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Recombination within the *Y* locus in *Ascobolus immersus*

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1. INTRODUCTION

Although there are many hypotheses concerning the mechanism(s) of recombination (Lederberg, 1955; Freese, 1957; Pritchard, 1960*a*, 1960*b*; Stadler & Towe, 1963; Bernstein, 1962, 1964; Whitehouse, 1963, 1965; Whitehouse & Hastings, 1965; Holliday, 1962, 1964) this basic genetic process is not fully understood.

We still need facts about intragenic recombination, especially concerning the frequency of non-reciprocal recombination (conversion), in one- and multi-point crosses. Octad analysis using spore colour mutants in fungi provides such information on a large enough scale. Conversion in such loci was studied in *Sordaria fimicola* (Olive, 1959; Kitani *et al.*, 1961, 1962; Kitani, 1962) and in *Ascobolus immersus* (Lissouba, 1961; Lissouba & Rizet, 1960; Rizet *et al.*, 1960; Lissouba *et al.*, 1962; Rizet & Rossignol, 1963; Rossignol, 1964; Makarewicz, 1964).

The present work is concerned with recombination analysis within one of the white spore loci (locus *Y*) in *A. immersus*. The chief aims of this analysis were:

(1) To establish the frequencies of conversion in both directions of different alleles in the *Y* locus in one-point crosses (i.e. basic conversion frequencies).

(2) To relate the basic conversion frequencies of different alleles with their position on the gene map.

(3) To compare the frequencies of conversion of the same allele in one- and two-point crosses in order to check any influence of the presence of one allele on the frequency of conversion of another.

(4) To establish the frequency of simultaneous conversion of some mutant pairs.

2. MATERIALS AND METHODS

The *Y* locus is located in chromosome IV, 10·8 map units from the centromere (Paszewski *et al.*, 1966). All mutants from this locus have colourless (white) ascospores, contrasted with the dark-brown colour of the wild type. All mutants used here are spontaneous mutations from the wild-type strain '*S*' and were obtained from Professor G. Rizet to whom we are deeply grateful for this generous offer.

Many mutations have been identified as belonging to the *Y* locus. Some of them belonged to a group of mutants giving no recombination when intercrossed. From this group were chosen *Y* and *183* to compare how two probably different mutants

from the same site behave in different crosses. The other mutants chosen were those which were easy to cross and gave only a small proportion of asci showing spurious segregation. Thus, only seven mutants are the object of the present study. These are: *794*, *Y*, *183*, *146*, *73*, *77*, *775*. All of them, except *73*, were tested for complementation in all possible combinations (Baranowska, 1964) with negative results.

The media used and the technique of cultivation are given by Paszewski *et al.* (1966).

Since after prolonged vegetative cultivation the strains lose the ability for sexual reproduction, all mutants were crossed from time to time with the wild type and re-isolated. This procedure had no influence on their conversion frequencies. The germination rate of isolated ascospores was about 80%.

The octads were tested using the backcross test of Mitchell (1955).

3. RESULTS

(i) *One-point crosses*

The seven mutants from the *Y* locus were crossed with the wild-type strain. Besides the majority (about 99%) of 4w:4d octads (w = white, i.e. mutant phenotype; d = dark, i.e. wild-type phenotype), also 2w:6d, 6w:2d, and 8w:0d were observed. Very rarely also 0w:8d and octads with odd segregation (5w:3d, 3w:5d, 7w:1d, 1w:7d) were found.

The preliminary testing of the least frequent types of octads showed that, in some of them, phenotypic segregation did not agree with the genotypic one. Other octads resulted from haphazard aggregation of spores from different octads, which often could be demonstrated by irregular segregation of the mating type. The octads in which phenotypes corresponded to genotypes and with regular segregation of the mating type, were so rare that they could also result from aggregation of spores from different octads. Thus if the odd and 0w:8d types of segregation do exist at all they must be extremely rare and were not further analysed.

The preliminary analysis of 8w:0d and 6w:2d octads has shown that nearly all 8w:0d octads and the majority of 6w:2d octads resulted from the simultaneous 4:4 segregation of the introduced white mutant and of another one with the same phenotype from another locus that had arisen spontaneously in either of the parental strains. Only a minority of 6w:2d octads resulted from the conversion of the wild-type allele to the mutant one. Thus, to establish the frequency of this type of conversion, the testing of a large number of 6w:2d octads was required.

The frequency of 2w:6d octads on the other hand, more or less corresponded to the frequency of conversion from the mutant allele to wild-type (Table 1). It can be seen that in the last four crosses the majority of 2w:6d octads resulted from conversion. In the crosses involving *Y* and *183*, two dark spores were paler than the rest in many of the 2w:6d octads, and this suggested that they were genotypically white, which was indeed proved by testing.

Table 1 shows that the seven mutants studied can be divided into three groups

Table 1. *Basic conversion frequencies to wild type of mutants of the* Y *locus (Standard errors were evaluated under the assumption that all recombinant octads were tested. Since only a proportion of 2w:6d octads were tested, the real standard errors are slightly smaller than those given in the table)*

Cross	No. of octads scored	2w:6d octads			Conversion octads		s.e.
		No.	Frequency $\times 10^3$	No. tested	No.	Frequency $\times 10^3$	
73 × +	55 046	5	0·09	1	0	< 0·09	
Y × +	61 556	16	0·26	8	1	0·03	± 0·03
183 × +	23 479	9	0·38	6	1	0·06	± 0·06
775 × +	39 828	45	1·13	27	26	1·09	± 0·21
794 × +	66 920	89	1·31	28	26	1·23	± 0·24
146 × +	39 345	61	1·55	18	18	1·55	± 0·37
77 × +	51 426	140	2·69	37	34	2·50	± 0·43

with statistically different frequencies of basic conversion. To the group with the lowest frequency (below $0{\cdot}09 \times 10^{-3}$) belong mutants *73*, *Y* and *183*. To the group with the intermediate conversion frequency (from $1{\cdot}09 \times 10^{-3}$ to $1{\cdot}55 \times 10^{-3}$) belong *775*, *794* and *146*. The highest frequency is shown by *77* mutant ($2{\cdot}5 \times 10^{-3}$).

