

A study on simultaneous conversions in linked genes in *Ascobolus immersus*

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(Received 4 August 1966)

1. INTRODUCTION

Stadler & Towe (1963), Whitehouse (1963) and Holliday (1964) proposed models of genetic recombination to overcome difficulties connected with the 'switch' model developed by Freese (1957) and its modifications. These new models assume that recombination, both conversion and crossing-over, takes place after meiotic DNA replication is completed (or nearly completed in the case of Stadler and Towe's model), perhaps in prophase when there is a visible pairing of chromosomes. According to these models conversion should occur in both chromatids of a chromosome at random, as is the case with crossing-over.

The purpose of this work was to check experimentally whether conversions do occur randomly in both chromatids of a chromosome. This was done by studying simultaneous conversions at two loci situated 40 crossover units apart in the same arm of the chromosome. It was assumed that conversions at these loci are independent of each other. An experiment was also carried out with two closely linked markers from different cistrons to see whether in this case conversions of both markers are independent. The experiments were carried out according to the scheme described in the next section.

2. THEORY

Studying the simultaneous conversions of markers in two loosely linked loci provides an opportunity to check whether conversion can occur randomly in either of the two chromatids of a chromosome, or is limited to one chromatid only.

Let a and b indicate mutants with the same phenotype in two linked loci and x , z and y be the frequencies of parental ditype (PD), non-parental ditype (NPD) and tetratype (T) asci, respectively, obtained in a cross $a + \times + b$. Assume that the frequencies of conversions of these mutants to their wild-type alleles are α and β , respectively. These frequencies can be estimated by tetrad analysis of crosses mutant \times wild-type. If the two loci in question are far apart, it is reasonable to assume that conversions of a and b to wild-type alleles are independent of each other. Thus, the frequency of simultaneous conversions of a and b should equal the product $\alpha\beta$.

Simultaneous conversions in the two loci in the same chromatid will result in asci with two mutant and six wild-type spores (the 2:6 asci—see Fig. 1A). However, in the case of crossing-over in the interval $a-b$ involving the sister chromatid of the one in which the conversions took place, an ascus with four mutant and four wild-type spores will result. This ascus is phenotypically indistinguishable from a PD ascus. In other words not all asci with simultaneous conversions in both loci

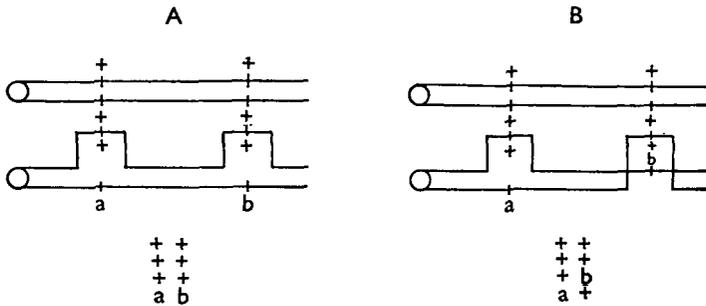


Fig. 1. Two possible cases of simultaneous conversions in linked genes.

can be recovered. The expected frequency of the 2:6 asci found in this cross is given by the formula:

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

if conversions in these two loci occur independently. Assuming, however, that conversion can occur in either chromatid of a chromosome at random (see Fig. 1B), the expected frequency of these asci will be:

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

which is approximately $V_1/2$.

It should be noted that formula (2) will apply both if conversion is equally likely in each of two sister chromatids, and if, when it occurs in the same chromatid, there is always 50% sister strand exchange, regardless of the distance between the markers.

3. MATERIAL AND METHODS

White-spored mutants of spontaneous origin in the normally dark-spored ascomycete *Ascobolus immersus* were used in this work. Except for mutant 84W they were offered to this laboratory by Professor G. Rizet. Mutants of this type are especially suitable for such experiments because they are easily distinguishable. Beside this, the fact that spores are discharged from asci in groups of eight and can be easily collected and examined on Petri dishes provides an opportunity of making tetrad analysis on a large scale. It may be noted that conversion is a

relatively rare phenomenon. Even if it is as high as 1% for each of the mutants studied, the frequency of simultaneous conversions of both will be at the most 0.0001, assuming the conversions to be independent.

