SECONDARY MUTATIONS AT THE *Bβ* INCOMPATIBILITY LOCUS OF *SCHIZOPHYLLUM* *

JOHN R. RAPER and MARJATTA RAUDASKOSKI †
Biological Laboratories, Harvard University, Cambridge, Massachusetts

Received 6.v.67

1. INTRODUCTION

MATING competence and sexual morphogenesis in a majority of higher Basidiomycetes, typified by *Schizophyllum commune*, are determined and controlled by a bifactorial or " tetrapolar " incompatibility system comprised of two series of factors, *A* and *B*, each constituted of two linked loci, α and β, with multiple alleles (Raper, 1966; Koltin, 1967). One of the persisting puzzles about this incompatibility system is the means by which the extensive series of alleles at each of the incompatibility loci could have evolved. It seems reasonable to assume that the series of alleles at the four loci are not static and that new alleles can originate only from existing alleles. These considerations led to a search for new alleles as the result of mutations induced in known alleles. To date, more than 20 mutations have been found in two (*Aβ* and *Bβ*) of the four basic incompatibility loci of *S. commune* and *Coprinus lagopus* (Day, 1963; Parag, 1962; Raper, Boyd and Raper, 1965; G. Simchen, unpublished). These mutations were not to new, normal alleles; instead, each had lost the discriminatory function of the affected factor and was self-fertile and compatible with all factors of the same series. A homokaryon having a mutant *Bβ* allele is thus a phenotypic mimic of the $A = B \neq$ heterokaryon. The subsequent recovery of four secondary *Bβ* mutations that restored normal morphology and self-sterility in the homokaryon and were compatible with the progenitor *Bβ* allele suggested the origin of new functional alleles from existing alleles by successive mutations (Raper, Boyd and Raper, 1965). These mutations, however, differed from wild-type alleles in details of the mating reaction.

This paper describes additional secondary *Bβ* mutations and considers their implications for the origin of the extensive series of alleles in nature.

2. MATERIALS AND METHODS

(i) *Origin of strains*

Four independent mutations in the *Bβ* locus of *S. commune* were used. Three, one spontaneous and two induced by HNO_2, were recovered in *B41 α3-β2* by Parag (1962) and one, induced by acriflavine, was recovered in *B127 α7-β7* by G. Simchen (unpublished). The first secondary mutations (Raper, Boyd and Raper, 1965) were derived from the original *Bβ* mutation recovered by Parag, who in 1960 provided us with the mutant strain. Parag and Simchen kindly made the three additional mutations available.

* This work was supported in part by U.S. Public Health Service Grant A106124.

† Fulbright Fellow from Finland, 1965-66. Present address: Department of Botany, University of Turku, Turku, Finland.

(ii) *Media*

Crosses and isolation of monosporous progeny were made on a complete medium (0·5 gm. $MgSO_4$, 0·46 gm. KH_2PO_4, 1·0 gm. K_2HPO_4, 2 gm. peptone, 2 gm. yeast extract, 20 gm. dextrose and 20 gm. agar per litre of distilled water). Mycelial matings to determine the interaction of *B* factors were made on minimal medium (0·5 gm. $MgSO_4$, 0·46 gm. KH_2PO_4, 1·0 gm. K_2HPO_4, 1·5 gm. asparagine, 120 μgm. thiamine-HCl, 20 gm. dextrose, and 20 gm. agar per litre of distilled water).

(iii) *Induction of mutations*

Strains carrying the primary *Bβ* mutations were grown in liquid culture at 23° C. for 4-7 days; they were then macerated, and the macerate was suspended in 0·05 M phosphate buffer at *p*H 8·0. Ethyl methane sulphonate, EMS, was added to the suspension to a concentration of 1 per cent., and the suspension was allowed to stand with gentle, continuous stirring for a sufficient period to achieve a mortality of about 50 per cent. (samples of EMS differed appreciably in toxicity; see table 1). Treatment was terminated by the addition to the reaction mixture of an equal volume of 0·4 M sodium thiosulphate. The treated macerate was then diluted and plated.

