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*MUTATIONS IN THE B INCOMPATIBILITY FACTOR OF SCHIZOPHYLLUM COMMUNE**

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Schizophyllum commune is a heterothallic, higher Basidiomycetous fungus in which the heterothallism, or the self-incompatibility, is genetically controlled by two incompatibility factors, A and B, each with a series of alternative states (A^1, A^2, A^3, etc., and B^1, B^2, B^3, etc.). Diploid basidia $A^1A^2B^1B^2$ produce four classes of basidiospores: A^1B^1, A^1B^2, A^2B^1 and A^2B^2. A mycelium of *mating type* A^1B^1 is incompatible with A^1B^1, A^1B^2 and A^2B^1, but it is compatible with A^2B^2 as well as A^2B^3, A^3B^3, etc., with which it establishes dikaryotic mycelia, e.g. (A^1B^1 + A^2B^2). The fruit bodies develop on the dikaryotic mycelium.[1–3] The A factor is composed of two subunits, α and β, which are two linked loci separable by crossing over. Preliminary results suggested a similar two-subunits model for the B factor. There is a multiple allelic series to each subunit. The combination of specific α and β alleles determines the specificity of the A or the B factor, so that difference in one subunit leads to difference in specificity, e.g., $B^{41}{}_{3-2}$ is different from $B^{c}{}_{3-1}$ (the superscript denotes the over-all B specificity; the subscripts denote the alleles of α and β subunits).[4, 5] A common-A mating, e.g., $A^1B^1 \times A^1B^2$ results in a *common-A heterokaryon* (A^1B^1:A^1B^2), which has typically aberrant morphology (*flat*) and is unilateral (i.e., when mated with another mycelium, it only donates nuclei but does not accept nuclei from the other mycelium).[2, 6] A common-B mating, e.g., $A^1B^1 \times A^2B^1$ gives, along the region of contact between the mycelia, a *common-B heterokaryon* (A^1B^1:A^2B^1). The latter is typified by pseudoclamps, which start to develop like clamp connections but fail to fuse with the subapical cell. The pseudoclamps may continue to grow as side branches, or they may stop growing; in the latter case they can be distinguished from true-clamp connections only under high magnification of a phase microscope.[7–9]

The existence of a multiple allelic series in nature stimulated efforts to obtain, via mutations, new incompatibility alleles in the laboratory. Kniep had allegedly reported such mutations in very high frequency among single-spore isolates of Schizophyllum, but these "mutations" were interpreted later as the result of inter-subunit crossing over.[2, 10] Quintanilha[11] demonstrated a change in mating specificity in *Coprinus fimetarius*, which could be a result of mutation in the B factor. The fact that no mutants have been detected in extensive analyses of basidiospores[3-6] and of vegetative mycelia of Schizophyllum and oidia of *Coprinus lagopus*[12] suggested that such mutations are rare and that selective techniques were needed. The apparent close association of the two nuclear types in a common-B heterokaryon suggested the latter as a tool for such a selection: a mutation $B^1 \rightarrow B^x$ in $(A^1B^1{:}A^2B^1)$ should lead to dikaryotic fruit bodies $(A^1B^1 + A^2B^x)$ which would be detectable on the background of the nonfruiting heterokaryon. Preliminary results of a mutation in the B factor obtained by this technique have been briefly reported.[8] Day gave a short description of mutations that affect the A factor and that were obtained by a similar technique.[13] The present communication reports an extension of the preliminary results previously published[8] on one spontaneous mutation, as well as more recent results dealing with two more mutations obtained following a treatment with chemical mutagens. These new results give clearer information about both the nature of these mutations and their locations on the chromosome in relation to the B subunits.

