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Postmeiotic segregation in locus '*46*' of *Ascobolus immersus*

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1. INTRODUCTION

Owing to gene conversion, intragenic recombination is predominantly non-reciprocal, i.e. in a two-point cross involving alleles the majority of asci with six mutant and two wild-type ascospores result from the segregation of one of the mutants in a 2:6 ratio (hereafter the first figure will indicate the mutant segregants). In the case of 3:5 segregation of one of the mutants an ascus with seven mutant and one wild-type ascospore is produced (Fig. 1). Postmeiotic segregation was observed in *Sordaria fimicola* (Kitani, Olive & El-Ani, 1962), *Neurospora crassa* (Case & Giles, 1964), and *Ascobolus immersus* (Lissouba, 1961; Lissouba, Mousseau, Rizet & Rossignol, 1962; Surzycki, 1964; Emerson & Yu-Sun, 1967). It appears from studies with *A. immersus* involving many alleles from a number of loci that mutants showing this type of segregation are rare.

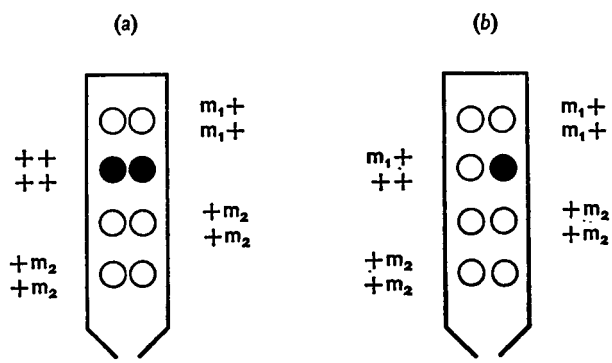

Fig. 1. Schematic representation of recombinant asci resulting from a cross between allelic mutants: (*a*) one of the mutants segregates in the 2:6 ratio and (*b*) one of the mutants segregates in the 3:5 ratio. Filled circles represent wild-type segregants.

Current models of genetic recombination (Whitehouse, 1963; Hastings & Whitehouse, 1964; Holliday, 1964) assume that formation of a heterozygotic region of DNA is a prerequisite for gene conversion and crossing-over. At a mutant site in this heterozygotic region there can exist some mispaired bases—a condition assumed to be unstable. This may be corrected by enzymic replacement of the abnormally paired bases by new ones with normal hydrogen bonding. Depending

on the direction of such a correction, the wild-type or mutant condition of the chromatid is restored. Processes of this type lead to marker segregation in the ratios 2:6 and 6:2. If the correction occurs in only one of the two chromatids with heterozygotic regions of DNA, postmeiotic segregation (3:5 or 5:3) is observed.

Intragenic recombination is usually polarized, i.e., within a gene or a part of it, alleles situated proximally to one end of this region convert in two-point crosses more frequently than those situated distally. According to the models mentioned above, polarization in intragenic recombination results basically from the unequal chances of various mutant sites to be involved in the heterozygotic region of DNA. Furthermore, the mutations themselves may influence this chance.

Polarization has been demonstrated so far for the 2:6 segregation (even type of segregation). The data in this paper concern polarization in the 3:5 segregation (postmeiotic segregation or odd segregation) in two-point crosses and are compared with polarization in the 2:6 segregation for the same pair of alleles.

2. MATERIAL AND METHODS

White-spored mutants of *Ascobolus immersus*, *63*, *1216* and *177* used in this work were kindly offered to this Laboratory by Professor G. Rizet. They belong to series '*46*' (Lissouba, 1961; Rossignol, 1964) and their order within the locus is as given above. The latter two when crossed with other mutants from the same series give some asci with seven mutant and one wild-type ascospore (7:1 asci), indicating postmeiotic segregation of the mutants involved.

The media used and the techniques of cultivation were exactly as previously described (Paszewski, Surzycki & Mańkowska, 1966) including some modification of those given by Lissouba *et al.* (1962).

3. RESULTS AND DISCUSSION

The results of crosses between the mutants and the wild-type strain are given in Table 1. The spores from individual asci are discharged in groups of eight and collected on an inverted Petri dish with a thin layer of agar. It happens sometimes

Table 1. *Numbers of various types of asci observed in one-point crosses between mutants 1216, 277, 63 and the wild type* (+)

	Number of asci								
Cross	4w:4d*	6w:2d	2w:6d	5w:3d	3w:5d	7w:1d	1w:7d	8w:0d	Total
1216 × (+)	39958	174	498	222	210	15	15	32	41134
277 × (+)	38113	537	654	141	338	19	31	57	39890
63 × (+)	22473	206	36	51	60	3	17	12	22858

* w, white; d, dark.

that spores from different asci are mixed, forming a group of eight resembling a native octad. Also, owing to some growth disturbance, the colour of spores does not always correspond to their genotype. In order to obtain accurate estimates of

the frequencies of various recombinant asci, it is necessary to analyse a sample of them. Data from such an analysis are presented in Table 2. Based on the results of ascus analysis, corrected frequencies were calculated (Table 3). In the case of

