MUTATIONAL ANALYSIS OF NATURAL ALLELES AT THE *B* INCOMPATIBILITY FACTOR OF *SCHIZOPHYLLUM COMMUNE*: *α2* AND *β6*[1,2]

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ABSTRACT

The *B* incompatibility factor of the fungus *Schizophyllum commune* having allelic specificity *α2–β6* was subjected to mutagenesis by X-irradiation. Five types of mutations were recovered, four of them new types previously unreported. All lead to loss of the *B*-factor regulatory function; three of the five have retained their allelic specificity. The mutations map in three closely linked sites: *Bα*, *Bβ*, and between *Bα* and *Bβ*.

IN recent years evidence has accumulated showing that enormous allelic variation exists for many genes in natural populations of many organisms. Consequently a great deal of interest has centered on the origin of such widespread variation. Tissue incompatibility systems in mammals and sexual incompatibility systems in plants represent some of the most extensive series of natural polymorphisms. In the higher fungi these systems are especially amenable to genetic analysis, and may serve as models for understanding the structure, mode of origin, and evolution of multiple alleles in nature.

The incompatibility system of the basidiomycete *Schizophyllum commune* consists of two unlinked factors, *A* and *B*. Each factor is composed of two linked loci, *α* and *β*, with a naturally occurring series of multiple alleles found at each locus. Studies based on the frequency and distribution of alleles in a worldwide sample have led to an estimate of nine alleles at the *Aα* locus (RAPER, BAXTER and ELLINGBOE 1960), 32 alleles at *Aβ* (STAMBERG and KOLTIN 1973a), and nine alleles each at the *Bα* and *Bβ* loci (PARAG and KOLTIN 1971). Factor specificity is conferred jointly by alleles of the *α* and *β* loci, an allelic difference at either locus being sufficient to make two factors unlike.

In a normal monokaryon the genes responsible for the various stages in the sexual progression leading to fruiting, diploidy, meiosis, and production of haploid spores, are not active. When two monokaryons having different *A* factor and *B* factor specificities interact, the genes controlling sexual morphogenesis begin to function. A fertile heterokaryon, the dikaryon, is formed. It has a character-

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istic morphology, both macroscopically and microscopically. Macroscopically, growth is dense and vigorous and fruiting bodies soon appear. Microscopically, a number of stages of the morphogenetic sequence leading to establishment of the dikaryon have been defined, including exchange of nuclei between the mates, migration of these nuclei through the host mycelium, and their subsequent divisions in synchrony with the host tip-cell nuclei. Lateral connections between the cells of the dikaryon are formed during the synchronous divisions and are characteristic of the dikaryotic mycelium. In matings between monokaryons that have different specificities for only one of the two incompatibility factors, infertile heterokaryons are formed. When the two monokaryons have the same *A*-factor and different *B*-factor specificities, the common-*A* heterokaryon which results has macroscopically sparse growth and few aerial hyphae. This morphology is referred to as "flat". Microscopically the hyphae are irregular and gnarled; nuclear distribution is irregular and cells may have from 0 to 6 nuclei, owing to apparently uncontrolled nuclear migration within the hyphae. When two monokaryons have the same *B*-factor and different *A*-factor specificities, the resulting common-*B* heterokaryon is characterized by incomplete clamps, known as "pseudoclamps", and binucleate cells at the line of confrontation between the two monokaryons. For further details of sexual morphogenesis and heterokaryon morphologies see RAPER (1966).

The developmental patterns in the heterokaryons described above imply that the incompatibility factors serve as regulators of the component stages of sexual morphogenesis. This interpretation is supported by the existence of mutations, in several of the loci of the incompatibility factors, which lead to constitutive operation of part of the morphogenetic sequence. Thus each component locus of the incompatibility factors has at least two functions: self-recognition, and the regulation of genes involved in morphogenesis.

