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Aberrant segregation patterns and gene mappability in *Ascobolus immersus*

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SUMMARY

Crosses between various types of mutant giving specific patterns of aberrant segregation were performed in the *b2* spore colour locus of *Ascobolus immersus*. The map of 41 mutations showing various patterns of aberrant segregation was established. The frequency of wild-type recombinants and the map additivity, map expansion and map contraction characteristics were shown to be strongly dependent upon the pattern of aberrant segregation of the mutations used. Mutations giving no postmeiotic segregation and an excess of conversion to wild type over conversion to mutant exhibit map expansion in small intervals and a strong map contraction in large intervals. Mutations giving postmeiotic segregations also exhibit map contraction in large intervals. Mutations giving no postmeiotic segregations and an excess of conversion to mutant over conversion to wild type show map additivity and thus provide a simple way for devising gene maps. The relationship between the mapping properties and the pattern of aberrant segregations is accounted for when considering parameters of gene conversion: frequency and distribution of hybrid DNA, frequency and direction of mismatch correction.

INTRODUCTION

Genetic mapping is based upon the additivity of prototroph frequencies in mutant × mutant crosses. However, such additivity is rarely observed in intragenic crosses. This absence of additivity is not surprising since most of the intragenic recombinants result from conversion, i.e. non-reciprocal recombination events corresponding to aberrant segregations for at least one mutant site. Aberrant segregations are very likely to result from hybrid DNA (hDNA) formation, associating two complementary strands of different parental origin, followed by an eventual correction of the mismatch(es) formed at the mutant site(s) (Pukkila, 1977, for review). Fincham & Holliday (1970) made a theoretical study of the

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characteristics of intragenic recombination frequencies. Two factors are supposed to contribute to intragenic recombination: hDNA ending and independent mismatch corrections when both sites are involved in hDNA. In the hypothesis where the mean size of the correction tract is smaller than the mean size of the hDNA segment, three phases are expected, according to the distance between sites:

(1) additivity, when the distance is smaller than the correction tract, preventing independent mismatch correction;

(2) map expansion when the distance increases, allowing independent corrections;

(3) map contraction when the sites are far enough to be always corrected independently, when involved in the same hDNA segment.

The two first expectations agree with several cases (*su-3* in *Schizosaccharomyces* and *ser-19* gene of *Ascobolus*, see Fincham & Holliday, 1970); nevertheless, strict additivities are rare (*ad-8* in *Neurospora*: Ishikawa, 1962; *ser-46* in *Ascobolus*: Lissouba *et al.* 1962). Actually, most of the intragenic maps do not present either additivity or clear map expansion. Striking cases of non-mappability on the basis of the meiotic frequency of wild-type recombinants were seen in *Ascobolus immersus* (Kruszewska & Gajewski, 1967), in *Neurospora crassa* (Fincham, 1967; Stadler & Kariya, 1969) and in *Saccharomyces cerevisiae* (DiCaprio & Hastings, 1976). Moreover, a lack of correspondence between physical distances and meiotic recombination frequencies was clearly indicated in the *cyc1* gene of *S. cerevisiae*, bringing to light the erroneous order of sites that could be inferred from meiotic gene mapping (Moore & Sherman, 1975).

The successful attempt to correlate in *Ascobolus immersus* the frequencies of conversion to wild type in one-point crosses with the frequencies of wild-type recombinants in two-point crosses (Kruszewska & Gajewski, 1967) strongly suggests that the correction pattern of the mutations might play a more important role than physical distances in the prototroph frequency. The study of prototroph frequencies on sets of mutations lying in the same gene and showing distinct conversion patterns (Leblon, 1972*a*; Yu-Sun, Wickramaratne & Whitehouse, 1977) is then essential to determine the relationship between the nature of the mutations and their mapping behaviour.

The *b2* locus, a gene which has been used extensively for meiotic recombination studies, was chosen for this purpose. Fourteen mutations giving numerous postmeiotic segregations (pms) also called type C mutations (Leblon, 1972*a*), 8 mutations giving no pms and an excess of 2-wild:6-mutant asci (2+6m) over 6+2m (type B mutations) and 19 mutations giving no pms and an excess of 6+2m over 2+6m (type A mutations) lie in this locus.

Type A mutations exhibit map expansion in small intervals and a strong map contraction in large intervals; type C mutations also exhibit map contraction in large intervals; type B mutations show map additivity providing a simple way for devising gene maps. An interpretation is proposed, using gene conversion parameters calculated by Paquette & Rossignol (1978); and a method for mapping genes is proposed, in which the additive phase of Fincham and Holliday is extended beyond

the co-correction length by using situations in which independent correction makes little contribution to wild-type recombinant frequencies.

MATERIAL AND METHODS

The culture, germinating and crossing media have been described by Rizet *et al.* (1960) and Lissouba *et al.* (1962). Crossing conditions were described by Rossignol & Paquette (1979). The *b2* mutants belong to stock 28 (Rizet, Rossignol

Table 1. *Conversion parameters of mutations in regions* F, E *and A*

Region	Type of conversion pattern	Mutation	Conversion parameters			
			γ	α	p	v
F	A	F13	0·39	0·92	1	0·74
	B	F0	0·39	0·92	1	0·38
	C	8E	0·36	0·94	0·64	0·59
E	A	E2	0·23	0·67	1	0·90
	B	E1	0·23	0·67	1	0·12
	C	G1	0·23	0·67	0·27	0·33
A	A	A6	0·17	0·28	1	0·73
	B	A0	0·17	0·28	1	0·18
	C	A4	0·17	0·28	0·25	0·43

All the mutations used but two (8E, G1) behave as intragenic suppressors.

γ, probability of hDNA formation per meiosis. α, probability that hDNA forms on only one of the two interacting chromatids. p, frequency of mismatch correction. v, probability that correction leads to a wild-type homoduplex.

The values given in the Table were obtained by Paquette & Rossignol (1978) and Paquette (1979).

& Lefort, 1969) and bear the *cv2A* alleles of the modifier of *b2* (Girard & Rossignol, 1974). Their origin is given by Leblon (1972*a*), Leblon & Paquette (1978) and Paquette & Rossignol (1978). Deletion mutants 10 and 138 were first described by Girard & Rossignol (1974).

