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Localized negative interference and its bearing on models of gene recombination

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1. INTRODUCTION

The use of techniques which permit automatic selection of rare recombinants in micro-organisms, and extensive use of tetrad analysis, have revealed properties of recombination which were not apparent from earlier studies.

For example, in the course of experiments in which selective techniques were used to establish the linkage relationships of a number of allelic mutants of *Aspergillus nidulans* (Pritchard, 1955) it was found that among recombinants with respect to very closely linked markers, the distribution of other markers on either side of the closely linked pair was of an unexpected type. The data were interpreted in terms of localized negative interference; that is to say, it was supposed that coincidence of recombination between the very closely linked markers and recombination in adjacent intervals occurred with greater than random frequency. The data indicated, on this hypothesis, that a positive correlation between recombination events was restricted to a few tenths of a map unit —i.e. the effect was highly localized—and its occurrence was therefore detected only because the markers used in this study were very closely linked.

That the effect is not confined to *Aspergillus* is suggested by earlier observations apparently of a similar nature in *Neurospora* (Giles, 1951, 1955) and *Drosophila* (Demerec, 1928; and possibly Sturtevant, 1951), and numerous other cases have been described more recently in bacteriophage (Streisinger & Franklin, 1956; Chase & Doermann, 1958), in *Neurospora* (St. Lawrence, 1956; Mitchell, 1956; Freese, 1957*a*, *b*; De Serres, 1958), in *Aspergillus* (Calef, 1957), in yeast (Leupold, 1958), and in *Drosophila* (Chovnick, 1958). The phenomenon seems to be a common one, although the basis for it may not be the same in all cases.

A second feature of recombination which has become apparent from extensive tetrad analysis in yeast (Lindegren, 1955; Roman, 1956; Leupold, 1958), *Neurospora* (Mitchell, 1955*a*, *b*; Case & Giles, 1958) and *Aspergillus* (Strickland, 1958*a*) is the rare occurrence of tetrads with 3 : 1 ratios with respect to one or more loci. The frequency of tetrads of this type, although low, is higher in heterozygotes than in homozygotes, and in some cases an interpretation of their origin on the basis of the known genetic mechanisms listed by Emerson (1956), such as polyploidy, is unsatisfactory. The occurrence of irregularities of this sort has also been inferred

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from analysis of half-tetrads following meiosis or mitosis (Roman, 1956; Leupold, 1958; Hexter, 1958). The occurrence of 3 : 1 ratios in tetrads has been attributed to hypothetical mechanisms distinct from crossing over and termed variously gene conversion (Lindegren, 1955), transmutation (Horowitz—see Beadle, 1957), transreplication (Glass, 1957). Roman (1956) uses the term 'non-reciprocal recombination' in connexion with his data: it should be noted that if among the four meiotic products of a cell heterozygous for two linked loci a 3 : 1 ratio occurs with respect to one of them, one of the four products will be a recombinant for which there is no complementary recombinant class.

Two questions arise from these observations. Firstly, do reciprocal recombinants and non-reciprocal recombinants result from two different mechanisms or are they the result of different aspects of a single mechanism of recombination between linked loci? Secondly, how can localized negative interference be interpreted in relation to current models of chromosome replication and recombination? In this connexion it should be pointed out that my interpretation of the data from *Aspergillus* in terms of localized negative interference has been questioned by some authors (Mitchell, 1957). The alternative is that if among a population of recombinants some were the consequence of a reciprocal recombination process (which we will call crossing over) not associated with negative interference and some the consequence of a different process, the non-reciprocal transfer of a marker from one chromosome to its homologue, the distribution of markers on either side of the interval in question might formally be described in terms of negative interference but would be significant only as an indication of the existence of more than one mechanism of recombination. The data already published (Pritchard, 1955; Roper & Pritchard, 1955), however, are difficult to reconcile with an interpretation of this sort.

In the present paper the evidence relating to localized negative interference is reviewed and added to and its implications are considered.

2. MATERIAL AND METHODS

For details of the origin of the mutant strains used in this work and the techniques employed in genetic analysis of *Aspergillus nidulans*, reference should be made to Pontecorvo, Roper, Hemmons, Macdonald & Bufton (1953), Pritchard (1955), Pontecorvo & Käfer (1958) and Käfer (1958).

3. MAP OF THE *ad8* CISTRON

Seven adenine-requiring mutations, all recessive and allelic by the *cis-trans* test, have now been mapped. Each occupies a different site in the cistron (Fig. 1).

The method of calculating the recombination fractions between these alleles has already been published (Pritchard, 1955). It is an indirect one involving a comparison of the number of colonies produced by a suspension of ascospores plated on different selective media at different densities. Two of the seven alleles are phenotypically distinguishable from the other five. Both (*ad16* and *ad20*) permit