Materials and Methods.—The strains used in this study came or were derived from the collection of Prof. J. R. Raper; their detailed histories have been previously described.[3, 14] Nomenclature and basic techniques (culturing, mating, testing for mating types, maceration of mycelia and media) have also been described elsewhere.[2, 4, 14] The markers used in addition to the incompatibility factors included *pol*^{r} (polymyxin resistance), *ura-1, nic-2,* and *pab* (requirements for uracil, niacin, and *p*-aminobenzoic acid, respectively). Heterokaryons were established by two methods:[7, 8] (a) isolation of heterokaryotic mycelia from the previously homokaryotic partner of *incompatible di-mon matings*,[2, 14] e.g. $(A^2B^2$ *ura-1 nic-2* $+ A^{41}B^{41}$ *pol*$^r) \times A^2B^{41}$ *ura-1 nic-2* $\rightarrow (A^{41}B^{41}$ *pol*$^r{:}A^2B^{41}$ *ura-1 nic-2*); (b) nutritional selection following transfers to minimal medium of mycelia from the region of confluence of mated homokaryons, e.g., $A^{41}B^{41}$ *pab* $\times A^2B^{41}$ *ura-1 nic-2* $\rightarrow (A^{41}B^{41}$ *pab*$:A^2B^{41}$ *ura-1 nic-2*).

In experiments involving only the untreated heterokaryons, agar blocks with mycelia were transferred to plates with fruiting medium. In others, several common-B heterokaryons were macerated and pour-plated following treatments with chemical mutagens. Untreated macerates served as controls. Treatment with nitrogen mustard proceeded as follows.[15] A heterokaryotic mycelium was macerated in liquid minimal medium, to which nitrogen mustard was added at a concentration of $0.0025M$. Thirty minutes later the macerates were washed and plated. Mycelial tissues from fruit bodies that appeared on the plates were isolated and those that fruited again were subjected to further genetic and microscopic studies.

Experimental Procedures and Results.—1. *A spontaneous mutation:* Nine common-B heterokaryons (A^2B^{41} *ura-1 nic-2*$:A^{41}B^{41}$ *pol*^{r}), independently isolated from illegitimate di-mon matings, were each inoculated to ten plates of fruiting medium and incubated for six weeks. A single cluster of fruit bodies appeared in one of the plates. Small pieces of mycelial tissue were isolated from a sterile portion of one of these fruit bodies. The mycelia that emerged from these isolates each had true-clamp connections (confirmed under high magnification of a phase microscope) and fruited within five days, i.e., it was a normal dikaryon. From the pattern of interactions of this mycelium with four testers, it was revealed to be indeed a dikaryon of genotype $(A^2B^m + A^{41}B^{41})$, with B^m indicating a B factor with possibly a new

TABLE 1

CROSSES INVOLVING MUTATED B FACTORS

a. Detailed results

Cross	Number of segregants	B factor	Morphology
Mutation S1			
1. $A^{41}B^{41} \times A^{2}B^{41} \xrightarrow{\text{mutation}} A^{41}B^{41} \times A^{2}B^{m}$	75	B^{m}	Flat
	102	B^{41}	Normal
2. $A^{2}B^{m} \times A^{41}B^{2}$	45	B^{m}	Flat
	37	B^{41}	Normal
3. $A^{2}B^{m} \times A^{41}B^{2}$	24	B^{m}	Flat
	11	B^{m}	Unilateral
	13	B^{2}	Unilateral
	19	B^{2}	Normal
4. $A^{41}B^{m}{}_{3-m} \times A^{43}B^{43}{}_{1-1}$	208	$B^{m}{}_{3-m}$*	Flat
	161	$B^{43}{}_{1-1}$	Normal
	1	$B^{c}{}_{3-1}$	Normal
5. $A^{41}B^{m}{}_{3-m} \times A^{39}B^{39}{}_{2-3}$	77	$B^{m}{}_{3-m}$*	Flat
	98	$B^{43}{}_{2-3}$	Normal
6. $A^{41}B^{m}{}_{3-m} \times A^{43}B^{43}{}_{1-1}$.	232	Parenteral B's*	
	3	$B^{c}{}_{3-1}$	Normal
Mutation NM3			
7. $A^{41}B^{41} \times A^{2}B^{41} \xrightarrow{\text{mutation}} A^{41}B^{41} \times A^{2}B^{m}$	16	B^{m}	Flat
	18	B^{41}	Normal
8. $A^{2}B^{m}{}_{3-m} \times A^{43}B^{43}{}_{1-1}$	174	$B^{m}{}_{3-m}$*	Flat
	167	$B^{43}{}_{1-1}$	Normal
	3	$B^{c}{}_{3-1}$	Normal
Mutation NM2			
9. $A^{41}B^{41} \times A^{2}B^{41} \xrightarrow{\text{mutation}} A^{41}B^{m} \times A^{2}B^{41}$	15	B_{m}	Flat
	19	B^{41}	Normal
10. $A^{2}B^{m}{}_{3-m} \times A^{43}B^{43}{}_{1-1}$	199	$B^{m}{}_{3-m}$*	Flat
	202	$B^{43}{}_{1-1}$	Normal
	8	$B^{c}{}_{3-1}$	Normal

b. Summary: recombination between the new mutations and the B region, the B subunits and the flat morphology