Three groups of mutations were previously defined by intragenic suppression in *b2* by Leblon & Paquette (1978): mutations in group *F* are located in the left end, mutations in group *E* are located in the middle and mutations in group *A* are located in the right end. We will call the regions where the mutations of the three intragenic suppression groups are located, regions *F*, *E*, *A*, respectively. The type of conversion pattern of these mutations is indicated in Table 1. Every mutation in a region will be named by its region, followed by its type of conversion pattern, (e.g., F13 which is located in region *F* and shows a type A conversion pattern will be named *F* A etc...).

When intercrossing mutants located in distinct regions (regions *F*, *E* and *A*), each pair of mutations (e.g. *F*A × *E*A etc...) was used several times, using different mutant strains. For example, in crosses given in Table 2 involving type A and type

B mutations, the *F*A and *F*B strains (F0 and F13) were isolated from one cross F0 × F13, the *E*A and *E*B strains (E2 and E1) were isolated from one cross E2 × E1 and the *A*A and *A*B strains (A6 and A0) were isolated from one cross A6 × A0. The same type of protocol was used for comparing type A × type C, type B × type C and type C × type C crosses (Table 3).

Table 2. *Recombinant asci in crosses involving type A and B mutants in regions* F, E, A

Mutation types	Type of ascus	Regions involved		
		F × *E*	*E* × *A*	*F* × *A*
B × B	2B 6W	689	195	915
	4B 4W	6	1	27
	6B 2W	1	0	0
	Total sample	9000	9000	9000
	FR	(*13–24*) 20	(*4–8*) 6	(*22–32*) 27
A × B	2B 6W	1038	615	1174
	4B 4W	27	21	38
	6B 2W	0	0	0
	Total sample	6000	6000	6000
	FR	(*36–54*) 46	(*24–33*) 28	(*40–69*) 52
B × A	2B 6W	326	209	362
	4B 4W	47	9	37
	6B 2W	0	0	1
	Total sample	3000	3000	3000
	FR	(*33–39*) 35	(*18–21*) 19	(*31–42*) 37
A × A	2B 6W	1095	843	1229
	4B 4W	272	291	284
	6B 2W	19	24	22
	Total sample	6000	6000	6000
	FR	(*49–89*) 71	(*54–71*) 63	(*54–98*) 78

Mutation type: conversion pattern of the left mutation × conversion pattern of the right mutation.

Mutants used (see Table 1). For each type of cross, several parental strains were used and a sample of 1000 asci was counted in every progeny; the numbers in parenthesis indicate extreme FR values found in each group of crosses.

FR: frequency of wild-type recombinant per 1000 ascospores.

RESULTS

(i) *Crosses between mutants in* F, E *and* A *regions*

(*a*) *Crosses between type A and B mutants*

The numbers of recombinant asci observed in the progeny of 72 crosses are given in Table 2. Asci with 4 wild-type ascospores (4B:4W asci, brown:white) and asci with 6 wild-type ascospores (6B:2W) may be numerous in some crosses (A × A),

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indicating that two and even three chromatids with a wild-type recombinant genotype for the *b2* gene can be formed in the same individual meiosis.

The frequencies of wild-type recombinants per 1000 ascospores (FR) show a relatively small intragroup variation. Much larger variations are observed when changing the type of mutation used or the interval. These variations lead to the following remarks:

Table 3. *Recombinant asci in crosses involving type C mutants in regions* F, E, A

Mutation types	Type of ascus	Regions involved		
		F × *E*	*E* × *A*	*F* × *A*
C × C	1B 7W	577	603	431
	2B 6W	634	116	837
	3B 5W	22	6	27
	4B 4W	9	0	14
	Total sample	6000	6000	6000
	FR	(*35–49*) 41	(*15–21*) 18	(*37–55*) 49
B × C (*a*)	1B 7W	297 (*b*)	344 (*a*)	252 (*b*)
or	2B 6W	655	235	688
C × B (*a*)	3B 5W	7	7	7
	4B 4W	9	0	13
	Total sample	6000	6000	5000
	FR	(*31–42*) 35	(*13–22*) 17	(*37–49*) 43
A × C (*c*)	1B 7W	425 (*c*)	649 (*d*)	330 (*c*)
or	2B 6W	1109	431	1168
C × A (*d*)	3B 5W	30	54	45
	4B 4W	18	12	41
	Total sample	*8000	6000	†7000
	FR	(*34–58*) 44	(*31–42*) 36	(*41–62*) 53

* 2 5B 3W, 1 6B 2W. † 3 5B 3W.
Legends: see Table 2. Mutations used: see Table 1.

(1) Within the same interval, the FR varies sharply when there is a change in the type of mutation involved in the cross. The most extreme situation is observed in the *E–A* interval, where the FR varies from 6 (*E*B × *A*B) to 63 (*E*A × *A*A) and gives intermediate values in *E*A × *A*B and *E*B × *A*A crosses. This means that physical distance cannot be related to FR as long as mutations with different patterns of aberrant segregation are used.

(2) When comparing the FR obtained in the *F–A* intervals with the sum of FRs obtained in the two subintervals *F–E* and *E–A*, apparent map expansion or map contraction may be observed when using mutations with different conversion patterns. When using flanking type A mutations and a median type B mutation, a weak map expansion is observed (78 observed versus 65 expected). A very strong map contraction is observed when using flanking type B mutations and a median type A mutation (27 observed versus 63 expected).

(3) When considering only mutations with identical patterns of aberrant segregations, map contraction is observed in A × A crosses (78 observed versus 134 expected), whereas B × B crosses exhibit a good additivity (27 observed versus 26 expected).