Much effort has been expended on attempts to clarify the mode of origin in nature of the multiple allele series found at the loci of the *A* and *B* factors. The experimental approach has been to try to derive new alleles from existing alleles by mutation and by intragenic recombination, but all attempts have failed. A variety of mutagenic agents has been used, and in fact 37 mutations were induced in the natural alleles of the *A* and *B* factors, but all led to loss of specificity or loss of regulatory function of the affected locus rather than to a new specificity (PARAG 1962; RAPER, BOYD and RAPER 1965; KOLTIN 1968; SIMCHEN, personal communication).

The wild-type *B* factor specificities can be divided into three classes on the basis of functional tests consisting of mating reactions with specific mutants, and frequency of recombination between $B\alpha$ and $B\beta$ (KOLTIN and RAPER 1967a, b; KOLTIN 1969). On the basis of the latter characteristic, the classes are referred to as "recombining" (Class I), "nonrecombining" (Class II), and "low-recombining" (Class III). The differences in mating behavior and in recombination frequency are explainable if the *B* factor specificities of classes II and III possess deletions for at least a part of the region between the $B\alpha$ and $B\beta$ of class I factors (KOLTIN 1969; PARAG and KOLTIN 1971). Furthermore, recombination studies

have led to the suggestion that certain class I $B\alpha$ and $B\beta$ alleles also possess smaller deletions (STAMBERG and KOLTIN 1973b). In an analysis of the recombinational behavior of the different alleles at $B\alpha$ and $B\beta$ of class I factors, it was found that certain α and β alleles fail to recombine with each other or with certain mutant *B* factors, although they recombine normally with most of the multiple series of alleles (STAMBERG and KOLTIN 1971, 1973). This led to the hypothesis that deletions rather than point mutations are associated with, and are responsible for the origin of, new incompatibility alleles in nature. A certain class I α allele and β allele may represent the original wild type alleles which gradually, through the occurrence of precise deletions, yielded a series of new allelic specificities. A prediction was made that the use of X-ray, a mutagenic agent known to induce deletions, on the original progenitor alleles would cause a deletion leading to a change in allelic specificity. The lack of success of the previous mutational studies with X-ray, it was suggested, could be due to the fact that one α, $B\alpha 3$, and one β, $B\beta 2$, were used almost exclusively.

We have, accordingly, begun a comparative study of the "mutational spectrum" for each of the naturally occurring alleles of the *B* factor, in the hope that the types of mutations recovered from each allele will eventually allow us to reconstruct the evolution of this polymorphic series. In this paper we report on mutations recovered after X-ray treatment of the alleles $B\alpha 2$ and $B\beta 6$.

MATERIALS AND METHODS

Strains: The two strains of *S. commune* used in this study were obtained from crosses between strains in our collection at Tel-Aviv University: Strain no. 14–2, *Aα2–β6 Bα2–β6 ade-5⁻*, and strain 14–13, *Aα8–β12 Bα2–β6 ura-1⁻*.

Selection and analysis of mutations: To survive on minimal medium, treated fragments of the two genotypes must interect to form a common-*B* heterokaryon in which the nutritional requirements of each strain are complemented. Any mutation in the *B* factor changing its specificity leads to formation of an easily recognizable dikaryon, with subsequent development of fruiting bodies. Dikaryotic regions were subcultured, and haploid spore samples were taken from the fruiting bodies and analyzed to determine the site of mutation. Detailed procedures of the analysis have been explained by KOLTIN (1968). Briefly, the steps include: (a) classifying a number of haploid progeny (50–75), for morphology, presence of auxotrophic markers (as a check against contamination by a different strain of *S. commune*), and compatibility with the parental strains; (b) testing of progeny showing changed compatibility with various other wild-type *B* factors from our collection, in an attempt to identify the changed specificity; (c) testing of progeny showing changed compatibility with certain "secondary mutant" strains in our collection, to test whether the $B\alpha$ specificity is retained. Strains carrying "secondary" mutations in $B\beta$ have been isolated and described by RAPER, BOYD and RAPER (1965), KOLTIN and RAPER (1966), RAPER and RAUDASKOSKI (1968), RAUDASKOSKI (1970), and RAPER and RAPER (1973). All possess the ability to discriminate between strains carrying the same and different $B\alpha$ alleles. A strain carrying $B\alpha 2$ and a secondary $B\beta$ mutation will not accept nuclei from a normal, wild-type strain carrying $B\alpha 2$ and any $B\beta$ allele, although it will accept nuclei from a wild-type strain having any other allele at the $B\alpha$ locus. Step (d) is the mapping of the mutation by a recombination test, consisting of a cross to a strain carrying a wild-type *B* factor. As detailed in Table 1, different classes of recombinant progeny are expected depending on whether the mutation occurred at the $B\alpha$ locus, at the $B\beta$ locus, outside the *B* region entirely, or between $B\alpha$ and $B\beta$.

