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The analysis of genetic recombination on the polaron hybrid DNA model

By H. L. K. WHITEHOUSE and P. J. HASTINGS

Botany School, University of Cambridge

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1. INTRODUCTION

A theory of genetic recombination has been proposed by Whitehouse (1963) in which recombination occurs by the breakage of single nucleotide chains from two homologous DNA molecules followed by rejoining by complementary base pairing. By this mechanism the newly formed strands consist, over a short region, of DNA derived from both parents. If these hybrid segments include sites of heterozygosity, a correction process could operate to make the molecule homozygous, the correction occurring in either direction independently in any chromatids involved, soth at non-reciprocal recombination could result. Failure of the correction process would lead to post-meiotic segregation.

Whitehouse proposed two configurations, both of which involve the synthesis of short lengths of extra nucleotide chains. In one configuration the points of primary breakage in the two chromatids are at random, while in the other they are homologous. The second configuration explains equally readily the occurrence of non-reciprocal recombination either with or without overall recombination. This configuration with homologous primary breaks has been modified (Hastings & Whitehouse, 1964) by postulating that the primary breaks occur at fixed points, corresponding to the linkage structures of the polaron model (Lissouba & Rizet, 1960). These points would be outside or at the ends of the genes, and might be the same as the points where dissociation is initiated when the gene functions, if separation of nucleotide chains over the length of the gene occurs in the formation of messenger RNA.

Rizet, Lissouba & Mousseau (1961) introduced the term linkage structure (une structure de liaison particulière) to describe the point or region between two polarons where crossing-over was possible, as distinct from *conversion* which constituted the only mechanism of recombination within a polaron. In the model discussed in this paper, two modifications are made to the concepts of polaron and linkage structure as originally proposed. A polaron is considered, as previously, to be a region of a chromosome between neighbouring linkage structures within which recombination is only by conversion, but it is recognized that conversion may give rise to reciprocal as well as to non-reciprocal recombination. Secondly, the linkage structure is identified with a point where crossing-over and non-crossover recombination are initiated by the primary breakage of nucleotide chains, but the process of crossing over is regarded as occupying a segment of the chromosome which may include mutant sites. Hence it would be misleading to say that crossing over is restricted to the linkage structures placed between the genes. The concept adopted here is that crossing-over and non-crossover recombination are initiated at these points, but extend into the polarons on one or both sides. Indeed, conversion is regarded as a process which takes place when a mutant site happens to lie within the region of a chromosome involved in a crossover or non-crossover event. The term *linkage point* rather than linkage structure is used in this paper, in order to indicate that the concept of the linkage structure has been defined more precisely as a fixed primary breakage-point from which crossover and non-crossover recombination events are initiated. No assumptions are made about the nature of these breakage-points, which may be determined by the code in the DNA, or by some other structural feature of the DNA or nucleoprotein.

Figure 1 shows the sequence of events visualized on the polaron hybrid DNA model. It is supposed that enzymatic breakage of complementary nucleotide chains one from each of two homologous chromatids takes place at a linkage point (*b* in Fig. 1 (i)) and is followed by dissociation and uncoiling of these chains on one or both sides of the break. DNA synthesis is then initiated from the points of breakage in one or both directions (Fig. 1 (ii)), and the newly synthesized chains uncoil (Fig. 1 (iii)). The double helices are formed again by the pairing and coiling of complementary strands one from each molecule (annealing) (Fig. 1 (iv)). The final step would be the breakdown of any unpaired nucleotide chains (Fig. 1 (v)). If the synthesis had taken place on one side only of the breakage-point, a crossover would result, while if it had taken place on both sides, there would be no overall crossing-over. In either event, lengths of hybrid DNA would be formed, which would give the opportunity for conversion if sites of heterozygosity were included. In this paper the whole series of events from primary breakage to breakdown of unpaired chains is called a *dissociation cycle*. It is to be noted that there is no net synthesis of DNA.

The frequency of conversion of any mutant would be related to the frequency with which it falls into hybrid DNA. This frequency would decrease as the distance from the initial breakage-point increases. The frequency of recombination between two well-spaced alleles would depend primarily on the frequency with which a length

of hybrid DNA ended between them. This would give distal polarity in recombination at the distal end of a polaron, and proximal polarity at the proximal end. That polarity within a gene is predominantly in one direction implies that hybrid DNA

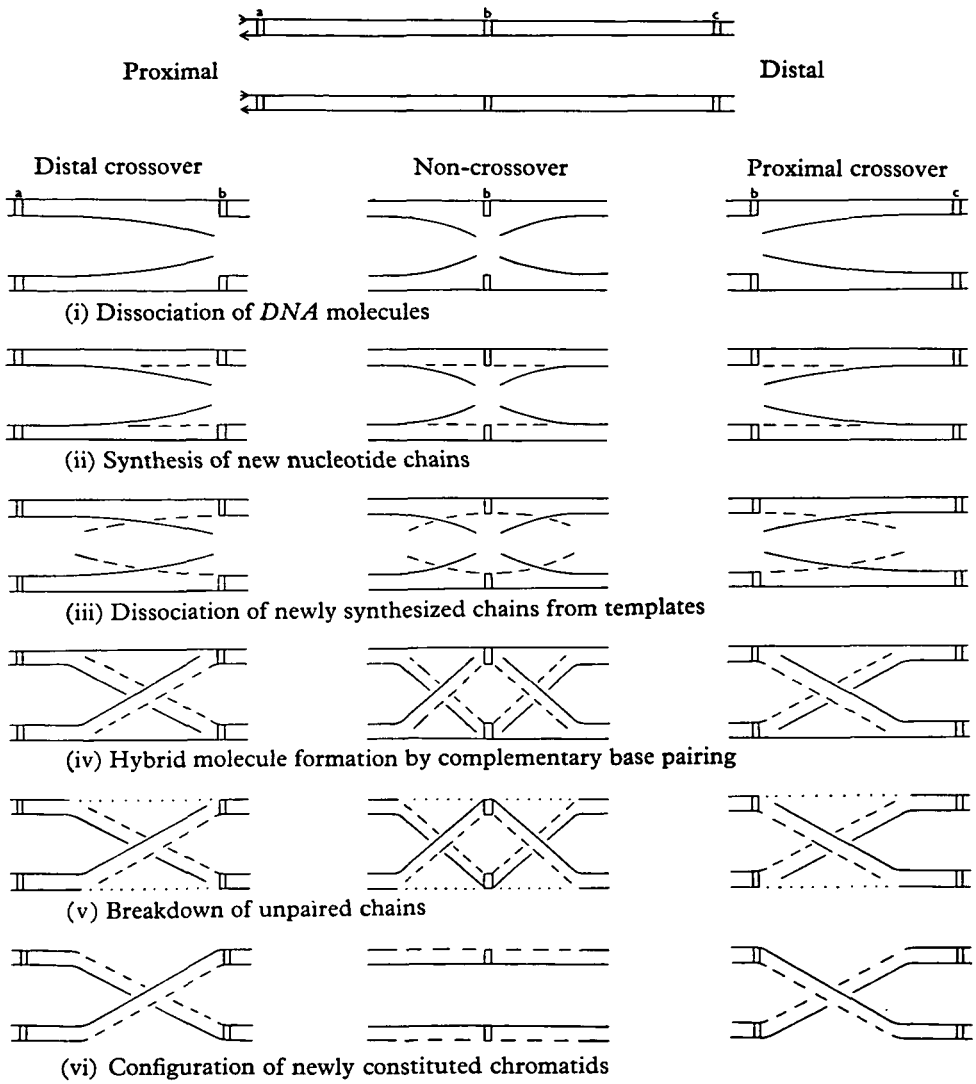

Fig. 1. Diagram to illustrate the polaron hybrid DNA model of genetic recombination. The lines represent the nucleotide chains of two homologous DNA molecules. The orientation of the chains is shown by arrows in the top diagram. The rectangles labelled *a*, *b* and *c* indicate the fixed points of primary breakage from which the dissociation is initiated. The broken lines represent newly-synthesized nucleotide chains, and the dotted lines chains which are breaking down.

from breaks at or beyond one end involves the mutant sites more often than hybrid DNA from breaks at or beyond the other end. It is suggested that the length of hybrid DNA formed in a dissociation cycle need not be equal in both chromatids

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involved, and that in a non-crossover event, hybrid DNA might sometimes be confined to one chromatid. The length of hybrid DNA formed is likely to be controlled primarily by the length of extra synthesis in each chromatid. Wimber & Prensky (1963) have shown by auto-radiography, following treatment with tritiated thymidine, that a limited amount of DNA synthesis occurs at about the pachytene stage of meiosis in males of *Triturus viridescens*, and Hotta & Stern (1961) found a minor peak at this stage in the capacity of pollen-mother-cells of *Trillium erectum* to phosphorylate thymidine—an ability present only at times of DNA synthesis.

The idea that conversion might be a consequence of the lateral association of nucleotide chains from the DNA of two parents was suggested by Taylor, Haut & Tung (1962). That conversion might result from the correction of heterozygosity caused by such association was first discussed by Holliday (1962), who has independently (Holliday, 1964) developed a model of recombination which has many features in common with that described above. The main point of difference is that Holliday's model does not involve synthesis of extra DNA. He postulates that identical nucleotide chains break at fixed points in homologous positions in homologous chromatids, uncoiling on one side of the break and then each associating with the unbroken nucleotide chain in the other chromatid. This chiasma at the nucleotide chain level may be resolved into separate molecules either by breakage of the previously unbroken chains, leading to recombination of outside markers, or by breakage of the same nucleotide chains as broke originally, leading to no overall recombination. In either case, two lengths of hybrid DNA would be formed, which allow conversion by a correction process. Since, in the absence of synthesis, the lengths of hybrid DNA in the two chromatids involved would be equal, the occurrence of conversion in one chromatid only is explained by postulating that the correction process has operated during a temporary hybrid association which breaks down because of the difficulty of complementary pairing across sites of heterozygosity.

In the account which follows, the available recombination data have been examined in terms of the polaron hybrid DNA model as proposed by Hastings & Whitehouse (1964). Data relating to a single site of heterozygosity within a gene are examined first, and then the more extensive results concerning two sites. The limited number of observations on the behaviour of three sites of heterozygosity within a gene (Case & Giles, 1959, 1960; Pritchard, 1960*a*; Martin-Smith, 1961; Lissouba *et al.*, 1962) have not been analysed.

2. ANALYSIS OF DATA RELATING TO A SINGLE SITE OF HETEROZYGOSITY WITHIN A GENE

In studies of recombination using single sites of heterozygosity within a gene, it has usually not been possible to fix precisely the positions of the individual recombination events. The only extensive set of data where, nevertheless, detailed information about events occurring at a particular site has been obtained is that of Kitani, Olive & El-Ani (1962). These authors took advantage of a spore character

difference in *Sordaria fimicola* to select the rare asci with spore colours in the ratios 6:2, 5:3 or abnormal sequences of 4:4 from a cross between a mutant called *gray* (*g*) and the normal black-spored fungus. They analysed 122 such asci with marker genes closely linked on each side of the locus for spore colour, and a further 126 asci where a marker gene was present on one side only. The marker genes used were *corona* placed 3·4 units distally of *g* on the linkage map, and either *milky* or *mat* placed 1·0 and 0·4 units away, respectively, on the proximal side. These mutants affected the morphology of the mycelium. The numbers which they observed of each kind of ascus with particular marker configurations are given in Table 1. The marker combinations are indicated by the letters CO for crossover, NCO for non-crossover, PCO for a crossover with its primary breaks on the proximal side of *g*, QCO for a crossover with its primary breaks on the distal side, and T for an apparent two-strand double crossover spanning the *g* site.

In all except 6 of the 122 aberrant asci with marker genes on both sides, the recombination in the segment of the chromosome so defined involved not more than two of the four pairs of spores in the ascus, or in other words not more than two of the four homologous chromatids at meiosis. If only two chromatids take part when crossing-over and conversion occur in proximity, these few instances where three or four chromatids have participated would be attributed to two separate recombination events occurring in the interval of the chromosome between the outside marker genes. However, the genotype of one of the 122 aberrant asci (marked with a dagger (†) in Table 1) appears to imply that three strands have participated in the same region. Furthermore, Kitani *et al.* (1962) observed an ascus showing a 7:1 ratio of spore colours, which also implies that at least three strands have been involved at the *g* site. This recombination involving more than two strands has been ignored in the present analysis, but is discussed in section 4 (vii).

The interpretation of the various aberrant asci on the hybrid DNA hypothesis is shown in Tables 2 and 3. If both the recombining chromatids have hybrid DNA at the mutant site, there are three possible genotypes for each, depending on whether the site remains heterozygous, or whether it converts to one or other allele (mutant or wild-type). Combining these in all possible ways gives the 9 genotypes shown in Table 2 where the outside markers have the parental combinations, and a further 9 shown in Table 3 with crossing-over between the markers. The T class asci are attributed to conversion in non-crossover hybrid DNA and not to a double crossover. If only one chromatid has hybrid DNA at the site of the spore character mutation, the other will have either a parental configuration (Table 2) or a crossover configuration (Table 3) for the outside markers.

In Tables 2 and 3 the expected frequencies of each of the 18 ascus genotypes are given in terms of the frequencies of conversion in the two directions at the spore mutant site, and of the occurrence of hybrid DNA in only one chromatid at the site. The frequency of remaining heterozygous is denoted by a, of conversion to + by b, and of conversion to *g* by c, where $a + b + c = 1$. These frequencies are assumed to be constant and to apply whenever the mutation occurs in hybrid DNA. The frequencies of its occurrence in hybrid DNA in only one chromatid are denoted by d when

Table 1. *The data of Kitani, Olive & El Ani* (1962) *for aberrant asci of* Sordaria fimicola *segregating for the grey-spored mutant* g. *The figures at the head of the columns show the numbers of their tables which are the source of the data. The outside marker genes used were* milky *and* corona (*their tables* 1 *and* 2), mat *and* corona (*their table* 3), *and* mat *alone* (*their tables* 4 *and* 5). *The data for* 4:4*s are based on their table* 6. *CO = Crossover between the outside markers. NCO = Non-crossover. PCO = Crossover with its primary breaks on the proximal side of the site of* g. *QCO = Crossover with its primary breaks on the distal side. T = An apparent two-strand double crossover spanning the* g *site*

Class of aberrant ascus +:*g*	Combination of marker genes: Parental — Sub-class	Tables (Kitani *et al.*) 1	2	3	Total	Non-parental — Sub-class	Tables (Kitani *et al.*) 1	2	3	Total	Unknown — Sub-class	Tables (Kitani *et al.*) 4	5	Total
4:4	NCO	—	—	2	2	CO	—	—	2	2	NCO or CO	—	5	9‡
5:3	NCO	6*	4	8†	18	QCO	5*	3	9	17	NCO or QCO	6	15	108
	T	4	4	7	15	PCO	5	0	8	13	T or PCO	5	19	
3:5	NCO	3	1	3*	7	QCO	0	0	0	0	NCO or QCO	1	4	20
	T	0	0	1	1	PCO	0	0	3	3	T or PCO	1	3	
6:2	NCO	9	6	17**	32	CO	5	1	2	8	NCO or CO	11	47	98
2:6	NCO	0	0	3	3	CO	0	0	1	1	NCO or CO	1	8	13
Total omitting 4:4s	—	22	15	39	76	—	15	4	23	42	—	25	96	239

* Each asterisk indicates an ascus which had a crossover involving a third chromatid, but in classifying these asci these crossovers have been ignored.

† One of these asci had a two-strand double crossover between the marker genes and involving a third chromatid, but this has been ignored in classifying the ascus.

‡ These 9 asci were from a total sample of 150 aberrant asci.

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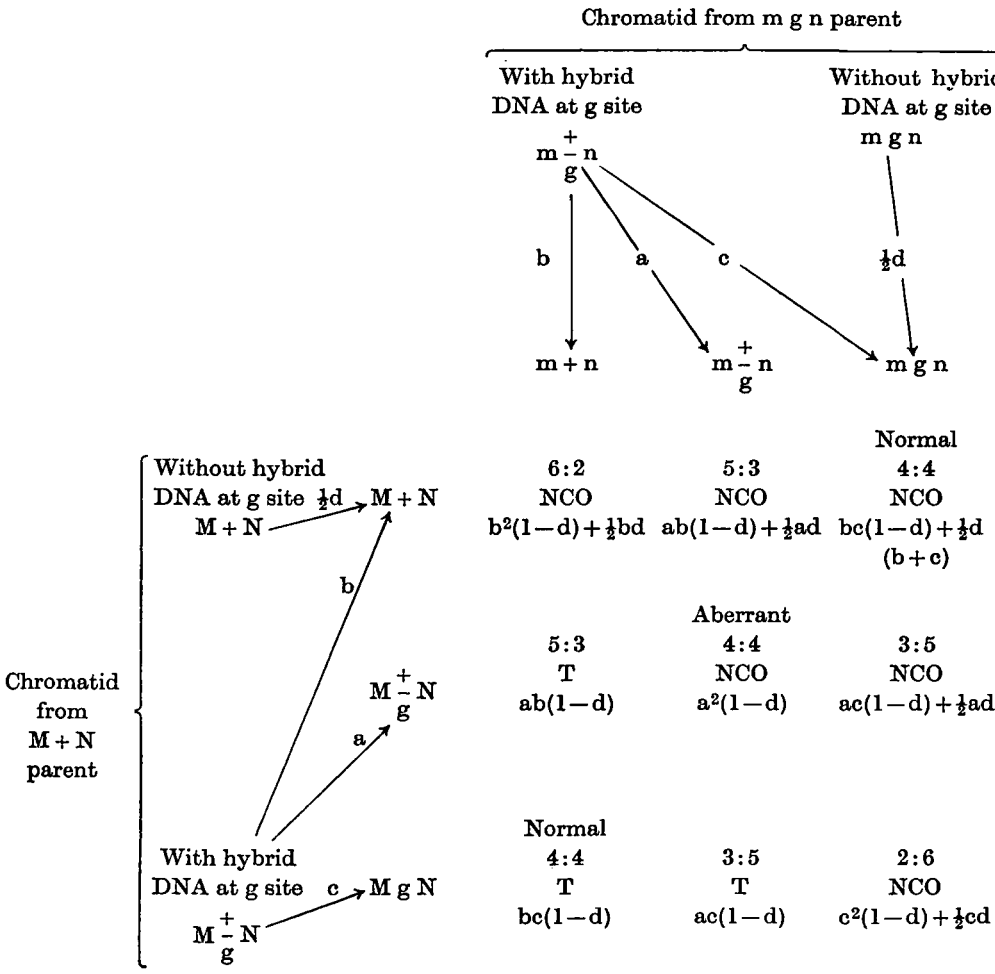
the outside markers show the parental combinations, e when the primary breaks of a crossover have occurred on the proximal side, and f when such breaks have occurred on the distal side. The frequencies with which the g site occurs in hybrid

Table 2. *Interpretation of the* 9 *ascus classes expected when hybrid DNA has formed at the site of a spore character mutation* (*grey*, g) *but outside marker genes* (*M/m and N/n*) *have not crossed over. For each ascus class, the number of black* (*wild-type*, +) *spores is given first and then the number of mutant* (*grey*, g) *spores. NCO = Non-crossover. T = An apparent two-strand double crossover spanning the site of* g



DNA in both chromatids would then be $1-d$ for the non-crossovers and $1-(e+f)$ for the crossovers. It is assumed that when there is hybrid DNA at the site of g in only one chromatid, if this strand has a parental combination of the outside

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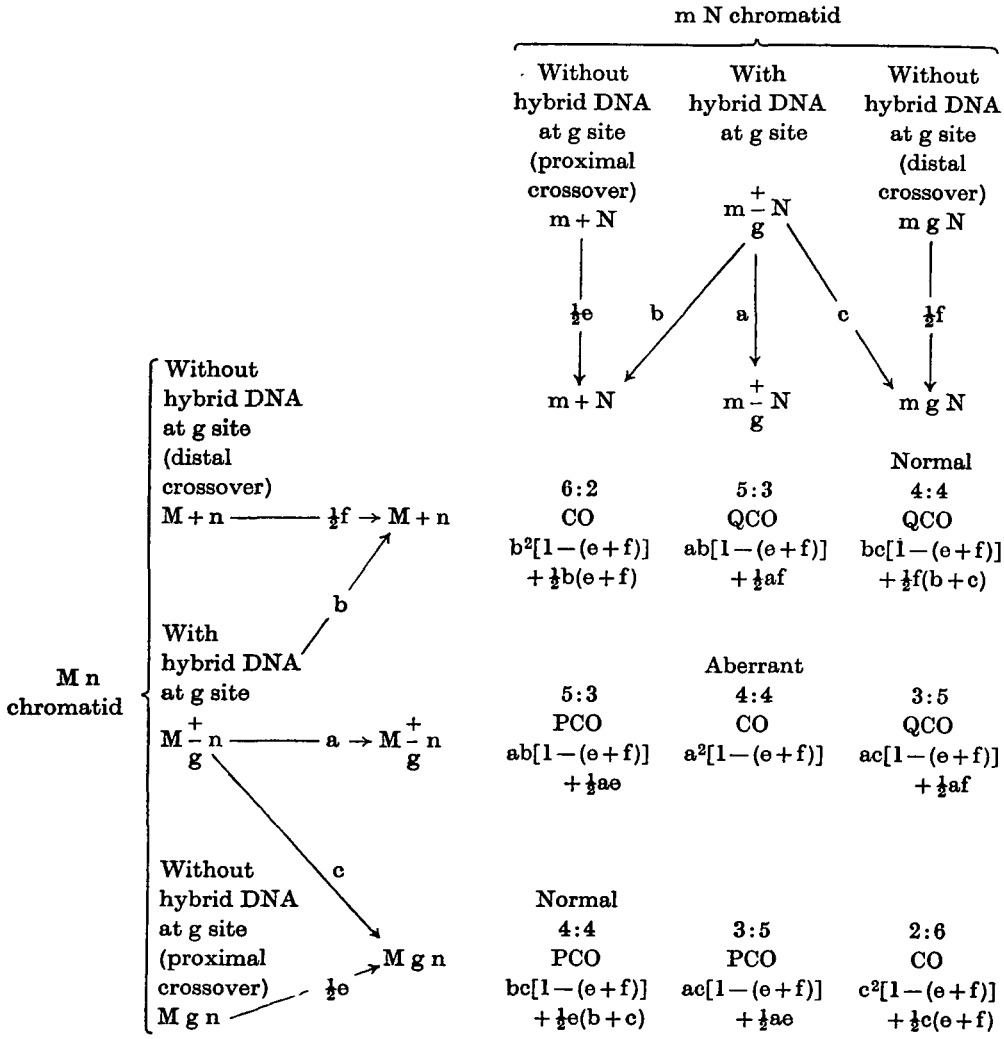
markers then it is equally likely to be that of either parent (frequency $d/2$ each). Similar assumptions are made for e and f.

