A *buff* spore colour mutant in *Sordaria brevicollis* showing high-frequency conversion

1. Characteristics of the mutant

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SUMMARY

A mutant, YS17, at the *buff* spore colour locus in *Sordaria brevicollis*, when crossed with wild type, gives rise to aberrant asci with a frequency over 10 times that of other *buff* mutants. Over 98 % of the aberrant asci have 6 wild type and 2 mutant spores. From tests with another *buff* mutant it is concluded that loss of the mutant spore colour when YS17 shows conversion to wild type is associated with loss of the high frequency conversion, and that both characters are caused by the same mutation. A methionine-requiring mutant (*met-1*) has been obtained that maps 5 units to the left of *buff*, and this, together with the nicotinamide-requiring mutant (*nic-1*) 2 units to the right, has provided flanking markers for *buff* that can be scored with complete reliability. Crosses between YS17 and 28 other *buff* mutants have revealed close linkage to three of them which map to its right on the basis of flanking marker behaviour, all the others mapping to its left. The frequency of post-meiotic segregation at the sites of *buff* mutants near to the site of YS17 is greatly increased in the presence of YS17, and occurs in the chromatid showing conversion to wild type at YS17.

From these and other results, obtained largely by ascus analysis, the following conclusions have been drawn.

(1) The YS17 mutation is probably acting as a recognition site for an endonuclease that initiates recombination, with the result that the frequency of heteroduplex DNA within the *buff* gene is much increased.

(2) The recombination initiated at YS17 is asymmetric (or at least predominantly so), with the YS17 site acting as a recipient of a nucleotide chain from the other parent, not a donor to it.

(3) The frequency of crossing over associated with conversion at YS17 is variable: about 30 % in crosses with most of the *buff* mutants, about half this value in crosses with wild type, and almost zero in crosses with closely-linked *buff* mutants.

(4) In about one third of the crossover asci in crosses between YS17 and other *buff* mutants the crossover is not adjacent to the site of YS17 but separated from it by the site of the allele, which shows normal 4:4 segregation.

(5) It seems necessary to revive the idea of more than one recombination event in proximity, a non-crossover conversion event sometimes leading to a second event – a crossover – in the vicinity. It is tentatively suggested that both might be controlled by a single enzyme aggregate.

1. INTRODUCTION

In the course of an investigation of conversion behaviour of spore colour mutants of *Sordaria brevicollis* induced with various mutagens, a mutant, YS17, at the *buff* spore colour locus was obtained that showed conversion almost entirely to wild type and with a frequency about 20 times that of any other *buff* mutant. The peculiarities of this mutant were mentioned by Yu-Sun, Wickramaratne & Whitehouse (1974). Its behaviour has now been investigated in detail and forms the subject of the present paper. The data have provided information about several aspects of the process of recombination. Some of the results were unexpected and conflict with current models.

During the investigation of the YS17 mutant a suppressor of its high frequency conversion was discovered. The buff spore colour of YS17 was unaffected. The results obtained from study of the suppressor will be given in a second paper.

A summary of the findings with YS17 and the suppressor has been prepared (MacDonald, Sang & Whitehouse, 1979).

2. MATERIALS AND METHODS

The spore colour mutants used in this work are at the *buff* locus, which is about 4 units from the centromere in the right arm of linkage group II of *Sordaria brevicollis*. Mutants C47, C53, C67, C69, C70, C79, C80, S6, S41, S44, S140, S156, S180 and YS9 were induced by irradiation with ultra-violet light; YS43 and YS100 were obtained using N-methyl-N'-nitro-N-nitrosoguanidine (NG); YS39, YS40, YS117 and YS132 using ethylmethane sulphonate (EMS); and YS14, YS17, YS19, YS20, YS61, YS69, YS77, YS85 and YS86 using the acridine mustard ICR170. Those mutants prefixed by the letter C were obtained by K.-C Chen at Columbia University and the remainder were obtained in this laboratory, S6 to S156 by M. H. V. Cooray, S180 by D. J. Bond, and those prefixed by the letters YS by C. C. Yu-Sun (Yu-Sun, Wickramaratne & Whitehouse, 1977). Mutant C80 was called *h* (hyaline) by Lamb (1969).

Two auxotrophic outside markers were used. The proximal marker *met-1* maps 5 units from *buff* in the left arm of the chromosome. It was U.V.-induced by one of us (M.V.M.) using the method developed in this laboratory by Wickramaratne (1976). The distal marker *nic-1* maps about 2 units from *buff* and has been used previously by Bond (1973). The *met-1* and *nic-1* mutants have provided flanking markers for *buff* that can be scored with complete reliability.

The crossing and germination media used were those reported by Yu-Sun *et al.* (1977). Glucose minimal medium of the following composition: 20 g D-glucose, 100 ml × 10 Westergaard salt solution, 15 g Difco plain agar, made up to 1 l. with distilled water, was used on its own and with a supplement of 0·01 g of either methionine or nicotinamide to test the auxotrophic mutants.

The *buff* mutants were crossed to wild type and also in pairwise crosses in the presence of flanking markers. The crosses were made by inoculating opposite sides

of a petri dish with the two strains. The plates were kept at 25 °C and perithecia were mature in about 10 days.

The conversion pattern of a mutant was obtained by transferring mature perithecia to a microscope slide and then crushing them in a drop of 8 % glucose. The clusters of asci were divided to prevent asci overlapping. The intact asci were counted and classified according to the different ratios of wild-type:mutant spores. Throughout this paper the number of wild-type spores is given before the colon and of mutant spores after it.

Ascospores were isolated from the crosses in one of two ways: either the eight ascospores were dissected out by hand from intact asci using a fine platinum needle, or the crosses were inverted over petri-dish lids containing 4 % agar. The shot spores adhered to the agar and the lids were scanned. The isolated spores were grown up separately and tested for flanking marker characters. The isolates were back-crossed to check their genotype.

3. RESULTS

As already pointed out, mutant YS17, when crossed with wild type, was found to give rise with high frequency to asci with 6 wild-type and 2 mutant spores. In order to study this behaviour crosses of a number of different kinds have been investigated.

(a) Crosses of YS17 × YS17

No wild-type spores have been observed among very large numbers of progeny ascospores from crosses of YS17 with itself. It is estimated that a total of approximately 1.5×10^6 spores have been scanned without finding any wild-type ones. It is concluded that the frequent occurrence of asci with 6 wild-type spores in crosses between YS17 and wild type results from conversion and not from mutation.

(b) Crosses of YS17 with wild type

When first obtained the YS17 mutant gave aberrant asci when crossed with wild type (crosses 1 and 2 in Table 1) with a frequency of $11.6 \pm 0.9 \%$. Re-isolates of YS17 from various crosses showed considerable variation in the frequency of aberrant asci, ranging from $3.8 \pm 0.4 \%$ to $19.7 \pm 2.1 \%$ without any clear indications of discontinuities of frequency. The counts for the various crosses are given in Table 1. Aberrant 4:4 asci are not included in the table, as observing them is obscured by spindle overlap or spore displacement.

Crosses 1 and 2 refer to perithecia of YS17 and of wild-type maternal parentage, respectively, from the same dish. There is no evidence for an effect of the maternal parent on aberrant ascus frequency.

Crosses 1–16 are of YS17 of A mating type crossed with wild type of a mating type, and crosses 17–34 are of wild type $A \times YS17a$. There is no evidence for an effect of the mating type of YS17 on total aberrant ascus frequency, but there are indications that the 2:6 and 5:3 frequencies may be influenced. With YS17A

there were no 2:6 asci and 11 5:3's, while with YS17a the corresponding figures were 8 and 2.

It is evident from Table 1 that there is a marked excess of 6:2 asci over all other kinds of aberrant asci in all the crosses. In the total data 6:2 asci constitute

Table 1. *Results of ascus counts in crosses between YS17 and wild type*

Cross no.	Wild-type:mutant spores							Aberrants		
	4:4	6:2	2:6	5:3	3:5	8:0	Total	Total	%	SE(±)
1	1221	159	—	—	—	1	1381	160	11·6	0·9
2	1148	147	—	—	—	3	1298	150	11·6	0·9
3	1894	207	—	1	—	3	2105	211	10·0	0·7
4	1988	167	—	—	—	1	2156	168	7·8	0·6
5	1978	116	—	1	—	1	2096	118	5·6	0·5
6	1776	235	—	—	—	4	2015	239	11·9	0·7
7	1873	146	—	—	—	—	2019	146	7·2	0·6
8	1758	162	—	1	—	1	1922	164	8·5	0·6
9	1762	312	—	5	—	1	2080	318	15·3	0·8
10	1909	167	—	—	—	—	2076	167	8·0	0·6
11	1933	110	—	—	—	1	2044	111	5·4	0·5
12	299	63	—	—	—	1	363	64	17·6	2·0
13	313	54	—	1	—	1	369	56	15·2	1·9
14	281	61	—	—	—	—	342	61	17·8	2·1
15	1714	271	—	2	—	3	1990	276	13·9	0·8
16	342	15	—	—	—	2	359	17	4·7	1·1
17	1683	165	1	—	—	3	1852	169	9·1	0·7
18	1854	251	—	1	—	3	2109	255	12·1	0·7
19	1739	223	2	—	—	2	1966	227	11·5	0·7
20	1985	120	1	—	1	—	2107	122	5·8	0·5
21	2032	89	1	—	—	1	2123	91	4·3	0·4
22	1898	73	—	—	—	2	1973	75	3·8	0·4
23	1665	246	—	1	—	4	1916	251	13·1	0·8
24	1907	222	—	—	—	4	2133	226	10·6	0·7
25	1875	266	—	—	—	2	2143	268	12·5	0·7
26	1877	230	1	—	—	1	2109	232	11·0	0·7
27	1925	81	—	—	—	1	2007	82	4·1	0·4
28	1767	95	—	—	—	—	1862	95	5·1	0·5
29	1960	101	—	—	—	—	2061	101	4·9	0·5
30	1939	100	2	—	—	—	2041	102	5·0	0·5
31	1841	249	—	—	—	4	2094	253	12·1	0·7
32	1892	173	—	—	—	1	2066	174	8·4	0·6
33	1895	101	—	—	—	—	1996	101	5·1	0·5
34	301	74	—	—	—	—	375	74	19·7	2·1
Total	54224	5251	8	13	1	51	59548	5324	8·9	—

98·6 % of them. The significance of this conversion pattern is discussed in section (*d*) below and in the Discussion, section (*b*)(iii).

