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Intragenic recombination pattern within the 164 locus of *Ascobolus immersus* in the presence of outside markers

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SUMMARY

Seven ascospore colour mutants of the 164 locus of *Ascobolus immersus* were mapped, and their basic conversion frequencies established. Polarization of the sum of the basic conversion frequencies was observed. The recombination frequencies from the two-point crosses (in repulsion) are nonadditive. The influence of one mutant on the frequency of conversion of another in crosses in repulsion and coupling was pronounced. The frequency of crossing over between a pair of mutants was higher in the cross in repulsion than in coupling. A total of 535 asci containing a pair of wild-type spores were analysed from five crosses in repulsion. 193 6w:2d asci originated from the conversion of the proximal allele, 43 6w:2d asci from conversion of the distal allele and 277 asci exhibited reciprocal recombination. 54 6w:2d asci from one cross in coupling were analysed. These asci were classified and assigned to four classes: proximal allele conversion, distal allele conversion, reciprocal recombination and simultaneous conversion of wild type-alleles to mutants. In all classes of recombinant asci analysed the frequencies of additional exchanges in the adjacent intervals were higher than in random samples of asci. The wild-type chromatid was involved in the additional exchanges in the majority of crosses with a frequency exceeding 60%.

1. INTRODUCTION

Intragenic recombination by means of tetrad analysis has been studied in *Ascobolus immersus* (Lissouba *et. al.* 1962; Rossignol, 1964; Makarewicz, 1964; Kruszewska & Gajewski, 1967; Emerson & Yu-Sun, 1967; Mousseau, 1967; Paszewski & Prażmo, 1969), *Sordaria fimicola* (Fields & Olive, 1967; Kitani & Olive, 1967; 1969), *Saccharomyces cerevisiae* (Fogel & Hurst, 1967; Fogel & Mortimer, 1969) and in *Neurospora crassa* (Stadler & Towe, 1963; Case & Giles, 1964).

The most extensive data on conversion frequencies in one-point, two-point and three-point crosses come from studies on *Ascobolus*. However, since no outside markers were available it was very difficult to compare these data with those obtained in other organisms.

The purpose of the present work was to study recombination within the 164 locus with the use of the morphological mutation *col2* as distal marker, while the

centromere with which the 164 locus is closely linked could be used indirectly (through segregation of the mating type in a tetrad) as proximal marker. Thus the results can be compared directly with other data concerning intra-locus recombination in *Ascobolus*, as well as with the data obtained on other fungi by means of tetrad or random spore analyses.

2. MATERIAL AND METHODS

The 164 locus is located in chromosome I, 8 map units from the centromere and 18 map units from the gene *col2* (Paszewski, Surzycki & Mankowska, 1966). Seven very closely linked and probably allelic mutations were used in the experiments: 24, 10, W173, K94, K140, K115 and K70. They have colourless (white) ascospores, contrasting with the dark colour of the wild type spores. All of them were of spontaneous origin. The first two were kindly offered us by Professor Rizet, while the others were obtained in this Department. The morphological mutant *col2* was obtained following ultraviolet irradiation of ascospores.

There is evidence (Makarewicz, 1964) that in *Ascobolus immersus* mating type is a centromere marker: it shows practically 100 % first-division segregation. Thus in an allelic cross of $a_1 \times a_2$ type, if in the tetrad no crossing over took place between the mutant sites and their centromere, all the a_1 segregants will be of the one mating type and all a_2 of the other. If, on the other hand, spore pairs carrying an a_1 mutant are of opposite mating types, and the same is true for a_2 mutants, this means that an exchange took place between the mutant sites and their centromere. In this indirect way the mating type could be used as a 'proximal marker' in crosses involving alleles from 164 locus.

The media and the techniques used were the same as described by Paszewski *et al.* (1966). Since after a prolonged vegetative growth the strains lose the ability for sexual reproduction, all mutants were periodically crossed with the wild-type strain and re-isolated. The germination of isolated ascospores was about 78 %. The octads were tested by the backcross tests.

3. RESULTS

(*a*) *Genetic map of* 164 *locus*

(i) *Gene conversion in one-point crosses*

Each of the seven mutants was crossed with the wild-type to determine the frequency and pattern of gene conversion. Besides the majority of 4 white : 4 dark (4w : 4d) octads, also 6w : 2d and 2w : 6d were observed. Very rarely 8w : 0d, 0w : 8d and 5w : 3d, 3w : 5d, 7w : 1d and 1w : 7d were found, but when tested all showed normal 4 : 4 segregation of mutant and wild type alleles. Samples of 6w : 2d and 2w : 6d asci from different crosses were tested. Some of them showed 4w : 4d segregation. Thus the basic frequencies of conversion from mutant to wild type and from wild type allele to mutant were established after having analysed samples of 6w : 2d and 2w : 6d asci from each cross (Table 1). Comparison of the frequencies

Table 1. *One-point crosses: the results of ascus analysis and basic conversion frequencies for mutants of the* 164 *locus*

Cross	No. of octads scored	No. of octads with segregation:		No. tested	No. of conversion octads	Basic conversion frequencies ($\times 10^3$)	Sum of basic conversion frequencies
W173 × +	145585	6:2	711	20	11	2·68 ± 0·13	3·02
		2:6	192	32	8	0·34 ± 0·05	
24 × +	140851	6:2	281	37	8	0·43 ± 0·05	2·23
		2:6	343	43	32	1·80 ± 0·11	
140 × +	136323	6:2	324	18	14	1·84 ± 0·12	2·94
		2:6	210	7	5	1·10 ± 0·09	
10 × +	54649	6:2	79	28	8	0·41 ± 0·09	0·94
		2:6	56	33	17	0·53 ± 0·10	
94 × +	76414	6:2	164	25	6	0·51 ± 0·08	1·15
		2:6	98	12	6	0·64 ± 0·09	
70 × +	53978	6:2	105	34	12	0·68 ± 0·11	0·84
		2:6	56	25	6	0·16 ± 0·05	
115 × +	116598	6:2	167	37	8	0·31 ± 0·05	0·80
		2:6	240	42	10	0·49 ± 0·06	