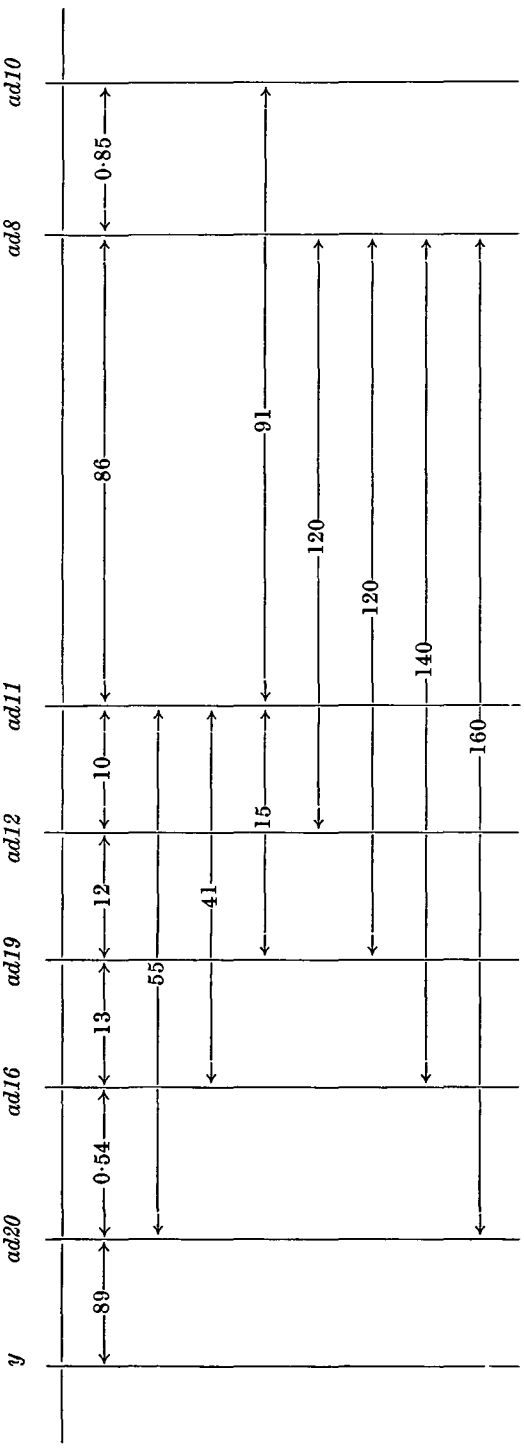

Fig. 1. Above: map of the *ad8* cistron showing recombination fractions $\times 10^5$. Below: map of chromosome I showing location of markers referred to in text. Nutritional requirements are *an*, aneurin; *paba*, *p*-amino-benzoic acid; *ad*, adenine; *bi*, biotin; and *y*, yellow conidia. Markers on other chromosomes used were *nic2*, nicotinic acid; *s12*, thiosulphate; *pyro4*, pyridoxin; *w3*, white conidia; and *Acr1*, resistance to acriflavine (see Käfer, 1958). Throughout this paper mutant alleles are referred to without the allele number except in the case of the *ad* alleles. Wild-type alleles are designated by superscript +.

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slow growth in the absence of an external supply of adenine, and *ad20* is suppressed by either of two mutant alleles at unlinked loci (*su1ad20* and *su2ad20*). The suppressors are specific for *ad20*.

4. THE OCCURRENCE OF LOCALIZED NEGATIVE INTERFERENCE

From fourteen crosses so far made of the general type y ad_x/ad_y *bi*, the majority of adenine-independent types recovered was associated with recombination between *y* and *bi*. One of the two possible recombinant classes was always in excess of the other, the recombinants being prevalently either *y bi* or y^+ bi^+.

The seven mutant alleles could be assigned a linear order on the basis of the majority recombinant class without any inconsistencies, and the map distances computed from the total frequency of adenine-independent segregants were approximately additive (Fig. 1).

The ability to obtain a map which is qualitatively and quantitatively consistent indicates that the seven sites are linearly arranged, not only with respect to each other, but also with respect to neighbouring loci. The data provide no support for models of the chromosome involving side-chains of the type proposed by Taylor (1957), since these require a branched linkage map. Evidence of this sort against a branched linkage structure has been known for some time, not, as maintained by Freese (1957*a*), only recently from experiments with bacteriophage. Thus, that allelic mutations were separable and linearly arranged with respect to each other, and to the rest of the linkage map, was first demonstrated by Lewis (1945) for alleles of '*Star*' in *Drosophila*, although Lewis did not at that time consider them to be alleles of one gene.

Ample confirmation of this observation has since been obtained from *Drosophila* (e.g. Green & Green, 1949; MacKendrick & Pontecorvo, 1952; Lewis, 1952; Green, 1954) and *Aspergillus* (Roper, 1950; Pritchard, 1955; Calef, 1957).

As to the nature of interallelic recombination, it was shown as early as 1952 by Lewis and later by Green (1954) that recombination between allelic mutants in *Drosophila* is generally reciprocal. This also appeared to be so in the case of recombination between allelic *ad* mutations in *Aspergillus* since it was shown (Roper & Pritchard, 1955) that from diploids heterozygous in *trans* for two *ad* alleles it was possible to recover adenine-independent types still doubly heterozygous, but now in *cis*, as a result of a single reciprocal event.

The unexpected feature of the data from crosses of the general type described was that the frequency of parental combinations *y* bi^+ and y^+ *bi* among adenine-independent segregants was in every case greater than 6%, the approximate frequency expected in the absence of interference. In some cases the frequency of parental combinations was as much as 40%.

The data were consistent with the view that the high frequency of parental combinations was due to a localized excess of multiple recombination events over the number expected from coincidence of independent events. The alternative hypothesis, that there was no negative interference but that recombinants were produced by two different mechanisms, crossing over and gene conversion, in

such a way as to simulate negative interference, is not supported by a comparison of the data from crosses involving different pairs of *ad* mutants.

On this hypothesis, among adenine-independent colonies obtained from crosses of the general type

$$\frac{y \quad ad_x \quad + \quad +}{+ \quad + \quad ad_y \quad bi},$$

those originating by crossing over between ad_x and ad_y would have the constitution $y^+ bi^+$, while those originating by conversion of ad_x and ad_y would have the constitution $y\, bi^+$ and $y^+ bi$ respectively. The frequency of adenine-independent colonies of each type can therefore be used to obtain an estimate of the relative frequency of the three types of event provided a correction is made for the expected occurrence of 6% recombination in the inverval ad_y–*bi* among all adenine-independent types, whatever their origin. A proportion of the crossovers between ad_x and ad_y would consequently have the constitution $y^+ bi$, and a proportion of those adenine-independent types due to conversion of ad_x and ad_y would have the constitution $y\, bi$ and $y^+ bi^+$ respectively. Recombination between *y* and ad_x can be ignored since its frequency in the absence of interference will be negligible.

The apparent frequency of conversion for each of the seven *ad* alleles was calculated in this way from two crosses, that yielding the lowest and that the highest frequency of adenine-independent types (Table 1).

Table 1. *Apparent frequency of gene conversion of seven different alleles compared in different crosses assuming no interference*

Alleles involved in cross	'Crossover' frequency ($\times 10^6$)	'Conversion' frequency ($\times 10^6$)						
		ad20	*ad16*	*ad19*	*ad12*	*ad11*	*ad8*	*ad10*
ad20 and *ad16*	2·60	0·56	0·82	—	—	—	—	—
ad8 and *ad10*	4·38	—	—	—	—	—	0·91	1·15
ad12 and *ad11*	69·7	—	—	—	5·15	11·5	—	—
ad19 and *ad12*	91·2	—	—	0·99	12·9	—	—	—
ad11 and *ad10*	543	—	—	—	—	41·6	—	140
ad19 and *ad8*	749	—	—	21·4	—	—	204	—
ad12 and *ad8*	922	—	—	—	25·9	—	113	—
ad16 and *ad8*	1080	—	30·0	—	—	—	131	—
ad20 and *ad8*	1260	81·0	—	—	—	—	89·0	—

If gene conversion were a mutational event of the type proposed by Lindegren (1955), its frequency might be expected to be specific for a particular allele, in heterozygous condition with the corresponding wild-type allele, and independent of the frequency of recombination between it and a second allele. The data in Table 1 indicate on the contrary that the apparent rate of conversion for a particular allele may differ by a factor of over 100 in different crosses and, in addition, that there is a correlation between the apparent frequency of conversion and the frequency of crossing over. This confirms the conclusion drawn previously

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(Pritchard, 1955), from a comparison of this sort between the crosses available at that time, that the data were inconsistent with the view that directed back-mutation makes a significant contribution to the formation of adenine-independent types.

A similar conclusion has been drawn more recently by Chase & Doermann (1958) from a comparison of this sort between crosses involving different rII mutants of bacteriophage, and by Freese (1957*a*) from a comparison of crosses involving different histidine mutants of *Neurospora*.