Asci with a 6:2 ratio from a cross between YS17 and wild type were analysed. The flanking markers were present. The asci were dissected, the spores germinated and the resulting cultures tested for the flanking marker characters. The results are given in Table 2. There are 6 possible ascus genotypes, *a–f*. Classes *a* and *b*

Table 2. *Analysis of asci from a cross between YS17 and wild type in the presence of flanking markers*

$$\text{Parents:} \frac{+ \ \ YS17 \ \ +}{met \ \ \ + \ \ \ nic}$$

Class	Genotype	Observed numbers of asci	Estimated numbers after allocating incompletely scored asci to classes a-f
a	+ YS17 + + + + met + nic met + nic	71	90
b	+ YS17 + + + nic met + + met + nic	17	21
c	+ + nic + + + met YS17 + met + nic	5	6
d	+ YS17 nic + + + met + + met + nic	0	0
e	+ + nic + + nic met + + met YS17 +	0	0
f	+ YS17 nic + + nic met + + met + +	0	0
a, c or d	—	20	—
b or d	—	4	—
Total		117	117

correspond to conversion without crossing-over, and conversion associated with crossing-over, respectively. The other genotypes (*c–f*) result from incidental crossovers in the interval between the flanking markers. The effects of such cross-overs are as follows.

(i) A non-crossover event at *buff* (class *a*) plus an incidental crossover on the proximal side will give rise to ascus genotype *b* if 2 chromatids, or one combination of 3 chromatids, are involved altogether, and to genotype *c* if 4 chromatids or the other combination of 3. The effects of an incidental crossover on the distal instead of the proximal side are similar, except that genotype *d* is substituted for *c*. It follows that, if there is no chromatid interference, incidental crossovers will give rise to genotype *b* as often as the sum of *c* and *d*.

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(ii) A crossover event at *buff* (class *b*) plus an incidental crossover on the proximal side will give rise to genotypes *a*, *b*, *c*, and *e*, depending on the chromatid relationship of the two events (2, one kind of 3, the other kind of 3, and 4 chromatids, respectively). The corresponding genotypes with a distal incidental crossover are *a*, *b*, *d*, and *f*.

Owing to poor germination of the *buff* spores some of the asci could not be fully scored (see Table 2). If these asci are ignored, there were 17 asci (class *b*) with crossovers apparently associated with the conversion out of 93, that is, 18 %. Using the argument in (i) above, however, it can be inferred that about 5 asci in class *b* resulted from incidental crossovers. The number with crossing-over associated with the conversion of YS17 is therefore estimated to be 12 out of 93, or 13 %. A similar result is obtained if the incompletely scored asci are allocated to classes *a–f* in proportion to the observed numbers (right-hand column of Table 2). The frequency of 13 % is one of the lowest ever recorded and only half the value found for 6:2 asci with other *buff* mutants (Sang & Whitehouse, 1979*a*).

Two asci showing postmeiotic segregation were also dissected from the same cross. One had a 5:3 and the other a 3:5 ratio of wild type:*buff* spores. Both asci were found to have a non-crossover genotype for the flanking markers and to be tritype, that is with the four products of meiosis comprising three different genotypes.

About 1 % of the aberrant asci in crosses between YS17 and wild type have an 8:0 segregation, that is, all 8 spores of wild-type genotype. The most likely explanation of their origin is the occurrence of two 6:2 events involving different chromatids. The expected number of 8:0 asci, on the assumption that the two 6:2 events occur independently, can be calculated approximately from the expression

$$(8:0) = (6:2)^2/4N$$

where brackets surrounding an ascus symbol, e.g. (6:2), indicate the number of such asci observed in a total count of N asci (Leblon, 1972*a*; Lamb & Wickramaratne, 1973).

The numbers of 8:0 asci in the individual crosses are insufficient for a test of agreement with expectation, but for the data as a whole the expected number of 8:0 asci is 116. This is significantly greater ($P < 0.01$) than the observed number of 51. It appears that two 6:2 events involving in aggregate 3 or all 4 chromatids are less frequent than would be expected by chance if the two events occurred independently.

(c) *High-frequency conversion and spore colour – one mutation or two?*

A question of central importance is whether the *buff* spore colour and the high-frequency conversion associated with YS17 are properties of a single mutation or two closely linked ones. In an attempt to distinguish these possibilities, wild-type spores resulting from conversion of YS17 were tested against another *buff* mutant to find out if its conversion frequency was raised. The supposition was that if two

separate mutations were involved, conversion of the spore-colour mutation to wild type would not necessarily be associated with conversion of the other mutant site. Spores that were wild type in colour but carrying a mutation giving high frequency conversion could then be detected.

The 6:2 asci that were dissected from a cross between YS17 and wild type in the presence of the flanking markers (section (*b*)) were used to investigate this question. The wild-type spores resulting from conversion could often be distinguished by their flanking marker genotype from those derived directly from the wild-type parent (see Table 2). The spores that were wild type as a result of conversion (including the parental ones where there was uncertainty) were crossed with mutant S156 and the conversion frequency examined. None showed a raised frequency. It is concluded that the spore colour and high frequency conversion shown by YS17 are properties of a single mutation. If two separate mutations are involved their sites must be so close together that co-conversion occurs in over 99 % of conversion events.

(d) *Crosses of other* buff *mutants with wild type*

In order to compare the behaviour of YS17 with that of the 28 other *buff* mutants available, each in turn (except S44 and S140) has been crossed with wild type, and progeny asci scored for the occurrence of aberrant asci (other than aberrant 4:4). The results are given in Table 3. The mutants are grouped by mutagen and within each group are placed in order of decreasing frequency of aberrant asci. It is evident that all the alleles have an aberrant ascus frequency at least an order of magnitude lower than YS17.

Leblon (1972*a*, *b*) showed that in *Ascobolus immersus* base-substitution mutants gave a much higher frequency of postmeiotic segregation than frameshift mutants. Furthermore, he found that a majority of the frameshifts fell into two classes, A and B. In class A the aberrant asci were predominantly with 6 wild-type and 2 mutant spores, and in class B the converse. From reversion studies the A and B classes were believed to correspond to deletions and additions of nucleotides, respectively.

Yu-Sun *et al.* (1977) demonstrated a similar relationship in *Sordaria brevicollis* between conversion pattern and mutagen. They found that mutants of the *grey-3*, *grey-4* and *grey-5* genes induced with ICR170 gave a low frequency of postmeiotic segregation (either class A or class B), while the majority of those induced with ultraviolet light and all those induced with EMS and NG gave a much higher frequency of postmeiotic segregation (class C). YS17 is clearly an A class mutant. With *buff* mutants other than YS17 the frequency of aberrant asci is so low that large numbers of asci need to be scored before individual mutants can be classified for conversion pattern. Counts for the UV-induced mutants S41, S6, S156, S180 and S44 by Ahmad (1975) indicated postmeiotic segregation frequencies of about 58 % of the aberrant asci for each of the mutants, that is, they were of C class. Counts reported by Sang & Whitehouse (1979*a*) indicate that ICR170-induced mutant YS20 and UV-induced mutants C69, YS9 and C53 are A class, ICR170-

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induced mutant YS85 is B class, and UV-induced mutants C47, C70 and C67 and EMS-induced mutants YS40, YS39 and YS132 are C class.

Table 3. *Results of ascus counts in crosses between* buff *mutants (other than YS17) and wild type*

| Mutagen | Mutant | Wild type:mutant | | | | | Other | | Total asci | Aberrants | | |
		4:4	6:2	2:6	5:3	3:5	Ratio	No.		Total	%	S.E.
ICR 170	YS77	3143	1	6	—	4	—	—	3154	11	0·35	0·11
	YS19	1892	1	3	1	1	—	—	1898	6	0·32	0·14
	YS61	3902	1	11	—	—	—	—	3914	12	0·31	0·09
	YS86	2038	1	2	—	3	—	—	2044	6	0·29	0·12
	YS85	4068	1	10	—	—	—	—	4079	11	0·27	0·08
	YS69	2339	—	2	—	1	—	—	2342	3	0·13	0·07
	YS20	2911	2	—	—	—	8:0	1	2914	3	0·10	0·06
	YS14	3212	—	—	—	—	—	—	3212	0	0	—
UV	C67	2271	—	4	—	4	—	—	2279	8	0·35	0·12
	S41	3845	1	2	—	8	—	—	3856	11	0·29	0·08
	C47	5902	4	7	2	2	8:0	1	5918	16	0·27	0·07
	C69	4065	7	2	—	1	8:0	1	4076	11	0·27	0·08
	YS9	3871	3	4	1	1	—	—	3880	9	0·23	0·10
	S6	3701	3	1	1	3	—	—	3709	8	0·22	0·08
	C70	4214	3	3	—	3	—	—	4223	9	0·21	0·07
	S156	5910	—	5	—	4	1:7	1	5920	10	0·17	0·05
	C53	2152	1	2	—	—	—	—	2155	3	0·14	0·08
	C80	2198	—	1	1	—	7:1	1	2201	3	0·14	0·08
	S180	4016	1	1	—	—	—	—	4018	2	0·05	0·04
	C79	2258	—	—	—	1	—	—	2259	1	0·04	0·04
EMS	YS40	1824	—	5	—	2	—	—	1831	7	0·38	0·14
	YS39	1672	2	3	—	1	—	—	1678	6	0·36	0·15
	YS132	4041	2	1	2	4	—	—	4050	9	0·22	0·07
	YS117	1941	1	1	—	—	—	—	1943	2	0·10	0·07
NG	YS100	1923	2	2	2	—	—	—	1929	6	0·31	0·13
	YS43	1802	1	4	—	—	—	—	1807	5	0·28	0·12

It is evident that the *buff* mutants show a similar relationship between conversion pattern and mutagen to that already demonstrated for other spore colour genes in *S. brevicollis*, namely, ICR170: A or B class; UV: A or C; EMS: C.

From the evidence provided by Leblon (1972*b*) for *Ascobolus* it would be inferred that YS17, being of A class, was a deletion frameshift. This conclusion is questioned in the Discussion, section (*b*)(iii).

(e) Crosses between YS17 and alleles

The YS17 mutant has been crossed in turn with each of the other *buff* mutants and asci from each cross scored for recombinants with wild-type spores. The results are given in Table 4.