TABLE 1

The induction by EMS of mutations restoring normal morphology in Bβ2 *mutant strains*

Genotype	Treated sample ($\times 10^{-5}$)	Time of treatment (min.)	Survivors (per cent.)	Mycelia with normal morphology (No.)
B α3-β2(I_2)	3·3	15	51	145
B α3-β2(I_3)	3·2	15	54	70
B α3-β7(I_1)	3·5	120	56	150

(iv) *Recovery of mutations*

The mutant-*B* strains clearly mimic the $A = B \neq$ heterokaryon (sparse growth, lack of aerial hyphae, irregularly branched hyphae, and frequent protoplasmic extrusions). Against this background, clumps of morphologically normal mycelia are readily discernible. For the selection of secondary mutations in *Bβ*, however, this system has two serious faults, one of which critically affects the type of mutations that can be recovered: (*a*) only mutations that are unilateral in their mating response, *i.e.* that block nuclear migration, can be expressed. A bilaterally reacting strain would interact with the original strain to form a $A = B \neq$ heterokaryon that would be indistinguishable from the mutant homokaryon. (*b*) Type IV modifier mutations (Raper and Raper, 1966), which lie outside the *B* factor but restore normal morphology to the mutant-*B* homokaryon, are efficiently selected and outnumber secondary *Bβ* mutations by about ten to one.

(v) *Testing procedures*

The two types of mutants are readily distinguished by mating each with two tester strains. The secondary *Bβ* mutations react unilaterally with their progenitor *B* factors but bilaterally with *B* factors carrying α and β alleles different from those of the progenitor factor, whereas type IV mutations

react unilaterally with all compatible *B* factors (compatible *A* factors in mutant and tester strains):

	Tester strains	
	B α3-β2	*B αx-βx*
B α3-β2mut-mut	−/+	+/+
B α3-β2mut MIV	−/+	−/+

"+" signifies dikaryosis, and "−", no reaction; symbols to left and right of the diagonal indicate reactions of strains at left and above, respectively.

To determine the interrelationships among secondary mutations, each mutant strain was outcrossed with an appropriate strain to obtain recombinants in which each secondary mutation was associated with two compatible *A* factors and two different auxotrophic markers. Nutritionally forced matings between strains carrying the different mutations, *e.g.* *A1 B α3-β2(1-1) ura*$^-$ × *A2 B α3-β7(1-1) ade*$^-$ were then used to determine the identity or non-identity of the mutations: if the result was $A \neq B$ = heterokaryosis, the two mutations were considered to be identical, *i.e.* to have the same specificity; if dikaryosis resulted, the two mutations were considered to have different specificities. The two strains were first mated on complete medium and incubated for a week or so, at which time small mycelial plugs were cut from the line of hyphal intermingling and transferred to minimal medium. The resulting mycelia were examined for the distinguishing features of the dikaryon (clamp connections) or $A \neq B$ = heterokaryon (pseudoclamp connections).

3. Experimental results

(i) *Induction and recovery of morphologically normal homokaryons*

Homokaryons carrying the three primary mutations of *Bβ2* were indistinguishable and were comparable in every way with strains carrying the primary mutation of *Bβ7*. As no distinction could be made among the four, they were provisionally assumed to be alike and were designated *B* $\beta 2(1_1)$, *B* $\beta 2(1_2)$, *B* $\beta 2(1_3)$ and *B* $\beta 7(1_1)$ to indicate primary mutations of a single kind (Koltin and Raper, 1966)—the subscripts to indicate their independent origins (see below, table 4).

Strains carrying the three mutations, *B* $\beta 2(1_2)$, *B* $\beta 2(1_3)$ and *B* $\beta 7(1_1)$, each in association with *Bα3*, were treated with EMS. Three hundred and sixty-five mycelia that appeared to be normal homokaryons were recovered from the three samples (table 1).

(ii) *Differentiation between secondary Bβ mutations and modifier mutations*

Each of the mutant strains was mated with tester strains having wild-type *B* factors that related to the progenitor wild-type *B* factor in three ways: (*a*) the same *Bα* and *Bβ* alleles, (*b*) the same *Bα* and a different *Bβ*, and (*c*) different *Bα* and *Bβ* alleles. In these tests, three patterns of interaction were observed (table 2).

1. Three hundred and sixteen isolates reacted unilaterally with all of the testers. Several isolates giving this mating response were earlier shown, in crosses with wild-type mates, to carry mutations at loci other than those of the *B* factor and were designated modifier mutations of type IV (Raper and Raper, 1966).

2. Forty-seven isolates interacted unilaterally with both testers having the common *Bα* alleles and bilaterally with the tester having different *Bα* and *Bβ* alleles. This pattern of interactions is the same as that of the secondary *Bβ* mutants earlier described (Raper, Boyd and Raper, 1965), and they were designated *B β2(1-1)* or *B β7(1-1)* to signify secondary mutations of type 1 induced in primary mutations of type 1. Identity of these mutations with those characterised earlier was later established (see below).