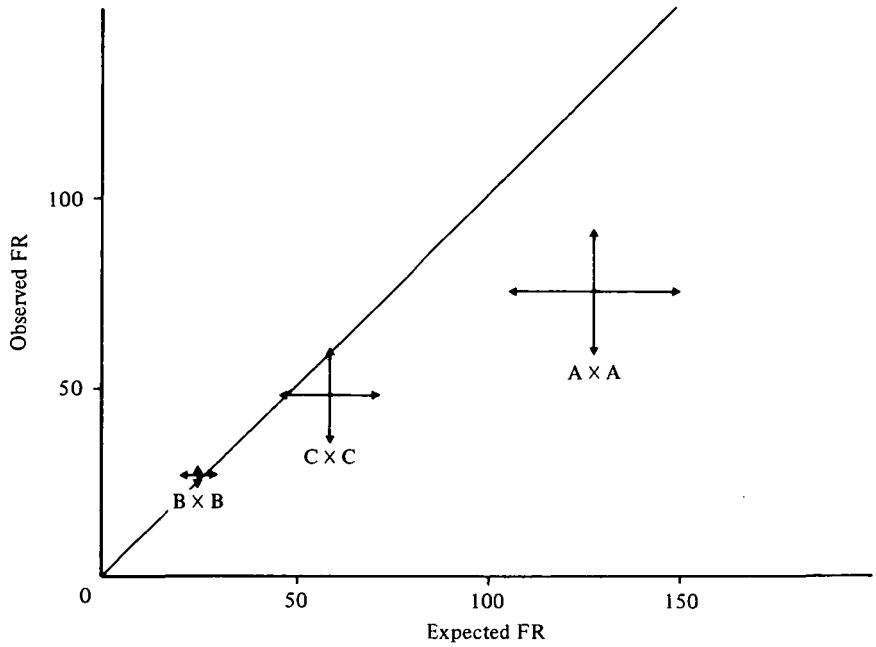

Fig. 1. Observed against expected frequencies of wild-type recombinants (FR) in B × B, C × C and A × A crosses (see Table 2). Observed values are those observed in the *F–A* interval: expected values correspond to the sum of the values found in *F–E* and *E–A* subintervals. The arrows indicate extreme observed and expected values.

(*b*) *Crosses involving type C mutants*

The numbers of recombinant asci observed in the progeny of these crosses are given in Table 3. Asci with 1B7W and 3B5W ascospores reflect the existence of postmeiotic segregations which are observed for type C mutations. The bearing of mutations type on FR is again observed: B × C crosses tend to give smaller FR than C × C crosses whereas A × C crosses tend to give larger FR. Map contraction is observed when considering C × C crosses (49 observed versus 59 expected).

Data for crosses involving identical mutation types are reported in Fig. 1. Increasing FR are observed in B × B, C × C and A × A crosses. Strong map contraction is observed in A × A crosses. Map contraction is still apparent, but weaker in C × C crosses whereas B × B crosses show a good additivity.

Table 4. *FR in crosses B × B and B × deletions*

	F0	B100	B17	E1	B79	B101	B4	A0	10	138
F0	0	9	17	20	22	23	26	24	0	46
	304·0†	*4·8*	*5·2*	*4·4*	*4·3*	*4·2*	*5·0*	*5·6*	*18·7*	*3·1*
	B100	0	7	13	18	18	19	18	0	41
		36	*2·0*	*4·5*	*1·9*	*4·4*	*3·3*	*4·3*	*11·4*	*3·1*
		B17	0	4	8	8	13	14	0	20
			18	*6·0*	*1·7*	*2·2*	*1·2*	*1·8*	*12·0*	*4·4*
			E1	0	3	5	8	6	0	8
				152‡	*6·8*	*5·3*	*2·2*	*5·0*	*12·6*	*4·1*
				B79	0	2	3	5	0	0
					0·7	*3·9*	*3·0*	*0·2*	*9·9*	*9·7*
					B101	0	1	4	0·1	0
						3·0	*6·9*	*5·1*	*9·3*	*10·0*
						B4	0	3	3	0
							24	*3·9*	*9·2*	*9·5*
							A0	0·01	5	0
								389·0‡	*6·4*	*3·0*

The total sample of asci observed ($\times 10^{-3}$) is given in italics below the FR.

† See Paquette, 1979. ‡ See Leblon (1974).

(ii) *Mapping of* b2 *locus*

(*a*) *Additivity when mapping type B mutations*

Eight type B mutants were intercrossed. The results are given in Table 4. They lead to the sequence drawn in Fig. 2. Crosses with the two deletion mutants 10 and 138 confirmed the order found in B × B crosses. Observed versus expected FRs in all intervals along the *b2* locus are presented in Fig. 3. Additivity is observed as a general feature in B × B crosses.

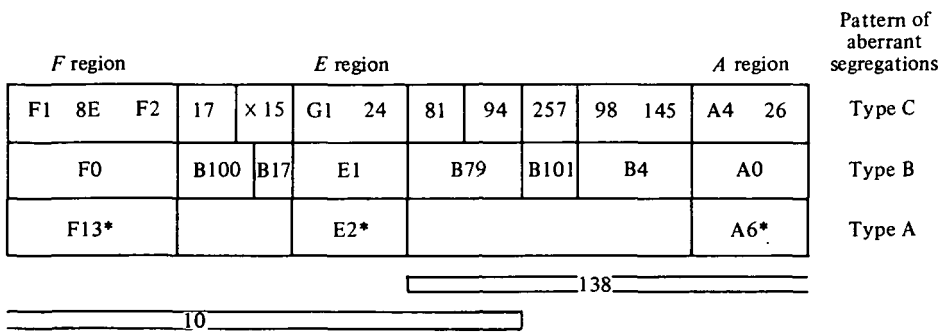

Fig. 2. Genetic map of locus *b2*. * 19 distinct type A mutations lie in regions *F*, *E* and *A* (13 in *F*, 2 in *E*, 4 in *A*).

(*b*) *Map expansion for type A mutations in small intervals*

Crosses between type A mutations lying in the same groups of intragenic suppression (*F* and *A*) were performed by Leblon & Paquette (1978): these crosses lead to map expansion as seen in Fig. 4.