Most of the crosses have given recombinant asci with a frequency of 2–11 %. These high frequencies are evidently attributable to YS17, because the corresponding frequencies for crosses between alleles other than YS17 rarely exceed

Table 4. *Results of ascus counts in crosses between* YS17 *and other* buff *mutants*

Mutagen	Mutant crossed with YS17	Wild-type:mutant spores							Total asci	Recombinants		
		0:8	1:7	2:6	3:5	4:4(Exp.)	5:3	6:2		Total	%	Range
ICR 170	YS77	10931	25	1101	—	8 (25·1*)	—	—	12056	1134	9·4	6·8 −11·9
	YS19	12354	3	276	—	1 (1·5)	—	—	12634	280	2·2	1·5 − 3·1
	YS61	11812	13	732	—	7 (10·7)	—	—	12562	752	6·0	4·3 − 7·5
	YS86	12402	17	444	1	1 (3·8)	—	—	12865	463	3·6	2·2 − 4·4
	YS85	11177	30	1151	—	11 (26·8*)	—	—	12369	1192	9·6	7·2 −11·5
	YS69	11453	10	819	1	6 (13·6)	—	—	12289	836	6·8	4·5 − 9·4
	YS20	11829	16	1119	—	36 (24·1)	—	—	13002	1171	9·0	5·4 −12·2
	YS14	10889	7	748	—	6 (12·0)	—	—	11650	761	6·5	3·7 − 7·7
UV	C67	11652	104	1074	7	8 (24·7*)	—	—	12845	1193	9·3	6·0 −13·4
	S41	11540	14	1305	—	15 (33·3*)	—	—	12874	1334	10·4	7·2 −12·8
	C47	11440	19	534	2	2 (5·9)	—	—	11998	557	4·6	3·8 − 5·5
	C69	10986	16	1365	1	130 (37·3*)	—	1	12499	1513	12·1	5·3 −15·2
	YS9	12402	10	1277	1	127 (29·5*)	—	1	13818	1416	10·3	7·0 −12·9
	S6	11970	21	820	—	10 (13·1)	—	—	12821	851	6·6	2·7 − 8·6
	C70	11737	146	1245	16	14 (29·5*)	—	—	13158	1421	10·9	6·6 −12·9
	S156	11312	9	826	1	4 (14·0*)	—	—	12152	840	6·9	4·0 − 9·5
	C53	12112	5	688	2	27 (9·2*)	—	1	12835	723	5·6	3·6 − 6·4
	C80	12371	3	94	—	3 (0·2)	—	—	12417	100	0·81	0·39− 1·23
	S180	11551	194	1135	7	8 (25·0*)	—	—	12895	1344	10·4	7·2 −12·3
	S44	12152	6	6	—	6 (0·0)	—	—	12170	18	0·15	<0·01− 0·32
	S140	12379	4	23	—	2 (0·0)	—	—	12408	29	0·23	0·05− 0·52
	C79	11155	24	943	—	14 (19·9)	—	—	12136	981	8·1	4·2 −12·8
EMS	YS40	11086	53	1191	6	24 (28·7)	—	—	12360	1274	10·3	8·0 −14·2
	YS39	11221	23	1328	34	50 (34·8)	—	1	12657	1436	11·3	6·6 −14·6
	YS132	11326	22	1123	7	23 (25·2)	1	1	12503	1177	9·4	6·0 −11·2
	YS117	11871	12	?22	21	23 (13·2*)	—	1	12750	879	6·9	5·2 − 8·7
NG	YS100	11985	14	749	4	11 (11·0)	—	—	12763	778	6·1	3·7 − 7·4
	YS43	11782	103	848	2	4 (14·1*)	—	—	12739	957	7·5	6·7 − 8·7

The expected frequencies of 4:4 asci are explained in the Results section (*h*) (iii). An asterisk indicates a significant difference (*P* < 0·01) between observed and expected.

0·25 %. All the crosses have been made several times using different isolates of YS17 and these have shown some significant variations in recombination frequency. This is expected in view of the heterogeneity in aberrant ascus frequency between crosses of YS17 and wild type (Table 1). The range of frequencies of recombinant asci for each two-point cross is given in Table 4.

Three of the *buff* mutants, namely, S44, S140 and C80, gave low frequencies of recombinant asci in crosses with YS17. The values are in the range 0·1–0·8 %. These three mutants are much more closely linked to YS17 than the remainder.

A total of 25310 recombinant asci are recorded in Table 4. The origin of the various kinds is discussed in section (*h*).

Owing to the low frequency of recombination between *buff* mutants other than YS17, and to the variability in recombination frequency of YS17 with alleles, mapping the *buff* mutants has been based primarily on flanking marker behaviour.

(f) Buff *mutant map*

The *buff* mutants have been mapped by making pairwise crosses between them in the presence of the flanking markers, and recording the numbers of the different flanking marker genotypes in the wild-type recombinant spores. This has involved

Table 5. *Classification of wild-type recombinant progeny from crosses of* buff *mutants with one another*

Class of cross	Flanking marker genotype of *buff* mutants crossed		Flanking marker genotype of wild-type recombinant progeny			
	Proximal	Distal	P1	P2	R1	R2
1	*met* +	+ *nic*	*met* +	+ *nic*	+ +	*met nic*
2	+ *nic*	*met* +	+ *nic*	*met* +	*met nic*	+ +
3	*met nic*	+ +	*met nic*	+ +	+ *nic*	*met* +
4	+ +	*met nic*	+ +	*met nic*	*met* +	+ *nic*

isolating these spores, either from those discharged on to the lid of the petri dish (random spore data) or from intact asci, germinating them, and testing their growth on minimal medium with and without methionine and nicotinamide added. The mapping is based on the relative numbers of the two recombinant flanking marker genotypes on the assumption that the genotype (R1 class) capable of originating from a relatively simple event (recombination between the sites of the two *buff* mutants only) will be more frequent than that which requires a complex event (recombination in all three marked intervals) to account for it (R2 class): see Table 5 and Discussion, section *(c)*(iv).

The random-spore results obtained from crosses not involving YS17 are given in Table 6 and for crosses with YS17 in Table 7. From the recombinant flanking marker data the map shown in Fig. 1 has been constructed. It is based primarily on the results of crosses involving 55 different combinations of the mutants, namely, those in Tables 6 and 7 in which R1 exceeded R2. The map is in keeping with data of the same kind reported by Bond (1973) for various combinations of seven of the mutants (C47, C70, C79, S6, S41, S156, S180) and by Sang & Whitehouse (1979*b*) for four of them (C47, C67, YS9, YS132). Only 5 crosses (C53 × YS85, YS86 × YS20, YS132 × YS20, S41 × YS17, and C67 × YS17) have shown an excess of R2 over R1 and hence are in conflict with the map, but in only one case (S41 × YS17) is the excess significant. The exceptional behaviour of this cross is discussed later (section *(c)*(iv) of Discussion).

The data on the relative frequencies of the R1 and R2 classes obtained from dissecting and scoring recombinant asci are given in section *(h)* below. Such ascus analyses were made for crosses between YS17 and 14 other *buff* mutants and have given R1 and R2 data in agreement with those in Table 7 from the random wild-type progeny.

The relative positions of the mutants whose identification symbols are placed

Table 6. *Flanking marker genotype of wild-type progeny from pairwise crosses of* buff *mutants other than YS17*

Mutants crossed		Class	Flanking marker genotype					Frequency per 10^5
Proximal	Distal	(Table 5)	P1	P2	R1	R2	Total	spores
C53	YS85	1	10	1	6	7	24	2·0
	YS132	4	6	6	3	0	15	1·3
C47	YS85	1	6	6	7	2	21	1·1
	C69	1	2	11	8	5	26	1·3
	YS132	2	4	22	21	10	57	2·9
	S180	2	3	14	12	8	37	2·3
	YS40	2	1	9	3	2	15	0·9
	YS9	2	2	29	28	19	78	2·4
	YS77	1	1	2	14	1	18	1·5
	C70	2	12	16	14	3	45	2·8
	YS20	2	5	15	8	6	34	0·8
	S6	2	1	6	7	3	17	1·1
	C67	1	0	2	6	3	11	1·4
	YS14	2	1	2	3	0	6	0·3
	YS39	2	2	17	11	6	36	2·3
	YS117	1	2	4	2	1	9	0·8
YS100	YS132	4	(58)*	6	8	2	(74)	—
	S180	2	7	7	7	0	21	0·9
	YS40	2	8	4	7	2	21	1·1
	YS20	2	2	8	6	1	17	0·9
S156	YS85	$\begin{cases}1\\2\end{cases}$	$\begin{matrix}1\\0\end{matrix}$	$\begin{matrix}6\\2\end{matrix}$	$\begin{matrix}7\\4\end{matrix}$	$\left.\begin{matrix}3\\0\end{matrix}\right\}$	23	1·0
S41	YS85	$\begin{cases}1\\2\end{cases}$	$\begin{matrix}2\\0\end{matrix}$	$\begin{matrix}2\\1\end{matrix}$	$\begin{matrix}2\\1\end{matrix}$	$\left.\begin{matrix}1\\1\end{matrix}\right\}$	10	0·2
C79	YS85	2	2	7	4	2	15	0·3
	S180	2	3	13	4	2	22	0·5
	YS9	2	0	9	4	3	16	0·7
YS19	YS132	$\begin{cases}1\\2\end{cases}$	$\begin{matrix}1\\1\end{matrix}$	$\begin{matrix}2\\7\end{matrix}$	$\begin{matrix}2\\3\end{matrix}$	$\left.\begin{matrix}0\\0\end{matrix}\right\}$	16	0·9
	YS9	1	4	9	8	8	29	1·5
YS86	YS85	2	3	6	4	4	17	0·4
	YS132	2	2	9	5	2	18	1·1
	YS20	2	2	8	1	2	13	0·2
	S6	2	1	1	5	1	8	0·3
YS132	C70	2	7	8	8	3	26	1·3
	YS20	2	2	8	1	2	13	0·5
	S6	$\begin{cases}2\\3\end{cases}$	$\begin{matrix}0\\0\end{matrix}$	$\begin{matrix}1\\1\end{matrix}$	$\begin{matrix}2\\4\end{matrix}$	$\left.\begin{matrix}0\\0\end{matrix}\right\}$	8	0·1
YS9	YS20	$\begin{cases}1\\2\end{cases}$	$\begin{matrix}4\\0\end{matrix}$	$\begin{matrix}0\\0\end{matrix}$	$\begin{matrix}1\\2\end{matrix}$	$\left.\begin{matrix}0\\0\end{matrix}\right\}$	7	0·3

* YS100 showed reversion to wild type in this cross.

