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The bearing of mutant and cross specificity on the pattern of intragenic recombination

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1. INTRODUCTION

In earlier papers concerning intragenic recombination the possibility of an influence of a particular mutant on the pattern of recombination has been scarcely taken into account. The various patterns observed in intragenic recombination were usually considered to result from a particular position of the mutated sites within a gene.

More recently, when allelic mutants were crossed with the wild type, it became apparent that their conversion rates differ considerably and for a number of loci there is no correlation between these rates and the position of mutations within a gene (Kruszewska & Gajewski, 1967; Mousseau, 1966, 1967; Rossignol, 1967; Fields & Olive, 1967). Differences in basic conversion rates, i.e. those observed in one-point crosses (Kruszewska & Gajewski, 1967) often, but not always, accounted for the frequency of recombination and its pattern observed in two-point and three-point crosses. It was found in a few cases that a particular mutant affected the conversion rate of another one or had a marked influence on the frequency of crossing-over, i.e. mutant specificity.

Here the effect of the mutants used on the recombination pattern observed in a cross, especially on the polarization and conversion/crossing-over relationship, is studied. It is confirmed that the pattern of intragenic recombination depends on mutant specificity, the position of the mutants within the gene and the way they are introduced into the cross (coupling or repulsion); that is, cross specificity. The data revealed the occurrence of a number of separate but highly correlated recombinational events within a gene. The bearing of the data on recombination models is discussed.

2. MATERIAL AND METHODS

Mutants of *Ascobolus immersus* incapable of producing pigment in ascospores were used in this work. They originated spontaneously from the wild-type strain S_{60}. A mutant, *84W*, isolated in this laboratory, was crossed with about 200 white-spored mutants. These mutants as well as the wild-type strain were generously offered us by Professor G. Rizet. Seven mutants allelic to *84W* were found: *696*, *1085*, *728*, *1025*, *870*, *936*, *1043* and *787* (Paszewski, 1965). The mutants *696*, *1043*, *728*, *1025* and *936* belong to a cluster of closely situated sites. The mutant *936*

was arbitrarily chosen from this group for further experiments involving also mutants *84W*, *787* and *1043*. Among the 59000 asci scored in a cross between *84W* and *787* no recombination was found. One additional white-spored mutant, *873*, from a locus located 39 units distally from *84W* (Paszewski, Surzycki & Mankowska, 1966) was used in crosses aimed to establish the order of sites with respect to the centromere in locus *84W*. Complementation tests (Mousseau, 1963) carried out with these four mutants all gave negative results so the mutants can be regarded as alleles of one functional unit. No wild-type ascospores were observed among 34400 asci scored in the cross *936* × *936*, 49400 asci in the cross *84W* × *84W*, 27344 asci in the cross *787* × *787* and 58038 asci in the cross *1043* × *1043*, indicating a low, if any, reversion rate of these mutants.

The media used and the technique of handling the organism were exactly the same as previously described (Paszewski *et al.* 1966).

3. EXPERIMENTAL

(i) *Establishment of the order of sites with respect to the centromere*

To establish the order of mutants *936* and *84W* with respect to the centromere two crosses were performed: (*a*) *936*.*873* × *84W* and (*b*) *936* × *84W*.*873*. If the order is *centromere–936–84W–873*, the frequency of detectable recombinant asci in the first cross should approach the expected value V_1 calculated from the formula:

$$V_1 = \beta\left(\frac{y}{2}+z\right)+\alpha\left(1-\frac{y}{2}-z\right), \tag{1}$$

and in the second cross should approach the expected value V_2 calculated from the formula:

$$V_2 = \beta\left(1-\frac{y}{2}-z\right)+\alpha\left(\frac{y}{2}+z\right), \tag{2}$$

where z and y are the frequencies of nonparental ditype and tetratype asci, respectively, found in the cross *84W* × *873*; α is the frequency of conversion of *84W* to its wild-type allele observed in the cross *84W* × *936* and β is the frequency of crossing-over between *936* and *84W* in the same cross.

If the order is *centromere–84W–936–873*, the expected frequencies of recombinant asci detected in the crosses in question should be equal to:

$$V_3 = \beta\left(1-\frac{y}{2}-z\right)+\alpha\left(1-\frac{y}{2}-z\right) \tag{3}$$

for cross (*a*) and

$$V_4 = \beta\left(\frac{y}{2}+z\right)+\alpha\left(\frac{y}{2}+z\right) \tag{4}$$

for cross (*b*).