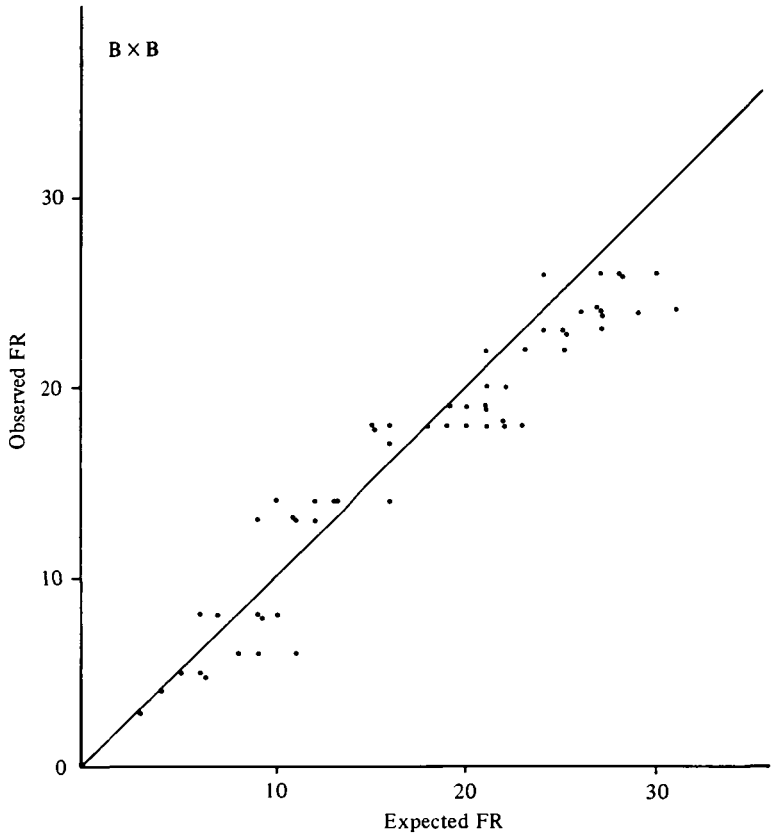

Fig. 3. Observed against expected FR values in B × B crosses (see Table 4). Expected values correspond to the sum of the two values found in the various pairs of possible subintervals (for example, observed value in cross F0 × B79 is compared to the sums of the values found in F0 × B100 and B100 × B79; F0 × B17 and B17 × B79; F0 × E1 and E1 × B79).

(*c*) *Mapping type C mutations*

C × *C crosses*. Fourteen type C mutants were intercrossed. The results are given in Table 5. FRs vary between 0 and 56. On the basis of FR, several groups of type C mutations exhibit relatively close linkage: F1, F2, 8E (FR smaller than 6) G1 and 24 (FR smaller than 1) 98 and 145 (no wild-type recombinant found), A4 and 26 (FR equal to 3). Most other crosses give relatively high FR and mapping these mutations from Table 5 is not possible. When choosing 3 mutations the 3 possible

crosses often show similar FR values, indicating map contraction. Observed versus expected FRs in all intervals are presented in Fig. 5. Additivity is observed for expected FR smaller than 20 and map contraction becomes unambiguous for expected FR equal to or larger than 50. For expected FR equal to 80 or more, the observed value is roughly half that expected, in other words, the FR in the

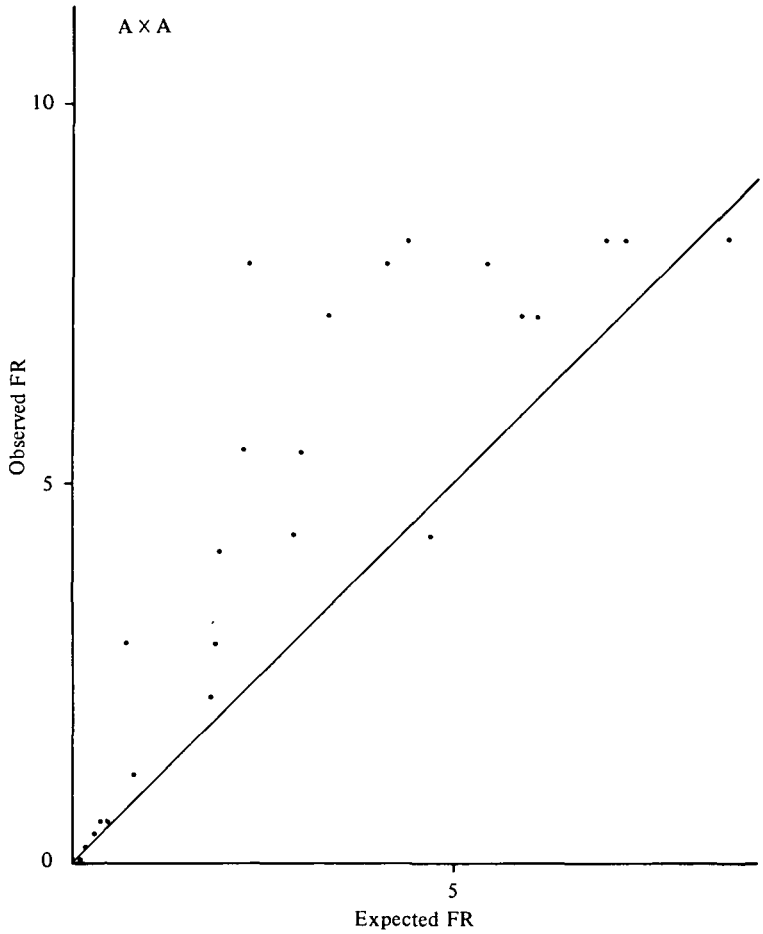

Fig. 4. Observed against expected FR values in A × A crosses (see Leblon & Paquette, 1978).

two subintervals are the same as the FR in the total interval. In conclusion, except for detecting very closely linked sites, C × C crosses cannot be used for setting up an intragenic map.

Crosses C × deletions. From the crosses with the two deletion mutants (Table 5), type C mutations were classified in three sets according to their ability to give recombination with deletion mutant 10 only, with deletion mutant 138 only or with neither (Fig. 2).

Table 5. *FR in crosses C × C and C × deletions*

(The total sample of asci observed ($\times 10^{-3}$) is given in italics below the FR.)

	17	X15	24	G1	81	257	98	145	A4	26	10
8E (F2)	18 *4·0*	28 *2·0*	45 *2·0*	28 *2·7*	41 *2·0*	34 *4·5*	40 *2·0*	—	46 *3·0*	—	0 (0) *1·2 (15·0)*
	17	—	41 *2·0*	21 *5·0*	43 *3·3*	31 *6·0*	43 *2·5*	—	39 *2·3*	—	0 *32·0*
		X15	31 *1·6*	18 *4·3*	39 *3·5*	41 *1·0*	56 *3·6*	—	36 *3·6*	—	0 *30·1*
			24	0·9 *3·0*	29 *4·5*	40 *1·0*	46 *1·7*	—	39 *2·1*	—	0 *20·0*
				G1	14 *0·5*	19 *1·3*	19 *1·6*	—	14 *6·0*	—	0 *6·8*
8E					81 (94)	22 *1·0*	30 *1·8*	—	26 *1·5*	—	0 (*0*) *16·0 (9·5)*
0·2 *3·3*	F1					257	9 *6·0*	19 *1·8*	13 *7·0*	27 *3·0*	0·6 *9·2*
5 *2·5*	6 *1·2*	F2					98	0 *1·5*	6 *1·6*	8 *0·5*	7 *2·7*
								145	6 *3·3*	10 *2·6*	4 *3·2*
									A4	3 *1·3*	7 *2·6*
										26	10 *3·9*