In order to perform the experiments two mutants from linked loci with relatively high frequency of conversion should be selected. A few such mutants were found among more than 200 examined and two of them, numbered 84W and 873, showed linkage. They are located in the same chromosome arm in the following order: 873-84W-centromere (Paszewski, Surzycki & Mankowska, in press). This pair was used as *a* and *b* mutants. Another pair of mutants used in this work consisted of closely linked mutants 231 and 186. The first one belongs to series '75' (Lissouba *et al.*, 1962), the second gives about 10% recombinant asci with 231, of which only one-fifth result from reciprocal recombination (Surzycki, in preparation). These two mutants belong to different cistrons (Baranowska, unpublished).

For technical details, see Lissouba (1961), Makarewicz (1961) and Lissouba *et al.* (1962).

4. EXPERIMENTAL

(i) *The determination of values α , β , γ and z*

Conversion frequencies of the mutants were found directly from crosses with the wild-type (Table 1). The 2:6 asci represent those with conversion of the mutant to its wild-type allele. From each cross some of these asci were tested to determine whether all dark spores are genotypically wild-type. This was done by

Table 1. *Crosses of mutants 84W, 936 and 873 with the wild strain*

Cross	Number of asci scored	Number of asci with segregation*			Frequency of 2:6 asci $\times 10^4$	Number of 2:6 asci tested	Genotypic 2:6 asci among tested	Corrected frequency of 2:6 asci $\times 10^4$
		6w:2d	8w:0d	2w:6d				
<i>84W</i> \times +								
1.	4649	102	24	59	127 \pm 16			
2.	3599	32	4	44	122 \pm 18			
Total	8248	134	28	103	124 \pm 12	13	13	124
<i>873</i> \times +								
1.	4534	72	10	83	191.8 \pm 20			
2.	6134	131	34	119	194 \pm 18			
Total	10668	203	44	206	193.1 \pm 13	26	25	185.6

* w—white, d—dark.

crossing each spore from the ascus with the wild-type. The corrected frequencies of conversions are given in the same table. They represent the experimental values for α and β . The numbers of the 6:2 asci (six mutant and two wild-type spores) are also given in this table. They result mostly from conversion of wild-type allele

to mutant, but partly they are recombinants between mutants used in the cross and new mutants affecting ascospore pigmentation originating in the strains. Not enough of these asci were tested to estimate this ratio.

From the cross $84W \times 873$, 1534 asci were scored. Of these 485 were PD, 171 NPD and 874 T, which gives a recombination frequency of 0.397 ± 0.0079 . The frequency of T asci (y) is 0.572 and of NPD asci (z) is 0.111. Similar frequencies (0.595 for T and 0.101 for NPD asci) were obtained from 4393 asci scored in a cross between 873 and 936 , an allele of $84W$ which has a conversion frequency of less than 0.001 (Paszewski, unpublished). This means that the relatively high conversion frequency of $84W$ does not appear to influence the frequencies of T and NPD asci. In further calculations approximate values of 0.6 for y and 0.1 for z are taken.

(ii) *Cross $84W.873 \times wild-type$*

Three experiments involving different isolates of the double mutant $84W.873$ were carried out (see Table 2). The asci with six dark and two white spores (the 2:6 asci) in the progeny of these crosses resulted from simultaneous conversions in the two loci (other types of asci are not indicated in the table). In the first two experiments the observed frequency of the 2:6 asci from the pooled data was

Table 2. *Crosses between double mutant $84W.873$ and wild strain ($bc \times + +$)*

Cross No.	Number of asci scored	Number of 2:6 asci	Frequency $\times 10^6$	Number of 2:6 asci tested	Genotypic 2:6 asci among tested	Corrected frequency $\times 10^6$
1.	125225	23	183			
2.	134131	24	181			
Total	259356	47	181	13	6	83 ± 17
3.	67767	12	177	12	5	73 ± 32

