THE GENETIC STRUCTURE OF THE INCOMPATIBILITY FACTORS OF SCHIZOPHYLLUM COMMUNE: THE B FACTOR*

BY YIGAL KOLTIN, JOHN R. RAPER, AND GIORA SIMCHEN

BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY, AND DEPARTMENT OF GENETICS, UNIVERSITY OF BIRMINGHAM, BIRMINGHAM, ENGLAND

Communicated November 21, 1966

Multiple-allelic incompatibility factors at one locus or at two unlinked loci were reported almost 50 years ago as the typical systems determining self-sterility and the patterns of interstrain mating of the higher Basidiomycetes.[1, 2] The concept of multiple series of alleles at one locus or at two independent loci was accepted for almost 30 years, and during this time, the origin of new, nonparental incompatibility factors was attributed to mutations.[3] This acceptance was challenged by Papazian,[4, 5] whose results, with the *A* incompatibility factor of *S. commune*, related the origin of nonparental factors to meiosis by the demonstration that new factors, when crossed, yield among their progeny the original factors at a low frequency. A later study[6, 7] showed beyond any doubt that nonparental factors originate through recombination, the nonparental factors of any cross always assignable to two classes, each self-sterile but cross-fertile with the other. The *A* incompatibility factor was found to be made up of two linked loci, each comprising a series of alleles, with compatibility of two *A* factors being dependent upon allelic difference(s) at either locus or at both loci. Later studies with *Coprinus lagopus*[8, 9] and *Collybia velutipes*[10] provided a similar picture.

A study of the *B* factor of *S. commune* was initiated in 1955 to test the *B* factor against the basic two-locus model found in the *A* factor,[6] and preliminary results with three *B* factors showed a high degree of structural similarity between the *A* and *B* factors. Analysis of the progency of additional pairings of *B* factors has brought this generalization into question, however, since (*a*) recombinants were detected in fewer (7 of 23) random crosses between *B* factors than was expected, and (*b*) the interrelations between these factors could not be explained simply on the basis of a two-locus model.[11] A close parallel to this case was found in *Pleurotus ostreatus*[12, 13] and *Pleurotus spodolecus.*[10, 14] Until recently, the further study of the structure of the *B* factor was impractical because of the low frequency of recombination, in the absence of any closely linked, bracketing markers that could afford some selective enrichment for intrafactor recombinations. The recovery and characterization of a secondary mutation in the $B\beta$ locus,[15] which gave one mating response when mated with a *B* factor having the same $B\alpha$ allele and a different response when mated with a *B* factor having an unlike $B\alpha$ allele, provided a new means of discrimination of *B*-factor structure and stimulated the renewal of the study. The availability and use of this mutant was expected to increase greatly the efficiency of the work, since it should permit the identification of alleles at the $B\alpha$ locus of the entire collection of *B* factors.

The main objective of this study was to clarify the structure of the *B* factor and to seek a general model for the structure and function of the factor.

Materials and Experimental Procedures.—The strains of *S. commune* used were either the original strains from a world-wide collection[16] or strains that were outcrossed for one or more

generations to free them from morphological mutations that interfere with mating interactions. A total of 59 interfertile *B* factors was employed.

Two mutations used for preliminary identification of the $B\alpha$ locus were both derived from a primary mutation of the $B\beta 2$ allele originally described by Parag[17] and kindly provided by him: (*a*) the secondary mutant in the $B\beta$ locus described by Raper *et al.*[15] henceforth designated as $B\beta 2(1\text{-}1)$ (*b*) a different secondary mutation in the $B\beta 2$ allele recovered from material treated with unfiltered 100-kv X ray (dosage *ca.* 180,000 r).[18] This secondary mutation will henceforth be referred to as $B\beta 2(1\text{-}2)$. (The first number in parentheses is the code number of a primary mutation; a second number, when present, is the code number for a mutation generated from a primary mutant allele.) Homokaryons carrying either of the two secondary mutations, $B\beta 2(1\text{-}1)$ or $B\beta 2(1\text{-}2)$, are morphologically normal, in contrast to those carrying the primary mutation, $B\beta 2(1)$, which mimic common-*A* heterokaryons. The two secondary mutations differ, in matings between strains having compatible *A* factors, however, in that $B\beta 2(1\text{-}2)$ is incompati-ble, i.e., forms a common-*B* heterokaryon, in interactions involving a homoallelic $B\alpha$ countepart, whereas $B\beta 2(1\text{-}1)$ is unilaterally compatible, i.e., acts only as a donor of nuclei to form a dikaryon, when mated witha ny strain having a different *B* factor that carries a common $B\alpha$ allele.[15]