Crosses C × B. Since type B mutations can be unambiguously ordered from B × B crosses, the mapping of type C mutations was tentatively performed by crossing type C with type B mutants. Type B and type C mutants giving recombination with mutant 10 were intercrossed and so were type B and type C mutants giving no recombination with mutant 10 (Table 6). In the left part of *b2* (top) FR are usually larger than in the right part (bottom): up to 35 in the left part and only up to 19 in the right part. If FR smaller than 10 per 1000 ascospores are taken as a criterion for close linkage, in the left part, F1, 8E and F2 are closely linked to F0; mutant 17 is closely linked to F0 and B100; X15 is closely linked to B100 and B17; and mutants 24 and G1 are closely linked to E1. If FR smaller than 4 per 1000 ascospores are taken as a criterion of close linkage in the right part, mutant 81 is close to B79, mutant 94 is close to B79 and B101, mutant 257 is close to B101 and B4 mutants 98 and 145 are close to B4 and A0 and mutants A4 and 26 are close to A0. This leads to the sequence given in Fig. 2.

DISCUSSION

The bearing of conversion patterns upon the mappability of mutations is well illustrated by these results. In the very same interval, either map expansion, map additivity or map contraction can be found, depending on the mutations used. Only type B mutations allow consistent mapping, based on the additivity of wild-type

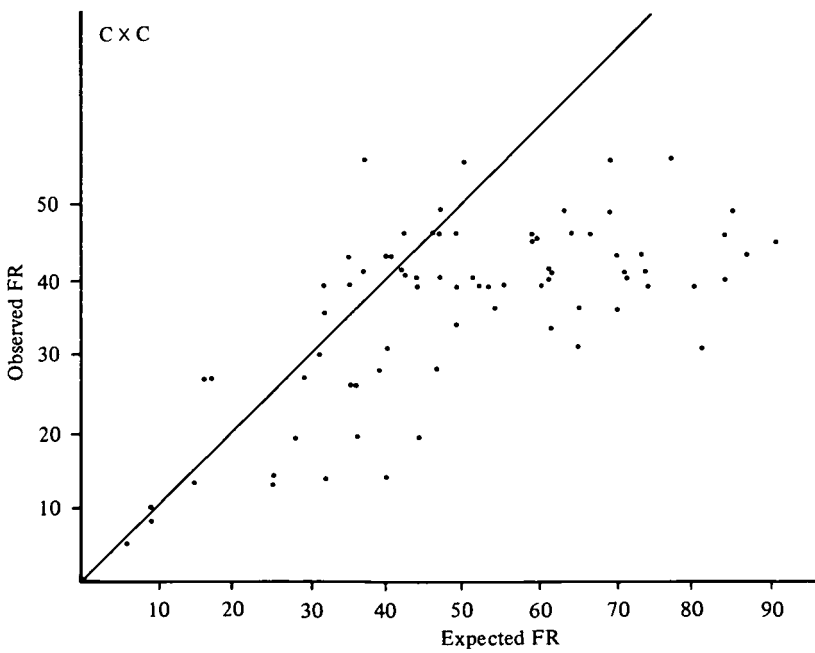

Fig. 5. Observed against expected FR values in C × C crosses (see Table 5).

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recombinant frequencies. The observed map agrees exactly with the results of the crosses involving the deletion mutants. Type A and C mutations show map contraction in large intervals and do not allow intragenic mapping.

The results show that conversion is a major source of wild-type recombinants in gene *b2*. Previous studies showed that type A, B and C mutations correspond to distinct mutational changes (Leblon, 1972*b*, 1979). Therefore, the differences

Table 6. *FR in crosses B × C*

	F1	8E	F2	17	X15	24	G1
F0	0·7*	0·4	1*	7	25	—	31
	8·6	*8·4*	*7·6*	*1·7*	*3·3*		*1·4*
B100	17	15	18	9	9	31	21
	3·8	*2·8*	*2·7*	*3·1*	*4·3*	*1·9*	*1·6*
B17	27	24	29	22	8	11	12
	1·2	*0·6*	*3·9*	*2·9*	*13·8*	*2·1*	*1·2*
E1	30	34	32	24	35	0	0·03
	2·5	*1·0*	*1·8*	*3·1*	*1·1*	*5·0*	*34·4*
	81	94	257	98	145	A4	26
B79	2	2	4	8	8	12	7
	5·0	*6·0*	*4·8*	*5·0*	*5·0*	*5·0*	*6·0*
B101	9	1	0·3	6	6	9	7
	2·4	*3·5*	*7·3*	*8·7*	*6·0*	*0·9*	*7·0*
B4	13	5	2	3	4	6	5
	14·2	*5·6*	*13·8*	*22·1*	*6·3*	*8·2*	*10·9*
A0	19	15	13	3	3	1*	1
	3·0	*3·7*	*5·6*	*5·0*	*5·9*	*2·0*	*5·0*

Upper part: crosses involving left hand mutations.
Lower part: crosses involving right hand mutations.
The total sample of asci observed ($\times 10^{-3}$) is given in italics below the FR.
* In crosses F0 × F1, F0 × F2 and A0 × A4, the double-mutant recombinants are screened together with the wild-type recombinants (Leblon & Paquette, 1978).

in FR can be related to differences in genetic alterations. An interpretation of the relationship between conversion pattern and mutational alteration has been suggested previously (Leblon, 1972*b*, Leblon & Rossignol, 1973, 1979). Hybrid DNA molecules are formed during recombination: when they span small addition/deletion mutations (corresponding to types A and B), a correction process is systematically triggered at the site of the mutation. The correction can extend in both directions by an excision-repair type process. Type C mutations frequently escape correction. This interpretation makes possible an estimation of the bearing of conversion on the FR obtained in 2-point crosses. The estimation takes into account the frequencies of hDNA molecules and their distribution in one (asymmetric) or two (symmetric) chromatids at each mutant site and the correction parameters of the markers used. It assumes that associated intragenic crossing-over has little effect upon the frequency of wild-type recombinants.