If the occurrence of hybrid DNA at the site of g in one chromatid was always accompanied by its occurrence at this site in the other, d, e and f would be zero and the formulae in Table 2 for the frequency of each ascus type become identical with

Table 3. *Interpretation of the 9 ascus classes expected when hybrid DNA has formed at the site of a spore character mutation (grey, g) and outside marker genes (M/m and N/n) show crossing-over. For each ascus class, the number of black (wild-type, +) spores is given first and then the number of mutant (grey, g) spores. CO = Crossing-over between M/m and N/n. PCO = Crossover with its primary breaks on the proximal side of the g site. QCO = Crossover with its primary breaks on the distal side of g.*



those for the corresponding type in Table 3. The outside marker combinations can then be ignored and the expected frequencies of asci with aberrant 4:4, and with

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5:3, 3:5, 6:2, and 2:6 ratios are given by the expressions a^2, $2ab$, $2ac$, b^2, and c^2, respectively. Applying these formulae to the total data of Kitani *et al.* (1962) given in the right-hand column of Table 1, the solution which gives the best fit appears to be $a = 0{\cdot}27$, $b = 0{\cdot}56$, and $c = 0{\cdot}17$. The expected numbers of the various types of asci for these values of a, b, and c are given in Table 4. Comparison with the observed numbers of 5:3, 3:5, 6:2 and 2:6 ratios shows that the difference is not significant ($\chi^2 = 5{\cdot}9$, n = 3, P = 0·2–0·1). Moreover, the number of aberrant 4:4 asci is also in agreement, as indicated in the footnote to Table 4. Nine such asci were observed out

Table 4. *The numbers of asci in the various classes observed by Kitani, Olive & El Ani* (1962) *with the* gray (g) *mutant of* Sordaria fimicola *are shown, together with the numbers expected when conversion to* + *and to* g *occur in* 56% *and* 17% *respectively of the occurrences of* g *in hybrid DNA, heterozygosity being maintained in the remaining* 27% (a = 0·27, b = 0·56, c = 0·17). *It is assumed that the hybrid DNA occurs in both the participating chromatids at the* g *site* (d = 0, e = 0, f = 0)

	Class of ascus + g	Observed numbers	Expected numbers	
Normal	4:4	—	61·8	
Aberrant	4:4	9*	23·7	
	5:3	108	98·1	
	3:5	20	29·8	$\chi^2 = 5{\cdot}9$
	6:2	98	101·7	n = 3
	2:6	13	9·4	P = 0·2–0·1
Total omitting	4:4s	239	239·0	
Grand total		—	324·5	

* The 9 aberrant 4:4 asci were in a total sample of 150 aberrant asci. The expected number in such a sample is $23{\cdot}7 \times (141/239) = 14{\cdot}0$.

of a total of 150 aberrant asci, and the expected number is 14. Furthermore, the observed number of aberrant 4:4 asci is probably below the true one, since some would probably be dismissed as due to overlap of the two central spindles at the third division. Their aberrant character would not be revealed unless they were dissected and scored for the outside markers.

It has been assumed in the analysis above that hybrid DNA never occurs in only one chromatid at the *g* site, but this is unlikely to be true. From the formulae in Table 2 for the expected frequencies of the various classes, it is evident that a direct estimate of the frequency d of non-crossover hybrid DNA at the *g* site can be obtained by comparing the frequency of the NCO classes of 5:3 and 3:5 asci with that of the T classes. From Table 1, the observed total in the NCO classes was 25 and in the T classes 16. This difference is not significant, but is in the direction expected if it were due to the occurrence of hybrid DNA in only one chromatid. Moreover, on a

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percentage basis, the difference is greater for the 3:5 asci than for the 5:3s. This is what would be expected if hybrid DNA in one chromatid is contributing to the NCO classes because it would contribute equally (by an amount equal to $\frac{1}{2}ad$) to both 5:3s and 3:5s. Since the overall frequency of 3:5s is lower than that of 5:3s, the percentage contribution of hybrid DNA in one chromatid to the 3:5s is expected to be greater than to the 5:3s.

In order to determine what fraction of the conversion at the *g* site is likely to be associated with hybrid DNA in only one chromatid, it has been assumed that the differences between the NCO and T classes of 5:3s and 3:5s arise from this cause. On the basis of the value of *a* obtained above, *d* can be calculated from the expression *ad* for the total difference between the NCO and T classes, which amounts to 9 asci (Table 1). Using the values for *b* and *c* also obtained above, the contributions to the other non-crossover ascus classes made by hybrid DNA in only one chromatid can then be determined. After deducting these values from the observed numbers, the formulae quoted above for the expected frequencies of the 5:3, 3:5, 6:2 and 2:6 classes due to hybrid DNA in both chromatids can be used to solve for *a*, *b* and *c*. These revised estimates of *a*, *b* and *c* were then used to obtain a new estimate for *d*, and hence further values for *a*, *b* and *c*. By this method of successive approximation, it has been found that a good fit with the observed numbers of both crossover and non-crossover asci is obtained if $a = 0{\cdot}4$, $b = 0{\cdot}5$, and $c = 0{\cdot}1$. This is on the assumption that *e* and *f* are zero, that is, that crossover hybrid DNA is never restricted to only one chromatid at the site of *g*. The expected numbers of asci in the various classes for these values of *a*, *b* and *c* are given in Table 5. For the 5:3, 3:5, 6:2 and 2:6 asci with parental combinations of the markers, $\chi^2 = 4{\cdot}1$, $n = 5$, $P = 0{\cdot}7$–$0{\cdot}5$; and for those with non-parental combinations $\chi^2 = 8{\cdot}6$, $n = 5$, $P = 0{\cdot}2$–$0{\cdot}1$. Thus, neither set of data differs significantly from the expected values. The observed numbers of aberrant 4:4 asci are also in agreement, as shown in the footnote to the table.

These values for *a*, *b* and *c* imply that for every 76 asci with 5:3, 3:5, 6:2 and 2:6 ratios and with parental combinations of the marker genes, another 14·9 asci with normal and 13·0 with aberrant 4:4 ratios are expected, in which conversion had occurred at the *g* site, making a total of 103·9. Hence $ad = 9/103{\cdot}9$, and with $a = 0{\cdot}4$, $d = 0{\cdot}216$. Thus, on the basis of the 9 asci by which the NCO classes of 5:3s and 3:5s exceed the T classes, about one-fifth of the conversion without crossing over can be attributed to hybrid DNA in only one chromatid. The contributions to the various classes are shown in the column headed 'One strand' in the non-crossover part of Table 5. It is evident that with more extensive data, if a significant excess of NCO over T in these classes were found, it would be possible to make a reliable estimate of the extent to which hybrid DNA in one chromatid was contributing to the non-crossover conversion at the *g* site.

In the analysis above it has been assumed that crossover hybrid DNA at the site of *g* never occurs in only one chromatid, that is, that *e* and *f* are zero. However, as with the non-crossovers, this is not necessarily true. It is evident from Table 3 that the aberrant 4:4 asci are the only crossover class to which hybrid DNA is one chromatid makes no contribution. Data on the frequency of this class relative to the others

would therefore be of particular value for determining the single chromatid frequency. Unfortunately, Kitani *et al.* (1962) obtained little information about the frequency of this class and so no attempt has been made to estimate e and f until more data are available. It is evident, however, that if e and f are large they must be approximately equal, since the total numbers of the PCO and QCO sub-classes of

Table 5. *The numbers of asci in the various classes observed by Kitani, Olive & El-Ani* (1962) *with the* gray (g) *mutant of* Sordaria fimicola *are shown, together with the numbers expected when conversion to* + *and to* g *occur in* 50% *and* 10% *respectively of the occurrences of* g *in hybrid DNA, heterozygosity being maintained in the remaining* 40% (a = 0·4, b = 0·5, c = 0·1), *and when hybrid DNA occurs at the site of* g *in both the participating chromatids in* 78·4% *of the parental and* 100% *of the non-parental marker combinations* (d = 0·216, e = 0, f = 0)

		Combination of marker genes									
		Parental					Non-parental				
Class of ascus +:g	Sub-class	Observed nos.	Expected numbers: Two strand	Expected numbers: One strand	Expected numbers: Total	χ^2	Sub-class	Observed nos.	Expected numbers: Two strand	Expected numbers: One strand	χ^2
Normal 4:4	NCO	—	4·07	6·75	10·82		QCO	—	2·84	0	
	T	—	4·07	—	4·07		PCO	—	2·84	0	
Aberrant 4:4	NCO	2*	13·03	—	13·03		CO	2*	9·06	—	
5:3	NCO	18	16·28	4·50	20·78	χ^2 =	QCO	17	11·35	0	χ^2 =
	T	15	16·28	—	16·28	4·1	PCO	13	11·35	0	8·6
3:5	NCO	7	3·26	4·50	7·76	n = 5	QCO	0	2·27	0	n = 5
	T	1	3·26	—	3·26	P =	PCO	3	2·27	0	P =
6:2	NCO	32	20·35	5·63	25·98	0·7–0·5	CO	8	14·19	0	0·2–0·1
2:6	NCO	3	0·82	1·12	1·94		CO	1	0·57	0	
Total omitting 4:4s	—	76	60·25	15·75	76·00		—	42	42·00	0	
Grand total	—	—	81·42	22·50	103·92		—	—	56·74	0	

* The 2 aberrant asci with 4:4 ratios and parental combinations, for the outside markers, and the 2 with non-parental combinations, were in a total sample of 49 aberrant asci. The expected frequencies in such a sample are 13·03 × (45/118) = 4·97, and 9·06 × (45/118) = 3·46, respectively.

the 5:3 and 3:5 asci are alike (16 and 17, respectively). On the other hand, if e and f are small, an inequality between them would not be detected without more extensive data. A reason is given in section 4 (iv) for expecting the values of e and f to be less than d, and there is evidence in agreement with this expectation from studies of intragenic recombination in other organisms (see section 4 (iv) and (v)).

Kitani *et al.* (1962) found that 5:3 asci showed a stronger association with crossing-

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over near the *g* site than did 6:2 asci. There appear to be two reasons for this. Firstly, conversion at the *g* site can simulate a two-strand double crossover spanning the site, but this effect cannot be detected in 6:2 asci. The classes of asci indicated by the letter T in Table 2 owe their origin to such conversion. Secondly, as was indicated above and is also evident from Table 2, hybrid DNA in only one chromatid makes no contribution to the T asci. Hence, the greater the proportion of non-crossover hybrid DNA which is confined to one chromatid at the *g* site, the fewer 5:3 asci in the T class which are expected, and hence the smaller the proportion of 5:3 asci with parental combinations of the outside markers, while 6:2 asci are not affected. The expected numbers given in Table 5 indicate a smaller difference between 5:3 and 6:2 asci, as regards associated crossing-over, than the observed numbers, but as already shown, the differences between the observed and the expected values are not significant.

The frequency of aberrant asci (5:3, 3:5, 6:2 and 2:6 ratios) at the *g* site was found by Kitani *et al.* (1962) to be 1 in every 865 asci. Taking the analysis in Table 5 as a guide, the 118 such asci were associated with another 42·6 in which hybrid DNA or conversion gave 4:4 ratios. It would thus appear that about one-quarter of the occurrences of hybrid DNA at the *g* site were not being detected, and hence that the hybrid molecules were occurring at this site in about one meiosis in every 650.

Kitani *et al.* (1962) found that other spore mutations showed different frequencies of aberrant asci, ranging from 1 in 250 for a hyaline-spored mutant to 1 in over 10,000 for a tan-spored mutant. They also found that the proportions of the different kinds of aberrant asci varied greatly for the different mutants. One called *mummy* showed approximately equal numbers of 6:2 and 2:6 asci but no 5:3s or 3:5s. On the hybrid DNA hypothesis, *a*, *b* and *c* would be 0, 0·5, and 0·5 respectively for this mutant, and half the occurrences of the site of the mutant in hybrid DNA would give rise to 4:4 asci. Since the total frequency of 6:2 and 2:6 asci for this mutant was about 1/1500, hybrid DNA would be occurring at the site in about 1 meiosis in every 750.

A green-spored mutant was found by Kitani *et al.* to give 5:3, 3:5, 6:2 and 2:6 asci with about equal frequency. Ignoring the possibility of single-chromatid hybrid DNA, these observations would imply that $a = 0{\cdot}2$, $b = 0{\cdot}4$ and $c = 0{\cdot}4$, and about one-third of the occurrences of hybrid DNA at this site would lead to 4:4s, either normal or aberrant. The aggregate frequency of 5:3, 3:5, 6:2 and 2:6 asci is described as being between that for the hyaline (1/250) and grey (1/865) mutants. Allowing for the undetected occurrences, it is probable that hybrid DNA occurs at the green-spore site in about one meiosis in every 300–400.

It seems clear that the differences in aberrant ascus frequency reflect real differences in the frequency of occurrence of hybrid DNA at different sites. Lissouba (1961) found indications that allelic mutations for spore colour in *Ascobolus immersus* differed in the frequency with which they gave rise to aberrant asci when crossed with wild-type, and furthermore the frequencies formed a graded series conforming to the position of each site on the linkage map of the alleles. In the alleles of series 46 the total frequencies of 6:2 and 2:6 ratios per 1000 asci were approximately 1 for

mutant no. 1604 at the left-hand end of the map (Rizet & Rossignol, 1964), approximately 8 for mutants nos. 63, 46 and W in the middle of the map, and about 16 for mutant no. 137 at the right-hand end (Lissouba, 1961). Similar results have been reported by Gajewski *et al.* (1963) for another series of alleles. Indications of the series 46 gradient are also shown by the data of Lissouba & Rizet (1960) from crosses between pairs of mutants in this series, since usually only the right-hand member of each pair shows conversion, and the frequencies of this conversion show a gradient corresponding with the position on the map. Lissouba (1961) also found that the frequencies of conversion in the two directions (to wild-type and to mutant) were not always equal.

The occurrence of post-meiotic segregation was discovered by Olive (1956, 1959) with the *g* mutant and with hyaline- and yellow-spored mutants of *Sordaria fimicola*, and is now known to occur widely. Lissouba *et al.* (1962) reported a number of examples in *Ascobolus immersus*: mutants nos. 277 and 1216 in series 46, mutant no. 60 in series 19, and several mutants (unnamed) in series 75, when crossed with allelic mutants gave occasional asci with 1:7 ratios of wild-type:mutant. Strickland (1961) crossed an inositol-requiring mutant (*inos*, allele no. 37401) of *Neurospora crassa* with an inositol-independent strain and obtained 1 ascus with 5:3 and 2 with 2:6 ratios of +:*inos* in 10,269 asci examined. Stadler & Towe (1963) found that allele no. 64 at the cysteine (*cys*) locus in *N. crassa* gave occasional 5:3 ratios, while other alleles did not. Out of 2240 asci from a cross between allele no. 17 and wild-type, there were 14 asci with 6+:2 *cys* and 1 with 2+:6 *cys* spores (total frequency 1/150), while allele no. 64 gave 5 asci with 6+:2 *cys* and 2 with 5+:3 *cys* spores in a sample of 1637 asci (total frequency of aberrant asci: 1/230). The technique used favoured recognition of asci with more than 4 wild-type spores rather than those with less than 4, so the conversion frequencies to wild-type and to mutant are not necessarily unequal.

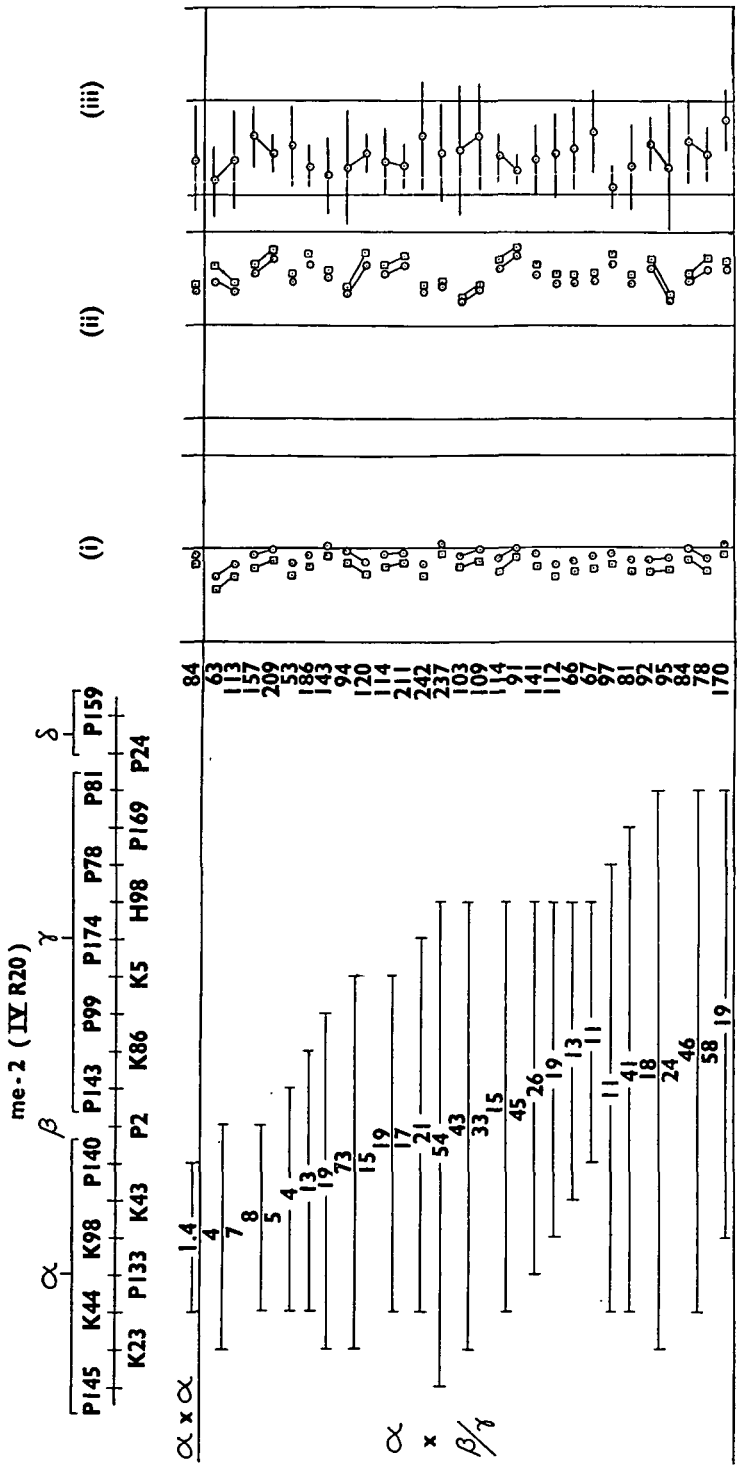
3. ANALYSIS OF DATA RELATING TO TWO SITES OF HETEROZYGOSITY WITHIN A GENE, AND TO TWO SITES WITHIN APPARENTLY NEIGHBOURING CISTRONS

(*a*) *Results from haploid recombinant selection*

(i) *Introduction*

Recombination within a gene has been studied by a number of authors by crossing allelic nutritional mutants and plating the progeny spores on minimal medium, thereby selecting for the rare recombinants lacking the nutritional requirement (prototrophs). Control experiments using the same alleles in both parents have in general shown no prototroph formation, or only at extremely low rates attributable to mutation. Linked marker genes on both sides of the nutritional locus have enabled recombination of the outside markers to be studied in conjunction with that of the alleles. This was first done by Giles (1952) and Pritchard (1955). The results of such studies are summarized in Figs. 2–8.

Let M/m represent the marker gene on the proximal (centromere) side of the alleles, and N/n be that on the distal side, and let 1 and 2 represent the alleles at the



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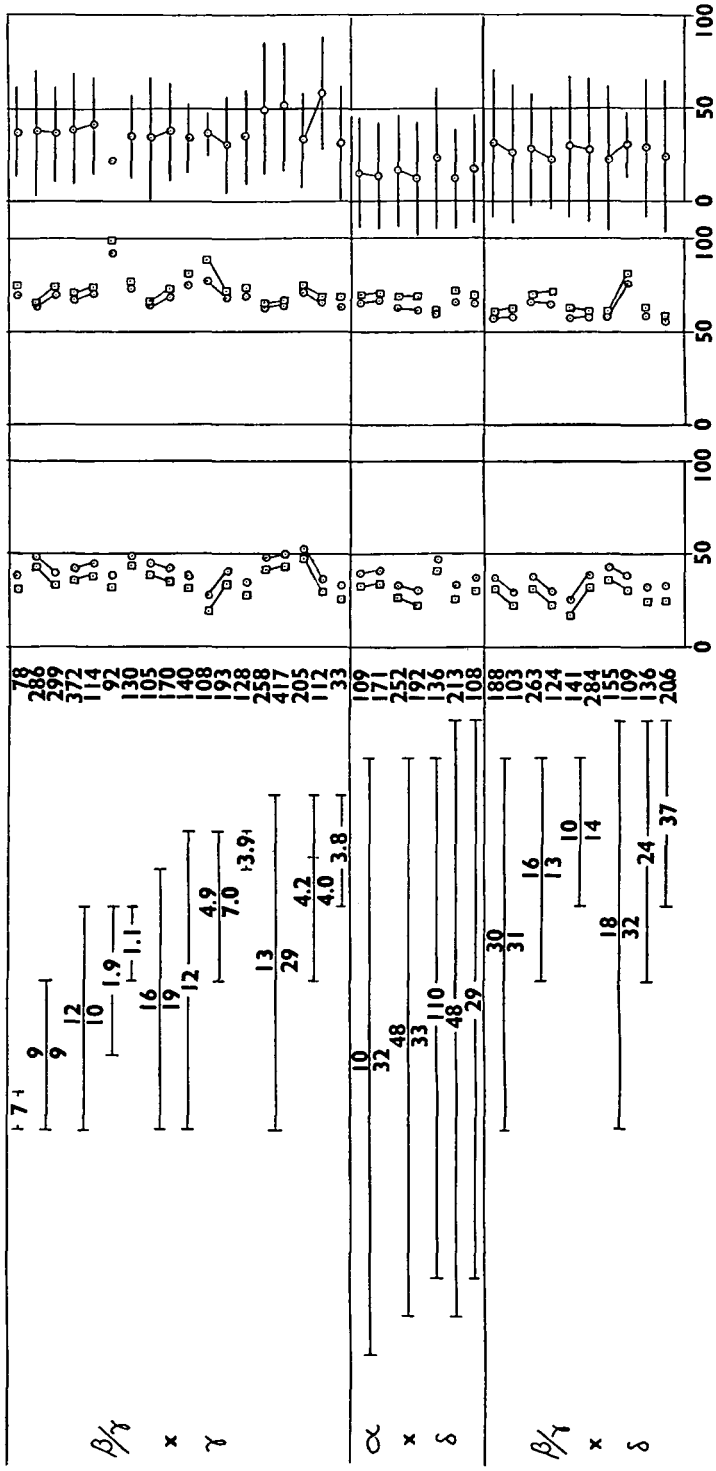

Fig. 2. The diagram shows the data of Murray (1960, 1963) for recombination between alleles at the methionine 2 (*me*-2) locus in *Neurospora crassa*. The diagram is explained in sections 3 (*a*) (ii) to (iv) of the text. The sequence of the α mutants is uncertain except that K44 is proximal to P140. In the γ mutants, the positions of P143, P99, and P174 are uncertain. The sequence of the two δ mutants is unknown. The recombination frequencies between *me*-2 and the proximal and distal outside markers were taken as 6% and 4% respectively.