Table 2. *Frequencies of recombinant octads in two-point crosses*

Cross	No. of octads scored	6w:2d octads		
		No.	frequency ($\times 10^3$)	S.E.
W173 × 24	85260	17	0·19	± 0·05
W173 × 140	62489	8	0·12	± 0·04
W173 × 10	72985	279	3·82	± 0·23
W173 × 94	56107	208	3·70	± 0·25
W173 × 70	79808	818	10·24	± 0·36
W173 × 115	69249	652	9·41	± 0·37
24 × 140	54485	32	0·58	± 0·10
24 × 10	61993	477	7·69	± 0·34
24 × 94	55151	481	8·72	± 0·40
24 × 70	47054	467	9·92	± 0·46
24 × 115	47884	394	8·22	± 0·41
140 × 10	96154	222	2·30	± 0·15
140 × 94	58069	187	3·22	± 0·24
140 × 70	127807	538	4·20	± 0·18
140 × 115	180691	2457	13·59	± 0·27
10 × 94	93475	0	0	
10 × 70	31671	99	3·12	± 0·31
10 × 115	28447	60	2·10	± 0·27
94 × 70	6571	9	1·36	± 0·46
94 × 115	37898	122	3·21	± 0·29
70 × 115	81864	369	4·50	± 0·23

of conversion in opposite directions shows that for some alleles such as W173, 24 and 70, they are significantly different, while for 140, 94 and 10 they are similar.

(ii) *Order of alleles (two-point and three-point crosses)*

Seven mutants of the 164 locus were crossed with each other and the frequencies of 6w:2d octads were scored (Table 2). In some crosses a few 5w:3d, 4w:4d and 7w:1d asci were observed. These octads, as well as 0w:8d, 8w:0d asci and asci with odd segregation from one-point crosses, were counted in the general pool of asci.

Table 3. *Frequencies of recombinant octads in three-point crosses*

Cross	No. of asci 8w:0d	6w:2d asci	
		No.	frequency ($\times 10^3$)
1. 24·115 × 70	50734	2	0·04
2. 24·115 × W173	12701	2	0·16
3. 24·115 × 10	16595	3	0·18
4. 24·10 × 115	16677	36	2·20
5. W173·70 × 24	20554	0	0
6. W173·70 × 140	15070	0	0
7. W173·70 × 10	18093	6	0·33

On the basis of recombination frequencies, in two-point crosses the alleles could not be ordered unequivocally. Therefore three-point crosses were carried out (Table 3). In their interpretation it was assumed that between these mutants reciprocal exchanges occurred. In the first and third cross the frequencies of 6w:2d asci are considerably lower than those found in two-point crosses involving mutants 24, 115, 70 and 10. This indicates that mutants 70 and 10 are located between 24 and 115. On the other hand, in cross No. 4 the frequency of 6w:2d asci is similar to that found in the two-point cross 10 × 115. This confirms the conclusion that mutant 10 is located between 24 and 115. In cross no. 2 the frequency of 6w:2d asci is almost identical with that observed in the two-point cross W173 × 24. This implies that W173 is not located between 24 and 115. The results of cross no. 5 point to the same conclusion. The results of crosses no. 6 and 7 suggest that mutants 140 and 10 are located between W173 and 70.

The sequence of alleles in respect to the centromere was determined in three crosses by the marker combinations found in asci containing wild-type and double-mutant (reciprocal recombinant) spores.

Cross	No. of spores	
	+ + +	+ + *col2*
24.*col2* × 115	22	43
24 × 115.*col2*	44	23
W173 × 70.*col2*	61	18

In the first cross the + + *col2* spores were more frequent than + + + spores, while in the second the situation was reversed. In the third cross the + + +

spores were more frequent than + +*col2*. It is evident that W173 and 24 mutants are proximal, while 70 and 115 are distal to the centromere.

The arrangement of alleles is shown on the map of the 164 locus (Fig. 1). There does not seem to be correlation between the basic conversion frequencies of mutant

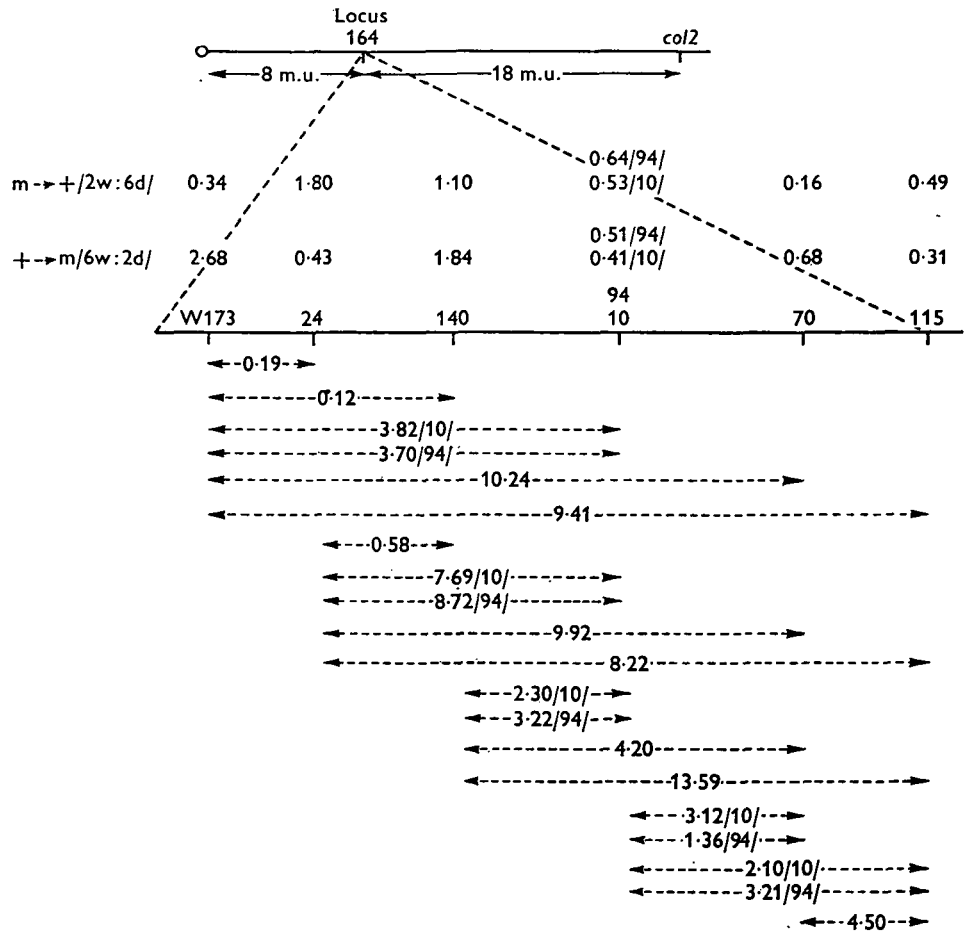

Fig. 1. Map of locus 164.

to wild type or in the opposite direction, and their positions on the map. However, the sums of basic conversion frequencies (frequencies of 6w:2d plus 2w:6d asci) for the successive mutants increase in the direction from *col2* to the centromere (Table 1).