All crosses, spore isolations, and test matings were performed on a medium containing 20 gm glucose, 2 gm peptone, 2 gm yeast extract, 0.46 gm KH_2PO_4, 1.0 gm K_2HPO_4, 0.5 gm $MgSO_4$, and 20 gm agar per liter of distilled water.

The crosses were incubated for a period of 72 hr at 30°C to hasten the establishment of the dikaryon and were then returned to room temperature until sporulating fruiting bodies were obtained. Prior to the isolation of spores, the fruiting cultures were incubated for a period of 18–24 hr at 30°C to increase the frequency of recombination.[7] The first spores shed after the incubation period were isolated and subjected to analysis. The mating types of the progeny were determined in matings with appropriate tester strains. Matings were incubated at 30°C and were scored at 72 hr, at which time clear interactions were obtained.

Identification of the allelic composition was performed in a number of sequential steps. In the following examples, the *A* factors are compatible and are omitted except where they play a role in the screening process, and a two-locus model for the *B* factor is assumed.

1. The secondary mutations were associated by recombination with different known $B\alpha$ alleles to identify the $B\alpha$ alleles among the unknown *B* factors:

	Mutant Testers	
Native *B* factors	$B\alpha x\text{-}\beta 2(1\text{-}1)$	$B\alpha x\text{-}\beta 2(1\text{-}2)$
$B\alpha x\text{-}\beta x$	+/−	B
$B\alpha x\text{-}\beta y$	+/−	B
$B\alpha y\text{-}\beta y$	+/+	+/+
$B\alpha y\text{-}\beta z$	+/+	+/+

"Plus" denotes a compatible *B* reaction; "minus," no reaction; and "B," a common *B* reaction. Symbols to the left and right of the diagonal specify the responses of the strain at left and of the tester strain, respectively.

Either secondary $B\beta$ mutation was then associated by recombination with $B\alpha$'s other than $B\alpha x$ i.e.,

$$B\alpha y\text{-}\beta y \times B\alpha x\text{-}\beta 2(1\text{-}1) \text{ or } (1\text{-}2),$$

to obtain the recombinant $B\alpha y\text{-}\beta 2(1\text{-}1)$ or $(1\text{-}2)$. This could then be employed in the manner described above to identify all *B* factors carrying $B\alpha y$. With knowledge of the $B\alpha$ alleles, crosses among *B* factors heteroallelic for $B\alpha$ could then be made with reasonable assurance of the recovery of recognizable recombinants. For example, among the four compatible *B* factors in the tabulation above, factor 1 is known to be heteroallelic for $B\alpha$ with factors 3 and 4; therefore, it must be heteroallelic for $B\beta$ with either 3 or 4 or with both.

2. In screening for nonparental *B* factors, the following test was used, e.g., for the progeny of the cross *A1 B1* × *A2 B2*:

Progeny	Tester Strains A1 B1	Tester Strains A2 B2	
A1 B1	−	+	
A2 B2	+	−	
A2 B1	−	F	
A1 B2	F	−	
A1 Bx	F	+	Nonparental *B* factor
A2 Bx	+	F	Nonparental *B* factor

"F" denotes a common-*A* interaction; other symbols as above. This test does not distinguish nonparental *A* factors.

3. The different classes of nonparental *B* factors among the progeny of a single cross were then more precisely identified by either of two procedures.