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nutritional locus. It is assumed that allele no. 1 is the more proximal and no. 2 the more distal of the two, and that the parental strains were

M 1+ N

and m +2 n,

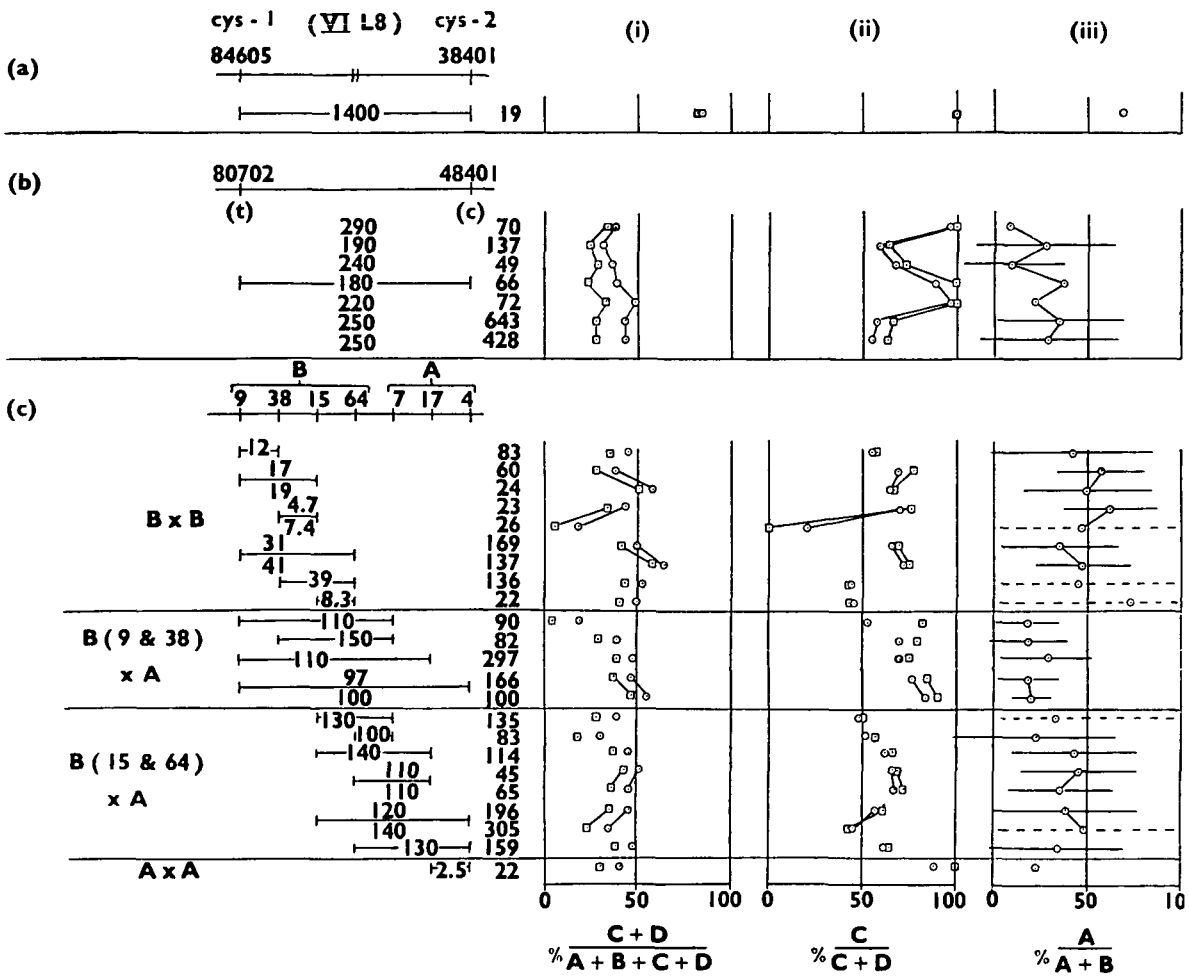

Fig. 3. The diagram shows the data of Stadler (1956, 1959*a*, *b*) and of Stadler & Towe (1963) for recombination between cysteine (*cys*) mutants in *Neurospora crassa*. The diagram is explained in sections 3 (*a*) (ii) to (iv) of the text. The relative positions of the mutants in parts (a), (b) and (c) of the diagram are not known, but all are situated in the same region of the left arm of linkage group VI. In (c), the allele map differs from that proposed by Stadler & Towe. The position of mutant no. 38 within the B group of alleles is particularly uncertain. In (a) and (b) the frequencies of prototrophs (given alongside the lines indicating the alleles crossed) are per 10^5 germinated spores, while in (c) they are per 10^5 total spores, whether germinated or not. The percentages of recombination between *cys* and the outside markers were as follows: (a), proximal 8, distal 4; (b), for the successive points in the graphs, reading downwards: proximal 3·8, 5·6, 6·2, 13, 17·5, 17, 17; distal 2·8, 2·1, 4·9, 8·1, 5·1, 5, 5; (c), proximal 9, distal 6.

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where + stands for the normal alleles at the nutritional locus. In the discussion which follows, the four possible genotypes for the prototrophs with respect to the outside markers, namely, M ++ N, m ++ n, m ++ N and M ++ n are denoted by the letters A, B, C and D respectively. In other words, those genotypes with parental marker combinations are represented as A and B, with A as the class in which the recombination additional to that between the alleles has occurred on the proximal side of them, and B as the class with the additional recombination on the distal side; and those genotypes with non-parental outside marker combinations are represented as C and

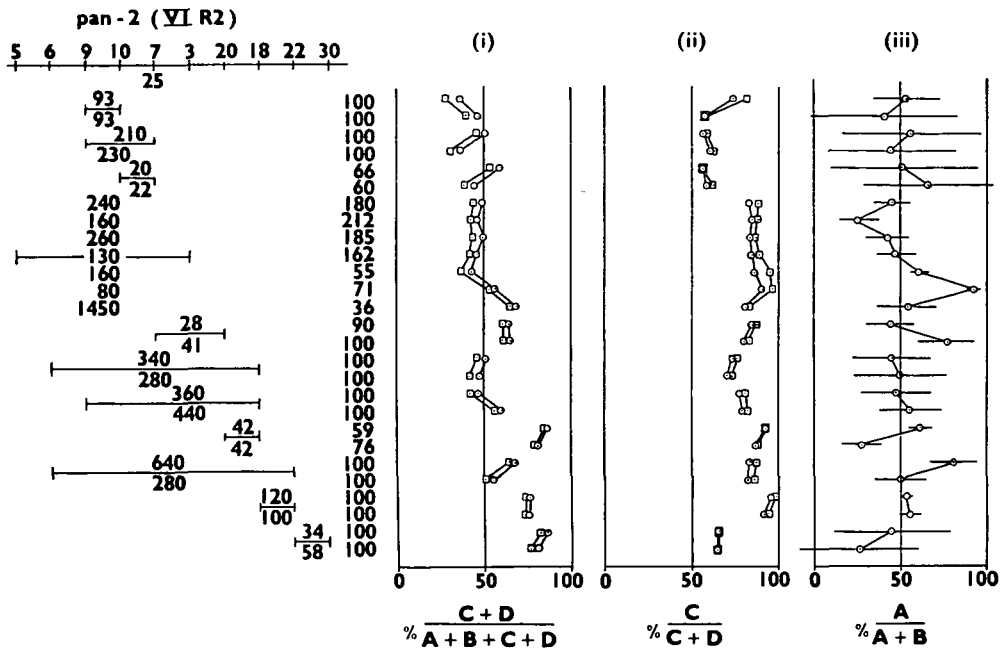

Fig. 4. The diagram shows the data of Case & Giles (1958, 1959) for recombination between alleles at the pantothenic acid 2 (*pan*-2) locus in *Neurospora crassa*. The diagram is explained in sections 3 (*a*) (ii) to (iv) of the text. The distal outside marker showed 8% recombination with *pan*-2. The proximal one showed 2% recombination, except for the data referring to 180 spores in the series of crosses between alleles nos. 5 and 3, where there was 4% recombination. Case & Giles (1960) give some results for additional alleles at the distal end of *pan*-2. These data have not been included in the diagram, but they agree with the general pattern obtained from the earlier work.

D, with C as the class in which there has been no additional recombination (such as would result if the only recombination was a crossover between the alleles), and D as the class with additional recombination on both proximal and distal sides.

(ii) *Explanation of Figs.* 2–8

In Figs. 2–8 the data of various authors are given relating to 10 genes in *Neurospora crassa*, 3 in *Aspergillus nidulans*, and 1 in *Saccharomyces cerevisiae*. In the diagrams the name of each gene is followed by its 'address' in the form of the

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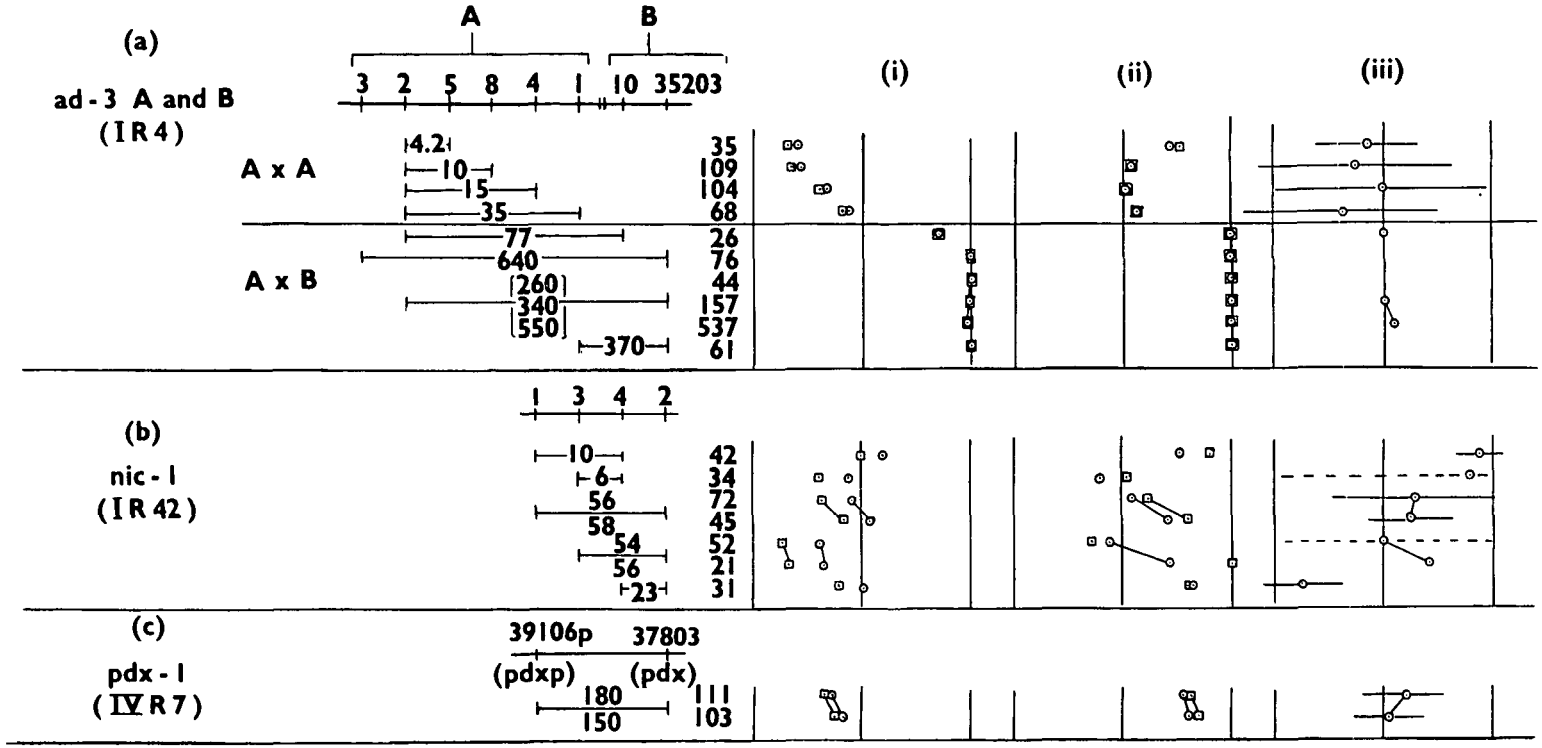

Fig. 5. The diagram shows the results of studies on recombination between closely-linked mutant sites at a number of loci in *Neurospora crassa*. The diagram is explained in sections 3 (*a*) (ii) to (iv) of the text.

(a) The data of de Serres (1956, 1960) and Giles, de Serres & Barbour (1957) for recombination within and between the adenine 3 (*ad*-3) cistrons A and B. The outside marker recombination percentages were 2 proximally and 3 distally, except for the data referring to 537 spores in the series of crosses between A2 and B35203, where they were 3 and 5, respectively.

(b) The data of St. Lawrence (1956) for the nicotinic acid 1 (*nic*-1) locus. The proximal outside marker gave 1% recombination and the distal 19% with *nic*-1.

(c) The data of Mitchell (1956) for the pyridoxin 1 (*pdx*-1) locus. The proximal and distal outside markers showed 1% and 4% recombination respectively with *pdx*-1.

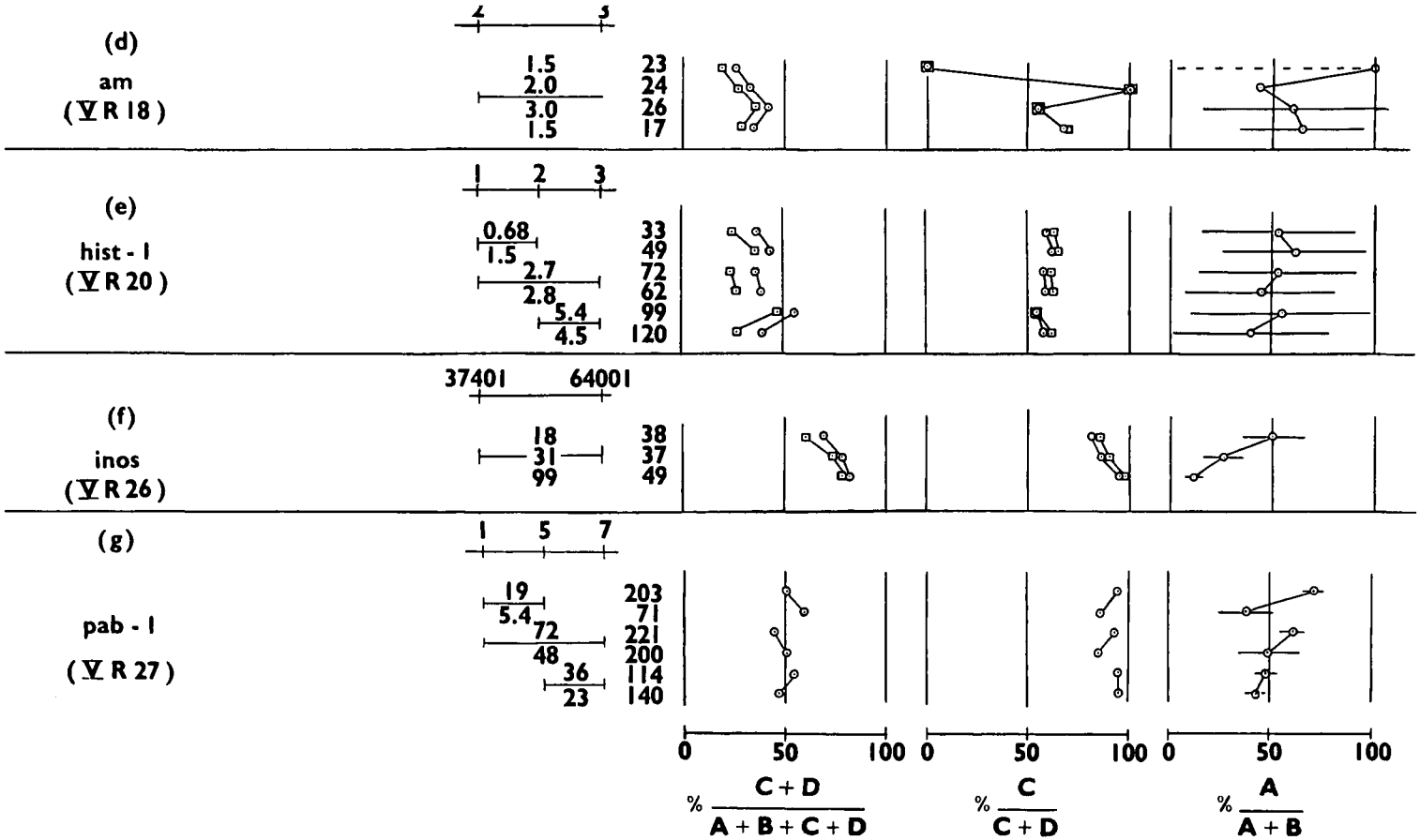

Fig. 5.—*continued*

(d) The data of Pateman (1960) for the amination (*am*) locus. The outside markers gave 6·5% and 2·5% recombination proximally and distally respectively.

(e) The data of Freese (1957*a*) for the histidine 1 (*hist*-1) locus. The relative positions of alleles 1 and 2 are uncertain. The marker recombination percentages were 10 on the proximal side and 6 on the distal side.

(f) The data of Giles (1956) for the inositol (*inos*) locus. The data of Giles (1952) have been omitted, as it has been assumed that they were included in those of the later publication. For the data referring to 38 and to 37 spores, the frequency of recombination between *inos* and the proximal outside marker was 17%, and for the data referring to 49 spores it was 7%. The distal marker gave 3% recombination throughout.

(g) The data of Freese (1957*b*) for the *para*-aminobenzoic acid 1 (*pab*-1) locus. The proximal and distal outside markers both gave 1% recombination with *pab*-1. In view of their proximity, marker distance correction has not been necessary.

linkage group as a Roman numeral, the arm (L for left, R for right), and the approximate distance from the centromere in crossover units as an Arabic numeral. In each diagram the linkage map of the alleles is shown in the upper left-hand part, with the more proximal alleles to the left and the more distal to the right. In several instances the sequence of some of the mutant sites is not known with certainty. Where there is particular doubt this is referred to in the legends to the individual diagrams.

Below the map in each diagram are horizontal lines indicating particular pairs of alleles which have been crossed. The figures alongside these lines show the frequency of prototrophs from each cross per 10^5 viable progeny spores (or total spores, whether germinated or not, in Fig. 3(c)). It is partly on the basis of these frequencies that the sequence of the alleles on the map has been determined. No attempt has been made to represent the prototroph frequencies to scale on the map. These frequencies vary greatly for different pairs of alleles, but the sites are shown as if equally spaced. Where a particular pair of alleles has been crossed more than once, usually with different parental combinations of the outside marker alleles, separate prototroph frequencies are given for each cross if this information is available.

The order in which the data for the various pairs of alleles are shown in the diagrams has in general been determined by the position of the more distal of the two alleles in each cross, and, within a group of crosses involving the same distal mutant, by the position of the more proximal allele. There are two exceptions to this sequence. First, in Figs. 2 and 3, crosses of extreme proximal with extreme distal alleles have been grouped together in order to reveal features which might otherwise be overlooked; and likewise crosses of alleles of intermediate position have been grouped together. The basis of the grouping is indicated down the left-hand side of the diagrams. In every other respect the sequence has been determined by the allele positions, as indicated above. Secondly, the data of Calef (1957) and Martin-Smith (1961) for the *ad*-9 locus in *A. nidulans*, given in the upper part of Fig. 6, are shown in the reverse sequence, that is, primarily by the position of the more proximal allele and secondarily by the distal one.

The column of figures to the right of the horizontal lines in each diagram shows the number of prototrophs which have been scored for the outside marker combinations from each cross.

The three graphs labelled (i), (ii), and (iii) in each diagram give the results of the marker scoring. Graph no. (i) in each diagram shows the total frequency of classes C and D, that is, of crossovers for the outside markers, plotted as a percentage of the total prototrophs. In the discussion which follows, this quantity, after correction for the marker distances, is called the *crossover coefficient*. Graph no. (ii) shows the frequency of class C as a percentage of C + D, that is, of the total crossovers; and graph no. (iii) shows the frequency of class A as a percentage of A + B.

(iii) *Marker distance correction*

In these graphs, in addition to plotting the observed proportions of the various classes, allowance has been made for the distance from the nutritional locus of the outside marker genes, since the occurrence of crossovers in these intervals will

affect the frequencies of the outside marker combinations in the prototrophs. In making a correction for the marker distances, it has been assumed that there is no interference between the occurrence of a crossover and the occurrence of conversion without crossing-over. Evidence for the lack of such interference was found by Stadler (1959*b*) from his observations on recombination in a separate but nearby region following selection for recombination between cysteine mutants in *Neurospora crassa*. The frequency of recombination events involving a crossover between two chromatids and conversion without crossing-over in a third chromatid in the immediate vicinity points to the same conclusion (see section 4 (vii)). On the hybrid DNA hypothesis, the negative interference between recombination events over short intervals of the linkage map is due to conversion, to the association between conversion and crossing-over, and to the association between conversion at one site and conversion at another nearby, and is not due to negative interference between crossovers. It is therefore assumed that in *N. crassa* and *Saccharomyces cerevisiae* there is positive position interference between crossovers over all the distances under study, and it has been considered justifiable to neglect the occurrence of double crossovers between the outside markers. In *Aspergillus nidulans*, on the other hand, there is no interference between crossovers, and it has therefore been necessary to assume that crossovers may have occurred in either of the intervals defined by the outside markers, irrespective of the events at the nutritional locus. Details of the corrections for marker distance which have been applied to the data are as follows.

Let A, B, C and D represent the expected frequencies of prototrophs in the respective classes, if the marker genes had been so close to the nutritional locus that crossing-over in the intervals was absent (apart from any crossovers directly associated with the recombination at the nutritional locus). Let a′, b′, c′ and d′ be the observed frequencies of the respective classes, when the marker distances are p on the proximal side and q on the distal side. It is intended that p and q should represent the recombination frequencies with the nutritional locus.