Table 7. *Flanking marker genotype of wild-type progeny from YS17 crossed with alleles*

Mutant crossed with YS17	Class (Table 5)	Flanking marker genotype				Total
		P1	P2	R1	R2	
Proximal to YS17						
C53	1	1	27	11	1	40
C47	2	0	40	13	3	56
YS100	2	0	39	12	7	58
S156	2	1	49	15	3	68
S41	1	3	141	7	23	174
	2	0	43	1	13	57
YS19	2	2	51	14	5	72
C79	2	8	113	37	17	175
YS86	2	3	69	36	5	113
YS85	2	0	94	20	13	127
C69	2	19	106	40	20	185
YS132	2	6	124	40	17	187
	3	5	61	15	9	90
S180	2	9	269	78	33	389
YS40	2	0	75	24	8	107
YS9	1	3	43	16	9	71
YS77	2	1	63	19	7	90
C70	2	1	37	13	9	60
YS61	2	1	44	16	5	66
YS20	2	0	61	20	5	86
S6	1	5	133	27	11	176
C67	2	0	99	15	21	135
YS43	2	1	52	19	1	73
YS14	1	0	82	16	6	104
YS39	1	5	21	19	0	45
YS69	2	0	71	18	2	91
YS117	2	5	56	22	10	93
Distal to YS17						
S44	2	9	0	0	0	9
C80	1	148	1	19	15	183

above one another in Fig. 1 are not known. Whenever mutants within these columns have been crossed wild-type spores have not been found. The crosses examined are indicated by vertical lines in Fig. 1 joining the mutant symbols. The estimated number of progeny spores from each cross examined for wild-type ones has been in the range 8×10^5 to 24×10^5 (and in one cross 48×10^5). Two mutants, YS77 and YS43, have shown no recombination with mutants in more than one column, as indicated in Fig. 1. It is possible that these mutants are deletions. Four mutants (symbols at the top of Fig. 1) have been mapped with less precision than the others, as indicated in the diagram.

The map of the *buff* mutants in Fig. 1, based on numbers of R1 and R2 wild-type progeny, gains some support from the recombination frequencies in Table 6.

Per 10^5 progeny, mutants in adjoining columns in Fig. 1 showed a mean recombination frequency of 0·5 (range 0·1–1·3), those separated by an intervening column 1·2 (range 0·5–2·0), and those separated by two or three columns 1·4 (range 0·2–2·9).
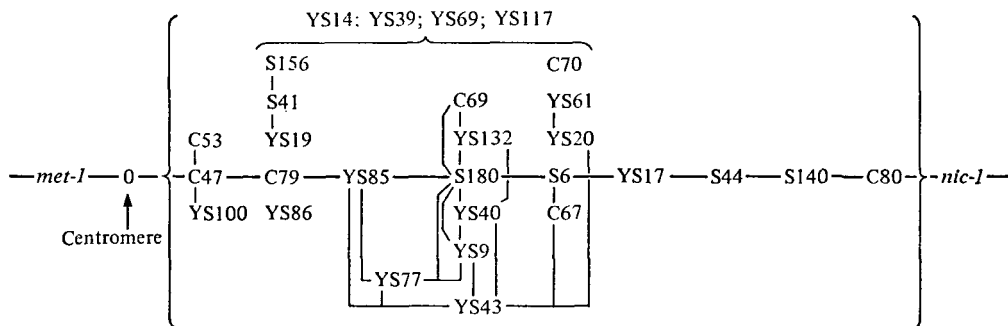
Fig. 1. Provisional map of *buff* mutants based primarily on flanking marker behaviour. Those within each column seem to be closely linked and their relative positions are not known. Those joined by vertical lines showed no wild-type recombinants in over 10^5 progeny. Mutants YS77 and YS43 may be deletions as no recombinants have been found with mutants in the regions indicated by the horizontal lines. The sites of mutants YS14, YS39, YS69 and YS117 have not yet been mapped with precision but appear to be in the region indicated by the bracket at the top of the diagram. Recombination frequencies between mutants are given in Tables 4 and 6.

The YS17 mutant maps towards the distal (right-hand) end of the *buff* gene, on the basis of the flanking marker behaviour (Table 7). Mutant C80, however, maps to the right of YS17 from the results of a large sample of random recombinant spores and of dissected asci. The sites of mutants S44 and S140 also seem to be to the right of that of YS17, though the evidence is slight, since wild-type progeny when these mutants are crossed with YS17 rarely have recombinant flanking markers (see section (*h*)). The evidence that S44 and S140 are to the right of YS17 rather than to the left is based, for S44, on a single peculiar 4:4 ascus (see section (h)(iii)), and, for S140, on two 2:6 asci (see section (*h*)(i)(4)).

The relative positions in Fig. 1 of the three mutants placed to the right of YS17 are based on their recombination frequencies with it (Table 4). It is of interest that these three alleles all show low frequencies of recombination with YS17, while the other 25, which map to its left, show high frequencies.

(g) *Polarity*

Polarity in recombination is shown in two-point crosses by an inequality in the frequency of the two parental flanking marker genotypes in the wild-type recombinant spores. From the data for crosses not involving YS17 (Table 6), it is evident that the mutants that map near the proximal (left-hand) end of the *buff* gene, that is, C53, C47 and YS100 (Fig. 1) show an excess of P1 over P2, or equality of these two classes, in some of the crosses with alleles. A comparable result to this was found by Bond (1973) with C47. On the other hand, almost all the other crosses show an excess of P2 over P1. This again is similar to Bond's findings.

The wild-type spores from crosses involving YS17 (Table 7) invariably show a marked excess of the flanking marker genotype corresponding to that of the YS17 parent, in other words, P2 for mutants mapping to the left of YS17 and P1 for those mapping to the right. This effect is of no help in mapping YS17, since if a mutant is wrongly placed on the map relative to YS17, its transfer from one side of it to the other will mean, not only that classes R1 and R2 are interchanged, but also P1 and P2. The over-riding effect of YS17 on the normal polarity in the *buff* gene was expected from its high conversion frequency.

(h) Analysis of recombinant asci from crosses between YS17 and alleles

Asci with wild-type spores were dissected from 16 crosses involving 14 different *buff* mutants crossed with YS17. The flanking marker genotypes of the parental strains used in these crosses are shown in Table 8. The spores from the dissected asci were germinated and their genotypes for the flanking markers ascertained by growth tests, and for *buff* by back-crossing to each parent and looking for wild-type recombinants.

Table 8. *Crosses between YS17 and alleles from which recombinant asci were analysed*

Allele (x) proximal				Allele (y) distal			
M + YS17 N				M YS17 + N			
m x + n				m + y n			
x	met	nic		y		met	nic
C47	M	n		S44	(i)	m	N
S41	M	n			(ii)	M	N
S156	M	n		S140	(i)	m	N
C79	M	n			(ii)	M	N
YS85	M	n		C80		M	n
C69	M	n					
S180	M	n					
YS40	M	n					
YS77	M	n					
C70	m	N					
YS20	M	n					

(i) 2:6 asci

In crosses between YS17 and alleles, the most frequent class of recombinant ascus, comprising in total 94% of them, has 2 wild-type and 6 mutant spores (Table 4). These asci would be the equivalent of those with 6 wild-type and 2 mutant spores in the cross of YS17 with wild type. The high frequency of 2:6 asci in the two-point crosses is therefore in agreement with the hypothesis that they result from conversion of YS17 to wild type. The expected genotypes for these asci, on this hypothesis, are shown in Table 9, both for a cross of YS17 with a proximal allele and with a distal allele. Three types of crossover event are considered: I, non-crossover; II, a crossover adjoining the YS17 site; and III, a crossover not adjoining this site. These alternatives give rise to the following flanking marker genotypes for the pair of wild-type spores: I, P2 if the mutant is

Table 9. *2:6 ascus genotypes from YS17 crossed with alleles*

Crossover event	Allele (x) proximal					Allele (y) distal			
	M	+	YS17	N		M	YS17	+	N
	m	x	+	n		m	+	y	n
I	M	+	YS17	N		M	YS17	+	N
	M	+	+	N (P2)		M	+	+	N (P1)
	m	x	+	n		m	+	y	n
	m	x	+	n		m	+	y	n
II	M	+	YS17	N		M	YS17	+	N
	M	+	+	n (R1)		M	+	y	n
	m	x	+	N		m	+	+	N (R1)
	m	x	+	n		m	+	y	n
III	M	+	YS17	N		M	YS17	+	N
	M	x	+	n		M	+	+	n (R2)
	m	+	+	N (R2)		m	+	y	N
	m	x	+	n		m	+	y	n

I, Non-crossover; II, crossover adjoining site of YS17; III, crossover not adjoining site of YS17.

The flanking marker genotype of the wild-type spores is indicated by the usual symbols, namely: P1, corresponding to proximal allele in parents; P2, corresponding to distal allele in parents; R1, corresponding to an exchange between the alleles; R2, requiring a triple exchange.

proximal to YS17, and P1 if it is distal; II, R1; and III, R2 (see Table 9). A fourth category, P1 for proximal mutants and P2 for distal, is not predicted. The numbers of asci in these four categories that were found in the 16 crosses are given in Table 10.

The data show several features of interest:

(1) A large majority of the 630 asci analysed fall into categories I and II, which are those expected with conversion at YS17. An interesting result, however, is the occurrence of 62 asci, or 10 % of the total, in category III. The occurrence in these asci of crossing-over at a site close to that showing conversion but separated from it by a mutant site showing normal 4:4 segregation is discussed below (Discussion, section (c)(iv)).

(2) When YS17 is crossed with an allele on its proximal side, the occurrence of asci with the P1 flanking marker genotype in the wild-type spores is not predicted if the 2:6 asci are arising from conversion at YS17 (Table 9), but would be expected if conversion occurred at the other mutant site. Only 7 asci, or about 1 % of the recombinants, were in the P1 category, and of the two fully analysed, both resulted from class II events with incidental crossovers responsible for the P1 genotype. There is no evidence, therefore, that any of the 2:6 asci resulted from conversion at the site of the allele with which YS17 was crossed. In the random spore data (Table 7) the numbers of P1 genotypes for proximal alleles, and P2 for distal ones, are also quite low. Furthermore, when correction is made for incidental crossovers using the formulae of Whitehouse & Hastings (1965) the number diminishes close

Table 10. *Numbers of 2:6 asci of different genotypes from crosses of YS17 with alleles*

Crossover event (Table 9)	Proximal alleles											Distal alleles				
												S44		S140		C80
	C47	S156	S41†	C79	YS85	C69	S180	YS40	YS77	C70	YS20	(i)	(ii)	(i)	(ii)	
I	(9)	(20)	(13)	(29)	17+(18)	(5)	35+2+(29)	(10)	(18)	47+4+(12)	(12)	16+(11)	(5)	6+(6)	11+1+(15)	47+2+(38)
II	(1)	(11)	(13)	(13)	8+(4)	(5)	16+1+(10)	(5)	(3)	9+1+(8)	(5)	—	—	(1)	1	13+(7)
III	(1)	(2)	(4)	(8)	4	(3)	6+(6)	(2)		10+(2)	(2)	—	—	—	—	6+2+(5)
P1 (prox.) or P2 (dist.)	(1)			(1)		(1)	2*	(1)		(1)		—	—	—	—	—
Total	12	33	17	51	51	14	107	18	21	94	19	32		41		120

Numbers after a plus sign but not in brackets indicate asci with an incidental crossover not affecting the flanking marker genotype of the wild-type spores. Figures in brackets are numbers of asci in which only the wild-type spores germinated or were isolated.