Mutagenesis: The two strains were grown individually on complete medium and macerated in an Omnimixer to obtain uninucleate fragments from the mycelia. The fragments were

TABLE 1

Recombination test to determine location of mutations
Cross: Mutant derived from strain *Bα2–β6* × wild-type strain *Bα3–β2*.

Possibility		Location of mutation	Recombinant progeny expected
1.	*Bα*	*α2mut* *β6*	*α3–β6* *α2mut–β2*
2.	*Bβ*	*α2* *β6mut*	*α2–β2* *α3–β6mut*
3.	outside of *B*	*α2* *β6* .... *mut*	*α2–β6* *α3–β2–mut* *α2–β2** *α3–β6–mut** *α2–β2–mut** *α3–β6**
4.	between *Bα–Bβ*	*α2* *mut* *β6*	*α2–β2** *α3–β6** *α2–mut–β2** *α3–mut–β6**

* Depending on exact linkage relationships between *Bα*, *Bβ*, and the mutated site, some of these types of recombinants may not be found.

irradiated by X-ray, using a Phillips MG 100 generator at 70 kV, at a plate current of 10 mA providing a dosage of 32,000 r/min. A total of 2.2×10^9 fragments were irradiated for times varying from 6–25 min. A total of 1×10^8 surviving fragments of both genotypes were plated together at a density of 20–50,000 per plate on minimal medium. Approximately one month after plating, a layer of minimal medium was added on top of the original medium, to allow for an additional period of growth. Dikaryotic fragments were selected from this layer. For composition of media used, see RAPER and MILES (1958) and SNIDER and RAPER (1958).

RESULTS

Six mutations were recovered from the total sample treated, giving a mutation frequency of 1.7×10^{-7}. As will be shown, these mutations were located in three separate but closely linked loci. They were of five different types, as summarized in Table 2.

Type 1: The dikaryon, upon fruiting, produced haploid progeny half of which had normal monokaryotic morphology and retained the parental *B*-factor specificity. The other half of the progeny had the "flat" phenotype characteristic of common-*A* heterokaryons and of previously described monokaryons carrying a mutation in the *B* factor causing lack of the *B*-factor regulatory function. These progeny were compatible with their progenitor and with all other *B* factors tested, indicating that the *B* factor had lost its previous specificity. The mutation was located in the *Bβ* locus, as shown by a cross to a wild-type strain *Bα3–β2* from which two recombinant types were recovered: *Bα2–β2*, having the normal monokaryotic phenotype and *Bα3–β6mut*, having the "flat" or mutant phenotype.

TABLE 2

Mutations obtained from Bα2–β6 *after X-irradiation*

Mutational type	Morphology	Interaction with progenitor *Bα2–β6*	Location of mutation	Designation	Allelic specificity and self-recognition	*B* regulatory function
Type 1	flat	+*	*Bβ*	*Bβ6(1)*	lost	lost
Type 2	flat	+	*Bα*	*Bα2(1)*	lost	lost
Type 3	aberrant	—	*Bβ*	*Bβ6(2)*	unchanged	lost
Type 4	flat [or aberrant]	—	*Bα*	*Bα2(2)*	unchanged	lost
Type 5	aberrant	—	between *Bα* and *Bβ*	*B-Su(1)*	unchanged	lost

* + indicates that the progenitor is dikaryotized by the mutant; — indicates that the progenitor is not dikaryotized.