For *N. crassa* and *S. cerevisiae* with positive interference between crossovers within chromosome arms, it is supposed that the observed frequencies (a′ and b′) of the non-crossover classes are slightly less than their true values (A and B) owing to crossovers in the marked intervals, which would have the effect of transferring these potentially A and B prototrophs into the C and D classes. Expressed algebraically

$$a' = [1-(p+q)]A,$$
$$b' = [1-(p+q)]B,$$
$$c' = C+pA+qB,$$

and

$$d' = D+qA+pB.$$

Hence

$$A = \frac{a'}{1-(p+q)}, \qquad B = \frac{b'}{1-(p+q)},$$

$$C = c' - \frac{pa'+qb'}{1-(p+q)}, \quad \text{and} \quad D = d' - \frac{qa'+pb'}{1-(p+q)}.$$

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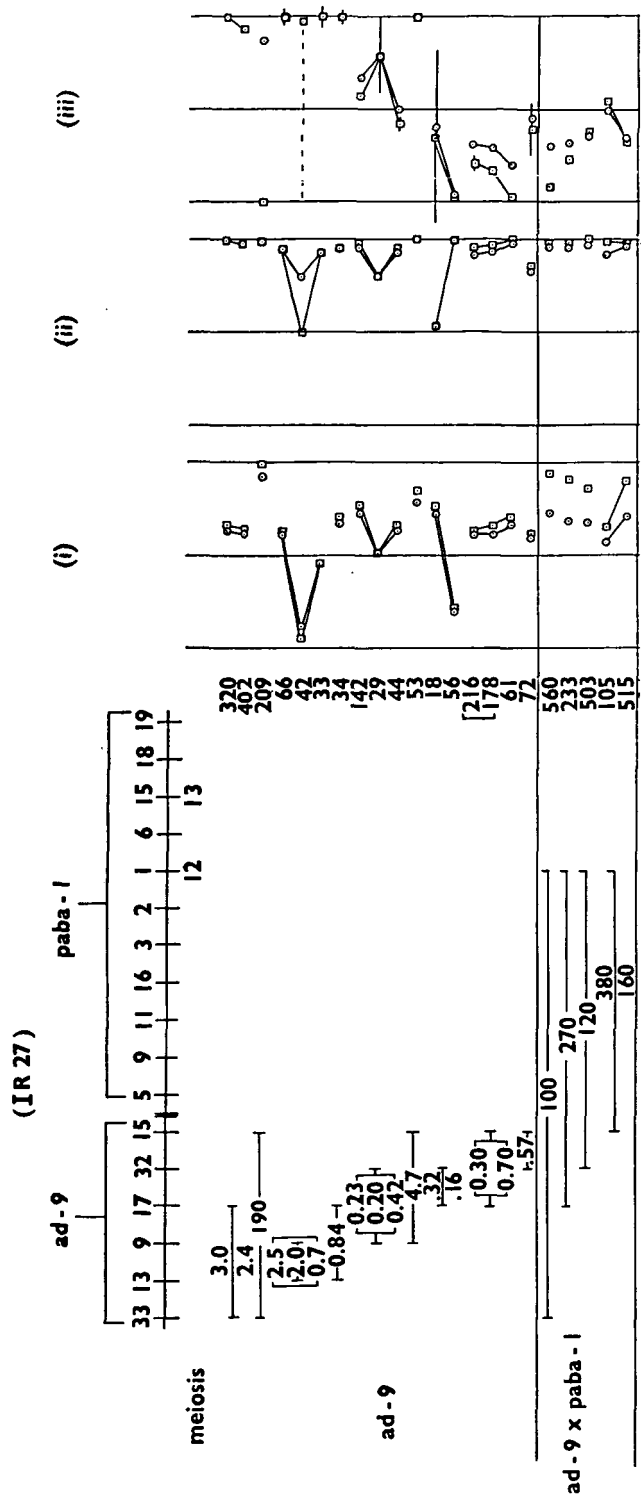

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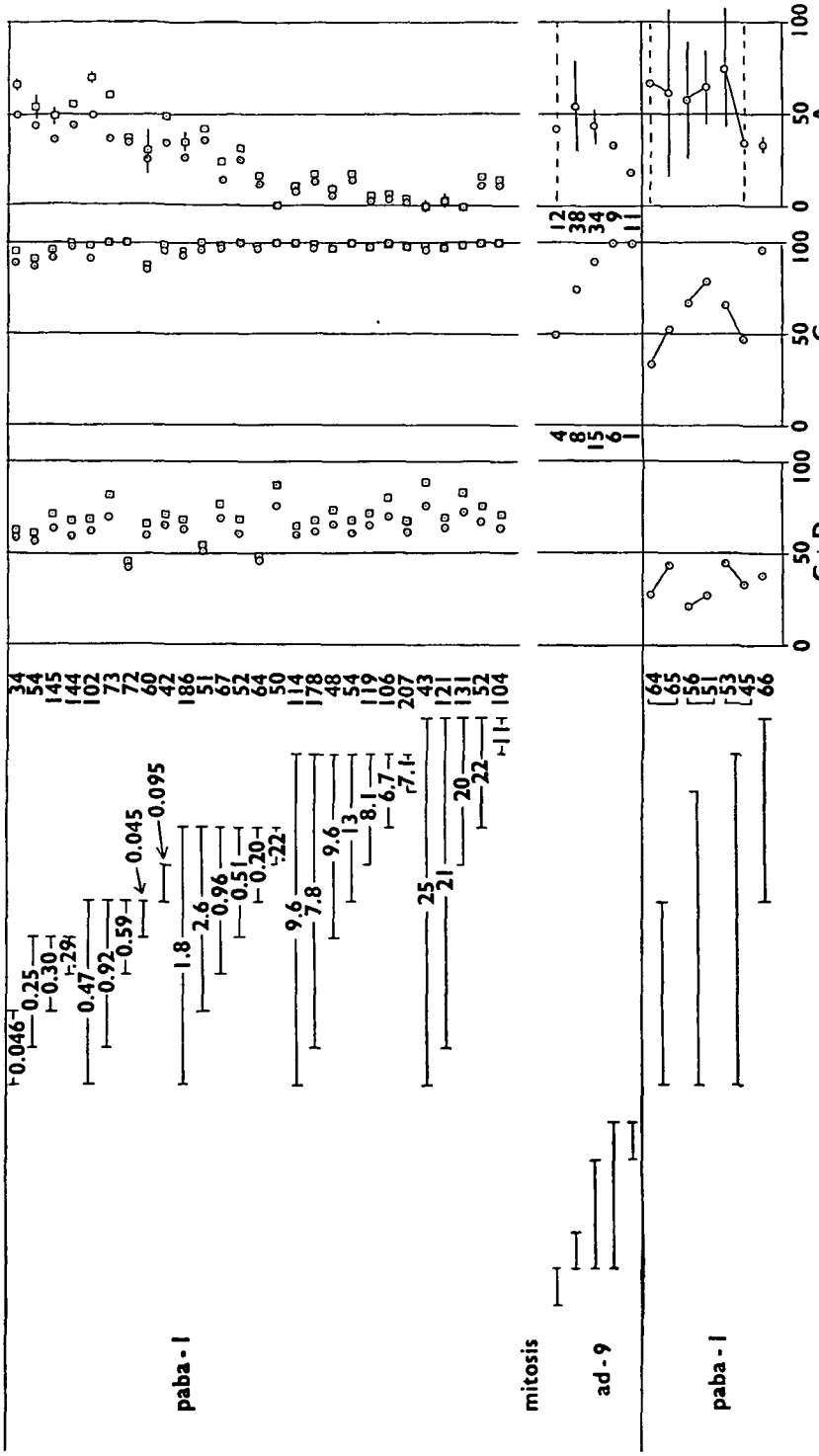

Fig. 6. The diagram shows the data of Calef (1957), Martin-Smith (1961), Siddiqi (1962) and Putrament (1964) for recombination at the adenine 9 (*ad*-9) and *para*-aminobenzoic acid 1 (*paba*-1) loci in *Aspergillus nidulans*. The diagram is explained in sections 3 (*a*) (ii) to (iv) of the text. For the meiotic data for *ad*-9, the outside marker recombination frequencies were taken to be 8% proximally and 0% distally. The *ad*-9 data referring to 216 and to 178 spores from the cross between alleles nos. 17 and 15 are due to Calef and the remainder to Martin-Smith. The recombination frequencies for the cross between *ad*-9 allele no. 17 and *paba*-1 were taken to be 9% proximally and 18% distally, and for the data relating to 105 spores from the cross between *ad*-9 allele no. 15 and *paba*-1 they were taken to be 11% and 14% respectively (Calef's data). For the other *ad*-9 × *paba*-1 crosses, which are due to Martin-Smith, they were taken as 8% and 16%, respectively. For the meiotic data for *paba*-1, which are due to Siddiqi, the marker recombination frequencies were assumed to be 0% proximally and 16% distally. The sequence of alleles nos. 5 and 9 of *paba*-1 is uncertain. The *ad*-9 mitotic data are due to Martin-Smith and the *paba*-1 mitotic data to Putrament.

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For *A. nidulans*, with no interference between crossovers, the corrections necessary in one direction for *N. crassa* and *S. cerevisiae* must be applied in both directions. In other words, crossovers in the marked intervals may transfer potential A and B prototrophs to C and D, and also transfer potential C and D prototrophs to A and B. Expressed algebraically

$$a' = [1-(p+q)]A + pC + qD,$$
$$b' = [1-(p+q)]B + qC + pD,$$
$$c' = [1-(p+q)]C + pA + qB,$$

and

$$d' = [1-(p+q)]D + qA + pB.$$

From these equations it can be shown that

$$A = \frac{E(Ga'+Fb')-(Jc'+Hd')}{G^2-F^2},$$

$$B = \frac{E(Fa'+Gb')-(Hc'+Jd')}{G^2-F^2},$$

$$C = \frac{E(Gc'+Fd')-(Ja'+Hb')}{G^2-F^2},$$

and

$$D = \frac{E(Fc'+Gd')-(Ha'+Jb')}{G^2-F^2},$$

where

$$E = 1-(p+q),$$
$$F = 2pq,$$
$$G = 2E+F-1,$$
$$H = Fp+Gq,$$

and

$$J = Gp+Fq.$$

If either p or q is zero, these expressions for A, B, C and D (with no interference between crossovers) become greatly simplified. Thus, if $p = 0$,

$$A = \frac{(1-q)a'-qd'}{1-2q}, \qquad B = \frac{(1-q)b'-qc'}{1-2q},$$

$$C = \frac{(1-q)c'-qb'}{1-2q}, \text{ and } D = \frac{(1-q)d'-qa'}{1-2q}.$$

Similarly, if $q = 0$,

$$A = \frac{(1-p)a'-pc'}{1-2p}, \qquad B = \frac{(1-p)b'-pd'}{1-2p},$$

$$C = \frac{(1-p)c'-pa'}{1-2p}, \text{ and } D = \frac{(1-p)d'-pb'}{1-2p}.$$

In the diagrams, the uncorrected data are shown as dots surrounded by a circle (⊙), and the corrected data, using the appropriate formulae above, by dots surrounded by a square (⊡). The values for p and q that have been used in making the

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marker distance corrections are given in the legends to the diagrams. Dots referring to the same pairs of alleles are joined by lines. No correction for marker distances has been applied to data for mitotic recombination in *A. nidulans* and *S. cerevisiae*, since it has been assumed that recombination events at mitosis are rare enough for correction to be unnecessary. However, events involving three chromatids are sufficiently frequent to make it questionable whether this assumption is justified

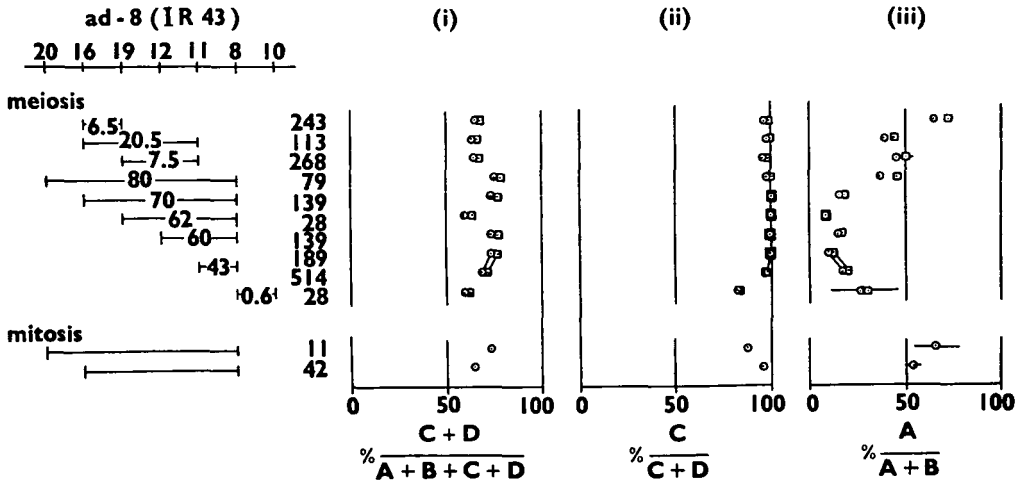

Fig. 7. The diagram shows the data of Pritchard (1955, 1960*a*,*b*) for recombination between alleles at the adenine 8 (*ad*-8) locus in *Aspergillus nidulans*. The diagram is explained in sections 3 (*a*) (ii) to (iv) of the text. For the meiotic data, the outside marker recombination frequencies were taken to be 0% and 6% in the proximal and distal directions respectively.

(but see section 4 (vii)). No corrections are shown to the *N. crassa* and *S. cerevisiae* data for the frequency of class A as a percentage of A + B (graph (iii) in the diagrams) because, as can be seen from the formulae above, with the assumptions made about interference this frequency is unaffected by the marker distances.

(iv) *Interpretation of classes A–D*

On the hybrid DNA hypothesis, the simplest explanations for the various prototroph genotypes (derived from a cross between M 1 + N and m + 2 n) are as follows.

A. The A genotype (M ++ N) could be the consequence of the proximal allele (no. 1) occurring in non-crossover hybrid DNA (+/1) and then converting to wild-type. The distal allele could have remained outside the region of hybrid DNA.

B. Conversely, the B genotype (m ++ n) could be due to non-crossover hybrid DNA occurring at the site of the distal allele (+/2) and converting to wild-type, while the proximal allele remained unaffected.

C. One possibility for the origin of the C genotype (m ++ N) is that an entire crossover, that is, the whole of its region of hybrid DNA, lay between the mutant sites, neither of which was included in the hybrid region. A second

possibility is that the crossover was on the proximal side of the alleles and its hybrid DNA extended to include the proximal but not the distal site. Conversion of + /1 to wild-type would then give the C genotype. A third possibility is the converse of this, namely, a distal crossover with hybrid DNA extending proximally to include the distal but not the proximal site, followed by conversion of + /2 to wild-type.

D. The D genotype (M ++ n), with crossing-over of the outside markers and with recombination (additional to that between the alleles) occurring on both sides of the alleles, could be the consequence of crossover hybrid DNA extending to both sites from either end, followed by conversion of both + /1 and + /2 to wild-type.

Genotypes A, B and C could also arise, like D, through the occurrence of hybrid DNA at both sites in the appropriate crossover or non-crossover chromatid, followed by conversion of both + /1 and + /2 to wild-type. However, if A, B and C always arose

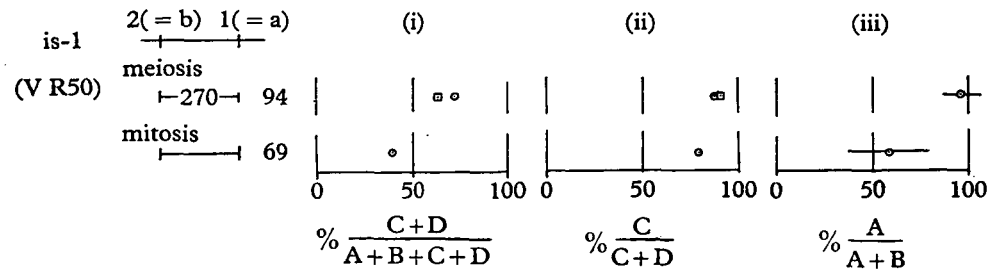

Fig. 8. The diagram shows the data of Sherman & Roman (1963) and Kakar (1963) for recombination between alleles at the iso-leucine 1 (*is*-1) locus in *Saccharomyces cerevisiae*. The diagram is explained in sections 3 (*a*) (ii) to (iv) of the text. For the meiotic data (Sherman & Roman), the recombination frequencies with the proximal and distal outside markers were taken to be 15% and 9% respectively.

in this way, as a consequence of hybrid DNA at both sites, followed by conversion to + at both of them, the frequencies of genotypes A and B would be expected to be equal, and likewise C and D would be expected to be equal. It is evident from graph (iii) in Figs. 2–8 that A and B are often unequal, and likewise from graph (ii) that C and D are often unequal. It follows that classes A, B and C must frequently be arising as a consequence of the occurrence of hybrid DNA at one site, as indicated above, rather than at both sites. An alternative explanation for the inequality of the C and D classes is through the first possibility mentioned above for the origin of the C class, namely, the occurrence of a region of hybrid DNA between the mutant sites but not extending to either of them. This possibility is discussed in section 4 (vi).

When crossover hybrid DNA occurs at both sites, followed by conversion to + at both, it is expected to give rise to classes C and D with equal frequency, whereas crossover hybrid DNA at only one site (either the proximal or the distal one), followed by conversion to +, will give rise to class C alone. Thus 2D, that is, twice the frequency of class D after correction for the marker distances, is a measure of the

frequency of crossover hybrid DNA at both sites, and C − D, that is, the corrected frequency of class C minus the corrected frequency of class D, is a measure of the crossover hybrid DNA frequency at only one site. Hence, the expression

$$\frac{2D}{C+D}$$

provides a measure of the relative frequencies of one- and two-site hybrid DNA associated with a crossover, and in the discussion which follows this is called the *site coefficient* for a particular pair of alleles. When this expression is zero, the crossover hybrid DNA never extends to both sites. When the value of the coefficient is 0·5, one- and two-site events are equally frequent. If the value of the coefficient is 1, single site events are absent and the crossover hybrid DNA is occurring entirely at both sites.

When non-crossover hybrid DNA occurs at both sites, followed by conversion to + at both, it is expected to give rise to classes A and B with equal frequency, whereas non-crossover hybrid DNA at only one site, followed by conversion to +, will give rise to class A if it is at the proximal site and class B if it is at the distal one. Thus class A is a measure of the frequency of non-crossover hybrid DNA at the proximal site alone, plus half the frequency of non-crossover hybrid DNA at both sites. Similarly, class B is equivalent to the distal single-site frequency plus half the double-site frequency. It is evident that there is no means of estimating the site coefficient in non-crossover hybrid DNA, except that, as already pointed out, a significant inequality between A and B must mean there have been some single-site events.

In analysing the data in Figs. 2–8, it has been assumed that for any given pair of alleles the non-crossover hybrid DNA has the same site coefficient as the crossover hybrid DNA. As indicated below, it is probable that this assumption is not always true. The horizontal lines extending on either side of the points in graph (iii) in Figs. 2–8 show the contributions to the A and B classes due to hybrid DNA at both sites, on this assumption that one- and two-site events occur in the same proportions irrespective of the occurrence of crossing-over. The total length of each line represents the site coefficient in crossover hybrid DNA for that pair of alleles. The coefficient is plotted with the half values, that is, D/(C+D), extending on each side of the point in the graph, since, as already indicated, double-site hybrid DNA is expected to contribute equally to the A and B classes. Hence, the distance from the left-hand end of each line to the left-hand axis of the graph, that is, to the line representing A/(A+B) = 0, shows the frequency of conversion at the proximal site alone. Similarly, the distance from the right-hand end of each line to the right-hand axis [A/(A+B) = 1] shows the frequency of conversion at the distal site alone.

In a few instances the horizontal lines in graph (iii) extend beyond one or other axis. This implies that no single-site hybrid DNA is contributing to that class (A or B, depending on which axis is crossed). These negative values for the proximal (or distal) single-site frequency may be due to sampling error, the true values being zero or slightly above. Alternatively, the negative value may mean that the assumption that crossover and non-crossover hybrid DNA have the same site

coefficient is unjustified. The occasional negative values for the proximal single site frequency seen in the upper part of Fig. 2 (iii) (Murray's data for *me*-2 in *Neurospora crassa*) are probably due to sampling error, but the consistently negative values at the bottom of the graph imply that for these allele combinations the non-crossover hybrid DNA has a lower site coefficient than the crossover hybrid DNA. This inference is discussed further in section 4 (iv).

There are several instances in Figs. 2–8 where class D slightly exceeds C. The site coefficient is then necessarily greater than unity, with the implication that conversion has occurred at both sites in all the prototrophs. This is indicated for the non-crossover classes by a broken line extending the whole width of graph (iii). Under such circumstances, the frequencies of the A and B classes are expected to be equal.

The relative frequencies of classes C and D provide a second method of constructing the linkage map of the alleles, in addition to the prototroph frequency referred to earlier. In some sets of data, for example, those for *Aspergillus nidulans* (Figs. 6 and 7), the C and D classes usually differ so widely in frequency that any ambiguity in the map based on prototroph frequencies can be readily resolved. In other instances, such as in some of the data for *N. crassa*, the C and D frequencies are so nearly equal that uncertainty about the sequence of the mutant sites on the map remains. In general, a site sequence map based on the assumption that C is greater than D agrees well with the site sequence map based on prototroph frequencies, but in a few instances there is a conflict of evidence. Examples of this are to be found in the data of Freese (1957) for *hist*-1 in *N. crassa* (Fig. 5(e)), and in some of the data of Stadler & Towe (1963) for *cys* alleles also in *N. crassa* (Fig. 3(c)). Where there is this uncertainty in drawing the map, greater weight has been given to the relative frequencies of classes C and D than to the prototroph frequencies. The reasons for this are discussed in section 4 (v). Reversal of the position of two mutant sites on the map has the effect of interchanging the frequency of class C with that of class D, and also of interchanging the frequency of class A with that of class B. With those sites where such reversal may be necessary, its effects on the general interpretation of the data will be slight, since C and D are necessarily nearly equal or otherwise no ambiguity would have arisen. Under these circumstances, as indicated above, A and B are also expected to be approximately equal. Because of this relatively slight effect of map errors, it has been thought justifiable to present maps of all the sites that have been studied at all extensively, even though the site sequence shown is sometimes little more than a guess.

(v) *Results from non-allelic crosses*

The data in Figs. 2–8 reveal a difference between the behaviour of allelic and non-allelic mutations. Three examples of crosses between closely-linked non-allelic mutations are available: *cys*-1 × *cys*-2 in *Neurospora crassa* studied by Stadler (1956) and shown in Fig. 3(a); *ad*-3A × *ad*-3B in *N. crassa* studied by de Serres (1956) and Giles, de Serres & Barbour (1957) and shown in Fig. 5(a); and *ad*-9 × *paba*-1 in *Aspergillus nidulans* studied by Calef (1957) and Martin-Smith (1961) and shown in Fig. 6. After correction for the marker distances, the results in all three instances are

similar, namely, a high frequency of prototrophs in class C, a low or very low frequency in classes A and B, and the absence of prototrophs in class D. On the other hand, the allelic crosses give much higher frequencies of classes A and B, and usually a number of class D prototrophs as well.

It seems likely that the high frequency of C class prototrophs from the non-allelic crosses is due to the occurrence of entire crossover events between the mutant sites. This has been confirmed by Giles, de Serres & Barbour (1957) for the A2 and B35203 mutations of the *ad*-3A and B cistrons, respectively, by means of tetrad analysis (see section 3 (*b*) (ii)). The absence of D class prototrophs would imply that the hybrid DNA of the crossover between the sites never extended to cover both of them. This is in agreement with the predictions of the polaron hybrid DNA hypothesis. With homologous primary breakage-points, a crossover requires hybrid DNA to be formed in only one direction from the initial breakage-points. It could not therefore extend to mutant sites on both sides, and hence no D class prototrophs would be expected. The A and B class prototrophs could arise through conversion at one or other or both sites in non-crossover hybrid DNA. This might have originated between the sites or on the proximal or distal side of them.

