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Mutagen specificity in conversion pattern in *Sordaria brevicollis*

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SUMMARY

A total of 39 mutants at the *grey-3*, *grey-4* and *grey-5* spore colour loci in *Sordaria brevicollis* have been investigated for conversion pattern by crossing them with wild type and counting aberrant asci. Twenty-one of the mutants were obtained with ICR170 and all showed postmeiotic segregation only rarely (0–8 % of the aberrant asci); two showed conversion predominantly to wild type (class A) and the other 19 predominantly to mutant (class B). Six mutants were obtained with ethylmethane sulphonate and one with nitrosoguanidine, and they all showed postmeiotic segregation frequently (14–54 % of the aberrant asci) and conversion usually about equally frequently in each direction, though with considerable diversity between mutants (class C). Eleven UV-induced mutants comprised one of class B and ten of class C. There was considerable variation in aberrant ascus frequency between alleles, but conversion pattern seemed to be independent of this frequency.

1. INTRODUCTION

Leblon (1972*a*) discovered that in the fungus *Ascobolus immersus* the conversion behaviour of a mutant was dependent on its molecular nature. This was revealed by obtaining spore colour mutants with various mutagens. Leblon found that frameshift mutants showed conversion almost entirely to wild type (class A) or to mutant (class B) and with little postmeiotic segregation, while substitution mutants showed a much higher proportion of postmeiotic segregation and without such a strong bias in the direction in which conversion occurred (class C). Using particular mutagens, several intragenic revertants of the frameshift mutants were obtained as a result of mutation at a second site (Leblon, 1972*b*), and from study of these Leblon concluded that the A mutants were deletion frameshifts and the B addition frameshifts.

By contrast with these results, Fogel, Hurst & Mortimer (1971) and Lawrence Sherman, Jackson & Gilmore (1975) reported that in the yeast *Saccharomyces*

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cerevisiae conversion to wild type and to mutant were approximately equally frequent for all the mutants that they had studied. Moreover, Fink & Styles (1974) and Fink (1974) have shown that deletions in the *histidine-4* gene of *S. cerevisiae* are similar in conversion behaviour to point mutations, with conversion to wild type and to mutant equally frequent. Lawrence *et al.* (1975) also found that a deletion (*cyc1-1*) showed conversion equally often in each direction. This deletion was extensive, covering the entire *cyc*1 gene (the structural gene for iso-1-cytochrome *c*) and extending into a neighbouring gene. The conversion frequency of the deletion was fivefold lower than that of allelic point mutants.

In view of this difference of behaviour between yeast (a Hemiascomycete) and *Ascobolus* (a Discomycete), it was of interest to examine the conversion behaviour of *Sordaria brevicollis* (a Pyrenomycete). An abstract of the results has been published (Yu-Sun, Wickramaratne & Whitehouse, 1974).

2. MATERIAL AND METHODS

Wild-type *Sordaria brevicollis* was provided by Professor L. S. Olive. For the production of microconidia and for making crosses the crossing medium recommended by Fields & Olive (1967) was used. This contains the following, made up to 1 l with distilled water: 2 g D-glucose, 5 g sucrose, 1 g yeast extract, 0·5 g potassium dihydrogen orthophosphate (KH_2PO_4) and 17 g Difco cornmeal agar. For germination of ascospores the germination medium that they recommended was used. It has the following composition: 20 g D-glucose, 30 g sucrose, 1 g yeast extract, 7 g sodium acetate, 10 g Difco cornmeal agar and 26 g Difco plain agar, made up to 1 l with distilled water.

Spore colour mutants were obtained using ultraviolet light (UV) or one of three chemicals: the acridine mustard ICR170, the nitroso compound *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG), and the alkylating agent ethylmethane sulphonate (EMS). The ICR170 was provided by Dr H. J. Creech, and the EMS and NG were obtained from Koch-Light Laboratories Ltd.

Microconidia were used for mutagen treatment. They were obtained by adding 3–4 ml of sterile distilled water (or pH 8·0 buffer) to a 5-day-old plate culture of one mating type, and detaching the microconidia with a bent glass rod. The microconidial suspension was then filtered through two layers of muslin to remove mycelial fragments and exposed to the mutagen.

For the UV treatment, the suspension was exposed for 1 min at a distance of 18 cm from a low-pressure mercury vapour discharge lamp which, according to the maker's specification, supplies 87 % of its energy between 260 and 270 nm. The dose-rate was 10000–12000 ergs/cm²/sec.

For the chemical treatments, which were carried out in the dark in a water-bath at 37°, the concentrations and times were as follows: EMS, 0·05 M in phosphate buffer (pH 7·0) for 25 min; ICR170, 10–40 μg/ml phosphate buffer (pH 7·0) for 2 h; NG, 1000 μg/ml phosphate buffer (pH 6·0) for 15 min. The NG treatment was based on the work of Bond (1969), who used it to obtain auxotrophs. The ICR170

treatment was first applied by Dr Lucy King. After the chemical treatments the microconidial suspensions were centrifuged for 5 min at 3500 rpm and the mutagen solution drained off. The microconidia were then washed in two changes of phosphate buffer at pH 8·0 and resuspended in sterile distilled water.