(iii) *Two-point crosses in repulsion*

Five crosses were made: 24.*col2* × 115, 24 × 115.*col2*, 24 × 10.*col2*, W173 × 70.*col2* and W173 × 10.*col2*. W173 and 70 mutants had the lowest basic frequencies of conversion to wild-type, mutant 24 had the highest, while mutants 115 and 10 were intermediate (Table 1).

Table 4. *Types of recombinant asci from crosses between* 164 *alleles in the presence of the* col2 *marker*

Ascus type	First spore-pair	Second spore-pair	Third spore-pair	Fourth spore-pair	No. of asci analysed from crosses: 24.*col2* × 115	24 × 115.*col2*	24 × 10.*col2*	W173 × 70.*col2*	W173 × 10.*col2*	Total
			6w:2d asci containing conversion of proximal allele							
I	+ + −	1 + −	+ 2 +	+ 2 +	27					75
	+ + +	1 + +	+ 2 −	+ 2 −		23	14	8	3	
II*	+ + −	1 + −	+ 2 +	+ 2 +	22					
	+ + +	1 + +	+ 2 −	+ 2 −		23	3	3	2	53
III	+ + −	1 + +	+ 2 +	+ 2 −	5					19
	+ + +	1 + −	+ 2 −	+ 2 +		1	10	3	0	
IV*	+ + −	1 + +	+ 2 +	+ 2 −	1					9
	+ + +	1 + −	+ 2 −	+ 2 +		3	2	3	0	
V	+ + +	1 + −	+ 2 −	+ 2 +	9					28
	+ + −	1 + +	+ 2 +	+ 2 −		10	4	5	0	
VI*	+ + +	1 + −	+ 2 −	+ 2 +	2					5
	+ + −	1 + +	+ 2 +	+ 2 −		0	3	0	0	
VII	+ + +	1 + +	+ 2 −	+ 2 −	1					1
	+ + −	1 + −	+ 2 +	+ 2 +		0	0	0	0	
VIII	+ + −	1 2 +	+ 2 +	+ 2 −	2					2
	+ + +	1 2 −	+ 2 −	+ 2 +		0	0	0	0	
IX	+ + −	1 2 −	+ 2 +	+ 2 +	1					1
	+ + +	1 2 +	+ 2 −	+ 2 −		0	0	0	0	
X	+ + +	1 + +	+ 2 +	+ 2 −	0					1
	+ + −	1 + −	+ 2 −	+ 2 +		1	0	0	0	
XI*	+ + −	1 2 −	+ 2 +	+ 2 +	0					1
	+ + +	1 2 +	+ 2 −	+ 2 −		0	0	1	0	
			6w:2d asci containing conversion of distal allele							
XII	+ + +	1 + −	1 + −	+ 2 +	0					12
	+ + −	1 + +	1 + +	+ 2 −		2	4	0	6	
XIII*	+ + +	1 + −	1 + −	+ 2 +	1					
	+ + −	1 + +	1 + +	+ 2 −		0	2	2	0	5

Table 4 (*cont.*)

Ascus type	First spore-pair	Second spore-pair	Third spore-pair	Fourth spore-pair	No. of asci analysed from crosses: 24.*col2* × 115	24 × 115.*col2*	24 × 10.*col2*	W173 × 70.*col2*	W173 × 10.*col2*	Total
XIV	+ + −	1 + −	1 + +	+ 2 +	2					10
	+ + +	1 + +	1 + −	+ 2 −		2	0	4	2	
XV*	+ + −	1 + −	1 + +	+ 2 +	1					3
	+ + +	1 + +	1 + −	+ 2 −		0	1	1	0	
XVI*	+ + +	1 + +	1 + −	+ 2 −	2					2
	+ + −	1 + −	1 + +	+ 2 +		0	0	0	0	
XVII	+ + −	1 + +	1 + −	1 2 +	0					1
	+ + +	1 + −	1 + +	1 2 −		1	0	0	0	
XVIII	+ + −	1 + +	1 + +	+ 2 −	1					6
	+ + +	1 + −	1 + −	+ 2 +		1	0	2	2	
XIX*	+ + −	1 + +	1 + +	+ 2 −	0					3
	+ + +	1 + −	1 + −	+ 2 +		2	0	0	1	
XX	+ + +	1 + +	1 + −	+ 2 −	0					2
	+ + −	1 + −	1 + +	+ 2 +		0	0	2	0	
			6w:2d asci containing reciprocal recombinants							
XXI	+ + −	1 + −	1 2 +	+ 2 +	20					89
	+ + +	1 + +	1 2 −	+ 2 −		23	17	24	5	
XXII*	+ + −	1 + −	1 2 +	+ 2 +	13					52
	+ + +	1 + +	1 2 −	+ 2 −		10	12	11	6	
XXIII	+ + +	1 + −	1 2 −	+ 2 +	7					25
	+ + −	1 + +	1 2 +	+ 2 −		6	3	9	0	
XXIV*	+ + +	1 + −	1 2 −	+ 2 +	8					17
	+ + −	1 + +	1 2 +	+ 2 −		1	3	3	2	
XXV	+ + +	1 + −	1 2 +	+ 2 −	3					
	+ + −	1 + +	1 2 −	+ 2 +		10	2	5	2	22
XXVI*	+ + +	1 + −	1 2 +	+ 2 −	2					
	+ + −	1 + +	1 2 −	+ 2 +		5	5	0	1	13
XXVII*	+ + −	1 + +	1 2 −	+ 2 +	2					
	+ + +	1 + −	1 2 +	+ 2 −		1	1	2	0	6

Table 4 (*cont.*)