Characters tested	Mutation	Expected types of recombinants	Observed frequency (recombinants/total)	Crosses involved
m versus B	S1	B^{41}	0/704	2, 3, 4, 5
	NM3	"	0/341	8
	NM2	"	0/409	10
m versus *flat*	S1	B^{m} with normal homokaryotic morphology or B^{x} flat†	0/804	1, 2, 4, 5
	NM3		0/385	7, 8
	NM2		0/443	9, 10
m versus B_{β}	S1	$B^{a}{}_{1-2}$ *or* $B^{42}{}_{2-2}$	0/780	4, 5, 6
	NM3	B	0/344	8
	NM2	"	0/409	10
m versus B_{α}	S1	$B^{c}{}_{3-1}$ or $B^{23}{}_{3-3}$	4/780	4, 5, 6
	NM3	B^{c}	3/344	8
	NM2	"	8/409	10

* Can also include recombinant B: $B^{m}{}_{1-m}$ (crosses 4, 6, 8, 10) or $B^{m}{}_{2-m}$ (cross 5), indistinguishable from the parental $B^{m}{}_{3-m}$.

† B^{x} = B^{41} in crosses 1, 7, 9; B^{2} in cross 2; B^{43} in crosses 4, 8, 10; B^{39} in cross 5.

mating specificity. Among a sample of 177 monosporous progeny from a single fruit body, 102 were incompatible with B^{41} and 75 were compatible with B^{41} (cross 1, Table 1); the latter ones are therefore inferred to have a new B, designated B^{m}, as a result of a mutation coded as S1. Among these progeny, 102 were tested for

A factors and for their biochemical requirements and polymyxin resistance, and the sample showed the segregation expected from regular meiosis in the diploid basidium. The mycelia with B^m had the *flat* morphology typical of the common-A heterokaryon, and were thus easily distinguishable from mycelia having a normal B. Also, like common-A heterokaryons, the B^m mycelia were all unilateral, and, therefore, could not be tested against each other to determine compatibility of A^1B^m with A^2B^m.

2. *Induced mutations:* Two apparent dikaryons with fruit bodies emerged from separate samples of (A^2B^{41} *ura-1 nic-2*:$A^{41}B^{41}$ *pab pol*^{r}) treated with nitrogen mustard. By the methods applied above these mycelia were also both found to be true dikaryons: one, with the mutation NM2, was ($A^2B^{41} + A^{41}B^m$), and the other, with the mutation NM3, was ($A^2 B^m + A^{41}B^{41}$). Monosporous isolates from fruit bodies produced by these mycelia also showed segregation for B^{41} with normal morphology and for B^m with *flat* morphology (Table 1, crosses 7, 9).

3. *Mapping:* All three mutations occurred in B^{41} strains. Therefore, if a mutation was a suppressor outside the B region, B^{41} should appear in crosses involving B^m and any B other than B^{41}:

$$B^x \times B^{41}\ su \rightarrow B^{41} \text{ (in addition to } B^x,\ B^x\ su, \text{ and } B^{41}\ su).$$

B^{41} did not appear among 1454 segregants of such crosses involving the three B_m mutations (Table 1, crosses 2, 3, 4, 5, 8, 10). These results indicate that the three mutations occurred within the B region. Tests were also performed in an attempt to locate each of the mutations in regard to the two established subunits of the B factor, B_α and B_β.[4] If the mutation is a suppressor located within the B region, two B recombinant classes are expected from a cross such as ${B^{41}}_{3-2}\ su \times {B^{43}}_{1-1}$, but only one of these classes is expected to be recognized if the mutation occurred in one of the two subunits:

$$
\begin{aligned}
{B^{43}}_{1-1} \times {B^{41}}_{3-2}\ su &\rightarrow {B^a}_{1-2} \text{ and } {B^c}_{3-1}\\
'' \times {B^m}_{m-2} &\rightarrow {B^a}_{1-2} \text{ and } {B^m}_{m-1}\\
'' \times {B^m}_{3-m} &\rightarrow {B^c}_{3-1} \text{ and } {B^m}_{1-m}
\end{aligned}
$$