Following the UV or chemical treatment the suspension of microconidia was transferred by pipette to a 5-day-old culture of the other mating type grown on crossing medium. Ascospores begin to be discharged from the perithecia *c.* 5 days later in cultures kept at 25°, and were collected on plain agar in the lids of the Petri dishes. If the mutagen treatment had given rise to an ascospore colour mutant, this would be revealed as a spore cluster containing many pale ones, since protoperithecia are normally fertilized by only one microconidium. All the asci in the resulting perithecium will then be expected to segregate for dark (wild-type) and pale (mutant) spores.

Ascospores from such clusters were transferred to germination medium. Many mutants were inviable and failed to germinate. For example, in one experiment, out of 203 UV-induced clusters of pale spores, 64 (32 %) had spores that had not developed normally and were clearly inviable. A further 108 (53 %) had spores of normal size but vacuolated, and only one out of 17 tested showed germination: it proved to be a slow-growth mutant with spores of normal colour. Spores from 14 of the remaining 31 clusters were tested for germination. One showed no germination and 2 had inviable sporelings which died soon after germination. Pale spores that germinated successfully were crossed with wild type in order to establish whether or not the pale colour arose from mutation. Of the 11 tested in the experiment just described, 9 proved to be mutants and 2 were evidently developmental abnormalities that were not inherited.

The conversion pattern of a mutant was obtained by crossing it with wild type and counting the eight-spored asci with different ratios of wild-type to mutant spores. The crosses were made by inoculating opposite sides of a Petri dish with the two strains. Perithecia mature about 10 days later at 25°. They were crushed in an 8 % glucose solution on a microscope slide and the perithecial fragments removed. Before adding the cover-slip, the cluster of asci from each perithecium was divided with fine tungsten needles so that not more than *c.* 40 asci remained together. When the glucose mounts dry up bubbles appear within the spores and it becomes difficult to distinguish mutant from wild type. The bubbles disappear, however, when the mount is irrigated. Only intact eight-spored asci were counted, any that were broken and had lost one or more spores being ignored. Likewise, asci with one or more aborted spores were not counted. Intact asci with abnormal numbers of spores, e.g. 4 instead of 8, are occasionally found and these also were ignored.

3. RESULTS

A total of 205 spore colour mutants were obtained with the four mutagens, distributed as shown in Table 1. The 85 mutants induced with ICR170 include two at the *yellow-9* locus in linkage group II obtained by Dr Lucy King, and the 31

mutants induced with NG include one at the *yellow-4* locus in linkage group V obtained by Mu'Azu (1973).

A majority of the 205 mutants have been assigned to one or other of 12 loci. Those showing high conversion frequency have been found (with one exception) to belong to three loci: *grey-3* (*g-3*), *grey-4* (*g-4*), and *grey-5* (*g-5*). The *g-4* and *g-5* loci were recognized by Chen (1965), who found they were in linkage groups II and IV respectively. The *g-3* locus has not been described before. We previously called it 'a granular grey gene' (Yu-Sun *et al.* 1974). Its linkage group has not yet been established with confidence, as mutants show *c.* 74% second division segregation, with no linkage to the markers available in any of the seven linkage groups. This lack of linkage is not surprising since most of the available markers are closely linked to their centromeres. Chen (1965) used the symbol *g-3* for one of the mutants he had obtained but, as Bond (1973) has pointed out, it is an allele of the *buff* locus, so *g-3* is available as a locus symbol.

Table 1. *Numbers of spore colour mutants obtained with various mutagens*

	Gene			
Mutagen	All	*Grey-3*	*Grey-4*	*Grey-5*
EMS	53	3	1	2
ICR170	85	6	4	11
NG	31	0	1	0
UV	36	2	3*	4*
Total	205	11	9	17

* Mutants C14 and C18, at the *g-4* and *g-5* loci, respectively, are not included in the Table, although used in the work described. They were induced by UV at Columbia University and provided by Professor L. S. Olive.

The numbers of *g-3*, *g-4* and *g-5* mutants obtained with the various mutagens are given in Table 1. In addition two mutants (C14 and C18) in the *g-4* and *g-5* loci respectively were provided by Professor L. S. Olive. These mutants were induced at Columbia University by irradiation with ultraviolet light.

Mutants at the three loci studied differ in colour, as indicated in Table 2. As wild-type spores mature their colour changes through a sequence from colourless to brownish black. Chen (1965) and Hackett & Chen (1976) described this sequence as: hyaline, yellow, beige, grey or grey-brown, and dark brown or brown-black. *Grey-3* mutants are indistinguishable from wild type at early stages of development but subsequently the phase when the spores have a granular appearance is abnormally prolonged and the spores remain yellower than wild type during most of the later stages of development. By contrast, *grey-4* mutants lack the yellow pigment and can be distinguished from wild type at an early stage. *Grey-5* mutants resemble wild type in early stages of development but remain pale when wild-type spores darken. There was some variation between alleles in spore colour. Also, mutant spores adjacent to a wild-type spore in the ascus are often darker in

colour than those further away. This darkening is presumably a consequence of pigment precursors diffusing from the wild-type to the mutant spore.