* Both of these asci were class II with an incidental crossover proximal to *buff* generating the P1 genotype of the wild-type spores.

† The exceptional behaviour of mutant S41 when crossed with YS17 is referred to in section (f) of the Results.

to zero in most instances. The conclusion is that conversion to wild type at the site of the allele with which YS17 is crossed usually make a negligible contribution to the 2:6 asci.

(3) In the crossover asci (classes II and III) one or both *buff* spores with the reciprocal flanking marker genotype to that of the wild-type spores germinated in 47 instances, comprising 7, 11, 15, 1 and 13 from the crosses with YS85, S180, C70, S140, and C80, respectively, but in no case was a double mutant found. It is concluded that reciprocal recombination of these pairs of alleles is rare.

(4) Recombinant flanking marker genotypes were absent from the YS17 × S44 crosses and very rare with YS17 × S140. This result is discussed later (see Discussion, section (c)(iii)).

(5) The frequency of crossing-over associated with the recombination events at *buff* in the crosses between YS17 and alleles other than S44 and S140 is 33 % on the basis of the ascus data in Table 10, and 28 % from the random spore data in Table 7. These values are higher than that obtained when YS17 was crossed with wild type (18 % – see section (b) above) and lower than that found in crosses of alleles other than YS17: the data in Table 6, omitting YS100 × YS132, give a crossover frequency of 48 %.

(ii) *1:7 asci*

As can be seen from Table 4, 1:7 asci occur regularly in the two-point crosses. Their frequency is usually about 1–4 % of the total recombinant asci but in six of the crosses (YS17 crossed with C67, C70, S44, S140, S180 and YS43) the frequency is much higher (9–33 % of the recombinant asci). Four of these mutants are known to be C class and the conversion pattern of the other two is not known. The significance of these higher frequencies is discussed later (see Discussion, section (a)(iv)).

Table 11. *Possible explanations of (a-c) 1:7 asci, (d, e) 4:4 asci, and (f, g) 3:5 asci from crosses of YS17 with an allele, x*

$$\text{Parents:}\ \dfrac{+\quad\text{YS17}}{x\quad+}$$

(a)		(b)		(c)		(d)		(e)		(f)		(g)	
+	YS17	+	YS17	+	YS17	+	YS17	+	+	+	YS17	+	+
+	YS17	+	YS17	+	YS17	+	YS17	+	+	+	YS17	+	+
+	YS17	+	YS17	+	+	+	+	+	+	+	+	+	+
+	YS17	+	+	x	+	+	+	+	+	+	+	x	+
+	+	x	+	x	+	+	+	x	+	+	+	x	+
x	+	x	+	x	+	+	+	x	+	x	+	x	+
x	+	x	+	x	+	x	+	x	+	x	+	x	+
x	+	x	+	x	+	x	+	x	+	x	+	x	+

Three possible explanations of the 1:7 segregation are shown in Table 11 (a–c). These alternatives are (a) a 5:3 segregation at the site of the mutant with which YS17 was crossed, the YS17 site showing normal 4:4 segregation; (b) the converse situation, with the 5:3 segregation at the YS17 site and normal 4:4 segregation at

Table 12. *1:7 ascus genotypes from YS17 crossed with alleles*

$$\frac{\text{M} + \text{YS17 N}}{\text{m} \; x \; + \; \text{n}}$$

YS17 crossed with an allele (x) proximal to it

Crossover event	(a)	(b)	(c)
I	M + YS17 N M + YS17 N m x/+ + n (P1) m x + n	M + YS17 N M + YS17/+ N (P2) m x + n m x + n	M + YS17 N M +/x + N (P2) m x + n m x + n
II	M + YS17 N M x/+ + n (R1) m + YS17 N m x + n	M + YS17 N M + YS17/+ n (R1) m x + N m x + n	M + YS17 N M +/x + n (R1) m x + N m x + n
III	M + YS17 N M + YS17 n m x/+ + N (R2) m x + n	M + YS17 N M x + n m + YS17/+ N (R2) m x + n	M + YS17 N M x + n m +/x + N (R2) m x + n

$$\frac{\text{M YS17} + \text{N}}{\text{m} \; + \; y \; \text{n}}$$

YS17 crossed with an allele (y) distal to it

Crossover event	(a)	(b)	(c)
I	M YS17 + N M YS17 + N m + +/y n (P2) m + y n	M YS17 + N M YS17/+ + N (P1) m + y n m + y n	M YS17 + N M + +/y N (P1) m + y n m + y n
II	M YS17 + N M YS17 + n m + +/y N (R1) m + y n	M YS17 + N M + y n m YS17/+ + N (R1) m + y n	M YS17 + N M + y n m + +/y N (R1) m + y n
III	M YS17 + N M + +/y n (R2) m YS17 + N m + y n	M YS17 + N M YS17/+ + n (R2) m + y N m + y n	M YS17 + N M + +/y n (R2) m + y N m + y n

I, Non-crossover; II, crossover outside gene adjoining site of aberrant segregation (or of YS17 if both sites involved); III, crossover outside gene on opposite side to site of aberrant segregation (or to YS17 if both sites involved. (*a*), (*b*), (*c*) as in Table 11.

the other site; and (*c*) a 6:2 segregation at YS17 in conjunction with a 3:5 at the other site. These possibilities can be distinguished by ascus analysis, as indicated in Table 12, where *a*, *b* and *c* indicate these alternatives, and I, II and III the crossover events described above in discussing the 2:6 asci. With a non-crossover event (I), alternative (*a*) can be distinguished immediately from (*b*) and (*c*) by the flanking marker genotype of the wild-type spore (P1 *v*. P2). On the other hand, to distinguish (*b*) from (*c*) requires identification of the *buff* mutant in the sister spore to the wild-type one, irrespective of the crossover event.

Table 13. *Numbers of 1:7 asci of different genotypes from crosses of YS17 with alleles*

Crossover event (Table 12)	Aberrant segregation (Table 11)	Proximal to YS17									Distal to YS17			Total
		C47	S156	S41†	C79	YS85	S180	YS40	C70	YS20	S44 (i)	S140 (i)	C80	
I	(b)/(c)	(2)	(2)	(2)	(3)	4+(3)	4+(5)	—	4+(4)	(1)	2+(1)	—	2+(1)	40 ⎫
	(b)	—	—	—	—	3	—	—	(1)	—	—	1	1	6 ⎬ 55
	(c)	—	—	—	—	—	6	—	3	—	—	—	—	9 ⎭
II	(a)/(b)/(c)	—	(1)	—	—	1+(1)	(4)	—	1+1*+(1)	—	—	—	—	10 ⎫
	(a)/(c)	—	—	—	—	—	2+(1)	—	—	—	—	—	—	3 ⎬ 20
	(b)/(c)	—	—	—	—	3	4	—	—	—	—	—	—	7 ⎭
III	(a)/(b)/(c)	—	—	(1)	—	—	—	(1)	—	—	—	—	(1)	3 ⎫ 4
	(b)/(c)	—	—	—	—	—	1	—	—	—	—	—	—	1 ⎭
	Total	2	3	3	3	15	27	1	15	1	3	1	5	79

Figures in brackets are numbers of asci in which only the wild-type spores germinated or were isolated.

* Incidental crossover on proximal side of *buff* locus involving postmeiotic-segregation chromatid and generating a P1 wild-type spore

† The exceptional behaviour of mutant S41 when crossed with YS17 is referred to in section (*f*) of the Results.

The results obtained from dissection of 79 1:7 asci from crosses of YS17 with 12 different alleles are given in Table 13. None of the non-crossover asci was in category (a), but both (b) and (c) were represented. With the crossover asci the possibility that some of them were in category (a) could not be eliminated, but this is unlikely in view of their absence from the non-crossover ones. It is possibly significant that category (c), which unlike (b) involves postmeiotic segregation at the site of the mutant with which YS17 is crossed, is more in evidence with the C class mutants S180 and C70 than with the B class mutant YS85. It is concluded that the 1:7 asci result sometimes from a 5:3 segregation at the site of YS17 with a normal 4:4 segregation at the other mutant site, and sometimes from a 6:2 segregation at YS17 associated with a 3:5 at the other site.

Two features noted with the 2:6 asci are also evident with the 1:7 asci, namely (1) the low frequency of crossing-over associated with recombination at *buff* in the crosses of YS17 with the closely-linked mutants S44 and S140, and (2) the origin of a P1 flanking marker genotype in a wild-type spore as a result of an incidental crossover with an event at *buff* that would otherwise have given the R1 genotype (Table 13).

(iii) *4:4 asci*

All the crosses between YS17 and other *buff* mutants have given 4:4 asci, usually with a frequency that ranges between 0·25 % and just over 1 % of the recombinant asci (Table 4). Four of the crosses gave higher frequencies: about 4 % for C53 and YS20 and about 10 % for C69 and YS9. These four mutants are all of A class. The significance of this finding is discussed below.

The 4:4 asci are likely to have originated in one or other of two ways:

(1) Conversion to wild type might have occurred at each mutant site in each of the two recombining chromatids (Table 11 d).

(2) Conversion to wild type might have occurred at the site of YS17 in both chromatids of this genotype, three or all four chromatids being involved altogether in the recombination event (Table 11 e).

The ascus genotypes expected with these methods of origin are shown in Table 14, both for a proximal and for a distal allele crossed with YS17, and for various crossover events at *buff*. It is evident that ascus analysis will distinguish the different alternatives even if only the wild-type spores germinate.

A total of 39 4:4 asci from crosses of YS17 with 8 different alleles have been analysed. The results are given in Table 15. It is evident that 4:4 asci are arising in both the ways anticipated, with differences in their relative frequencies depending on the mutant. The high frequency of 4:4 asci with the A class mutants referred to above is likely to be a consequence of larger numbers of 4:4 asc resulting from conversion at both sites (Table 11 d) with these mutants.