0.000181. The 2:6 asci should be tested because it happens sometimes that genotypically white spores are phenotypically dark through being joined to dark ones due to some growth disturbances. Forty-seven 2:6 asci were found, but, because of low spore germination, only thirteen were fully tested. Among these thirteen only six were genotypically of the 2:6 type. The percentage germination of spores from the 2:6 asci was 62.25 (41.37 for white spores and 69.2 for dark ones). A similar low percentage germination was found in non-recombinant (4:4) asci (36.7 for white spores and 72.45 for dark ones). Thus, it is clear that there was no factor affecting spore germination differentially in the 2:6 asci. Assuming the same fraction of genotypically 2:6 asci among all the forty-seven asci found as in the thirteen asci tested, the corrected frequency of 2:6 asci is $83 \pm 17 \times 10^{-6}$.

In the third experiment only 67,767 asci were examined, but this time spore germination was much better so that all twelve 2:6 asci found could be tested.

Among these twelve, five asci were genotypically of the 2 : 6 type. Their frequency equals $73 \pm 32 \times 10^{-6}$.

The expected frequency of the genotypic 2 : 6 asci in this cross can be calculated from formulae (1) and (2):

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

$$V_1 = 0.0124 \times 0.01856 (1 - 0.3 - 0.1)$$

$$= 138 \times 10^{-6}$$

$$V_2 = \frac{V_1}{2} = 70 \times 10^{-6}$$

It is evident that the corrected frequency in the three experiments, and especially in the third one, is close to the value V_2 and not to the V_1 .

(iii) *Cross 231.186 × wild-type*

Surzycki (in preparation) found that the frequencies of conversion of mutants *231* and *186* to their wild-type alleles are 0.0317 and 0.0569, respectively. Thus, the expected frequency of the simultaneous conversion of these two mutants is either 0.0018 or 0.0009 as calculated from formula (1) or (2), respectively. Among 9899 asci scored in this cross, 128 asci were found with simultaneous conversion of *231* and *186* to wild-type (2 : 6 asci). Their frequency (0.0129) is markedly higher than both predicted values. This means that conversions in these two closely linked sites are not independent of each other.

5. DISCUSSION

The experiments presented above showed a high correlation of conversions occurring in two closely linked cistrons. The frequency of simultaneous conversion of the two markers studied was seven times higher than the expected value calculated on the assumption that these conversions are independent. The correlation may be even higher if it is not limited to one of two sister chromatids.

This means that a recombination event, at least of conversion type, can span over more than 1 crossover unit and involve more than one cistron. The same may be true for crossing-over which has often, although not always, been found to be associated with conversion (see review by Whitehouse & Hastings, 1965) and according to the new recombination models mentioned above is a different consequence of a common mechanism.

On the other hand, conversions in two loosely linked loci were found to be independent of each other and distributed randomly in both chromatids, as is the case with crossing-over. One can assume that conversions can occur in one chromatid only, as is predicted in conservative DNA replication models, and then randomized by sister strand exchange. Models of this type seem unlikely, especially in view of the recent data by Chiang (1965) showing a semi-conservative pattern of

meiotic DNA replication in *Chlamydomonas*, and the finding that DNA synthesis occurs before haploid nuclei fuse in the ascus primordium (Rossen & Westergaard, 1966). In this situation an alternative interpretation of the results obtained in this work, that is, that conversion can occur in either chromatid of a chromosome, seems much more reasonable.

SUMMARY

White-spored mutants of *A. immersus* were used in experiments carried out to study the relationship between conversions occurring simultaneously in linked genes, and to check whether conversion occurs at random in the two chromatids of a chromosome.

Conversions in two loosely linked loci were shown to be independent of each other and randomly distributed between sister chromatids. On the other hand, conversions in two closely linked loci showed a high correlation of occurrence.

I wish to thank Dr S. Surzycki for help in the selection of mutants. Thanks are due to Professor W. Gajewski for stimulating discussion during the course of this work. I am very much indebted to Drs J. R. S. Fincham and P. J. Hastings for critical reading of the manuscript and their most valuable remarks.

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