(vi) *Results from allelic crosses*

A number of remarkable features of intragenic recombination are revealed in Figs. 2–8. Murray's data for *me*-2 in *Neurospora crassa*, shown in Fig. 2, indicate a progressive decline in the crossover coefficient (graph (i)) and in C/(C+D) (graph (ii)) as one moves distally within the gene, while the relative frequencies of A and B (graph (iii)) show A always less than B but with distinct differences depending on the cluster (α, β/γ, or δ) in which each site is situated. The data of Stadler (1959*a*, *b*) and of Stadler & Towe (1963) for *cys* in *N. crassa*, given in Fig. 3(b) and (c), show rather similar patterns to those found by Murray for *me*-2, except that at the proximal end of the gene classes A and B are equal. There is much variation in Fig. 3(b) in the relative frequencies of C and D for the same pair of alleles in different experiments. These differences were associated with different genetic backgrounds (Stadler 1959*a*). On the other hand, variation shown in Fig. 4 between different experiments using alleles nos. 5 and 3 of *pan*-2 in *N. crassa*, studied by Case & Giles (1958, 1959), has affected the A and B frequencies rather than C and D. In these 5×3 crosses, the genetic background was more uniform than in Stadler's work. The data of Fig. 4 show a trend in the crossover coefficient in the opposite direction to that in Murray's data (Fig. 2), and the frequencies of C/(C+D) and A/(A+B) (graphs (ii) and (iii)) show less regular variation. The data in Fig. 5(a–g) due to de Serres (1960), St. Lawrence (1956), Mitchell (1956), Pateman (1960), Freese (1957*a*), Giles (1956) and Freese (1957*b*) and referring to *ad*-3A, *nic*-1, *pdx*-1, *am*, *hist*-1, *inos*, and *pab*-1, respectively, in *N. crassa* show similarity in most respects to the more extensive data in Figs. 2–4, except that Giles' data for *inos* and Freese's for *pab*-1 indicate unusually high frequencies for the crossover coefficient (graph (i)) and for C/(C+D) (graph (ii)). It is of interest that one of the alleles studied by Giles (no. 37401) was found by Strickland (1961) to show post-meiotic segregation (see section 2).

The data for *ad*-9 and *paba*-1 in *Aspergillus nidulans* shown in Fig. 6 and due to Calef (1957), Martin-Smith (1961), Siddiqi (1962) and Putrament (1964) reveal higher frequencies for the crossover coefficient (graph (i)) and much lower frequencies for class D (graph (ii)) than have usually been found in *N. crassa*, but at mitosis in *A. nidulans* the findings are similar to meiosis in *N. crassa*. Trends in the relative frequencies of A and B in different parts of the genes are clearly evident (graph (iii)). Pritchard's data for *ad*-8 in *A. nidulans* given in Fig. 7 show similar features to the data in Fig. 6. The limited data for *Saccharomyces cerevisiae* given in Fig. 8 resemble the results for *A. nidulans*.

To sum up, the outstanding features of allelic recombination revealed by Figs. 2–8 are

(a) the progressive changes within some genes in the frequencies of the quantities plotted in graphs (i), (ii) and (iii) as one proceeds from one end of a group of alleles to the other,
(b) the marked differences in the frequencies of prototrophs of classes C and D in *N. crassa* (Figs. 2–5) compared with *A. nidulans* (Figs. 6 and 7), and
(c) the contrast between the frequencies of these classes in meiotic and mitotic recombination in *A. nidulans*.

The significance of these features will be discussed in section 4, after the results from diploid and tetrad recombinant selection have been analysed.

(*b*) *Results from diploid and tetrad recombinant selection*

(i) *Introduction*

When conversion occurs at the sites of one or both of two alleles (1 and 2), and when outside markers (M/m and N/n) show the parental combinations, there are 16 possible genotypes for the tetrad at the end of meiosis, on the assumption that a mutant site in hybrid DNA does not remain heterozygous but always converts to wild-type or to mutant. These genotypes are listed in Table 6. It has been assumed that only two chromatids have taken part in the conversion, and that no other recombination events have occurred in the interval between M and N. Two of the four products of meiosis will then have the parental genotypes (M 1 + N and m + 2 n). The genotypes of the other two products are listed in the third column of the table. With the same assumptions, a further 16 genotypes are possible when the conversion is associated with crossing-over, and these are shown in Table 7.

Out of these 32 genotypes, there are 14 which include wild-type progeny for the character represented by the pair of alleles. These 14 are the only ones which will be readily detected experimentally, and in the fourth columns of Tables 6 and 7 they are indicated by letters which refer to their classification in Table 8. In this classification, the letters A–D have the same meaning as in the previous section of this paper and refer to the outside marker genotype of the wild-type chromosome. The letters P–S refer to the allele genotype of the homologous chromosome, which has

Table 6. *The table shows the* 16 *tetrad genotypes possible when conversion occurs at the sites of one or both of two alleles* (1 *and* 2), *and when outside markers* (*M*/*m and N*/*n*) *have the parental combinations. The expected frequency of each genotype is given in terms of the frequency of hybrid DNA at either or both sites in one or both chromatids* (*see section* 3 (b) (*i*) *of the text*). *It has been assumed that conversion takes place in each direction with equal frequency. The frequencies relating to tetrads with wild-type spores are indicated by an asterisk.*

Type of recombination for alleles	No.	Tetrad genotype (parental strands M 1+ N and m +2 n) Genotype of other two strands	Reference letters (wild-types)	Frequency of hybrid DNA at sites: One chromatid v_{pn}	v_{qn}	w_n	Both chromatids x_{pn}	x_{qn}	y_{pn}	y_{qn}	z_n
None	1	M +1 N m +2 n	—	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{16}$
	2	M +2 N m 1+ n	—	—	—	—	—	—	—	—	$\frac{1}{16}$
Conversion at proximal site	3	M ++ N m +2 n	AP	$\frac{1}{4}$*	—	$\frac{1}{8}$*	$\frac{1}{4}$*	—	$\frac{1}{8}$*	$\frac{1}{16}$*	$\frac{1}{16}$*
	4	M 1+ N m 12 n	—	$\frac{1}{4}$	—	$\frac{1}{8}$	$\frac{1}{4}$	—	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{16}$
	5	M +2 N m ++ n	BP	—	—	—	—	—	—	$\frac{1}{16}$*	$\frac{1}{16}$*
	6	M 12 N m 1+ n	—	—	—	—	—	—	—	$\frac{1}{16}$	$\frac{1}{16}$
Conversion at distal site	7	M ++ N m 1+ n	AQ	—	—	—	—	—	$\frac{1}{16}$*	—	$\frac{1}{16}$*
	8	M +2 N m 12 n	—	—	—	—	—	—	$\frac{1}{16}$	—	$\frac{1}{16}$
	9	M 1+ N m ++ n	BQ	—	$\frac{1}{4}$*	$\frac{1}{8}$*	—	$\frac{1}{4}$*	$\frac{1}{16}$*	$\frac{1}{8}$*	$\frac{1}{16}$*
	10	M 12 N m +2 n	—	—	$\frac{1}{4}$	$\frac{1}{8}$	—	$\frac{1}{4}$	$\frac{1}{16}$	$\frac{1}{8}$	$\frac{1}{16}$
Reciprocal conversion	11	M ++ N m 12 n	AR	—	—	—	$\frac{1}{4}$*	—	$\frac{1}{8}$*	—	$\frac{1}{16}$*
	12	M 12 N m ++ n	BR	—	—	—	—	$\frac{1}{4}$*	—	$\frac{1}{8}$*	$\frac{1}{16}$*
Conversion at both sites (parental)	13	M 1+ N m 1+ n	—	—	—	$\frac{1}{8}$	—	—	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{16}$
	14	M +2 N m +2 n	—	—	—	$\frac{1}{8}$	—	—	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{16}$
Conversion at both sites (recombinant)	15	M ++ N m ++ n	ABS	—	—	—	—	—	$\frac{1}{16}$*	$\frac{1}{16}$*	$\frac{1}{16}$*
	16	M 12 N m 12 n	—	—	—	—	—	—	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{16}$
		Total (wild-types)		$\frac{1}{4}$*	$\frac{1}{4}$*	$\frac{1}{4}$*	$\frac{1}{2}$*	$\frac{1}{2}$*	$\frac{7}{16}$*	$\frac{7}{16}$*	$\frac{7}{16}$*

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Table 7. *The table shows the* 16 *tetrad genotypes possible when conversion occurs at the sites of one or both of two alleles* (1 *and* 2), *and when outside markers* (*M*/*m and N*/*n*) *show crossing-over of the same two chromatids. The expected frequency of each genotype is given in terms of the frequency of hybrid DNA at either or both sites in one or both chromatids* (*see section* 3 (b) (*i*) *of the text*). *It has been assumed that conversion takes place in each direction with equal frequency. The frequencies relating to tetrads with wild-type spores are indicated by an asterisk*

Type of recombination for alleles	No.	Tetrad genotype (parental strands M 1+ N and m +2 n) Genotype of other two strands	Reference letters (wild-types)	Frequency of hybrid DNA at sites: One chromatid v_{pc}	v_{qc}	w_{pc}	w_{qc}	Both chromatids x_{pc}	x_{qc}	y_{pc}	y_{qc}	z_c
None	17	M +2 n / m 1+ N	—	$\frac{1}{2}$	—	$\frac{1}{4}$	—	$\frac{1}{4}$	—	—	—	$\frac{1}{16}$
	18	M 1+ n / m +2 N	—	—	$\frac{1}{2}$	—	$\frac{1}{4}$	—	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{16}$
Conversion at proximal site	19	M +2 n / m ++ N	CP	$\frac{1}{4}$*	—	$\frac{1}{8}$*	—	$\frac{1}{4}$*	—	—	$\frac{1}{16}$*	$\frac{1}{16}$*
	20	M 12 n / m 1+ N	—	$\frac{1}{4}$	—	$\frac{1}{8}$	—	$\frac{1}{4}$	—	—	$\frac{1}{16}$	$\frac{1}{16}$
	21	M ++ n / m +2 N	DP	—	—	—	$\frac{1}{8}$*	—	—	$\frac{1}{8}$*	$\frac{1}{16}$*	$\frac{1}{16}$*
	22	M 1+ n / m 12 N	—	—	—	—	$\frac{1}{8}$	—	—	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{16}$
Conversion at distal site	23	M 1+ n / m ++ N	CQ	—	$\frac{1}{4}$*	—	$\frac{1}{8}$*	—	$\frac{1}{4}$*	$\frac{1}{16}$*	$\frac{1}{8}$*	$\frac{1}{16}$*
	24	M 12 n / m +2 N	—	—	$\frac{1}{4}$	—	$\frac{1}{8}$	—	$\frac{1}{4}$	$\frac{1}{16}$	$\frac{1}{8}$	$\frac{1}{16}$
	25	M ++ n / m 1+ N	DQ	—	—	$\frac{1}{8}$*	—	—	—	$\frac{1}{16}$*	—	$\frac{1}{16}$*
	26	M +2 n / m 12 N	—	—	—	$\frac{1}{8}$	—	—	—	$\frac{1}{16}$	—	$\frac{1}{16}$
Reciprocal conversion	27	M 12 n / m ++ N	CR	—	—	—	—	$\frac{1}{4}$*	$\frac{1}{4}$*	—	$\frac{1}{8}$*	$\frac{1}{16}$*
	28	M ++ n / m 12 N	DR	—	—	—	—	—	—	$\frac{1}{8}$*	—	$\frac{1}{16}$*
Conversion at both sites (parental)	29	M 1+ n / m 1+ N	—	—	—	$\frac{1}{8}$	$\frac{1}{8}$	—	—	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{16}$
	30	M +2 n / m +2 N	—	—	—	$\frac{1}{8}$	$\frac{1}{8}$	—	—	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{16}$
Conversion at both sites (recombinant)	31	M ++ n / m ++ N	CDS	—	—	—	—	—	—	$\frac{1}{16}$*	$\frac{1}{16}$*	$\frac{1}{16}$*
	32	M 12 n / m 12 N	—	—	—	—	—	—	—	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{16}$
		Total (wild-types)		$\frac{1}{4}$*	$\frac{1}{4}$*	$\frac{1}{4}$*	$\frac{1}{4}$*	$\frac{1}{2}$*	$\frac{1}{2}$*	$\frac{7}{16}$*	$\frac{7}{16}$*	$\frac{7}{16}$*

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undergone hybrid DNA formation to give the wild-type chromosome, with or without crossing-over. The letter P indicates that the allelic constitution of the homologue is + 2, implying that conversion to + has occurred at the proximal site. Q means the genotype is 1+, indicating distal conversion to +. R implies that reciprocal recombination has occurred between the alleles, the homologous chromosome

Table 8. *Classification of the* 14 *tetrad genotypes possible when a wild-type chromosome arises from a cross between alleles* 1 *and* 2, *and when crossing-over, if it occurs in the interval between the marker genes M/m and N/n, is confined to the same two chromatids as the conversion. The parental genotype is* (*M* 1 + *N*)/(*m* + 2 *n*). *It is assumed that hybrid DNA does not remain heterozygous at the mutant sites* (1 *and* 2), *but that conversion occurs in one or other direction*

Allele genotype of homologous chromosome which has undergone recombination with wild-type chromosome		Marker genotype of wild-type chromosome			
		A	B	C	D
		M ++ N	m ++ n	m ++ N	M ++ n
		M 1+ N	M 1+ N	M 1+ N	M 1+ N
P	+2	M ++ N	M +2 N	M +2 n	M ++ n
		m +2 n	m ++ n	m ++ N	m +2 N
		m +2 n	m +2 n	m +2 n	m +2 n
		M 1+ N	M 1+ N	M 1+ N	M 1+ N
Q	1+	M ++ N	M 1+ N	M 1+ n	M ++ n
		m 1+ n	m ++ n	m ++ N	m 1+ N
		m +2 n	m +2 n	m +2 n	m +2 n
		M 1+ N	M 1+ N	M 1+ N	M 1+ N
R	12	M ++ N	M 12 N	M 12 n	M ++ n
		m 12 n	m ++ n	m ++ N	m 12 N
		m +2 n	m +2 n	m +2 n	m +2 n
		M 1+ N		M 1+ N	
S	++	M ++ N		M ++ n	
		m ++ n		m ++ N	
		m +2 n		m +2 n	

carrying both mutations (12). The genotypes indicated by the letter S have a second wild-type chromosome (++), implying that conversion to + has occurred at the site of both alleles.

In Tables 6 and 7, the expected frequency of each genotype is given in terms of the frequency of occurrence of hybrid DNA at one or other or both sites in one or both chromatids. These expectations have been calculated on the assumption that conversion takes place in each direction (to wild-type and to mutant) with equal

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frequency. This assumption is clearly not justified in some individual instances (see section 2), but it might be expected to apply to the average of a number of mutations.

The letters v and w refer to the occurrence of hybrid DNA in only one chromatid at the mutant sites, v being its frequency at one site alone, and w at both. The letters x, y and z refer to the occurrence of hybrid DNA in both chromatids, x being its frequency at one site in each, y at both sites in one chromatid and one site in the other, and z at both sites in both. The suffixes c, n, p and q refer to the source of the hybrid DNA (proximal or distal), and whether or not there has been crossing-over: c stands for crossover, n for non-crossover, p for proximal, and q for distal source. The letters v to z with two-letter suffixes, for example, v_{pn}, represent individual frequencies of the appropriate category of hybrid DNA. With single-letter suffixes, for example, v_n, they represent the appropriate total; and v to z without suffixes represent the grand totals. Thus $v = v_n + v_c$, $v_n = v_{pn} + v_{qn}$ and $v_c = v_{pc} + v_{qc}$, and similarly for w, x, y and z.

To illustrate how the expected frequencies have been determined, v_{pn} represents the frequency of occurrence of non-crossover hybrid DNA at the proximal site alone in one chromatid. In half the instances this chromatid will have the genotype $M\ ^{+}/_{1} + N$. The chromatid with which it has undergone recombination will have the parental genotype m + 2 n. Conversion to + will then give genotype no. 3 and conversion to 1 will give genotype no. 1. In the other half of the occurrences of non-crossover hybrid DNA at the proximal site, the genotype of the chromatid will be $m\ ^{+}/_{1}\ 2\,n$, and its partner will be M 1 + N. Conversion to + and to 1 will give genotypes nos. 1 and 4 respectively. Hence, the expected frequencies of genotypes nos. 1, 3 and 4 are $\frac{1}{2}v_{pn}$, $\frac{1}{4}v_{pn}$, and $\frac{1}{4}v_{pn}$ respectively, as indicated in the column headed v_{pn} of Table 6.

The frequencies relating to the tetrads with wild-type spores are indicated by asterisks in Tables 6 and 7, and their totals are given at the bottom of each table. The wild-type tetrads in the R and S genotypes require hybrid DNA to have occurred in both chromatids at one or other or both sites, whereas the P and Q tetrads, other than AQ and BP, do not. The theory demands that there shall be hybrid DNA in both chromatids in order that a crossover may form between them, but the hybrid region need not extend for an equal distance in each chromatid from the primary breakage-points. A similar argument applies to non-crossover hybrid DNA, which moreover might sometimes be confined to one chromatid. From Tables 6 and 7,

$$P + Q = \tfrac{1}{4}v + \tfrac{1}{4}w + \tfrac{1}{4}x + \tfrac{1}{4}y + \tfrac{1}{4}z,$$

and

$$R + 2S = \tfrac{1}{4}x + \tfrac{1}{4}y + \tfrac{1}{4}z,$$

where P, Q, R and S represent the frequency of occurrence of the tetrads of corresponding class. From these equations, it is evident that the total frequency of the P and Q tetrads is made up of $\frac{1}{4}(v + w)$ from hybrid DNA in only one chromatid at the site nearest the source, that is, nearest to the points of primary breakage; and $\frac{1}{4}(x + y + z)$ from hybrid DNA in both chromatids at this site. This means that,

whatever the pattern of distribution of hybrid DNA as regards the sites, hybrid DNA at the site nearest to the linkage point in only one chromatid and hybrid DNA at this site in both chromatids will contribute equally to the P and Q tetrads. On the other hand, hybrid DNA in one chromatid (v, w) will make no contribution to the R and S tetrads, whereas hybrid DNA in both chromatids (x, y, z) will contribute equally to P + Q and to R + 2S. It follows that the expression

$$\frac{R+2S}{P+Q}$$

is a measure of the relative frequencies with which hybrid DNA occurs in one chromatid or in both at the site nearest the linkage point. In the discussion below, this quantity is called the *strand coefficient* for the near site, using strand in the sense of chromatid. If hybrid DNA always occurs in only one strand at the sites, R and S will be zero and the coefficient will be zero. On the other hand, if hybrid DNA never occurs in only one strand at the site nearest the linkage point, v and w will be zero, and the value of the coefficient will be 1. If hybrid DNA occurs equally often in one chromatid and in two at the nearer site, $v+w = x+y+z$, and the coefficient will be 0·5.

(ii) *Results from tetrad analyses with outside markers*

Tables 9–13 show the data that are available from tetrad analysis in crosses between allelic mutants or mutants in apparently neighbouring cistrons, with outside markers on both sides. All the data refer to *Neurospora crassa*. The classification corresponds to that in Table 8.

Table 9. *Classification of* 6 *asci of* Neurospora crassa *showing recombination between mutants in apparently neighbouring cistrons, from the data of Giles, de Serres & Barbour* (1957) *for mutant A*2 *of* ad-3*A crossed with mutant B*35203 *of* ad-3*B. The recombination frequencies between* ad-3 *and the proximal and distal marker genes were* 2% *and* 3% *respectively*

	A	B	C	D
P	0	0	0	0
Q	0	0	0	0
R	0	0	5 + 1*	0
S	0		0	

* Another crossover occurred distal to the *ad*-3 cistrons, and involving the double mutant (*ad*-3A *ad*-3B) chromatid, but not the prototroph chromatid (that is, a three-strand double crossover).

Table 9 gives the results of a cross between mutants in the adjacent cistrons *ad*-3A and *ad*-3B studied by Giles, de Serres & Barbour (1957). Six asci with prototroph spores were found in a total of 646. All belong to class CR, implying crossing-over for the outside markers (C) and reciprocal recombination between the mutant sites (R). One of the six had a second crossover in the marked interval. As mentioned in section 3 (*a*) (v), it appears from these results that recombination between the mutant

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sites concerned (A2 and B35203) is taking place through the occurrence of entire crossover events in the interval between the sites, such that the hybrid DNA had not reached either site, with the result that no conversion took place. It is evident from Fig. 5(a) that about 2% of the prototroph spores from this cross are arising through non-crossover conversion, but at this frequency none would be expected in the ascus sample studied.

Mitchell (1955) was the first to study allelic recombination by tetrad analysis,

Table 10. *Classification of* 4 *asci of* Neurospora crassa *showing recombination between the alleles* pdxp *and* pdx *at the* pdx-1 *locus from the data of Mitchell* (1955). *The recombination frequencies between* pdx-1 *and the proximal and distal marker genes were* 2% *and* 4% *respectively*

	A	B	C	D
P	2	0	0	0
Q	1	0	0	0
R	0	0	0	0
S	0		1	

using outside markers. The 4 asci with prototroph spores which she found in a total sample of 585 asci from a cross between alleles at the *pdx*-1 locus are classified in Table 10. A strikingly different pattern of recombination is revealed from that shown by the non-allelic cross of Table 9. Reference to Tables 6 and 7 indicates that

Table 11. *Classification of* 2 *asci of* Neurospora crassa *showing recombination between alleles nos.* 43 *and* 17 *of* pyr-3 *from the data of Suyama, Munkres & Woodward* (1959). *The recombination frequencies between* pyr-3 *and the proximal and distal marker genes were* 1% *and* 16% *respectively*

	A	B	C	D
P	0	0	0	0
Q	1	1	0	0
R	0	0	0	0
S	0		0	

two of the asci (those in classes AQ and CDS) require hybrid DNA to have occurred in both chromatids at the site nearest the primary breakage-point.

The 2 prototroph asci found by Suyama, Munkres & Woodward (1959) from a cross between *pyr*-3 alleles are classified in Table 11. Here also there is an ascus in class AQ, implying that the site nearest the source must have been in hybrid DNA in both chromatids.

Table 12 shows the classification of 16 asci from crosses involving alleles nos. 5 and 3 of *pan*-2 from the data of Case & Giles (1958, 1959) and Cooke (unpublished). In addition to the asci shown in the table, Case & Giles found a further 5 belonging to

Table 12. *Classification of* 16 *asci of* Neurospora crassa *showing recombination between alleles nos.* 5 *and* 3 *at the* pan-2 *locus. The upper figure for each class shows the data of Case & Giles* (1958), *the second figure those of Case & Giles* (1959), *and the third figure those of Cooke* (*unpublished*). *The percentages refer to the total data. The data of Case & Giles* (1959) *refer to a cross between the double mutant for the alleles and a pantothenic-independent strain. In order to include these in the table, it has been necessary to transpose the distal mutant* (*no.* 3) *and its normal allele* (+). *The recombination frequencies between* pan-2 *and the proximal and distal marker genes were* 2% *and* 8% *respectively. In addition to the asci classified in the table, Case & Giles* (1958) *found* 3 *in class* 14 *of Table* 6, *and* (1959) 1 *in class* 14 *and* 1 *in class* 30 (*Table* 7). *These* 5 *asci had no recombination between the alleles, but at both sites conversion had occurred in the direction of one of the parental strains*

	A	B	C	D
P	0 1 + 1‡ 0 12·5%	1† 1 0 12·5%	3 0 1 25%	0 0 1 6%
Q	0 0 0 0%	1 + 1* 1 0 19%	0 1 0 6%	0 1 0 6%
R	0 0 0 0%	0 0 0 0%	2 0 0 12·5%	0 0 0 0%
S		0 0 0 0%		0 0 0 0%

‡ Both chromatids from one parent showed conversion to + at the site of the proximal allele (no. 5).

† A crossover had occurred not involving the wild-type chromatid, but between the two chromatids mutant for the distal allele (no. 3).