Ascus type	First spore-pair	Second spore-pair	Third spore-pair	Fourth spore-pair	No. of asci analysed from crosses: 24.*col2* × 115	24 × 115.*col2*	24 × 10.*col2*	W173 × 70.*col2*	W173 × 10.*col2*	Total
XXVIII	+ + −	1 + +	1 2 −	+ 2 +	4					24
	+ + +	1 + −	1 2 +	+ 2 −		2	3	14	1	
XXIX	+ + −	1 + +	1 2 +	+ 2 −	3					16
	+ + +	1 + −	1 2 −	+ 2 +		3	1	8	1	
XXX*	+ + −	1 + +	1 2 +	+ 2 −	1					9
	+ + +	1 + −	1 2 −	+ 2 +		4	2	2	0	
XXXI*	+ + +	1 + +	1 2 −	+ 2 −	1					
	+ + −	1 + −	1 2 +	+ 2 +		1	0	1	0	3
XXXII	+ + +	1 + +	1 2 −	+ 2 −	1					1
	+ + −	1 + −	1 2 +	+ 2 +		0	0	0	0	
XXXIII	+ + −	1 2 −	1 2 +	+ 2 +	0					2
	+ + +	1 2 +	1 2 −	+ 2 −		1	1	0	0	
XXXIV	+ + +	1 2 +	1 2 −	+ 2 −	2					2
	+ + −	1 2 −	1 2 +	+ 2 +		0	0	0	0	
XXXV	+ + −	1 2 +	1 + −	1 + +	0					2
	+ + +	1 2 −	1 + +	1 + −		2	0	0	0	
XXXVI	+ + −	1 2 +	+ 2 −	+ 2 +	0					1
	+ + +	1 2 −	+ 2 +	+ 2 −		0	0	1	0	
XXXVII	+ + +	1 + −	1 2 +	1 2 −	0					1
	+ + −	1 + +	1 2 −	1 2 +		1	0	0	0	
XXXVIII*	+ + −	1 + +	1 2 −	1 + +	0					1
	+ + +	1 + −	1 2 +	1 + −		0	0	0	1	
Total										525

The crosses were of two types:

$$0\text{—}\ \frac{1+}{+2}\ \frac{col2}{+} \quad \text{and} \quad 0\text{—}\ \frac{1+}{+2}\ \frac{+}{col2},$$

where 1 and 2 are the proximal and distal alleles, respectively. For each ascus type the upper row gives the genotypes of spores from the first type of crosses (proximal mutant in coupling with *col2*), while in the lower row the genotypes of spores from the second type of crosses (distal mutant in coupling with *col2*).

* Second-division segregation of the mutants.

Table 5. *Distribution of additional exchanges in the recombinant asci described in Table 4*

$$0 \text{—} \frac{1 \quad +}{+ \quad 2} \text{—} \frac{col2}{+}$$

Interval: I II III

Number and relative frequencies of asci with:

	Conversion of proximal allele					Conversion of distal allele					Reciprocal recombination						
		With crossing over in interval					With crossing over in interval					With crossing over in interval					
Cross	Without crossing over	I	III	IaIII	Total	Without crossing over	I	III	IaIII	Total	Without crossing over	I	I	IaIII	Total	No. of other asci	Total
24.*col2* × 115	27	22	17	3	69	0	2	3	2	7	20	13	18	14	65	5	146
	0·39	0·32	0·25	0·04			0·28	0·43	0·28		0·31	0·20	0·28	0·21			
24 × 115.*col2*	23	23	12	3	61	2	0	3	2	7	23	10	21	12	66	8	142
	0·38	0·38	0·20	0·05		0·28		0·43	0·28		0·35	0·15	0·32	0·18			
24 × 10.*col2*	14	3	14	5	36	4	2	0	1	7	17	12	9	11	49	2	94
	0·39	0·08	0·39	0·14		0·57	0·29		0·14		0·35	0·25	0·18	0·22			
W173 × 70.*col2*	8	3	8	3	22	0	2	8	1	11	24	11	36	8	79	6	118
	0·36	0·14	0·36	0·14			0·18	0·72	0·10		0·30	0·14	0·46	0·10			
W173 × 10.*col2*	3	2	0	0	5	6	0	4	1	11	5	6	4	3	18	1	34
	0·60	0·40				0·60		0·30	0·10		0·28	0·33	0·22	0·17			
Total	75	53	51	14	193	12	6	18	7	43	89	52	88	48	277	22	535
	0·39	0·27	0·26	0·07		0·28	0·14	0·42	0·16		0·32	0·19	0·32	0·17			

From these crosses a total of 535 complete 6w:2d asci were tested. 525 of them could be roughly grouped into three classes characterized by (1) conversion of the proximal allele: asci containing one pair of wild-type ascospores, one pair carrying a proximal allele and two pairs of ascospores carrying a distal allele (Table 4, types I–VII and X); types VIII, IX and XI are formally included in this class, since they show conversion of a proximal mutant, but they carry also a double mutant pair of spores, and in this respect resemble asci of class (3); (2) conversion of the distal allele: asci containing one pair of wild-type ascospores, one pair carrying a distal allele and two pairs of ascospores carrying a proximal allele (types XII–XX); (3) reciprocal recombinants: asci containing one pair of wild-type ascospores, one pair of double-mutant spores and two pairs carrying each of the two parental mutants (types XXI–XXXII). In asci belonging to types XXXIII–XXXVIII both, reciprocal and non-reciprocal events took place. The most frequent were type XXI (89 asci) derived from reciprocal recombination, and type I due to the conversion of the proximal allele (75 asci), without any additional exchanges. For each tetrad a chromatid scheme was constructed and additional exchanges are postulated in the adjacent intervals to account for the observed genotypes. The results of exchange distribution for each cross and for each of the three classes of asci are presented in Table 5.

In the first two crosses, the conversion of the proximal allele is as frequent as reciprocal recombination; in the remaining three crosses asci due to reciprocal recombination are more frequent. Distal allele convertants are less frequent than proximal allele convertants, with the exception of the last W173 × 10.*col2* cross.

(iv) *Two-point crosses in coupling*

The double mutant strains 24 115 and W173 10 carrying also the *col2* marker, were crossed with the wild-type strain (Table 6). Here, of course, the most frequent were apparently non-recombinant 4w:4d asci. The 2w:6d octads could result only

Table 6. *Frequency of recombinant asci in two-point crosses (in coupling)*

		No. and frequency (× 10^3) of asci			
		6w:2d			2w:6d
Cross	No. 4w:4d asci	Scored	Tested	Recombinant	Scored
24.115.*col2* × + + +	103·531	234 2·25 ± 1·47	79	54 1·54 ± 0·071	41 0·39 ± 0·061
W173.10.*col2* × + + +	16·773	10 0·59 ± 0·18			4 0·23 ± 0·12

from simultaneous conversion of both mutants to their wild-type alleles. These were not analysed, because they were too rare and in most of them two dark spores grown into one. A total of 79 6w:2d octads from 24.115.*col2* × + + + cross were analysed. Twenty-five of them were probably due to a physiological disturbance of

spore colouration, since they showed in fact 4w:4d segregation. The remaining 54 asci represent various recombinational events. They were grouped into 25 types (Table 7), belonging to four major classes:

(1) Conversion of the wild-type allele to the proximal mutant (+→ 24): asci containing a pair of wild-type spores, two pairs of double mutant spores and one pair carrying the proximal mutant (frequency $0{\cdot}37 \times 10^{-3}$).

