A NOTE ON THE FREQUENCY DISTRIBUTION OF TETRADS BY RANK IN DROSOPHILA MELANOGASTER

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TETRAD frequencies for free X chromosomes, reasonably well marked for their entire genetic length, have been computed by WEINSTEIN (1936). A typical set of values are: no-exchange tetrads (E_0) = 0.056, one-exchange tetrads (E_1) = 0.485, two-exchange tetrads (E_2) = 0.429, and tetrads of rank greater than two (E_3) = 0.030. In tetrad frequencies obtained from structurally abnormal X chromosomes, e.g., heterozygous inversions, exchange is reduced. This is indicated, generally, by an increased frequency of zero (and, depending on the amount of the reduction, perhaps single) exchange tetrads at the expense of the multiple exchange classes. There are, however, two structurally aberrant chromosomes, the exchange values from which do not accord with any others known; namely, the reversed acrocentric compound X chromosome and the reversed compound ring X chromosome. The former is structurally similar to an attached-X chromosome with the centromere subterminal instead of medial, and the latter is also similar to an attached-X chromosome, but with the free chromosome ends connected by a heterochromatic segment. The two chromosomes, then, are structurally very much alike both in the way they can synapse and in that they both contain a heterochromatic segment between the two component chromosomes of the compound. Both chromosomes give a frequency distribution of tetrads characterized by a high frequency of tetrads of ranks zero and two (or perhaps greater than two) with a low frequency of single exchange tetrads (SANDLER 1954, 1957). Such a distribution is manifestly very strange.

Now, of course, the first possibility that must be considered is that this distribution is only apparent; that is, some mistaken assumption has been used in analyzing the data and arriving at the exchange values. The possibility would exist, for example, that single exchange products from these chromosomes are not recoverable, or that chromatid interference operates in these compounds. Although it is certainly not possible to eliminate all such possibilities, the reports on those chromosomes show that they are rather unlikely, and that the most reasonable supposition at the present time is that the strange distribution inferred from the data is approximately the true one. Existing data are unfortunately not precise enough to distinguish between a low frequency of single exchanges and an absence of singles.

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If single exchanges are completely absent, it would seem that the explanation required is a physical (as opposed to a stochastic) model according to which it would physically be extremely difficult, or impossible, for a single exchange to occur in these compounds. If, on the other hand, there is really some low frequency of singles (as indeed the data would appear to indicate), then it would seem that a model is required that renders singles, not impossible, but only relatively improbable. In this note, we propose to explore one such model. It should be emphasized that this is not meant to imply that the physical model will not be required—we merely wish to consider the second alternative.

THE PROPOSED MODEL

Consider first the case of normal, free, X chromosomes. Imagine that the physical basis of crossing over (chromosome replication, for instance) starts at one end of the chromosome arm (which end is not, for the moment, important) and proceeds toward the other end. We suppose that in every genetic unit of length, there is some constant probability of an exchange, α. After an exchange has occurred, chromosome replication continues in the same direction, but the per-unit probability of an exchange is now different, say β. After a second exchange, the probability reverts to α, after a third exchange, to β again, and so forth. Now, if it is possible to consider the frequency distribution of tetrads from normal X chromosomes as being generated by these two probabilities, it seems likely that $\alpha > \beta$. If this is so, then the distribution from the reversed acrocentric and reversed ring compounds might be generated by this same model but with the two probabilities (α and β) interchanged. This might mean, for example, that the interstitial heterochromatin of the compound is synaptically equivalent to the first exchange. The required distribution could come about because the probability of the first exchange is low (β), giving rise to an appreciable number of no-exchange tetrads, but, given an exchange, the probability of a second exchange is high (α), which could mean that most of the single-exchange tetrads would be converted to the double-exchange class.

It is now required that we express the probabilities of the different rank tetrads (for normal chromosomes) in terms of the two parameters, α and β. We must, however, first consider the matter of interference. Ideally, we should like to incorporate into the probability expressions a function of the distance from any exchange, such that the probability of any exchange (except the first) at any point would be given by the appropriate parameter (β, if, for example, the exchange being considered is the second one) multiplied by the function evaluated at the point being considered. This approach was not used because the incorporation of such functions (even relatively simple ones) lead to complex products which are, in general, intractable. Instead, the true function, whatever it may be, has been approximated by a step function whose value is zero for i units after every exchange and one thereafter. This can be handled reasonably easily.