The frequency of conversion from wild type to mutant allele was determined for only five alleles (Table 2). To minimize the occurrence of 6w:2d octads resulting from independent mutations, newly re-isolated strains were used. Only the conversion frequency for the $+^{77}$ allele differs significantly from the rest.

Table 2. *Basic conversion frequencies from wild-type alleles to mutants in the* Y *locus Standard errors as in the Table 1*

Cross	No. of octads scored	6w:2d octads			Conversion octads		s.e.
		No.	Frequency $\times 10^3$	No. tested	No.	Frequency $\times 10^3$	
73 × +	55 046	64	1·16	46	24	0·61	± 0·12
Y × +	61 556	61	0·99	43	17	0·39	± 0·09
183 × +	23 479	16	0·68	12	10	0·57	± 0·18
775 × +	39 828	57	1·43	44	24	0·80	± 0·16
77 × +	22 111	51	2·31	37	29	1·81	± 0·34

The comparison of the frequencies of conversion in opposite directions shows that for some alleles such as $775 - +^{775}$ and $77 - +^{77}$, they are statistically the same, whereas for the alleles $Y - +^{Y}$, $183 - +^{183}$ and $73 - +^{73}$ they are significantly different and the conversion in the direction from mutant to wild-type allele is many times lower.

(ii) *Two-point crosses (in repulsion)*

Seven mutants of the *Y* locus were crossed with each other and the frequencies of 6w:2d octads were checked (Table 3). Besides the 6w:2d octads, two 4w:4d and

three 5w:3d octads were observed, but these were not tested. On the basis of recombination frequencies in two-point crosses the alleles could not be ordered unambiguously. Two clusters, however, one with *794*, *183* and *Y* mutants and the second with *146*, *73*, *77* and *775* mutants could be distinguished—with recombination frequencies much lower within each cluster than between them. Only in the crosses *Y* × *73* and *183* × *73* were the recombination values as low as within a cluster.

Table 3. *Frequencies of recombinant octads in two-point crosses (in repulsion)*

Cross	No. of octads scored	6w:2d octads		
		No.	Frequency × 10^3	S.E.
794 × *Y*	51 956	5	0·10	± 0·04
794 × *183*	147 654	31	0·21	± 0·04
794 × *146*	58 410	154	2·64	± 0·21
794 × *73*	55 201	77	1·39	± 0·16
794 × *77*	55 693	172	3·09	± 0·24
794 × *775*	56 013	127	2·27	± 0·20
Y × *183*	149 560	0	—	—
Y × *146*	65 889	110	1·67	± 0·16
Y × *73*	84 531	25	0·30	± 0·06
Y × *77*	64 667	175	2·71	± 0·20
Y × *775*	38 449	42	1·09	± 0·17
183 × *146*	92 543	61	0·66	± 0·08
183 × *73*	49 807	3	0·06	± 0·03
183 × *77*	54 784	130	2·37	± 0·21
183 × *775*	119 517	94	0·79	± 0·08
146 × *73*	27 535	1	0·036	± 0·036
146 × *77*	97 559	23	0·24	± 0·05
146 × *775*	157 565	52	0·33	± 0·05
73 × *77*	138 965	0	—	—
73 × *775*	76 449	2	0·026	± 0·018
77 × *775*	108 591	33	0·30	± 0·05

The testing of 6w:2d octads shows that they are of three different types (Table 4). One resulted from reciprocal recombination and two from conversion of one or the other of the two mutants crossed. The great majority (94·8%) of the 6w:2d octads tested resulted from conversion. The octads resulting from crossing-over were observed in nearly all crosses between alleles from different clusters. Except in the cross *73* × *Y* they were much less frequent than those resulting from conversion.

Conversion octads could be of two kinds, depending on which of the two mutants converted. Thus it is possible to distinguish three types of crosses:

(1) Those where only one mutant converted.

(2) Those where both mutants converted but with significant differences in frequency.

(3) Those where both mutants converted with statistically the same frequencies.

Table 4. *Types of 6w:2d octads in two-point crosses (in repulsion)*

Cross $a \times b$	No. of octads tested	Crossover octads	Conversion octads		
			Total	Conversion of a mutants	Conversion of b mutants
794 × *Y*	3	0	3	3	0
794 × *183*	15	0	15	15	0
794 × *146*	39	2	37	8	29
794 × *77*	26	1	25	2	23
794 × *73*	21	2	19	19	0
794 × *775*	30	2	28	12	16
Y × *146*	27	1	26	0	26
Y × *77*	30	1	29	0	29
Y × *73*	9	6	3	1	2
Y × *775*	31	1	30	0	30
183 × *146*	19	0	19	0	19
183 × *77*	29	0	29	0	29
183 × *775*	31	2	29	0	29
146 × *77*	9	0	9	1	8
146 × *775*	14	0	14	5	9
77 × *775*	11	0	11	9	2
Total	344	18	326	75	251

(iii) *Two-point crosses (in coupling)*

The double mutant strains obtained from crossover octads were crossed with wild type. In such crosses the 2w:6d octads can result only from simultaneous conversion of both mutants to their wild-type alleles (Table 5), whereas 6w:2d octads can be of several different origins. These were not analysed. All double mutant crosses involving *Y* give similar values of 2w:6d octad frequency and this is about ten times lower than in the crosses involving mutant *794*.