(*a*) The nonparental-*B*-factor progeny of the cross, e.g., $B\alpha x$-$\beta x \times B\alpha y$-βy, were mated in all combinations to differentiate the classes of progeny (two loci assumed):

Recombinant factors	$B\alpha x$-βy	$B\alpha y$-βx
$B\alpha x$-βy	B	+
$B\alpha y$-βx	+	B

(*b*) The secondary mutations used for the identification of the $B\alpha$ alleles in wild factors could also be employed for the recognition of $B\alpha$ alleles in recombinant-*B* classes:

Recombinant factors	Mutant Testers $B\alpha x$-$\beta 2(1\text{-}1)$	Mutant Testers $B\alpha x$-$\beta 2(1\text{-}2)$
$B\alpha x$-βy	+/−	B
$B\alpha y$-βx	+/+	+/+

Strains that gave identical responses to the mutant testers were then mated among themselves to detect more subtle differences that might be attributed to additional loci.

4. Recombinants originating in crosses between different pairings of *B* factors were intermated (*a*) to identify common recombinant classes and (*b*) to ascertain that the above-mentioned procedures detect all relevant genetic differences. It was assumed that additional differences might result in compatible interactions between strains with common alleles at the two major loci, $B\alpha$ and $B\beta$.

5. The recombinant *B*'s were mated with all available *B* factors to identify additional native $B\alpha$-β combinations.

Experimental Results.—The association of $B\alpha 1$, $B\alpha 2$, $B\alpha 3$, and $B\alpha 6$ with $B\beta 2(1\text{-}1)$ and with $B\beta 2(1\text{-}2)$ permitted the identification of the $B\alpha$ alleles of about half of the 59 *B* factors tested. Difficulties were encountered in the incorporation of the mutant $B\beta$ alleles with $B\alpha$ alleles other than those cited. Spore samples large enough to provide resolution of 0.3 per cent recombination yielded none of the expected recombinants, and experiments are presently being conducted to resolve this problem. A possible reason for these difficulties will be discussed later.

The identification of the $B\alpha$ alleles made possible studies of recombination between factors heteroallelic for $B\alpha$ and thus the identification of the $B\beta$ alleles present (Table 1). Certain of the recombinants obtained in these crosses proved to be incompatible with a number of additional native factors. The allelic constitutions of 30 *B* factors have been established by two methods, recombination and incompatibility with newly generated recombinants. The wide variation in frequency of recombination between the loci of the *B* factor, 0.1–8.0 per cent, with a mean of 2.3 per cent, is reminiscent of previous reports of comparable variation in the *A* factor of *S. commune*.[6, 7] The samples used throughout these studies pro-

TABLE 1

ALLELIC CONSTITUTION OF NATIVE *B* FACTORS OF *Schizophyllum commune*

Par. 1 αx-βx		Par. 2 αy-βy	Origin	Sample	Recombinants αx-βy	Recombinants αy-βx	Per cent	Factor Constitution αy	Factor Constitution βy
B43			Alabama					1	1
*"	×	*B42*	Mass.	416	2	3	1.2	2	2
*"	×	*B41*	Mass.	236	3	1	1.3	3	2
B41	×	*B10*	Calif.	298	3	1	1.3	1	4
"	×	*B105*	Argentina	305	2	2	1.3	1	5
"	×	*B39*	Mass.	314	1	3	1.2	2	3
"	×	*B69*	N. Carolina	300	3	4	2.3	2	5
"	×	*B4*	Illinois	174	5	6	6.3	2	6
"	×	*B38*	Mass.	308	8	7	4.8	2	7
"	×	*B56*	Mass.	243	2	2	1.6	4	6
"	×	*B118*	N. Carolina	366	3	8	3.0	4	7
"	×	*B95*	Ghana	468	2	1	0.6	5	5
"	×	*B9*	Calif.	198	1	2	1.5	5	6
"	×	*B3*	New York	371	9	6	4.0	5	7
"	×	*B50*	Wisconsin	568	1	4	0.8	6	1
"	×	*B65*	Penna.	242	4	1	2.0	6	4
"	×	*B79*†	Mozambique	692	0	1	0.1	6	5
"	×	*B53*	Mass.	392	5	5	2.5	6	6
"	×	*B97*	Costa Rica	200	6	10	8.0	6	7
"	×	*B77*	Germany	580	4	4	1.3	7	4
"	×	*B127*†	England	379	3	0	0.8	7	7
B97	×	*B23*	Mass.	333	1	5	1.8	3	3
B23	×	*B24*	Mass.	295	2	1	1.0	1	2
B42	×	*B26*	Mass.	305	3	2	1.6	3	1
B4	×	*B6*	Wisconsin	153	7	3	6.5	3	7
B$\alpha 3$-$\beta 2$*(1-2)*	×	*B32*	Mass.	266	1	1	0.7	6	2
		B37‡ = αx-βy, *B41* × *B69*						3	5
		B14‡ = αx-βy, *B41* × *B4*						3	6
		B111‡ = αy-βx, *B42* × *B9*						5	2
		B76‡ = αy-βx, *B41* × *B77*						7	2