If double events (Table 11 e) are making an appreciable contribution to the 4:4 asci, as the data in Table 15 indicate, the expected contribution from this source can be estimated on the assumption that the two events occur independently.

The expression:

$$(4\!:\!4) = (2\!:\!6)^2/4N$$

can be used, where brackets surrounding an ascus symbol indicate the number of such asci observed in a total count of N asci. This formula is comparable to that used in section (*b*) above to estimate the expected frequency of 8:0 asci in crosses of YS17 with wild type. On this basis, the expected number of 4:4 asci has been

Table 14. *4:4 ascus genotypes from YS17 crossed with alleles*

Class of 4:4 ascus (Table 11)	Crossover events (Table 9)	YS17 crossed with an allele (*x*) proximal to it: M + YS17 N				YS17 crossed with an allele (*y*) distal to it M YS17 + N			
		m	*x*	+	n	m	+	*y*	n
(*d*)	I	M	+	YS17	N	M	YS17	+	N
		M	+	+	N (P2)	M	+	+	N (P1)
		m	+	+	n (P1)	m	+	+	n (P2)
		m	*x*	+	n	m	+	*y*	n
	II or III	M	+	YS17	N	M	YS17	+	N
		M	+	+	n (R1)	M	+	+	n (R2)
		m	+	+	N (R2)	m	+	+	N (R1)
		m	*x*	+	n	m	+	*y*	n
(*e*)	I and I	M	+	+	N (P2)	M	+	+	N (P1)
		M	+	+	N (P2)	M	+	+	N (P1)
		m	*x*	+	n	m	+	*y*	n
		m	*x*	+	n	m	+	*y*	n
	I and II	M	+	+	N (P2)	M	+	+	N (P1)
		M	+	+	n (R1)	M	+	*y*	n
		m	*x*	+	N	m	+	+	N (R1)
		m	*x*	+	n	m	+	*y*	n
	I and III	M	+	+	N (P2)	M	+	+	N (P1)
		M	*x*	+	n	M	+	+	n (R2)
		m	+	+	N (R2)	m	+	*y*	N
		m	*x*	+	n	m	+	*y*	n

For explanation of P1, P2, R1 and R2 see Table 9.

calculated from the observed number of 2:6 asci for each cross, and is given in brackets in Table 4 after the observed number of 4:4 asci. There is a well-marked tendency for the 4:4 frequency to increase with increasing 2:6 frequency, as expected if double events are making a substantial contribution to the 4:4 class. But four of the crosses (with C69, YS9, C53, and YS117) show significantly more 4:4 asci ($P < 0.01$) than expected, and eight (YS17 crossed with YS77, YS85, C67, S41, C70, S156, S180, and YS43) significantly less. A higher frequency than predicted by double events is not surprising in view of the known contribution of conversion at both sites (Table 11*d*) to the 4:4 asci. A lower frequency than is predicted by double events suggests that, as with the 8:0 asci in crosses of YS17 with wild-type (section (*b*) above), two 6:2 events at YS17, involving in aggregate

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three or all four chromatids, are often less frequent than would be expected if the two events were independent.

One of the 4:4 asci from the cross YS17 *nic* × *met* S44 is of special interest. As indicated in a footnote to Table 15, two spore-pairs had the genotype of the S44 parent, and the other two, wild type for spore colour, were *met nic*. Conversion to wild type at YS17 in both the YS17 chromatids was associated with conversion to *met* in the same chromatids. Simultaneous conversion of widely-spaced mutants

Table 15. *Numbers of 4:4 asci of different genotypes from crosses of YS17 with alleles*

Class of 4:4 ascus (Table 11)	Crossover event (Table 14)	Proximal alleles					Distal alleles			
		C79	YS85	C69	S180	C70	S44 (i)	S140 (i)	S140 (ii)	C80
(d)	I	—	(1)	(1)	—	—	—	—	—	—
	II or III	—	2 + (1*)	—	1	1	—	—	—	—
(e)	I and I	(1)	2 + (7)	—	—	—	7† + (2)	(3)	1	3 + (3)
	I and II	—	(1)	—	—	—	—	—	—	1
	I and III	—	—	—	—	—	—	—	—	(1)
	Total	1	14	1	1	1	9	4		8

Figures in brackets are numbers of asci in which only the wild-type spores germinated or were isolated.

* The possibility cannot be ruled out that this ascus was class (e) with crossover events II and III.

† One of these asci had both pairs of wild-type spores of *met nic* genotype (R1), apparently as a result of conversion to *met* in both the YS17 chromatids along with the conversion of YS17 to wild type, also in both.

has been reported by Bandiera, Armaleo & Morpurgo (1973) and Esposito (1978) for mitotic recombination in *Aspergillus nidulans* and *Saccharomyces cerevisiae*, respectively. If the events at YS17 and *met* were linked, this is evidence that S44, which was not involved, is to the right of YS17.

(iv) *Other ratios*

Small numbers of asci with a 3:5 segregation have been found in some of the two-point crosses (Table 4). Likely explanations of how such asci originate are shown in Table 11 (*f*) and (*g*). These alternatives are in a sense the counterparts of the two proposed explanations for the 4:4 asci (Table 11*d*, *e*). One possibility is conversion to wild type at YS17 in conjunction with postmeiotic segregation at the other mutant site in the other recombining chromatid (Table 11*f*). The alternative is a double event at YS17 to give an 8:0 ratio at that site and a 3:5 segregation at the other mutant site (Table 11*g*).

The A or B class mutants (YS85, YS20, C69, YS9, and C53) gave only 4 asci with a 3:5 ratio out of a total of 5917 recombinant asci, that is, a frequency of 0·07 %, whereas the C class mutants (S41, C47, S6, C70, S156, S180, S44, C67, YS40, YS39, and YS132) gave 3:5's ten times more often (80 in 11435 recombin-

ants, or 0·7 %). This difference is in agreement with the suggestion that the post-meiotic segregation is at the site of the mutant with which YS17 is crossed, rather than at YS17 itself. One 3:5 ascus has been analysed from the YS40 cross (Table 8). It was found to correspond to Table 11 (*g*).

Asci with 5:3 or 6:2 segregations have occasionally been found in the two-point crosses (Table 4). To account for them requires both explanations offered for the 3:5 and 4:4 ratios. In other words, a recombination event at *buff* has involved conversion to wild type at YS17 and either postmeiotic segregation (Table 11 *f*) or conversion to wild type (Table 11 *d*) in the other recombining chromatid, and a second event has led to conversion to wild type in the other YS17 chromatid (Table 11 *e* or *g*).

4. DISCUSSION

The results will be discussed under three headings, namely, evidence that mutant YS17 acts as a recognition site for an endonuclease that initiates recombination, comparison with similar mutants in other organisms, and information about the recombination mechanism.

(*a*) *YS17 as a recognition site*

Four pieces of evidence fit the hypothesis that the YS17 mutation is a recognition site for an endonuclease that initiates recombination.

(i) The frequency of aberrant segregation at the site of YS17, in crosses with wild type, is more than 10 times greater than that for any of the 28 other *buff* mutants available.

(ii) The pattern of polarity in recombination within the *buff* gene is modified in the presence of YS17 in the way expected if it is an initiation site for recombination. As originally reported by Bond (1973), the polarity in the *buff* gene is predominantly distal, that is, a higher conversion frequency at the distal site of a pair. The exception to this is when the site of one of the mutants crossed is close to the proximal end of the gene. In the presence of YS17, however, conversion at its site greatly exceeds that of any of the alleles, whether they map on its proximal or distal side.

(iii) An attempt to separate the YS17 mutation causing buff spore colour from that causing high frequency conversion has failed. The wild-type spores resulting from conversion at YS17 in 117 asci were tested against another *buff* mutant, but in every case loss of the buff spore colour was associated with loss of the high frequency conversion. It seems likely, therefore, that the effects on spore colour and on conversion frequency are properties of the same mutation. In other words, the results are in agreement with the hypothesis that the YS17 mutation has not only led to an abnormal product of the *buff* gene, but has also given rise to a nucleotide sequence that is recognised by an endonuclease that initiates recombination. This argument is not invalidated by the discovery of a suppressor of the high frequency conversion at YS17 without affecting the mutant spore colour (manuscript in preparation).

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(iv) As pointed out in section (h)(ii) of the Results, many of the 1:7 asci found in crosses of YS17 with alleles result from conversion to wild type at the site of YS17 in conjunction with postmeiotic segregation at the site of the other mutant. When the C class mutants S180 and C70 were crossed with YS17, out of 10 1:7 asci where the nature of the recombination event was determined, 9 arose in this way and one from postmeiotic segregation at the site of YS17 (Table 13). In these crosses 1:7 asci constituted 14 % and 10 %, respectively, of the recombinant asci (Table 4). The absolute frequencies were 1·5 % and 1·1 %. These frequencies are much greater than the frequency of postmeiotic segregation found at the sites of S180 and C70 when crossed with wild type: taking the two mutants together, there were 3 examples in 8241 asci, or 0·04 % (Table 3). Allowing for the fact that about 10 % of the 1:7 asci in the crosses with YS17 may have resulted from postmeiotic segregation at the site of YS17, it is evident that the frequency of postmeiotic segregation at the sites of C70 and S180 was nevertheless substantially increased in the presence of YS17. Furthermore, at least in the non-crossover asci, the post-meiotic segregation at the sites of C70 and S180 and the conversion at the site of YS17 have taken place in the same chromatid: see Table 12, column c (allele x), row I. It is evident that in these crosses the conversion to wild type at YS17 is often associated with hybrid DNA formation at the allelic site in the same chromatid. This is expected if YS17 is an initiation site for recombination. There would have been donation to YS17 by the other parent of a nucleotide chain covering both mutant sites, followed by mismatch repair to wild type at YS17 but no repair at the other site. The postmeiotic segregation is then at the site of the mutant other than YS17, but its high frequency compared with a cross to wild type is evidently a consequence of the formation of hybrid DNA with high frequency by YS17.

It may be significant that the six *buff* mutants (C67, C70, S44, S140, S180 and YS43) with high proportions of 1:7 asci among the recombinant asci in crosses with YS17 (Table 4 and section (h)(ii) of Results) all map relatively close to YS17 (Fig. 1). On the supposition that the hybrid DNA is originating at the site of YS17, this result implies that the hybrid DNA extends less often to more distant sites in *buff*. Another observation supports this conclusion. The frequency of recombinant asci in most of the crosses between YS17 and other *buff* mutants (Table 4) is as high as in crosses of YS17 and wild type (Table 1). If hybrid DNA often extended from YS17 to the allelic site, co-conversion would be expected to be frequent. This would not give rise to asci with wild-type spores. The frequency of such asci would therefore be expected to be correspondingly reduced compared with the frequency of 6:2 asci in crosses of YS17 with wild type.