The frequency of simultaneous conversions of two mutants in such crosses is not negatively correlated with the distances between the mutants. It seems to be related rather with the basic conversion frequencies of the mutants involved.

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Table 5. *Frequencies of simultaneous conversions of some pairs of mutants in two-point crosses (in coupling)*

Cross	No. of octads scored	2w:6d octads			Conversion octads		
		No.	Frequency $\times 10^3$	No. tested	No.	Frequency $\times 10^3$	S.E.
Y 146 × + +	58 238	2	0·03	1	1	0·03	± 0·03
Y 77 × + +	27 061	3	0·11	0	0	< 0·11	
Y 775 × + +	50 427	3	0·06	2	2	0·06	± 0·04
794 77 × + +	40 234	11	0·27	8	8	0·27	± 0·10
794 775 × + +	52 364	16	0·30	6	6	0·30	± 0·12

Table 6. *Frequencies of recombinant octads in three-point crosses*

Cross	No. of octads scored	6w:2d octads		S.E.
		No.	Frequency $\times 10^3$	
Y 77 × *794*	266 213	3	0·011	± 0·007
Y 77 × *146*	201 939	1	0·005	± 0·005
Y 77 × *775*	165 104	9	0·055	± 0·018
Y 775 × *794*	56 150	1	0·02	± 0·02
Y 775 × *146*	66 692	3	0·04	± 0·03
Y 775 × *77*	137 134	1	0·007	± 0·007
183 775 × *794*	103 489	17	0·16	± 0·04
183 775 × *77*	11 938	1	0·08	± 0·08
794 146 × *Y*	116 230	0	—	—
794 146 × *183*	79 360	2	0·025	± 0·018
794 146 × *77*	51 431	10	0·19	± 0·06
794 146 × *775*	52 690	15	0·28	± 0·07
794 77 × *Y*	50 626	0	—	—
794 77 × *183*	110 699	0	—	—
794 77 × *146*	38 050	1	0·03	± 0·03
794 77 × *775*	28 436	7	0·25	± 0·09
794 73 × *146*	159 243	0	—	—
794 73 × *775*	42 227	2	0·05	± 0·03
794 775 × *Y*	205 484	0	—	—
794 775 × *183*	119 685	0	—	—
794 775 × *146*	193 108	14	0·07	± 0·02
794 775 × *77*	113 870	4	0·035	± 0·018

(iv) *Three-point crosses*

The results of three-point crosses are given in Table 6. The order of the alleles on the map was deduced chiefly from these crosses. When in the cross $ab \times c$ the

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recombination frequency is higher than in the cross $ac \times b$ the assumed order of the mutants is abc.

In all cases the recombination frequencies in two configurations differed by about one order of magnitude and so the ordering was, on this basis, unambiguous. When the comparison of the recombination frequencies from the crosses $ab \times c$ and $ac \times b$ suggested the abc order, then in the majority of cases the frequency of recombination from the cross $ab \times c$ was similar to the recombination frequency in two-point cross $b \times c$. This was further confirmation that the sequence of mutants was abc. The established order of the seven mutants from the Y locus is given in Fig. 1.

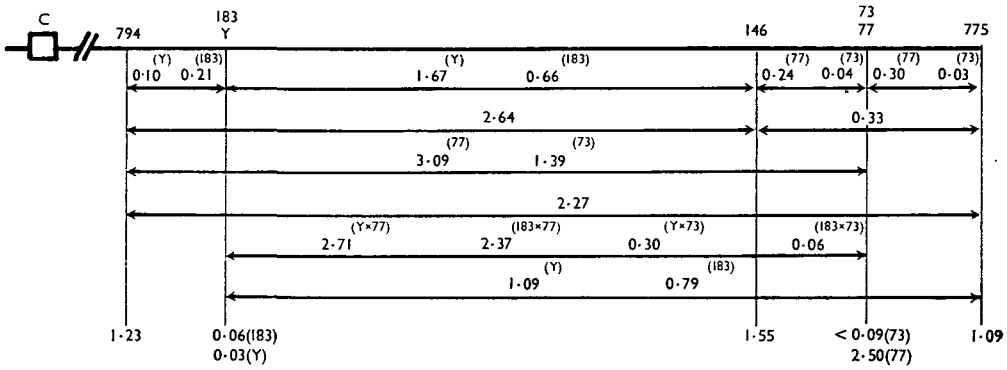

Fig. 1. Map of the Y locus. The sequence of mutants has been established on the basis of three-point crosses. Figures above the map and those in the brackets refer to mutant isolation numbers. Figures above the horizontal lines correspond to 6w : 2d octad frequencies (in %) from two-point crosses in repulsion. Figures below refer to the basic conversion frequencies (in %) of corresponding mutants. c—centromere.

In some of the three-point crosses (Table 6) of the $ac \times b$ type (sequence abc) no 6w : 2d octads were found, in others like *794 146* $\times$ *183*, *794 77* $\times$ *146*, *794 775* $\times$ *146* and *794 775* $\times$ *77* such octads were observed. If the frequency of 6w : 2d octads in such crosses is the product of frequencies of such octads from the two corresponding two-point crosses $a \times b$ and $b \times c$, they should not be expected at all. For instance, in the cross *794 775* $\times$ *146* the frequency found was $0{\cdot}07 \times 10^{-3}$ and the calculated product of frequencies from corresponding two-point crosses was $0{\cdot}00092 \times 10^{-3}$, thus about one hundred times lower. Similar differences were true for other crosses mentioned above.

