Approximated from 1/2 the Tetratype or Second-Division Segregation Frequency, to Recombinant Map Distances Obtained from a Single-Strand Analysis

Tetratype or 2nd Division Segregation Frequency	1/2 Tetratype or 2nd Division Segregation Frequency	Recombination Frequency	Difference in Map Units
0.0	0.0	0.0	0.0
0.1	0.05	0.056	0.6
0.2	0.10	0.106	0.6
0.3	0.15	0.165	1.5
0.4	0.2	0.228	2.8
0.5	0.25	0.302	5.2
0.6	0.3	0.392	9.2
0.67	0.335	0.501	16.6

* after Spiegelman 1952

A formula for the conversion of tetratype or second division segregation frequencies to recombination frequency under the assumption of zero interference is given by Spegelman (1952):

Recombination frequency $=\frac{1}{2}\ [1-(1-3/2x)^{2/3}]$ *Equation 11.*

Where: x=tetratype or second-division segregation frequency

Barratt, Newmeyer, Perkins and Garnjobst (1954) have discussed mapping functions which assume interference levels between zero and complete interference, and have compared the calculated effect of several levels of interference with actual data from *Drosophila* and *Neurospora*. It appears from their calculations that if one is going to chose one of the two extreme situations (zero interference or complete interference) as an assumption for calculating map distance that the complete interference situation would be the best choice (Equation 10).

THE DETECTION OF LINKAGE AND INDEPENDENCE IN TETRAD ANALYSIS

As pointed out by Perkins (1953) single strand analyses are a more efficient method (per strand) of detecting linkage than are tetrad analyses. However, since one may obtain other information from tetrad analysis, one may prefer to detect linkage also by tetrad analysis. The comparison of the numbers of the two types of ditype tetrads (PD and NPD) is the most efficicient and accurate method of linkage detection in tetrad analysis (Perkins 1953). As diagrammed in figure 4, there should be an approximately equal number of PD and NPD tetrads when two genes are non-linked. On the other hand, linked genes should produce an excess of PD over NPD tetrads, as illustrated in figure 2. A significant deviation in one direction from a 1:1 ratio of PD to NPD tetrads indicates linkage. The deviation would theoretically always be toward an excess of PD over NPD. Figure 9 gives the ratios of PD to NPD tetrads which give evidence of linkage at different significance levels.

If there is no significant deviation from a 1:1 ratio of PD: NPD tetrads, the two genes can be considered to be independent. There is another criterion for gene independence in tetrad analyses. It is unlikely that two genes are linked if the NPD: T ratio significantly exceeds 1:4 (Perkins 1953; Barratt, Newmeyer, Perkins and Garnjobst 1954). This relationship can best be understood by referring to figure 7. Two distantly linked genes would have a high multiplicity of exchanges between them, and therefore would produce a tetratype frequency of 2/3 (figure 7). The remaining tetrads (1/3) would be ditypes, half of which (1/6) would be non-parental dityes. Therefore, with distantly linked genes 1/6 of the tetrads would be NPD, and 4/6 (=2/3) would be tetratypes (1 NPD: 4 T ratio). Genes which are not linked, on the other hand, would be expected to produce a higher ratio of NPD: T tetrads (see figure 4).

CONFIDENCE LIMITS ESTIMATED EXCHANGE FREQUENCIES

The determination of standard sampling errors for estimated exchange frequencies for regions between two linked genes or between a gene and a centromere in an

ordered tetrad is relatively simple, since the estimated exchange frequencies are obtained directly from observed values (tetratype frequencies or second-division segregation frequencies, respectively). Barratt, Newmeyer, Perkins and Garnjobst (1954) have discussed this subject, and have produced a graph (their figure 8) from which the confidence limits can be read directly.

Figure 9*

Smallest Numerical Ratios Showing Significant Deviation in One Direction from 1:1