The possibility that the rates of both crossing over and gene conversion are dependent on some other condition, such as 'intimate association' between chromosomes (Mitchell, 1956), and that this is responsible for the correlation found, is unlikely in this case since the difference in the total frequency of adenine-independent types from crosses involving different pairs of *ad* alleles is clearly related to their relative locations on the linkage map.

The directed mutation of one allele under the influence of an homologous allele in a heterozygote is not the only conceivable mechanism, distinct from crossing over, which might be responsible for tetrads with 3 : 1 ratios and apparent negative interference in random strand analysis. An alternative possibility is that a segment of the genetic material might be copied twice on one of the two alternative templates in a heterozygote (Lederberg, 1955). A mechanism of this sort is favoured by Roman (1956) to account for non-reciprocal recombination in yeast.

Whether an event of this type occurred in *Aspergillus* with a frequency high enough to simulate negative interference could not easily be determined by random strand analysis; it might be by tetrad analysis. In the present case tetrad analysis of crosses between pairs of *ad* mutants could not easily be undertaken, owing to poor ascospore viability in crosses involving these mutants. The reciprocal products of recombination can nevertheless be recovered if they occur by making use of mitotic recombination in heterozygous diploids (Roper & Pritchard, 1955).

Mitotic analysis has certain limitations which make quantitative treatment of the data unreliable. The chief limitations are: firstly, that only two of the four homologous daughter strands produced at mitosis are recovered and some assumption must be made as to the distribution of these to the two daughter cells; and secondly, that the frequency of mitotic recombination is less than that of meiotic recombination by a factor of about 10^4. This means that the frequency of recombination between alleles at mitosis approaches the spontaneous back-mutation rate of the *ad* mutants used in this work.

The genotypes of adenine-independent diploids obtained from two adenine-dependent diploids heterozygous in *trans* for *ad16* and *ad8* have already been described. Data from a third diploid heterozygous for *ad20* and *ad8* are given in Table 2. The results obtained from the three diploids are summarized in Table 3.

A total of fifty-two adenine-independent diploids was obtained. The genotypes of twelve of these (e.g. types 5 and 6 in Table 2) were consistent with an origin by back-mutation of one or other *ad* allele. The number of diploids of this type was about that expected from the relative frequencies of mitotic recombination (*ca.* 10^{-7}) between, and back-mutation (*ca.* 10^{-8}) of, the *ad* mutants used.

Table 2. *Genotypes of adenine-independent diploid types derived from a diploid heterozygous for* ad20 *and* ad8

Intervals ...	...	**a**	**b**	**c**	**d**	**e**			
Parent diploid	...	*an* / +	*paba* / +	*y* / +	*ad20* / +	+ / *ad8*	+ / *bi*	+ / *nic*	*pyro* / +	*Acr* / +

Recombinant type	Genotype†	Inferred origin	No. obtained
1	*an paba y ad20 ad8 bi* / + + + + + +	Reciprocal products of single exchange in **d**	2
2	*an paba y ad20* + + / + + + + + +	Single exchange in **d**. Reciprocal products not recovered	3
3	*an paba y ad20 ad8* + / + + + + + +	3-strand double in **d** and **e**. Reciprocal products of exchange in **d** recovered	1
4	*an paba y ad20* + *bi* / + + + + + +	3- or 4-strand double* in **d** and **e**. Reciprocal products of neither exchange recovered	1
5	*an paba y ad20* + + / + + + + + *bi*	2- or 3-strand double in **d** and **e**. Reciprocal products of neither exchange recovered. Or mutation of *ad8*	1
6	*an paba y* + + + / + + + + *ad8 bi*	2- or 3-strand double in **c** and **d**. Reciprocal products not recovered. Or mutation of *ad20*	2
7	*an paba y* + + *bi* / + + + + *ad8 bi*	?	1

* Alternatively, this type might conceivably have arisen by a single inexactly reciprocal exchange as illustrated in Fig. 3.

† All remained heterozygous *nic*/+, *pyro*/+ and *Acr*/+.

Table 3. *Summary of types of adenine-independent mitotic recombinant diploids obtained from three diploids heterozygous* ad *in* trans

No. of exchanges	Reciprocal products of exchange between alleles	
	Recovered	Not recovered
One	10	19
Two	2	4
2- or 3-strand double or back-mutation	—	12
Three or more exchanges, or mutation + exchange	—	5
Total	12	40

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Recombination was clearly involved in the origin of the remaining forty diploids. The genotypes of twenty-nine of these were those expected following a single reciprocal exchange between *ad* alleles (e.g. types 1 and 2 in Table 2). We should expect to recover both products of the exchange in half these provided the occurrence of a mitotic exchange did not influence the segregation of the chromatids involved. Both products were recovered in only ten of the twenty-nine. The deviation from expectation is not statistically significant. If it is nevertheless real, two possible causes might be (*a*) that recombination at mitosis is in some cases not reciprocal, or (*b*) that the reciprocal products of a mitotic exchange segregate preferentially to different daughter nuclei (a non-disjunctional type of segregation is ruled out since all adenine-independent diploids remained heterozygous at the *paba* locus). Since in all nineteen individuals in which reciprocal products were not recovered the wild-type chromosome with respect to adenine requirement was a recombinant in the interval *y–bi* whereas the other chromosome was not, it appears that only one of the two strands involved in the recombination event was recovered and an explanation of the second type appears the more probable.

Of the remaining eleven adenine-independent diploids, six had genotypes consistent with coincidence of two mitotic exchanges, one between the *ad* alleles and one in a neighbouring interval. Reciprocal products of the mitotic exchange between the *ad* alleles were recovered in two of these and reciprocal products of both exchanges in one.

The remaining five diploids could be accounted for only by coincidence of three or more mitotic exchanges (e.g. type 7 in Table 2). In none of these were both products of an exchange between the *ad* alleles recovered. It is not unreasonable to suppose that one or more non-reciprocal events were involved in the origin of some of these individuals, but a larger sample would be necessary to establish this.

Mitotic analysis, then, indicates that recombination at mitosis between allelic mutants is frequently a reciprocal process, and the data are not inconsistent with the possibility that it is almost always so. It is also clear that localized negative interference operates at mitosis as it has been inferred to do at meiosis. This is apparent even if only those diploids are considered in which the reciprocal products of a mitotic exchange between the *ad* alleles were recovered. In two out of twelve diploids of this type recombination between the *ad* alleles was associated with recombination in an adjacent interval. The occurrence of two doubles in so small a sample should be compared with the data of Pontecorvo & Käfer (1958) which show that coincidence of mitotic recombination in two intervals, when these are long, is exceedingly infrequent as would be expected from the overall incidence of recombination at mitosis.