From the cross *794 775* $\times$ *146* five octads were partially tested. In all of them only two of the six white ascospores were single mutant *146*. The genotypes of the remaining four white spores were not completely established. It is known, however, that all of them include mutation *794* and thus are probably of the double mutant parental type. Hence it seems that this type of octad results from the conversion of mutant *146*. This conversion should not be regarded as two independent exchanges but as one event or two interdependent events.

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4. ANALYSIS OF THE RESULTS AND DISCUSSION

(i) *Reciprocal and non-reciprocal recombination*

As the majority (94·8%) of the recombinant octads studied resulted from non-reciprocal recombination (conversion) so our further discussion is mainly concerned with it. In the majority of crosses, the crossover octads were not found at all or were very infrequent, except in the cross $Y \times 73$ in which a majority of the octads tested were of the crossover type. Perhaps in this case also the absolute frequency of crossover octads is not higher than in the other crosses between mutants from different clusters, and their high frequency among tested 6w:2d octads was due only to the low frequency of basic conversion frequencies of both these mutants. However, the relations between the frequencies of these two types of recombination and any considerations concerning their mutual relationship require much more numerous data.

(ii) *Basic conversion frequencies in opposite directions*

From the comparison of Tables 1 and 2 it can be seen that the basic conversion frequencies in opposite directions are equal or similar for some alleles (as $775 - +^{775}$, $77 - +^{77}$) or different as for $Y - +^{Y}$, $183 - +^{183}$, $73 - +^{73}$ alleles, where the frequencies of conversion to the mutant allele are about ten times higher than in the opposite direction. The same situation was found in the *46* locus in *Ascobolus immersus* (Rizet & Rossignol, 1963; Rossignol, 1964) where *1604* and *63* alleles had higher conversion frequencies to mutant alleles than in the opposite direction, and *137* mutant had the same conversion frequency in both directions.

Basic conversion frequency in *Sordaria fimicola* for the *g* mutant was many times higher toward wild type (Kitani *et al.*, 1961, 1962).

(iii) *Basic conversion frequency and position on the map*

Inspection of the map (Fig. 1) shows that there is no correlation between the basic conversion frequency of a mutant and its position on the map, as observed for the mutants of the *46* locus in *Ascobolus immersus* (Lissouba, 1961; Rossignol, 1964). In the *Y* locus, mutants with similar basic conversion frequencies such as *794*, *146* and *775* are in different parts of the map separated by mutants with higher or lower basic conversion frequencies. On the other hand, the *77* and *73* mutants with probably the same position on the map, have completely different conversion frequencies.

If the differences in the efficiency of transformation among different markers are comparable with differences in basic conversion frequencies, then our results are similar to the results obtained for *amiA* mutants of *Pneumococcus* in one-point crosses (Ephrussi-Taylor *et al.*, 1965; Lacks, 1966). In this locus no correlation was observed between the efficiency of transformation and the position of the allele on the map.

(iv) *Asymmetry of conversion in two-point crosses*

The comparison of conversion in two-point crosses with corresponding basic frequencies (Table 7) shows that:

(1) Octads resulting from conversion of the same mutant were found only in crosses between mutants with the lowest basic conversion frequencies (*Y*, *183*, *73*) and the remaining ones. In these octads the mutant converted was always that with the higher basic conversion frequency.

Table 7. *Relation between basic conversion frequencies and conversion in two-point crosses* (*in repulsion*)

		6w:2d conversion octads				
			Mutant converted		Ratio I*	Ratio II*
Type of cross	Cross $a \times b$	Total octads	a	b	$\frac{6:2(a\rightarrow+)(a\times b)}{6:2(b\rightarrow+)(a\times b)}$	$\frac{f\,2:6(a\rightarrow+)(a\times+)}{f\,2:6(b\rightarrow+)(b\times+)}$
1	*Y* × *794*	3	0	3	0/3	0·03/1·23
	Y × *146*	26	0	26	0/26	0·03/1·55
	Y × *77*	29	0	29	0/29	0·03/2·50
	Y × *775*	30	0	30	0/30	0·03/1·09
	183 × *794*	15	0	15	0/15	0·06/1·23
	183 × *146*	19	0	19	0/19	0·06/1·55
	183 × *77*	29	0	29	0/29	0·06/2·50
	183 × *775*	29	0	29	0/29	0·06/1·09
	73 × *794*	19	0	19	0/19	< 0·09/1·23
2	*794* × *77*	25	2	23	2/23	1·23/2·50
	146 × *77*	9	1	8	1/8	1·55/2·50
	775 × *77*	11	2	9	2/9	1·09/2·50
	794 × *146*	37	8	29	8/29	1·23/1·55
3	*794* × *775*	28	12	16	12/16	1·23/1·09
	146 × *775*	14	5	9	5/9	1·55/1·09
	Y × *73*	3	1	2	1/2	0·03/< 0·09

(2) Octads resulting from conversion of either mutant, but with different frequencies, were found in crosses between mutants with medium basic conversion frequencies and the *77* mutant (which showed the highest basic conversion frequency). It was always *77* mutant that converted more frequently. To this group belongs also the cross *794* × *146*, although here the frequencies of the basic conversion were similar for both mutants.

(3) Octads resulting from conversion of either mutant with similar frequencies were found in all crosses between mutants with similar basic conversion frequencies except the one just mentioned.