All the *g-3*, *g-4* and *g-5* mutants in which the conversion pattern has been investigated have been found to be stable. Isolates of each mutant of opposite mating type have been crossed with one another and the progeny ascospores allowed to be discharged on to plain agar in the lid of the Petri dish. In this way many thousands of spores can be quickly scanned, but no revertants were seen with any of the mutants.

Table 2. *Spore colour at successive stages of ascus development*

Wild type	*Grey-3*	*Grey-4*	*Grey-5*
Colourless	Colourless	Colourless	Colourless
Yellow	Yellow	Colourless	Yellow
Yellow and granular	Yellow and granular	Colourless and granular	Yellow and granular
Grey	Yellowish grey and granular	Light grey	Yellowish grey
Brown	Grey and granular	Light grey	Yellowish grey
Brownish black	Brownish grey	Grey	Yellowish grey

Owing to third-division spindle overlap non-sister and sister spores often alternate in the ascus, and preclude direct observation of aberrant 4:4 asci. Counts of aberrant asci have therefore been restricted to those with other than four wild-type spores. In addition to the four primary classes of aberrant asci, that is, with the narrower ratios 6:2, 2:6, 5:3 or 3:5 wild-type:mutant spores, asci have occasionally been observed with the wider ratios 7:1, 1:7. 8:0 or 0:8. The 0:8 category, however, often cannot be recognized with confidence because an ascus with eight mutant spores is similar to an immature ascus with normal 4:4 segregation.

Confirmation that the spore colour is an accurate guide to genotype has been obtained by dissecting asci, isolating and germinating the spores, and testing their genotype by crossing with wild type. A total of 120 wild-type and 26 mutant spores from *g-5* crosses showed no exceptions to the genotype deduced from spore colour. Small numbers of progeny from *g-3* and *g-4* crosses also gave no exceptions.

The numbers of asci observed with various ratios of wild-type:mutant spores for the *g-3*, *g-4* and *g-5* mutants are shown in Tables 3–5 respectively. The total frequencies of aberrant asci are also given. There was considerable variation between alleles in these frequencies, which ranged from 3·9% to 10·3% for *g-3*, 1·3% to 4·0% for *g-4*, and 1·4% to 6·0% for *g-5*.

With *g-5*, the UV-induced mutants gave higher frequencies of aberrant asci (4·1 to 6·0%) than those induced with other mutagens (ICR170 or EMS, 1·4 to 2·7%). There was no comparable effect, however, in *g-3* or *g-4*, where a relationship between mutagen and total frequency of aberrant asci was not evident. The UV-induced *g-5* mutant that gave the highest frequency of aberrant asci (mutant B7, frequency 6·0%) was found to give lower values, comparable to those of the

Table 3. *Numbers of aberrant asci from crosses between* grey-3 *mutants and wild type*

Class	Mutagen	Mutant	Asci (wild-type:mutant spores) 4:4	6:2	2:6	5:3	3:5	7:1	1:7	8:0	0:8	Total	Aberrant asci Total	% ± S.E.
B	ICR170	YS50	2630	2	274	0	12	0	3	0	11	2932	302	10·3 ± 0·6
		YS46	2988	2	235	11	9	0	1	0	4	3250	262	8·1 ± 0·5
		YS48	3333	0	272	5	7	0	1	0	4	3622	289	8·0 ± 0·5
		YS47	3092	11	222	0	3	0	0	0	9	3337	245	7·3 ± 0·5
		YS45	2521	1	181	0	10	0	1	0	0	2714	193	7·1 ± 0·5
		YS49	2821	11	137	1	7	0	0	0	0	2977	156	5·2 ± 0·4
	UV	YS94	2919	3	178	0	13	0	1	0	2	3116	197	6·3 ± 0·4
C		RW9	2608	9	63	18	17	0	0	0	0	2715	107	3·9 ± 0·4
	EMS	YS51	2706	91	31	54	36	2	3	1	0	2924	218	7·5 ± 0·5
		YS52	2868	80	23	45	46	0	2	0	0	3064	196	6·4 ± 0·4
		YS53	2928	79	20	65	23	0	1	1	0	3117	189	6·1 ± 0·4

Table 4. *Numbers of aberrant asci from crosses between* grey-4 *mutants and wild type*

Class	Mutagen	Mutant	Asci (wild-type:mutant spores) 4:4	6:2	2:6	5:3	3:5	7:1	1:7	8:0	0:8	Total	Aberrant asci Total	% ± S.E.
B	ICR170	YS67	3574	10	131	5	2	0	0	0	0	3722	148	4·0 ± 0·3
		YS22	3333	12	115	5	2	0	0	0	1	3468	135	3·9 ± 0·3
		YS23	3486	19	101	4	2	0	0	0	1	3613	127	3·5 ± 0·3
		YS32	3259	17	87	4	2	0	0	0	2	3371	112	3·3 ± 0·3
C	UV	B8	2721	28	51	20	11	0	0	1	0	2832	111	3·9 ± 0·4
		YS10	3158	84	12	24	1	0	0	1	0	3280	122	3·7 ± 0·3
		B17	3102	13	14	19	13	0	0	0	0	3161	59	1·9 ± 0·2
		C14	3187	16	9	15	2	0	0	0	0	3229	42	1·3 ± 0·2
	EMS	YS112	3062	25	31	22	15	1	0	0	0	3156	94	3·0 ± 0·3
	NG	YS7	3256	22	14	34	3	1	0	1	0	3331	75	2·3 ± 0·3