(From a sample of 256 spores tested, 4 of the former and 9 of the latter type of recombinant appeared, giving 5.1% recombination). The *Bα3–β6mut* recombinants were universally compatible with all *B* factors, including the series of *B* factors having *Bα3* and *β1* through 7. Thus the mutation that occurred in this strain in the *Bβ* locus has caused the locus to lose its former specificity but no new specificity has been gained. In every respect this mutation is identical to the mutations recovered by PARAG (1962) and later by KOLTIN (1968) in *Bβ2* and designated *Bβ2(1)*. Following this notation, our mutation can be designated as *Bβ6(1)*.

Type 2: Progeny which were obtained from this dikaryon were of two types: half were monokaryotic in phenotype and carried the parental *B*-factor specificity, whereas the other half were flat and compatible with the parental *B* factor as well as with all other *B* factors tested. The universal compatibility indicated that the previous *B*-factor specificity was lost in this mutant. Test matings were made between this mutant and the secondary mutant strains carrying *Bα2*, and in all cases the secondary mutants accepted nuclei. This result is indicative that the *Bα* locus in the newly generated mutant has lost its former specificity and is no longer recognized as *Bα2* by the secondary mutant testers. In a cross between the new mutant and a wild-type strain with *Bα3–β2*, 275 of the monokaryotic-appearing progeny were tested and 6 (2.2%) were found to be recombinants of type *Bα3–β6*. This confirms that the *Bβ* locus is unchanged. We have here the first case where a mutation in the *α* locus of a class I *B* factor has been recovered, and we designate it as *Bα2(1)*.

Type 3: Two independent mutations appeared from different dikaryons, which gave identical results in all of the following tests. Each dikaryon, upon fruiting, gave progeny of two types: Approximately half were normal in morphology and carried the parental *B* factor; the other half were "weakly flat" in morphology. Macroscopically they were typically flat-looking, but microscopic examination showed that most hyphae were normal in appearance and only a few had the plasmal extrusions and gnarled appearance typical of the flat mycelium; a large number of cells exhibited false clamps. Cytological examination indicated that more than 30% of the cells were anucleate, approximately half of the cells had

one or two nuclei, and almost 20% were multinucleate. This irregular nuclear distribution is typical of the flat phenotype. This morphology has previously been described by KOLTIN (1968) in mutants of class II and III *B* factors and designated "aberrant". It probably represents a modification of the typical flat morphology due to the presence in the genome of various "modifier" genes affecting stages in sexual morphogenesis (RAPER and RAPER 1966). The aberrant progeny did not dikaryotize their progenitor although they did dikaryotize strains having a different *Bα* or different *Bβ*. In tests between the aberrant progeny and secondary mutant strains carrying *Bα2*, the secondary mutants did not accept nuclei. Thus it appears that the *α* specificity is unchanged; and in fact the specificity of the entire *B* factor is unchanged since it recognizes its progenitor and does not interact with it to form a fertile dikaryon. Recombination tests were made with a wild-type strain carrying *Bα3–β2* and two types of recombinants were recovered: normal monokaryotic progeny of genotype *Bα2–β2* and progeny with aberrant morphology of genotype *Bα3–β6mut.* (Recombination frequency in each case was about 5%.) The latter class of recombinants did not dikaryotize a wild-type strain carrying *Bα3–β6*, although all wild type strains with different *α*'s or *β*'s were readily dikaryotized. Thus the mutation is located at the *Bβ* locus or very close to it and, in combination with an *Bα* allele, recognizes the wild-type strain having the progenitor *Bβ6* and the same *Bα*, and fails to interact with it. This mutation has lost the *Bβ* regulatory function (as shown by its aberrant morphology) but still retains self-recognition. KOLTIN (1968) found mutations with similar properties in class II and class III *B* factors, but this is the first case of such a mutation in a class I factor, and it can be designated *Bβ6(2)*.