TABLE 2

*Reactions of morphologically normal mutants selected from treated mutant-*Bβ(1) *strains*

Assigned genotype	No.	Tester strains with normal *B* factors: *B α3-β2* or *B α3-β7*	*B α3-β1*	*B α1-β1*
B α3-β2(I_2) MIV	130	−/+	−/+	−/+
B α3-B2(I_3) MIV	62	−/+	−/+	−/+
B α3-β7(I_1) MIV	124	−/+	−/+	−/+
	316			
B α3-β2(I_2-1)	13	−/+	−/+	+/+
B α3-β2(I_3-1)	8	−/+	−/+	+/+
B α3-β7(I_1-1)	26	−/+	−/+	+/+
	47			
B α3-β2(I_2-3)	2	−/+	+/+	+/+

3. Two isolates interacted unilaterally only with the tester strain having the progenitor wild-type *B* factor; their interactions with the two other tester strains were bilateral and normal. Both isolates were later shown to carry secondary *Bβ* mutations, and these were designated *B β2(1-3)*, *B β2(1-2)* having been pre-empted earlier (Koltin and Raper, 1966).

In hundreds of subsequent matings, the patterns of interactions caused by these secondary mutations, *B β(1-1)* and *B β(1-3)*, have been invariable: *B β(1-1)*, unilateral mating when the mutant and wild *B* factors have the same *Bα*, regardless of the allele at *Bβ*, and bilateral when the *B* factors have different *Bα*'s; *B β(1-3)*, unilateral with strains carrying the single *B* factor having the same *Bα* associated with the progenitor *Bβ* allele and bilateral with all others.

Each isolate with a presumed secondary *Bβ* mutation was crossed with a strain carrying a normal *B* factor and auxotrophic mutations (*a*) to ascertain that the new mutation was located in the *B* factor—progeny with the aberrant morphology of the primary mutant-*B* parent would be recovered if the new mutation lay outside the *B* factor—and (*b*) to obtain the new mutation with the same *Bα* allele in strains with different *A* factors and different auxotrophic markers. Progenies from crosses involving representatives of each mutant type were analysed to locate more accurately the site of the mutations. The procedure used for and the results obtained from the cross,

B α3-β2(I₂-3) × B α1-β1, are detailed in table 3. The recovery of reciprocal *B*-factor recombinants, the failure to recover the primary *Bβ* mutations, *i.e.* progeny having the mutant-*B* phenotype, and the same mating response in the mutant parent and in the recombinant carrying the *Bβ* allele of the mutant parent show that the new mutations are in or near the *Bβ* locus.

TABLE 3

Location of secondary mutation by recovery of α-β recombinants

Cross: *B α3-β2(I₂-3) × B α1-β1*

Progeny	No.	Testers			
		B α3-β2	*B α3-β1*	*B α1-β2*	*B α1-β1*
B α3-β2(I₂-3)	294	−/+	+/+	+/+	+/+
B α1-β1	271	+/+	+/+	+/+	com-*B**
B α1-β2(I₂-3)	5	+/+	+/+	−/+	+/+
B α3-β1	3	+/+	com-*B*	+/+	+/+

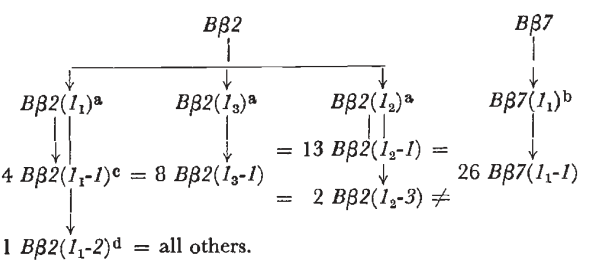
* com-*B* designates *A*≠*B*= interaction.

(iii) *Determination of relationships among secondary Bβ mutations*

The relationships between the secondary *Bβ* mutations of different types and different origins were determined in matings on minimal medium that opposed strains carrying the various mutations in association with the same *Bα* allele, different *A* factors, and at least one pair of non-allelic auxotrophic markers.

TABLE 4

Derivation and interrelations of secondary B*β mutations*



"=" incompatible, *i.e.* same specificity; "≠" compatible, *i.e.* different specificities.