In these formulae the conversion of *936* to its wild-type allele and simultaneous

conversions to the wild-type alleles at sites *84W* and *873* are not taken into account because of the very low frequency of these events (see Table 3 and Paszewski, 1967). Because of the large distance between the loci *84W* and *873*, the possibility of a correlation between conversion at these loci and crossing-over between them can be neglected.

Table 1. *Observed and expected frequencies of recombinant (6:2) asci in crosses* 936 × 84W . 873 *and* 936 . 873 × 84W

Cross ...	*936 × 84W . 873*	*936 . 873 × 84W*
No. of asci scored	59 813	28 298
No. of 6:2 asci	603	328
Frequency of 6:2 asci (× 10^3)	$10 \pm 0{\cdot}8$	$11{\cdot}6 \pm 1{\cdot}2$
Expected frequency (× 10^3) of 6:2 asci calculated assuming following order of sites:		
centr–936–84W–873	9·6	11·2
centr-84W–936–873	8·3	12·4

Experimental values for the parameters included in the above equations are: 0·6 for y and 0·1 for z (Paszewski, 1967), and $14{\cdot}4 \times 10^{-3}$ for α and $6{\cdot}44 \times 10^{-3}$ for β (see Table 4). Observed frequencies of recombinant asci found in both crosses together with the expected values calculated from the formulae given above are presented in Table 1. The data evidently give support to the order *centromere–936–84W–873*. Also the results of analysis of a sample of recombinant asci from both crosses are in agreement with this arrangement. Four types of ascus were found in a sample of 24 from the cross *936 × 84W . 873*, namely:

A			B			C			D		
936	+	+	*936*	+	*873*	*936*	+	+	*936*	+	*873*
936	+	*873*	*936*	+	*876*	*936*	*84W*	*873*	*936*	*84W*	*873*
+	+	+	+	+	+	+	+	+	+	+	+
+	*84W*	*873*	+	*84W*	+	+	*84W*	*873*	+	*84W*	+

There were 14 asci of type A, 4 of type B, 5 of type C and 1 of type D. The ratio of asci of type A to those of type B should equal $(y/2):z$; that is, 3:1. The results are in agreement with this prediction.

Seven recombinant asci were analysed from the cross *936 . 873 × 84W*. Five were of type E and two of type F:

E			F		
936	+	*873*	*936*	+	*873*
936	+	*873*	*936*	+	+
+	+	+	+	+	+
+	*84W*	+	+	*84W*	*873*

Theoretically the ratio of these two types should equal $x:(y/2)$ (x is the frequency of parental ditype asci in the cross *84W × 873*, and equals 0·3); that is, 1:1.

The results of intragenic crosses described in the following section indicate that

1043 is situated distally from *84W*. Thus the order of sites is *centromere–936–84W(787)–1043*.

(ii) *Analysis of intragenic recombination*

The numbers of asci scored in various crosses in the course of this work are given in Table 2. It should be noted that in some of these crosses rare asci with odd segregation were found during visual scoring of octads. A sample of 24 of such asci from different crosses was tested, i.e. the genotype of ascospores was checked by backcrossing. Among these asci 17 were found to give only phenotypically odd segregation, the mating-type segregation of 4 was different from 4:4 and 2 asci can possibly be interpreted as a result of odd segregation. These two asci, however, most likely resulted from random aggregation of spores from different octads like the four with irregular segregation of mating-type. Thus, it appears that the

Table 2. *Results of intragenic crosses*

Cross	No. of asci scored				
	4w:4d*	6w:2d	2w:6d	8w:0d	Total
Mutant × wild type					
84W × +	27 666	321	301	.	28 288
936 × +	35 574	164	26	.	35 764
787 × +	55 507	217	217	.	55 941
1043 × +	23 968	241	243	.	24 452
Mutant × mutant					
936 × *84W*	9	699	.	30 680	31 388
936 × *787*	4	265	.	19 308	19 577
936 × *1043*	21	1 319	.	51 651	50 311
84W × *1043*	.	1 095	.	87 095	88 192
787 × *1043*	.	370	.	52 544	52 174
Double mutant × wild type					
936.84W × + +	14 912	309	13	.	15 234
936.1043 × + +	32 540	772	89	.	33 401
84W.1043 × + +	53 970	836	128	.	54 442
Three-point crosses					
936.1043 × *84W*	.	774	.	65 085	65 859
936.1043 × *787*	.	144	.	54 637	54 781
936.84W × *1043*	.	93	.	15 429	15 522
936.787 × *1043*	.	184	.	24 907	25 091
936 × *84W.1043*	6	1 079	.	116 370	117 453
936.84W.1043 × + + +	46 459	1 270	21	.	47 750

* w, white; d, dark.

mutants studied here give odd (postmeiotic) segregation, if any, only exceptionally, and so asci with such segregation were neglected in the further classification. The same refers to rare 8:0 octads observed in crosses of mutant by wild type. Some of them are simple immature asci and others result from new mutations affecting ascospore pigmentation which originated from the wild type.