Table 5. *Numbers of aberrant asci from crosses between* grey-5 *mutants and wild type*

Class	Mutagen	Mutant	Asci (wild-type:mutant spores) 4:4	6:2	2:6	5:3	3:5	7:1	1:7	8:0	0:8	Total	Aberrant asci Total	% ± S.E.
A		YS25	2102	47	3	0	0	0	0	2	0	2154	52	2·4 ± 0·3
		YS33	2667	36	2	0	3	0	0	5	0	2713	46	1·7 ± 0·2
B	ICR170	YS15	3656	9	85	0	8	0	0	0	1	3759	103	2·7 ± 0·3
		YS93	2196	0	59	0	0	0	0	0	1	2256	60	2·7 ± 0·3
		YS89	2313	5	54	0	0	0	0	0	0	2372	59	2·5 ± 0·3
		YS16	1976	0	48	0	0	0	0	0	0	2024	48	2·4 ± 0·3
		YS29	2192	3	45	0	1	0	0	0	0	2241	49	2·2 ± 0·3
		YS13	1245	4	18	1	0	0	0	0	0	1268	23	1·8 ± 0·4
		YS162	2013	0	34	0	1	0	0	0	0	2048	35	1·7 ± 0·3
		YS168	1127	0	18	0	0	0	0	0	0	1145	18	1·6 ± 0·4
		YS11	1315	0	18	0	0	0	0	0	1	1335	19	1·5 ± 0·3
C	UV	B7	3536	98	52	7	51	0	15	3	0	3762	226	6·0 ± 0·4
		YS4	2038	44	31	1	34	0	8	2	0	2158	120	5·6 ± 0·5
		B4	2234	49	24	3	32	0	5	2	0	2349	115	4·9 ± 0·4
		YS3	5469	80	75	4	103	0	16	0	0	5747	278	4·8 ± 0·3
		C18	1619	28	17	2	20	0	2	0	0	1688	69	4·1 ± 0·5
	EMS	YS35	5539	74	35	13	29	1	0	0	0	5691	152	2·7 ± 0·2
		YS133	4078	20	30	5	3	0	0	0	0	4136	58	1·4 ± 0·2

alleles induced with the other mutagens, when it was crossed with wild-type reisolates obtained by crossing the original wild-type strains of opposite mating type with one another. More limited data were obtained for two other UV-induced *g-5* mutants, YS3 and YS4, and they also gave significantly lower aberrant ascus frequencies when crossed with reisolated wild types. This effect of the wild-type parent requires further study but it seems likely that there is segregation for genes affecting the frequency of recombination within the *g-5* locus. Mu'Azu (1973) found there was genetic variation by a factor of two in the frequency of recombination in the interval between the *buff* and *yellow-9* spore colour genes in linkage group II. From his data he inferred that the heterogeneity was caused by at least three heritable factors. It seems possible that a similar situation exists within *g-5*. The high frequency of aberrant asci shown by the UV-induced mutants, when compared with the alleles induced with other mutagens, might then be fortuitous and have a cause external to the mutants themselves.

Taking the three genes (*g-3*, *g-4* and *g-5*) together, the 39 mutants were found to exhibit three different conversion patterns corresponding to the A, B and C classes recognized by Leblon (1972*a*) with *Ascobolus immersus*. There are two class A mutants (postmeiotic segregation rare; conversion predominantly to wild type), both of which are *g-5* mutants induced with ICR170. There are 20 class B mutants (postmeiotic segregation rare; conversion predominantly to mutant) of which one was induced with UV and the remainder with ICR170. The other 17 mutants belong to class C (postmeiotic segregation frequent) and comprise 10 induced with UV, 6 with EMS and one with NG. In Tables 3–5 the mutants of each gene are grouped according to the conversion pattern and the mutagen. Within each group the mutants are listed in order of decreasing frequency of aberrant asci, but the conversion pattern seems to be independent of this frequency.

4. DISCUSSION

(*a*) *Class A and class B mutants*

Leblon (1972*b*) concluded that the class A and class B mutants in *Ascobolus immersus* were frameshifts because he found that they could revert by intragenic suppression, with the suppressors located close to the suppressed mutant site and giving a mutant spore colour when separated from the mutant they suppressed.

He inferred that the A mutants were deletions of one or two nucleotides, and the B additions of one or two nucleotides. This conclusion was based on three observations:

(1) ICR170 produced B mutants only and caused intragenic reversion (either by back-mutation or suppression) of an A mutant readily but of a B mutant only rarely.

(2) EMS produced A and B mutants (and also C) and caused intragenic reversion of a B mutant with high frequency.

(3) An intragenic suppressor of an A mutant, when separated from the mutant, behaved as a B spore colour mutant; and three intragenic suppressors of a B

mutant, when separated from it, were found to be A mutants (a fourth was of class C).