Total Numbers	Ratios Attaining Significance Level (One-Sided)			Total Numbers	Ratios Attaining Significance Level (One-Sided) 5%
	5%	2½%	1%		
5	5:0			51	33:18
6	6:0	6:0		52	33:19
7	7:0	7:0	7:0	53	34:19
8	7:1	8:0	8:0	54	34:20
9	8:1	8:1	9:0	55	35:20
10	9:1	9:1	10:0	56	35:21
11	9:2	10:1	10:1	57	36:21
12	10:2	10:2	11:1	58	36:22
13	10:3	11:2	12:1	59	37:22
14	11:3	12:2	12:2	60	38:22
15	12:3	12:3	13:2	61	38:23
16	12:4	13:3	14:2	62	39:23
17	13:4	13:4	14:3	63	39:24
18	13:5	14:4	15:3	64	40:24
19	14:5	15:4	15:4	65	40:25
20	15:5	15:5	16:4	66	41:25
21	15:6	16:5	17:4	67	41:26
22	16:6	17:5	17:5	68	42:26
23	16:7	17:6	18:5	69	43:26
24	17:7	18:6	19:5	70	43:27
25	18:7	18:7	19:6	71	44:27
26	18:8	19:7	20:6	72	44:28
27	19:8	20:7	20:7	73	45:28
28	19:9	20:8	21:7	74	45:29
26	20:9	21:8	22:7	75	46:29
30	20:10	21:9	22:8	76	46:30
31	21:10	22:9	23:8	77	47:30
32	22:10	22:10	23:9	78	47:31
33	22:11	23:10	24:9	79	48:31
34	23:11	24:10	25:9	80	49:31
35	23:12	24:11	25:10	81	49:32
36	24:12	25:11	26:10	82	50:32
37	24:13	25:12	26:11	83	50:33
38	25:13	26:12	27:11	84	51:33
39	26:13	27:12	28:11	85	51:34
40	26:14	27:13	28:12	86	52:34
41	27:14	28:13	29:12	87	52:35
42	27:15	28:14	29:13	88	53:35
43	28:15	29:14	30:13	89	54:35
44	28:16	29:15	31:13	90	54:36
45	29:16	30:15	31:14	91	55:36
46	30:16	31:15	32:14	92	55:37
47	30:17	31:16	32:15	93	56:37
48	31:17	32:16	33:15	94	56:38
49	31:18	32:17	34:15	95	57:38
50	32:18	33:17	34:16	96	57:39
				97	58:39
				98	58:40
				99	59:40
				100	59:41

* after Perkins (1953)

The estimated exchange frequencies in regions between genes and centromeres in unordered tetrads, however, are not directly observed, but are calculated values. These values are calculated by means of equation 6, 7, and 8 from the observed tetratype frequencies for each gene-gene relationship. Since standard sampling errors must be based on the sample, one must base the standard error on the observed number of tetratypes for each gene-gene relationship ($t_{a,b}$; $t_{b,c}$; $t_{a,c}$), and the total number of tetrads observed for each gene-gene relationship ($n_{a,b}$; $n_{b,c}$; $n_{a,c}$).

Figure 10

Example of the Application of the Formulae for Standard Sampling Error for Exchange Frequencies Calculated from Simultaneous Equation(1).

Gene-gene relationship(2).

	$a-b$	$b-c$	$a-c$
n	390	479	444
t	40	43	65
v(3)	0.00074	0.00051	0.00104

Exchange frequencies (from equations 12, 13, and 14):

x=*nic-1* and centromere=0.0843

y=*pab-1* and centromere=0.0209

z=*thi-1* and centromere=0.0711

Standard Errors (equations 15, 16, and 17):

$$\sigma(x) = \left(1/3-\frac{0.0843}{2}\right)\sqrt{0.00074+0.00051+0.00104} = 0.0139$$

$$\sigma(y) = \left(1/3-\frac{0.0209}{2}\right)\sqrt{0.00074+0.00051+0.00104} = 0.0155$$

$$\sigma(z) = \left(1/3-\frac{0.0711}{2}\right)\sqrt{0.00074+0.00051+0.00104} = 0.0142$$

Gene-Centromere Region	Exchange Frequency	Map Distance
nic-1	0.0843±0.0139	8.43±1.39
pab-1	0.0209±0.0155	1.04±1.55
thi-1	0.0711±0.0142	3.55±1.42

(1) Method from Whitehouse (1957), data from Gowans (1960).

(2) a=*nic*-1, b=*pab-1*, c=*thi-1*

(3) Calculated from equations 18. 19, and 20.

The following formulae, given by Whitehouse (1957) can be used for the calculation of standard sampling error for estimated exchange frequencies obtained by the use of simultaneous equations:

$$\sigma(x)=(1/3-x/2)\ (v_{a,b}+v_{b,c}+v_{a,c}) \qquad \textit{Equation 12.}$$

$$\sigma(y)=(1/3-y/2)\ (v_{a,b}+v_{b,c}+v_{a,c}) \qquad \textit{Equation 13.}$$

$$\sigma(z)=(1/3-z/2)\ (v_{a,b}+v_{b,c}+v_{a,c}) \qquad \textit{Equation 14.}$$

Where:

$$v_{a,b}=\frac{9t_{a,b}(n_{a,b}-t_{a,b})}{n_{a,b}(2n_{a,b}-3t_{a,b})^2}$$

$$v_{a,c}=\frac{9t_{a,c}(n_{b,c}-t_{b,c})}{n_{a,b}(2n_{b,c}-3t_{b,c})^2}$$

$$v_{a,c}=\frac{9t_{a,c}(n_{a,c}-t_{a,c})}{n_{a,c}(2n_{a,c}-3t_{a,c})^2}$$

And: $\sigma(x)$, $\sigma(y)$, and $\sigma(z)$ are the standard sampling errors, respectively, for the calculated exchange frequencies x, y, and z from equations 6, 7, and 8.

Whitehouse (1957) gives an example of the application of these formulae to the data of Sager (1955). In figure 10 the formulae are applied to the data of Gowans (1960).

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