[a] Parag and Raper (1960); Parag (1962).
[b] G. Simchen, unpublished.
[c] Raper, Boyd and Raper (1965).
[d] Koltin and Raper (1966).

The results of these tests (table 4) may be summarised as follows:

Intercrosses among all *B β(1-1)* mutations and between *B β2(1-1)* and *B β2(1-3)* mutations resulted in the establishment of *A* ≠ *B* = heterokaryons. Matings between the two *B β2(1-3)* mutations and the *B β7(1-1)* mutations resulted in each case in the establishment of a normal dikaryon in the *B β2(1-3)* strain and no reaction in the *B β7(1-1)* mate.

A sample of 174 progeny of one of these dikaryons was analysed, and the *B*-factor genotype of each strain was determined; the two parental *B* factors segregated normally: 82 *B α3-β2(1-3)* and 92 *B α3-β7(1-1)*. A sample of 353 progeny of a similar cross but heteroallelic for *Bα*, *B α1-β2(1-3)* × *B α3-β7(1-1)*, was also analysed; segregation of the parental *B* factors was again normal (184 and 173), and one *α-β* recombinant of the genotype *B α3-β2(1-3)* was recovered.

TABLE 5

Comparative interactions of wild-type and mutant alleles of B*β*2 *with wild* B *factors*

	Testers		
Bβ allele	*B α3-β2*	*B α3-β1*	*B α1-β1*
B α3-β2	com-*B*	+/+	+/+
B α3-β2(1)	−/+	−/+	−/+
B α3-β2(1-1)	−/+	−/+	+/+
B α3-β2(1-2)	com-*B*	com-*B*	+/+
B α3-β2(1-3)	−/+	+/+	+/+

Although all secondary mutations recovered in this study were assignable to two types, *B β(1-1)* and *B β(1-3)*, a third type, *B β(1-2)*, was earlier described by Koltin and Raper (1966). *B β2(1-2)*, like *B β2(1-1)* and *B β2(1-3)*, restores normal morphology in the homokaryon. Its mating response differs from those of these mutations, however, in that it is incompatible (rather than unilaterally compatible) with " recombining " factors that contain a common *Bα* allele (Koltin, Raper and Simchen, 1967).

The origins and interrelationships of the various secondary *Bβ* mutations are given in the pedigree presented in table 4, and the interactions of *Bβ2* and all known mutant alleles derived from *Bβ2* with wild-type *B* factors are compared in table 5.

4. DISCUSSION

The members of either series of factors, *A* and *B*, were early recognised to be alternate and equivalent, and each factor was considered as having a distinct specificity. When the factors proved to be complex, the numerous alleles at each locus were also found to be specific, *i.e.* each is self-incompatible and cross-compatible with all others. Self-incompatibility and cross-compatibility have thus become accepted as the criteria for the definition of allelic specificity. By this definition *B β2(1-1)* and *B β2(1-3)* are identical, as in intercrosses they elicit only $A \neq B =$ interactions; yet they are demonstrably different, for, by the same definition, *B β2(1-1)* is identical to *B β7(1-1)*, whereas *B β2(1-3)* is different from *B β7(1-1)*. To confuse the issue further, *B β2(1-2)* is identical to all wild-type *Bβ*'s, its progenitor allele, *Bβ2*, included, if the single criterion of incompatibility is used to define allelic identity. These facts at least indicate (*a*) that no single final specificity is endowed in the primary mutation and (*b*) that, from a given wild *Bβ* allele, different " specificities " can be generated, albeit the differences in specificity obtained to date are insufficient to permit their direct interaction in the manner of wild *Bβ* alleles.

These " unnatural " interrelationships of *Bβ* alleles relate in a basic manner to the structure and function of the *Bβ* locus. Parag (1965), in a

consideration of the genetic structure(s) of the four incompatibility loci in *S. commune*, pondered the causes for the total distinctiveness and equivalence of all known incompatibility alleles and the apparent lack of overlapping specificities among the alleles at any of the loci. Put another way, no case is known in which, for example, two compatible wild $B\beta$ alleles are both incompatible with a third. Relationships among wild alleles of different origins are invariably

$$\begin{matrix} B\beta 1 \neq B\beta 2 \\ \neq \quad \neq \\ B\beta 3 \end{matrix} \quad \text{or} \quad \begin{matrix} B\beta 1 = B\beta 1 \\ \diagdown\!\!\diagup \\ B\beta 1 \end{matrix}, \quad \text{never} \quad \begin{matrix} B\beta 1 \neq B\beta 2 \\ \diagdown\!\!\diagup \\ B\beta 3 \end{matrix},$$