* Results obtained in earlier studies.[6]
† Although only one recombinant class was obtained, the factor was identified later by a common-*B* interaction with a newly derived factor.
‡ Allelic constitution identified by incompatibility with recombinant factors.

vided a minimal resolution of 0.28 per cent, almost ten times greater than the average frequency of recombination.

In each case in which recombinants were detected, it was possible to classify them into two intrasterile, interfertile classes. This fact supports the view that the *B* factor, like the *A* factor, is basically constituted of two linked loci. This view was further substantiated by the results of crosses between factors with known allelic constitution. In each of many such crosses, the identities of the recombinant progeny were correctly forecast, e.g.:

Parents	Recombinants
B4 $\alpha 2$-$\beta 6$ × *B118* $\alpha 4$-$\beta 7$	**B38 $\alpha 2$-$\beta 7$** and *B56* $\alpha 4$-$\beta 6$
B69 $\alpha 2$-$\beta 5$ × *B97* $\alpha 6$-$\beta 7$	**B38 $\alpha 2$-$\beta 7$** and *B79* $\alpha 6$-$\beta 5$
B42 $\alpha 2$-$\beta 2$ × *B6* $\alpha 3$-$\beta 7$	**B38 $\alpha 2$-$\beta 7$** and *B41* $\alpha 3$-$\beta 2$

To rule out the possibility of the involvement of additional loci, the *B* $\alpha 2$–$\beta 7$ factors derived in the three different crosses between six distinct factors were mated among themselves; in each case, the result was a common-*B* interaction. This test was applied to all recombinants possessing common α and β alleles (Table 2). All the interactions among strains having common alleles at both loci produced common-*B* interactions. Thus, among the crosses that yielded recombinants, no exception to the two-locus model has been detected.

TABLE 2

ALLELIC CONSTITUTION OF RECOMBINANTS DERIVED FROM CROSSES OF DIFFERENT NATIVE FACTORS

Cross	No. recombinants tested	Allelic Constitution of Tested Recombinants α	β	α	β
B10 α1-β4 × *B41 α3-β2*	3	3	4	1	2
B65 α6-β4 × "	5	"		6	2
B77 α7-β4 × "	4	"		—	
B105 α1-β5 × "	3	3	5	1	2
B69 α2-β5 × "	7	"		2	2
B95 α5-β5 × "	3	"		5	2
B4 α2-β6 × "	10	3	6	2	2
B56 α4-β6 × "	4	"		4	2
B9 α5-β6 × "	3	"		5	2
B53 α6-β6 × "	10	"		6	2
B38 α2-β7 × "	15	3	7	2	2
B118 α4-β7 × "	10	"		4	2
B3 α5-β7 × "	15	"		5	2
B97 α6-β7 × "	1	"		—	
B127 α7-β7 × "	2	"		—	
B50 α6-β1 × "	4	6	2	—	
B79 α6-β5 × "	1	"		—	

TABLE 3

REPRESENTATIVE CROSSES INVOLVING "NONRECOMBINING" *B* FACTORS

"Nonrecombining" factors	"Recombining" factors		Sample
B2 × *B1*			986
" × *B18*			374
" × *B13*			384
" × *B16*			347
"	× *B42 α2-β2*		461
"	× *B41 α3-β2*		329
"	× *B α3-β2(1)*		288
"	× *B α3-β2(1-1)*		2069
"	× *B α3-β2(1-2)*		371
		Total	5609

The sample of 30 *B* factors from which recombinants have been obtained contains 7 *Bα* alleles and 7 *Bβ* alleles, all of which have been identified through recombinational studies.