We now write down the probabilities of exchanges of ranks 0, 1, 2, and more

than 2—P_0, P_1, P_2, and P_3. It follows directly from the model that, for a chromosome N units long, $P_0 = (1 - a)^N$.

To obtain P_1, we consider separately all the possible cases; that is, that the one required exchange in the tetrad occurs in each of the N genetic units. Thus, for the first $(N - i)$ units we have,

Unit in which the exchange occurs	*Probability*
1	$(1-a)^0\, a\, (1-\beta)^{N-i-1}$
2	$(1-a)^1\, a\, (1-\beta)^{N-i-2}$
3	$(1-a)^2\, a\, (1-\beta)^{N-i-3}$
.	.
.	.
.	.
k	$(1-a)^{k-1}\, a\, (1-\beta)^{N-i-k}$
.	.
.	.
.	.
$N-i$	$\dfrac{(1-a)^{N-i-1}\, a\, (1-\beta)^0}{a \sum_{k=1}^{N-i} (1-a)^{k-1}\, (1-\beta)^{N-i-k}}$

,

and for the remaining i units, in which the probability of a second exchange is zero by the definition of i, we have,

Unit in which the exchange occurs	*Probability*
$N-i+1$	$(1-a)^{N-i}a$
$N-i+2$	$(1-a)^{N-i+1}a$
.	.
.	.
.	.
r	$(1-a)^{N-i+r}a$
.	.
.	.
.	.
N	$\dfrac{(1-a)^{N-1}a}{a \sum_{r=0}^{i-1} (1-a)^{N-i+r}}$

.

In total then,

$$P_1 = a \sum_{k=1}^{N-i} (1-a)^{k-1}\, (1-\beta)^{N-i-k} + a \sum_{r=0}^{i-1} (1-a)^{N-i+r} \quad .$$

By the same type of argument,

$$P_2 = \alpha \sum_{s=1}^{N-2i-1} (1-\alpha)^{s-1} \left[\beta \sum_{k=1}^{N-2i-s} (1-\beta)^{k-1} (1-\alpha)^{N-2i-k-s} + \beta \sum_{r=0}^{i-1} (1-\beta)^{N-2i-s+r} \right]$$

$$+ \alpha \beta \sum_{t=0}^{i-1} (1-\alpha)^{N-2i-1+t} \sum_{x=0}^{i-t-1} (1-\beta)^{x} ,$$

and, finally,

$$P_3 = 1 - P_0 - P_1 - P_2.$$

Evaluating the indicated summations, we find that

$$P_0 = (1-\alpha)^N$$

$$P_1 = \frac{\alpha(1-\beta)^{N-i} - \beta(1-\alpha)^{N-i}}{\alpha-\beta} - P_0$$

$$P_2 = (1-\alpha)^{N-2i-1} \left[1 + \frac{(N-2i-1)\,\alpha\beta}{\beta-\alpha} \right] + \frac{\alpha^2 (1-\beta)}{(\beta-\alpha)^2}$$

$$\left[(1-\beta)^{N-2i-1} - (1-\alpha)^{N-2i-1} \right] - (P_0 + P_1)$$

$$P_3 = 1 - P_0 - P_1 - P_2.$$

According to the proposed model, the frequency distribution of tetrads from the reversed acrocentric and reversed ring compounds is generated by these same equations with α replaced by β, and β replaced by α.

Some numerical estimates of α and β

Typical tetrad frequencies for free X chromosomes are given in Table 1, and for reversed acrocentric chromosomes in Table 2. Reversed rings behave very much like reversed acrocentrics, but certain complications (see SANDLER 1957) do not allow a direct computation of exchange values.

TABLE 1

The frequency distribution of tetrads by rank for free X chromosome tetrads heterozygous for sc ec cv ct v g f

	Experiment A		Experiment B	
Rank of tetrad	Number	Relative frequency	Number	Relative frequency
0	1,709	0.06	1,522	0.09
1	17,982	0.64	10,038	0.62
2	8,076	0.28	4,464	0.28
3	472	0.02	112	0.01

In experiment B, the chromosomes were actually heterozygous for *s* instead of *g*, and in addition *car* and *bb*. The experiment has been corrected to include only the *sc* to *f* region so that it is comparable with experiment A (data from WEINSTEIN 1936).