where "$\neq$" and "$=$" signify compatibility and incompatibility, respectively. Allelic differences that include dominance and overlapping function are known in the *S* alleles of certain incompatibility systems in higher plants (Lewis, 1949), but comparable allelic differences have not been reported for wild alleles in any multiple-allelic-incompatibility system in the fungi. Functional overlapping, however, is very common among mutations induced at single loci involved in the synthesis of essential metabolites, in which cases it is expressed as the failure of interallelic complementation (Giles, 1958; Lacy and Bonner, 1961; Costello and Bevan, 1964; Dorfman, 1964). This paper reports the first case in a fungal multiple-allelic-incompatibility system in which the alleles are less than totally distinct in their specificities.

If incompatibility loci are continuously mutating in nature, imperfect alleles like those described here should occur in nature. That they have not been found may reflect their rarity. Primary mutations of $A\beta$ as well as of $B\beta$ have been induced at frequencies in the order of 10^{-7} per treated nucleus, and the frequency of their spontaneous mutation may be even lower by some orders of magnitude. Although the primary mutations have a very slight selective advantage in terms of outbreeding efficiency (Raper, 1966) and would probably be maintained, they would continuously be mutating to secondaries, a process that occurs, at least under laboratory conditions, at a frequency about a hundred times higher. The selective system that has been used for the recovery of secondary mutations, however, allows expression only of unilaterally mating derivatives; nothing is known of other more nearly or completely normal allelic types that might be simultaneously generated, and these, for which no selective system has yet been devised, might well be *the* significant products of the mutative process.

Anomalous alleles probably exist in the natural population at a very low frequency. By dint of their occasional interincompatibility, they would suffer a very slight selective disadvantage and ultimately be lost, an eventuality predicted by Parag (1965) for any overlapping alleles that might occur.

The evidence to date points to a definitive number of alleles at each incompatibility locus, which, with the possible exception of the alleles of $B\alpha$, seem to occur in the natural population in approximately equal frequency (Raper, Baxter and Ellingboe, 1960; Koltin, Raper and Simchen, 1967). Raper (1966) suggested the possibility that the structure of the incompatibility loci is such as to restrict the number of alternate, functional alleles and that existing alleles could be converted, by whatever means, only into other previously existing alleles.

These considerations, however, ignore an alternate possible explanation of the interrelationships of the anomalous alleles described here. Koltin

(1967) suggests that allelic specificity may not be the determining factor in the mating interactions elicited by the mutant $B\beta$ alleles. Three separate functions must be attributed to the $B\beta$ locus or its product: (*a*) interaction between sister $B\beta$ alleles, (*b*) interaction with all $B\alpha$ alleles, and (*c*) jointly with the $B\alpha$ allele, control of the *B* morphogenetic sequence. Only the interaction between $B\beta$ alleles is dependent upon allelic specificities, whereas, alterations in either of the other two functions could conceivably lead to the minute deviations from normal, *i.e.* wild-type, behaviour characteristic of these mutations. Complementarity or the lack of it between sites involved in these functions could thus determine compatibility or incompatibility between two factors, whether their $B\beta$ alleles were interacting or not. The fact that the patterns of interactions with wild *B* factors characteristic of the *B* β(*1-1*), *B* β(*1-2*) and *B* β(*1-3*) mutations are strictly dependent upon the identity of the associated $B\alpha$ alleles might well be interpreted in such a way as to discount the importance of allelic specificity (*i.e.* in the $B\beta x$-$B\beta y$ interaction) in these mating responses.

The basic objectives of the study—to generate new, functional alleles from existing alleles and to explain their origin in nature—have not been realised. The secondary mutations encountered in this study, are clearly not equivalent to wild-type alleles. The two *B* β*2*(*1-3*) mutations represent the nearest approach to this goal, but even these mutations fail in the critical test that opposes them with the progenitor allele in association with a common $B\alpha$. Successive mutations in the $B\beta$ locus, however, should not be discounted as the route by which new alleles originate until at least two additional approaches are adequately explored. (*a*) An attempt will be made to devise a system for the selection of bilateral secondary mutations, so that an intensive search can be made for such mutations. (*b*) A system for the selection of bilateral derivatives induced in the secondary, *B* β(*1-1*) or *B* β(*1-3*), has been developed, and a hunt is now under way for tertiary $B\beta$ mutations. Either of these procedures, if successful in yielding mutant derivatives with the characteristics of wild alleles, could provide a feasible, and even possibly the correct, explanation of the origin of new $B\beta$ alleles and the extensive series in which they occur in nature.