In the following discussion, for two mutants when crossed with one another, the ratio of the number of asci showing conversion to wild type at one site to the number

* For explanation of the symbols see the footnote on page 168.

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showing conversion to wild type at the other site is called ratio I, and the corresponding ratio when they are crossed individually to wild type is called ratio II. In the majority of crosses no statistically significant differences were found between the ratios I and II (Table 7). As the numbers of 6w:2d octads tested from each cross were relatively small and the basic conversion frequencies were estimated only approximately, the similarities of the ratios I and II are still not firmly established. Since in two-point crosses of the type $a \times b$ only a proportion of conversion is detectable, the evidence for exact proportionality of the conversion frequencies in one- and two-point crosses can be estimated only from the following equation:

$$\frac{6:2(a \rightarrow +)(a \times b)}{6:2(b \rightarrow +)(a \times b)} = \frac{f\,2:6(a \rightarrow +)(a \times +) - x}{f\,2:6(b \rightarrow +)(b \times +) - y} \qquad (1)^*$$

where x is the unknown frequency of undetectable conversion of mutant $a \rightarrow +$ accompanied by simultaneous conversion of $+ \rightarrow b$, and y is the corresponding value of conversion of mutant $b \rightarrow +$ accompanied by simultaneous conversion of $+ \rightarrow a$.

Statistically significant differences between the ratios I and II were observed for two pairs of mutants, i.e. *794*, *77* and *794*, *146*. Probably here the conversion frequencies of the mutants in one- and two-point crosses are not proportional indeed.

There is another possibility: Only the frequencies of detectable conversions in two-point crosses are not proportional to the corresponding frequencies in one-point crosses. Total conversion frequencies (i.e. both detectable and undetectable) in two-point crosses are proportional to the corresponding frequencies in one-point crosses; in other words ratios I and II are different, but equation (1) is fulfilled. This possibility, however, seems to be much less probable.

The comparison of ratios I and II from Table 7 shows that symmetry or more or less pronounced asymmetry in the conversion of two mutants was due chiefly to their similar or different basic conversion frequencies. The crosses *794* × *77* and *794* × *146* show, however, that other factors can also be involved.

As the asymmetry of conversion in two-point crosses mainly resulted from differences between basic conversion frequencies which are not evidently correlated with the positions on the map, no consistent direction of asymmetry (polarization) was observed.

Similar results in *Ascobolus* were obtained for series *75* (Lissouba *et al.*, 1962) and *726* (Makarewicz, 1964). The series *75* is not strictly comparable, since it consists of more than one cistron. For *Ascobolus* in the *46* locus (Lissouba, 1961; Rossignol, 1964) and *19* (Lissouba *et al.*, 1962) polarity was demonstrated at least for a part of the gene map. Asymmetry and polarization of conversion were obtained also for different loci of *Neurospora crassa* (Stadler & Towe, 1963; Murray, 1961, 1963) and

* In this formula and in the following ones the symbols used should be read as follows: $6:2(a \rightarrow +)(a \times b)$ means the number of 6w:2d tetrads among tested ones resulted from the conversion of mutant a to its wild-type allele in the cross $a \times b$; $f\,2:6(a \rightarrow +)(a \times +)$ means the conversion frequency of mutant a to its wild-type allele in the cross $a \times +$ (i.e. basic conversion frequency of a).

in *Aspergillus nidulans* (Siddiqi, 1962; Siddiqi & Putrament, 1963; Putrament, 1964).

(v) '*Marker effect*' *on the conversion frequency*

In the majority of the two-point crosses the frequencies of recombination between mutants from different clusters are approximately equal to the sum of their basic conversion frequencies, i.e.:

$$f\,6{:}2\,(a\times b)\approx f\,2{:}6\,(a\to +)(a\times +)+2{:}6\,(b\to +)(b\times +) \qquad (2)$$

This suggests that in these instances frequencies of undetectable conversion are relatively low and the frequencies of conversions of each mutant in one- and two-point crosses are equal or similar if the ratios I and II are also similar. The relation (2) is fulfilled for all crosses between two clusters except for the crosses *73* × *Y* and *183* × *146*. For the first cross this result could be explained by the exceptionally high frequency of crossover octads among 6w:2d tetrads. Taking into account only octads of conversion type the relation is perhaps fulfilled. The second cross will be discussed later on.

For the crosses *794* × *77* and *794* × *146* the relation (2) is fulfilled, but as the ratios I and II differ significantly it seems that the conversion frequencies of these mutants in two-point crosses are different from those in one-point crosses.

In the remaining two-point crosses between mutants from different clusters the conversion frequencies are probably near or the same as the basic conversion frequencies. This means that in these instances there is probably no influence (or only very small) of the presence of one mutant on the conversion frequency of the second one.

In the crosses within the cluster the frequencies of 6w:2d tetrads are evidently lower than the sums of basic conversion frequencies of mutants. The difference is even bigger than in the cross *183* × *146*.

Failure to fulfil the relation (2) does not indicate that there are differences in the conversion frequencies in one- and two-point crosses. Such differences could arise if in the crosses within the cluster a relatively high fraction of the total conversion $a\to +$ and $b\to +$ is undetectable (high values of x and y). Only if the following equation is not fulfilled,*

$$f\,6{:}2\,(a\times b)=f\,2{:}6\,(a\to +)(a\times +)+f\,2{:}6\,(b\to +)(b\times +)-(x+y) \qquad (2a)$$

would the influence of the presence of one mutant on the conversion frequency of another be proved. Unfortunately the values of x and y are unknown and difficult to ascertain. One could determine at least their maximal values from the conversion frequencies of two alleles in both directions if the presence of one mutant is not stimulating the conversion of another allele at a different site.

