Genet. Res., Camb. (1974), **24**, *pp.* 251–279
With 7 text-figures
Printed in Great Britain

Genetic analysis of recombination at the *g* locus in *Sordaria fimicola*

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(*Received* 10 *June* 1974)

SUMMARY

Interallelic crosses of mutants at the *grey* (*g*) spore colour locus in *Sordaria fimicola*, heterozygous for flanking markers, give rise to a large number of aberrant ascus genotypes, 45 of which can arise through relatively simple events and have been chosen for study. These genotypes comprise 50–75% of the aberrant asci, depending on the mutants crossed.

Comparison of the results from 10 pairwise crosses involving 7 alleles reveals that linked postmeiotic segregation and co-conversion decrease rapidly in frequency with increasing separation of the mutant sites.

The data from reciprocally recombinant asci, from asci with normal segregation at one of the two mutant sites, and from flanking marker behaviour in one- and two-point crosses, agree with the Holliday–Sobell formulation, with the following additional features:

(1) The nuclease, which nicks homologous polynucleotides and then degrades one of the two nicked chains when a mutant enters the hybrid DNA structure, can show preferential degradation of the mutant (or the wild-type) chain. In addition, a second nuclease is involved in the excision-repair process that introduces an additional preferential (marker specific) bias in the degradation of the mutant (or the wild-type) chain. This could explain why asci with odd-ratio conversion (5:3 and 3:5 ratios) sometimes show a different bias, as first reported by Emerson for *Ascobolus*, from those with even-ratio conversion (6:2 and 2:6 ratios), since the latter but not the former require, in addition, the action of a mismatch correction enzyme to account for them.

(2) The migratory hybrid DNA structure which enters the gene at one end may be of a different size from that which enters from the other end.

(3) Mismatch correction at the end of the hybrid DNA structure leads to a non-recombinant outside marker genotype and modifies the 1:1 ratio of parental:recombinant flanking markers that is otherwise found.

1. INTRODUCTION

Data were presented in the preceding paper (Kitani & Whitehouse, 1974*b*) on ascus genotypes from crosses of mutants at the *g* locus in *Sordaria fimicola* with wild type and with one another. Some 128 different genotypes were recorded among the aberrant asci from the two-point crosses when the behaviour of the outside (flanking) markers, *mat* and *corona*, is taken into account. Some of these genotypes require complex events to explain their origin. The present paper is

limited to a discussion of ascus genotypes which can be explained rather simply. It is planned later to extend the analysis to the more complex recombination events.

From the tables of ascus genotypes, particularly those for the more widely separated mutants (Kitani & Whitehouse, 1974*b*, Tables 11–18), it is evident that the favoured genotypes are often asymmetrically distributed, particular genotypes at *g* being associated predominantly with particular outside marker genotypes. It is this asymmetry which has provided information about the sequence of the mutant sites within the *g* locus. Many of the favoured kinds of aberrant asci could have originated, as already suggested, from rather simple events at the *g* locus. For example, the preponderance of asci in the bottom left-hand corner of the upper part and in the top right-hand corner of the lower part of Tables 13–18 of Kitani & Whitehouse (1974*b*) is explained if events at *g* affecting only the left-hand mutant site are responsible for these genotypes, because the right-hand mutant in these asci showed normal 4:4 segregation and a lack of recombination with the right-hand flanking marker (*corona*). These asci are discussed in detail in sections 8 and 9 below.

The idea that the hybrid DNA does not always extend to the second mutant site within the *g* locus is not necessarily in conflict with the finding that all crosses give the same total frequency of aberrant asci whether between *g* mutants and wild-type or between one *g* mutant and another (see Tables 2 and 7 of Kitani & Whitehouse, 1974*b*). Sobell (1972) suggested that in eukaryote recombination a hybrid DNA structure containing two Holliday (1964)-type cross strand exchange connexions was formed and that this could migrate by rotary diffusion along the pair of recombining chromatids in either direction from the point where it was initiated. According to Sobell's model, this hybrid DNA structure arises by first nicking symmetric regions (postulated to be at ends of genes or operons) on strands of opposite polarity at or near the same point on homologous DNA molecules. This allows the initiation of hydrogen-bonding between homologous DNA molecules and the subsequent propagation of the hybrid DNA structure through branch migration. In the presence of polynucleotide ligase, one can then form the migratory hybrid DNA structure. Sobell further suggested that entry of a site of mutation into a hybrid DNA structure, giving rise to regions of mismatch in both chromatids, could trigger endonuclease attack, nicking homologous polynucleotides, and fixing the starting-point of the hybrid DNA in the final recombinant molecules. If hybrid DNA structures normally migrate right through the *g* locus when it is homozygous, all crosses would give the same total frequency of aberrant asci, as observed, and the failure of hybrid DNA often to extend to the second mutant site in two-allele crosses would be a consequence of the enzyme action triggered when the first site entered the hybrid DNA structure. Other features of Sobell's hypothesis are discussed in sections 4 and 10.

No attempt is made to relate the *g* locus data to hypotheses other than the Holliday–Sobell model, for the following reasons:

(1) Fungal recombination data generally show more conversion of the left-hand

allele than of the right-hand one in two-point crosses when the left-hand mutant site is near the left-hand end of the gene and, conversely, more conversion of the right-hand allele than the left if the right-hand mutant site is near the right-hand end of the gene. Whitehouse & Hastings (1965) interpreted this polarity as a consequence of hybrid DNA extending into the gene for a variable distance from fixed starting-points at (or beyond) each end of the gene. This hypothesis predicts that in one-point crosses mutants situated near gene ends will show a higher conversion frequency than mutants located in mid-gene. The constant total frequency of aberrant asci in the *g* locus one-point crosses contradicts this prediction. Nevertheless, many asci in the two-point crosses, as indicated above, can be interpreted as the result of hybrid DNA ending in mid-gene. Kitani & Olive (1969) resolved this dilemma by postulating that, despite appearances to the contrary, the hybrid DNA extends right through the gene whenever an aberrant ascus originates, but the mismatched nucleotides are then often corrected back to the parental genotype, so giving the appearance of a shorter extent of hybrid DNA. For correction to restore a parental genotype more often than might be expected by chance, it is necessary to suppose that the correcting enzyme can distinguish a parental from a non-parental polynucleotide. This might be possible if all the events gave rise only to non-recombinant outside marker genotypes, because the parental chain, unlike the non-parental, might be unbroken (as in Holliday's model). Many aberrant asci, however, are associated with flanking marker recombination. For these asci Kitani & Olive used the genotype of the proximal outside marker to distinguish correction of the restoration type (giving a 'parental' genotype) from correction of the substitution type (giving a 'non-parental' genotype) but this was an arbitrary procedure and the behaviour of the distal outside marker could have been used with equal plausibility. With flanking marker recombination it is not clear how the action of the correction enzyme could give rise to the observed results, unless elaborate postulates are made.

(2) Direct support for the Holliday–Sobell model is provided by three features of the *g* locus data: (i) the occurrence of 50% outside marker recombination in aberrant 4:4 asci from one-point crosses (section 13); (ii) evidence for the determination of parental *versus* recombinant flanking marker genotypes by events at the ends of the hybrid DNA segments (section 13); and (iii) evidence that the two mutant polynucleotides, one in each duplex of a hybrid DNA structure heterozygous for wild type and mutant, differ in molecular polarity (section 14). This feature is less well established than the other two.

In view of the high frequency of occurrence of ascus genotypes which can be explained relatively simply, 45 such genotypes have been chosen for study in detail. They are listed in Table 1 and the genotypes of their recombinant chromatids are given in Table 2 for a trans heterozygote.

Exact numbers of asci of these genotypes in each cross are rarely known, owing to incomplete scoring. This is chiefly the consequence of bursting of some of the spores. When this happens to the paler mutant spores, the segregation pattern for the darker mutant cannot be fully determined (see Kitani & Whitehouse,

Table 1. *Ascus genotypes of simple origin*

(The numbers refer to the chromatid genotypes in Tables 8–18 of Kitani & Whitehouse, 1974*b*.)

Event	Class	Flanking markers: Parental *MN* chromatid	Parental *mn* chromatid	Recombinant *Mn* chromatid	Recombinant *mN* chromatid
Linked postmeiotic segregation (*lp*)	*lp*1	5	5	5	5
	*lp*2	3	5	3	5
	*lp*3	5	7	5	7
	*lp*4	5	3	5	3
	*lp*5	7	5	7	5
Co-conversion (*cc*)	*cc*1	3	3	3	3
	*cc*2	7	7	7	7
Distal asymmetric events (*da*)	*da*1	3	4	3	4
	*da*2	6	7	6	7
	*da*3	3	1	3	1
	*da*4	9	7	9	7
Distal symmetric events (*ds*)	*ds*1	6	4	6	4
	*ds*2	9	4	9	4
	*ds*3	6	1	6	1
	*ds*4	9	1	*	*
Proximal asymmetric events (*pa*)	*pa*1	2	7	7	2
	*pa*2	3	8	8	3
	*pa*3	1	7	7	1
	*pa*4	3	9	9	3
Proximal symmetric events (*ps*)	*ps*1	2	8	8	2
	*ps*2	2	9	9	2
	*ps*3	1	8	8	1
	*ps*4	1	9	*	*
Reciprocal recombination (*rr*)	*rr*	—	—	9	1

* The *ds*4 and *ps*4 genotypes at the *g* locus associated with recombinant flanking markers are included in the *rr* class.

Simplest events implied by the chromatid genotypes:

1. Conversion to wild type (trans configuration) or mutant (cis configuration) at distal site (*da* and *ds*), or conversion to wild type at proximal site (*pa* and *ps*) or hybrid DNA between sites (*rr*).
2, 8. Postmeiotic segregation at proximal site.
3. Parental genotype (*MN* chromatid), or co-conversion (*mn* chromatid), or recombination on distal side of *g* (*Mn* chromatid), or on proximal side (*mN* chromatid).
4, 6. Postmeiotic segregation at distal site.
5. Postmeiotic segregation at both sites.
7. Co-conversion (*MN* chromatid), or parental genotype (*mn* chromatid), or recombination on proximal side of *g* (*Mn* chromatid), or on distal side (*mN* chromatid).
9. Conversion to mutant (trans configuration) or wild type (cis configuration) at distal site (*da* and *ds*), or conversion to mutant at proximal site (*pa* and *ps*), or hybrid DNA between sites (*rr*).

1974*b*). Germination was usually sufficient, however, to enable the ascus genotype frequencies to be estimated with reasonable precision. The estimation depends on the assumption, justified by experimental counts (Y. Kitani, unpublished), that the paler mutant, for example mutant no. 4 in the cross 1×4, germinates with the same frequency as the double mutant, that is, 1 and 4 in this instance. For each ascus phenotype the ungerminated spores can then be allocated to the alternative genotypes in the same proportions as those that have germinated.