In the generation of wild-type recombinants by conversion, two situations must be considered:

First, *the hybrid molecules include only one out of the two sites* (Fig. 6). If hDNA forms on the chromatid mutant for this site, non-correction or correction to wild-type leads to wild-type recombinants. If hDNA forms on the wild-type chromatid, wild-type recombinants cannot be generated. Intragenic crossovers associated with hybrid DNA are not shown in Fig. 6 for the sake of simplicity. When these intragenic crossovers are associated with asymmetric hDNA, they can

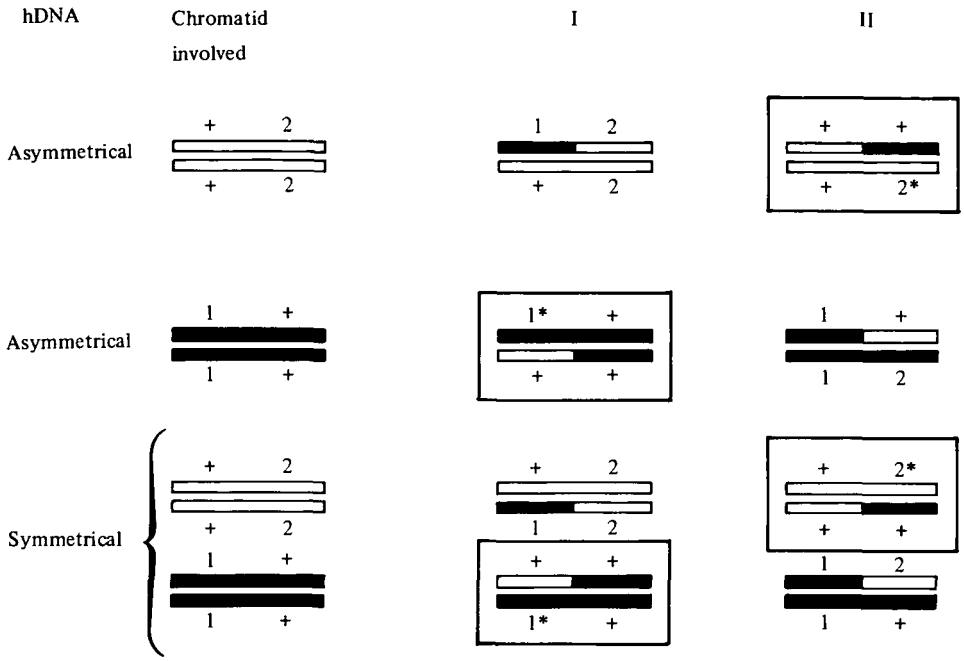

Fig. 6. Bearing of the various patterns of hDNA formation upon wild-type recombinants in a $1+\times+2$ cross. I, Site 1 alone is included in hDNA. II, Site 2 alone is included in hDNA. In a square: events that may lead to wild-type recombinants. Wild-type recombinants can be generated every time the hDNA forms at the mutant site on the chromatid involved.

* No correction gives one wild-type recombinant; correction to wild-type gives two wild-type and correction to mutant gives no wild-type recombinants.

lead only to additional wild-type recombinants. The size of this contribution depends on the frequency of this configuration. Intragenic crossing-over associated with pms was detected in *b2* (Rossignol & Haedens, 1980), but the frequency of association between intragenic crossing-over and asymmetrical hDNA is not known. Second, the *hybrid molecules include both sites* (Fig. 7): then, only corrections to wild-type triggered on at least one site without overlapping the other site will lead to wild-type recombinants. The existence of hDNA spanning several sites was shown in *b2* for tightly linked sites (Leblon & Rossingol, 1973, 1979; Rossignol & Haedens, 1978) and for distant sites (Rossignol & Haedens, 1980).

Mutations located in regions *F*, *E* and *A* were chosen to estimate the contribution of conversion to wild-type recombinants in two-point crosses. Most of the corrections triggered in each of these regions do not span the two others (Kalogeropoulos & Rossignol, 1980). Furthermore, the frequencies of symmetrical and asymmetrical hDNA are known in each of these regions (Table 1). It is possible to estimate the probability of hDNA distribution at both sites involved in the cross by assuming the three following postulates:

(1) Polarity in *b2* results from hDNA initiation at the left end of the gene (Paquette & Rossignol, 1978; Paquette, 1979; Hamza, Haedens, Mekki-Berrada,

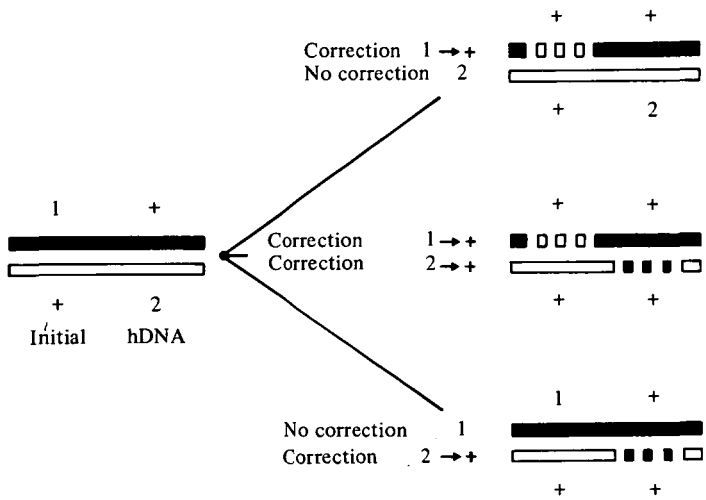

Fig. 7. Bearing of correction to wild-type upon wild-type recombinants when hDNA includes both sites in an $1+ \times +2$ cross.

Rossignol, unpublished data). We will assume that this initiation *always* starts at the left end of the gene; in other words, any hybrid DNA involving the right hand site necessarily involved the left hand one.

(2) Continuity was shown between symmetrical hDNA on the right of the gene and asymmetrical hDNA on the left (Rossignol & Haedens, 1980). We will assume that there is never symmetrical hDNA on the left associated with asymmetrical hDNA on the right.

(3) Ending of hDNA within gen *b2* was shown for both distributions: asymmetric and symmetric (Rossignol & Haedens, 1980). We will assume that, for the same interval, the probability of ending is the same for these two types of hDNA distribution.