${B^m}_{m-1}$ or ${B^m}_{1-m}$ is expected to be indistinguishable from the mutated parental B,${B^m}_{m-2}$ or ${B^m}_{3-m}$. From 780 segregants of crosses involving S1 (Table 1, crosses 4, 5, 6), four recombinants, all of one class, ${B^c}_{3-1}$, were found. From 308 segregants from a cross involving NM3, only three recombinants were found, also of the above class (Table 1, cross 8). From 409 segregants from a cross involving NM2, eight recombinants were found, again only of the class ${B^c}_{3-1}$ (Table 1, cross 10). These results indicate that the three mutations occurred in the B_β subunit.

4. *The nature of the mutations:* The mutant S1 was tested with 55 different B's and was found to be compatible with all of them. The mutants NM2 and NM3 were each compatible with 55 B's with which they were tested. Since only about 64 B's are estimated in natural populations,[3] if B^m was of the same nature as the natural B factors, there is more than a fair chance that the mutated B should be identical with one of these B's. The fact that all three mutants are different from natural B's suggests that this difference is significant. The following experiments are designated to test the hypothesis that the mutation did not change B^{41} into

another B factor, but rather caused the loss of specificity of the B-factor, so that self-incompatibility in strains with B^m is controlled only by the A factor.

a. *The flat morphology:* As previously stated, B^m strains also had the *flat* morphology characteristic of common-A heterokaryons. That this is not due to accidental heterokaryotic or disomic isolates was shown by several tests. (1) No segregation occurred between B^m and *flat* among 804 progeny of crosses involving S1 (Table 1, crosses 1, 2, 4, 5), among 385 progeny of crosses involving NM3 (Table 1, crosses 7, 8), and among 443 progeny of crosses involving NM2 (Table 1, crosses 9, 10). An apparent exception occurred in cross 3 (Table 1), in which 11 B^m segregants with homokaryotic morphology were recovered. All 11 of these strains were unilateral, as were 13 B^2 strains. This suggests that a new genic expression, epistatic to B^m (in respect to morphology), is also segregating. Subsequent crosses were made between each of seven B^m segregants of cross 3 with a B^2 strain: four of these segregants were phenotypically homokaryotoic, and three were *flat*. All crosses in which *flat* mycelium was involved gave a 1:1 segregation for *flat* versus homokaryotic morphology; the other crosses in which the homokaryotic segregants were involved gave a 1:3 segregation for *flat* versus homokaryotic morphology. This again indicates that the *flat* mycelium is typical of B^m unless an epistatic gene for unilaterality is involved. Such unilateral mutations are common in *S. commune*;[6, 16] a few of them appeared independently in other B^m strains and converted them from *flat* to normal homokaryotic mycelia. (2) If somehow A^1B^m is converted to $(A^1B^x{:}A^1B^y)$ by the instability of B^m, uninucleate cells isolated from the *flat* mycelium should give rise to stable homokaryons A^1B^x or A^1B^y. Six B^m *flat* strains were successively transferred four times: at each transfer, single-celled hyphal tips were isolated from hyphae that appeared to have the morphology of the normal homokaryon. All the hyphal tips isolated in these successive transfers gave rise to *flat* mycelia. Another attempt was made to isolate one or the other component of a suspected common-A heterokaryon by dedikaryotization of a derived dikaryon ($B^m + B^2$). A dikaryon (A^2B^m pol^r + $A^{41}B^2$) was established. It was assumed that for this dikaryon each cell would contain only two nuclei and, if the A^2B^m were heterokaryotic, only one of its components would therefore exist in an individual cell of the dikaryon. When plated on polymyxin medium, the resistant A^2B^m has been shown to sector as a homokaryon.[7, 14] Nine hyphal tips were isolated from the dikaryon, and the resulting mycelia, when plated on polymyxin medium, sectored to give *flat* mycelia. These results suggest that B^m strains are self-compatible, so $A^1B^m \equiv (A^1B^x{:}A^1B^y)$, i.e., the B^m homokaryons truly have the features of common-A heterokaryons.