2. LINKED POSTMEIOTIC SEGREGATION

By linked postmeiotic segregation is meant the occurrence in a chromatid of hybrid DNA having one parental genotype at both mutant sites in the *g* locus in one nucleotide chain and the other parental genotype in the other nucleotide chain or, in other words, the trans hybrid DNA configuration from a trans heterozygote or the cis configuration from a cis heterozygote. Remarkably, a single instance was found of a cis configuration for postmeiotic segregation arising from a trans heterozygote (see Table 13 of Kitani & Whitehouse, 1974*b*). This genotype requires a complex series of events to explain its origin and will not be discussed further at present.

Five relatively simple ascus genotypes involving linked postmeiotic segregation have been selected for study in the category with parental outside markers, and the corresponding 5 with flanking marker recombination (Tables 1 and 2). The total frequency of these 10 genotypes out of the total number of recombinant asci is plotted in Fig. 1 for each cross. In many cases the frequencies are not known with precision owing to incompletely scored asci, and it has been possible to calculate the standard errors for only two crosses. However, in most of the crosses the range of possible values for the total frequency is small. Only in the cross of mutants 1×2 is there real uncertainty about the frequency of these linked postmeiotic segregation (*lp*) events, but the maximum possible frequency is known and is shown in Fig. 1.

It is evident from Fig. 1 that the frequency of these *lp* events declines rapidly with increasing distance between the mutant sites from perhaps a quarter of all the events with the shortest interval to about 1% for the longest. The absolute frequency declines similarly, since the total frequency of aberrant asci of all kinds is relatively constant for all the crosses (see Fig. 1 of Kitani & Whitehouse, 1974*b*).

The higher frequency of *lp* events in 6×5 than 1×5 implies that the site of mutant 6 is probably to the right of that of mutant 1 on the map and hence nearer to that of mutant 5. When mutants 1 and 6 were crossed, the frequency of asci with wild-type spores was found to be about one quarter of that obtained when either is crossed with mutant 5 (Kitani & Whitehouse, 1974*b*). If the 6–5 and 1–5 intervals span the modal length of hybrid DNA in the final recombinant molecules, this would account for the difference in the *lp* frequencies of 6×5 and 1×5, despite the low frequency of recombination between 1 and 6.

Although the absolute distances between the mutant sites in terms of nucleo-

Table 2. *Genotypes of the recombinant chromatids in simple events in a trans heterozygote*

$\left(\text{The parental genotype is } \frac{\text{M 1 + N}}{\text{m + 2 n}}\right)$.

Class	Flanking markers: Parental	Flanking markers: Recombinant
*pa*1	$\text{M}\frac{1}{+}\text{+N}$	M + 2 n
	m + 2 n	$\text{m}\frac{1}{+}\text{+N}$
*pa*2	M 1 + N	$\text{M}\frac{1}{+}$ 2 n
	$\text{m}\frac{1}{+}$2 n	m 1 + N
*pa*3	M + + N	M + 2 n
	m + 2 n	m + + N
*pa*4	M 1 + N	M 1 2 n
	m 1 2 n	m1 + N
*ps*1	$\text{M}\frac{1}{+}\text{+N}$	$\text{M}\frac{1}{+}$ 2 n
	$\text{m}\frac{1}{+}$2 n	$\text{m}\frac{1}{+}\text{+N}$
*ps*2	$\text{m}\frac{1}{+}\text{+N}$	M 1 2 n
	m 1 2 n	$\text{M}\frac{1}{+}\text{+N}$
*ps*3	M + + N	$\text{M}\frac{1}{+}$ 2 n
	$\text{m}\frac{1}{+}$2 n	m + + N
*ps*4	M + + N m 1 2 n	—
rr	—	M 1 2 n m + + N
*lp*1	$\text{M}\frac{1\ +}{+\ 2}\text{N}$	$\text{M}\frac{1\ +}{+\ 2}\text{n}$
	$\text{m}\frac{1\ +}{+\ 2}\text{n}$	$\text{m}\frac{1\ +}{+\ 2}\text{N}$
*lp*2	M 1 + N	M 1 + n
	$\text{m}\frac{1\ +}{+\ 2}\text{n}$	$\text{m}\frac{1\ +}{+\ 2}\text{N}$
*lp*3	$\text{M}\frac{1\ +}{+\ 2}\text{N}$	$\text{M}\frac{1\ +}{+\ 2}\text{n}$
	m + 2 n	m + 2 N

Class	Flanking markers: Parental	Flanking markers: Recombinant
*da*1	M 1 + N	M 1 + n
	$\text{m}+\frac{+}{2}\text{n}$	$\text{m}+\frac{+}{2}\text{N}$
*da*2	$\text{M 1}\frac{+}{2}\text{N}$	$\text{M 1}\frac{+}{2}\text{n}$
	m + 2 n	m + 2 N
*da*3	M 1 + N m + + n	M 1 + n m + + N
*da*4	M 1 2 N m + 2 n	M 1 2 n m + 2 N
*ds*1	$\text{M 1}\frac{+}{2}\text{N}$	$\text{M 1}\frac{+}{2}\text{n}$
	$\text{m}+\frac{+}{2}\text{n}$	$\text{m}+\frac{+}{2}\text{N}$
*ds*2	M 1 2 N	M 1 2 n
	$\text{m}+\frac{+}{2}\text{n}$	$\text{m}+\frac{+}{2}\text{N}$
*ds*3	$\text{M 1}\frac{+}{2}\text{N}$	$\text{M 1}\frac{+}{2}\text{n}$
	m + + n	m + + N
*ds*4	M 1 2 N m + + n	—
*cc*1	M 1 + N m 1 + n	M 1 + n m 1 + N
*cc*2	M + 2 N	M + 2 n
	m + 2 n	m + 2 N
*lp*4	$\text{M}\frac{1\ +}{+\ 2}\text{N}$	$\text{M}\frac{1\ +}{+\ 2}\text{n}$
	m 1 + n	m 1 + N
*lp*5	M + 2 N	M + 2 n
	$\text{m}\frac{1\ +}{+\ 2}\text{n}$	$\text{m}\frac{1\ +}{+\ 2}\text{N}$

https://doi.org/10.1017/S0016672300015275 Published online by Cambridge University Press

tides are not known, certain of the crosses are of special interest because the interval between the sites is the sum of that in other crosses. Thus 1×5 can be compared with 1×2 and 2×5; $1 \times 4b$ with 1×5 and $5 \times 4b$; and 1×4 and $1{,}4 \times +$ with 1×5 and 5×4. In each case the *lp* frequency for the longer interval is less – often much less – than that for either of the shorter intervals.

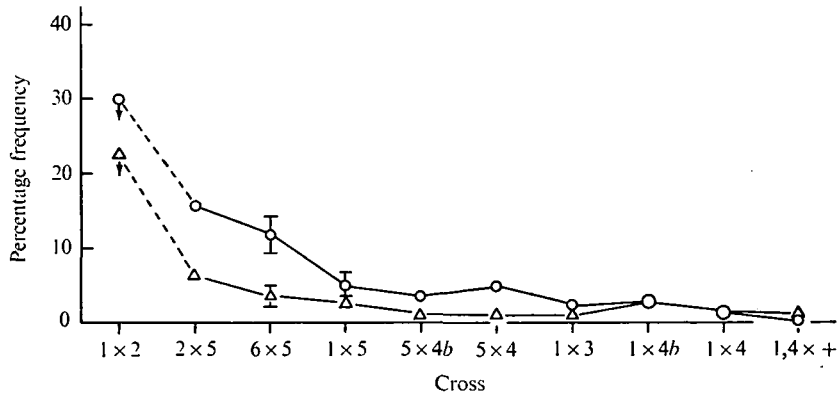

Fig. 1. The total frequencies of asci showing linked postmeiotic segregation (○) and of those showing co-conversion (△) are plotted as percentages of all aberrant asci for each cross. The crosses are in order of increasing separation of the mutant sites, as far as this is known. Standard errors are given where possible. The points plotted in the 1×2 cross are the maximum values and are probably in excess of the true ones.

There are two likely explanations for the decline in *lp* events with increasing separation of the mutant sites. One possibility is that hybrid DNA often fails to reach the second site when these are far apart. This was suggested in section 1 as an explanation for some of the ascus genotypes. Another possibility is that hybrid DNA initially extended to both sites, but subsequently the mispairing was corrected at one or both of them. If such correction was more probable with distant sites, the observed decline in *lp* with distance between the sites would be explained. To assess the contribution, if any, of this second possibility to the observed decline requires study of the complex recombination events observed in the various crosses. This study is reserved for a later publication.

A notable feature of the *lp* data is the rarity of class *lp*1, involving linked postmeiotic segregation in both chromatids. Even with the closest sites (1×2) there were not more than 5 such asci in the 293 recombinant asci investigated. Linked postmeiotic segregation in one chromatid and a parental genotype at *g* in the other chromatid involved in the recombination event (*lp*2–5) was in general much more frequent. This is an example of the phenomenon, so often encountered in eukaryote recombination data, of the asymmetry within the gene in the events in the two chromatids involved. Possible explanations are that hybrid DNA is of unequal extent in the two chromatids, or that correction of mismatched bases is more frequent in one chromatid than the other. The possibility that the asymmetry arises through the action of an exonuclease is discussed in section 4.

3. CO-CONVERSION

Co-conversion (*cc*), or linked conversion, is typically revealed when both sites at the *g* locus have the same parental genotype in both of the chromatids involved. The total frequencies of the *cc* events listed in Table 1 are plotted in Fig. 1. As with *lp* events, precise frequencies are rarely known, and with the 1×2 cross only the maximum possible value can be given. Like *lp* events, the *cc* events decline steadily in frequency with increasing separation of the sites and, except for the longest intervals, are usually about half as frequent as the *lp* events. Co-conversion, however, is less easily observed than linked postmeiotic segregation. Thus, the genotypes *lp*4 and 5 may have involved co-conversion in the chromatid not giving rise to postmeiotic segregation.

The decline in co-conversion frequency with increasing distance between the sites is in agreement with the findings of Fogel *et al.* (Fogel & Mortimer, 1969; Fogel, Hurst & Mortimer, 1971) for the *arginine-4* locus in *Saccharomyces cerevisiae* and of Gutz (1971) for the *adenine-6* locus in *Schizosaccharomyces pombe*. The relatively low frequency of co-conversion for all except the shortest intervals in the *g* locus of *Sordaria fimicola*, compared with the yeast data, may imply either that the modal length of polynucleotide excised in the correction of mismatched bases in *S. fimicola* is much shorter than in the yeasts, or that the *g* locus is much larger than the yeast genes investigated.

In trans heterozygotes involving the longer intervals (Tables 13–17 of Kitani & Whitehouse, 1974*b*) the *cc*1 genotype (total frequency 1) is less frequent than *cc*2 (total frequency between 5 and 18). The *cc*1 genotype in trans heterozygotes is associated with conversion to mutant at the left-hand site and to wild type at the right-hand site, and *cc*2 has the converse pattern (Table 2). The left-hand mutants (1 and 5) in these crosses show more 6:2 than 2:6 wild type:mutant ratios in one-point crosses, while the right-hand mutants (3, 4*b* and 4) show the converse effect (see Table 4 of Kitani & Whitehouse, 1974*b*). The bias in favour of *cc*2 is therefore probably another manifestation of the differences between the mutants evident in the one-point crosses. It is not known whether these peculiarities of the individual mutants are a consequence of their molecular structure or whether their position in the gene plays a part.