The occurrence in *Aspergillus* of a mechanism of recombination distinct from crossing over is not, of course, ruled out by the data available at present. What is clear is that such a mechanism will not provide an adequate explanation for localized negative interference.

5. THE PATTERN OF LOCALIZED NEGATIVE INTERFERENCE

(*a*) *Introduction*

Coincidence values greater that unity with respect to two intervals would be observed if the occurrence of recombination in one directly increased the probability of recombination in the second. Alternatively (Weinstein, 1918; Sturtevant, 1955), if the distribution of exchanges between the two intervals was a random one but a population of cells undergoing meiosis was heterogeneous with respect to the total incidence of recombination, coincidence values greater than unity would necesssarily result.

A model of the second type was used to account for localized negative interference in *Aspergillus* (Pritchard, 1955). One of the conditions necessary for recombination to take place was called 'effective pairing'. Effectively paired segments were assumed to be very short and discontinuously, although not necessarily randomly, distributed such that only a small fraction of the genome was effectively paired in any one zygote. In this case coincidence of recombination in two intervals would occur with greater than random frequency if these intervals were short enough and close enough to be frequently included within one effectively paired segment.

The term 'effective pairing' was used to differentiate it from pairing observable cytologically. There need be no connexion between the two, and there probably is none. It has been shown that duplication of DNA may occur long before the onset of the cytological events associated with meiosis (see review of Taylor, 1957). If recombination occurs at the time of DNA replication, effective pairing would also occur before the onset of cytologically observable pairing.

Chase & Doermann (1958) have made use of a similar model to account for localized negative interference in bacteriophage; their 'switch regions' would correspond to regions of effective pairing in *Aspergillus*. Discontinuous pairing has been used to account for negative interference extending over larger sections of the genome in *Escherichia coli* (Rothfels, 1952; Cavalli-Sforza & Jinks, 1956) and *Drosophila* (Sturtevant, 1955). The occurrence of negative interference across an inversion in *Drosophila subobscura* (Spurway & Philip, 1952) may also have the same basis.

(*b*) *Experimental method*

An attempt has been made to determine some of the properties of effective pairing segments, such as their mean length, whether they are randomly distributed along chromosomes, and the recombination frequency per unit length. The cross used is shown in Table 4 and has provided preliminary answers to these questions.

The cross was between two strains, one carrying *ad8* and *ad20*, the other carrying *ad11*. Since *ad20* will grow slowly on minimal medium it was possible to select for recombination between *ad8* and *ad11* and follow the segregation of *ad20* among the selected progeny.

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Samples of ascospores from a single suspension were plated on two different media, one lacking biotin and nicotinic acid and the other lacking adenine. Colonies obtained from selection I (Table 4) were used to establish control recombination fractions for intervals **a, b,** and **g**. Control values for intervals **c** and **d** could not be obtained from this cross and values obtained from other crosses were therefore used.

Table 4. *Genotypes of recombinants from a cross involving* ad20, ad11 *and* ad8

				g					
Interval ...	**a**		**b**	**c**	**d**	**e**	**f**		
Cross 1 ...	*an*	*paba*	*y*	*ad20*	+	*ad8*	+	*nic*	*Acr*
	+	+	+	+	*ad11*	+	*bi*	+	+

Genotype	Selected types: IIa *ad20 ad11* + *ad8* +* (exchange in **e** but not **d**)		IIb *ad20* + *ad11* + *ad8* +* (exchange in **d** and **e**)		I *bi* + *nic* + (control)	
	Interval	No. of colonies	Interval	No. of colonies	Interval	No. of colonies
an paba y bi	**0**	133	**c**	8		
+ *paba y bi*	**a**	76	**ac**	1		
+ + *y bi*	**b**	20	**bc**	0		
an + *y bi*	**ab**	9	**abc**	1		
+ + + *bi*	**c**	5	**0**	69		
an + + *bi*	**ac**	2	**a**	34		
an paba + *bi*	**bc**	1	**b**	21		
+ *paba* + *bi*	**abc**	1	**ab**	4		
an paba y +	**f**	57	**cf**	1	**0**	232
+ *paba y* +	**af**	24	**acf**	1	**a**	93
+ + *y* +	**bf**	5	**bcf**	0	**b**	26
an + *y* +	**abf**	4	**abcf**	0	**ab**	15
+ + + +	**cf**	0	**f**	14	**g**	9
an + + +	**acf**	2	**af**	12	**ag**	4
an paba + +	**bcf**	0	**bf**	2	**bg**	1
+ *paba* + +	**abcf**	0	**abf**	7	**abg**	2
	Total	339		175		382

* Accurate counts of the number of colonies of each type on a number of plates gave 2804 IIa : 108 IIb or 3·85% IIb.

Colonies obtained from selection II were of two types, slow growers (*ad20*) and normal growers. The former are derived by recombination in interval **e**, and the latter by recombination in both **d** and **e**. From the relative frequency of the two types (Table 4) the recombination fraction in **d** among crossovers in **e** can be directly obtained and compared with the control value (Table 5). Colonies of both types were isolated and classified for the remaining marked loci (Table 4), but the

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number of each type fully classified in this way was not proportional to their relative frequencies in selection II. The results are summarized in Table 5.

Comparing first selection IIa with selection I it is apparent that selection for recombination in **e** results in a dramatic increase in the frequency of recombination in intervals **c**, **d**, and **f**. (The difference in length between interval **g** in selection I and **f** in selection II will be very small and a comparison between the two can therefore legitimately be made.) The absence of an increase in interval **b** indicates the degree of localization of negative interference.

Table 5. *Recombination fractions calculated from the data given in Table 4*

					g				
Interval ...		**a**	**b**	**c**		**d**	**e**	**f**	
Cross 1 ...	*an* / +	*paba* / +	*y* / +	*ad20* / +	+ / *ad11*	*ad8* / +	+ / *bi*	*nic* / +	*Acr* / +

	Interval					
Selection	**a**	**b**	**c**	**d**	**e**	**f** or **g**
I. *bi*$^+$ *nic*$^+$	29·8 ± 2·34	11·5 ± 1·60	*0·089*	*0·055*	0·25	4·55 ± 0·91*
II. *ad11*$^+$ *ad8*$^+$	—	—	—	3·85 ± 0·36	—	—
IIa. *ad20* (exchange in **e** but not **d**)	34·8 ± 2·59	11·8 ± 1·75	3·24 ± 0·96	—	—	27·1 ± 2·37
IIb. *ad20*$^+$ (exchange in **d** and **e**)	34·3 ± 3·60	20·0 ± 3·02	6·86 ± 1·91	—	—	21·1 ± 3·09
IIa and IIb weighted and pooled data	34·8	12·1	3·38	3·85	—	26·9

Values in italics were obtained from other crosses.

* This fraction is based on data from 382 colonies shown in Table 4 and a further 145 classified as to *y* or *y*$^+$ only.