The basic frequencies of conversion from mutant to wild-type alleles and in the opposite direction were established in one-point crosses after having analysed a

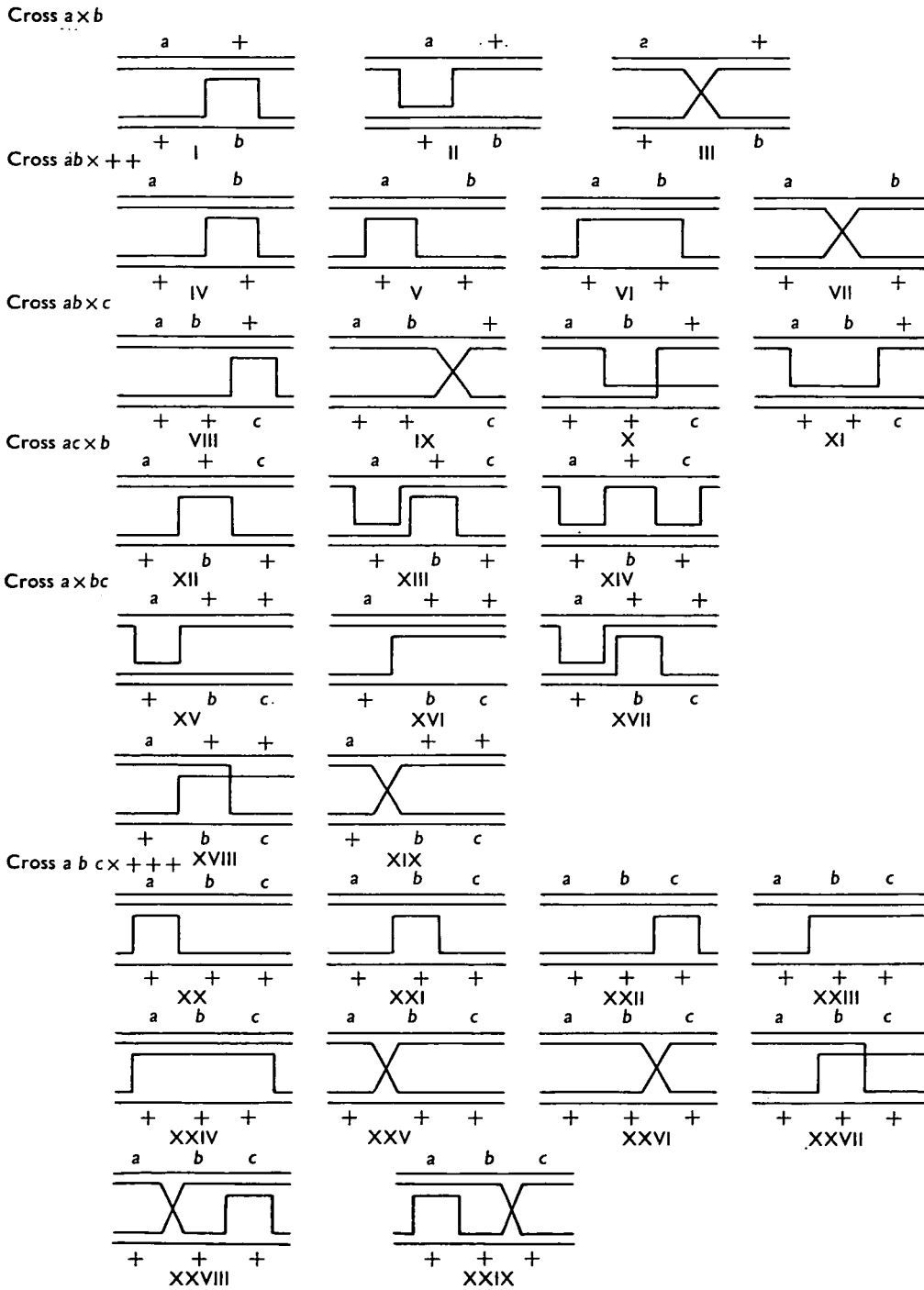

Fig. 1. Schemes showing types of recombination observed in various crosses.