4. DISTAL ASYMMETRIC EVENTS

The expression *distal asymmetric events* refers to ascus genotypes in which one chromatid involved in the event has a parental genotype at the *g* locus and the other shows aberrant segregation only at the distal site with respect to the centromere, that is, the right-hand site on the map. Furthermore, the asci in this category show no recombination on the proximal side of *g*, that is, between the left-hand mutant site at the *g* locus and the left-hand flanking marker (*mat*). Four genotypes with parental outside markers and four with flanking marker recombination fit this description (Tables 1 and 2). The numbers observed of each kind (ignoring

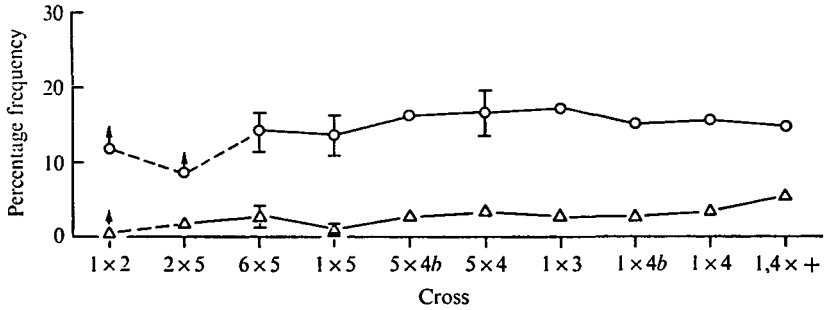

Fig. 2. The total frequencies of distal asymmetric (○) and distal symmetric (△) asci are plotted as percentages of all aberrant asci for each cross. The crosses are in order of increasing separation of the mutant sites, as far as this is known. Standard errors are given where possible. In the 1 × 2 cross the minimum values are plotted (the precise value being unknown) and in the 2 × 5 cross the *da*2 and *da*4 genotypes of distal asymmetric events were not recorded so the point plotted is probably below the true value.

Table 3. *Numbers of asci of* da *and* ds *genotypes*

(See Tables 1 and 2 for an explanation of the ascus genotypes. The figures are the pooled data for parental and recombinant outside markers, except with *ds*4 where the flanking markers always have the parental genotypes because the corresponding event at *g* with recombinant flanking markers gives asci of the *rr* genotype (see Table 1). Where the numbers of asci are uncertain owing to incomplete scoring, the range of possible values is given since the uncertainty is sometimes great, for example, with some of the genotypes in cross 1 × 2.)

Cross	Number of asci analysed	Ascus genotypes							
		*da*1	*da*2	*da*3	*da*4	*ds*1	*ds*2	*ds*3	*ds*4
1 × 2	293	27	0–29	7	0–49	1–18	0–18	0–2	0–3
2 × 5	134	2–7	—	1–7	—	1–6	0–1	0–7	0–3
6 × 5	170	14	0	10	0	1	2–3	1	0
1 × 5	162	12	1	8	1	0	0–1	1	0
5 × 4*b*	209	22	3–7	5	0–1	2–5	1–3	0	0
5 × 4	211	26	3–5	4	1	1–5	0–4	1	0–1
1 × 3	241	19	9–17	1	1–8	0–4	0–2	1	0–2
1 × 4*b*	154	7	4–16	1	0–2	0–2	0–3	0–1	0
1 × 4	154	8	3–12	3	0–4	0–3	1	0–1	0
1,4 × +	102	1–4	6	0–1	4	1–5	1	1	1

the outside markers) are given in Table 3 for each cross, and the total frequency of the 8 genotypes is plotted in Fig. 2 for each cross. In some crosses the frequency has had to be estimated but, apart from the 1 × 2 cross, the range of possible values is small. The *da*2 and *da*4 genotypes were not recognized in the 2 × 5 cross, which has the paler mutant as the left-hand one. Ignoring these uncertainties, the frequency of *da* asci is constant for all the crosses and forms about 15% of the recombinant asci. This constancy is discussed in section 9.

The simplest explanation of how the *da* genotypes arise is for hybrid DNA to reach the *g* locus from the distal side and to reach only the distal mutant site and

only in one chromatid. In the *da*1 and *da*2 genotypes there is postmeiotic segregation at the distal site in one chromatid, and in the *da*3 and *da*4 genotypes there is conversion at this site either to wild type (*da*3 trans and *da*4 cis) or to mutant (*da*3 cis and *da*4 trans). A remarkable feature, however, of the distal asymmetric events is the low frequency of *da*2 compared with *da*1 in the crosses 6×5, 1×5, $5 \times 4b$ and 5×4 (see Table 3). If *da*1 and *da*2 arise through hybrid DNA in only one chromatid, they would be expected to occur with equal frequency.

Sobell (1972) suggested that the endonuclease which he postulated as nicking homologous polynucleotides when a mutant site enters a migratory hybrid DNA structure (see section 1 above) also possesses exonucleolytic activity, degrading one of the two nicked chains. This molecule would subsequently be repaired by a DNA polymerase. The consequence of such exonuclease action would be that hybrid DNA would be confined to one molecule. If this is the explanation for the *da* asci, it is necessary to postulate that the enzyme acts non-randomly, degrading the nicked polynucleotide that is mutant at the distal site more often than the other one (wild type at the distal site), in order to account for the inequality of *da*1 and *da*2 in the crosses mentioned above. Sobell (1973) discussed the possibility of one heteroduplex being eroded more often than the other as a consequence of the kind of mismatch influencing the affinity of the enzyme.

Some implications of preferential degradation are discussed in section 5.

5. ODD- AND EVEN-RATIO CONVERSION

Sobell (1973), in discussing exonucleolytic activity following endonuclease attack at a mutant site in a hybrid DNA structure, suggested that preferential degradation could explain the asymmetry in half-chromatid (odd-ratio) conversion. Whole chromatid (even-ratio) conversion requires an additional excision-repair step, and this may introduce a further bias in the conversion patterns. The fungal conversion data are most readily interpreted if one postulates these two steps in conversion, catalysed by two different enzymes. These will be referred to as the degradation and the correction enzymes, respectively. The degradation enzyme would act in one duplex only and would result in hybrid DNA being confined to the other molecule. The correction enzyme, on the other hand, could act at the same site in both molecules if the hybrid DNA in one of them had not been lost through the action of the degradation enzyme. This hypothesis is illustrated in Fig. 3. The existence of aberrant 4:4 asci is evidence that polynucleotide degradation does not occur in every aberrant ascus. Information about the frequency of degradation is discussed in section 6.

Preferential degradation would affect the relative frequencies of asci showing 5:3 segregation and those showing a 3:5 ratio of wild type : mutant spores in a cross between the mutant and wild type. The correction enzyme would also affect their relative frequencies, but only in those asci in which degradation had not occurred at the mutant site. On the other hand, the relative frequencies of asci showing 6:2 and 2:6 ratios would depend, to a greater degree than the odd

ratios, on the activity of the correction enzyme, since the exonuclease alone could not generate such genotypes. Thus the odd-ratio asci could be generated by either enzyme alone, while the even-ratio asci would require the correction enzyme acting in one molecule and either the degradation enzyme or the correction enzyme in the other.

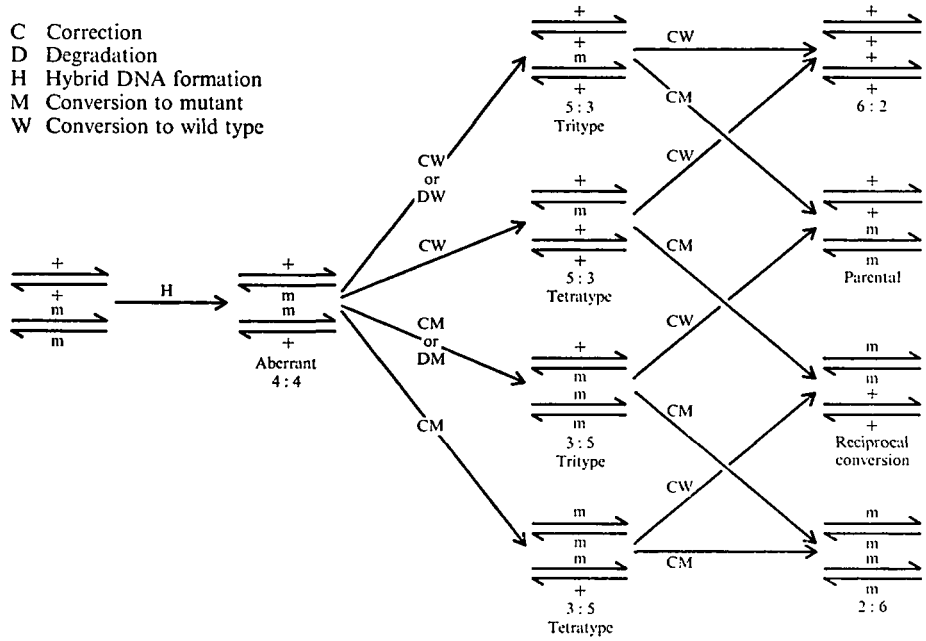

Fig. 3. The conversion pathways according to the two-enzyme hypothesis. The expressions tritype and tetratype refer to the ascus genotypes when the flanking markers are non-recombinant. Only the two chromatids involved in recombination are shown.

This two-enzyme hypothesis of conversion, with its different explanation for odd- and even-ratio asci, might explain how these asci can have biasses in opposite directions. Emerson (1966) found 6:2 asci more frequent than 2:6 but 5:3 less frequent than 3:5 with the *w*-62 mutant in *Ascobolus immersus*. He attributed this to a different mismatch in the two chromatids (which Holliday's model predicts) and a differential response by the correction enzyme. This possibility is discussed in section 14. The two-enzyme hypothesis might also account for a number of other differences that have been observed between odd- and even-ratio conversion (see Kitani & Whitehouse, 1974*a*).

As mentioned in section 1, Kitani & Olive (1969) recognized two types of correction of mismatched bases which they called restoration, meaning restoration of the genotype existing before hybrid DNA formation, and substitution, that is, correction that leads to the genotype of the other parent. According to the two-enzyme hypothesis of conversion, the degradation enzyme can give rise only to the restoration type of base correction, whereas the correction enzyme can generate either type. Kitani & Olive found variation in the frequencies of the restoration and

substitution events between one mutant and another, between one-point and two-point crosses for the same mutant, and between one chromatid and the other involved in the event. These sources of variation can all be attributed on the present hypothesis to an effect on the activity of both the degradation and correction enzymes of the molecular nature of the mismatch, including an influence of the second mutant site on enzyme behaviour at the first site. The latter possibility is discussed in section 10.