Numerous attempts have been made, with samples providing resolution of 0.25–0.06 per cent, to analyze the remaining 29 factors, but all efforts have thus far failed. A total of 81 crosses among these 29 factors and with factors known to recombine have been analyzed. The summed sample of these crosses was 30,572, or an average of 377 progeny per cross. Representative crosses are detailed in Table 3. In practice, the *B* factors thus belong to two categories, "recombining" and "nonrecombining."

Any assumption about the existence of two separate types of *B* factors, however, must reconcile the number of factors in the population and the number of alleles identified at the two loci. An estimate of the first can be derived from the random sample of *B* factors previously collected for the estimation of the number of *A* and *B* factors in the total natural population[16] plus some 13 additional factors that have since been added; an estimate of the second can be derived from the recombinational studies given above. The method used here for the estimation of the number of factors in the population is based directly on the Poisson distribution rather than on Wright's formula,[19] which has been previously applied to a similar problem.[16] The estimates obtained by this method, although somewhat higher, are in reasonable

TABLE 4

ESTIMATION OF THE NUMBER OF B FACTORS IN THE POPULATION BASED ON A SAMPLE OF 126 NATIVE STRAINS OF *S. commune*

	Observed						Estimated	
	n_1	n_2	n_3	n_4	n_5	n_6	N	Range
All B factors	36	13	8	6	2	1	90	83–99
"Recombining" B's	16*	6	4	5	2	0	42	36–50
"Nonrecombining" B's	20*	7	4	1	0	1	50	39–72

* These groups include seven B factors that were included in the initial survey and estimate[16] but have been lost. Because of the almost equal frequency of the "recombining" and "nonrecombining" groups, the seven were added, three to the former and four to the latter.

agreement with those calculated with Wright's formula; the present method is preferred, however, as it avoids the difficulty of estimating sample variance. The general population is regarded as having NB factors at approximately equal frequencies. In the sample obtained in the laboratory, n_i factors are represented i times, that is, n_1 are represented once, n_2 twice, and so on. Thus the n_i frequencies are expected to be distributed as a Poisson series except for the n_0 frequency, which is unknown. The latter value represents the factors which are not represented in the sample. Since $N = \sum_{i=0}^{\infty} n_i = n_0 + n_1 + n_2 + n_3 + \ldots$, we can estimate N by assigning a value to n_0 that will conform with a Poisson distribution. All whole numbers from 0 to 99 are successively taken as n_0, and the goodness of fit of each n_i distribution to a Poisson series is then examined by calculation of the appropriate chi-squares. This procedure has been carried out separately for the "recombining," the "nonrecombining," and the total sample of B factors, as given in Table 4. In the calculation of chi-squares, grouping of the values from n_3 upward was necessary as the expected values fell below 5. Thus chi-square values for two degrees of freedom were obtained, the minimal of which pointed to the best estimate of N (Fig. 1); the "range" in Table 4 contains all the estimates of N which do not give a significant chi-square at the 5 per cent level.

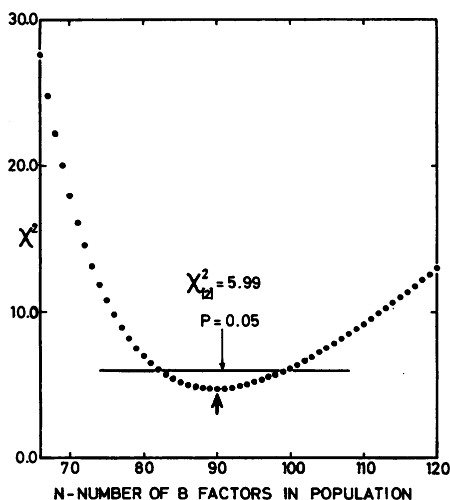

FIG. 1.—Estimation of total B factors in the population by method of minimal chi-squares. The heavy arrow indicates the best estimate of N (the minimal value of chi-square). For details, see text.