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sample of 6:2 and 2:6 asci from each cross. This analysis was found necessary as it happens that for some mutants phenotypic segregation disagrees with the genotypic one. The results of ascus analysis from one-point crosses and the conversion frequencies based on this analysis are presented in Table 3.

Table 3. *One-point crosses: the results of ascus analysis and basic conversion frequencies for mutants* 936, 787, 84W *and* 1043

	6w:2d asci			2w:6d asci		
Cross	No. of asci tested	Genotype confirmed	Frequency (10^3)	No. of asci tested	Genotype confirmed	Frequency (10^3)
84W × +	29	27	10·5 (9·2–12·0)*	26	23	9·4 (8·4–10·6)
936 × +	41	27	3·0 (2·7–3·3)	13	8	0·44 (0·25–0·72)
787 × +	26	19	2·8 (2·3–3·3)	33	24	2·9 (2·5–3·4)
1043 × +	40	32	7·9 (6·8–9·0)	38	36	9·3 (8·1–10·6)

* 95% confidence interval.

The results of ascus analysis from two-point and three-point crosses, both in coupling and repulsion, are summarized in Table 4. In this table the various types of recombinant 6:2 asci are classified according to the schemes shown in Fig. 1 and their respective frequencies are given. It should be noted that the schemes in Fig. 1 are meant to represent a result of recombination and not its mechanism.

Some 2:6 asci in crosses in coupling were observed (Table 2). These resulted from simultaneous conversion of the mutants to wild-type alleles. Seventy such asci were tested from the cross *84W*.*1043* × wild type and 27 from the cross *936*.*84W* × wild type. The genotype was confirmed in 38 and 4 asci, respectively. The frequency of simultaneous conversions *84W*.*1043* → + + calculated on this basis equals 2·3 $(1{\cdot}9\text{–}2{\cdot}8) \times 10^{-3}$ and *936*.*84W* → + + equals 0·12 $(0{\cdot}03\text{–}0{\cdot}29) \times 10^{-3}$. Eight 2:6 asci from the cross *936*.*1043* × wild type were analysed and the genotype was confirmed in one of them, so it is difficult to establish the real frequency of these asci in that cross.

4. ANALYSIS OF RESULTS AND DISCUSSION

It is evident that for the four mutants studied there is no correlation between the position they occupy on the map of the gene and their basic conversion frequencies. Mutants *84W* and *787*, which do not recombine when crossed with each other, showed a marked difference in their basic conversion frequencies. Both findings are in excellent agreement with the results obtained by Kruszewska & Gajewski (1967) for locus *Y* of the same organism. No correlation between the position of a mutant and its basic conversion rate could be found in series *19*

Table 4. *Types and frequencies of 6:2 asci in two-point and three-point crosses*