b. *Spontaneous dikaryosis:* If the above conclusion is correct, it might be expected that a similar mutation in the A factor would lead to complete self-fertility resulting in spontaneous dikaryosis of single-spore isolates. Truly, many segregants from crosses with S1 sooner or later became dikaryotic-like and fruited as normal dikaryons. Single-spore isolates from such a fruit body were all genetically identical and all were dikaryotic-like and fruited. Although it was later demonstrated that this was due not to a mutation in the A factor but to an *A-suppressor* unlinked to the A factor,[17] and that the mycelia had pseudoclamps instead of trueclamp connections,[9] it remains clear that A^{41} *su-A* B^m is phenotypically identical to (A^{41} *su-A* B^H: A^{41} *su-A* B^y). This again indicates that B^m is self-fertile.

c. $B^{m} \times B^{m}$ *matings:* Direct mating of $A^{1}B^{m} \times A^{2}B^{m}$ would, of course, constitute a critical test, but the unilaterality of B^{m} strains earlier prevented such tests. It was observed, however, that young segregants do not show the *flat* morphology for at least the first three days (23°). If unilaterality accompanying this morphology behaves in a similar manner, a direct test would be feasible. Well-separated germinating spores were marked 20 hr after the spreading of the spores. Twenty-eight hr later half of the young mycelium from each spore was mated with another one, and the other half was transferred for testing of its mating type. From 44 such pair matings—of which the mating type of both partners later became known—four were $A^{2}B^{m} \times A^{43}B^{m}$, and all four gave dikaryons with fruit bodies. The mycelia had true-clamp connections and binucleate cells. One fruit body analyzed gave a segregation of 10 $A^{2}B^{m}$ and 17 $A^{41}B^{m}$. This gave the direct evidence that B^{m} strains are self-fertile as far as the B factor is concerned.

5. *Relation of B^{m} to B^{41}:* All the three mutations discussed above appeared in B^{41}, and they are compatible with it as well as with all the B factors tested, which are the vast majority of native B factors. The mating reaction of B^{m} with B^{41}, however, is in most cases clearly distinct from those with all other B's. The dikaryotization of B^{41} by B^{m} is clearly retarded. In most matings of B^{m}, when mycelia with other B's are already completely dikaryotized, the periphery of the freshly dikaryotized B^{41} is predominantly homokaryotic.

Discussion.—The three B-mutations have clearly arisen independently. Each is very probably located in the B_{β} locus. The significant point is that they all lead to the loss of the control of self-incompatibility by the B factor. Since the recognizable function of the B factor is to impose self-incompatibility, it can be said that the mutation leads to the loss of the function of the B factor. Several models for the mechanism of action of the incompatibility factors have been suggested.[18] At the genetic level, these models can more or less be assigned to two groups, or to two genetic models. One is basically intragenic complementation (two alleles, each biochemically inactive, complement in compatible matings). The other genetic model is based on interallelic specificity (all alleles are equally active, but the active products are specifically different and act differently with products of identical alleles versus products of all other alleles). A differentiation between these two genetic models may eventually be made possible by the number of types and the nature of mutations that occur in the factor-subunits. Mutations resulting in the loss of factor function would be expected by either model, whereas new subunit-specificities, hence new factor-specificities, would be unique to the latter. The three mutated B's considered here are nonfunctional, and the hoped-for differentiation can be achieved only after new active alleles are obtained via mutations or after enough information is accumulated to suggest that new alleles cannot be obtained. It is expected that the use of mutagens, like base analogues and nitrous acid that cause single-base changes in the DNA molecule, would increase the chance of obtaining "missense"[19] mutations—consequently mutations with new B specificities—if it is at all possible. The present study, therefore, included preliminary treatments of common-B heterokaryons with nitrous acid, 2-amino purine, and 5-bromouracil (negative results to date). In further study effort will be concentrated on the use of these mutagens.