(*c*) *The distribution of effective pairing segments*

The possibility had been considered that effective pairing segments might correspond in position and length with the functional units (or cistrons) of a chromosome. Such a situation would provide circumstantial evidence for a physical discontinuity along chromosomes corresponding to the functional discontinuity. If this were the case, the increase in the recombination fractions in **c** and **f** over the control values should be the same in selections IIa and IIb. This would not be the case if pairing segments were randomly distributed. In this case selection for an exchange in both **d** and **e** would sample a population in which effective pairing segments would have a mean position proximal to that of a similar population of segments sampled by selection for an exchange in **e** and not in **d**. The recombination fraction in interval **c** should therefore be greater in the former

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Table 6. *Three crosses, involving different pairs of alleles, showing non-random distribution of additional exchanges on either side of a selected exchange*

Cross 2 ...

	a	b	c	d	e		
	an	*paba*	+	*ad16*	+	*bi*	*pyro*	+
	+	+	*y*	+	*ad19*	+	+	*s12*

f

Selection	Interval a	b	c	d	e or f
I. *pyro* + *s12* +	30·9 ± 3·43	12·7 ± 2·47	*0·048*	—	7·73 ± 1·99
II. *ad16* + *ad19* +			24·6 ± 2·08	0·013	
IIa. *y* (exchange in **d** but not **c**)	37·0 ± 3·55	14·7 ± 2·60	—	—	16·3 ± 2·72
IIb. *y* + (exchange in **d** and **c**)	37·2 ± 4·39	17·3 ± 3·44	—	—	9·92 ± 2·72

Cross 3 ... ...

	b	c	d	e		
	paba	*y*	+	*ad11*	+	*pyro*
	+	+	*ad19*	+	*bi*	+

f

Selection	Interval b	c	d	e or f
I. *pyro* + *bi* +	12·6 ± 2·57	?	—	3·43 ± 0·77
II. *ad19* + *ad11* +	—	14·6 ± 1·57	0·015	—
IIa. *y* (exchange in **d** but not **c**)	11·9 ± 2·64	—	—	23·7 ± 3·48
IIb. *y* + (exchange in **d** and **c**)	15·5 ± 4·29	—	—	9·86 ± 3·54

Cross 4 ... ...

	b	c	d	e		
	paba	*y*	*ad20*	+	+	+
	+	+	+	*ad11*	*bi*	*pyro*

f

Selection	Interval b	c	d	e or f
I. *paba* + *pyro* +	10·1 ± 1·64	?	—	8·04 ± 1·48
II. *ad20* + *ad11* +	—	15·7 ± 1·36	0·055	—
IIa. *y* + (exchange in **d** but not **c**)	12·4 ± 2·06	—	—	12·4 ± 2·06
IIb. *y* (exchange in **d** and **c**)	23·3 ± 6·44	—	—	4·65 ± 3·21

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population than in the latter. In interval **f**, the reverse should be true—the recombination fraction should be less in the former selection than in the latter.

The results are in agreement with the second alternative. Thus the recombination fraction in **c** is significantly greater in selection IIb than in IIa while the recombination fraction in **f** shows the reverse relationship, although in this case the difference is not significant. In addition, it is apparent that in selection IIb the region of negative interference is extended into interval **b** since the recombination fraction in this interval is significantly greater in IIb than in IIa although that in IIa does not differ from the control value.

In order to see if the same type of correlation could consistently be obtained, similar selections were made in three other crosses. The data are given in Table 6. These crosses involved only two alleles. Recombination fractions in intervals **b** and **e** were compared among crossovers in **d** and among crossovers in both **c** and **d**. The results were in each cross similar to those obtained from cross 1; shifting the point of selection towards *y* increases the recombination fraction proximal to *y* and decreases it in the interval distal to the *ad8* region.

Stated another way, the data from four crosses indicate that among recombinants between very closely linked markers the distribution of correlated exchanges on either side of the selected exchange is not a random one. There is a positive correlation (or negative interference) between additional exchanges in adjacent intervals on the same side of the selected exchange, and a negative correlation (or positive interference) between intervals on different sides of the selected exchange.

It is not necessary to assume that the distribution of exchanges within effective pairing segments is other than a random one to account for these correlations. Indeed, it would be difficult to explain them on the basis of a non-random distribution of exchange events. Rather it seems simpler to assume that they result from variability in the position of these segments.

(*d*) *The length of effective pairing segments*

Data at present available are not sufficiently precise to determine whether effective pairing segments are variable in length. From an analysis of localized negative interference in a different part of the map of *Aspergillus nidulans*, Calef (1957) concluded that effective pairing segments were variable in length and suggested that a proportion of them were very long (at least 15 map units). In cross 1 (Table 5) and in cross 2 (Table 6) the effect of selection for an exchange in the *ad8* cistron on the recombination fraction in the interval *an–paba* (15 map units away) could be followed. In both crosses selection for an exchange in the *ad8* cistron resulted in an increase in the recombination frequency between *an* and *paba*. The increase was in neither case statistically significant, but the trend was sufficiently consistent to suggest that it was a real one.

For two reasons it seems unlikely that this increase can be accounted for by extreme variability in length of effective pairing segments such that a proportion of them extends over a large fraction of the length of chromosome I. Firstly, the

increase in the recombination frequency in the interval *an–paba* in selection IIa compared with the control value was in both crosses proportionately greater than the corresponding increase in the interval *paba–y*. Secondly, the increase in the recombination frequency in the interval *paba–y* in selection IIb compared with IIa is not associated with a corresponding increase in the interval *an–paba*.

The correlation between recombination in the *ad8* cistron and recombination in the interval *an–paba* has therefore a different basis from the localized negative interference which can be accounted for by discontinuous effective pairing. Presumably such long-range correlations result from heterogeneity in the total frequency of recombination per zygote (Sturtevant, 1955). Similar long-range correlations have been found by Elliott (1959) and have been studied in greater detail by him.

(*e*) *The frequency of recombination in effectively paired segments*

The data from cross 1 can be used to obtain an estimate of the amount of recombination per unit map length of the genetic material that is effectively paired, in a manner analogous to that used by Chase & Doermann (1958) for bacteriophage. It may be assumed that in individuals in which an exchange occurred in both intervals **c** and **e** both exchanges involved the same effective pairing segment. In this case, all individuals of this type should be effectively paired across interval **d** which lies between **c** and **e**. The proportion of individuals of this type which also have an exchange in **d** will therefore give a measure of the recombination fraction per unit map length of chromosome which is effectively paired.