If the basic conversion frequency $a\to +$ is higher than the basic conversion frequency $+\to b$, then the value of x (the frequency of simultaneous conversions

* This equation is also simplified since we assume that all 6w:2d tetrads result from conversion.

$a \rightarrow +$ and $+ \rightarrow b$ in the cross $a \times b$) could not be higher than the basic conversion frequency $+ \rightarrow b$. That is, if

$$f\,2{:}6\,(a \rightarrow +)(a \times +) > f\,6{:}2\,(+ \rightarrow b)(b \times +)$$

then

$$x \leqslant f\,6{:}2\,(+ \rightarrow b)(b \times +)$$

Similarly, for the value of y, if

$$f\,6{:}2\,(+ \rightarrow a)(a \times +) > f\,2{:}6\,(b \rightarrow +)(b \times +)$$

then

$$y \leqslant f\,2{:}6\,(b \rightarrow +)(b \times +)$$

Substituting the maximal values of x and y in the equation (2a) this equation transforms into the following inequality:

$$f\,6{:}2\,(a \times b) \geqslant f\,2{:}6\,(a \rightarrow +)(a \times +) + f\,2{:}6\,(b \rightarrow +)(b \times +) - f\,6{:}2\,(+ \rightarrow b)\,(b \times +) - f\,2{:}6\,(b \rightarrow +)\,(b \times +)$$

$$f\,6{:}2\,(a \times b) \geqslant f\,2{:}6\,(a \rightarrow +)(a \times +) - f\,6{:}2\,(+ \rightarrow b)(b \times +) \qquad (3)$$

Corresponding values for the pairs of mutants *77*, *775* and *775*, *73* are known. (Mutant a is the one with higher basic conversion frequency to wild type.) Inserting them in the inequality (3) we obtain:

77 and *775*	$0{\cdot}30 \geqslant 2{\cdot}50 - 0{\cdot}80$		*775* and *73*	$0{\cdot}03 \geqslant 1{\cdot}09 - 0{\cdot}61$	
	$0{\cdot}30 \geqslant 1{\cdot}70$			$0{\cdot}03 \geqslant 0{\cdot}48$	

In both cases the inequality (3) is not satisfied. For the pairs of mutants *794* and *Y*, *794* and *183*, *146* and *73* the basic conversion frequencies $+ \rightarrow a$ are unknown. Probably also in these instances the frequency $(+ \rightarrow a)(a \times +)$ is higher than $(b \rightarrow +)(b \times +)$ and thus the data for these three pairs of mutants can also be inserted in the inequality (3). We obtain:

794 and *Y*	$0{\cdot}10 \geqslant 1{\cdot}23 - 0{\cdot}39$		*146* and *73*	$0{\cdot}04 \geqslant 1{\cdot}55 - 0{\cdot}61$	
	$0{\cdot}10 \geqslant 0{\cdot}84$			$0{\cdot}04 \geqslant 0{\cdot}94$	
794 and *183*	$0{\cdot}21 \geqslant 1{\cdot}23 - 0{\cdot}57$				
	$0{\cdot}21 \geqslant 0{\cdot}66$				

It can be seen that also in these instances the inequality is not fulfilled. If the frequency of conversion $(+ \rightarrow a)(a \times +)$ is lower than $(b \rightarrow +)(b \times +)$ then

$$y \leqslant f\,6{:}2\,(+ \rightarrow a)(a \times +)$$

When this maximal value of y and the previously discussed maximal value of x are inserted in the equation (2a) the following inequality will be obtained:

$$f\,6{:}2\,(a \times b) \geqslant f\,2{:}6\,(a \rightarrow +)(a \times +) + f\,2{:}6\,(b \rightarrow +)(b \times +) - f\,6{:}2\,(+ \rightarrow b)(b \times +) - f\,6{:}2\,(+ \rightarrow a)(a \times +) \qquad (4)$$

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The inequality (4) will not be fulfilled even more strongly than the inequality (3). In all six cases this difference is too large to be ascribed only to the errors of determination of basic conversion frequencies.

The fact that the inequality (3) is not satisfied could indicate one of the following possibilities:

(1) The presence of one mutant diminishes the conversion frequency of another.

(2) The presence of one mutant can stimulate the conversion frequency at another site in the opposite direction (higher values of x and y than maximal values previously accepted).

It seems that the first supposition is much more probable. In any case the fact that the inequality (3) is not satisfied points to the influence of the presence of one mutant on the conversion frequency at another site.

The 'marker effect' was also evident from the comparison of some two- and three-point crosses. When in the cross $b \times c$ the total conversion frequency of b and c mutants (both detectable and undetectable) is equal to total frequency in the cross $ab \times c$, then the frequency of the 6w:2d tetrads in the cross $b \times c$ should be equal to the frequency of 6w:2d octads in the cross $ab \times c$, minus some value z. The value z is the frequency of conversion of b and c—which is undetectable because it is masked by mutant a. This value should be near to the frequency of 6w:2d octads in the cross $ac \times b$. When this frequency is relatively low one could expect that the frequency of 6w:2d octads in the cross $ab \times c$ should be approximately equal to the frequency of 6w:2d octads from the corresponding two-point cross. This was true for all compared cases (Table 8 upper part) except those including the Y mutant. When the Y mutant is introduced in the position a or b of our scheme the frequency of recombination in the cross $ab \times c$ is about ten times lower than in the corresponding two-point cross (Table 8 lower part). Such striking differences could not be explained by the increase of the frequency of undetectable conversion in three-point crosses compared with two-point ones. Such results suggest again that the presence of an additional marker can change the conversion frequency of other markers involved in the same cross.