5. Summary

1. The interrelations, *i.e.* compatibility or incompatibility, were determined for 54 secondary mutations in the $B\beta$ incompatibility locus of *Schizophyllum commune*.

2. Five secondary mutations derived from a primary mutation of $B\beta 2$ were available from previous studies. Forty-nine additional secondary mutations, induced by ethyl methane sulphonate, were recovered from two independent primary mutations of $B\beta 2$ and from one of $B\beta 7$.

3. The 54 mutations belong to three types: 51 *B* β*2*(*1-1*) and *B* β*7*(*1-1*), 1 *B* β*2*(*1-2*), and 2 *B* β*2*(*1-3*). Matings among all of the mutations resulted in incompatible reactions except those of *B* β*2*(*1-3*) $\times$ *B* β*7*(*1-1*), which resulted in dikaryosis of the *B* β*2*(*1-3*) mates.

4. All of the mutations save one (*B* β*2*(*1-2*)) are compatible with their wild-type progenitors and have most, but not all, of the attributes of new wild-type alleles. The major differences between them probably reflect the selective system used rather than any necessary characteristics of the secondary mutations.

5. New alleles at the basic incompatibility loci may originate from existing alleles by a series of successive mutations such as that described here; the selective systems used, however, did not permit the recovery of fully normal alleles.

6. References

COSTELLO, W. P., AND BEVAN, E. A. 1964. Complementation between *ad-5/7* alleles in yeast. *Genetics, 50,* 1219-1230.

DAY, P. R. 1963. Mutations affecting the *A* mating-type locus in *Coprinus lagopus. Genet. Res. Camb., 4,* 55-65.

DORFMAN, B. 1964. Allelic complementation at the *ad-5/7* locus in yeast. *Genetics, 50,* 1231-1243.

GILES, N. H. 1958. Mutations at specific loci in *Neurospora. Proc. X. Intern. Congr. of Genetics, 1,* 261-279. Montreal.

KOLTIN, Y. 1967. Structure and function of the *B* factor of *Schizophyllum commune.* Thesis, Harvard University.

KOLTIN, Y., AND RAPER, J. R. 1966. *Schizophyllum commune*: new mutations in the *B* incompatibility factor. *Science, 154,* 510-511.

KOLTIN, Y., RAPER, J. R., AND SIMCHEN, G. 1967. Genetics of the incompatibility factors of *Schizophyllum commune*: the *B* factor. *Proc. Nat. Acad. Sci. (U.S.), 57,* 55-62.

LACY, A. M., AND BONNER, D. M. 1961. Complementation between alleles of the *td* locus in *Neurospora crassa. Proc. Nat. Acad. Sci. (U.S.), 47,* 72-77.

LEWIS, D. 1949. Incompatibility in flowering plants. *Biol. Rev., 24,* 472-496.

PARAG, Y. 1962. Mutations in the *B* incompatibility factor in *Schizophyllum commune. Proc. Nat. Acad. Sci. (U.S.), 48,* 743-750.

PARAG, Y. 1965. Genetic investigation into the mode of action of the genes controlling self-incompatibility and heterothallism in Basidiomycetes. In *Incompatibility in Fungi* (K. Esser and J. R. Raper, eds.), pp. 80-98. Springer-Verlag, Berlin.

PARAG, Y., AND RAPER, J. R. 1960. Genetic recombination in a common-*B* cross of *Schizophyllum commune. Nature (London), 188,* 765-766.

RAPER, C. A., AND RAPER, J. R. 1966. Mutations modifying sexual morphogenesis in *Schizophyllum. Genetics, 54,* 1151-1168.

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

RAPER, J. R., BAXTER, M. G., AND ELLINGBOE, A. H. 1960. The genetic structure of the incompatibility factors in *Schizophyllum commune*: the *A* factor. *Proc. Nat. Acad. Sci. (U.S.), 46,* 833-842.

RAPER, J. R., BOYD, D. H., AND RAPER, C. A. 1965. Primary and secondary mutations at the incompatibility loci in *Schizophyllum. Proc. Nat. Acad. Sci. (U.S.), 53,* 1324-1332.