Since all adenine-independent types were recombinants in **e**, the appropriate fraction is

$$\frac{\mathbf{cd}}{\mathbf{c}+\mathbf{cd}}.$$

The total incidence of recombination in **c** was 0·0338 (Table 5, bottom row). Among recombinants in **d** the incidence of recombination in **c** was 0·0686, but since recombinants in **d** represented only 3·85% of all adenine-independent types, the overall incidence of **cd** recombinants was

$$0{\cdot}0686 \times 0{\cdot}0385 = 0{\cdot}00264;$$

hence

$$\frac{\mathbf{cd}}{\mathbf{c}+\mathbf{cd}} = \frac{0{\cdot}00264}{0{\cdot}0338} = 0{\cdot}0781.$$

A recombination fraction of 0·0781 corresponds to a crossover frequency of 0·0849 (Haldane, 1919).

Since the map length of interval **d** is 0·055 the crossover frequency per unit map length of effectively paired chromosome is

$$\frac{0{\cdot}0849}{0{\cdot}00055} = 154.$$

This admittedly crude calculation implies that the recombination fraction in an

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interval that is effectively paired is about 150 times greater than the same fraction in the population as a whole.

(*f*) *The mean length of effectively paired segments*

This can be estimated from the increase in map length in adjacent intervals associated with selection for recombination in a very short interval. For example, in cross 1 the recombination fractions in intervals **b**, **c**, **d**, and **f** in both selection I and selection II (using in this case the weighted and pooled data from selections IIa and IIb—Table 5) were converted crossover fractions (Haldane, 1919). The values in selection II subtracted from those in selection I give the exchange frequency attributable to effective pairing segments encompassing interval **e**. This works out at 41%. Since it has been calculated that the exchange frequency per unit length of effectively paired segments is 154, this increase corresponds to 0·27 as the minimum estimate of the mean length of effectively paired regions. Each effective pairing segment must include all or part of interval **e**. In cross 1 this had an estimated length of 0·25 map unit, but a previous estimate of the length of this interval was nearer 0·1, which is more consistent with other values in the map of the *ad8* cistron. We can therefore make a provisional estimate that the mean length of effective pairing segments is about 0·4 map unit. The mean exchange frequency per segment will therefore be $0{\cdot}004 \times 150$, or 0·6.

(*g*) *The degree of negative interference in different crosses*

If recombination is restricted to effectively paired segments, it may be predicted than an inverse relationship should be found between the length of the interval being used for selection and the degree of negative interference in adjacent intervals, as Calef (1957) and Chase & Doermann (1958) have pointed out. The appropriate data from fourteen crosses, in which the size of the interval used for selection differed by as much as a factor of 300, are listed in Tables 7 and 8. The largest interval available, that between *ad20* and *ad8*, is still much shorter than the estimated mean length of effective pairing segments, and any variation in the degree of negative interference is not therefore expected to be large.

In Table 7, crosses in which the proximal *ad* allele was identical have been listed in order of decreasing recombination fraction between this allele and a second. The last column gives the recombination fraction in the interval between *y* and the proximal *ad* allele among exchanges in the interval between the two *ad* alleles. With two exceptions the data agree with the prediction.

In Table 8 a similar comparison is made with respect to the interval distal to the interval used for selection. In this case the crosses can be compared as a whole since differences in the position of the distal *ad* allele will not alter the length of this interval appreciably. It is clear that in this interval there is no correlation of the type predicted. The reason for this may perhaps be found in the observation that the control recombination fractions in the interval *ad–bi* are heterogeneous. Differences in the degree of negative interference may therefore be obscured by heterogeneity between different crosses.

Table 7. *The relationship between the recombination fraction between different* ad *alleles, and the recombination fraction in the interval between* y *and the proximal* ad *allele*

Cross involving	Recombination fraction ($\times 10^5$)	Recombination fraction between *y* and proximal *ad* allele ($\times 10^2$)
ad20 and *ad8*	160	10·1 ±3·39
ad20 and *ad11*	55	14·3 ±2·02
ad20 and *ad16*	0·5	21·0 ±9·35
ad16 and *ad8*	140	4·32±1·72
ad16 and *ad11*	41	19·3 ±5·28
ad16 and *ad19*	13	24·6 ±2·08
ad19 and *ad8*	120	3·57±3·51
ad19 and *ad11*	15	14·6 ±1·57
ad19 and *ad12*	12	1·67±1·65
ad12 and *ad8*	120	4·32±1·72
ad12 and *ad11*	10	10·0 ±3·87
ad11 and *ad10*	91	9·18±2·92
ad11 and *ad8*	86	2·74±0·85

Table 8. *The relationship between the recombination fraction between two* ad *alleles, and the recombination fraction in the interval between the distal* ad *allele and* bi

Cross involving	Recombination fraction ($\times 10^5$)	Recombination fraction between distal *ad* allele and *bi* ($\times 10^2$)	Control value ($\times 10^2$)*	Difference
ad20 and *ad8*	160	16·4± 4·17	—	10·4 ± 4·17
ad16 and *ad8*	140	22·3± 3·53	—	16·3 ± 3·53
ad12 and *ad8*	120	23·0± 3·57	—	17·0 ± 3·57
ad19 and *ad8*	120	35·7± 9·05	—	29·7 ± 9·05
ad11 and *ad10*	91	35·7± 4·84	6·25±2·02	29·4 ± 6·11
ad11 and *ad8*	86	24·0± 3·19	—	18·0 ± 3·19
ad20 and *ad11*	55	11·3± 1·83	8·04±1·48	3·26± 2·36
ad16 and *ad11*	41	35·7± 6·71	5·80±1·62	29·9 ± 6·90
ad19 and *ad11*	15	21·8± 2·57	3·43±0·77	18·4 ± 2·69
ad16 and *ad19*	13	14·8± 2·28	7·73±1·99	6·07± 3·02
ad19 and *ad12*	12	25·0± 5·59	4·86±1·79	20·1 ± 5·88
ad12 and *ad11*	10	31·7± 3·98	14·2±2·23	17·5 ± 6·38
ad8 and *ad10*	1	39·3± 9·23	—	33·3 ± 9·23
ad20 and *ad16*	0·5	31·6±10·7	7·80±1·82	23·8 ±10·8

* In some crosses a control value could not be obtained and the standard map value of 6·0 units for the interval *y–bi* has been used.

6. DISCUSSION

In the introduction the question was raised whether a process different from recombination of the classical sort was responsible for irregular tetrad ratios, or whether a single mechanism could account for both reciprocal and non-reciprocal

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events. There is general agreement (Roman, 1956; Freese, 1957*a*; Case & Giles, 1958) that 3 : 1 ratios in tetrads have a recombinational rather than mutational origin. Since 'gene conversion' is frequently not associated with recombination between markers spanning the locus segregating irregularly, it follows that the frequent simultaneous occurrence of two exchanges, or switches, in a very short chromosome segment must occur and this might be taken as evidence that 'gene conversion' obeyed different rules from crossing over. But the present work suggests that correlated exchanges in very short segments of the genetic material are a property of recombination in general and cannot therefore provide the basis for a distinction between crossing over and 'gene conversion'. The hypothesis that 'gene conversion' is an intra-genic process and crossing over an inter-genic process (Beadle, 1957) is also untenable, as Pontecorvo (1958) has pointed out.