6. TRITYPE AND TETRATYPE ODD-RATIO CONVERSION

In a cross between a spore colour mutant and wild type, asci showing a 5:3 or a 3:5 ratio of wild-type:mutant spores, and with a parental combination of outside markers, can be of two kinds, *tritype* and *tetratype*. If the parental genotypes are *M g N* and *m + n*, where *M/m* and *N/n* are the flanking markers, the 4 meiotic chromatids that give rise to these asci have the following genotypes:

tritype 5:3	*M g N*, *M g*/+*N*,	*m* +*n*, *m*+*n*;
tritype 3:5	*M g N*, *M g* *N*,	*m g*/+*n*, *m*+*n*;
tetratype 5:3	*M g N*, *M* +*N*,	*m g*/+*n*, *m*+*n*;
tetratype 3:5	*M g N*, *M g*/+*N*,	*m g* *n*, *m*+*n*;

where *g*/+ indicates hybrid DNA, and hence postmeiotic segregation. To explain their origin, the tetratype asci require hybrid DNA in both chromatids at the spore colour mutant site, followed by conversion in one of them, whereas the tritype asci can be accounted for by hybrid DNA in only one chromatid (Fig. 3).

The data for *g* locus mutants crossed with wild type, with the exception of the 3:5 asci from crossed DG (mutant 2 in the presence of guanine) and K (mutant 3), gave totals of 453 tritype and 226 tetratype odd-ratio asci (see Table 5 of Kitani & Whitehouse, 1974*b*). On the assumption that hybrid DNA in both chromatids gives tritype and tetratype asci with equal frequency, the 227 excess tritypes are attributable to hybrid DNA in only one chromatid, implying that polynucleotide degradation, on Sobell's hypothesis, occurs in 33·4% of cases. The exceptional crosses (DG and K) gave 64 tritype and only 5 tetratype 3:5 asci.

Stadler & Towe (1971), in their investigation of mutants at the *w*17 locus in *Ascobolus* found 53 tritype and only 1 tetratype odd-ratio conversion ascus. This indicates degradation in 96% of the recombinant asci.

7. DISTAL SYMMETRIC EVENTS

Distal symmetric events are those in which there is evidence that hybrid DNA has reached the distal mutant site within the *g* locus in both chromatids involved in the event, but the proximal site shows normal 4:4 segregation and there has been no recombination between the proximal mutant site and the proximal flanking marker (*mat*). In *ds*1 (Tables 1 and 2) there is postmeiotic segregation at the distal site in both chromatids. In the other *ds* genotypes there are various

combinations of postmeiotic segregation in one chromatid and conversion in the other, or conversion in both, in each case involving only the distal site.

The numbers of *ds* asci observed in each cross are given in Table 3. The total frequency of the *ds* genotypes is constant at about 2·5% of the recombinant genotypes in all the crosses (Fig. 2). The constancy of *ds* frequency, like that of *da*, is discussed in section 9.

8. PROXIMAL ASYMMETRIC EVENTS

In this class of asci hybrid DNA appears to have reached the proximal mutant site in the *g* locus in only one chromatid, and there is no recombination between the distal site, which shows normal 4:4 segregation, and the distal flanking marker (*corona*). The 8 genotypes in this category (*pa* genotypes in Tables 1 and 2) are the counterparts of the 8 in *da*.

Table 4. *Estimated percentage frequencies of* pa, ps *and* rr *genotypes out of total aberrant asci for each cross*

(Brackets indicate less certain estimates. See Tables 1 and 2 for an explanation of the ascus genotypes. The figures are based on the pooled data for parental and recombinant outside markers, except for *ps*4 (always parental) and *rr* (always recombinant): see Table 1.)

	Ascus genotypes								
Cross	*pa*1	*pa*2	*pa*3	*pa*4	*ps*1	*ps*2	*ps*3	*ps*4	*rr*
1 × 2	(3)	—	(3)	—	(0)	(0)	(0)	(0)	(0)
2 × 5	3·4	3·1	4·5	4·7	1·7	0	0	0	0
6 × 5	6·4	—	11·1	—	0·6	0	0·6	0·6	2·9
1 × 5	6·8	—	12·3	—	0	0	0	0	0
5 × 4*b*	17·1	—	5·4	—	14·3	1·1	4·0	0	4·1
5 × 4	21·0	—	4·0	—	9·7	1·1	7·1	0	4·0
1 × 3	(15)	—	(19)	—	(5)	(1)	(11)	(0)	(0–1·6)
1 × 4*b*	20·1	—	16·6	—	2·7	0	5·5	0	0
1 × 4	6·8	—	11·5	—	8·1	2·7	14·2	2·6	10·1
1,4 × +	—	6·3	—	0	7·7	9·3	19·3	5·9	10·3

The estimated frequencies of the *pa* genotypes (ignoring the flanking markers) are given in Table 4 and the total frequency of *pa* events is plotted in Fig. 4 for each cross. In trans heterozygotes with the distal allele the paler one (Tables 8, 11–17 of Kitani & Whitehouse, 1974*b*) the *pa*2 and *pa*4 genotypes cannot be recognized. When the proximal allele is the paler one (cross 2 × 5, Table 9 of Kitani & Whitehouse, 1974*b*) all the *pa* genotypes can be recognized. Two points are therefore plotted for this cross: the total frequency of *pa* events, and the frequency of *pa*1 and *pa*3 alone for comparison with the other crosses. In a cis heterozygote with the distal allele the paler (cross 1,4 × +, Table 18 of Kitani & Whitehouse, 1974*b*) *pa*2 and *pa*4 can be recognized but not *pa*1 and *pa*3.

Although *pa* frequencies are rarely known with precision, such that the standard error can be given in only one instance (Fig. 4), nevertheless there is no doubt that, in general, the frequency of *pa* events (*pa*1 and *pa*3), unlike *da*, rises

with increasing separation of the mutant sites, except in the crosses of mutants 1 and 4. Since with the longer intervals the *pa* asci contribute substantially to the asci with wild-type spores, it could be argued that the evidence for increased separation of the sites depends on the increase in *pa* asci. However, as Kitani & Whitehouse (1974*b*) indicate, the evidence for greater site separation of some mutants than others does not depend solely on the frequency of asci with wild-type spores. Thus, the 1–4*b* interval is believed to be longer than either the 1–5 or 5–4*b* intervals because the flanking marker behaviour in the pairwise crosses points to the site sequence 1–5–4*b*.

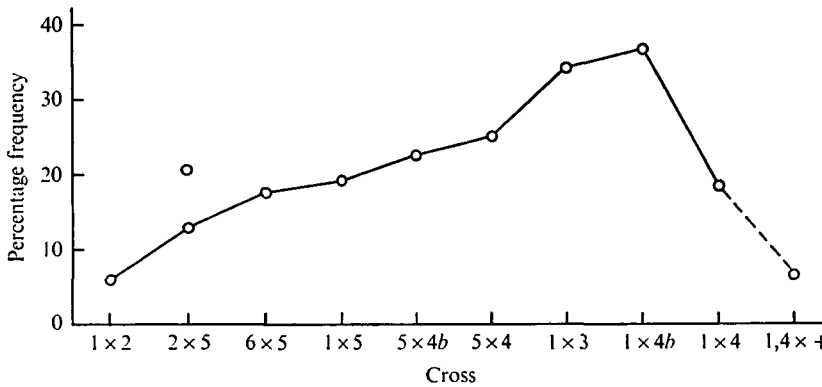

Fig. 4. The total frequency of the proximal asymmetric genotypes *pa*1 and *pa*3 is plotted as a percentage of all aberrant asci for each cross. The crosses are in order of increasing separation of the mutant sites, as far as this is known. The upper point in the 2 × 5 cross shows the total frequency of *pa* events (that is, including *pa*2 and *pa*4 as well as *pa*1 and *pa*3). This frequency is not known for the other crosses. In the 1,4 × + cross the point plotted is the total frequency of *pa*2 and *pa*4 asci, *pa*1 and *pa*3 not being recorded in this cross.

The significance of the rise in *pa* frequency with increasing site separation, and the exceptional behaviour of the crosses of mutants 1 and 4, are discussed in sections 9 and 10.

9. PROXIMAL SYMMETRIC EVENTS

The counterparts of distal symmetric events are proximal ones (*ps*) in which hybrid DNA appears to have reached the proximal mutant site at the *g* locus in both chromatids, but the distal site shows normal 4:4 segregation and no recombination with the distal flanking marker (*corona*). The *ps* genotypes are listed in Tables 1 and 2 and their estimated frequencies (ignoring the outside markers) are given in Table 4 for each cross. The totals are plotted in Fig. 5(*a*). It is evident that the *ps* frequency is low (0–2 % of the recombinant asci) for the closer sites, rising abruptly to about 20 % for most of the more distant ones. The 1 × 4*b* cross is exceptional and is discussed in section 10.

The site separation at which *ps* frequency rises abruptly might correspond to the modal length of the migratory hybrid DNA structure postulated by Sobell

(1972) since, according to his ideas, the end of the structure would be fixed in position at the site of the first mutant it encountered. Another possibility is that this site separation corresponds to the length of polynucleotide degraded exonucleolytically (see section 4), proximal events with closer sites appearing as *pa* instead of *ps*. It is evident from Table 4 and Fig. 4 that *pa* events are still frequent with these close sites. These explanations for the abrupt rise in *ps* events are not mutually

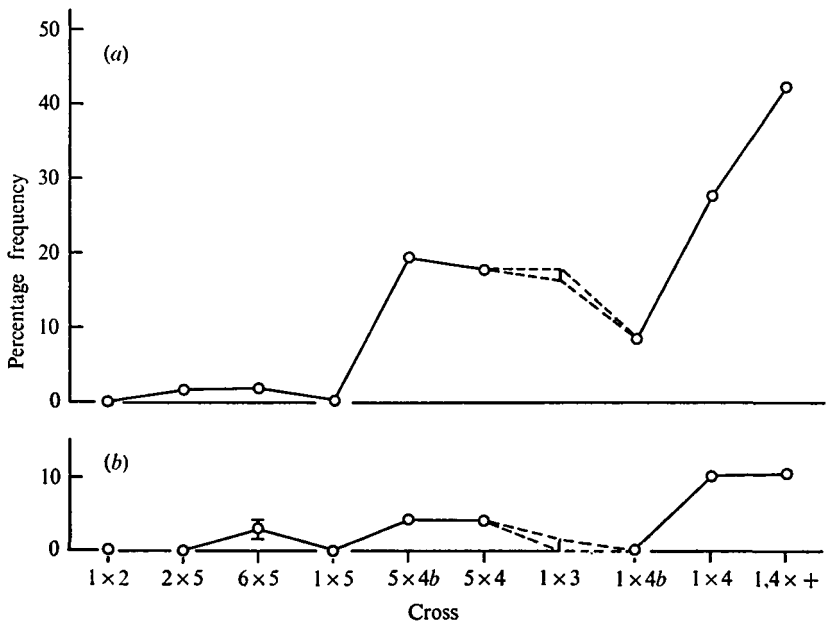

Fig. 5. The total frequencies of (*a*) proximal symmetric (*ps*) asci and (*b*) reciprocal recombination (*rr*) asci are plotted as percentages of all recombinant asci for each cross. The crosses are in order of increasing separation of the mutant sites, as far as this is known. Standard errors are given where possible. In the 1 × 3 cross the frequencies are uncertain and lines indicate the most likely values.

exclusive and it may be that both contribute because, as indicated in section 6, evidence from crosses of *g* mutants with wild type suggests that polynucleotide degradation (if that is the explanation for *pa* and *da* events) occurs in only one third of the aberrant asci.