* A crossover had occurred proximal to the alleles and not involving the wild-type chromatid.

classes 14 and 30 of Tables 6 and 7, that is, with conversion at both sites in the direction of the same parental strain. As with the data in Tables 10 and 11, certain of the asci require that hybrid DNA shall have formed in both chromatids at the site nearest the linkage point.

The more extensive data of Stadler (1959*a*) and Stadler & Towe (1963) for cysteine mutants, shown in Table 13, reveal a rather different pattern of recombination from

the other *N. crassa* mutants for which tetrad information is available. The data comprise 3 asci from a cross between alleles *t* and *c*, 6 from a cross between alleles 9 and 64, 5 from 38 × 64, 6 from 38 × 4, and 28 from 64 × 17. Of the total of 48 asci, all except one (in class AQ) can be explained most readily on the assumption that hybrid DNA occurred in only one chromatid at the sites. These are the only tetrad data which are sufficiently extensive for the frequency of the various patterns of

Table 13. *Classification of* 48 *asci of* Neurospora crassa *showing recombination between alleles at the* cys *locus from the data of Stadler* (1959a) *and Stadler & Towe* (1963). *Within each class the figures refer, in descending order, to alleles* t *and* c (*total* 3 *asci*), *nos.* 9 *and* 64 (6 *asci*), *nos.* 38 *and* 64 (5 *asci*), *nos.* 38 *and* 4 (6 *asci*), *and nos.* 64 *and* 17 (28 *asci*). *The percentages refer to the total data. The recombination frequencies between* cys *and the proximal and distal marker genes were* 6% *and* 2% *respectively for alleles* t *and* c, *and* 9% *and* 6% *respectively for the other pairs of alleles*

	A	B	C	D
	2	0	0	0
	1	0	0	2
P	1	0	1	0
	1	0	0	0
	8* + 1†	0	1	2
	29%	0%	4%	8%
	0	0	1	0
	0	1	2	0
	0	1	2	0
Q	0	2 + 1†	2	0
	1	8 + 1‡	6	0
	2%	29%	27%	0%
R	None	None	None	None
S	None		None	

* One of these 8 asci showed post-meiotic segregation for allele no. 64 (1+:7 mutant spores).

† A crossover occurred distal to the alleles and not involving the wild-type chromatid.

‡ A crossover occurred proximal to the alleles and not involving the wild-type chromatid.

hybrid DNA at the sites to be estimated with any precision. On the basis of the expressions in Table 6 and 7 for the expected frequencies of the various alternatives, it is evident from the lack of R and S tetrads (Table 13) that x, y and z must be small, or in other words, as indicated above, that hybrid DNA rarely occurs in both chromatids at the sites of the mutations. It follows that, for the non-crossover tetrads,

$$\text{AP} = \tfrac{1}{4}v_{pn} + \tfrac{1}{8}w_n = 14,$$

and

$$\text{BQ} = \tfrac{1}{4}v_{pn} + \tfrac{1}{8}w_n = 14.$$

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From these expressions, the values of v_{pn}, v_{qn} and w_n cannot be obtained, but it is clear that non-crossover hybrid DNA in one chromatid is occurring equally often at the proximal site alone (v_{pn}) and at the distal site alone (v_{qn}). The prototroph spore selection data in Fig. 3 (iii) show that, with a larger sample, an excess of class B over class A, and hence of v_{qn} over v_{pn}, is to be expected for several of the pairs of alleles crossed.

From the crossover tetrads, more information can be obtained, since

$$\begin{aligned} \text{CP} &= \tfrac{1}{4}v_{pc} + \tfrac{1}{8}w_{pc} = 2, \\ \text{DQ} &= \tfrac{1}{8}w_{pc} = 0, \\ \text{CQ} &= \tfrac{1}{4}v_{qc} + \tfrac{1}{8}w_{qc} = 13, \\ \text{and} \quad \text{DP} &= \tfrac{1}{8}w_{qc} = 4. \end{aligned}$$

Hence $v_{pc} = 8$, $v_{qc} = 36$, $w_{pc} = 0$ and $w_{qc} = 32$. In other words, about 90% of the crossover hybrid DNA is of distal origin extending to one site or to both about equally often, while the 10% that is of proximal origin extends only to one site and never to two.

These conclusions are only tentative because

(a) 48 asci is not enough to draw firm conclusions,
(b) the data refer to 5 pairs of alleles which haploid prototroph selection has shown to give different patterns of recombination (Fig. 3(b) and (c)), but which nevertheless have been pooled in the above analysis in order to provide sufficient data, and
(c) the assumption that conversion frequencies in each direction are equal may not be true.

Nevertheless, the data point to a much stronger distal polarity in crossover than that in non-crossover hybrid DNA, such as would be expected if in crossing-over the nucleotide chains more often became dissociated on one side of a primary break than on the other side, or alternatively if DNA synthesis were more often restricted to one side than the other. In this instance, it is presumed that at the linkage points on both sides of *cys*, crossover hybrid DNA forms on the proximal sides more often than on the distal sides (cf. Hastings & Whitehouse, 1964). The distal polarity in non-crossover hybrid DNA (Fig. 3 (iii)) presumably implies that the mutant sites under study are nearer to the distal than to the proximal linkage point, and the difference in site coefficient between the crossover hybrid DNA of proximal and of distal origin could have the same cause. Another possibility, referred to in section 4 (ii), is that dissociation-cycles are initiated at one linkage point more often than another.

(iii) *Results from diploid analyses*

Information about recombination between alleles has been obtained by several authors from the analysis of diploid prototrophs following mitotic recombination in a diploid heterozygous for recessive nutritional alleles. The information obtained from such diploids is necessarily less complete than from tetrad analysis.

The 20 genotypes that are possible for such a diploid prototroph, when recombination occurs at the four-strand stage but is confined to two of the strands in the interval between the outside markers, are classified in Table 14. The classification is in terms of the outside marker combination (A–D) in the prototroph strand, and the allele combination (P–S) in the strand with which it has undergone recombination, and so is essentially the same as the tetrad classification in Table 8. The

Table 14. *Classification of the* 20 *diploid genotypes possible when a wild-type chromosome arises by mitotic recombination in a diploid heterozygous for alleles* 1 *and* 2, *and when crossing-over, if it occurs in the interval between the marker genes M/m and N/n, is confined to the same two chromatids as the conversion. The parental genotype is* (*M* 1+ *N*)/(*m* +2 *n*). *It is assumed that hybrid DNA does not remain heterozygous at the mutant series* (1 *and* 2), *but that conversion occurs in one or other direction*

Genotype of homologue of wild-type chromosome	Marker genotype of wild-type chromosome			
	A	B	C	D
	M ++ N	m ++ n	m ++ N	M ++ n
P +2	M ++ N m +2 n	M +2 N m ++ n	M +2 n m ++ N	M ++ n m +2 N
Parental (M/m)		M 1+ N m ++ n	M 1+ N m ++ N	M ++ n m +2 n
Q 1+	M ++ N m 1+ n		M 1+ n m ++ N	M ++ n m 1+ N
R 12	M ++ N m 12 n	M 12 N m ++ n	M 12 n m ++ N	M ++ n m 12 N
Parental (M/M or m/m)	M 1+ N M ++ N	m ++ n m +2 n	m ++ N m +2 n	M 1+ N M ++ n
S ++	M ++ N m ++ n		M ++ n m ++ N	

differences arise merely from the fact that in the diploid only two of the four chromatids that were present at the time of recombination are recovered. Some of the diploids will consist of the prototroph strand and a parental strand, that is, one which has not participated in recombination. Such diploids are classified in the table according to whether they are heterozygous or homozygous for the proximal outside marker. Homozygosity for this marker is likely to be rare, since it requires crossing over to have occurred proximal to it, in addition to the recombination at the

nutritional locus. As is indicated in the table, diploids of classes AP and BQ cannot be distinguished from diploids with the same prototroph strand and one of the parental strands. The reason for this is apparent by reference to Table 8 where it can be seen that tetrads AP and BQ each have two of the four products of meiosis alike, whereas the other 12 tetrad genotypes have all four different from one another.

Data from the analysis of diploid prototrophs obtained from diploids heterozygous for a pair of nutritional alleles are summarized in Tables 15–18, where the classification corresponds to that in Table 14. In Table 15 are given the results obtained

Table 15. *Data of Pritchard* (1955, 1960a) *from the analysis of mitotic recombination between alleles at the* ad-8 *locus in diploid* Aspergillus nidulans. *In each class, the upper figure refers to alleles nos.* 20 *and* 8 (11 *prototrophs analysed*) *and the lower to alleles nos.* 16 *and* 8 (41 *prototrophs*). *The percentages refer to the total data. The outside markers used in classifying the prototrophs were* y (*yellow conidia*) *and* bi-1 (*biotin* 1), *which on the meiotic map show* 0·2% *and* 6% *recombination with* ad-8 *on the proximal and distal sides respectively. Since* y *may be a neighbouring cistron to* ad-8, *it may be unreliable as an outside marker. Re-classifying the prototrophs using the more distant proximal marker* paba-1 *has the effect of transferring the two diploids in the 'Parental (M/M or m/m)' classes to the B and C 'Parental (M/m)' classes*

	A	B	C	D
P		0 0 0%	0 0 0%	0 0 0%
	2 4 11%			
Parental (M/m)			3 16 37%	1 0 2%
		1 5 11%		
	0		1	0
Q	1 + 1*		2	0
	4%		6%	0%
	0	0	2 + 1*	0
R	0	1	8	0
	0%	2%	21%	0%
	0	0	0	0
Parental (M/M	1	0	0	1
or m/m)	2%	0%	0%	2%
		0		0
S		1		0
		2%		0%

* Homozygous for the distal outside marker.

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by Pritchard (1955, 1960*a*) for alleles at the *ad*-8 locus in *Aspergillus nidulans*. The upper figure for each class refers to 11 prototrophs analysed from a heterozygote for alleles nos. 20 and 8, and the lower to 41 prototrophs from a heterozygote of alleles nos. 16 and 8. The percentages refer to the total data (52 prototrophs). Table 16 gives the results obtained by Martin-Smith (1961) for alleles at the *ad*-9 locus in *A. nidulans*. The figures refer to four different pairs of alleles, as indicated above the table. The percentages relate to the total data (79 fully analysed prototrophs, and a further 47 incompletely analysed). The prototrophs studied by Martin-Smith were a selected sample in which the outside markers showed the dominant phenotype. In consequence, there are none in the six classes with a parental second chromosome, since these would have one or both outside markers homozygous. (A single such diploid which was analysed because it happened to be heterozygous for one outside marker and homozygous for the dominant allele of the other, has been omitted from the table.)

In Table 17, the extensive data of Putrament (1964) for alleles at the *paba*-1 locus in *A. nidulans* are given. Reading downwards within each class of the table, the first two figures refer to alleles nos. 5 and 2 (128 prototrophs analysed), the second two to alleles nos. 5 and 15 (106 prototrophs), the third two to alleles nos. 5 and 18 (94 prototrophs), and the seventh figure to alleles nos. 2 and 19 (65 prototrophs). The percentages refer to the total data (393 prototrophs). In classifying her data, *ad*-9 was used as the proximal outside marker. From evidence given in section 4 (ii), this marker may adjoin *paba*-1. It is therefore possible that in selecting for recombinants at the *paba*-1 locus one may also be selecting to some extent for conversion at the *ad*-9 site. This possibility can be tested, since a more distant outside marker, proline 1 (*pro*-1), which at meiosis shows about 8% recombination with *ad*-9 and *paba*-1, was also used in the experiments. When the 393 prototrophs are re-classified using *pro*-1 as proximal outside marker and ignoring *ad*-9, it is found that all except 21 of them are unaffected, but 16 out of the 22 diploids classified in Table 17 as 'Parental (M/M or m/m)' are transferred to classes in the upper half of the table. It seems probable that many of these, notably those which are heterozygous for the more distant outside markers and with parental combinations of them (the 5 in class no. 23 and the 4 in class no. 29 of Putrament's Table 1), owe their homozygosity at the *ad*-9 locus to conversion. This would mean that conversion had occurred in both *ad*-9 and *paba*-1 in the formation of these diploids, in keeping with the hypothesis that non-crossover hybrid DNA extends in both directions from the linkage point.

Comparison of the *A. nidulans* mitotic data in Tables 15–17 with the *Neurospora crassa* meiotic data for allelic crosses in Tables 10–13 shows a general similarity in the relative frequencies of the various tetrad genotypes, when allowance is made for the diversity that is evident within both species. The occurrence of R and S genotypes in *A. nidulans* resembles the *N. crassa* findings with *pdx*-1 and *pan*-2 (Tables 10 and 12) rather than with *cys* (Table 13). The *A. nidulans* markers that have been studied evidently have a comparatively high strand coefficient, but estimation of its value is complicated by the high frequency of diploids of CR genotype found in some

Table 16. *Data of Martin-Smith* (1961) *for mitotic recombination between alleles at the* ad-9 *locus in* Aspergillus nidulans. *The diploid adenine prototrophs analysed were a selected sample in which the outside markers showed the dominant phenotype. In consequence, diploids homozygous for the outside markers were rarely analysed and are omitted from the table. Reading downwards within each class the figures refer as follows: alleles nos.* 33 *and* 13 (16 *prototrophs analysed*); *alleles nos.* 13 *and* 9 (16 *prototrophs analysed plus* 30 *incompletely analysed*); *alleles nos.* 13 *and* 32 (32 *analysed plus* 17 *incompletely*); *and alleles nos.* 13 *and* 15 (15 *analysed*). *The percentages refer to the total data* (79 *completely analysed prototrophs and* 47 *incompletely*), *but, owing to the omission of* 6 *classes, are not directly comparable with the percentages in Tables* 15, 17 *and* 18

	A	B	C	D
P	3 8 + 10 10 + ?* 3 31%	0 1 + ?† 0 + 0 0 1%	0 1 + ?§ 1 + ?′ 0 3%	1 0 + ?‡ 0 + 0 0 1%
Parental (M/m)		7 4 + ?† 13 + 5	—	—
Q	2 0 + 1 0 + ?* 0 3%	6 36%	1 1 + ?§ 1 + ?′ 2 6%	1 0 + ?‡ 1 + 0 0 2%
R	0 0 + 1 1 + ?* 0 2%	0 1 + ?† 1 + 0 0 2%	1 0 + ?§ 4 + ?′ 4 11%	0 0 + ?‡ 0 + 0 0 0%
Parental (M/M or m/m)	—	—	—	—
S	0 0 + 1 0 + 0 0 1%		0 0 + 0 0 + 1 0 1%	

* Incompletely analysed: AP + AQ + AR = 4
† ,, ,, BP + BQ + BR = 11
§ ,, ,, CP + CQ + CR = 4
‡ ,, ,, DP + DQ + DR = 2
′ ,, ,, CP + CQ + CR = 7

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Table 17. *Data of Putrament* (1964) *for mitotic recombination between alleles at the* paba-1 *locus in* Aspergillus nidulans. *Reading downwards within each class the figures refer as follows: alleles nos.* 5 *and* 2, *cross no. D*15 (64 *prototrophs analysed*); *the same alleles, cross no. D*14 (64 *prototrophs*); *alleles nos.* 5 *and* 15, *cross no D*7 (55 *prototrophs*); *the same alleles, cross no. D*5 (51 *prototrophs*); *alleles nos.* 5 *and* 18, *cross no. D*3 (50 *prototrophs*); *the same alleles, cross no. D*6 (44 *prototrophs*); *and alleles nos.* 2 *and* 19, *cross no. D*20 (65 *prototrophs*). *This is the same sequence as the data are given in Fig.* 6. *The percentages refer to the total data* (393 *prototrophs*). *The classification has been made using the apparently neighbouring cistron* ad-9 (*allele no.* 9) *as proximal outside marker. When the* paba-1 *prototrophs are re-classified using the more distant gene* pro-2 *as proximal outside marker, a majority of those in the 'Parental (M/M or m/m)' classes are transferred elsewhere* (*see section* 3 (b) (*iii*) *of the text*)

	A	B	C	D
P		0	2	2
		0	2	2
		0	1	0
		1*	1	0
		0	1	2
	31	0	0	0
	17 + 3*	0	1	0
	26		2%	1·5%
	24	0·3%		
Parental (M/m)	18 + 2*		2	7
	8		10	6
	11		4	2
			6	3
	35·6%	14 + 1*	6	1
		10 + 1*	2	7
		17	12	0
		11 + 1*	10·7%	6·6%
		5		
Q	0	16 + 2*	0	2 + 1‡
	1†	26	3	0
	0		0	0
	0	26·5%	1	0
	0		0	1
	0		1	1
	1*		5	0
	0·5%		2·5%	1·3%
R	0	0	1	0
	0	0	0	3
	0	1*	2	0
	0	0	2	0
	0	1*	5	0
	0	0	3	0
	0	0	1	1
	0%	0·5%	3·6%	1%

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Table 17.—*Continued*

	A	B	C	D
	0	0	1	0
	1′	1+1′	0	2
	0	0	0	1
	0	0	1	0
Parental (M/M	1	0	2	2
or m/m)	1	1	1	0
	1′	0	5	0
	1%	0·8%	2·5%	1·3%

	A/B	A/C	C/D
	0	0	0
	1	0	0
	0	0	1
	0	0	0
S	0	1*	2
	0	1*	0
	1	0	0
	0·5%	0·5%	0·8%

* Homozygous for the distal outside marker.
† Homozygous for the proximal outside marker.
‡ Homozygous for both outside markers.
′ Heterozygous for the distal outside marker.

of the data. It is evident from Table 7 that CR is not expected to exceed the sum of CP and CQ, yet in Pritchard's data for *ad*-8 it accounts for 11 of the prototrophs compared with 3 in CP + CQ, and in Martin-Smith's data for alleles nos. 13 and 32, and nos. 13 and 15 of *ad*-9 there were altogether 8 diploids in CR but only 4 in CP + CQ. Similarly, in Putrament's data for alleles 5 and 18 of *paba*-1, 8 of the prototrophs were in CR compared with only 2 in CP + CQ. The remainder of Putrament's data do not show this feature. The significance of this excess of CR genotypes is discussed in section 4 (vi).

The strand and site coefficients in crossover hybrid DNA for the *paba*-1 allele pairs 5 and 2, 5 and 15, and 2 and 19 have been estimated from Putrament's data in the following way. From Table 7, it is evident that

$$v_c(= v_{pc} + v_{qc}) = 4[(CP + CQ + DR) - (CR + DP + DQ)],$$
$$w_c(= w_{pc} + w_{qc}) = 8[(DP + DQ) - (DR + CDS)],$$
$$x_c(= x_{pc} + x_{qc}) = 4(CR + DR - 2CDS),$$

and $$y_c + z_c(= y_{pc} + y_{qc} + z_c) = 16CDS,$$

where CP, CQ, etc. stand for the frequencies of these classes. Taking Putrament's total data for alleles 5 and 2, 5 and 15, and 2 and 19, given in Table 17, CP = 7, DP = 4, CQ = 9, DQ = 2, CR = 6, DR = 4 and CDS = 1. A prototroph in DQ which was homozygous for both outside markers has been omitted, since in its formation at

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least three chromatids must have been involved in recombination in the interval between the outside markers. Such multiple-stranded events are discussed in section 4 (vii). Applying the expressions above to the observed frequencies of the various classes gives $v_c = 32$, $w_c = 8$, $x_c = 32$, and $y_c + z_c = 16$. Since v and w represent only one chromatid in hybrid DNA at the nearer site, while x, y and z represent two, the strand coefficient is given by $x + y + z$ as a fraction of the total, or 55%. The other method of estimating this, using the expression $(R + 2S)/(P + Q)$, gives the same value.

Within the single chromatid conversions, v represents hybrid DNA extending to only one site, and w to both, hence the site coefficient is given by w as a fraction of the total, or 20%. Similarly, within the double chromatid conversions, x represents hybrid DNA extending to only one site, and y and z to both (at least in one chromatid). Hence, the site coefficient is given by $y + z$ as a fraction of the total, or 33%. No attempt has been made to estimate the frequencies of proximal and distal events within each category (v, w, x, y) as the data are hardly adequate for this. Indeed, the estimates of the strand and site coefficients are only tentative, because the data referring to several different pairs of alleles have been pooled, although they clearly do not all behave alike, and it has been assumed that conversion frequencies to wild-type and to mutant are equal. An analysis of the strand coefficient in non-crossover hybrid DNA has not been attempted since it would be necessary to make assumptions about the sizes of the AP and BQ classes.

In Table 18, the data of Kakar (1963) for a pair of alleles at the isoleucine 1 (*is*-1) locus in *Saccharomyces cerevisiae* are given. The upper figure for each class in the table shows the numbers of isoleucine prototrophs obtained from two crosses in which tryptophan requirement (the distal outside marker) was in coupling with the distal allele (cross I—total 43 diploids). The second figure for each class shows the numbers obtained from the reciprocal cross in which the outside markers were reversed with respect to the alleles, so that the tryptophan requirement was in repulsion with the distal allele (cross II—total 26 diploids). The data from these crosses are biased because among the isoleucine prototrophs obtained, a larger proportion of those requiring tryptophan were analysed than of the tryptophan-independents. Moreover, there was evidence that there had been inadvertent selection in favour of tryptophan-independence in obtaining the isoleucine prototrophs. The effects of this non-randomness with respect to the distal outside marker have been eliminated in the following way. Out of 29 tryptophan-independent isoleucine prototrophs from cross I, Kakar found that 10 or 34·5% were homozygous for tryptophan-independence, while the corresponding figures from cross II were 2 out of 13 or 15·4%. If r, s and t represent the frequencies of occurrence of the 3 tryptophan genotypes + +, + *tr* and *tr tr* respectively from cross I, their expected frequencies from cross II will be t, s and r respectively. Hence $r/(r + s) = 0{\cdot}345$ and $t/(s + t) = 0{\cdot}154$, whence $r = 0{\cdot}308$, $s = 0{\cdot}586$ and $t = 0{\cdot}106$. The total numbers of r, s and t prototrophs analysed from the two crosses were 23, 30 and 16 respectively, and from the above calculation the expected numbers are 21·2, 40·5 and 7·3. The r, s and t frequencies for each class have, therefore, been multiplied by 21·2/23,

40·5/30 and 7·3/16, respectively, with the results shown by the third figure in each class in the table. The percentages quoted are based on these corrected figures.

Kakar's data for *S. cerevisiae* show a striking difference from the *A. nidulans* data by the absence of any R class diploids. Roman & Jacob (1959), using the same alleles, obtained 5 diploids in class S and none in class R out of a total sample of 52. Wilkie

Table 18. *Data of Kakar* (1963) *for mitotic recombination between alleles nos.* 2 *and* 1 *at the isoleucine* 1 (is-1) *locus in* Saccharomyces cerevisiae. *Reading downwards within each class, the figures refer to cross I, to cross II, and to the corrected total, to which the percentages also refer* (*see section* 3 (b) (*iii*) *of the text*)

	A	B	C	D
P	10 + 2* 4 + 1* 18·9 + 2·7* 31%	3* 0 1·4* 2%	2 1* 2·7 + 0·9* 5%	0 0 0 0%
Parental (M/m)		4 + 1* 7 + 1* 14·9 + 1·0* 23%	5 11 14·7 21%	8 0 3·6 5%
Q	3* 0 2·8* 4%		2 0 2·7 4%	1 + 2* 0 1·3 + 0·9* 3%
R	None	None	None	None
Parental (M/M or m/m)	None	None	None	None

	A/B	A/C	C/D
S	0 0 0 0%	0 1* 0·5* 1%	0 0 0 0%

* Homozygous for the distal outside marker.