For instance, at least in the majority of cases, 6w:2d octads arising in the cross

Table 8. *Frequencies of 6w:2d octads from three-point crosses of* a b × c (*or* a × b c) *type compared with corresponding two-point crosses*

Cross $ab \times c$ or $a \times bc$	Frequency of 6w:2d octads	S.E.	Cross $b \times c$ or $a \times b$	Frequency of 6w:2d octads	S.E.
183 775 × 794	0·16	± 0·04	*183 × 794*	0·21	± 0·04
794 146 × 77	0·19	± 0·06	*146 × 77*	0·24	± 0·05
794 146 × 775	0·28	± 0·07	*146 × 775*	0·33	± 0·05
794 77 × 775	0·25	± 0·09	*77 × 775*	0·30	± 0·05
794 73 × 775	0·05	± 0·03	*73 × 775*	0·026	± 0·018
Y 77 × 794	0·011	± 0·007	*Y × 794*	0·10	± 0·04
Y 775 × 794	0·02	± 0·02	*Y × 794*	0·10	± 0·04
Y 77 × 775	0·055	± 0·018	*77 × 775*	0·30	± 0·05

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794 × *Y* result from the conversion of *794* mutant. In the corresponding cross *794* × *Y 775* the frequency of 6w:2d octads is about five times lower. Hence the presence of the *775* marker together with *Y* decrease the conversion frequency of the *794* mutant. It is remarkable that for the *183* mutant no such effect was observed in the analogous configuration, notwithstanding the fact that *Y* and *183* are not separable as to their position on the map and behave in a similar way in two-point crosses.

A similar 'marker effect' was observed by Ravin & Iyer (1962) in transformation of different markers from the erythromycin resistance locus in *Pneumococcus*. Three-point crosses revealed that particular configurations of the markers strongly changed the frequencies of exchanges in certain regions of the studied locus.

(vi) *Non-additivity of recombination frequencies*

The recombination frequencies from the two-point crosses (in repulsion) are not only non-additive but they cannot be used to construct a linear map of the *Y* locus. The non-additivity of recombination values results mainly from their dependence on the basic conversion frequencies specific for different mutants. For the interval *Y–73* the recombination value is lower than for the interval *Y–146* although *146* seems to be located between *Y* and *73*. This probably results from the fact that the basic conversion frequency of *146* is much higher than that of *73*. For the same reasons the recombination values of mutants from cluster 1 are higher with the nearer *77* mutant than with the more distant *775* mutant.

The same relationships can be observed when some crosses between mutants from the same cluster are compared. The recombination frequency in the cross *146* × *775* is lower than the sum of recombination frequencies from the crosses *146* × *77* and *77* × *775*, which could be explained by the high basic conversion frequency of the *77* mutant.

The striking differences in the recombination frequencies within and between the cluster suggest some kind of cross-specificity depending probably also on distance. This is not due only to the probably high frequency of undetectable conversion in crosses between closely located mutants. An additional cause of the low frequency of recombination between closely located mutants could be due to the inhibiting influence of one mutant on the conversion frequency of another. The dependence on distance does probably exist, but is not simple and is affected by other factors. Thus the frequency of recombination in the two-point crosses cannot be used as a measure of distance between the mutants of the *Y* locus.

We should expect better additivity in such loci where the mutants have rather similar basic conversion frequencies. This is true for the *46* locus in *Ascobolus*, at least in the neighbouring mutants which do not differ greatly in their basic conversion frequencies.

Fine structure analysis of several loci in fungi has shown that the linear ordering of mutant sites based on recombination frequencies from two-point crosses is often difficult or impossible, as in the *Y* locus. However, in the majority of loci studied

linear ordering was possible but the additivity was often very poor. Only Ishikawa (1962) with the *ad*-8 locus in *Neurospora* has found nearly ideal additivity.

Very different degrees of additivity are observed in viruses and bacteria. In the rII region of the phage T4 fine structure analysis with recombination frequencies above $10^{-2}\%$ showed approximate additivity, but Tessman (1965) has shown that when the resolving power of the recombinational analysis was much increased the additivity disappeared.

Also, in fine structure analysis of many loci by transduction in bacteria, a departure from additivity was observed (Yanofsky & Crawford, 1959; Smith, 1961; Balbinder, 1962; Margolin, 1963). In the majority of cases there were observed single mutants with abnormally high or low recombination in crosses with the other mutants. Ignoring them, the rest of the mutants could be easily mapped. Recently, Sicard & Ephrussi-Taylor (1965) have shown, that in the *amiA* locus in *Pneumococcus*, the frequencies of recombination are additive provided all of the distances are estimated with recipients of the same transformation efficiency class.

(vii) *The present results and different models of recombination*

The copy-choice theory in its simplest form does not explain the following facts established for the *Y* locus.

Inequality of basic conversion frequencies of some mutants and their wild-type alleles.

Lack of correlation between basic conversion frequencies of different mutants and their positions on the map.

Lack of correlation between frequencies of simultaneous conversion of two mutants and their distance on the map.

It is also difficult to explain on the basis of the copy-choice theory the influence of one mutant on the frequency of conversion of another observed in the present work.