Type 4: A dikaryon fruited and produced progeny half of which had monokaryotic morphology and the parental *B* factor, the other half of which were flat in morphology (expression of the flat morphology among the progeny ranged from aberrant to the typical flat phenotype, possibly due to the segregation of a modifier gene), but did not dikaryotize their progenitor, although they dikaryotized strains with a different *Bα* or different *Bβ*. This implies that the regulatory function of the *B* factor has been lost (hence the aberrant morphology), but the *B*-factor specificity or self-recognition is unchanged. When test matings were made between this new mutant strain and the secondary mutants carrying *Bα2*, in all cases the secondary mutants failed to accept nuclei from the newly mutant strain. This suggests that the *Bα* in the new mutant still retains its specificity as *α2*.

A recombination test was made by crossing the mutant to a wild-type strain with *Bα6–β7*. Only the monokaryotic-appearing progeny were tested; in a sample of 243 such progeny, 20 recombinants (8.2% recombination) were identified and all were of genotype *Bα6–β6*. In addition, in a cross between the mutant and wild-type strain *Bα3–β2*, two reciprocal recombinant classes of progeny (giving 4.7% recombination) were identified in a total sample of 255: (a) monokaryotic-appearing progeny of genotype *Bα3–β6*, and (b) flat or aberrant progeny which did not dikaryotize the wild-type strain *Bα2–β2* but did dikaryotize *Bα2–β6*. The results of both recombination tests indicate that the mutation is in the *Bα* locus

or very close to it, and the mutated *Bα* retains its former specificity but has lost the regulatory function. We designate this mutation *Bα2(2)*. In all respects except its location it appears identical to *Bβ6(2)*.

Type 5: The mutant progeny from one dikaryon were identical, in morphology and in mating reactions, to the progeny described above which carried type 3 and 4 mutations. Recombination tests with a wild-type strain carrying *Bα3–β2*, however, produced three recombinant types among the progeny: normal monokaryons carrying *Bα3–β6*, progeny with the aberrant phenotype and genotype *α3–β6*, and phenotypically aberrant progeny of genotype *α2–β2*. The findings that the mutant phenotype can segregate with either the *α2* or the *β6*, and that *β6* (and probably also *α2*) can be recovered in non-mutant condition, indicate that the mutated locus is not *α* or *β* itself but lies in between these two loci: *Bα– mut–Bβ*. Crossing over between *Bα* and *mut* or between *mut* and *Bβ* would generate the recombinant types found. Since the mutation suppresses the normal regulatory function of the *B* factor we designate it *B–Su(1)*.

DISCUSSION

Among the six mutations recovered in this study, four new types are represented. Type 1, designated *Bβ6(1)*, is identical in appearance and behavior to the *Bβ2(1)* mutation described by PARAG (1962) and represents a loss of both allelic specificity and the *B* regulatory function. Type 2, called *Bα2(1)*, is very similar to these mutants and represents the first case of a mutation in a class I*α* allele. (RAPER and RAPER [1973] reported the recovery of mutations of class I *B* factors in which both the *α* and *β* locus were affected; these probably result from large deletions of most or all of the *B* factor. The assignment of the mutated site to the *Bα* locus was deduced by functional studies.) In our mutant *Bα2(1)*, as in the case of *Bβ6(1)*, allelic specificity and regulatory function are simultaneously lost.

Types 3, 4 and 5 are mutations which have lost the *B* regulatory function but retained allelic specificity, i.e. self-recognition. The behavior of each is similar but the mutations are respectively located in *Bβ*, *Bα*, and between *Bα* and *Bβ*. Mutations similar to types 3 and 4 have been obtained in class II and III *B* factors by KOLTIN (1968) but have not previously been found in class I.