Table 7. *Recombinant 6w:2d asci from the cross* 24.115.col2 × *wild-type strain*

Ascus type	First spore-pair	Second spore-pair	Third spore-pair	Fourth spore-pair	Number of asci
6w:2d asci due to conversion of the wild-type allele to the proximal mutant					
A	+ + +	1 2 −	1 2 −	1 + +	5
B	+ + +	1 2 −	1 2 +	1 + −	2
C*	+ + +	1 2 −	1 2 +	1 + −	1
D	+ + −	1 2 +	1 2 −	1 + +	1
E	+ + −	1 2 +	1 2 +	1 + −	2
F*	+ + −	1 2 −	1 2 +	1 + +	1
G*	+ + +	1 2 +	1 2 −	1 + −	1
H	+ + −	1 2 −	1 2 −	1 + +	1
6w:2d asci due to conversion of the wild-type allele to the distal mutant					
I	+ + +	1 2 −	1 2 −	+ 2 +	2
J	+ + −	1 2 +	1 2 −	+ 2 +	2
K	+ + −	1 2 +	1 2 +	+ 2 −	1
L*	+ + +	1 2 −	1 2 −	+ 2 +	2
M*	+ + −	1 2 +	1 2 −	+ 2 +	2
N*	+ + −	1 2 +	1 2 +	+ 2 −	1
O	+ + +	1 2 −	1 2 +	+ 2 −	2
P*	+ + +	1 2 −	1 2 +	+ 2 −	3
R	+ + −	1 2 +	+ 2 −	+ 2 +	1
S*	+ + +	1 + −	1 + +	+ 2 −	1
6w:2d asci due to simultaneous conversion of the wild-type alleles to mutants					
T	+ + +	1 2 −	1 2 −	1 2 +	6
U*	+ + +	1 2 −	1 2 −	1 2 +	7
W	+ + −	1 2 −	1 2 +	1 2 +	1
6w:2d asci due to reciprocal recombinants					
X*	+ + +	1 2 −	1 + +	+ 2 −	4
Y	+ + +	1 2 −	1 + +	+ 2 −	3
Z*	+ + −	1 2 −	1 + +	+ 2 +	1
Ż	+ + −	1 2 +	1 + +	+ 2 −	1
Total					54

* Second-division segregation of the mutants.

(2) Conversion of the wild-type allele to the distal mutant (+→ 115): asci containing a pair of wild-type spores, two pairs of double-mutant spores and one pair carrying the distal mutant (frequency $0{\cdot}45 \times 10^{-3}$).

(3) Simultaneous conversion of two wild-type alleles to mutants (+ +→ 24 115): asci containing a pair of wild-type spores and three pairs of double-mutant spores (frequency $0{\cdot}40 \times 10^{-3}$).

(4) Reciprocal recombinants: asci containing a pair of wild-type spores, a pair of double-mutant spores, a pair of spores carrying the proximal mutant and a pair of spores carrying the distal mutant (frequency $0{\cdot}28 \times 10^{-3}$).

The distribution of exchanges observed in the 54 6w:2d asci from this cross is shown in Table 8. It was impossible to distinguish whether the tetrads belonging to the U type originated from an exchange in interval I or in interval III.

Table 8. *Distribution of exchanges in 6w:2d asci from 25.115.col2 × + + + cross*

$$0 \text{——} \frac{24}{+} \quad \frac{115}{+} \quad \frac{col2}{+}$$

Interval: I II III

Ascus class	Number and frequency of asci with crossing over in interval: I	III	I and III	Without crossing over	Total
Conversion of proximal allel + → 24	0	5 0·35	3 0·21	6 0·43	14
Conversion of distal allele + → 115	3 0·17	6 0·35	6 0·35	2 0·12	17
Reciprocal recombinants	4 0·50	1 0·10	1 0·10	3 0·30	9
Simultaneous conversion + + → 24 115	←———	8 0·57	———→	6 0·43	14
Total					54

(*b*) *Analysis of results*

(i) *Comparison of conversion frequencies in one-point and two-point crosses*

Analysis of the 6w:2d asci from two-point crosses in repulsion provided estimates of the conversion frequencies of the proximal and distal mutants, as well as the reciprocal recombination frequencies. In Table 9 these frequencies are compared with the basic conversion frequencies of the same mutants.

In two-point crosses analysed in detail in the present work the conversion frequencies of the proximal mutants (either 24 or W173) were higher than in the respective one-point crosses (Table 9). The conversion frequency of the distal mutant 115 was slightly lower in crosses with 24 than in the one-point cross. The distal mutant 10 had practically an identical conversion frequency in the one-point cross and in a cross with 24, but converted twice as frequently in a cross with W173. The distal mutant 70 converted four times as frequently in a cross with W173 as in the one-point cross. A similar tendency was observed in the frequencies of + → m conversions in one- and two-point crosses in coupling.

In the cross 24.115.*col2* × + + + the overall frequencies of conversion of the wild-type allele to the proximal or distal mutant are the sum of a 'single' conversion frequency (+ → m), and the simultaneous conversion frequency of the wild-type alleles to both mutants. It was found that the frequency of conversion + → 24 was: $0{\cdot}37 \times 10^{-3} + 0{\cdot}40 \times 10^{-3} = 0{\cdot}77 \times 10^{-3}$, while the frequency of con-

version $+ \rightarrow 115$ was: $0{\cdot}45 \times 10^{-3} + 0{\cdot}40 \times 10^{-3} = 0{\cdot}85 \times 10^{-3}$. The basic conversion frequencies of both these mutants are lower (see Table 1). The frequency of the reciprocal recombination in coupling ($0{\cdot}28 \times 10^{-3}$) is considerably lower than in repulsion ($3{\cdot}97 \times 10^{-3}$). Thus the arrangement of the mutants on the chromosome seems to affect the frequencies of all types of exchanges in which they are involved.