The constancy of distal events (*da* and *ds*) with increasing site separation compared with the rise in proximal events (*pa* and *ps*) may imply a hybrid DNA structure of larger size for the distal events. The frequency of *da* and *ds* events for all the crosses corresponds to that of *pa* and *ps* events for the shorter intervals before the abrupt rise in *ps* shown at the 5–4*b* and 5–4 separation. If the hybrid DNA structure of distal origin frequently extended to the second site, the events of distal origin corresponding to *pa* and *ps* of proximal origin would often appear as complex events involving hybrid DNA at both sites. Conversely, a relatively small hybrid DNA structure of proximal origin might explain why the *pa* and *ps* events increase in frequency with increasing site separation, the likelihood of the

second site not being reached by the hybrid DNA increasing with increasing distance between the mutant sites. The length of the promoter region of DNA to which recombination proteins may bind in the initiation of eukaryote recombination (Sobell, 1972) might determine the size of the hybrid DNA structure initiated at that region. The idea that the distal hybrid DNA structure at the *g* locus is larger than the proximal is discussed further in section 10.

10. RECIPROCAL RECOMBINATION

The expression *reciprocal recombination* (*rr*) refers to the ascus genotype in Tables 1 and 2 in which each allele shows a normal 4:4 segregation, but with recombination between them and no additional recombination between the alleles and the flanking markers on either side. The simplest explanation of *rr* asci is that the hybrid DNA of a crossover was confined to the interval between the sites of the alleles. Another possibility is reciprocal conversion in association with a crossover: if the hybrid DNA of the crossover had reached one of the mutant sites in both chromatids, conversion to wild type might have occurred in one chromatid and to mutant in the other. Reciprocal conversion at the proximal and distal sites, respectively, and associated with parental outside marker combinations, is presumed to be the explanation for the *ps*4 and *ds*4 genotypes (see Table 1).

The frequency of *rr* asci can be estimated with reasonable precision in all the crosses except 1×3, although the standard error can be calculated in only one instance. The frequencies are given in Table 4. The numbers of *ds*4 asci found in each cross are given in Table 3. It is clear that *ds*4 asci are rare in all the crosses, only one certain example having been found. It is likely, therefore, that reciprocal conversion at the distal site can be neglected as a source of *rr* asci. The estimated frequency of *ps*4 asci is given in Table 4. Comparing the *ps*4 and *rr* frequencies it seems likely that only in the crosses of mutants 1 and 4 has reciprocal conversion at the proximal site contributed appreciably to the *rr* asci. In these crosses it is likely that the 10 % of *rr* asci comprised about 3 % arising by reciprocal conversion at the proximal site (the counterpart, with recombinant flanking markers, of the *ps*4 genotype) and the other 7 % by interallelic crossing-over.

The frequency of *rr* asci shows similarity to the frequency of *ps* events, as can be seen from Table 4 and Fig. 5. The reduced frequency of both in the $1 \times 4b$ cross compared with the crosses of 1 and 4 is of particular interest in view of the origin of mutant 4*b* from mutant 4 (see Table 1 of Kitani & Whitehouse, 1974*b*). The $1 \times 4b$ cross, when compared with the 1–4 crosses, shows:

(1) fewer *ps* events (about 8 % instead of 30 % – see Fig. 5*a*),
(2) fewer *rr* events (none instead of 10 % – see Fig. 5*b*),
(3) more *pa* events (about 35 % instead of 20 % – see Table 4 and Fig. 4), and
(4) less conversion of mutant 1 to wild type in the proximal events.

The latter difference may relate primarily to the change in the proportion of *pa* and *ps* events, because there is less conversion of mutant 1 to wild type in *pa* events in $1 \times 4b$ than in *ps* events in 1×4. Thus, in $1 \times 4b$ the *pa*1 genotype

(postmeiotic segregation) and *pa*3 (conversion) are equally frequent, whereas in the 1–4 crosses *ps*1 (a counterpart of *pa*1: see Table 2) is less frequent than *ps*3 (a counterpart of *pa*3): see Table 4.

Sobell (1973) suggested that interallelic crossing-over could be accounted for by a migratory hybrid DNA structure and I had made (Whitehouse, 1967) a rather similar suggestion. The hybrid DNA might have reached the region between the alleles from the proximal or the distal end of the gene. Considering first the possibility of proximal entry, and applying this idea to the 1×4 cross, it must be supposed that when mutant 1 enters the hybrid DNA structure the nuclease attack triggered by the mismatched bases occurs on the distal side of the site of the mutant. Continued movement distally of the hybrid DNA structure would then restore homoduplexes on the proximal side of the nicks, with the result that the hybrid DNA structure would come to lie between the sites of the alleles but not extending to either of them. This is on the assumption that the distance between the sites of mutants 1 and 4 is greater than the length of the hybrid DNA structure. The primary difference between interallelic crossing-over and a *ps* event would be that the endonuclease attack triggered by the entry of mutant 1 into the hybrid DNA structure would occur proximal to the site of 1 in *ps* events and distal to it in interallelic crossing-over. To explain the lack of interallelic crossing-over in the $1 \times 4b$ cross, it would then be necessary to suppose that the presence of mutant 4*b* instead of 4 alters the behaviour of the nuclease. For the second mutant to influence the course of events the hybrid DNA structure must temporarily reach the second site (4 or 4*b*). An alternative possibility is that mutant 4*b* is a deletion such that there is insufficient space between its site and that of mutant 1 to accommodate the hybrid DNA structure. This explanation, however, seems untenable because the $5 \times 4b$ cross shows interallelic crossing-over, despite the fact that mutant 5 is nearer to the site of 4*b* than is mutant 1.

Considering secondly the possibility of distal entry of a hybrid DNA structure, a series of events complementary to those described for proximal entry can be envisaged. The difference between the 1×4 and $1 \times 4b$ crosses as regards interallelic crossing-over can now be attributed to a direct effect of the 4 and 4*b* heteroduplex mismatches on the activity of the nuclease, nicking proximal to 4 and distal to 4*b*. This explanation, however, is unsatisfactory, for the following reasons:

(1) It predicts that the events in the $1 \times 4b$ cross corresponding to interallelic crossing-over in 1×4 will be distal events (*da* or *ds*). The $1 \times 4b$ cross, however, shows no increase in these classes compared with the crosses of 1 and 4 (Fig. 2).

(2) It fails to explain why mutants 4 and 4*b* behave alike when crossed with mutant 5, both crosses giving about 4% of interallelic crossing-over.

It is tentatively concluded that the *rr* asci at the *g* locus result from proximal entry of a hybrid DNA structure and that whether or not interallelic crossing-over takes place depends on the molecular nature of both mutations as well as on their distance apart. If the position of the hybrid DNA structure can oscillate by rotary diffusion, both mutants could influence the course of events even though neither

is ultimately included in the structure. Such an explanation, besides accounting for the mutant specificity of reciprocal recombination of alleles, first demonstrated with *Ascobolus immersus* (Lissouba *et al.* 1962), will explain in general terms such peculiarities as the finding of interallelic crossing-over in the 5×4, $5 \times 4b$ and 1×4 crosses, but not in $1 \times 4b$. The similarity of the *rr* and *ps* graphs (Fig. 5) is also accounted for if the *rr* events are of *ps* origin. The occurrence of *pa* events in $1 \times 4b$ in place of the *ps* and *rr* events of 1×4 may imply that the exonucleolytic

Table 5. *Data of Fields & Olive (1967) for the* hyaline-1 *locus of* Sordaria brevicollis

(A total of 385 asci with wild-type spores from 10 pairwise crosses were scored for flanking markers. Eight of the asci required more complex events to explain their origin and are excluded from the table. All the asci classified as *da* or *ds* could also have arisen from more complex events. Owing to the use of the centromere as proximal outside marker, parental and recombinant outside marker genotypes could not be distinguished in the *pa* and *ps* asci. For an explanation of the ascus genotypes see Table 2.)

Ascus genotype	Total	Ascus genotype	Flanking markers: Parental	Flanking markers: Recombinant
*pa*1	19	*da*1	9	0
*pa*3	148	*da*3	58	10
*ps*1	0	*ds*1	0	0
*ps*2	0	*ds*2	0	0
*ps*3	0	*ds*3	0	1
*ps*4 or *rr*	130	*ds*4	2	—

Table 6. *Data of Fogel & Hurst (1967) for the* histidine-1 *locus of* Saccharomyces cerevisiae

(A total of 1081 asci with wild-type spores from 3 pairwise crosses were scored for flanking markers. 112 asci required more complex events to explain their origin and are excluded from the table. For an explanation of the ascus genotypes see Table 2.)

Ascus genotype	Flanking marker genotype: Parental	Recombinant	Uncertain
*da*3	46	77	3
*pa*3	462	268	12
*ds*4	0	—	—
*ps*4	4	—	3
rr	—	94	

activity of the nuclease can also be influenced, either directly or indirectly, by the same forces that determine the final position of the hybrid DNA structure. The exceptionally low frequency of *pa* events in the 1–4 crosses (Fig. 4), and the exceptionally high frequency of *ps* and *rr* asci in these crosses (Fig. 5), support this conclusion. Furthermore, the difference between *pa* events (in $1 \times 4b$) and *ps* events (in 1×4) in the frequency of conversion of mutant 1 to wild type may

imply that the exonucleolytic activity of the nicking enzyme and the activity of the mismatch correction enzyme are interrelated.

It was suggested in section 9 that the hybrid DNA structure entering the *g* locus from the distal end might be larger than that of proximal origin. This hypothesis might also explain the apparent absence of *rr* asci of distal origin. It is known that reciprocal recombination of alleles is rare or absent with closely linked mutants (Rossignol, 1967; cf. Fig. 5) and a likely explanation (Sobell, 1973) is that the mutants are situated too close together to accommodate an entire hybrid DNA structure. If hybrid DNA structures from opposite ends of a gene differ in size, the minimum site separations allowing interallelic crossing-over would also differ for proximal and distal origins.

The data of Fields & Olive (1967) for the *hyaline-1* locus of *Sordaria brevicollis* (Table 5) and of Fogel & Hurst (1967) for the *histidine-1* locus of *Saccharomyces cerevisiae* (Table 6) reveal lower frequencies of *ds* and *ps* asci than were found in the *g* locus of *Sordaria fimicola*. The *rr* ascus frequencies, however, were comparatively high* though, as usual, with much variation in frequency from one cross to another. It seems likely, therefore, that in these genes degradation of a polynucleotide in one molecule is very frequent when a mutant enters a hybrid DNA structure, but that inclusion of the entire structure between the sites of the alleles is also frequent.