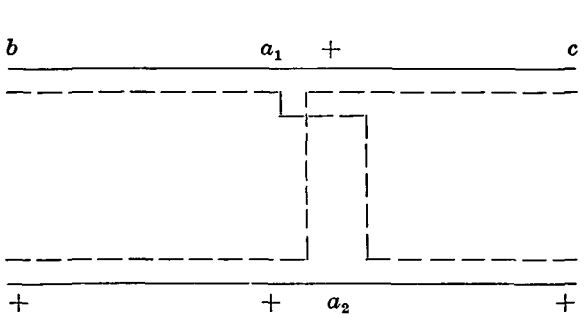
Several authors (e.g. Roman, 1956; Freese, 1957*a*; Chase & Doermann, 1958) have made use of the idea that 3 : 1 ratios in tetrads could be accounted for if recombination occurred by copy-choice provided replication along two mated templates was not necessarily synchronous. Lack of synchrony would permit one template to be vacated by its copy and used to make a second. A specific model of this type was proposed by Freese (1957*a*). He supposed that, within an effectively paired region, switching of the copy from one template to the other occurs repeatedly but is not correlated with a reverse switch, so that within such regions recombination will be generally of the non-reciprocal type. For such a model to have general application the behaviour of all organisms in which tetrad analysis, or half-tetrad analysis, is possible must be taken into account. In yeast in two out of three cistrons analysed (Roman, 1956; Leupold, 1958), recombination between different sites of a cistron appears to be predominantly non-reciprocal. No comparable situation is found in *Aspergillus* (Strickland, 1958*a*, *b*) or *Drosophila*, where non-reciprocal events are very much the exception to the rule. Thus in *Aspergillus* Strickland classified a total of 1245 asci in which all four products of meiosis were recovered, and a further 380 in which three of the four products were recovered. The crosses he used were heterozygous at several loci, and the total number of cases in which any exception from a 2 : 2 ratio would have been detected if it had occurred was 7165 for the complete tetrads and 2119 for the incomplete tetrads, involving a total of 16 loci. Only four exceptional tetrads were found for which orthodox explanations such as contamination and extra mitosis were unlikely. Three of these tetrads involved the same mutant (*bi1*), and in each case a $3bi1^+ : 1bi1$ ratio was found. In the other exceptional ascus, recombination between two very closely linked loci was associated with a 3 : 1 ratio for one of them and in addition a 3 : 1 ratio at a second locus 9 map units distant. A segment of chromosome at least 9 units long was represented three times in the tetrad; it had, apparently, been duplicated twice.

Neurospora appears to occupy an intermediate position with respect to the frequency of tetrads with 3 : 1 ratios (Mitchell, 1955*a*, *b*; Giles *et al.*, 1957; Case & Giles, 1958).

The relative frequencies of reciprocal and non-reciprocal events is clearly

variable from case to case and perhaps from species to species. A model which took account of this variation would be one which postulated a correlation between a switch from one template to a second with a switch in the opposite direction, although the two switches need not necessarily be at precisely the same point, i.e. that recombination is generally a reciprocal process but not necessarily always and precisely so. On such a model a 3 : 1 ratio associated with recombination between spanning markers would require a single event (Fig. 2) instead of three independent events, as on the Freese model. Furthermore, differences in the degree of synchrony of replication would permit variation in the relative frequencies of reciprocal and non-reciprocal events.

A consequence of a unitary model of this type is that in single-strand analysis, among recombinants between two closely linked markers, the frequency of parental combinations with respect to spanning markers should not be much greater than 50%. An excess over 50% is possible if the mean exchange frequency per effective



Products

b	a_1	+	*c*
b	a_1	+	+
+	+	+	*c*
+	+	a_2	+

FIG. 2. Proposed origin of a tetrad with a 3 : 1 ratio associated with recombination between spanning markers as a result of a single inexactly reciprocal exchange. Dotted lines represent newly formed products.

pairing segment is not sufficiently great as to randomize the segregation of the spanning markers, since a negative correlation will then occur between exchanges on different sides of the selected exchange due to variability in the position of effective pairing segment (cf. cross 1). Data from the crosses between *pdx* mutants (Mitchell, 1956) and between *pan* mutants (Case & Giles, 1958) of *Neurospora* are in accord with this prediction (Table 9).

These examples are chosen because tetrad analysis in both cases revealed a high frequency of non-reciprocal recombinants. In both cases an excess of parental combinations occurred, but inequality of the two recombinant classes indicated that the frequency of exchange per effective pairing segment was not high. The data of Roman (1956), on the other hand, suggest that 3 : 1 ratios in yeast are not, in the cases studied, associated with recombination. If his results are confirmed they will provide more convincing evidence for more than one mechanism of recombination. At present the evidence is not sufficiently decisive to warrant a definite conclusion.

A further question posed by the experiments discussed in this paper is the significance of localized negative interference. It seems possible that its occurrence may provide an explanation for a number of difficulties encountered in attempting to relate the experimental evidence concerning the mechanism and time of recombination with the cytologically observable sequence of events during nuclear division.

It was an essential feature of postulated mechanisms of recombination involving breakage and reunion of chromosomes that it occurred after zygotene pairing and during or after chromosome duplication (Darlington, 1935). Chromosome duplication was supposed to occur at the end of pachytene. Models of this type have

Table 9. *The relative frequency of different marker combinations among recombinants with respect to closely linked mutants of* Neurospora

Cross	Types selected	Relative frequency of marker combinations (%)					
		Crossovers			Non-crossovers		
		pyr co	+ +	Total	*pyr* +	+ *co*	Total
$\frac{pyr\ pdx\ +}{+\ pdxp\ co}$	Pyridoxin independent	28	8	36	25	39	64
$\frac{pyr\ pdxp\ +}{+\ pdx\ co}$	Pyridoxin independent	8	33	41	31	28	59
		+ +	*ad tryp*		+ *tryp*	*ad* +	
$\frac{+\ +\ B3\ tryp^*}{ad\ B5\ +\ +}$	Pantothenate independent	40	7	47	39	14	53
		ad +	+ *tryp*		+ +	*ad tryp*	
$\frac{ad\ +\ B3\ tryp^*}{+\ B5\ +\ +}$	Pantothenate independent	38	5	43	35	22	57

* *B3* and *B5* determine requirement for pantothenic acid.
References: Mitchell, 1956; Case & Giles, 1958.

been repeatedly questioned for reasons summarized and added to recently by Pontecorvo (1958). A model in which recombination is the result of copy-choice at the time of replication is now generally preferred, but in its simplest form such a model has its own difficulties. Recombination by copy-choice requires homologous contact between chromosomes at the time of replication, but replication of DNA had been shown to occur well before zygotene pairing in a number of organisms (see Taylor, 1957).