Table 9. *Conversion frequencies of five mutants in one- and two-point crosses*

Cross	Conversion frequency ($\times 10^3$) m $\rightarrow$ + of: Proximal mutant: Basic conversion frequency	Proximal mutant: Conversion frequency in two-point crosses	Distal mutant: Basic conversion frequency	Distal mutant: Conversion frequency in two-point crosses	Frequency ($\times 10^3$) of reciprocal recombination
24 *col2* × 115 24 × 115 *col2*	1·80 ± 0·11	3·56 ± 0·29	0·49 ± 0·06	0·38 ± 0·09	3·97 ± 0·28
24 × 10 *col2*	1·80 ± 0·11	2·94 ± 0·20	0·51 ± 0·10	0·57 ± 0·09	4·18 ± 0·33
W173 × 70 *col2*	0·34 ± 0·05	1·90 ± 0·15	0·16 ± 0·05	0·92 ± 0·10	7·42 ± 0·23
W173 × 10 *col2*	0·34 ± 0·05	0·52 ± 0·08	0·51 ± 0·10	1·00 ± 0·13	2·01 ± 0·16

(ii) *Additional exchanges in recombinant asci*

The exchanges observed in the I and III intervals in each class of asci were compared with the expected numbers of exchanges. The expected frequency of exchanges in the III interval is the frequency of NPD and T asci in a random sample from the 164 × *col2* cross and is equal to 0·36. The expected frequency of exchanges in interval I is the frequency of the second division segregation of the 164 gene (Paszewski *et al.* 1966), and is equal to 0·16.

The comparisons of the expected frequencies of exchanges with those observed in the 6w : 2d asci from five two-point crosses are shown in Table 10. In all but one cross conversion of either the proximal or distal mutant within the 164 locus enhanced about 2-fold the exchanges in the adjacent proximal interval. Increased numbers of exchanges in the distal (III) interval were observed in the third and fourth crosses in asci showing conversion of a proximal mutant, and in the first, second and fourth crosses showing conversion of a distal mutant. In one cross (24 × 10.*col2*) there was a considerable decrease of additional exchanges in interval III in asci showing conversion of the distal mutant. Thus an increased number of additional exchanges in adjacent intervals (i.e. negative interference) was observed in convertant asci in the majority of crosses. However, the intensity of the negative interference was different in each cross. There does not seem to be any correlation between the intensity of negative interference, and the basic conversion frequencies of the mutants involved.

In the asci with reciprocal recombination, the frequency of additional recombination increased in the proximal interval about 2·5-fold in all crosses, and in the distal interval only slightly, in the first, second and fourth cross (Table 10).

Table 10. *Relative frequencies of additional exchanges in three classes of 6w:2d asci (data from Table 5)*

$$0 \text{——} \frac{1 \quad +}{+ \quad 2} \quad \frac{col2}{+}$$

Interval: I II III

Cross	Frequency of asci with: Conversion of proximal mutants: Without crossing over	Conversion of proximal mutants: With crossing over in interval I	Conversion of proximal mutants: With crossing over in interval III	Conversion of distal mutants: Without crossing over	Conversion of distal mutants: With crossing over in interval I	Conversion of distal mutants: With crossing over in interval III	Reciprocal recombination: Without crossing over	Reciprocal recombination: With crossing over in interval I	Reciprocal recombination: With crossing over in interval III
24.*col2* × 115	0·39	0·36	0·29	0	0·56	0·71	0·31	0·41	0·49
24 × 115.*col2*	0·38	0·43	0·25	0·28	0·28	0·71	0·35	0·33	0·50
24 × 10.*col2*	0·39	0·22	0·53	0·57	0·43	0·14	0·35	0·47	0·40
W173 × 70.*col2*	0·36	0·28	0·50	0	0·28	0·82	0·30	0·24	0·56
W173 × 10.*col2*	0·60	0·40	0	0·60	0·10	0·40	0·28	0·50	0·39
Frequency of crossing over in random sample of asci	—	0·16	0·36	—	0·16	0·36	—	0·16	0·36

As seen in Table 10 (see also Fig. 1) the frequencies of exchanges between the centromere and the 164 locus showed a tendency to increase with the decrease of recombination frequency within the interval of selection. On the other hand the reverse dependence between the length of the interval under study and the frequency of the additional crossing-over was found for the distal interval. In Table 11 the simultaneous exchanges expected and observed in the I and III intervals are presented. The expected values were calculated by multiplying the frequencies of

Table 11. *Frequency of simultaneous exchanges in the intervals flanking the* 164 *locus*

$$0 \text{——} \frac{1 \quad +}{+ \quad 2} \frac{col2}{+}$$

Interval: I II III

	Frequency of simultaneous exchanges in intervals I and III in asci with:					
	Conversion of proximal mutant		Conversion of distal mutant		Reciprocal recombinations	
Cross	Expected	Observed	Expected	Observed	Expected	Observed
24.*col2* × 115	0·10	0·04	0·40	0·28	0·20	0·21
24 × 115.*col2*	0·11	0·05	0·20	0·28	0·17	0·18
24 × 10.*col2*	0·12	0·14	0·06	0·14	0·19	0·22
W173 × 70.*col2*	0·14	0·14	0·23	0·10	0·13	0·10
W173 × 10.*col2*	0	0	0·04	0·10	0·19	0·17

Table 12. *Frequencies of additional exchanges in four classes of* 6*w*:2*d asci from* 24.115.*col2* × + + + *cross*

$$0 \text{——} \frac{24 \quad 115}{+ \quad +} \frac{col2}{+}$$

Interval: I II III

	Frequency of asci:		
	Without crossing over	With crossing over in interval: I	III
Ascus class			
Conversion of proximal allele + → 24	0·38	0·23	0·61
Conversion of distal allele + → 115	0·12	0·50	0·76
Reciprocal recombinants	0·30	0·60	0·20
Frequency of crossing over in random sample of asci	—	0·16	0·36

exchanges actually found in intervals I and III (see Table 10). In convertant asci the values found are either lower than, or equal to those expected, while in asci in which reciprocal exchanges within the 164 locus took place the values found are always equal to the anticipated ones.

In the cross 24.115.*col2* × + + + (Table 12), in the asci resulting from conversion

of wild-type to mutant allele, the additional exchanges in interval III were twice as frequent as in non-selected asci, while in the asci resulting from the reciprocal recombination, positive interference was found in interval III. In all recombinant asci the frequencies of additional exchanges in interval I were increased.