11. POLARITY IN ODD- AND EVEN-RATIO EVENTS

Bond (1973) discovered from crosses between mutants of the *buff* gene in *Sordaria brevicollis* that distal events produce more asci with one wild-type spore than with two, while proximal events produce approximately equal numbers. This information was obtained from the relative numbers of the two parental outside marker genotypes in the wild-type spores. He pointed out that a similar polarity in odd- and even-ratio events is apparent in the 1×3, $1 \times 4b$ and 1×4 crosses for the *g* locus of *S. fimicola*, but that the effect here, unlike the *S. brevicollis* data, may be attributable to marker effects, the distal alleles happening to show more postmeiotic segregation than the proximal ones.

Among the simpler events at the *g* locus of *S. fimicola*, the chief sources of asci with one wild-type spore are the *da*1, *pa*1 and *ps*1 genotypes and with two wild-type spores *da*3, *pa*3 and *ps*3 (see Table 2). In all the crosses *da*1 is more frequent than *da*3, on average by a factor of three (Table 3). On the other hand, in the crosses showing the odd-even polarity (1×3, $1 \times 4b$, 1×4), *pa*1 and *pa*3 are equally frequent and *ps*1 is less frequent than *ps*3 (Table 4). This difference between proximal and distal events in the relative frequency of postmeiotic segregation (giving one wild-type spore) and conversion to wild-type (giving two wild-type spores) seems to be the primary source of the odd-even polarity in these crosses.

* In the *Sordaria brevicollis* data *rr* cannot be distinguished from *ps*4, but the absence of *ps*1-3 makes it likely that the asci in question were *rr* and not *ps*4.

How far the difference is attributable to marker effects is uncertain. Mutant 5 as a distal mutant (crosses 2×5, 6×5, 1×5) showed *da*1 about 1·5 times as frequent as *da*3 (Table 3), while as a proximal mutant (crosses $5 \times 4b$, 5×4) it showed *pa*1 about 4 times as frequent as *pa*3 (Table 4). The difference in behaviour is probably significant as it is based on the genotypes of over 100 asci, and there was consistency within the two groups of crosses. It is possible therefore that the odd-even polarity may arise, partly at least, from a difference in the activity of the degradation and correction enzymes in the hybrid DNA structures of proximal and distal origin, respectively. Some such difference in enzyme activity seems necessary, in any case, to explain the *S. brevicollis* data, as Bond has implied. It has already been suggested (section 10) that the difference in behaviour of mutant 1 in the 1×4 and $1 \times 4b$ crosses may result from variation in the activity of the degradation and correction enzymes in relation to the position of the hybrid DNA structure.

12. FREQUENCY OF SIMPLE EVENTS

The simple recombination events listed in Table 1 comprise about 70–80 % of the total events for both the shortest and longest intervals studied, but only about 50 % for intermediate site separations. (The frequencies are obtained by

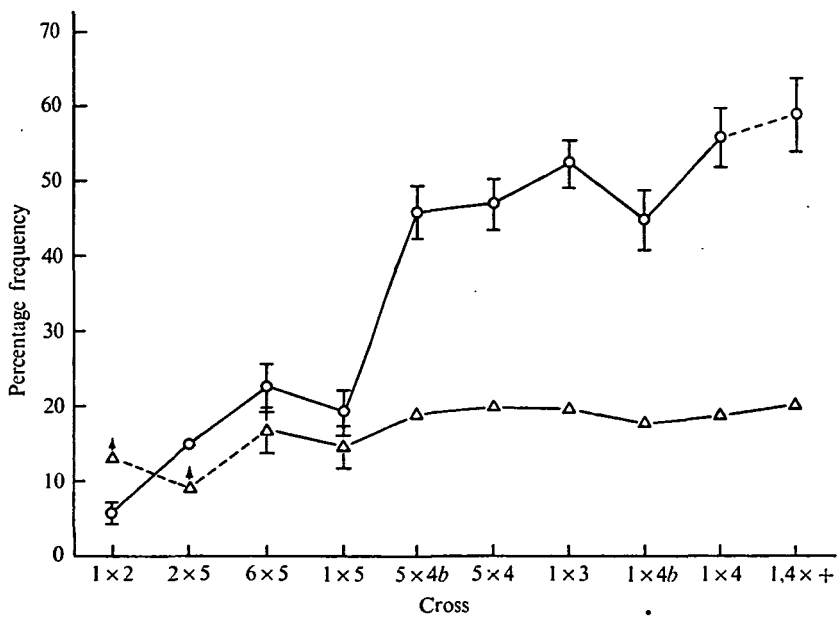

Fig. 6. The total frequencies of simple proximal events – *pa*1, *pa*3, *ps* and *rr* – (○) and of simple distal events – *da* and *ds* – (△) are plotted as percentages of all aberrant asci for each cross. The crosses are in order of increasing separation of the mutant sites, as far as this is known. Standard errors are given where possible. In the $1,4 \times +$ cross the value for proximal events includes *pa*2 and *pa*4 instead of *pa*1 and *pa*3. In the graph of distal events the minimum value is plotted for the 1×2 cross, and the *da*2 and *da*4 genotypes are omitted from the value for the 2×5 cross.

summing the graphs in Figs. 1 and 6.) The reason for this pattern is that with closely located sites the linked events (linked postmeiotic segregation and co-conversion) predominate, while with widely spaced sites simple proximal events (*pa* and *ps*) predominate. The total frequency of the latter, together with reciprocal recombination (also believed to be proximal in origin – see section 10) is plotted in Fig. 6. It is possible to give standard errors for most of these frequencies because in crosses with the distal allele the paler one, that is, all the crosses except 2×5, the simple proximal events in the aggregate form a discrete ascus phenotype. The rise in simple proximal events with increasing site separation is thus seen to be significant, while the total frequency of simple distal events (*da* and *ds*, also plotted in Fig. 6) is constant. The total frequencies of events of proximal and distal origin do not necessarily differ, however, from one cross to another, because there may be compensating changes in the frequencies of linked and of complex events, where the hybrid DNA origin usually cannot be determined.

13. PARENTAL AND RECOMBINANT FLANKING MARKER GENOTYPES

The numbers of aberrant asci with parental and with recombinant flanking marker genotypes obtained from crosses between mutants at the *g* locus and wild type are given in Table 5 of Kitani & Whitehouse (1974*b*). For the aberrant 4:4

Table 7. *Frequencies of aberrant asci with recombinant flanking marker genotypes from crosses between mutants at the* g *locus and wild type*

(The data are derived from Table 5 of Kitani & Whitehouse, 1974*b*.)

Cross	Aberrant segregation (wild type: mutant)	Flanking marker genotype: Parental	Recombinant	Total	Percentage recombinant and standard error
All	4:4	294	283	577	49·0 ± 2·1
All except R(w)	5:3	459	388	847	45·8 ± 1·7
	3:5	263	205	468	43·8 ± 2·3
	Total odd	722	593	1315	45·1 ± 1·4
R(w)	Odd	26	5	31	16·1 ± 6·6
All except A-1	6:2	336	241	577	41·8 ± 2·1
	2:6	59	38	97	39·2 ± 5·0
	Total even	395	279	674	41·4 ± 1·9
A-1	Even	36	8	44	18·2 ± 5·8
All	Total	1473	1168	2641	44·2

asci the outside marker recombination frequency does not differ significantly from 50% in any of the crosses nor in the total data. The odd-ratio asci (5:3 and 3:5) give a slightly lower value (45·1%) and the even-ratio conversion asci (6:2 and 2:6) show a still lower outside marker recombination frequency (41·4%). These

frequencies exclude crosses (R(w) for the odd ratios and A-1 for the even ratios) which differ significantly from the remainder (see Table 7). The difference between the even-ratio conversion asci and the aberrant 4:4 asci in flanking marker behaviour is significant ($\chi_1^2 = 7{\cdot}4$, $P < 0{\cdot}01$). The difference in flanking marker behaviour of different types of aberrant asci was pointed out by Kitani & Olive (1967).

Table 8. *Numbers of aberrant asci with parental (P) and with recombinant (R) flanking marker genotypes from two-point crosses*

(The segregation pattern refers to the paler mutant of the two in each cross, as indicated in the left-hand column.)

			Segregation (wild type:mutant)															
		Table no. (Kitani & Whitehouse, 1974*b*)	Aberrant 4:4		5:3		3:5		6:2		2:6		Normal 4:4		Total		P or R	Total
Mutant	Cross		P	R	P	R	P	R	P	R	P	R	P	R	P	R		
2	1 × 2	8	23	15	42	29	44	38	14	5	31	19	14	6	168	112	13	293
	2 × 5	9	8	9	8	9	6	5	5	2	2	3	4	2	33	30	1	64
	2 × 5	10	12	12	13	8	3	2	3	5	—	—	8	4	39	31	0	70
5	6 × 5	11	13	10	22	25	14	12	13	7	6	1	25	18	93	73	4	170
	1 × 5	12	13	7	16	21	16	16	17	8	3	0	30	12	95	64	3	162
3	1 × 3	15	10	8	15	10	27	12	1	1	6	5	76	60	135	96	10	241
4*b*	5 × 4*b*	13	16	11	21	15	12	13	8	1	0	3	59	48	116	91	2	209
	1 × 4*b*	16	8	5	14	7	20	15	1	1	7	2	36	33	86	64*	4	154
4	5 × 4	14	10	7	28	19	17	8	7	4	2	1	56	48	120	87	4	211
	1 × 4	17	8	4	12	4	13	8	3	1	5	3	41	49	82	69	3	154
	1,4 × +	18	6	1	7	5	8	3	3	3	3	1	31	30	58	43	1	102
		Total	127	89	198	152	180	132	75	38	65	38	380	310	1025	760*	45	1830

* Including an ascus of uncertain type of aberrant segregation.

The numbers of aberrant asci with parental and with recombinant flanking marker genotypes observed in the two-point crosses are given in Table 8. The segregation patterns refer to the paler mutant of the two in each cross as indicated in the table. It is not possible to give comparable results for the darker mutant because not all the asci were fully scored for this. The data for each type of aberrant segregation are individually homogeneous. Even-ratio conversion, however, gives a lower frequency of flanking marker recombination than odd-ratio events (see Table 9), the difference being significant at the 1 in 20 level ($\chi_1^2 = 4{\cdot}0$, $P = 0{\cdot}05$–$0{\cdot}02$).

The asci with normal 4:4 segregation for the paler mutant (the aberrant segregation in these asci being shown by the darker mutant of the two) are heterogeneous for flanking marker behaviour. From crosses with relatively short intervals between the mutant sites (1 × 2, 2 × 5, 6 × 5 and 1 × 5) there was significantly less outside marker recombination ($\chi_1^2 = 7{\cdot}0$, $P < 0{\cdot}01$) than with the longer intervals (5 × 4*b*, 5 × 4, 1 × 3, 1 × 4*b*, 1 × 4 and 1,4 × +) – see Table 9. There

seem to be several factors contributing to this difference, one being the increased occurrence of interallelic crossing-over with longer intervals: see sections 10 and 14.