The fact that mutations of class I *B* factors have now been recovered in which loss of the regulatory function occurs without loss of allelic specificity indicates that the component loci of class I *B* factors have a bifunctional role. This has previously been pointed out by KOLTIN (1968) for class II and III *B* factors. Furthermore the derivation of identical mutant phenotypes by mutation in the *Bα* and in the *Bβ* loci suggests a basic similarity in the two loci. This finding is in contrast to the previous claim that "the two loci must . . . differ in mutability, size, or function" (SIMCHEN, STAMBERG and KOLTIN 1969), a claim which was based on the mutational data available at that time, and which may reflect only the characteristics of the alleles then studied.

TABLE 3

Primary mutations in the B *factor*

Alleles	Location of mutation	Designation	No. repeats of same mutation	Allelic specificity	Regulatory function	Mutagenic treatment	Reference
Class I							
Bα3–β2	*β*	*Bβ2(1)*	1	lost	lost	none (spontaneous)	PARAG (1962)
	β	*Bβ2(1)*	2	lost	lost	nitrogen mustard	PARAG (1962)
	β	*Bβ2(1)*	4	lost	lost	X-ray	KOLTIN (1968)
Bα7–β7	*β*	*Bβ7(1)*	1	lost	lost	acriflavin	SIMCHEN, pers. comm.
Bα2–β6	*β*	*Bβ6(1)*	1	lost	lost	X-ray	This paper
	β	*Bβ6(2)*	2	unchanged	lost	X-ray	This paper
	α	*Bα2(1)*	1	lost	lost	X-ray	This paper
	α	*Bα2(2)*	1	unchanged	lost	X-ray	This paper
	between *α* and *β*	*B-Su(1)*	1	unchanged	lost	X-ray	This paper
Class II†							
Bα1–β′1	*β*	*Bβ′1(1)*	3	unchanged	lost	X-ray	KOLTIN (1968)
Class III							
Bα′1–β4	*α*	*Bα′1(1)*	1*	unchanged	lost	X-ray	KOLTIN (1968)
	β	*Bβ4(1)*	2*	lost	lost	X-ray	KOLTIN (1968)
Bα′2–β4	?	?	?*	unchanged	lost	X-ray	KOLTIN (1967, 1968)

* A total of 15 mutations in class III were isolated. The precise locations of only 3 were determined.
† The designations *α′* and *β′* refer to unique *α* and *β* alleles found only in class III or II *B* factors, respectively (PARAG and KOLTIN 1971).

Type 5, called *B–Su(1)*, represents the first instance in which a mutation to loss of the *B* regulatory function has been located outside of the *Bα* or *Bβ* locus. The mutation is located between these two loci, in a region where at least one gene affecting nuclear migration is found (KOLTIN and STAMBERG 1972). A change in such a gene causing it to be insensitive to regulation by the *B* factor itself, could lead to constitutive function—i.e., continuous nuclear migration.

Thus, the six mutations recovered in this study, like all previous mutations reported (see Table 3), are characterized by loss of the *B* factor regulatory function. Why no mutation has yet been found that is characterized by a change to a new allelic specificity without a loss of regulatory function is not clear. The selective system used permits detection of such mutant types, as shown in reconstruction experiments by RAPER, BOYD and RAPER (1965), and by KOLTIN and STAMBERG (unpublished). If, as we have postulated, the natural alleles of *Bα* arose directly from each other by mutation (point or deletion), and likewise those of *Bβ*, then it should be possible to recover strains carrying mutations leading to changed specificity but unaltered regulatory function.

To date, only two natural alleles at the *Bα* locus and two at the *Bβ* locus have been extensively studied by means of mutagenesis. The progenitor alleles we have postulated to exist among the polymorphic series at *Bα* and *Bβ* may be among those alleles as yet unexamined. The search for new alleles is presently continuing in our laboratory by analysis of the mutational spectrum of additional natural alleles.