Taylor (1957) concluded that any hypothesis requiring chromosome duplication after zygotene pairing was untenable. This led him to suggest a branched structure for chromosomes and two mechanisms of recombination, one occurring at the time of replication by copy-choice and involving the side chains, and one occurring after replication by breakage and reunion and involving the chromosome backbone.

But the evidence against a branched linkage structure is convincing and Taylor's model is therefore unsatisfactory.

If recombination is the result of copy-choice it becomes necessary to conclude that it occurs before, and independently of, zygotene pairing as Pontecorvo (1958) has suggested.

The discovery of the extreme discontinuity in the distribution of recombination events in *Aspergillus* (Pritchard, 1955) suggested how this apparent paradox might be resolved. It was pointed out then that effective pairing, a condition for recombination, was not necessarily identical with pairing observed cytologically. A provisional estimate now indicates that the frequency of exchange per effective pairing segment is about 0·6, corresponding to a recombination fraction of about 35%. This means that the frequency of recombination between loosely linked markers is determined principally by the frequency of effective pairing between them, i.e. that effective pairing is a limiting factor in recombination.

Thus homologous chromosomes need necessarily be in contact over only a small fraction of their total length to account for the recombination fractions observed. Such chromosomes need not be paired in the cytologically visible sense. All that would be necessary would be occasional points, or very short segments, of contact between them. Chiasmata would be produced at the time of replication (before cytologically visible pairing) but observed only at diplotene.

It is assumed in this argument that effective pairing is synonymous with homologous contact. Although this idea is attractive it is clear that discontinuous contact is not the only source of discontinuity along chromosomes that could lead to clustering of recombination events. There is nevertheless evidence which at first sight seems to support this view. It is well known that the frequency of chiasmata in triploids and trisomics is greater than in the corresponding diploid (e.g. Darlington, 1934, 1941; Upcott, 1935), and there is evidence for a corresponding increase in the recombination frequency in individuals of this type in *Drosophila*, although the effect of recombination on disjunction in triploids complicates the interpretation of these results (see Schultz & Redfield, 1951). A similar situation seems to exist in trisomic *Aspergillus* (Pritchard & Siddiqi, unpublished).

If contact between homologues were not a limiting factor in recombination these observations could only be interpreted in terms of an unspecified effect of the presence of a third homologue. If homologous contact were limiting, on the other hand, an increase would be predicted simply as a result of the increased probability of contact between two homologous points when three are present. This was pointed out by Darlington (1941) for the case of an organism in which cytologically visible pairing was incomplete. The present hypothesis is that homologous contact is always incomplete—more precisely, discontinuously so—irrespective of the pattern of cytologically visible pairing.

It is not, of course, suggested that all observations of this sort can be accounted for entirely in this way. Sturtevant's (1951) observation of an increase in recombination in chromosome IV of *Drosophila melanogaster* in diplo-IV triploids is a case in point.

It is assumed, then, that the probability of recombination between loosely linked loci has three components: the frequency of effective pairing, the length of effectively paired segments, and the frequency of exchange per unit length of such segments. If this is correct, it suggests how large differences in the frequency of recombination per unit of physical length of the genetic material in different organisms could arise, although the underlying mechanism of recombination was the same in all of them. A comparison between *Aspergillus* and bacteriophage will make this clear.

We can use the cistron as an approximate standard of physical length in view of its presumed direct or indirect role as a template for synthesis of a polypeptide, and Pontecorvo & Roper's (1956) estimate that in organisms as different as *Drosophila* and phage the amount of DNA per cistron is probably similar. The genetic length of the *ad8* cistron is about 0·2 map unit. This value seems typical for *Aspergillus* (e.g. Roper, 1950). The genetic length of the rIIA and rIIB cistrons in phage T4, on the other hand, is about 3 and 5 units respectively (Chase & Doermann, 1958), which also seems to be typical (e.g. Streisinger & Franklin, 1956). We can calculate what these values would correspond to if effective pairing were complete in the two organisms. The *ad8* cistron would become 30 ($0{\cdot}2 \times 150$) units long, and the rIIA and rIIB cistrons 30 (3×10) and 50 (5×10) units respectively. A conversion factor of 10 is used for phage, since Chase & Doermann found that if only those individuals in which a given interval was effectively paired were considered the recombination fraction was 6–14 times that in the population as a whole. Thus the 20-fold difference in the probability of recombination per unit physical length of the genetic material in the two organisms may turn out to be due largely to differences in the frequency or length of effective pairing segments.

In comparison with these values the high frequency of double exchanges required for the incorporation of very small transducing or transforming segments of DNA by copy-choice no longer seems remarkable. Indeed, there is at present no compelling evidence against the view that fundamentally the same mechanism is involved in recombinational processes apparently as different as transformation and crossing over.

SUMMARY

In the analysis of recombination in *Aspergillus nidulans* coincidence values of about 1 are found in 3-point tests using markers more than a few map units apart. In comparable tests in which the marked intervals were very short (0·1 map unit or less), coincidence values of over 100 had been found. To account for this difference it was proposed that a necessary condition for recombination, termed 'effective pairing', was realized at any particular point on the chromosome in only a small fraction of a population of cells at meiosis. It was supposed that when effective pairing occurred it extended over a very short segment of the chromosome and that the probability of recombination in the effectively paired segment was high, i.e. about 1. Coincidence values greater than unity would be

a necessary consequence of such a situation provided the intervals in question had a total length not much greater than that of effective pairing segments.

The experiments described in this paper were undertaken in an attempt to measure the mean length of effectively paired segments, their distribution, and the frequency of exchange within them. The data suggest a mean length of about 0·4 map unit, a mean exchange frequency of about 0·6, and a distribution which is variable, perhaps random.

The occurrence of localized negative interference suggests a way in which a number of difficulties encountered in relating the experimental evidence concerning the time and mechanism of recombination with the cytological evidence concerning the sequence of events at meiosis might be resolved. The data indicate that the frequency of recombination between linked loci is a measure principally of the frequency of effective pairing between them. If effective pairing is synonymous with homologous contact between chromosomes, and evidence is presented which suggests this may be the case, it becomes possible to construct a simple model which is compatible with the view that recombination takes place before chromosomes are paired, in the cytologically observable sense (i.e. before zygotene), at meiosis.

The recombination events occurring within effectively paired regions are generally, although possibly not exclusively, reciprocal. Non-reciprocal recombinants have been encountered in *Aspergillus* and other organisms, characterized by the occurrence of 3 : 1 ratios in tetrads. On the basis of evidence currently available it does not seem necessary to invoke a special mechanism of recombination, distinct from crossing over, to account for the formation of non-reciprocal recombinants. A single mechanism of recombination of the copy-choice type which, although primarily a reciprocal process, is nevertheless not necessarily exactly so or always so in detail, will account for the observed results.

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