(b) *Comparison of YS17 with similar mutants in other organisms*

Three sites with similarities to YS17 will be discussed.

(i) *The* cog *site in* Neurospora crassa

The *cog* site in *Neurospora crassa* described by Angel, Austin & Catcheside (1970)

(see also Catcheside, 1977) shows similarities to YS17. Angel *et al.* discovered, in different wild stocks, variants of a *recognition* locus (*cog*) associated with the *histidine-3* (*his-3*) gene and mapping just distal to it in the right arm of linkage group I. Recombination in this gene is affected by *rec-2*, a recombination repressor in linkage group V. In the absence of the dominant allele *rec-2+*, recombination in *his-3* is derepressed. The frequency of recombination is then affected by the variants *cog+* and *cog*. In the presence of the dominant allele, *cog+*, recombination frequencies between *his-3* mutants are 6–8 times those observed in its absence. Angel *et al.* concluded that *cog* was a recognition site for an enzyme that initiates recombination, with the two alleles differing in their response to the enzyme. Support for this hypothesis was provided by studies, reported more fully by Catcheside & Angel (1974), with a *his-3* structural mutant called TM429.

The YS17 mutation in the *buff* gene of *Sordaria brevicollis* is similar to *cog* in promoting recombination in its vicinity. It differs in being located within a gene, having evidently arisen as a result of treatment with ICR170.

(ii) *The* K/P/91 *site in* Ascobolus immersus

Emerson & Yu-Sun (1967) discovered that two closely-linked white-spored mutants, *w-10* and *w-78*, of *Ascobolus immersus* showed different frequencies and patterns of aberrant asci depending on to which of two wild strains, *K* and *P*, they were crossed. Lamb & Helmi (1978) investigated this situation further and identified control factors, *K* and *P*, responsible for the variation in behaviour. These factors were closely linked to the *w-10* locus, occasionally showing co-conversion with the spore colour mutants. Lamb & Helmi detected a third control factor, *91*, and isolated two more spore colour mutants mapping at the *w-10* locus. The three control factors behaved as alleles. From their results, Lamb & Helmi concluded that the *K/P/91* site may be an initiation-point for recombination. On this hypothesis, the variations in conversion pattern of the mutants at the *w-10* locus, depending on the *K/P/91* genotype, would be comparable to the differences in behaviour of *buff* mutants such as C70 and S180 in the presence and in the absence of YS17. This comparison assumes that YS17 is acting as an initiation site for recombination.

(iii) *The M26 mutant in* Schizosaccharomyces pombe

The results which most closely resemble the behaviour of YS17 are those described by Gutz (1971) for the X-ray induced M26 mutant in the *adenine-6* (*ade-6*) gene of *Schizosaccharomyces pombe*. This mutant mapped at an internal position in the gene and gave wild-type recombinants when crossed with distant alleles with a frequency approximately 13 times that shown by a closely linked allele, M375, when crossed with them. M26 showed conversion with a frequency of about 5 % in crosses with wild type, conversion to wild type being about 12 times as frequent as to mutant. Three other *ade-6* mutants tested gave aberrant ascus frequencies of 0·3 to 0·9 %, with no significant excess of conversion in one direction over the other, but the frequency of conversion of closely linked alleles was

greatly increased in the presence of M26, and its predominant direction was determined by M26, being to mutant in *trans* and to wild type in *cis* crosses.

The similarity between M26 and YS17 is striking. Both map within a gene, show a conversion frequency an order of magnitude higher than alleles and with a marked bias in favour of wild type. No test has yet been made for co-conversion with YS17, but the occurrence in the conversion chromatid of a much increased frequency of postmeiotic segregation at the allelic site has been demonstrated (see (*a*)(iv) above) and is a comparable phenomenon. Gutz (1971) suggested that the DNA at the M26 site preferentially undergoes single-strand breakage, thereby initiating recombination at this site, and our hypothesis to explain the behaviour of YS17 is similar.

There is one feature of the similarity between M26 and YS17 that is of special interest. It was pointed out (Results, section (*d*)) that the conversion pattern of YS17 was that of a deletion frameshift. There is no evidence, however, that the A, B and C patterns characteristic of *Ascobolus immersus* and *Sordaria brevicollis* apply to yeasts. In *Saccharomyces cerevisiae* the conversion patterns of large numbers of mutants have been examined without finding inequality of frequency of conversion in the two directions, that is, to wild type and to mutant (Fogel, Hurst & Mortimer, 1971; Lawrence *et al.* 1975) and even deletions have been found to show such parity (Fink & Styles, 1974; Fink, 1974). It may be, therefore, that the marked bias in favour of conversion to wild type shown by M26 and YS17 relates to the initiation of recombination at these sites. This possibility is strengthened by evidence presented by Gutz (1971) that M26 is not a deletion frameshift. He found that the adenine requirement of M26 was suppressed by some super-suppressors and he inferred that it was probably a polypeptide-termination mutant. This result implied that it had arisen by base substitution. Furthermore, in the presence of the suppressor of the high frequency conversion of YS17, its conversion pattern is different and is of C instead of A class (manuscript in preparation). It seems unlikely that the suppressor causes mutation at YS17, and a probable explanation of the change of conversion pattern is that it depends on whether or not recombination is being initiated at the site.

The idea that conversion predominantly to wild type by M26 and YS17 may be related to initiation of recombination at these sites is discussed in section (*c*)(i) below.

(c) *Mechanism of recombination*

The investigation of the YS17 mutant has given information about four aspects of the recombination mechanism, namely, the initial steps in the process; the question of whether hybrid DNA formation is symmetrical, that is, occurs in both recombining chromatids, or is asymmetrical, that is, in only one chromatid; and the frequency and the position of crossing-over associated with the conversion at the YS17 site.

(i) *The initial steps*

According to the hypothesis proposed by Meselson & Radding (1975) the initial step in recombination is the formation of a single-strand break in a DNA molecule. This becomes the site of strand displacement by a DNA polymerase. The displaced strand pairs with the complementary nucleotide sequence in another DNA molecule and induces breakage in its other strand. In a cross between YS17 and wild type, if YS17 is recognised by an endonuclease that induces a single-strand break at its site, according to the Meselson-Radding hypothesis a YS17 chromatid would act as donor and a wild-type chromatid as recipient of a single strand. The donor molecule would be repaired and a heteroduplex would be confined to the recipient molecule. Assuming that the site of the initial nicking was such that the YS17 nucleotide sequence was transferred to the recipient molecule, if the resulting mismatch was not repaired an ascus with a 3:5 ratio of wild-type: mutant spores would be produced. Correction would generate a 2:6 ratio or restore normal 4:4 segregation, depending on its direction, but could not give rise to the observed marked excess of 6:2 asci. To reconcile this result with the model it would be necessary to add an extra step in which the donor and recipient become transposed. For example, after the wild-type strand has been broken it would be necessary to postulate that it forms a heteroduplex with the YS17 molecule, the broken strand of which would be eroded exonucleolytically. This is comparable to the postulate by Gutz (1971) that, following breakage of one strand at the site of M26, there is exonucleolytic breakdown of this strand.

Support for the idea that the molecule broken initially acts as recipient, not donor, is available from other sources in addition to the M26 and YS17 data. Angel *et al.* (1970) found with *Neurospora crassa* that when recombination was derepressed (*rec-2* homozygote), wild-type recombinants with non-crossover flanking marker genotypes in pairwise crosses of *his-3* mutants were always predominantly of the *cog*+ parent if the other was of *cog* genotype. In other words, the *cog*+ chromatid, which is the favoured one for the initiation of recombination, is also favoured for conversion of its *his-3* allele to wild type. This is the result expected if the *cog*+ chromatid is the recipient of a nucleotide chain from the other parent, not the donor of a strand to it.

Friedman & Sobell (1978) reported additional evidence for sites of initiation of recombination acting as recipients of genetic information. Their data refer to mitotic recombination in *Saccharomyces cerevisiae* induced by irradiating one parent with gamma rays. They also quote results obtained by several authors with phage lambda of *Escherichia coli*.

If the A class conversion pattern of YS17 is a consequence of initiation of recombination at this site (see (*b*)(iii) above), the implication would be that mismatch repair is strongly favoured in the initiation region. This would explain the very low frequency of postmeiotic segregation shown by YS17, even though the molecular nature of the mutation may be such that failure of repair would be frequent if the mutant site were in hybrid DNA that had originated elsewhere. There is no information as to whether or not mismatch repair at YS17 is biassed in favour of

wild type or mutant, becase repair to mutant would restore a normal 4:4 segregation and so would not be detected. If there is no bias, recombination must be initiated at YS17 twice as often as 6:2 asci are seen in a cross with wild type.

(ii) *Symmetrical versus asymmetrical hybrid DNA*

Two results indicate that the recombination process associated with conversion of YS17 to wild type is predominantly asymmetrical.

First, if the conversion were associated with hybrid DNA formation in both recombining chromatids, this hybrid DNA might be expected sometimes to extend from YS17 to a nearby site in both of them. If there was no mismatch correction, aberrant 4:4 segregation would arise at this site, and this would be revealed as two non-sister wild-type spores differing in flanking marker genotype. Although 630 asci with a 2:6 segregation were analysed from crosses between YS17 and alleles (Table 10), none were of this $(1+1):6$ category, as it might be called. Without exception the wild-type spores formed a pair with the same flanking marker genotype. The occurrence of 1:7 asci as a result of hybrid DNA extending to the second site (section (h)(ii) of Results) leads one to expect that some $(1+1):6$ asci would occur if hybrid DNA formation was symmetrical.