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LITERATURE CITED

KOLTIN, Y., 1967 The structure and function of the *B* incompatibility factor of *Schizophyllum commune*. Ph.D. thesis, Harvard University. ——, 1968 The genetic structure of the incompatibility factors of *Schizophyllum commune*: comparative studies of primary mutations in the *B factor*. Mol. Gen. Genet. **102**: 196–203. ——, 1969 The structure of the incompatibility factors of *Schizophyllum commune*: class II factors. Mol. Gen. Genet. **103**: 380–384.

KOLTIN, Y. and J. R. RAPER, 1966 *Schizophyllum commune*: New mutations in the *B* incompatibility factor. Science **154**: 510–511. ——, 1967a The genetic structure of the incompatibility factors of *Schizophyllum commune*: three functionally distinct classes of *B* factors. Proc. Natl. Acad. Sci. U.S. **58**: 1220–1226. ——, 1967b The genetic structure of the incompatibility factors of *Schizophyllum commune*: the resolution of class III *B* factors. Mol. Gen. Genet. **100**: 275–282.

KOLTIN, Y. and J. STAMBERG, 1972 Suppression of a mutation disruptive to nuclear migration in *Schizophyllum* by a gene linked to the *B* incompatibility factor. J. Bacteriol. **109**: 594–598.

PARAG, Y., 1962 Mutations in the *B* incompatibility factor of *Schizophyllum commune*. Proc. Natl. Acad. Sci. U.S. **48**: 743–750.

PARAG, Y. and Y. KOLTIN, 1971 The structure of the incompatibility factors of *Schizophyllum commune*: Constitution of the three classes of *B* factors. Mol. Gen. Genet. **112**: 43–48.

RAPER, C. A. and J. R. RAPER, 1966 Mutations modifying sexual morphogenesis in *Schizophyllum*. Genetics **54**: 1151–1168. ——, 1973 Mutational analysis of a regulatory gene for morphogenesis in *Schizophyllum*. Proc. Natl. Acad. Sci. U.S. **70**: 1427–1431.

RAPER, J. R., 1966 *Genetics and Sexuality of the Higher Fungi.* Ronald Press, New York.

RAPER, J. R. and P. G. MILES, 1958 The genetics of *Schizophyllum commune.* Genetics **43:** 530–546.

RAPER, J. R. and M. RAUDASKOSKI, 1968 Secondary mutations at the *Bβ* incompatibility locus of *Schizophyllum.* Heredity **23:** 109–117.

RAPER, J. R., M. G. BAXTER and A. H. ELLINGBOE, 1960 The genetic structure of the incompatibility factors of *Schizophyllum commune*: the *A* factor. Proc. Natl. Acad. Sci. U.S. **46:** 833–842.

RAPER, J. R., D. H. BOYD and C. A. RAPER, 1965 Primary and secondary mutations at the incompatibility loci in *Schizophyllum.* Proc. Natl. Acad. Sci. U.S. **53:** 1324–1332.

RAUDASKOSKI, M., 1970 A new secondary *Bβ* mutation in *Schizophyllum* revealing functional differences in wild *Bβ* alleles. Hereditas **64:** 259–266.

SIMCHEN, G., J. STAMBERG and Y. KOLTIN, 1969 The origin of two-locus incompatibility factors in the tetrapolar fungi. Am. Naturalist **103:** 399–405.

SNIDER, P. J. and J. R. RAPER, 1958 Nuclear migration in the Basidiomycete *Schizophyllum commune.* Am. J. Botany **45:** 538–546.

STAMBERG, J. and Y. KOLTIN, 1971 Selectively recombining *B* incompatibility factors of *Schizophyllum commune.* Mol. Gen. Genet. **113:** 157–165. ——, 1973a The organization of the incompatibility factors in higher fungi: the effect of structure and symmetry on breeding. Heredity **30:** 15–26. ——, 1973b The origin of specific incompatibility alleles: a deletion hypothesis. Am. Naturalist **107:** 35–45.

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