An interesting feature of the B mutants is their mimicry of the common-A hetero-

karyon. This should be expected if the B factor lost its specificity. A single-spore isolate has one A factor and an inactive B factor, as though the strain has one A and at least two different B's: $A^1B^m = A^1B^1B^2 = (A^1B^1{:}A^1B^2)$. In spite of the unilaterality of these *flat* single-spore isolates, in certain conditions crosses $A^1B^m \times A^2B^m$ were successful, and the segregation of mating types was that of *bipolar* fungi (i.e., self-incompatibility is controlled by one factor). Only two mating types were found in such a cross: A^1B^m and A^2B^m. Does this mean that the mutation converted a tetrapolar organism into a bipolar one? The answer is clearly negative. Homokaryons of bipolar fungi do not have the characteristics of common-A heterokaryons as do these B-mutants. The latter apparently are still genetically informed that they are basically tetrapolar, in spite of the loss of the function of the B factor. Similarly, the mating behavior of a strain carrying a suppressor for A (e.g., $A^1 su\ A\ B^1$) is different from the mating behavior of a bipolar fungus.[17] A strain carrying both a mutated B and an A-suppressor (e.g., $A^1\ su\ A\ B^m$) is similarly quite different from either a homokaryon or a dikaryon of a homothallic (self-fertile) fungus.[9] The lack of complete correspondence between such factor-deficient tetrapolar strains and wild strains of bipolar and homothallic species suggests that bipolarity is different from tetrapolarity not only by having one less incompatibility factor and that homothallism is different from both not only by lacking both factors. It is possible that tetrapolarity versus bipolarity versus homothallism is controlled by gene(s) other than the incompatibility factors in a manner similar to the control of heterothallism versus homothallism in yeast.[20] The available mutated-B's and A-suppressors can be used for further investigations of the interrelationships between tetrapolarity, bipolarity and homothallism.

It is not yet clear why young mycelia of B^m strains can accept nuclei whereas more mature B^m strains cannot. It is even uncertain that the age of mycelia is responsible. That unilaterality can be overcome at least by using young segregants has practical significance: since crosses $B^m \times B^m$ are now feasible, they can be used for selecting intragenic recombinants in the B_β locus. If, for example, S1 and NM3 occurred in different sites of the locus B_β, rare recombinants can be selected on the basis of their wild-type morphology. One of the recombinant classes resulting from crossing over between these sites is expected to have a normal B factor, therefore, a normal homokaryotic morphology. This might open the way to investigations of the fine structure of B_β gene, thus providing information about the genetic basis of the mode of action of the B factor.

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GENETIC STUDIES ON MUTANT ENZYMES IN MAIZE, II. ON THE MODE OF SYNTHESIS OF THE HYBRID ENZYMES

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Maize tissue heterozygous for two different alleles of the *E* gene, which controls the specificity of a particular esterase enzyme, always forms a new enzyme in addition to the enzyme types specified by the two alleles when in homozygous condition.[1] These hybrid enzymes are found only in the heterozygotes. The esterase types specified by the different alleles are distinguishable by their electrophoretic mobilities. Three alleles, designated E^F, E^N, and E^S, were described in the earlier publication, and the three heterozygous combinations E^F/E^N, E^F/E^S, and E^N/E^S each form a different hybrid enzyme having an electrophoretic migration rate intermediate between that of the enzyme types specified by the parental alleles. Comparison on the relative intensities of the three enzyme bands of the heterozygotes in endosperm and seedling tissue, where the dosage relations of the two alleles can be varied, suggested that the enzymes are composed of two subunits. The subunits are considered to be identical in the enzyme types formed in homozygotes (*FF*, *NN*, and *SS*) but different in the hybrid enzymes (*FN*, *FS*, or *NS*). Since in our studies the heterozygotes are usually formed as F_1's by crossing two homozygotes, the *FF*, *NN*, and *SS* enzyme bands are referred to as the *parental types* for simplicity.

This paper deals with the timing of the event responsible for the doubled nature of the enzyme molecule; does it precede or follow synthesis? Specifically, the question asked is as follows: Is the enzyme synthesized as a double molecule or is it synthesized as a monomer with dimerization of two monomers occurring after synthesis is completed? The study was made possible by our finding that a part of