TABLE 2

The frequency distribution of tetrads by rank for reversed acrocentric compound X chromosomes

Rank of tetrad	Experiment A		Experiment B		Experiments A + B	
	Number	Relative frequency	Number	Relative frequency	Number	Relative frequency
0	1,059	0.39	1,399	0.48	2,458	0.44
1	454	0.17	222	0.08	676	0.12
2+	1,200	0.44	1,304	0.44	2,504	0.44

Experiments A and B are two different reversed acrocentric lines, both of which are marked in the same way, and both of which carry a homolog for the compound. (Data from SANDLER 1954).

With respect to numerical values of α and β, a few points must be considered. First of all, the exchange values given in both Tables 1 and 2 must have rather high errors attached to them because the method of tetrad analysis (especially for reversed acrocentrics) involves the propagation of statistical error. Moreover, since the equations given here are not algebraically solvable, any iterative solutions obtained involve a further propagation of this error. Furthermore, experimentation with these equations shows that they are rather sensitive to changes in α and β even in the third decimal place. For example, the value of α for the case of free X chromosomes comes directly from the equation for P_0. In experiment A of Table 1, $\alpha = 0.047$; for experiment B, $\alpha = 0.041$. Although the results from these two experiments are significantly different, the experiments are genetically the same and are certainly as comparable as either is with the reversed acrocentric experiments. Thus it would seem that, whereas the iterative solutions to the equations require a high degree of accuracy of α and β, this accuracy is, for the most part, biologically meaningless. A third factor is that only integral values of i have been used in these solutions, which must add to the imprecision in the estimates of α and β. Finally, it should be noted, that whereas the value of N (taken here to be the genetic length of the chromosome) is known fairly well for free X chromosomes ($N = 57$ for the region from *sc* to *f*), only a very rough guess can be made for the case of the reversed acrocentric. The value chosen here was 67 units. It is clear that estimates of i will be affected by the lack of precision in N. It should be noted that although the estimates of α and β themselves do, of course, depend on N, the ratio, $\beta:\alpha$ is, for small α and β (in the range of interest here) only very slightly dependent on N, and it is this ratio that is of primary theoretical interest.

For these reasons it seems that the most that can be expected is a general agreement in the values of α, β, and i as computed from the different sets of data. The results of such computations are given in Tables 3 and 4, which show that, for α between 0.04 and 0.05 and for β between 0.01 and 0.02, all the data are approximately satisfied.

The value of i obtained for all cases is reasonable. The difference between the value for reversed acrocentrics (11) and normal chromosomes (17) may very well not be real. If it is real, it could be that interference is simply different in

TABLE 3

Numerical estimates of α, β and i from data from free X chromosomes

Experiment	Rank	Observed frequency	Calculated* frequency	Estimates of parameters
A	0	0.06	0.06	$\alpha = 0.047$
	1	0.64	0.64	$\beta = 0.017$
	2	0.28	0.26	$i = 17$
	3	0.02	0.04	
B	0	0.09	0.09	$\alpha = 0.041$
	1	0.62	0.63	$\beta = 0.018$
	2	0.28	0.25	$i = 17$
	3	0.01	0.03	

The region controlled extends from *sc* to *f*, and hence $N = 57$. The data for the two experiments are given in Table 1.

* The method of approximating the values of α, β, and i from the equations involved forcing a fit to the observed values for ranks 0 and 1, and then refining the values for ranks 2 and 3. Thus almost all the imprecision occurs in the last two values.

TABLE 4

Numerical estimates of α, β, and i from data from reversed acrocentric compound X chromosomes

Experiment	Rank	Observed frequency	Calculated frequency	Estimates of parameters
A	0	0.39	0.42	$\alpha = 0.050$
	1	0.17	0.21	$\beta = 0.013$
	2	0.44	0.37	$i = 11$
A + B	0	0.44	0.48	$\alpha = 0.048$
	1	0.12	0.20	$\beta = 0.011$
	2	0.44	0.32	$i = 11$

The estimate of N used here is 67. The data used are given in Table 2. (Ranks 2 and 3 are not distinguishable in these experiments and hence are combined).

normal and reversed acrocentric chromosomes, or it may be that the difference arises because the estimate of N is poor for the reversed acrocentric chromosome.