Even if we accept that mutants such as *Y*, *183* and *73* are multisite mutations of some kind, the interpretation of the data obtained in terms of copy-choice theory still presents many difficulties. If we assume that the *Y* mutant has some length then the switch giving conversion to the wild-type allele must include the whole segment corresponding to this mutation. On the other hand, conversion towards the mutant could result from a switch anywhere within the length of the *Y* mutant. Thus we should expect the last value to be higher than the former as indeed was found. The same supposition could also explain the lack of correlation between basic conversion frequencies and the position of mutants on the map. According to such an interpretation, however, we should expect the conversion of the Y mutant to be more frequent than the simultaneous conversion of the *794* and *775* mutants, provided the latter was also due to two switches only, since the interval *794–775* includes the *Y* mutant. This prediction was not supported by our data, since the conversion of *Y* was about ten times lower than the simultaneous conversion of *794* and *775*. We could also assume that the conversion of *794* and *775* was due to

four switches, but this is inconsistent with the results from three-point crosses. Also the assumption that *73* is a multisite mutation does not explain the low frequency of recombination in the cross $Y \times 73$. This value should be higher than the frequency of recombination in the cross $Y \times 146$, since *146* is located between *Y* and *73*.

There are other facts observed in the present work which are not directly explicable with copy-choice theory. Their explanation would require many additional assumptions difficult to prove or to disprove.

It seems to be easier to interpret most of the present results when hybrid DNA models recently developed by Whitehouse and Holliday (Whitehouse, 1963, 1965; Hastings & Whitehouse, 1964; Whitehouse & Hastings, 1965; Holliday, 1962, 1964) are accepted. According to these models the basic conversion frequencies of the mutant and its wild-type allele should not necessarily be equal. They will depend only on the molecular nature of the initial mutational step.

The polarity of intracistronic recombination is explained by these models by an assumption of a fixed point of primary DNA strand breakage. The probability that a given site will be included in hybrid DNA will depend to some extent on its distance from this point. However, according to the models discussed, this relation may be obscured by different frequencies of correction in hybrid DNA for different mutants. Another factor, according to Holliday, could be the influence of the different mutations on the effective pairing and the resulting formation of hybrid DNA. It is still easier to explain the lack of correlation between basic conversion frequencies and the positions of mutants on the map by assuming, for instance, that primary breakage and subsequent hybrid DNA formation can occur on either side of the *Y* locus.

The results of two-point crosses in coupling are also much easier to interpret with hybrid DNA models. The probability of the simultaneous inclusion of the *794 775* double mutant into a hybrid DNA segment could be similar to or equal to *Y 775*. However, if the homozygotization of the type $Y \rightarrow +^{Y}$ is much lower than $794 \rightarrow +^{794}$ then the frequency of simultaneous conversion in the cross $Y\,775 \times + +$ will be much lower than in the cross $794\,775 \times + +$.

If the occurrence of 2w : 6d octads in the cross $794\,775 \times + +$ is due to the formation of heterozygous DNA which includes both mutants on two chromatids, followed by correction to the wild type in the four heterozygous points, then the frequency of such octads should be equal to the frequency of 4w : 4d octads in the cross 794×775. Since in the cross 794×775 such octads have never been observed, this could indicate that the formation of 2w : 6d octads in the cross $794\,775 \times + +$ is, as a rule, due to the occurrence of hybrid DNA on a single chromatid only.

It is also possible, however, that the occurrence of the 2w : 6d octads under discussion results from the formation of hybrid DNA on both chromatids, but the correction of the two sites in the same hybrid DNA molecule occurs, as a rule, in the same direction. It has been shown (Pettijohn & Hanawalt, 1964; Setlow & Carrier, 1964) that the excision of a non-complementary base in DNA is usually followed by enzymatic removal of adjacent nucleotides. The gap thus formed could include the sites of both mutants. Then the completion of the gap according to the un-

changed strand would result in simultaneous conversion of both mutants in coupling in the same direction.

Lack of additivity and difficulties with linear arrangement of mutants, impossible to explain on the basis of copy-choice theory, are fully compatible with hybrid DNA models.

The influence of one marker on the conversion frequency of another in multi-point crosses can be explained by Holliday's assumption of their influence on the effective pairing and hybrid DNA formation.

There are still facts that are difficult to explain with hybrid DNA models. For instance, in all three-point crosses (except those involving Y) of the type $ab \times c$ the frequency of 6w:2d octads was the same as in the corresponding two-point $b \times c$ crosses. If the 6w:2d octads are due mainly to the conversion of the c mutant, this could be easily explained in terms of hybrid DNA models. In crosses such as *794* 77×775 and 77×775, however, where 6w:2d octads are due to conversion of both *77* and *775*, we should expect much lower values for the corresponding three-point crosses. It is still difficult to explain this without further assumptions.

SUMMARY

Mutants of the Y locus differed appreciably in their basic conversion frequencies (frequencies of conversion in one-point crosses) to wild type. The differences in the basic conversion frequencies in the opposite direction, i.e. from corresponding wild-type allele to mutant, were in general not pronounced. For some alleles frequencies of conversion in both directions were similar, but for the others they differed markedly. No evident correlation between the position of mutants on the map and their basic conversion frequencies was observed.

In two-point crosses in repulsion, the great majority of recombinant octads were of conversion type. In these crosses symmetry or asymmetry of conversion depended mainly on similarity or differences in basic conversion frequencies of mutants crossed. In crosses between mutants from different clusters the recombination frequencies were near to the sums of their basic conversion frequencies. Such 'mutant specificity' makes it impossible to establish the linear order of mutants on the basis of recombination frequencies in two-point crosses.

The results of two-point crosses in repulsion between mutants within clusters pointed to the influence of one allele on the frequency of conversion of another one. This 'marker effect' was also evident in some three-point crosses.

The frequencies of simultaneous conversions in two-point crosses in coupling did not show negative correlation with the distances between the mutants involved.

It seems that many of the data presented here are most easily explained by recently developed hybrid DNA models.

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