	No. of asci tested	Genotype confirmed	No. of various types of asci and their frequencies ($\times 10^3$)		
(1) *936 × 84W*	79	78	I, 58 14.4 (11·8–16·5)	II, 5 1·4 (0·28–2·6)	III, 22 6·4 (4·2–8·3)
(2) *936 × 787*	43	43	I, 26 8·1 (6·2–10·0)	II, 2 0·62 (0–1·25)	III, 15 4·7 (2·8–6·6)
(3) *936 × 1043*	44	43	I, 25 14·8 (11·2–18·3)	III, 18 10·7 (7·6–13·5)	.
(4) *84W × 1043*	30	30	I, 5 2·1 (0·4–3·7)	II, 21 8·7 (5–12·4)	III, 4 1·6 (0–3·3)
(5) *787 × 1043*	42	41	I, 10 2·0 (0·5–3·1)	II, 31 5·0 (4·3–6·3)	.
(6) *936.84W* × + +	37	31	IV, 23 12·6 (9·9–15·3) VII, 3 1·6 (0·3–4·3)	V, 3 1·6 (0·3–4·3) .	VI, 2 1·1 (0·1–3·7) .
(7) *936.1043* × + +	29	24	IV, 14 11·0 (8·5–13·4) VII, 3 2·4 (0·5–6·2)	V, 6 4·7 (1·5–7·8) .	VI, 1 2·4 (0–4·0) .
(8) *84W.1043* × + +	84	77	IV, 25 5·1 (3·8–6·5) VII, 3 0·6 (0·2–1·8)	V,22 4·5 (3·2–5·8) . .	VI, 27 5·5 (4·9–6·9) . .
(9) *936.1043 × 84W*	32	32	XII, 31 11·2 (7·2–12·6)	XIII, 1 0·36 (0–1·8)	.
(10) *936.1043 × 787*	40	40	XII, 38 2·5 (2·3–2·6)	XIV, 2 0·13 (0–0·44)	.
(11) *936.84W × 1043*	36	34	VIII, 30 8·8 (7·7–10) XI, 1 0·3 (0–1·5)	IX, 2 0·6 (0–2·0) .	X, 1 0·3 (0–1·5) .
(12) *936.787 × 1043*	23	19	VIII, 19 6·0 (5·1–7·0)	.	.
(13) *936 × 84W.1043*	33	33	XV, 9 2·5 (1·2–3·7) XVIII, 1 0·3 (0–1·4)	XVI, 9 2·5 (1·2–3·7) XIX, 13 3·6 (2·1–5·2)	XVII, 1 0·3 (0–1·4) . .
(14) *936.84W.1043* × + + +	113	103	XX, 9 2·2 (0·5–3·9) XXIII, 24 5·4 (3·7–7·3) XXVI, 2 0·5 (0–1·7) XXIX, 1 0·25 (0–1·3)	XXI, 18 4·2 (2·4–6·0) XXIV, 5 1·2 (0·4–2·7) XXVII, 2 0·5 (0–1·7) .	XXII, 23 5·3 (3·5–7·3) XXV, 19 4·5 (2·6–6·3) XXVIII, 1 0·25 (0–1·3) .

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(Mousseau, 1966, 1967) and series *75* (Rossignol, 1967) in *Ascobolus immersus*. The same seems to be true for a series of hyaline mutants in *Sordaria brevicollis* (Fields & Olive, 1967).

The results of two-point crosses indicate polarization of recombination within the locus studied. In crosses of mutant *936* with the three others, the polarization is easily explained by the differences in the basic conversion rates of the mutants involved. However, this is not the case in crosses between *1043* and *84W* where the position of the mutant probably plays an important role. The pronounced influence of mutant *936* on the conversion rate of *84W* and especially of *787* should be noted. In cross *936* × *787* the conversion frequency of *787* is almost three times higher than its basic conversion frequency. Also in cross *787* × *1043* the conversion frequency of *787* is higher than the basic value. An increase of the conversion rate of a mutant caused by another mutant was observed in one case in series *19* (Mousseau, 1967) and in series *164* (Baranowska, unpublished). A mutant can also cause a decrease of conversion frequency of another one (Kruszewska & Gajewski, 1967; Mousseau, 1967) but this may be due to spanning of conversion on the two sites involved so that conversion to wild type of one mutant is accompanied by conversion of a wild-type allele to the mutant at the second site. It is very likely that a number of conversions of *1043* to its wild-type allele are not detected because of simultaneous conversions at *84W* (or *787*). That such simultaneous conversions in this region do occur with a high frequency is evident from the results of the cross *84W* . *1043* × wild type. In this cross the polarization observed in the cross *84W* × *1043* does not occur: each mutant contributes here about one-half of its conversions to the class of simultaneous conversions. The high frequency of such conversions suggests that they result from a common recombinational event. It is interesting, however, that the frequency of simultaneous conversions to the wild type is one half that of the conversions in the opposite direction in spite of the nearly equal basic conversion frequencies of the mutants involved in both directions (see Table 4).

Crossing-over was observed in both regions studied; that is, in *936–84W* and *84W–1043*. However, when *787* was used instead of *84W*, no crossing-over was found in the second region in both crosses *787* × *1043* and *936* . *787* × *1043*. This suggests that the appearance of crossing-over may be mutant-specific. Marked differences in the crossing-over frequency were observed between crosses in coupling and in repulsion involving the same pairs of mutants. Compare asci of type III from crosses 1, 3 and 4 with those of type VII from crosses 6, 7 and 8. In all cases crossing-over was more frequent in repulsion than in coupling crosses.