Clearly the A and B mutants are of complementary molecular structure, with ICR170 favouring the induction of B and the reversion of A, and EMS often inducing A and reverting B. Imada *et al.* (1970) showed that the acridine proflavin preferentially induces addition frameshifts in phage T4 of *Escherichia coli*. Leblon concluded that the acridine mustard ICR170 likewise induces chiefly additions. EMS is known to produce base substitutions and also frameshifts, and the latter are believed often to be deletions since EMS reverts T4 mutants that have been induced with proflavin (see review by Drake, 1970). Leblon (1972*b*) concluded that the A mutants were deletion frameshifts and the B additions.

We found with *Sordaria brevicollis* that ICR170 induced 9 % of class A mutants, the remainder being of class B. This difference from the results obtained by Leblon (1972*a*) with *Ascobolus* is not unexpected since, as already mentioned, he found that ICR170 would induce intragenic reversion of a B mutant, though not efficiently.

Out of a total of 25 *Ascobolus* spore colour mutants induced with ICR170, Leblon (1972*a*) found that 3 were intermediate between A and B, with conversion to wild type and to mutant about equally frequent (and postmeiotic segregation rare). One out of a total of 6 spontaneous frameshifts was also an AB intermediate. The molecular nature of these mutants, which might be called class AB, is not clear. No such mutants were found among the 22 presumed frameshift mutants of *S. brevicollis* that we have investigated. This can be seen from Figs. 1–3, where the frequencies of 6:2 and 2:6 asci are plotted for each mutant as a percentage of the total number of aberrant asci.

(*b*) *Class C mutants*

Leblon (1972*b*) inferred that the class C mutants which he obtained in *Ascobolus immersus* originated from base-pair substitution. This conclusion was based on the following observations.

(1) A class C mutant induced with NG was found to revert mainly by external suppression. Two of the external suppressors were tested for suppression of other spore colour mutants and found to show allele specificity but not gene specificity, with certain class C mutants suppressed but none of class B. These external suppressors thus behaved like nonsense suppressors resulting from a mutant transfer RNA.

(2) The class C mutants were induced either by NG (all the NG mutants), EMS (68 % of the EMS mutants) or spontaneously (40 % of the spontaneous mutants), and none were obtained with ICR170 as mutagen. Leblon (1972*b*) pointed out that the identification of the genetic alterations induced by NG or EMS in other organisms has led to the conclusion that both preferentially induce base-pair substitution (for reviews, see Drake (1970), Fincham & Day (1971) and Auerbach (1976)).

The 17 class C mutants which we investigated at the *g-3*, *g-4* and *g-5* loci of *Sordaria brevicollis* included all the mutants of these genes induced with NG and

EMS (although only a single NG mutant was available). Thus, as far as our data extend, they are comparable to those of Leblon for *Ascobolus*.

Leblon (1972*a*) recognized a fourth class of *Ascobolus* mutants which he called D type. His C and D type mutants differed in showing conversion predominantly

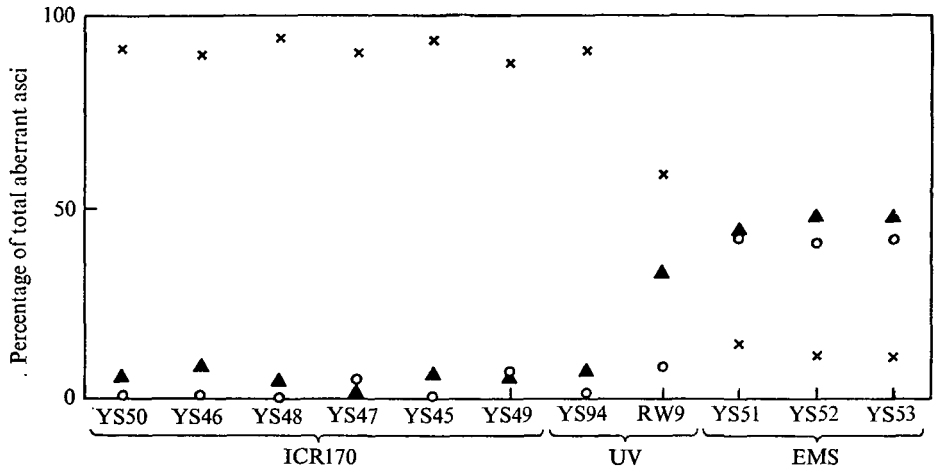

Fig. 1. The percentage frequency among the *grey-3* aberrant asci of those with 6:2 ratios of wild-type:mutant spores (○), with 2:6 ratios (×), and with postmeiotic segregation, that is, 5:3, 3:5, 7:1 and 1:7 ratios (▲).