& Lewis (1963) suggest that Roman and Jacob's S class diploids arose through crossing-over occurring at the two-strand stage, but this idea obtains no support from Kakar's data, where the single S class diploid evidently arose following crossing-over between one pair of chromatids, and non-reciprocal recombination with a third chromatid. Kakar's data resemble those of Stadler (1959*a*) and Stadler & Towe (1963) for *cys* in *N. crassa* (Table 13), and it is evident that, like the *cys* alleles, the yeast *is*-1 alleles have a very low strand coefficient, the hybrid DNA being practically confined to one chromatid even at the site nearest to the linkage point where

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dissociation was initiated. From Table 18, omitting the diploids homozygous for the distal outside marker, since these require at least three chromatids to have participated in recombination, CP = 2·7, CQ = 2·7, DQ = 1·3, and the other C and D classes are zero. Using the expressions given earlier in this section, $v_c = 16{\cdot}4$, $w_c = 10{\cdot}4$, $x_c = 0$, $y_c = 0$ and $z_c = 0$. Hence, in crossover hybrid DNA, the strand coefficient is zero, and the site coefficient is $10{\cdot}4/26{\cdot}8 = 39\%$. In other words, the crossover hybrid DNA is confined to one strand even at the nearer site, and rather more often than not it fails to reach the farther site.

(iv) *Results from tetrad analyses without outside markers*

Lissouba, Mousseau, Rizet & Rossignol (1962) have obtained extensive data on allelic recombination in *Ascobolus immersus* using spore colour mutants. Marker genes are not yet available, so the tetrads with wild-type recombinants, that is, asci with 1 or 2 pairs of black spores in a cross between white-spored mutants, can be classified only in terms of the allele genotype of the homologous recombinant chromosome (P, Q, R and S). In the absence of information about the position of the centromere, P is used in the discussion below for conversion at the left-hand site on the map, and Q for conversion at the right-hand site. As before, R implies reciprocal recombination, and S means a second wild-type chromosome, or in other words, a 4:4 ratio of black: white spores in the ascus instead of the 2:6 of P, Q and R.

The data of Lissouba *et al.* refer to three series called 19, 46 and 75. In the absence of linked markers, the map of the mutants based on the frequency of wild-type spores cannot be confirmed by means of the relative sizes of classes C and D. An alternative is provided in the relative frequencies of classes P and Q, but this is unreliable for mapping purposes, since if polarity is present it is not necessarily constant in direction. Thus, Calef's and Martin-Smith's data for *ad*-9 in *Aspergillus nidulans* in Fig. 6 (iii) show a progressive change in the relative frequencies of classes A and B, implying a similar change in P and Q.

In series 46, where polarity is extremely well-marked in all pairs of mutants tested, the map of the mutants based on the frequencies of wild-type recombinants, which range from 13 to 520 per 10^5 progeny, agrees well with the map based on polarity. The majority of these series 46 alleles, when crossed two at a time, give only Q tetrads, but mutant no. 277 as right-hand marker gives about 60% Q, 20% R, 0·5% S and about 20% of asci showing a 1:7 ratio of black: white spores, implying post-meiotic segregation. When the recombinants are confined to the Q class, it appears that hybrid DNA is restricted to one chromatid and to one site. Mutant no. 277 evidently raises the strand coefficient from zero to approximately $\frac{1}{3}$. Contrary to the map position given by Lissouba *et al.* (1962), mutant no. 277 is regarded, on the evidence of their data, as being to the left of no. 137, and the series 46 mutants are evidently all in one polaron. It is an interesting question whether the persistence of heterozygosity in the DNA through meiosis at the site of mutant no. 277, giving the 1:7 ratios, and the effect of this mutant on the strand coefficient, giving the R and S class asci, are causally related in some way. That mutant no. 60 in series 19 should also show post-meiotic segregation and give a high frequency of R class tetrads in

2-point crosses (Lissouba *et al.*, 1962; see below) suggests that the two phenomena are related.

The spore mutants of series 75 give wild-type recombinants with frequencies ranging from 260 to 1670 per 10^5 spores. These relatively high frequencies, and the frequent occurrence of asci showing reciprocal recombination (R class) in some of the crosses, support the idea that more than one polaron may be involved in this series. However, some P and Q class asci have been found in every cross and their aggregate frequency has always been 23% or more of the total recombinant asci. This implies that appreciable conversion is occurring at both sites in every cross, though not necessarily at both sites in any individual meiosis. Indeed, the rarity of S class asci (4:4 ratio of black:white spores) suggests that the site coefficient is low. It seems likely that accurate analysis of this series will not be possible until outside markers are available.

Analysis of the data for the series 19 mutants is also handicapped by the lack of outside markers. This series combines features shown by the other two. Some mutants when crossed give only Q class recombinants like series 46, others give low frequencies of P class and high frequencies of reciprocal recombinant asci (R class), like series 75. There are strong indications that the direction of polarity is reversed at one end of the series from the other, since a map of the sites based on polarity is in marked disagreement with a map based on the wild-type recombinant frequencies, which range from 1 to 350 per 10^5 spores. Thus, a map based on polarity would place mutants nos. 19, 60 and 55 in the sequence quoted, but the wild-type recombinant frequency of nos. 60 and 55 is $300/10^5$ while that of nos. 19 and 55 is only $37/10^5$, suggesting that no. 55 is nearer to no. 19 than to no. 60. Of 134 recombinant asci from a cross between mutants nos. 60 and 55, no less than 88, or 66%, were in class R. This represents a significant excess in this class, since R is not expected to exceed P + Q. It thus appears as if the excess of CR recombinants found with certain allele combinations in *A. nidulans* (see section 3 (*b*) (iii)) may also apply to certain combinations in *Ascobolus immersus*. The origin of these CR asci is discussed in section 4 (vi).

4. DISCUSSION

(i) *Introduction*

In sections 2 and 3 of this paper the quantitative predictions of the polaron hybrid DNA theory have been applied to the relevant recombination data. With the single exception of the excess of CR tetrads in some of the data, there has been agreement throughout between observation and theory wherever quantitative tests were possible. Thus, the numerical results for the relative frequencies of various classes of recombinants obtained by Kitani, Olive & El-Ani (1962) for the *gray* (*g*) mutant in *Sordaria fimicola*, by Stadler & Towe (1963) for the cysteine (*cys*) alleles in *Neurospora crassa*, and by Putrament (1964) for the *para*-aminobenzoic acid 1 (*paba*-1) alleles at mitotic recombination in *Aspergillus nidulans*, are predicted by the theory, when suitable values are chosen for the various parameters. A remarkable feature of the recombination data is the great variability that appears to exist not only between species, but also between genes within a species, and between alleles within

a gene. This variability concerns all aspects of the recombination process: in particular, the frequencies of crossover and non-crossover hybrid DNA, of proximal or distal origin, extending to one site or to two, on one chromatid or both, and at each site either remaining heterozygous or converting in one or other direction.

(ii) *Polarity*

The contrast between the behaviour of allelic and non-allelic mutations discussed in sections 3 (*a*) (v) and (vi) and 3 (*b*) (ii) supports the idea that the primary breakage-points of nucleotide chains of DNA, prior to recombination, are at fixed points outside or at the ends of the genes and not within them. The pattern of recombination within genes gives further support to this idea. The data of Siddiqi (1962) for *paba*-1 in *Aspergillus nidulans* (Fig. 6 (iii)) show a remarkable gradation in the relative frequencies of A and B class prototrophs as one moves from one end of the series of alleles to the other. At the proximal end there is a slight excess of class A over class B, implying that conversion is occurring at the proximal site rather more often than at the distal one. At the distal end of the series of alleles, on the other hand, the B class is markedly in excess of the A, implying that conversions occur more often at the distal than at the proximal site. These findings would be explained if the primary breaks associated with recombination can occur at either end of the gene, but not between the alleles, and if breaks at one end of the gene are for some reason favoured and therefore predominate over breaks at the other end, except near that end.

Essentially similar patterns can be seen in the data for a number of other genes, for example, *ad*-8 in *A. nidulans* (Fig. 7) studied by Pritchard (1955, 1960*a*, *b*), *ad*-9 in *A. nidulans* (Fig. 6) studied by Calef (1957) and Martin-Smith (1961), and *cys* in *Neurospora crassa* (Fig. 3(c)) studied by Stadler & Towe (1963). In *ad*-8 and *paba*-1 of *A. nidulans* and in *cys* of *N. crassa*, the distal polarity predominates, while the data of Martin-Smith (1961) for *ad*-9 of *A. nidulans* reveal predominantly proximal polarity in this gene.

A second pattern of intragenic polarity is shown by the extensive data of Murray (1960, 1963) for *me*-2 in *N. crassa* (Fig. 2). Here all the recombination appears to have been initiated from the distal end of the gene, since the horizontal lines in graph (iii) extend to the axis on the left-hand side, but not on the right. In other words, when the double-site contribution to conversion is deducted from the A and B values, B remains positive but no single-site A contribution is left. The double-site hybrid DNA is then likely also to be all of distal origin, since from whichever end it extends it is expected to contribute equally to the A and B classes.

The evidence just given for the existence of two different patterns of intragenic polarity in recombination refers to non-crossover events. It is possible that crossover hybrid DNA may show a different pattern from non-crossover. Indeed, evidence was presented in section 3 (*b*) (ii) from Stadler & Towe's data for *cys* alleles in *N. crassa* that distal polarity was stronger in crossover than in non-crossover hybrid DNA. Reasons will be given in section 4 (v) for believing that in *ad*-8 and *paba*-1 of *A. nidulans* the crossover hybrid DNA is predominantly or perhaps entirely of distal origin. This coincides with the predominant direction for non-

crossover hybrid DNA. In *me*-2 of *N. crassa* the crossover hybrid DNA is thought to be of distal origin like the non-crossover (see section 4 (v)). The data of Case & Giles for *pan*-2 in *N. crassa* (Fig. 4) show approximate equality between the A and B classes throughout the pairs of alleles studied. This result would be accounted for if non-crossover hybrid DNA reached the mutant sites about equally often from each end of the gene. Evidence will be given in section 4 (iv) for believing that crossover hybrid DNA is predominantly of proximal origin in this gene.

In Table 19 a number of genes in five species of fungi are classified on the basis of their intragenic recombination. It is suggested that genes might be described as *unipolar* when the hybrid DNA all appears to originate from the same end, and *bipolar* when at least the non-crossover hybrid DNA extends sometimes from one end and sometimes from the other. It may be that the polaron, that is, the region of a chromosome lying between neighbouring points of primary breakage and within which recombination is by conversion, sometimes coincides with the cistron, and in other instances contains several cistrons. A gene in which the intragenic recombination was bipolar might represent an entire polaron, whereas genes showing unipolar recombination patterns might represent only a part of a polaron placed towards one end.

As regards non-crossover recombination, the predominance of polarity in bipolar cistrons from one end of a gene over that from the other might reflect the relative distances of the two ends of the polaron. However, as can be seen from Fig. 6 (iii), the rather weak distal polarity at the distal end of *ad*-9 in *A. nidulans* appears to match the rather weak proximal polarity at the proximal end of *paba*-1, and suggests that these genes may be in neighbouring polarons and that dissociation cycles may be initiated less often at the intervening linkage point than at the other end of each polaron. Unipolar cistrons may therefore really be bipolar but with one linkage point showing dissociation so rarely as to have been overlooked. As regards crossovers, the predominant direction of crossover hybrid DNA within a cistron is thought to be a consequence of differences in the frequencies with which it is formed in the proximal and in the distal directions from the primary breakage-points. These differences might be characteristic of individual linkage points.

(iii) *Crossover, site and strand coefficients*

In addition to information about polarity, the approximate mean values for the other parameters in recombination, that is, the crossover, site and strand coefficients, are given in Table 19 for each gene, as far as known. Where there is evidence for an excess of CR class tetrads this is also indicated in the table. All the information tabulated is derived from the data of various authors given earlier in this paper. For *Sordaria fimicola*, the crossover and strand coefficients are based on the data in Tables 1 and 5 respectively; for *Neurospora crassa*, *Aspergillus nidulans* and *Saccharomyces cerevisiae*, the crossover and site coefficients are taken from graphs (i) and (ii) respectively of Figs. 2–8, and the strand coefficients from the data in Tables 10–13 and 15–18; and for *Ascobolus immersus* the information is in section 3 (*b*) (iv) of the text.

Table 19. *Classification of genes in terms of the pattern of recombination within them. The name of each gene is followed by its 'address' if known (see section 3 (a) (ii) of the text).*

Polarity	Gene	Crossover coefficient	Site coefficient	Strand coefficient
		(see p. 46)	(see p. 53)	(see p. 61)
Unipolar: Recombination always initiated from the same end of the gene (distal end for *me*-2 in *Neurospora crassa*)	*Ascobolus immersus* series 46	?	Low	Low
	Neurospora crassa *me*-2 (IV R 20)	25–45% decreasing distally	Intermediate to high and increasing distally	?
Bipolar: Recombination initiated sometimes from one end of the gene and sometimes from the other. Distal polarity predominates in *paba*-1 and *ad*-8 of *Aspergillus nidulans* and in *cys* of *Neurospora crassa*, and proximal polarity predominates in *ad*-9 of *A. nidulans* and in *pan*-2 of *N. crassa*. The proximal polarity in *pan*-2 is in crossover hybrid DNA and is not shown by non-crossover events.	*Ascobolus immersus* series 19	?	?	Variable, often high, and probably with CR excess for certain alleles
	Aspergillus nidulans			
	ad-9 (I R 26·5) meiosis	60–80%	Usually very low	?
	ad-9 (I R 26·5) mitosis	?	High except at distal end	High and with CR excess for certain alleles
	paba-1 (I R 27) meiosis	60–80%	Very low and decreasing distally	?
	paba-1 (I R 27) mitosis	25–40%	High except at distal end	High and with CR excess for certain alleles
	ad-8 (I R 43) meiosis	60–80%	Very low and decreasing distally except at distal end	?
	ad-8 (I R 43) mitosis	65%	Low	High and with CR excess
	Neurospora crassa			
	nic-1 (I R 42)	15–50%	Intermediate to high	?
	cys (VI L 8)	20–50%	Intermediate to high	Low
	pan-2 (VI R 2)	35–80%, increasing distally	High at proximal end, decreasing distally except at distal end	Low or intermediate

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Table 19—*Continued*

Polarity	Gene	Crossover coefficient	Site coefficient	Strand coefficient
Pattern of polarity unknown	*Ascobolus immersus* series 75	?	?	Variable, often high
	Neurospora crassa			
	ad-3A (I R 4)	15–40% increasing distally	High	?
	pdx-1 (IV R 7)	35%	Intermediate	Probably high
	pyr-3 (IV R 14)	?	?	Probably high
	am (V R 18)	30%	High	?
	hist-1 (V R 20)	25–40%	High	?
	inos (V R 26)	70%	Rather Low	?
	pab-1 (V R 27)	50%	Low	?
	Saccharomyces cerevisiae			
	is-1 (V R 50) meiosis	65%	Low	?
	is-1 (V R 50) mitosis	40%	Intermediate	Low
	Sordaria fimicola			
	g (I R 60)	35%	?	High

From Table 19 it is evident that there is great diversity in the values of the three coefficients and that this variation appears to be largely independent of the pattern of polarity which prevails. However, it appears that there may be systematic differences between the genes of different species, and also between the same genes under different conditions (meiosis and mitosis), in the values for the coefficients, and furthermore these values appear to be interrelated. The outstanding features shown in Table 19 may be summarized as follows:

(1) At meiosis, *Aspergillus nidulans* shows a higher crossover coefficient and a much lower site coefficient than *N. crassa*.

(2) At mitosis, both *A. nidulans* and *S. cerevisiae* tend to show a lower crossover coefficient and a higher site coefficient than at meiosis. In other words, in these respects mitosis in *A. nidulans* and *S. cerevisiae* is like meiosis in *N. crassa*. On the other hand, *A. nidulans* shows a high strand coefficient at mitosis, while *N. crassa* at meiosis usually shows a low or intermediate value. In this respect, therefore, *A. nidulans* mitosis and *N. crassa* meiosis differ.

(3) The sizes of the crossover and site coefficients are inversely related, both within and between genes. For example, within *me*-2 of *N. crassa* the crossover coefficient decreases distally and the site coefficient increases, while the converse is true of *pan*-2. The closely-linked genes *inos* and *pab*-1 in *N. crassa* have an exceptionally high crossover coefficient for the species and an unusually low site coefficient. The inverse relationship also holds for a gene under different conditions (meiosis and mitosis): see (2) above.

(4) There are indications that the sizes of the site and strand coefficients may often be inversely related, although the data on which this conclusion is based are

rather slight. Thus, *ad*-8 at mitosis in *A. nidulans* shows a low site coefficient and a high strand coefficient, while *cys* at meiosis in *N. crassa* and *is*-1 at mitosis in *S. cerevisiae* approach the converse situation. This inverse relationship, in conjunction with the inverse relationship referred to in (3) above, means there is a direct relationship between the crossover and strand coefficients.

(iv) *The hypothesis of a constant amount of newly-synthesized DNA*

The relationships between the various coefficients outlined above would be explained if the total length of hybrid DNA were more or less constant in each dissociation-cycle, irrespective of how it is distributed. This is most easily explained by supposing that the amount of newly-synthesized DNA is limited. If this postulate were true, it would follow that:

(1) Crossover hybrid DNA would in general extend further from the primary breakage-points than non-crossover, since non-crossover hybrid DNA is expected to extend in both directions from these points, and crossover hybrid DNA in only one direction. Crossover hybrid DNA would then be expected (a) to show a higher site coefficient than non-crossover hybrid DNA, and (b) over a certain range of distances from the linkage point to show a higher strand coefficient than non-crossover hybrid DNA.

(2) If the hybrid DNA tended to be of equal length in the two chromatids, it would not extend so far (at least in one chromatid) as if it tended to be of unequal length. An inverse relationship between the site and strand coefficients would then be expected, such as has been observed.

Predication (1a) is not easily tested directly since there is no means of estimating the site coefficient in non-crossover hybrid DNA, except that if A and B are unequal there must be some single-site events (see section 3 (*a*) (iv)). However, two pieces of evidence suggest that this prediction may be true. First, at the distal end of *me*-2 in *Neurospora crassa*, as already mentioned in section 3 (*a*) (iv), consistently negative values are obtained for the proximal single-site frequency (Fig. 2 (iii)), when the values for D (which are equivalent to half the frequency of crossover hybrid DNA at both sites) are subtracted from the values for A (which are equivalent to the frequency of non-crossover hybrid DNA at the proximal site alone, plus half the frequency of non-crossover hybrid DNA at both sites). These negative values imply that the non-crossover hybrid DNA has a lower site coefficient than crossover hybrid DNA.

Secondly, the crossover coefficient in *me*-2 decreases distally (Fig. 2 (i)). Since this gene appears to be a unipolar cistron with distal polarity, this gradient in the crossover coefficient implies that towards the distal end, that is, nearer the linkage point, a larger proportion of the recombinations detected are non-crossover. This is the pattern expected if crossover hybrid DNA is of greater average length, measured proximally from the distal end of the polaron, than non-crossover hybrid DNA. The increase in the site coefficient towards the distal end of *me*-2 can be accounted for on

the supposition that nearer the linkage point the crossover hybrid DNA more often reaches the second site than it does when farther from the source, that is, at sites nearer the proximal end of the gene. The inverse relationship between the crossover and site coefficients in *me*-2 would then be because both are related to distance from the linkage point, but affected in opposite ways.

It is likely that the inverse relationship found with other genes referred to in Table 19 has a similar cause, but since they are bipolar cistrons, the situation is more complex. The gene *pan*-2 in *N. crassa* studied by Case & Giles (1958, 1959, 1960) shows an increase distally in the crossover coefficient and a decrease distally in the site coefficient (except at the extreme distal end). This pattern would be expected if crossover hybrid DNA was predominantly of proximal origin and of greater average length than the proximal non-crossover hybrid DNA. The tetrad data (Table 12) support the idea that the crossover hybrid DNA is chiefly proximal since, with the inclusion of Cooke's data, there were 4 asci in class CP but only 1 in class CQ.

Prediction (1b) that mutant sites at a certain distance from the linkage point are expected to show a higher strand coefficient in crossover than in non-crossover hybrid DNA is borne out by two pieces of evidence. First, the data of Kitani *et al.* (1962) for *g* in *Sordaria fimicola* fit the expectations when there is appreciable non-crossover hybrid DNA in only one chromatid ($d = 0{\cdot}216$), but crossover hybrid DNA is always in both chromatids ($e = 0, f = 0$) (see Table 5). Secondly, from Tables 15–17 it is evident that in mitotic recombination in *Aspergillus nidulans*, R class tetrads (which imply hybrid DNA in both chromatids) are relatively more frequent with a crossover (C and D) than without (A and B) compared to P and Q class tetrads. In section 4 (v) it is predicted that the part of the *me*-2 gene of *N. crassa* in which the crossover strand coefficient is likely to exceed the non-crossover is the γ region.

The hypothesis of a relatively constant amount of DNA synthesis per dissociation cycle will thus account not only for the interrelations of the three coefficients, but will also explain a number of observed differences between crossover and non-crossover recombination.

The difference between *A. nidulans* and *N. crassa* in their intragenic recombination patterns at meiosis would be explained if in *A. nidulans* synthesis tends to occur for an equal distance in the two chromatids, while in *N. crassa* it tends to extend unequally. The synthesis in one chromatid will necessarily be in the opposite direction chemically to the synthesis in the other, if both proceed in the same direction physically. If synthesis is initiated at different times in the two chromatids involved, or if synthesis in the two chemical directions is at different rates, different lengths of hybrid DNA would result, giving the high site coefficient, low strand coefficient, and hence predominantly non-reciprocal recombination found within cistrons of *N. crassa*. Conversely, the low site coefficient and high strand coefficient, and hence more frequent reciprocal recombination found in *A. nidulans* cistrons, would be due to similar time of initiation or similar rates of synthesis in the two chromatids.

The difference between the patterns of meiotic and mitotic recombination both in *A. nidulans* and *Saccharomyces cerevisiae* would be explained if the amount of DNA synthesis permitted were greater at mitosis than at meiosis. This would account for

the higher site coefficient found at mitosis compared with meiosis. It would also explain why the strand coefficient is high in mitotic recombination in *A. nidulans*. The difference between the recombination patterns of *A. nidulans* at mitosis and *N. crassa* at meiosis would then be that, in the former, sufficient synthesis could occur for hybrid DNA to extend frequently to both sites in both chromatids, while in the latter, if it reached both sites in one chromatid it would rarely reach either in the other.

(v) *Allele maps*

If recombination frequency within a polaron depended merely on the frequency of correction of heterozygosity in DNA, it would bear no relationship to the distance between sites. However, the frequency with which the heterozygosity arises at a given site must also be taken into account. If there is variation in the length of hybrid DNA segments from the end of a polaron, sites near the end will occur in hybrid DNA more often than those further away, giving a gradient of conversion frequency with distance from the end. The recombination frequency of a pair of mutants within a polaron will depend on the frequency with which either site (or both) lies within a hybrid DNA segment. The further the mutants are apart, the nearer they will be to the ends of the polaron, and hence, if hybrid DNA can arise from either end, the greater the recombination frequency. It will therefore be expected that recombination frequencies will bear some relation to the physical distance apart of the mutant sites, but a linear relationship would not be expected.