(iii) *Participation of the 'wild-type' chromatid in the additional exchanges*

In the random sample of asci each of the sister chromatids may be expected to be involved in one-half of the total number of exchanges which took place in a given interval. Could that be true also for the chromatid which became 'wild-type' owing to a recombinational event within the 164 locus? Unfortunately only

Table 13. *Frequency with which the 'wild-type' chromatid was involved in crossing over in region III*

Cross	Conversion of: Proximal mutant (%)	Distal mutant (%)	Reciprocal recombination (%)
24.*col2* × 115	75	60	68
24 × 115.*col2*	67	100	70
24.115.*col2* × + + +	75	58	50
24 × 10.*col2*	37	100	65
W173 × 70.*col2*	45	78	41
W173 × 10.*col2*	0	100	71

additional exchanges in interval III could be considered, since it was impossible to identify which of the two sister chromatids participated in the exchanges in interval I. As seen in Table 13, among proximal allele convertants, the 'wild-type' chromatid was involved in additional exchanges in more than 50 % in the three first crosses, and less than randomly in the remaining crosses. In all crosses the 'wild-type' chromatid which originated from conversion of a distal allele participated in additional exchanges in interval III more often than randomly, up to 100 %. Also in the asci due to reciprocal recombination within the 164 locus, the 'wild-type' chromatids participated in the additional exchanges in interval III more often than randomly.

(iv) *Chromatid interference*

Chromatid interference results from nonrandom distribution of double cross-overs among the chromatids, i.e. where the proportions of two-strand, three-strand and four-strand double exchanges deviate from a 1:2:1 ratio. Negative chromatid interference occurs when two-strand exchanges are favoured.

Chromatid interference was studied in intervals II and III in 6w:2d asci due to reciprocal recombination. Table 14 presents the numbers of two-, three and four-strand double exchanges for five crosses. Out of a total of 143 asci, 45 two-strand, 69 three-strand and 29 four-strand double exchanges were scored. There was a

statistically significant excess of two-strand over four-strand double exchanges, indicating that the same two chromatids were involved in successive exchanges more often than randomly. A frequent involvement of a 'wild-type' chromatid in an exchange in the III region caused an excess of two-strand over four-strand double exchanges.

Table 14. *Chromatid interference*

	Number of double exchanges		
Cross	2-str	3-str	4-str
24.*col2* × 115 24 × 115.*col2*	25	32	14
24 × 10.*col2*	6	11	3
W173 × 70.*col2*	12	22	11
W173 × 10.*col2*	2	4	1
Total	45	69	29

Among the three-strand double exchanges two types could be distinguished: those in which the second exchange involves the chromatid which is wild-type in respect to spore colouring, and those in which it involves the double mutant chromatid. These two types of asci occurred with similar frequency (38 and 31, respectively). It does not seem unreasonable to assume that higher than random additional exchanges described as negative chromatid interference are 'by-products' of the recombinational events which occurred within the 164 locus.

4. DISCUSSION

Studies on recombination between very closely linked mutants having identical phenotypes (and often shown to be allelic) have been carried out by means of tetrad analysis in *Ascobolus immersus*, *Sordaria*, yeast and *Podospora*. In some respects each locus has its particular features. Thus, in *Sordaria* (Kitani & Olive, 1967, 1969) postmeiotic segregation seems to occur with high frequency, while in *Ascobolus* the overwhelming majority of apparent postmeiotic segregation is due to some kind of disturbances in spore colouration. In some loci the recombinant asci are due almost exclusively to conversion. Indeed, in certain crosses no intra-locus reciprocal exchanges were found (e.g. locus 46, Lissouba *et al.* 1962; Rossignol, 1964). In others, both reciprocal and nonreciprocal events are found, although the latter predominate (e.g. locus Y, Kruszewska & Gajewski, 1967; locus 726, Makarewicz, 1964.) In loci 75 (Rossignol, 1967) and 19 (Mousseau, 1967) in *Ascobolus* and in gene *hi1* in yeast (Fogel & Hurst, 1967) the proportion of intragenic reciprocal events is relatively high. Some crosses within loci 75 and 19 seem to produce predominantly reciprocal events. The same is true for crosses involving mutants 24, 10, W173 and 70 in the present work (Table 5).

When two mutants considered as very closely linked are crossed, the recovered conversion frequencies of each of them are highly reduced as compared with their respective basic conversion frequencies. This can be attributed to recombinational events undetectable in *Ascobolus*, such, for instance, as those which can be dis-

tinguished and were observed in *Podospora* (Picard, 1969): conversion of one mutant to its wild-type allele accompanied by conversion in the opposite direction of the other mutant involved in a cross. In consequence the map distances between the mutants calculated from the frequencies of wild-type recombinants are strikingly lower as compared with such pairs of mutants, detected conversions of which do not interfere with each other. This phenomenon was described by Holliday (1964, 1967) as map expansion. In the 164 locus a recombination fraction in two-point crosses (in repulsion) lower than the sum of basic conversion frequencies of the mutants involved was found only in three crosses (Fig. 1). Here, however, a kind of 'map contraction' was observed (Kruszewska & Gajewski, 1967; Rossignol, 1967; Paszewski & Prażmo, 1969). The sum of (recoverable) recombination fractions between two pairs of adjacent mutants, i.e. W173 × 24 and 24 × 140, was higher than the recombination fraction between the flanking mutants, i.e. W173 and 140. The same is true for 94 × 70 and 94 × 115 crosses.

No wild-type recombinants were found in a cross 10 × 94. Such pairs of mutants are usually considered as single-site, or overlapping deletions, but the possibility cannot be ruled out that they occupy different sites but conversion of one of them so interferes with conversion of the other that no wild-type recombinant can occur. In other words such pairs of mutants may represent an extreme case of map expansion.

In all other crosses a striking 'marker effect' was observed. The increased conversion frequency of certain mutants in crosses with some other mutants was observed previously (Kruszewska & Gajewski, 1967; Mousseau, 1967; Paszewski & Prażmo, 1969; Kitani & Olive, 1969), but never to such an extent as in locus 164.

Mousseau (1967) considered the possibility that such differences in conversion frequencies in one- and two-point crosses might be attributed to different genetic backgrounds of the strains used (Catcheside, Jessop & Smith, 1964; Jessop & Catcheside, 1965; Catcheside, 1966; Smith, 1966).