The fact that a mutant which is located in the central part of the region studied is the one which converts most often is not consistent with an hypothesis connecting the points of primary breakages of DNA chains with the ends of the gene (Whitehouse & Hastings, 1965; Whitehouse, 1966). If the site at which conversion occurs most frequently is the one which is located near the primary breakage point, it would mean that this point is in the vicinity of *84W* (or *787*) regardless of whether these mutants are crossed with *936* or *1043*. In cross *936* . *1043* × *787* the frequency

of conversion of the central mutant is equal to its basic conversion frequency. This suggests that often the conversions at *84W* involve a fraction of DNA which does not include sites *936* and *1043*; that is, the whole recombinational event involves only the central part of the region studied (if conversions involve *936* or *1043* they are not detected).

No reciprocal conversions were found in crosses *936.1043* × *84W* and *936.1043* × *787*. This suggests that the reciprocal recombinants observed in two-point crosses involving mutants *84W* and *787* resulted from crossing-over and not from reciprocal conversion (without closely linked flanking markers it is impossible to establish whether the double mutant in two-point cross results from reciprocal

Table 5. *Observed and expected frequencies of asci resulting from multiple intragenic exchanges*

Cross no. (from Table 4)	Type of ascus	Observed frequency ($\times 10^3$)	Expected frequency ($\times 10^3$)
10	XIV	0·13	0·002
14	XXVIII	0·25	0·025
14	XXIX	0·25	0·0025

conversion or crossing-over). Recombinations which can be interpreted as reciprocal conversions occur very rarely. Thus Whitehouse (1967) considers a situation where hybrid DNA is confined to one chromatid only at a given site to be the rule and not an exception as it was suggested in the earlier versions of the DNA hybrid models (Whitehouse; 1963; Whitehouse & Hastings, 1965; Holliday, 1964). It is very difficult, however, to find a logical explanation of the assumption that hybrid DNA is formed in one chromatid only (at least at a mutant site) whereas the primary events leading to the formation of such hybrid DNA occur in two chromatids. Thus the questions arise of whether they really occur in two chromatids and whether hybrid DNA is always formed.

Data from tetrad analysis of intragenic recombination from a number of loci in different fungi strongly suggest that conversion is an *asymmetrical* event in the sense that two chromatids are not equally involved in this type of recombination. One can assume that conversion starts by the breakage of one chromatid (or half-chromatid) only, possibly followed by degradation of a fraction of DNA leaving single DNA chains with free ends. This situation triggers off an involvement of the second chromatid (from the homologous chromosome) into recombinational processes. It can serve as a template or a material donor or both for the repair of the gap in the first chromatid. This possibility will be discussed in detail elsewhere. Here it may be noted, however, that following this line of argument one arrives at the conclusion that recombination is basically of the conversion type and conversion may occasionally lead to crossing-over. This contradicts the idea that hybrid DNA formation in both chromatids is a prerequisite of any recombinational event as postulated by the current models.

Analysis of recombinant asci revealed that a number of distinct but highly

correlated recombinational events can occur within a gene (ascus types XIV, XXVIII, XXIX). Table 5 presents the frequencies of asci showing such a complex recombination pattern along with the expected frequencies of these asci calculated on the assumption of independent occurrence of the exchanges observed in them. Especially interesting are asci of types XXVIII and XXIX found in cross *936 . 84W . 1043* × wild type; in the absence of marker *84W* in these asci they would be classified as ascus types corresponding to *X* or *XVIII*, that is as asci with inexactly reciprocal crossing-over. Thus, it is possible that in many cases recombinations described as inexactly reciprocal crossing-over represent a more complex recombinational pattern than is supposed. This conclusion is supported by the fact that the frequencies of classes XXVII and XXVIII + XXIX are equal.

SUMMARY

Four white-spored allelic mutants of *Ascobolus immersus* were used to study the effect of mutant and cross specificity on the recombination pattern in intragenic crosses.

In the locus studied no correlation was found between the position of mutants on the map and their basic conversion frequencies. One of the mutants evidently caused an increase of conversion frequency of the two others. Crossing-over in intragenic recombination may be mutant-specific as revealed by using two mutants which give no recombinants when crossed with each other. The frequency of crossing-over was higher in crosses in repulsion than in coupling involving the same pairs of mutants. Polarization observed in two-point crosses was due in some instances predominantly to differences in basic conversion frequencies of the mutants used, and in others to the relative position of the mutated sites. Mutants located in the central part of the studied region were those which converted most frequently in two-point crosses. No reciprocal conversions were observed.

A number of recombinant asci resulting from two or more separate but highly correlated recombinational events within a gene were found.

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