Seven alleles were recovered at each of the two loci constituting the "recombining" B factors. The $B\beta$'s appear to be equally frequent, but the $B\alpha$'s depart from equal distribution (Table 5). Therefore, the likelihood of having another $B\beta$ that is not represented in the 67 "recombining" factors analyzed is not very high. On the other hand, another $B\alpha$ will not be surprising, as the representation of $\alpha 4$, for instance, is already poor. This argumentation yields 49 (7×7) or perhaps 56 (7×8) "recombining" B factors, the former figure being within the range given in Table 4 and the latter being just within the upper limit at the 1 per cent level. This result and the agree-

TABLE 5

DISTRIBUTION OF ALLELES IN A SAMPLE OF 67 "RECOMBINING" *B* FACTORS

Bα or *Bβ* alleles	1	2	3	4	5	6	7	$\chi^2_{(6)}$*	*P*
B factors with *Bα*	12	13	19	2	6	10	5	20.6	0.01–0.001
B factors with *Bβ*	8	17	6	9	10	10	7	8.1	0.2–0.3

* χ^2 calculated to test whether the alleles are equally frequent in the population.

ment between the three sets of estimates given in Table 4 suggest that the "nonrecombining" *B* factors cannot be accounted for by combinations of the α's and β's that constitute the "recombining" group, and therefore are likely to be structurally different. That these classes may represent two distinct types of *B* factors is further suggested by differences in the interactions of "recombining" and "nonrecombining" factors with factors containing the mutations *Bβ2(1–1)* and *Bβ2(1–2)*. Details of these interactions will be reported elsewhere.

Discussion.—The results presented here suggest the existence of two types of *B* factors, which at present can be characterized as "recombining" and "nonrecombining." The "recombining" factors show a high degree of similarity to the structure and function of the *A* factor both in *S. commune*[7] and *C. lagopus*.[8, 9] Both *A* and *B* factors are composed of two linked loci, α and β, that jointly determine the mating competence of the factor, and compatibility between factors of either series is determined by different alleles at either or at both of the loci. The frequency of recombination varies among the factors when crossed with a common parent and analyzed under identical conditions. The wide variation in frequency of recombination may explain the inability to associate the secondary mutations, *Bβ2(1–1)* and *Bβ-2(1–2)*, with *Bα4*, *Bα5*, and *Bα7*. A clear distinction, however, must be made at this point between this case and that of "nonrecombining" factors. Factors containing the secondary mutations, like other "recombining" factors, have yielded intrafactor recombinants in most crosses, while the "nonrecombining" factors have not yielded such recombinants in any combination in the current study or in previous studies.[11] Additional clues to these difficulties may be provided by results obtained on numerous occasions with a few strains that have given the highest yield of recombinants. These strains consistently give a high frequency of intrafactor recombination with certain strains, but the frequency of recombination is sharply decreased to levels below 1 per cent in crosses with other strains. Recent studies both with bacteria[20, 21] and fungi[22, 23] have shown the existence of genetic control of recombination. Genetic control of recombination has also been demonstrated in the *A* factor region[24, 25] and in the *B* factor region[25] in *S. commune.* If this is the cause for the difficulties, it can be resolved genetically, and present efforts are directed toward this goal.

The lack of recombination in crosses involving the "nonrecombining" factors appears more basic than the situation described above. It would be premature to conclude that some *B* factors, although they control the same functions as do the recombining factors, may be structurally different. It is conceivable, however, in view of the possible evolution of the incompatibility system, that such a situation exists. This situation might reflect the present state of the population in terms of adaptability of the organism and a possible transition of the genes controlling the sexual process. If this were a reflection of the adaptability of local populations, however, one would expect that both *B* factors recovered from a dikaryon would be

of the same type, either "recombining" or "nonrecombining." No such correlation could be shown to exist.