Table 2. *Analysis of recombinant asci from crosses of mutants 1216, 277 and 63 with the wild type*

Cross	Type of ascus	No. of asci tested	Genotype confirmed Mating-type segregation in ratio		Genotype not confirmed Mating-type segregation in ratio	
			4:4	Other	4:4	Other
1216 × wild type	6w:2d	23	20	1	2	—
	2w:6d	26	9	—	16	1
	5w:3d	14	13	—	1	—
	3w:5d	15	13	1	1	—
	7w:1d	10	1	3	6	—
	1w:7d	6	2	1	3	—
277 × wild type	6w:2d	11	8	—	3	—
	2w:6d	10	6	1	3	—
	5w:3d	16	11	—	5	—
	3w:5d	16	11	1	4	—
	7w:1d	7	2	2	3	—
63 × wild type	6w:2d	11	7	—	4	—
		19*	15	—		
	2w:6d	14	2	2	10	—
		23*	2	—		
	5w:3d	10	4	2	4	—
	3w:5d	12	3	4	5	—
	7w:1d	3	—	1	2	—
	1w:7d	8	1	1	6	—

* From Rossignol (1964).

Table 3. *Frequencies of recombinant asci found in crosses between mutants 1216, 277, 63 and the wild type*

Cross	Frequencies ($\times 10^3$) of asci			
	6w:2d	2w:6d	5w:3d	3w:5d
1216 × wild type	3·7 (3·1–4·3)*	4·2 (3·6–4·9)	5·0 (4·3–5·8)	4·4 (3·8–5·1)
277 × wild type	9·8 (8·7–10·8)	9·8 (8·7–10·8)	2·4 (2·0–3·0)	5·8 (5·1–6·6)
63 × wild type	6·6 (5·6–7·7)	?	?	?

* * 95 % confidence interval.

mutants *1216* and *277*, a high proportion of the 1:7 and 7:1 asci had mating-type segregation different from 4:4, showing that these octads were made up from spores of different asci. It is likely that the asci with 4:4 segregation in these

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classes could have arisen in the same way. Thus, it is very difficult to establish whether mutants *1216* and *277* produce 7:1 and 1:7 asci, and if they do, what is their frequency. The same reasoning applies to all but the 6:2 types of recombinant asci observed in cross *63* × wild type. Because of this the frequencies of these ascal types were not calculated. The 8:0 asci are predominantly those in which the ascospores were discharged from the asci before pigment synthesis was completed.

Table 4. *Recombination data from two-point crosses*

Cross	No. of asci scored	6w:2d asci		7w:1d asci	
		Number	Frequency	Number	Frequency
1216 × *63*	161009	200	1·2 (1·1–1·4)	27	0·17 (0·11–0·24)
1216 × *277*	65845	266	4·0 (3·6–4·6)	30	0·45 (0·30–0·65)

Table 5. *Genetic structure of recombinant asci from two-point crosses*

Cross	Type of ascus	No. of asci analysed	Crossing-over	Conversion to the wild type of the	
				Left parent	Right parent
1216 × *63*	6w:2d*	30	1	29	—
	7w:1d	10	—	10	—
1216 × *277*	6w:2d*	32	4	6	22
	7w:1d	17	—	3	14

* From Rossignol (1964).

As seen from Table 3 in the case of mutant *1216* the frequencies of 6:2, 2:6, 5:3 and 3:5 asci are very similar. The situation is somewhat different with mutant *277*, where the frequencies of the 6:2 and 2:6 asci are higher than those of the 5:3 and 3:5 asci. Results of crosses between *1216* and *63* and between *1216* and *277* are given in Table 4 and data from the analysis of recombinant asci in these crosses in Table 5. (Some data of Rossignol (1964) are included in the tables as indicated.)

Polarization is clearly seen in both two-point crosses. In cross *1216* × *63* all the recombinant asci resulted from conversion of *1216*. This is expected in view of the very low frequency, if any, of conversion of *63* to its wild-type allele. In cross *1216* × *277* the ratio of the 6:2 asci with conversion at *277* to those with conversion at *1216* was 4·66, i.e. very similar to the corresponding proportion in the 7:1 asci, which was 3·66. This result gives support to the recombination models mentioned earlier.

There is, however, another fact which is not easily accounted for by the proposed models, namely the pronounced differences in the frequencies of the 6:2 and 7:1 asci, in both two-point crosses.

According to Whitehouse's model the most probable interpretation of the

recombination pattern observed in the two-point crosses in the locus studied is that formation of hybrid DNA is usually confined to one site and one chromatid (Whitehouse & Hastings, 1965; Whitehouse, 1967). There is no reason to believe that in one-point crosses the situation is basically different. Thus, if we consider only the chromatid originally carrying a mutant allele at a given site, it appears that, for mutant *1216*, heterozygotic DNA is corrected to the wild-type allele as frequently as it remains uncorrected. For mutant *277*, about twice as much heterozygotic DNA is corrected to the wild type as remains uncorrected (Table 3, columns 2 and 4). In the case of mutant *63*, formation of hybrid DNA in the chromatid carrying the mutant allele is rare, but if it does occur, it is nearly always corrected to restore the mutant allele. In terms of Whitehouse's model it is necessary to assume that hybrid DNA can be formed only in one chromatid at a given site, to account for the ratios of recombinant asci found in mutant *277* where $6:2 = 2:6$ but $3:5 > 5:3$. Alternatively, if hybrid DNA in two chromatids is postulated, one must assume that the rates of correction in these two chromatids differ. This is exactly the approach of Emerson (1966).