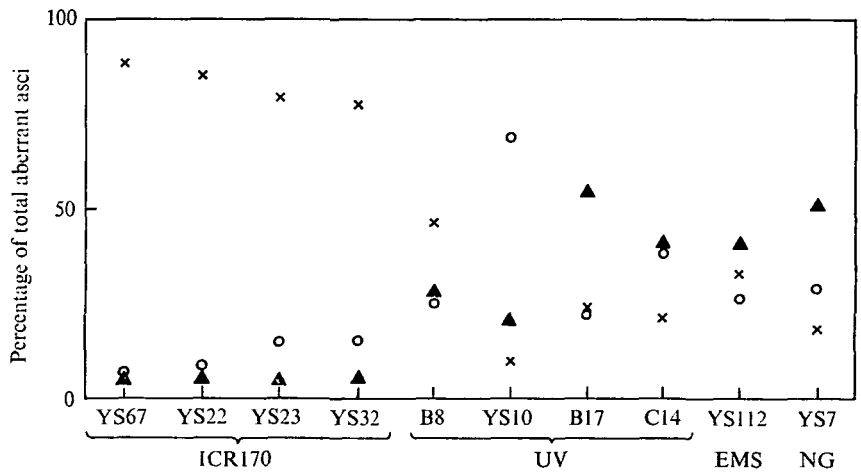

Fig. 2. The percentage frequency among the *grey-4* aberrant asci of those with 6:2 ratios of wild-type:mutant spores (○), with 2:6 ratios (×), and with postmeiotic segregation, that is, 5:3, 3:5, 7:1 and 1:7 ratios (▲).

to wild type and to mutant respectively, that is, 6:2 plus 5:3 significantly greater or significantly fewer than 2:6 plus 3:5. But he also found many mutants that were intermediate in behaviour: out of a total of 35 there were 17 of C type, 2 of D type and 16 intermediate, that is, with no significant difference in numbers of asci between 6:2 plus 5:3 and 2:6 plus 3:5. His data show no evidence for a

bimodal distribution: the C and D types are merely the extremes of a continuous range of variation. The situation is unlike that of the class A and class B mutants, the majority of which are sharply differentiated from one another. In view of the lack of a clear distinction between C type and D type, they have been treated here as a single category and called class C.

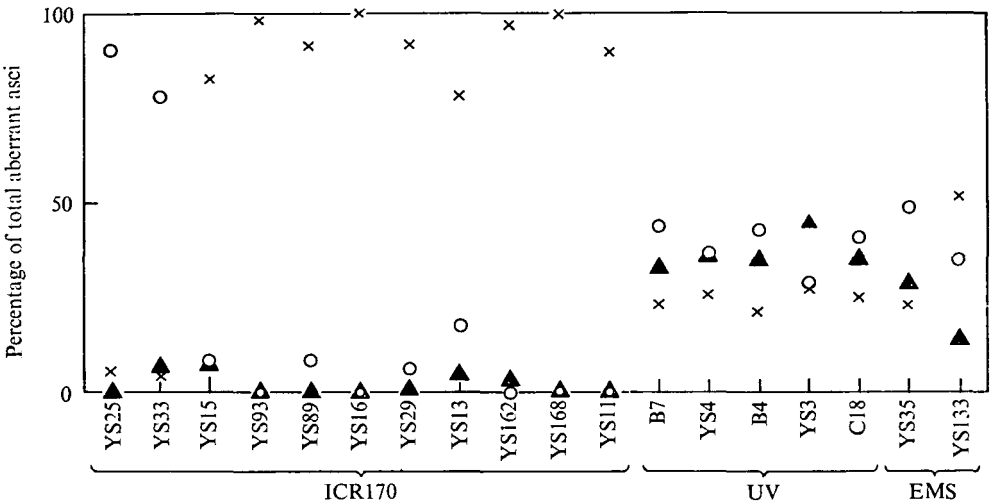

Fig. 3. The percentage frequency among the *grey-5* aberrant asci of those with 6:2 ratios of wild-type:mutant spores (○), with 2:6 ratios (×), and with postmeiotic segregation, that is, 5:3, 3:5, 7:1 and 1:7 ratios (▲).

Further evidence that C type and D type intergrade is revealed by the work of Lamb & Wickramaratne (1975) on the effect of temperature. Four *Ascobolus* genotypes carrying spore colour mutants presumed to have arisen by base-pair substitution were studied in crosses to wild type. The frequency of asci with 6 wild-type: 2 mutant spores increased with rise of temperature from 10° to 17½° to 22½°, but 2:6 asci were of maximum frequency at the intermediate temperature (17½°). As a consequence, all the genotypes were of C type at 22½° but some were of D type at the lower temperatures.

The diversity of behaviour of the class C mutants in *S. brevicollis* is shown in Figs. 1–3 where the frequencies of 6:2 and 2:6 asci are plotted for each mutant as percentages of the numbers of aberrant asci. The extremes are shown by YS10 (*g-4*, Fig. 2, with 69% of the aberrants having a 6:2 ratio and 10% 2:6) and RW9 (*g-3*, Fig. 1, with 8% 6:2 and 59% 2:6). The cause of this diversity of behaviour, which was also found in *Ascobolus* by Leblon, is not known.