In addition, evidence can be found in the published data for an effect of heterozygosity on the formation of hybrid DNA. In series 46 in *Ascobolus immersus* the right-hand member of a pair of mutations shows frequent conversion, but the same mutation as the left-hand member of a pair usually shows none. It is evident that the right-hand member is shielding the left-hand one from entering hybrid DNA, presumably by stopping its extension towards the left. That the effect is at the level of the formation of the hybrid DNA is indicated by the behaviour of mutant no. 277, which shows post-meiotic segregation when it is the right-hand member of a pair, for example, when paired with mutant no. 63 or mutant W, but not when it is the left-hand member, for example, when paired with mutant no. 137. That no. 277 is situated to the left of no. 137 was indicated in section 3 (*b*) (iv). Holliday (1964) has pointed out that the frequent occurrence of map expansion, that is, intervals in allele maps which are more than the sum of the intervening shorter intervals, is also evidence that the mutations are interfering with recombination. So the effect of heterozygosity is seen to reduce the formation of hybrid DNA both by restricting it in most cases to one site only, and also by stopping the formation before either site is included. Since Holliday's analysis shows that the last effect is dependent on the distance apart of the markers, marker interference with hybrid DNA formation could modify the pattern of recombination in a way that would allow an allele map to be made from the data.

An alternative mechanism which would have a similar effect in this respect is suggested by the results of Setlow & Carrier (1964). They found that the enzyme

which excised thymine dimers from the DNA of *Escherichia coli* after ultra-violet irradiation removed a considerable length of the nucleotide chain at the same time. Since this enzyme has many of the properties of the postulated correction enzyme, it is possible that this, too, removes a length of nucleotide chain after recognizing a site of heterozygosity. If this length included a second site of heterozygosity, both sites would be corrected to the same parental genotype, and recombination would not occur on that chromatid. Since the frequency of this linked correction would depend on the length of nucleotide chain excised, and on the distance apart of the markers, recombination frequency would also depend on their distance apart. If the length excised were long compared with the marker separation, the major contribution to recombination would come from tetrads in which the length of hybrid DNA had ended between the markers.

In view of the variation in the site and strand coefficients known to occur among alleles (see section 3), the values of v–z in Tables 6 and 7 are likely to differ for each pair. It is not surprising, therefore, that inconsistencies are found when an attempt is made to draw an allele map on the basis of the frequencies of wild-type recombinants from crosses between them. It is for this reason that, when the map based on wild-type frequencies differs from that based on the relative sizes of classes C and D, greater weight has been given to the latter (see section 3 (*a*) (iv)). It has been tentatively assumed in the following analysis of hybrid DNA distribution within cistrons, that the clustering of sites on allele maps reflects variation in the mapping unit rather than in the physical separation of the mutant sites.

In Fig. 9 is shown an example of the distribution of hybrid DNA within a distal unipolar cistron having a low crossover, high site and low strand coefficient, such as might apply to *me*-2 in *Neurospora crassa* studied by Murray (1963). The curves in Fig. 9 have been constructed in the following way. It has been assumed that the sites of the *me*-2 mutations are uniformly spaced, and their positions have been plotted on this basis along one axis. Starting at the proximal end of the gene, the prototroph frequency of a pair of alleles has been plotted as ordinate against the position of the distal member of the pair on the equal-spacing map as abscissa. Assuming that the distal member of this pair has been used as proximal member in another cross, the prototroph frequencies from the two crosses have been added and plotted against the position of the most distal of the three sites; and similarly with further crosses. In this way a series of points has been obtained which are thought to give an approximate indication of the relative conversion frequencies (or hybrid DNA frequencies) at each site. Approximate estimates of the frequencies of crossover and non-crossover conversion for each marker have been obtained from the data in Fig. 2, and the conversion rate for each marker divided in these proportions. The uncertainties in the method are such that only the general shape of the curves is shown.

The two regions of steep slope in the graphs for crossover and for non-crossover hybrid DNA would correspond to their respective mean lengths in the two chromatids. The nearly horizontal regions in the curve for total hybrid DNA would correspond to the site clusters on the map in Fig. 2, α being to the left and farthest

from the linkage point, and δ to the right and nearest to it. The site clusters β and γ are hardly separable by recombination pattern (Fig. 2), and the isolated β mutant is found to be on the slope where the map based on prototroph frequencies is relatively expanded at the left-hand end of the central (γ) horizontal region. The evidence for a greater length of crossover than non-crossover hybrid DNA, measured from

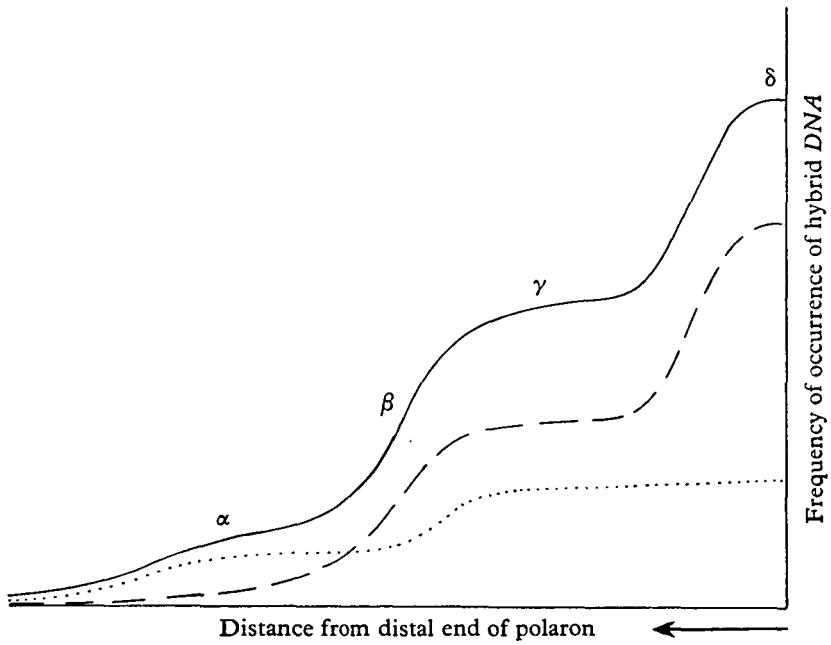

Fig. 9. Graphs to show the estimated distribution of hybrid DNA within a unipolar cistron with low crossover, high site and low strand coefficients. The curves are based on the data of Murray (1963) for the methionine 2 (*me*-2) locus in *Neurospora crassa* (Fig. 2). The dotted line shows the crossover hybrid DNA, the broken line the non-crossover hybrid DNA, and the continuous line shows the total. The Greek letters refer to Murray's site clusters, as given in Fig. 2.

the linkage point, implies that the strand coefficient in the γ region is higher in crossover than in non-crossover hybrid DNA (see section 4 (iv)).

In Fig. 10 is shown an example of the distribution of hybrid DNA within a predominantly distal bipolar cistron with a high crossover, low site and high strand coefficient, such as might apply to *ad*-8 or *paba*-1 at meiosis in *Aspergillus nidulans* studied by Pritchard (1955, 1960*a*, *b*) and Siddiqi (1962), respectively. The graphs have been constructed in a similar way to Fig. 9, using the data in Figs. 6 and 7. The method has been, first, to find the marker which appears to be nearest to the point of reversal of the polarity, and then to proceed in the manner outlined for Fig. 9, first in one direction from this point and then in the other. The crossovers are shown all of distal origin, since there appears to be no increase at the proximal end of the gene in the frequency of crossover hybrid DNA. The graphs are more difficult to construct than for a unipolar cistron, since the proportions of proximal and distal

events are uncertain without tetrad data. Unlike the previous example, only a single region of steep slope is apparent on each graph. This is because, with the low site and high strand coefficients, the mean lengths of the hybrid DNA in the two chromatids appear to be approximately equal, although longer for crossover than for non-crossover events. The longer intervals on the prototroph frequency maps towards the distal ends of *ad*-8 and *paba*-1 (Figs. 6 and 7) correspond to the slope towards the right-hand end of Fig. 10, while the very short interval at the extreme distal end of

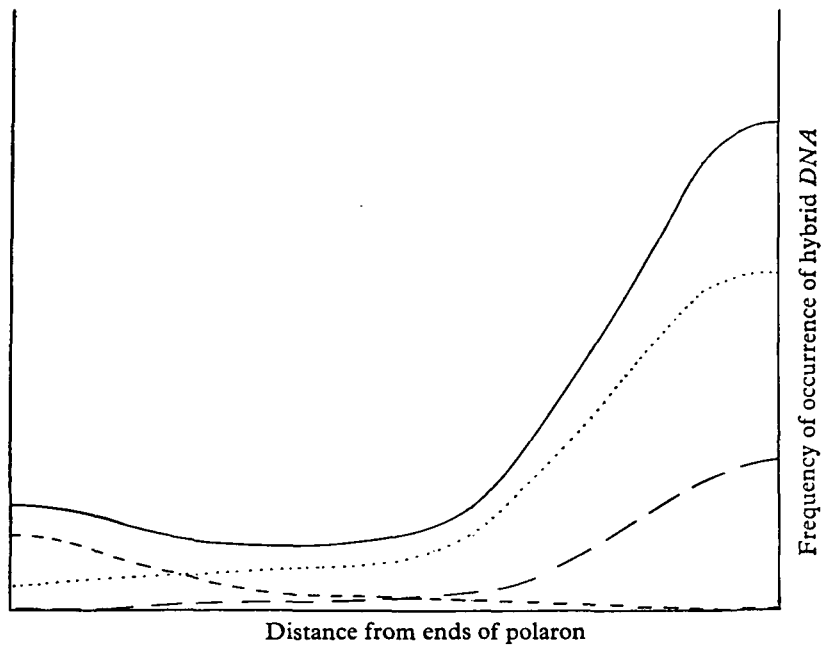

Fig. 10. Graphs to show the estimated distribution of hybrid DNA within a bipolar cistron with high crossover, low site and high strand coefficients. The curves are based on the data of Pritchard (1955, 1960*a*,*b*) for *ad*-8 and of Siddiqi (1962) for *paba*-1 in *Aspergillus nidulans* (Figs. 6 and 7). All crossovers are assumed to have originated from the distal linkage point. The dotted, broken and continuous lines have the same meaning as in Fig. 9. The short dashes (– – – –) indicate proximal and the longer dashes (— — — —) distal origin for the non-crossover hybrid DNA.

ad-8, associated with a high frequency of D class prototrophs, is represented in Fig. 10 by the nearly horizontal region at the extreme right-hand end.

Sites near a linkage point will in general be expected to show a higher strand coefficient than those further away. This may in part explain the wide variation found in *Ascobolus immersus* between the frequencies of reciprocal and non-reciprocal recombination shown by different pairs within a group of alleles.

(vi) *The excess of CR class tetrads*

As was indicated in sections 3 (*b*) (iii) and (iv), there are several instances in

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Aspergillus nidulans and *Ascobolus immersus* of the number of R class tetrads exceeding the sum of the P and Q class tetrads, or more specifically, of CR exceeding CP + CQ. The excess occurs in some of the data of Pritchard (1955, 1960*a*), Martin-Smith (1961) and Putrament (1964) for mitotic recombination in *Aspergillus nidulans* and of Lissouba *et al.* (1962) for meiotic recombination in *Ascobolus immersus*. Although the excess is barely significant in individual instances, it is too frequently encountered to be attributed to sampling error. If it were due to an inequality in conversion frequencies in opposite directions (to wild-type and to mutant), a similar excess in one or both R classes would be expected with non-crossover recombination, but a high frequency of AR or BR tetrads has not been observed.

The distribution of the occurrence of an excess of CR tetrads is indicated in Table 19. It appears to be associated with a low site and a high strand coefficient. Furthermore, reference to Tables 15–17 suggests that the occurrence of an excess of CR tetrads at mitotic recombination in *Aspergillus nidulans* is specific to particular combinations of alleles, and similarly the data of Lissouba *et al.* (1962) for meiotic recombination in *Ascobolus immersus* clearly indicate that an excess of R class tetrads is not a general phenomenon, but is a specific feature of crosses involving particular alleles, such as no. 60 in series 19. If the CR excess had been of general occurrence, it might have been explained by postulating a secondary process of crossing-over, with random breakage-points, occurring independently of and in addition to the postulates for the polaron hybrid DNA model. However, that the distribution of the CR excess should be site-specific precludes such an explanation. In *Ascobolus immersus*, for example, most crosses between series 46 mutants and a number of crosses between series 19 mutants are entirely devoid of R class tetrads.

The association between the CR excess and a low site and high strand coefficient suggests that the excess is related in some way to the behaviour of hybrid DNA. Furthermore, that the excess should be site specific suggests that either the nature of the heterozygosity or the position of the mutation plays a part. Since it appears, from the evidence given in section 4 (v), that mutant sites can interfere with the formation of hybrid DNA, it seems likely that CR tetrads arise when the hybrid DNA is of short and equal length in both chromatids (that is, a low site and high strand coefficient) and when the mutation, because of its structure or position, prevents the hybrid DNA from reaching the site in either strand. It is assumed that with the low site coefficient the hybrid DNA has also failed to reach the other allelic site. Thus neither mutation would be in hybrid DNA, but there would be a region of hybrid DNA lying between them, and hence a CR tetrad would result, if in a cross-over configuration the complementary base pairing (annealing) began at the ends of the newly-synthesized chains, that is, at the end away from the linkage point. If the annealing began at the ends of the old chains, that is, at the end nearest to the linkage point, a parental configuration for the alleles would result, which would not be detected in an allelic recombination selection experiment. The CR frequency might therefore depend on the direction in which the hybrid DNA was formed after the newly-synthesized chains had dissociated from their templates (Fig. 1 (iii)–(iv)).

(vii) *Multi-stranded recombination events*

In all the more extensive sets of data on recombination investigated by tetrad or diploid analysis, a proportion of the recombination events have involved more than two of the four chromatids in the interval between the outside markers. This occurrence is indicated by asterisks, etc. (*, †, ‡) in the tables of data in this paper. At meiosis in *Sordaria fimicola* their frequency was 6 out of 122, or 4·9% (Table 1), and in *Neurospora crassa* there were 7 out of 76, or 9·2% (Tables 9–13). At mitosis in *Aspergillus nidulans* their frequency was 23 out of 445, or 5·2% (Tables 15 and 17), but 4·5% if the 3 diploids marked ′ in Table 17 owe their homozygosity at the *ad*-9 locus to conversion (see section 3 (*b*) (iii)). At mitosis in *Saccharomyces cerevisiae* there were 10·2 multi-stranded events out of 69, or 14·8% (Table 18).

The frequencies at meiosis are in agreement with expectation if the multi-stranded events (with a few exceptions discussed below) arise from a crossover occurring in the interval between the outside markers independently of a non-crossover dissociation cycle at the inside locus under study. This agreement is on the assumption that there is no interference as regards position or strands between a crossover and a non-crossover dissociation cycle (see section 3 (*a*) (iii)). With the converse situation, of a crossover at the inside marked locus, a non-crossover dissociation cycle elsewhere between the outside markers will not be detected. In the data for *Sordaria fimicola* (Table 1) there were 78 asci with non-crossover events at the *g* site, and the outside markers were approximately 4 crossover units apart. Hence in 8% of the asci a crossover would be expected in this interval. One quarter of the 8% would be expected to involve the same two chromatids as the non-crossover event occurring in the same interval. Hence, the expected number of multi-stranded events is 6% of 78, or 4·7. The observed number was 4, corresponding to the asterisks (*) in the part of Table 1 headed 'Parental combination of marker genes'. The ascus marked with a dagger (†) is omitted—see below. Similarly, the weighted mean distance between the outside markers in the *N. crassa* allelic crosses (Tables 10–13) is approximately 13, the number of asci showing non-crossover recombination (classes A and B) was 41, hence the expected number of multi-stranded events is $\frac{3}{4} \times \frac{26}{100} \times 41 = 8{\cdot}0$. The observed number was 5, corresponding to those marked with an asterisk (*) or dagger (†) in Tables 12 and 13.

The following asci require at least three chromatids to have been involved in recombination at the same site during meiosis:

(1) The ascus of *N. crassa* marked ‡ in Table 12 (data of Case & Giles (1959) for *pan*-2).
(2) The ascus of *Sordaria fimicola* marked with a dagger (†) in Table 1 (data of Kitani *et al.* (1962) for *g*).
(3) Another ascus of *Sordaria fimicola* (described by the same authors and referred to in section 2) which had 7 black and 1 grey spore.

It seems likely that in these instances two dissociation cycles have occurred successively in time from the same linkage point, but involving either a different pair of chromatids or one the same and one different, so that in total 3 or all 4 chromatids

took part. With a bipolar cistron, another possibility is that the second dissociation cycle took place at the other end of the polaron.

In contrast to the position at meiosis, the occurrence of multi-stranded events at mitotic recombination is unexpected. More than one mitotic crossover in a chromosome arm is of very uncommon occurrence (Pontecorvo & Käfer, 1958). Consequently, it might be expected that there would rarely be more than one dissociation cycle of either kind, whether crossover or non-crossover. Yet, relative to two-stranded recombination, multi-stranded events appear to be as frequent as at meiosis, or perhaps more so in *Saccharomyces cerevisiae*.

(viii) *Interference*

According to the polaron hybrid DNA hypothesis the primary breakage-points at which dissociation cycles are initiated in a particular meiosis may be randomly distributed along the chromosome, but the resulting crossovers may show interference as a result of a non-random selection of those cycles in which hybrid DNA is formed on only one side of the linkage point. If the total new DNA synthesized is limited in amount, it may form on only one side, that is, either in the proximal or the distal direction, because of a difference in the time of the beginning of synthesis, or in the rate of synthesis, in the two physical directions. However, synthesis to the left in one chromatid would be similar chemically to synthesis to the right in the other, and might be expected to be controlled by the same enzyme. It may be, therefore, that whether a particular dissociation-cycle shall be a crossover or a non-crossover is determined by the pattern of the initial dissociation of nucleotide chains (as illustrated in Fig. 1), rather than by the pattern of subsequent synthesis of new chains.

The occurrence of a crossover coefficient of approximately $\frac{2}{3}$ at meiosis in *Aspergillus nidulans* (Figs. 6 and 7), where there is no interference between crossovers, and of a crossover coefficient of about $\frac{1}{3}$ in most of the genes studied in *Neurospora crassa* (Figs. 2–5), where there is a positive interference within chromosome arms, suggests that interference may be associated with a higher proportion of non-crossover events, and hence that dissociation cycles in the neighbourhood of a crossover are influenced by it and switched to the non-crossover path. The apparent lack of interference between the positions of crossover and non-crossover events in *N. crassa* (see sections 3 (*a*) (iii) and 4 (vii)) is in agreement with this suggestion. The influence of an event at one point in a chromosome on the behaviour of neighbouring parts, such as this hypothesis of interference would require, may be compared with the influence of heterochromatin on neighbouring euchromatic regions.

(ix) *Conclusion*

The polaron hybrid DNA model, as proposed by Hastings & Whitehouse (1964), has been found capable of explaining the large body of relevant data on genetic recombination. The great diversity of intragenic recombination patterns known to occur has all been accounted for. The ancillary hypothesis of a relatively constant amount of nucleotide-chain synthesis per dissociation cycle provides an explanation

for several otherwise puzzling features of the data. The patterns of conversion prevailing in individual genes appear to be determined by the frequencies with which dissociation cycles are initiated at each linkage point, and by the relative frequencies of crossover and non-crossover events extending into the polaron from those linkage points. For any site within a polaron, the recombination will be determined by the length of this crossover and non-crossover hybrid DNA in each of the two chromatids, and for any particular mutation at that site by the frequencies of conversion in each direction.

The predictions of the model could be further tested by tetrad, or preferably octad, analysis using allelic spore mutants and outside marker genes. It would also be particularly valuable to study the recombination of mutations near the adjoining ends of neighbouring polarons. Study of recombination within a group of functionally related and adjacent genes, such as Giles (1964) has described for the terminal steps of histidine synthesis in *Neurospora crassa*, would test the possibility that polarons may coincide with operons. The transforming properties of hybrid DNA, prepared *in vitro* by the method described by Marmur & Lane (1960) of heating and slow cooling a mixture of the DNA from two bacterial strains, might provide information about the interference in hybrid DNA formation caused by heterozygosity, and about the process of conversion. Ravin & Iyer (1962) found that allelic mutations in *Diplococcus pneumoniae* may differ in their recombination behaviour when studied in this way. Comparison of the behaviour of mutations obtained with different mutagens would be of particular interest.

5. SUMMARY

According to the polaron hybrid DNA model, the initial nucleotide-chain breakage leading to genetic recombination takes place only at the *linkage points* which define the ends of each *polaron*. The term *dissociation cycle* is proposed for the postulated series of events from primary breakage at a linkage point to the final breakdown of unpaired chains. At mutant sites, the mispairing in hybrid DNA may persist and give rise to post-meiotic segregation, or a correction process may operate by which molecular homozygosity is restored. This causes *conversion*, which may be evident as reciprocal or as non-reciprocal recombination.

The implications of this model are that a crossover occupies a segment of the chromosome, and that conversion is a process which takes place when a mutant site happens to lie within such a segment. Although crossovers appear to be initiated at fixed points outside or at the ends of the genes, they extend into the gene on one side or the other. The negative interference between recombination events over short intervals of the linkage map is attributed to the association between crossing-over and the conversion which is likely to occur at any mutant sites which happen to lie within the crossover. In the same way, non-crossover hybrid DNA can also lead to conversion, and hence to negative interference.

The relevant data on genetic recombination have been found to fit this model, and have led to the following main conclusions:

(1) The polaron may coincide with the cistron, or in some instances may possibly include more than one cistron. As a corollary to this, there appear to be two kinds of cistrons: *unipolar*, where all the recombination is initiated from the same end, and *bipolar*, where it is sometimes initiated from one end and sometimes from the other.

(2) In bipolar cistrons there is usually a preponderance of recombination initiated from one end over that from the other. In five genes where the orientation with respect to the centromere is known, two show a preponderance in favour of the proximal end and the other three in favour of the distal end. It seems possible that this asymmetry within the gene may reflect intrinsic differences in the frequencies with which the dissociation of the DNA molecules is initiated at different linkage points.

(3) The hypothesis of a fairly constant amount of newly-synthesized DNA per dissociation cycle, irrespective of how it is distributed along the four templates from a linkage point, leads to a number of predictions for which there is evidence in support. These concern the detailed pattern of crossover and non-crossover hybrid DNA within the gene.

(4) Specific differences in intragenic recombination are attributed to differences in the pattern of DNA synthesis. The predominantly non-reciprocal recombination found within cistrons of *Neurospora crassa* would be explained if synthesis extends unequally from the linkage point in the two chromatids. The higher frequency of reciprocal recombination found in *Aspergillus nidulans* is attributed to more equal extension.

(5) Differences between meiotic and mitotic intragenic recombination both in *A. nidulans* and in *Saccharomyces cerevisiae* are explained on the supposition that more DNA synthesis per dissociation cycle occurs at mitosis than at meiosis.

(6) Clustering of sites in maps of alleles based on recombination frequencies is attributed to a rather limited range of variation in the lengths of the newly-synthesized nucleotide chains.

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