The small number of alleles at both loci of the *B* factor was expected from the results of the earlier survey of the *B* factor, in which a high number of repeated factors in the random sample was found.[16] The apparent symmetry of the distribution of the alleles at the two loci, however, is in sharp contrast to the findings in the *A* factor.[7] The significance of these differences as well as the large number of alleles in the entire system is poorly understood.

In the study reported here, a part of an extended analysis of the structure and function of the incompatibility factors, we hoped to resolve the structure of the *B* factor to make possible the application of a single general model for its structure and function. At present we can with certainty apply a two-locus model similar to that of the *A* factor to 30 *B* factors. The remaining factors remain essentially an unsolved puzzle, although preliminary results have shed some light on the problem. Continuing work will be directed toward the complete characterization of this group.

The authors are indebted to Miss Anne Valerie Farnham for technical assistance, to Professor Jerome A. Schiff and Brandeis University for the use of X-ray equipment, and to Dr. M. J. Lawrence and Miss Mary Deneen, University of Birmingham, for help in programing the calculations for the computer.

* This work was supported by a National Institutes of Health grant, AI 06124.

[1] Kniep, H., *Verh. phys. med. Ges. Wurzburg*, **46**, 1 (1920).

[2] Brunswik, H., *Bot. Abh. K. Goebel*, **5**, 1 (1924).

[3] Kniep, H., *Z. indukt. Abstamm.-u. Vererb.-L.*, **31**, 170 (1923).

[4] Papazian, H. P., *Botan. Gaz.*, **112**, 143 (1950).

[5] Papazian, H. P., *Genetics*, **36**, 441 (1951).

[6] Raper, J. R., M. G. Baxter, and R. B. Middleton, these PROCEEDINGS, **44**, 889 (1958).

[7] Raper, J. R., M. G. Baxter, and A. H. Ellingboe, these PROCEEDINGS, **46**, 833 (1960).

[8] Day, P. R., *Genetics*, **45**, 641 (1960).

[9] Day, P. R., *Genet. Res. Camb.*, **4**, 55 (1963).

[10] Takemaru, T., *J. Okayama Univ.*, **7**, 133 (1961).

[11] Raper, J. R., *Genetics of Sexuality in Higher Fungi* (New York: The Ronald Press Co., 1966). The single cross *B47* × *B39*, that previously gave an unexpected class of recombinants and indicated a more complex *B* factor, has yielded no recombinants in the present study.

[12] Terakawa, H., *Sci. Papers Coll. Gen. Educ. Univ. Tokyo*, **7**, 61 (1957).

[13] *Ibid.*, **10**, 65 (1960).

[14] The distinction between *P. ostreatus* and *P. spodolecus* is difficult, and it is possible that in both cases *P. ostreatus* was studied; see Imazeki, R., and S. Taki, *Bull. Govt. Forestry Expt. Sta. (Japan)*, **53**, 69 (1952).

[15] Raper, J. R., D. H. Boyd, and C. A. Raper, these PROCEEDINGS, **53**, 1324 (1965).

[16] Raper, J. R., G. S. Krongelb, and M. G. Baxter, *Am. Naturalist*, **92**, 221 (1958).

[17] Parag, Y., these PROCEEDINGS, **48**, 743 (1962).

[18] Koltin, Y., and J. R. Raper, *Science*, **154**, 510 (1966).

[19] Dobzhansky, T., and S. Wright, *Genetics*, **26**, 23 (1941).

[20] Clark, A. J., and A. D. Margulies, these PROCEEDINGS, **53**, 451 (1965).

[21] Howard-Flanders, R., and L. Theroit, *Genetics*, **53**, 1137 (1966).

[22] Jessop, A. P., and D. G. Catcheside, *Heredity*, **20**, 237 (1965).

[23] Smith, B. R., *Heredity*, **20**, 257 (1965).

[24] Simchen, G., in press.

[25] Stamberg, J., and J. R. Raper, *Am. J. Botany*, **53**, 626 (1966).