The recognition of class C mutants seems nevertheless to be unambiguous on the basis of the frequency of postmeiotic segregation. In Figs. 1–3 the total frequency of asci with 5:3, 3:5, 7:1 and 1:7 ratios is plotted for each mutant as a percentage of the total number of aberrant asci. For the A and B mutants this frequency ranges from 0% to 8% and for the C mutants from 14% to 54%. Leblon (1972*a*) found that 0–5% of the aberrant asci showed postmeiotic segregation in A, AB or B

mutants of *Ascobolus*, and a much higher proportion for C mutants (45–88 % in gene *b1*, 27–64 % in gene *b2*). Thus, our results are similar, though not with such a wide separation of the C mutants from the others.

(*c*) *Mutants induced with ultraviolet light*

Leblon (1972*a*) did not investigate the conversion pattern of UV-induced mutants. Our results for UV-induced mutants of *Sordaria brevicollis* indicate that one is of class B (YS94, *g-3*, Table 3 and Fig. 1) and 10 of class C. The diversity of molecular structure implied by these results is expected with UV, which is believed to derive its mutagenicity from error-prone repair of damage to the DNA. Results with phase T4 of *Escherichia coli* and with *Neurospora crassa* have indicated that both base substitutions and frameshifts occur – see review by Auerbach (1976).

Kitani & Whitehouse (1974) reported aberrant ascus data for two UV-induced mutants, g1 and h5, at the *grey* (*g*) spore colour locus in *S. fimicola*. The total frequency of asci with 5:3 and 3:5 ratios, out of the total number of 5:3, 3:5, 6:2 and 2:6 asci observed in crosses with wild type, was 31–63 % for g1 and 73 % for h5. Thus, both mutants belong to class C. The corresponding frequencies for five X-ray induced mutants at this locus were h2, 85 %; h3, 87 %; h4, 85 %; g6, 65 %; g7, 47–74 %, so all belong to class C. It is provisionally concluded that all the *g* locus mutants that have been investigated in *S. fimicola* originated by base-pair substitution.

(*d*) *Frequency of aberrant asci*

The data for *Sordaria brevicollis* reveal four features of particular interest concerning aberrant ascus frequency:

(1) The frequency is to some degree gene specific, with *g-3* mutants (Table 3) giving higher values, in general, than *g-4* or *g-5* (Tables 4, 5), and the other 9 (or more) spore colour loci lower values. Comparable findings were made by Fogel, Hurst & Mortimer (1971) and Lawrence *et al.* (1975) with *Saccharomyces cerevisiae*, and by Leblon (1972*a*) with *Ascobolus immersus*, where mutants of gene *b2* usually showed aberrant ascus frequencies four to five times larger than mutants of gene *b1*.

(2) There was considerable diversity of aberrant ascus frequency between alleles (Tables 3–5), but with no clear evidence for a relationship between the frequency and the mutagen. The one apparent exception to this (high frequency for UV-induced *g-5* mutants – Table 5) has already been discussed.

(3) As already mentioned, the conversion pattern seems to be independent of the frequency of aberrant asci (compare Figs. 1–3 with Tables 3–5 respectively). There is no indication of trends of conversion pattern with changing frequency.

(4) It may be significant that the three spore colour genes in *S. brevicollis* with high aberrant ascus frequencies (*g-3*, *g-4*, *g-5*) also show high frequencies of second division segregation (*c.* 74 %, 70 % and 40 % respectively) while the remaining spore colour loci show low aberrant ascus frequencies and relatively low second-division segregation frequencies (0–30 %). The strong centromere linkage of the majority of loci in *S. brevicollis* may imply distal localization of chiasmata. The

high conversion frequency of distal genes would then be expected if they are located close to the mean chiasma position.

(*e*) *Aberrant asci with wider ratios*

The occurrence of aberrant asci showing the wider ratios 7:1, 1:7, 8:0 and 0:8 is attributed to two recombination events occurring independently and involving three or all four chromatids altogether. Thus, asci with 1:7 ratios of wild-type: mutant spores occur appreciably only when 2:6 and 3:5 ratios are frequent (Tables 3, 5): the simplest explanation of a 1:7 ascus is the occurrence of both a 2:6 and a 3:5 event. Likewise, 8:0 asci were recorded only when 6:2 asci were frequent, in agreement with the supposition that they result from two 6:2 events. Similarly, 0:8 asci were found only in crosses in which 2:6 asci were frequent.

The expected frequencies of asci with the wider ratios, on the assumption that these ratios result from two events of the appropriate kinds occurring independently, are obtained to a first approximation from the product of the observed frequencies of these events. Allowance must be made, however, for the fact that if two opportunities are available for events to occur, the narrower ratios could result from an event at either. Also, with two dissimilar events, e.g. 2:6 and 3:5, either may be regarded as the first event. The expected numbers of asci with the wider ratios are therefore given by the following expressions (Leblon, 1972*a*; Lamb & Wickramaratne, 1973), where brackets surrounding an ascus symbol, e.g. (6:2), indicate the number of such asci observed in a total count of N asci:

$$(7:1) = \frac{(6:2) \times (5:3)}{2N}$$

$$(1:7) = \frac{(2:6) \times (3:5)}{2N}$$

$$(8:0) = \frac{(6:2)^2}{4N}$$

$$(0:8) = \frac{(2:6)^2}{4N}$$

In Table 6 are shown the observed and expected numbers of these asci for each class of mutant in each gene. The data have been grouped in this way because the results for the individual mutants are inadequate. Surprisingly, in view of the scoring difficulty, the 0:8 asci were in agreement with expectation throughout. The numbers of 7:1 asci also agree with expectation. In a total of six cases, however, the observed numbers of 1:7 and 8:0 asci are significantly greater than those expected. The largest excess is a 28-fold one for the 1:7 asci found with the class C mutants of *g-5*. From Table 5 it can be seen that this excess is confined to the UV-induced mutants, where the excess is 30-fold. These mutants also showed a five-fold excess of 8:0 asci. The high frequency of aberrant asci shown by these mutants was discussed in section 3.