The calculated frequencies per meiosis of the various types of hDNA distribution along *b2* are given in Table 7. From these frequencies, the probability per chromatid of *F*, *E*, and *A* region being included in hDNA was calculated (Table 8).

The FR in two-point crosses were calculated according to the above assumptions

and assuming that asymmetric hDNA equally involves the two chromatids. The mismatch correction parameters used are given for each mutation in Table 1. Calculation procedure is examplified in the legend of Table 9.

The FR expected in crosses involving mutations with the same pattern of aberrant segregations are given in Table 9. This table shows that wild-type recombinants resulting from hDNA overlapping both sites are a map contraction

Table 7. *Probability per meiosis of the various possible types of hybrid DNA distribution*

Region involved by hDNA		
Asymmetric	Symmetric	Probability per meiosis, $\times 10^3$
—	*F*	$(1-\alpha_F)(\gamma_F-\gamma_E) = 11{\cdot}9$
—	*F, E*	$(1-\alpha_F)(\gamma_E-\gamma_A) = 5{\cdot}2$
—	*F, E, A*	$(1-\alpha_F)\gamma_A = 12{\cdot}6$
F	—	$\alpha_F(\gamma_F-\gamma_E) = 145{\cdot}1$
F, E	—	$\alpha_E(\gamma_E-\gamma_A) = 45{\cdot}8$
F, E, A	—	$\alpha_A\gamma_A = 46{\cdot}1$
F, E	*A*	$(\alpha_E-\alpha_A)\gamma_A = 65{\cdot}6$
F	*E, A*	$(\alpha_F-\alpha_E)\gamma_A = 41{\cdot}7$
F	*E*	$(\alpha_F-\alpha_E)(\gamma_E-\gamma_A) = 17{\cdot}1$

factor; indeed, the frequency in the longest interval is lower than the sum of the frequencies in the corresponding subintervals. Wild-type recombinants resulting from non-overlapping hDNA are an additivity factor: the frequencies observed in the largest interval are usually close to the sum of the frequencies in the composing subintervals. The mappability of the mutations depends on the relative importance of these two factors: when the additivity factor predominates (3–4 times more than non-additive contribution), the mutations become mappable. The observed frequencies are close to the calculated ones. When they differ, they are slightly higher than expected. This could possibly be due to the contribution of intragenic crossovers that was neglected in the calculations.

The calculations concerning crosses between mutants with different types of conversion patterns and located in regions *A*, *E* and *F* were performed: again, the results are consistent with the observed values.

Table 8. *Probability per chromatid of region* F, E *and* A *being included in hDNA.*

Region	Probability per chromatid of region being included in hDNA, $\times 10^3$
F, E, A	$\gamma_A(2-\alpha_F)/4 = 44{\cdot}6$
F	$(\gamma_F-\gamma_E)(2-\alpha_F)/4 = 42{\cdot}2$
F, E	$(\gamma_E-\gamma_A)(2-\alpha_F)/4 = 18{\cdot}3$
E, A	$(\alpha_F-\alpha_E)\gamma_A/4 = 10{\cdot}4$
A	$(\alpha_E-\alpha_A)\gamma_A/4 = 16{\cdot}4$
E	$(\alpha_F-\alpha_E)(\gamma_E-\gamma_A)/4 = 4{\cdot}3$

Values of γ and α are given in Table 1.

In the situation studied, most correction lengths do not overlap more than one marker. In the *b2* locus there are two regions where correction triggered at one site can span the neighbouring sites: the *A* group of suppression (Leblon & Rossignol, 1973) and the *F* group (Paquette, 1979). Fincham & Holliday (1970) expect an additive phase when corrections always span the neighbouring sites, followed by an expansion phase when correction may or may not span the neighbouring sites. In group *F* of intragenic suppression, when type A mutations are considered, expansion is observed (Fig. 4). The appearance of expansion is correlated with the appearance of non-overlapping corrections (Paquette, 1979). Additivity is actually observed for all mutation types when very small intervals are considered (Fig. 3–5).

Table 9. *Expected and observed FR taking into account the parameters of gene conversion of the mutations used*

(Column *a*: expected contribution of hybrid DNA including both sites. Column *b*: expected contribution of hybrid DNA involving only one site.)

Crosses	*a*	*b*	Expected	Observed
$F_A \times E_A$	42	22	64	49–89
$E_A \times A_A$	36	16	52	54–71
$F_A \times A_A$	24	32	56	54–98
$F_B \times E_B$	3	9	12	13–24
$E_B \times A_B$	1	3	4	4–8
$F_B \times A_B$	3	14	17	22–32
$F_C \times E_C$	5	13	25	35–49
$E_C \times A_C$	5	9	14	15–21
$F_C \times A_C$	8	21	30	37–55

For $FA \times EA$ cross, taken as example, calculation is as follows:

$$a = [p_F v_F(1-p_E)/2 + (1-p_F)p_E v_E/2 + p_F v_F p_E v_E]\,[\gamma_A(2-\alpha_F)/4 + (\gamma_E-\gamma_A)\,(2-\alpha_F)/4]$$

with p_F, v_F, p_E, v_E, γ_A, α_F, γ_E respectively equal to 1, 0·74, 1, 0·9, 0·17, 0·92, 0·23 as given in Table 1.

$$b = [((1-p_F)/2 + p_F v_F)\,(\gamma_F-\gamma_E)\,(2-\alpha_F)/8] + [((1-p_E)/2 + p_E v_E)\,(\alpha_F-\alpha_E)\gamma_A/8 + (\alpha_F-\alpha_E)\,(\gamma_E-\gamma_A)/8]$$

with p_F, v_F, γ_F, γ_E, α_F, p_E, v_E, α_E, γ_A respectively equal to 1, 0·74, 0·39, 0·23, 0·92, 1, 0·90, 0·67, 0·17.

It is probable that in these cases we work within co-correction length, as suggested by Fincham and Holliday. This additive phase is extended beyond the co-correction length by using situations in which independent correction makes little contribution to wild-type recombinants, i.e. type B mutations where correction is predominantly to mutant: then, the greatest part of the recombinants arising from hDNA including the two sites are double mutants. Our data give good support for the belief that this theoretical basis is correct. Extensive use of type B mutations was also successfully performed for mapping several genes in the stock 50 of *Ascobolus immersus* (Decaris, 1981; Kouassi, 1981).