Secondly, if hybrid DNA extended from the site of YS17 to the second site in both chromatids, followed by mismatch repair to mutant in one of them, a 1:7 ascus would result (6:2 segregation at YS17 and 3:5 segregation at the other site as in Table 11c). If the ascus were non-crossover, the flanking marker genotype of the wild-type spore would depend on which chromatid had undergone the repair. So if 1:7 asci were arising in this way, half would be expected to have one parental genotype for the wild-type spore and half the other. One of these possibilities corresponds to ascus genotypes

$$\left.\begin{array}{llll}
\text{M} & + & \text{YS17} & \text{N} \\
\text{M} & +/x & + & \text{N (P2)} \\
\text{m} & x & + & \text{n} \\
\text{m} & x & + & \text{n}
\end{array}
\quad \text{or} \quad
\begin{array}{llll}
\text{M} & \text{YS17} & + & \text{N} \\
\text{M} & + & +/y & \text{N (P1)} \\
\text{m} & + & y & \text{n} \\
\text{m} & + & y & \text{n}
\end{array}\right\} \quad (1)$$

for YS17 crossed with an allele (x) proximal to it, or an allele (y) distal to it, respectively, using the same symbols as in Table 12. These genotypes are those shown in the columns headed (c), row no. I, of the table. The other possibility would have the ascus genotypes

$$\left.\begin{array}{llll}
\text{M} & + & \text{YS17} & \text{N} \\
\text{M} & x & + & \text{N} \\
\text{m} & +/x & + & \text{n (P1)} \\
\text{m} & x & + & \text{n}
\end{array}
\quad \text{or} \quad
\begin{array}{llll}
\text{M} & \text{YS17} & + & \text{N} \\
\text{M} & + & y & \text{N} \\
\text{m} & + & +/y & \text{n (P2)} \\
\text{m} & + & y & \text{n}
\end{array}\right\} \quad (2)$$

Out of 55 non-crossover 1:7 asci analysed (Table 13) none had a wild-type spore with the flanking marker genotype of the mutant that was crossed with YS17, that is, corresponding to genotype (2) above. Some of the 1:7 asci resulted from postmeiotic segregation at the site of YS17 (Table 11b) and so are not relevant to the question of hybrid DNA distribution at the second site. For mutants S180

and C70 (where Table 11*b* rarely applies) there was a total of 9 asci of genotype (1) above and none of genotype (2). The numbers in these two classes should be equal on a symmetric model. It is evident that, at least for these crosses, hybrid DNA formation in both chromatids is rare.

If hybrid DNA extended from the site of YS17 to the second site in both chromatids, followed by mismatch repair to wild type in one of them, a 3:5 ascus would result, as in Table 11*f*. The rarity of such asci (Table 4 and Results, section *h* iv) confirms the rarity of symmetrical hybrid DNA.

The existence, when YS17 is crossed with alleles, of 4:4 asci involving conversion to wild type at each mutant site (Table 11*d*) implies hybrid DNA formation in both recombining chromatids. The occurrence of such asci (Table 4 and 15), even though of low frequency, indicates that hybrid DNA formation is not entirely asymmetrical.

The hypothesis proposed by Meselson & Radding (1975) predicts that hybrid DNA formation will be asymmetrical near the initiation site but may become symmetrical further from it. The predominant asymmetry of the hybrid DNA near YS17 is therefore in agreement with the Meselson–Radding model, if YS17 is an initiation site. Evidence is lacking, however, that symmetrical hybrid DNA is more frequent at greater distances from the initiation point. Such evidence has been obtained for the *b2* locus in *Ascobolus immersus* by Paquette & Rossignol (1978). In the *buff* gene in the absence of YS17, and therefore presumably with a more distant initiation point than in its presence, Sang & Whitehouse (1979*a*) found that recombination was still predominantly asymmetrical.

(iii) *Frequency of crossing-over associated with conversion at YS17*

It was pointed out in section (*b*) of the Results that when YS17 was crossed with wild type only 18 % of the asci showing conversion to wild type had a crossover associated with the conversion. When allowance was made for incidental crossovers in the marked interval, this figure dropped to 13 %, assuming no chromatid interference between the conversion of YS17 and the incidental crossover. Sang & Whitehouse (1979*a*) found that 6:2 asci from crosses of other *buff* mutants with wild type gave a crossover frequency of 32 % or, if corrected for incidental crossovers, 27 %.

In the recombinant asci from pairwise crosses of *buff* mutants, higher frequencies of crossing-over have been found, but again crosses involving YS17 have shown lower values (28–33 %, omitting YS17 × S44 and YS17 × S140: see Results, section (*h*)(i)(5)) than other crosses (48 %: data of Table 6). Bond (1973) obtained a similar result for crosses not involving YS17 (mean value 44 %), though the data were heterogeneous with frequencies ranging from 27 % ± 7 % to 58 % ± 7 % for different pairs of alleles.

The crosses between YS17 and the two mutants, S44 and S140, that are most closely linked to it have given very low frequencies of flanking marker crossing-over (Tables 7, 10, 13 and 15): 0 % for S44 (ignoring the peculiar ascus referred to in the footnote to Table 15) and 4 % for S140. This result is similar to that found

by Carlson (1971) for mutants at the locus for *rudimentary* wings in *Drosophila melanogaster*. He found that with closely linked alleles the frequency of flanking marker recombination was lower than with more distant ones. Indeed, there were indications that with closely-linked mutants the frequency of associated crossing-over became progressively lower the shorter the interval between the mutants. It is in keeping with Carlson's results that YS17 × S44 shows lower values than YS17 × S140 for both recombination frequency (Table 4) and flanking marker crossover frequency.

The significance of these remarkable variations in crossover frequency, depending on the allele or combination of alleles, will be discussed after considering the positions of the crossovers.

(iv) *Positions of crossovers associated with conversion at YS17*

A surprising result from the analysis of 2:6 asci in crosses between YS17 and alleles is the occurrence of crossing-over, not alongside the converted site (YS17), but separated from it by the site of the other allele, which itself shows normal 4:4 segregation (asci in class III of Table 10). This result cannot be explained away by supposing that the map of the *buff* mutants is incorrect, because many crossovers did occur in proximity to YS17 (class II in Table 10). Transposition of the mutant sites merely transposes classes II and III. Moreover, class III asci were found with several different alleles crossed with YS17.

The occurrence of these asci would not be surprising if there was evidence that hybrid DNA often extended to the second site, because the normal 4:4 segregation at this site could then be of secondary origin. In other words, hybrid DNA might have formed and then normal 4:4 segregation been restored through mismatch repair. Such an occurrence has been inferred by Rossignol & Haedens (1978) for crosses involving closely-linked alleles at the *b2* spore colour locus in *Ascobolus immersus*. The frequency with which hybrid DNA extends to the second site from YS17 appears to be too low to explain the frequency of class III 2:6 asci. Some of the 1:7 asci result from hybrid DNA at the second site (Table 11*c*) but their frequency (Tables 4 and 13) is much lower than that of the 2:6 asci (Table 4). Moreover, the 1:7 asci are rarely of class III (Table 13). With class C mutants, class III 1:7 asci, that is, corresponding to Table 11*c* and with a crossover as in row III of Table 12, would be expected to be as frequent as class III 2:6 asci, if they originate in the same way (but with mismatch repair to wild type in the 2:6 asci). Yet, with such mutants (S180 and C70) this category of 2:6's appears to be at least 40 times as frequent as the corresponding 1:7's.

The occurrence in *Sordaria brevicollis* of crossovers separated from the site of conversion by a site showing normal 4:4 segregation is not peculiar to YS17, as it has been found in crosses of other *buff* mutants (Sang & Whitehouse, 1979*b*). A similar result was reported by Fogel & Hurst (1967) from pairwise crosses of *histidine-1* mutants of *Saccharomyces cerevisiae*. Fincham (1974) found it necessary to resurrect the idea that recombinational events are clustered in order to explain his data for the *amination-1* (*am-1*) gene in *Neurospora crassa*. Clustering had been

proposed by Pritchard (1960). This idea conflicts with current models, however, such as that of Meselson & Radding (1975).

If the conversion and the associated crossing-over represent discrete events that may be separated from one another by a region of the genome not involved in hybrid DNA, the basis for mapping alleles by flanking marker behaviour is undermined. The theory of this mapping, as used to map the *buff* mutants (section (*f*) of Results), is that in pairwise crosses the two recombinant flanking marker genotypes (R1 and R2: see Table 5) in wild-type recombinant spores have unequal probabilities of arising that depend on the sequence of the sites. The R1 genotype can result from hybrid DNA extending only to one of the two sites, followed by mismatch repair to wild type, whereas R2 requires hybrid DNA to extend to both sites, followed by mismatch repair independently at each of them to wild type. The R1 genotype can also arise in this more complex way. But, provided the hybrid DNA does not always extend to both sites, R1 will be more frequent than R2, thus establishing the site sequence. If R2 exceeds R1 one would conclude that the sequence was incorrect: reversal of the sequence will interchange R1 and R2. It was equality of R1 and R2, but inequality of the parental flanking marker genotypes (P1 and P2), that led Fincham (1974) to favour clustering of recombinational events at the *am-1* locus in *Neurospora*. Inequality of P1 and P2 implies hybrid DNA often reaching one site only, but then R1 should be less than R2.

The analysis of recombinant asci from crosses of YS17 with alleles has shown that, with the 2:6 and 1:7 asci, the R1 and R2 flanking marker genotypes in the wild-type spores arise from class II and class III events, respectively (Tables 10, 13). The relative frequencies of R1 and R2 will therefore depend on how often a crossover occurs alongside or separate from the site showing conversion. The mapping will be reliable only if crossing-over alongside (giving R1) is consistently more frequent than crossing-over beyond the site showing normal 4:4 segregation (giving R2). Anomalies in the map will be expected if such consistency is lacking. This might explain the discrepancy when the R1/R2 data from the S41 × YS17 crosses (Table 7) are compared with the results of the other crosses involving these mutants that have led to the map in Fig. 1. Some further inconsistencies, that have been revealed in two-point crosses involving YS17 with its high frequency conversion suppressed, might have a similar explanation (manuscript in preparation).

According to the dual-event hypothesis outlined above, conversion and its associated crossing-over represent two separate, though interrelated, events. They are interrelated because they occur in proximity, though not necessarily adjacent, and involve the same two chromatids. The variations in crossover frequency described in the previous section would be attributed to differences in the frequency of occurrence of the second event, if that is the crossover. Sang & Whitehouse (1979*a*) found that 5:3 and 3:5 asci, in crosses of *buff* mutants with wild type, were associated with a lower frequency of flanking marker crossing-over than 6:2 and 2:6 asci. They suggested that an enzyme aggregate might control

recombination, with the result that there were interactions between different steps. Extending this idea to explain the crossover variations, it would be necessary to postulate that the high-frequency conversion of YS17, or other peculiarities of it as an initiation site, reduce the likelihood of a second event. A further reduction occurs when there is recombination in the short interval between the site of YS17 and the sites of the mutants S44 and S140 that are closely linked to it.

At present, the hypothesis of an enzyme aggregate can explain only in general terms how modifications of the primary event (assumed to be the conversion) might affect the likelihood of the second one (the crossover).

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