The two-point data give lower flanking marker recombination frequencies than the one-point for every type of aberrant ascus (compare Tables 7 and 9), and for aberrant 4:4 asci the difference is significant at the 1 in 20 level ($\chi^2_1 = 3{\cdot}9$, $P = 0{\cdot}05$).

Table 9. *Frequencies of aberrant asci at the* g *locus with recombinant flanking marker genotypes from two-point crosses*

(The data, which refer to the paler mutant only in each cross, are derived from Table 8. With the normal 4:4 segregations short intervals refer to crosses 1 × 2, 2 × 5, 6 × 5 and 1 × 5 and long intervals to the other two-point crosses.)

		Flanking marker genotype			
Segregation (wild-type: mutant)	Cross	Parental	Recombinant	Total	Percentage recombinant and standard error
Aberrant 4:4	All	127	89	216	41·2 ± 3·3
5:3	All	198	152	350	43·4 ± 2·6
3:5	All	180	132	312	42·3 ± 2·8
Total odd	All	378	284	662	42·9 ± 1·9
6:2	All	75	38	113	33·6 ± 4·4
2:6	All	65	38	103	36·9 ± 4·8
Total even	All	140	76	216	35·2 ± 3·2
Normal 4:4	Short intervals	81	42	123	34·1 ± 4·3
	Long intervals	299	268	567	47·3 ± 2·1
	Total	380	310	690	44·9
Total	All	1025	760*	1785*	42·6

* Including an ascus (see Table 16 of Kitani & Whitehouse, 1974*b*) that could not be referred to a particular class of aberrant asci.

The equality of parental and recombinant flanking marker genotypes in the aberrant 4:4 asci from the one-point crosses is predicted by the model proposed by Holliday (1964): see Sigal & Alberts (1972). The progressive decline in flanking marker recombination from aberrant 4:4 to odd to even conversion ascus types in the one-point data would be accounted for if the occurrence of conversion can deflect a potential recombinant flanking marker event into one associated with a parental genotype for the markers. This would also explain why the recombination frequencies are lower in the two-point crosses, because many of the asci will have undergone conversion at the other site.

The two-point data can be examined to see if particular combinations of events at the two sites are associated with peculiarities of outside marker behaviour. There are six genotypes listed in Tables 1 and 2 which are associated with 6:2 or 2:6 ratios for one or both of the *g* mutants: *cc*1, *cc*2, *pa*3, *pa*4, *da*3 and *da*4. The

co-conversion genotypes, omitting those crosses (2×5 for *cc*1, and 1×2, 1×5, $5 \times 4b$, 1×3, $1 \times 4b$ and 1×4 for *cc*2) where incomplete scoring has led to uncertainty, reveal only 5 asci (18·5 %) with recombinant outside markers in a total of 27. No useful information is available for the *pa*4 genotype of proximal asymmetric events, but *pa*3 data are available from Tables 9–14 of Kitani & Whitehouse (1974*b*) and indicate outside marker recombination within the range 16 (23·2 %) out of 69 to 19 (33·9 %) out of 56. The distal asymmetric events associated with conversion (*da*3 and *da*4), omitting crosses with uncertain counts (see Table 3), reveal 13 asci (28·9 %) with flanking marker recombination in a total of 45.

It is evident that the even-ratio conversion in these *cc*, *pa* and *da* asci is associated with less flanking marker recombination (24·1–28·9 %) than is shown by even-ratio conversion in the two-point data as a whole (35·2 %: see Table 9), though the difference is not significant. On the assumption, however, that the effect is real, since the outside marker recombination frequency in the *cc*, *pa* and *da* asci (taking the highest value) is significantly less than in even-ratio conversion asci in the one-point data ($\chi^2_1 = 7{\cdot}0$, $P < 0{\cdot}01$), it is instructive to consider what might be the peculiarity of the conversion in these asci that distinguishes it from the generality of even-ratio conversion. As already indicated, *pa* and *da* events are most simply interpreted as hybrid DNA that has reached only one of the two mutant sites (proximal and distal, respectively), and furthermore the hybrid DNA may be confined to only one chromatid. The peculiarity of conversion in these asci might then be that it was occurring near one or other end of the segment of hybrid DNA. In the co-conversion asci, excision and resynthesis (if that is the conversion mechanism) is evidently extensive and on that account might also reach an end of the hybrid DNA segment, though there is no direct evidence for this.

The hypothesis that conversion at the end of a region of hybrid DNA is associated with less recombination of flanking markers than conversion elsewhere in the hybrid DNA receives support from the work of Carlson (1970, 1971) on the *rudimentary* (*r*) locus in *Drosophila melanogaster*. He studied recombination in this gene by making pairwise crosses between *r* mutants and scoring wild-type recombinants for flanking markers. Some 45 *r* mutants were studied. When the mutants were well spaced (4 or more intervening mutant sites, or recombination frequencies above 2·5 per 10^5 progeny) the flanking marker recombination approximated to 60 %, but with closely-linked mutants in any part of the gene this frequency declined progressively as the interval was shortened (Fig. 7). With the shortest intervals, that is, mutants giving 0·5 or fewer wild-type recombinants per 10^5 progeny, 6 such recombinants were obtained and all had a parental combination of outside markers. With the short intervals it is reasonable to assume that the recombinants arose by conversion at one of the two mutant sites. It is likely that in many cases the other site remained outside the hybrid DNA, as in the *pa* and *da* asci. In other words, in selecting wild-type recombinants with progressively shorter intervals between the mutants one is selecting for conversion which on average occurred progressively nearer the end of the hybrid

DNA segments. The fall in outside marker recombination, as the interval between the sites is shortened, is therefore in agreement with the hypothesis that conversion at the end of a hybrid DNA segment favours an event that gives a parental outside marker genotype. Indeed, the fact that the flanking marker recombination frequency seems to fall to zero as the interval is shortened (Fig. 7) implies on this hypothesis that conversion at the end of a hybrid DNA segment is always associated with a parental genotype for the outside markers.

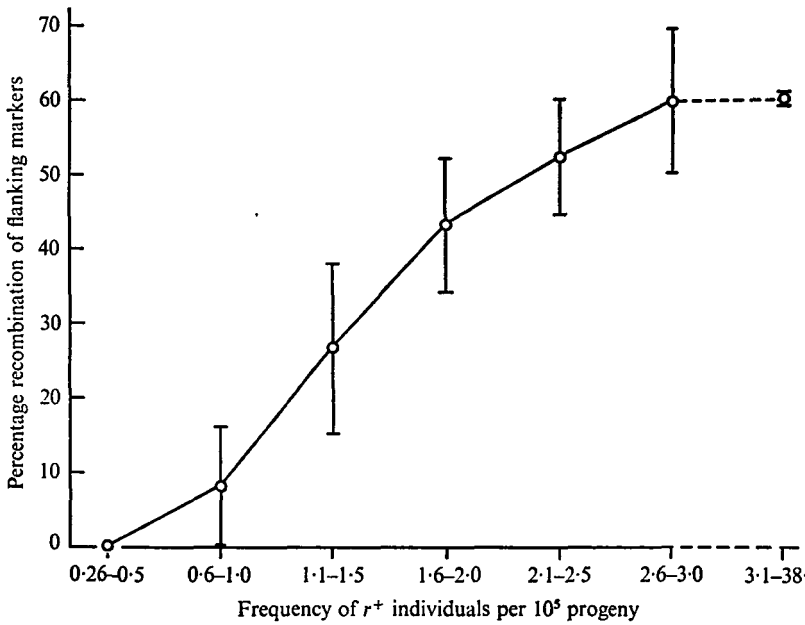

Fig. 7. The percentages of recombinant flanking markers in r^+ progeny from inter-allelic crosses of *r* mutants (*rudimentary* wings) of *Drosophila melanogaster* are plotted against the recombination frequency, from the data of Carlson (1970, 1971).

That events at the ends of a region of hybrid DNA should affect the flanking marker genotype is in agreement with the Holliday–Sobell model, which attributes this genotype (parental or recombinant) to the relationship between the nucleotide chains cut at each end of the hybrid DNA structure.

On Sobell's hypothesis the initial nicking of the two duplexes, although triggered by the entry of a mutant into hybrid DNA, need not mean that the hybrid DNA ends close to the site of the mutant. This is inferred from the study by Gutz (1971) of the behaviour of mutants of the *ad-6* gene of *Schizosaccharomyces pombe*. He found that mutant M26 of this gene showed conversion about 12 times as often as the other alleles he investigated, and moreover, unlike them, with a bias in favour of conversion to wild type. A corresponding increased frequency of conversion and bias in direction was imposed on these mutants, however, through co-conversion, if M26 was also present. Evidently M26 is recognized more often than the other mutants when it enters a hybrid DNA structure. Despite this

evidence that M26, when present, acts as the trigger for much of the conversion in this gene, Gutz found from a three-point cross that M26 often showed co-conversion simultaneously with mutants M216 and L52 whose sites are located one on each side of that of M26. It is not necessary to suppose, therefore, that a mutant which triggers endonuclease attack will necessarily be situated close to the end of the hybrid DNA in the final recombinant molecule.

Table 10. *Numbers of* pa*1 and* da*1 asci with parental and with recombinant flanking marker genotypes in crosses involving short intervals in the* g *locus*

	*pa*1			*da*1		
Cross	Parental	Recom-binant	Either	Parental	Recom-binant	Either
1 × 2	0–7*	0	1	16	10	1
2 × 5	7	8	0	1–6*	1	0
6 × 5	8–9*	2	0	9	4	1
1 × 5	3–9*	2	0	3	9	0

* These numbers are likely to be near the top of the range, because the alternative genotypes (*ps*1 and *ps*2 for *pa*1, and *ds*1 and *ds*2 for *da*1) have always been rare with short intervals whenever their number has been known at all accurately (see Tables 3 and 4).

It seems possible that degradation of a polynucleotide by the nicking enzyme may favour the parental flanking marker genotype when such degradation extends to the end of the hybrid DNA, in the same way as has already been suggested for mismatch correction. The evidence for this possibility comes from the *Sordaria fimicola g* locus data for the *pa*1 ascus genotype (see Table 10). There was a marked preponderance of the parental combination of outside markers (only 13–31 % with recombination) in *pa*1 asci when the interval between the mutant sites was short (crosses 1 × 2, 6 × 5 and 1 × 5), though cross 2 × 5 was an exception and there was also no marked excess of parental genotypes in the *da*1 asci. This diversity is discussed in section 14.

14. POLARITY IN FLANKING MARKER RECOMBINATION FREQUENCY AND THE OCCURRENCE OF FREQUENCIES ABOVE 50 %

In section 13 it was suggested that 50 % recombination frequency for flanking markers in aberrant asci was basic and that this frequency could be reduced by conversion. In the data for the *r* locus in *Drosophila melanogaster* (Carlson, 1971), however, it was pointed out that the outside marker recombination frequency was 60 % in the r^+ progeny from all the crosses except those with very low recombination frequencies (Fig. 7).