Predictions from the model

Since it is clear that, if this model is a reflection of some real physical situation, α is greater than β, and hence in a given region an exchange might be the third exchange in the tetrad more often than be the second. For example, let the chromosome be divided into three regions, 1, 2, and 3, and choose all tetrads with an exchange in region 2. Now we can compute the proportion which also has an ex-

change in region 3 when there was an exchange in region 1 and when there was not. According to the usual ideas about crossing over, these two proportions either ought be equal or, if an exchange in region 1 increases the interference between regions 2 and 3, an exchange in regions 3 (and 2) would be less frequent when there was an exchange in region 1 than when there was not. According to the assumptions used here, however, since α is greater than β, the contrary should obtain. Moreover, until this point we have not distinguished one end of the chromosome from the other, but now, if the physical basis of exchange starts more frequently from one end than the other, this comparison ought change depending on the direction on the chromosome that we use.

All such comparisons are subject to two technical limitations: (1) interference between regions 2 and 3 would tend to obscure any difference caused by the difference between α and β, and (2) the number of triple exchanges in specified regions is small, which will of course mean that the error on the ratios is high. For these reasons the following restrictions have been used: (1) region 3 must be at least 25 genetic units long, and (2) there must be at least 5 observed triple crossovers in the specified regions. With these limitations only two comparisons are possible, both of which come from experiment A (Table 1): in one case region 1 is the region from *sc* to *ec*, region 2 from *ec* to *cv*, and region 3 from *cv* to *f*; in the other case region 1 is from *f* to *g*, region 2 from *g* to *v*, and region 3 from *v* to *sc*. This is, fortunately, the same over-all region but proceeding in two different directions—the first from the tip to the centromere and the second from the centromere to the tip.

The precise comparison is

$$\frac{2,3}{2+2,3} \text{ vs. } \frac{1,2,3}{1,2+1,2,3}.$$

Proceeding from the tip to the centromere, this comparison is 0.53 (2888/5490) *vs.* 0.71 (48/68). In the other direction it is 0.48 (2888/6068) *vs.* 0.24 (64/268). These numbers suggest that the proper direction is from the tip toward the centromere. It might be noted that this is what one would expect because the interstitial heterochromatin of the reversed acrocentric and reversed ring compounds, which we suppose to be the synaptic equivalent of the first exchange, is distal in these compounds.

A second prediction from this model suggests itself. If a given genetic region is moved from a proximal (i.e. near the centromere) position to a distal one, crossing over in that region ought to increase. This is because when a region is distal, the per-unit probability of an exchange in the region would most often be α, whereas the same region in a proximal position would (a good fraction of the time, at least) be so placed that the per-unit probability of an exchange would be β. That this prediction is realized experimentally in Drosophila has, of course, been known for many years and is generally termed the "centromere effect" (BEADLE 1932).

One final point should perhaps be made. The data presented here could very

likely be considered, with respect to this model, in other and perhaps more precise ways. It is thought, however, that owing to the imprecision of the available data—most particularly the data from the reversed acrocentric compounds, which are at best crude—such analyses are not justifiable at present.

SUMMARY

A model is proposed that attempts to rationalize the frequency distribution of tetrads by rank from free X chromosomes with that of reversed acrocentric and reversed ring compounds. The former distribution is characterized by a high frequency of tetrads of ranks 1 and 2 with a lower frequency of tetrads of ranks 0 and 3, whereas the latter distribution shows a high frequency of ranks 0 and 2, with a low frequency of tetrads of rank 1 (the frequency of tetrads of rank 3 or more is indeterminate).

The model proposed is built on the supposition that the physical basis of exchange (e.g., chromosome replication) starts at one end of the chromosome (some evidence presented here suggests that this is the distal end) and proceeds toward the other end with a per-unit probability of an exchange equal to α. After an exchange has occurred, however, this probability changes (say to β); a third exchange changes it back to α, a fourth exchange causes it to revert to β, and so on. For the reversed acrocentric and reversed ring compounds this same model applies except that the compounds are assumed to start the process with the β probability instead of the α probability. This is physically the same as supposing that the heterochromatic connection between the component chromosomes of the compound is the synaptic equivalent of the first exchange.

With some reasonable approximations to account for chiasma interference, for $0.04 < \alpha < 0.05$ and for $0.01 < \beta < 0.02$, the data from normal chromosomes and from the compound chromosomes are approximated.

It is suggested that this notion will account for certain peculiar relationships between exchanges manifest in data from normal X chromosomes in Drosophila (see text), and also can account for the well known "centromere effect" phenomenon.

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