In the present work this possibility cannot be ruled out, but it does not seem to be very likely for at least two reasons. First, in the course of the work each mutant strain was periodically outcrossed with a wild-type strain and reisolated, to increase its fertility. Yet, no pronounced strain-dependent differences in recombination frequency were observed. In particular, crosses 24 × 115 were carried out with reciprocal arrangements of the *col2* marker, yet the recombination frequencies and patterns obtained were strikingly similar. Secondly, assuming that an independently segregating gene influenced conversion frequencies of the mutants discussed, one should assume further that either it was present in all one-point crosses and led to a decrease of the basic conversion frequencies, or it was present in all two-point crosses and led to increased conversion frequencies of four mutants (24, W173, 10, 70), while decreasing the conversion of one mutant (115). Thus, it seems more likely that the actual 'marker effect' is responsible for the striking differences in conversion frequencies of the same mutants in one- and two-point crosses. The data presented in Table 5 show a definite asymmetry, in four crosses the proximal mutant converting 2–6 times as frequently as distal, while in the

fifth cross the reverse asymmetry is apparent. This asymmetry seems to be due to the 'marker effect' and it may result from conversion frequencies of the mutants used.

In crosses 24 × 10 and 10 × 115 as well as 24 × 70 and 70 × 115 the frequencies of asci carrying wild-type recombinants are much higher than the sums of the basic conversion frequencies of the pairs of mutants involved (Table 2). This suggests that in two-point crosses conversions of 24 and 115 do not interfere with conversions of 10 and 70. The conversion of mutants 10 and 70 in three-point crosses may be unrecoverable, because of some simultaneously occurring additional recombinational events, for instance reciprocal exchanges in the 24–115 interval. Such events were found in *Ascobolus* in three-point crosses (Rossignol, 1967; Paszewski & Prażmo, 1969). They occurred with low frequency in both works cited, but they may be more frequent within the 164 locus where reciprocal recombination occurs often.

Thus, knowing the basic conversion frequency of a mutant, it is still impossible to predict its behaviour in two- and three-point crosses. In some cases the presence of the second mutant can stimulate conversion of the first, while in other crosses either its conversion is suppressed, or undetectable. For these reasons the additivity of map distances would be an exception rather than the rule.

In the present work an interesting aspect of polarity was observed. In one-point crosses neither conversion m → + nor + → m was dependent on the map position of a mutant, but the sum of conversions in both directions was polarized, increasing from distal to proximal mutants (Table 1). It may be supposed that in the 164 locus the conversion frequency of a mutant to its wild-type allele, and that in the opposite direction are predominantly mutant specific, while entry of a mutant into any recombinational event may depend also on its position within the locus.

Double-site conversions seem to be fairly common in fungi. They were found in *Ascobolus* (Mousseau, 1967; Rossignol, 1967; Paszewski & Prażmo, 1969), yeast (Fogel & Mortimer, 1969), *Podospora* (Picard, 1969) and in the half-tetrads analysed in *Aspergillus* (Putrament, 1964; 1967).

In the present work 12 asci exhibited 1:3 segregation of one mutant, and 3:1 segregation of the other mutant involved in a two-point cross (Table 4; types VIII, IX, XI, XXXIII–XXXVI, XXXVIII; Table 7, type R), i.e. about 2% of the total number of asci analysed. The actual number of such asci might be much higher since only part of such recombinational events leads to 6w:2d segregation, while some of the double-site conversions would be undistinguishable from intragenic reciprocal recombination.

In all crosses analysed here, in tetrads carrying wild-type recombinants, additional exchanges either in the proximal or the distal adjacent interval (or in both) were observed. This was the case for convertant as well as crossover asci. In the asci showing conversion of a distal mutant, and in the crossover asci it was preferentially, or even exclusively, the wild-type chromatid which was involved in the additional exchanges in the distal interval. However, in a number of asci (89) carrying a pair of double-mutant ascospores no exchanges of the *col2* marker were

observed. The frequency of such asci was in the present work much higher than found in *hi1* locus in yeast (Fogel & Hurst, 1967). Unfortunately, with the centromere used indirectly as a proximal marker, it was impossible to distinguish which of the sister chromatids participated in the additional exchanges in the proximal interval. This has been formally calculated as a negative chromatid interference (Table 14).

Neither the length of 164 locus nor the positions of the mutants in respect to its ends are known, so that it is impossible to establish whether the additional recombinational events observed were limited to the locus, or some of them occurred outside it. Both alternatives seem to be equally possible, since multiple recombinational events within a locus were found in three-point crosses in *Ascobolus* (Rossignol, 1967; Paszewski & Prażmo, 1969), while simultaneous recombinations within two adjacent or very closely linked cistrons were found in *Neurospora* (Murray, 1968) and *Aspergillus* (Putrament, 1967).

The present data, in agreement with earlier ones (Putrament, 1964; 1967; Fogel & Hurst, 1967), show that on the basis of marker arrangements in wild-type chromatids it is impossible to deduce whether the recombination within a gene was reciprocal or not.

According to the generally known cytological evidence (discussed in detail by Whitehouse, 1969) in a given region only two chromatids are involved in chiasmata, which indicates that only two chromatids are involved in exchanges. This conclusion has been confirmed by extensive tetrad analyses. However, more and more data accumulate which cannot be easily explained by any kind of exchange between two chromatids only. Some of the abundantly marked recombinant half-tetrads analysed in *Aspergillus* (Putrament, 1964, 1967) were attributed to recombinational events in which three chromatids participated within a very short segment of genetic material. The origin of at least two classes of recombinant tetrads in yeast (Classes 4 and 5, Fogel & Hurst, 1967) may be explained only by assuming that the wild-type chromatid originated from a single exchange between two mutant alleles, the second recombinant chromatid was due to a simple conversion $+ \rightarrow 1$ mutant, while the third recombinant chromatid is reciprocal with the wild-type one in respect to the interval including the pair of allelic mutants. In three-point crosses involving allelic mutants (Rossignol, 1964) at least seven tetrads have a three-strands recombinant.

In the present work the recombinant asci belonging to types VIII, IX, XI have both sister strands converted; thus at least one non-sister strand must have been a 'donor' for the conversions to take place. The asci belonging to types XXXV–XXXVII have three chromatids showing such exchanges, which must have involved the same segment of the locus 164. Although the actual number of such asci is low, their frequencies are much higher than if they were due to coincidences of two independent recombinational events. Moreover, such independent events are hardly probable at all because the time for them to occur is limited by the course of meiosis, while space is limited by the distance between allelic mutants involved in a cross.

The present data seem neither to prove nor to disprove any of the current hypotheses concerning the molecular mechanism of recombination (Whitehouse, 1963, 1964, 1965, 1966, 1967; Whitehouse & Hastings, 1965; Holliday, 1964, 1967; Stahl, 1969). Thus it seems premature to discuss them in detail here.

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