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REFERENCES

DECARIS, B. (1981). Les mutants instables d'*Ascobolus immersus*; le rôle dans l'instabilité de l'insertion et de l'excision d'éléments transposables. Thèse de Doctorat d'Etat, Université Paris-Sud, Orsay.

DICAPRIO, L. & HASTINGS, P. J. (1976). Gene conversion and intragenic recombination at the Sup6 locus and the surrounding region in *Saccharomyces cerevisiae*. *Genetics* **84**, 697–721.

FINCHAM, J. R. S. (1967). Recombination within the am gene of *Neurospora* crassa. *Genetical Research* **9**, 49–62.

FINCHAM, J. R. S. & HOLLIDAY, R. (1970). An explanation of fine structure map expansion in terms of excision repair. *Molecular and General Genetics* **109**, 309–322.

GIRARD, J. & ROSSIGNOL, J.-L. (1974). The suppression of gene conversion and intragenic crossing-over in *Ascobolus immersus*: evidence for modifiers acting in the heterozygous state. *Genetics* **76**, 221–243.

ISHIKAWA, T. (1962). Genetic studies of ad8 mutants in *Neurospora crassa*. I. Genetic fine structure of the ad8 locus. *Genetics* **47**, 1147–1161.

KALOGEROPOULOS, A. & ROSSIGNOL, J.-L. (1980). Evidence for independent mismatch corrections along the same hybrid DNA tract during meiotic recombination in *Ascobolus*. *Heredity* **45**, 263–270.

KOUASSI, A. (1981). Contribution à l'étude de la structure du gène *b1* et analyse des phénomènes d'instabilité au niveau de ce gène chez *Ascobolus immersus*. Thèse de 3ème cycle, Université Paris-Sud, Orsay.

KRUSZEWSKA, A. & GAJEWSKI, W. (1967). Recombination within the Y locus in *Ascobolus immersus*. *Genetical Research* **9**, 159–177.

LEBLON, G. (1972*a*). Mechanism of gene conversion in *Ascobolus immersus*. I. Existence of a correlation between the origin of mutants induced by different mutagens and their conversion spectrum. *Molecular and General Genetics* **115**, 36–48.

LEBLON, G. (1972*b*). Mechanism of gene conversion in *Ascobolus immersus*. II. The relationships between the genetic alterations in *b1* or *b2* mutants and their conversion spectrum. *Molecular and General Genetics* **116**, 322–335.

LEBLON, G. (1979). Intragenic suppression at the *b2* locus in *Ascobolus immersus*. II. Characteristics of the mutations in groups A and E. *Genetics* **92**, 1093–1106.

LEBLON, G. & PAQUETTE, N. (1978). Intragenic suppression at the *b2* locus in *Ascobolus immersus*. I. Identification of three distinct groups of suppression. *Genetics* **90**, 475–488.

LEBLON, G. & ROSSIGNOL, J.-L. (1973). Mechanism of gene conversion in *Ascobolus immersus*. III. The interaction of heteroalleles in the conversion process. *Molecular and General Genetics* **122**, 165–182.

LEBLON, G. & ROSSIGNOL, J.-L. (1979). The interaction during recombination between closely linked allelic frameshift mutant sites in *Ascobolus immersus*. II. A and B type mutant sites. *Heredity* **42**, 337–352.

LISSOUBA, P., MOUSSEAU, J., RIZET, G. & ROSSIGNOL, J.-L. (1962). Fine structure of genes in *Ascobolus immersus*. *Advances in Genetics* **11**, 343–380.

MOORE, C. N. & SHERMAN, F. (1975). Role of DNA sequences in genetic recombination in the iso-1-cytochrome c gene of yeast. I. Discrepancies between physical distances and genetic distances determined by five mapping procedures. *Genetics* **79**, 397–418.

PAQUETTE, N. (1979). Polarité multiple de la recombinaison génétique dans le locus *b2* d'*Ascobolus immersus*. Thèse Doctorat d'Etat, Université Paris-Sud, Centre d'Orsay, 321 pp.

PAQUETTE, N. & ROSSIGNOL, J.-L. (1978). Gene conversion spectrum of 15 mutants giving PMS in the *b2* locus of *Ascobolus immersus*. *Molecular and General Genetics* **163**, 313–326.

PUKKILA, P. J. (1977). Biochemical analysis of genetic recombination in eukaryotes. *Heredity* **39**, 193–217.

RIZET, G., ENGELMAN, N., LEFORT, C., LISSOUBA, P. & MOUSSEAU, J. (1960), Sur un ascomycète intéressant pour l'étude de certains aspects du problème de la structure du gène. *Comptes rendus de l'Académie des Sciences (Paris)* **270**, 2050–2052.

RIZET, G., ROSSIGNOL, J.-L. & LEFORT, C. (1969). Sur la variété et la spécificité des spectres

d'anomalies de ségrégations chez *Ascobolus immersus*. *Comptes rendus de l'Académie des Sciences* (*Paris*) **269**, 1427–1430.

ROSSIGNOL, J.-L. & HAEDENS, V. (1978). The interaction during recombination between closely linked allelic frameshift mutant sites in *Ascobolus immersus*. I. A (or B) and C type mutant sites. *Heredity* **40**, 405–425.

ROSSIGNOL, J.-L. & HAEDENS, V. (1980). Relationship between asymmetrical and symmetrical hybrid DNA formation during meiotic recombination. *Current Genetics* **1**, 185–191.

ROSSIGNOL, J.-L. & PAQUETTE, N. (1979). Disparity of gene conversion in frameshift mutants located in locus *b2* of *Ascobolus immersus*. *Proceedings of the National Academy of Sciences* **76**, 2871–2875.

STADLER, D. R. & KARIYA, B. (1969). Intragenic recombination at the *mtr* locus of Neurospora with segregation at an unselected site. *Genetics* **63**, 291–316.

YU-SUN, C. C. (1964). Biochemical and morphological mutants of *Ascobolus immersus*. *Genetics* **50**, 987–998.

YU-SUN, C. C., WICKRAMARATNE, M. R. T. & WHITEHOUSE, H. L. K. (1977). Mutagen specificity in conversion pattern in *Sordaria brevicollis*. *Genetical Research* **29**, 65–81.