A possible explanation for this recombination frequency above 50% is the occurrence of interallelic crossing-over, evidence for which Carlson obtained from experiments using attached-*X* strains. This explanation would also account for the high frequency of outside marker recombination observed in asci which

showed reciprocal recombination at the *g* locus. Those with parental flanking marker combinations have the *ds*4 and *ps*4 genotypes and those with recombinant flanking markers have the *rr* genotype. Reference to Tables 3 and 4 shows that *rr* is often more frequent than the sum of *ds*4 and *ps*4 – indeed it was this difference that led to the idea (section 10) that most of the *rr* asci resulted from interallelic crossing-over. The *ds*4 and *ps*4 genotypes are the counterparts of *rr* asci when the latter arise by reciprocal conversion, but the counterpart of *rr* asci which result from interallelic crossing-over is non-crossover hybrid DNA confined between the sites of the pair of alleles, and this cannot be detected without a third allele. This genotype was discovered by Rossignol (1967) with gene 75 of *Ascobolus immersus*: he found that flanking alleles of a mutant showing conversion could have normal 4:4 segregation and a non-recombinant genotype with one another. Fogel *et al.* (1971) found similar examples at the *arg-4* locus in *Saccharomyces cerevisiae* and confirmed, moreover, that outside markers as well as flanking alleles have a parental genotype with one another. It is evident that the occurrence of outside marker recombination frequencies above 50% in two-point crosses when the alleles show reciprocal recombination may be a consequence of the failure to detect the corresponding non-crossover event.

Nevertheless, flanking marker recombination frequencies above 50% cannot always be explained in this way. Fogel & Hurst (1967) found 62·6% flanking marker recombination in 123 *da*3 asci from three crosses of mutants of the *his-1* locus in *S. cerevisiae* (Table 6). There was no such excess in 730 *pa*3 asci from the same crosses (36·7% recombination). This polarity in outside marker recombination frequency may also apply to the *buff* locus in *Sordaria brevicollis* (Bond, 1973). What may be the same phenomenon has also been detected in prototroph selection experiments from pairwise crosses of allelic auxotrophic mutants in *Neurospora crassa*, recombinant flanking markers being more frequent with mutants at the proximal than at the distal end of *me-2* (Murray, 1963), *his-5* (Smith, 1965) and *am-1* (Fincham, 1967) and more frequent at the distal than at the proximal end in *pan-2* (Case & Giles, 1959) and *his-3* (Webber, 1965). Outside marker recombination frequencies above 50% were shown by prototrophs from pairwise crosses of mutants at the proximal end of *his-5* and at the distal end of *pan-2* and *his-3*. Frequencies above 50% are also regularly found in similar crosses in *Aspergillus nidulans* (review: Whitehouse & Hastings, 1965).

The occurrence of more than 50% flanking marker recombination with conversion genotypes (as distinct from reciprocally recombinant ones) has not been observed in the *g* locus in *S. fimicola*. There are indications, however, of polarity in outside marker recombination frequency in the *pa*1 and *da*1 genotypes when the interval between the mutant sites is short (Table 10). The *da*1 genotype seems to be associated with more flanking marker recombination than *pa*1, except in the 2 × 5 cross. A possible explanation is that the exonucleolytic action of the nicking enzyme may be non-random in action, not only between wild-type and mutant polynucleotides (see sections 4 and 5) but also between the two mutant (or the two wild-type) ones. This would be comparable to the finding by Spatz & Trautner

(1970) that mismatch correction, as revealed by single burst experiments in transfection studies using phage *SPP1* of *Bacillus subtilis*, may favour wild type in one heteroduplex and mutant in the reciprocal one for the same mutant. This is the explanation proposed by Emerson (1966) – see section 5 – for different biasses in odd- and even-ratio conversion.

On the degradation hypothesis, the *pa*1 and *da*1 genotypes require erosion of one of the mutant polynucleotides to account for their origin. The two mutant polynucleotides will differ in molecular polarity on the Holliday–Sobell model, so degradation of one will mean excision in the proximal direction and of the other in the distal direction. Calling these the *p* and *d* mutant polynucleotides, respectively, if the *d* mutant chain was degraded more often than *p* with mutants 1 and 6, the enzyme activity might often extend to the end of the hybrid DNA structure in *pa*1 events when one or other of these mutants was the proximal allele and the site interval was short. The short interval implies that the hybrid DNA ends near the site of the mutant, since it does not reach the second site. According to the hypothesis proposed in section 13, this excision to the end of the hybrid DNA would lead to a parental flanking marker genotype. The polarity in outside marker recombination frequency between *pa*1 and *da*1 would then be accounted for, unless the distal allele likewise lowered the recombination frequency in *da*1 events (as a result of preferential excision of the *p* mutant chain). The preferential excision hypothesis predicts that some mutants may show a reversed polarity (preferential degradation of *p* mutant chains instead of *d*) and furthermore, that mutants such as 1 and 6, which give a low flanking marker recombination frequency in *pa*1 asci when either of them is the proximal allele in a short-interval two-point cross, will not give a low value in *da*1 asci when either of them is the distal allele in a similar cross, since preferential degradation of the *d* mutant chain which might bring excision to the end of the hybrid DNA in a *pa* event will take it away from the end in a *da* event.

The high frequency of the parental outside marker genotype shown by the *da*1 asci in Fields & Olive's (1967) data for the *hyaline-1* locus in *S. brevicollis* (Table 5) might have a similar explanation to that proposed above for *pa*1 asci in certain *S. fimicola g* locus crosses.

Preferential degradation of *p* or *d* mutant chains, if it occurs, will be expected also to affect the *pa*3 and *da*3 asci, but the interpretation of polarity here in the flanking marker recombination frequency, as in Fogel & Hurst's (1967) data for the *his-1* locus in *Saccharomyces cerevisiae* (Table 6), is complicated by the presumed activity, in the origin of these genotypes, of both the degradation and correction enzymes, each of which may show preferential excision for particular mutants. A further complication in the yeast data is the presence of a mechanism that allows certain conversion asci (*da*3) to show more than 50% flanking marker recombination.

I am grateful to Dr Y. Kitani and Professor H. M. Sobell for their critical reading of this paper in manuscript, and to Professor P. S. Carlson for permission to refer to data in his thesis. Support from Science Research Council grant no. B/SR/8848 is acknowledged.

REFERENCES

Bond, D. J. (1973). Polarity of gene conversion and post-meiotic segregation at the *buff* locus in *Sordaria brevicollis*. *Genetical Research* **22**, 279–289.

Carlson, P. S. (1970). A genetic analysis of the *rudimentary* locus of *Drosophila melanogaster*. Ph.D. Dissertation, Yale University.

Carlson, P. S. (1971). A genetic analysis of the *rudimentary* locus of *Drosophila melanogaster*. *Genetical Research* **17**, 53–81.

Case, M. E. & Giles, N. H. (1959). Recombination mechanisms at the *pan-2* locus in *Neurospora crassa*. *Cold Spring Harbor Symposia on Quantitative Biology* **23**, 119–135.

Emerson, S. (1966). Quantitative implications of the DNA-repair model of gene conversion. *Genetics* **53**, 475–485.

Fields, W. G. & Olive, L. S. (1967). The genetics of *Sordaria brevicollis*. III. Gene conversion involving a series of hyaline ascospore color mutants. *Genetics* **57**, 483–493.

Fincham, J. R. S. (1967). Recombination within the *am* gene of *Neurospora crassa*. *Genetical Research* **9**, 49–62.

Fogel, S. & Hurst, D. D. (1967). Meiotic gene conversion in yeast tetrads and the theory of recombination. *Genetics* **57**, 455–481.

Fogel, S., Hurst, D. D. & Mortimer, R. K. (1971). Gene conversion in unselected tetrads from multipoint crosses. *Second Stadler Symposium, Columbia, Missouri*, pp. 89–110.

Fogel, S. & Mortimer, R. K. (1969). Informational transfer in meiotic gene conversion. *Proceedings of the National Academy of Sciences of the U.S.A.* **62**, 96–103.

Gutz, H. (1971). Site specific induction of gene conversion in *Schizosaccharomyces pombe*. *Genetics* **69**, 317–337.

Holliday, R. (1964). A mechanism for gene conversion in fungi. *Genetical Research* **5**, 282–304.

Kitani, Y. & Olive, L. S. (1967). Genetics of *Sordaria fimicola*. VI. Gene conversion at the *g* locus in mutant × wild type crosses. *Genetics* **57**, 767–782.

Kitani, Y. & Olive, L. S. (1969). Genetics of *Sordaria fimicola*. VII. Gene conversion at the *g* locus in interallelic crosses. *Genetics* **62**, 23–66.

Kitani, Y. & Whitehouse, H. L. K. (1974*a*). Effect of the proportion of parental nuclei in a heterokaryon on the pattern of gene conversion in *Sordaria fimicola*. *Molecular and General Genetics* **131**, 47–56.

Kitani, Y. & Whitehouse, H. L. K. (1974*b*). Aberrant ascus genotypes from crosses involving mutants at the *g* locus in *Sordaria fimicola*. *Genetical Research* **24**, 229–250.

Lissouba, P., Mousseau, J., Rizet, G. & Rossignol, J.-L. (1962). Fine structure of genes in the ascomycete *Ascobolus immersus*. *Advances in Genetics* **11**, 343–380.

Murray, N. E. (1963). Polarized recombination and fine structure within the *me-2* gene of *Neurospora crassa*. *Genetics* **48**, 1163–1183.

Rossignol, J.-L. (1967). Contribution à l'étude des phénomènes de recombinaison intragénique. Thèse, Université de Paris. 167 pp.

Sigal, N. & Alberts, B. (1972). Genetic recombination: the nature of a crossed strand-exchange between two homologous DNA molecules. *Journal of Molecular Biology* **71**, 789–793.

Smith, B. R. (1965). Interallelic recombination at the *his-5* locus in *Neurospora crassa*. *Heredity* **20**, 257–276.

Sobell, H. M. (1972). Molecular mechanism for genetic recombination. *Proceedings of the National Academy of Sciences of the U.S.A.* **69**, 2483–2487.

Sobell, H. M. (1973). Symmetry in protein-nucleic acid interaction and its genetic implications. *Advances in Genetics* **17**, 411–490.

Spatz, H. C. & Trautner, T. A. (1970). One way to do experiments on gene conversion? Transfection with heteroduplex *SPP*1 DNA. *Molecular and General Genetics* **109**, 84–106.

Stadler, D. R. & Towe, A. M. (1971). Evidence for meiotic recombination in *Ascobolus* involving only one member of a tetrad. *Genetics* **68**, 401–413.

Webber, B. B. (1965). Genetical and biochemical studies of histidine-requiring mutants of *Neurospora crassa*. IV. Linkage relationships of *hist-3* mutants. *Genetics* **51**, 275–283.

Whitehouse, H. L. K. (1967). Secondary crossing-over. *Nature* **215**, 1352–1359.

Whitehouse, H. L. K. & Hastings, P. J. (1965). The analysis of genetic recombination on the polaron hybrid DNA model. *Genetical Research* **6**, 27–92.