Since the frequencies of the 2:6 and 3:5 asci are equal in cross *1216* × *wild type* and differ less than two times in cross *227* × *wild type*, one should not expect the frequency of the 6:2 asci in cross *1216* × *277* (segregation of one of the mutants in the ratio 2:6) to be more than twice as high as the frequency of the 7:1 asci (segregation of one of the mutants in the 3:5 ratio). In fact, the frequency of the 6:2 asci in this cross is nine times higher than the frequency of the 7:1 asci. A similar situation is found in cross *1216* × *63*.

To explain these results one can assume that:

(*a*) in the case of postmeiotic (odd) segregation the fragments of DNA which undergo heterozygotization are usually longer than those connected with normal (even) conversion and so they often span over both sites involved in a cross and remain uncorrected or are corrected to one parent at both sites; or

(*b*) there is some other type of 'marker effect' besides that affecting the chance of a second site being involved in the heterozygotic fragment of DNA which influences the process of correction.

The lower frequency of the 7:1 asci as compared with the frequency of the 6:2 asci could be explained by assuming that hybrid DNA is formed at a given site in two chromatids and there are different rates of correction in either chromatid (Emerson, 1966). In Table 6 the numbers of recombinant asci observed in the cross *277* × *wild type* are listed together with the numbers expected according to Emerson's method. It is clear that the observed numbers fit only the expectation based on the assumption of different rates of correction in the two chromatids. Considering the two-point cross, we can assume that the chromatid in which the wild-type recombinant appeared is that with a higher rate of repair and thus very few 7:1 asci are formed. This, however, would result in a much higher ratio of 6:2 to 7:1 asci than is actually observed.

It would seem that none of the models of recombination provides a really satisfactory explanation of the results presented above.

It is an interesting fact that among a few hundred white-spored mutants of *Ascobolus immersus* (Rizet's strain) studied so far by tetrad analysis, only very few showed postmeiotic segregation. Some of them gave only phenotypically odd types of segregation, as established by ascus analysis (Lissouba, 1961; Kruszewska and Gajewski, unpublished).

Table 6. *Observed and expected frequencies of recombinant asci in cross 277 × wild type (latter derived according to Emerson,* 1966)

Type of ascus	Observed numbers	Expected numbers (*a*)	Expected numbers (*b*)
6w:2d	390	370	211
2w:6d	390	370	566
3w:5d	233	244	211
5w:3d	97	99	122
	χ^2(3D.F.)	2·68	208
	P	0·5–0·3	< 0·001

	(*a*) Relative rates of repair different in two chromatids: 1st	(*a*) Relative rates of repair different in two chromatids: 2nd	(*b*) Relative rates of repair the same in two chromatids
m/+ repaired to +/+	0·178	0·784	0·559
m/+ repaired to *m*/*m*	0·712	0·196	0·340
m/+ unrepaired	0·11	0·02	0·1

The situation seems different in the Pasadena strain of *Ascobolus immersus* studied by Emerson & Yu-Sun (1967) but it is most surprising that they did not obtain any 7:1 asci in crosses between some pairs of mutants showing 3:5 segregation when crossed with the wild type.

At present there is good evidence that only four mutants in *A. immersus* (Rizet's strain), namely *60* from series '*19*' (Lissouba *et al.* 1962), *186* (Surzycki, 1964) and the two studied in the present work, show postmeiotic segregation. The last three mutants also produce 'selfers' (Paszewski & Surzycki, 1964; Lissouba, 1961; Rossignol, 1964), i.e. give 6:2 asci when selfed. 'Selfers' are as rare a phenomenon as postmeiotic segregation among white-spored mutants of *Ascobolus*, so the appearance of both phenomena in these three mutants is remarkable. Since in selfers the wild-type spores always form a pair, postmeiotic segregation cannot be simply a complex product of 'normal' conversion and selfers. It is possible that the difference between odd and even segregation in asci cannot be fully explained in terms of corrected and uncorrected hybrid DNA. Perhaps the as yet unexplained phenomenon of selfer formation is in some way connected with postmeiotic segregation.

SUMMARY

White-spored mutants of *Ascobolus immersus* were used to study postmeiotic segregation within a gene. It was found that in two-point crosses between mutants showing postmeiotic segregation, polarization in recombinant asci resulting from this type of segregation resembled that in normal (even-type) conversion. However, the ratio of recombinant asci with even segregation (6:2 asci) to those with odd segregation (7:1 asci) was much higher than might have been expected on the basis of the frequencies of various recombinant asci found in crosses between the same mutants and the wild-type strain. It seems that none of the current models of recombination gives a satisfactory explanation of the results obtained.

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