Leblon (1972*a*) found that the frequency of *Ascobolus* asci with wider ratios fitted the hypothesis of two independent events, but Lamb & Wickramaratne

Table 6. *Observed and expected numbers of asci with 7:1, 1:7, 8:0 and 0:8* ratios *of wild-type:mutant spores*

Gene	Class of mutant	7:1		1:7			8:0			0:8	
		Observed	Expected	Observed		Expected	Observed		Expected	Observed	Expected
Grey-3	B	0	0·0	7	*	2·1	0		0·0	30	26·6
	C	2	2·3	6	*	0·6	2		1·7	0	0·5
Grey-4	B	0	0·0	0		0·1	0		0·1	4	3·4
	C	2	0·7	0		0·2	3	*	0·7	0	0·4
Grey-5	A	0	0	0		0·0	7	*	0·4	0	0·0
	B	0	0·0	0		0·1	0		0·0	3	2·0
	C	1	0·3	46	*	1·6	7	*	1·8	0	0·7

An asterisk (*) indicates a significant difference ($P < 0{\cdot}01$) between observed and expected values.

(1973) reported examples of the second event being more frequent and, in other instances, less frequent than expected.

(*f*) *Diagrammatic representation of conversion pattern*

Some of the main features of the conversion pattern of mutants are revealed by plotting the frequency of postmeiotic segregation (5:3, 3:5, 7:1 and 1:7 asci) among the aberrant asci against the frequency of conversion to wild type (6:2 and 8:0 asci) among the even-ratio conversion asci, that is, with 6:2, 2:6, 8:0 and 0:8 ratios.

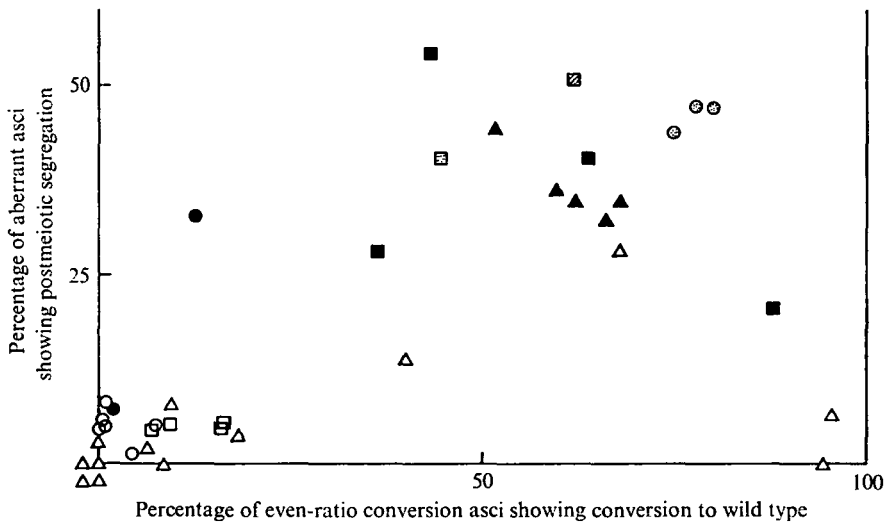

Fig. 4. The percentage of aberrant asci showing postmeiotic segregation (5:3, 3:5, 7:1 and 1:7 ratios) plotted for each mutant against the percentage of even-ratio conversion asci (6:2, 2:6, 8:0 and 0:8 ratios) that showed conversion to wild type, that is, 6:2 and 8:0 ratios. Key to symbols:

	Mutagen			
Gene	ICR170	EMS	UV	NG
g-3	○	⊗	●	
g-4	□	⊞	■	▨
g-5	△	◬	▲	

The *Sordaria brevicollis* data are plotted in this way in Fig. 4. In the diagram the three classes of mutants (A, B and C) are sharply differentiated from one another: the two class A mutants are in the bottom right-hand corner, the 20 class B mutants in the bottom left-hand corner, and the 17 class C mutants are dispersed over the centre of the graph. The much greater diversity, both in postmeiotic segregation frequency and in frequency of conversion in one direction, shown by the C mutants compared with A and B is evident. The various symbols in the diagram distinguish the mutagens and the loci. It is clear that there is a considerable

degree of mutagen specificity (as already discussed) but apparently no gene specificity in conversion pattern. It should be noted, however, that the diagram does not reveal the relative numbers of 5:3 and 3:5 segregations. There are some indications here of gene specificity, 5:3 being favoured over 3:5 with the C mutants of *g-4* (Table 4) and the converse with *g-5* (Table 5).

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