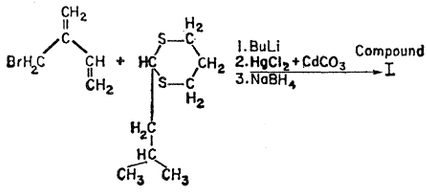

Fig. 2. Synthesis of compound I.

retention time 13.5 to 16 minutes; 1.5 mg of pure compound II was collected. A small portion (25 μg) of compound III was chromatographed again for bioassay under the following conditions: 4 percent Ucon polar on Chromosorb G; aluminum tubing, 3 m by 0.3 cm; column temperature, 110°C; detector temperature, 130°C; 7 cm^3 of He per minute; retention time 54 to 58 minutes.

Evidence for the identity of compound I has been presented (*2*).

The mass spectrum of compound II showed the following diagnostic peaks (mass/charge, *m/e*): 152 (P), 137 (P−CH_3), 134 (P−H_2O), 119 (P−33). The base peak was 43. The infrared spectrum (CCl_4 solvent) showed the following diagnostic peaks (μ): 2.98 (OH), 6.04 (C=C), 9.65 and 9.90 (C–OH), 7.27 [notched, $C(CH_3)_2$]. The nuclear magnetic resonance (NMR) spectrum (CCl_4, τ) was: 4.73 (broadened, =CH), 5.71 (broadened, CHOH), 7.5 to 8.2 (multiplet, four protons), 8.30 (singlet, =C–CH_3), 8.68 (singlet, CH_3), 8.77 (singlet, OH), 8.97 (singlet, CH_3). The ultraviolet spectrum (hexane) was: λ 215 mμ, ϵ 4000; optical rotation (0.1 percent in acetone): $[\alpha]_D^{21} = +4 \pm 3°$, and the melting point was 69°C.

The mass spectrum of compound III showed the following diagnostic peaks: 152 (P), 134 (P−H_2O), 119 (P−CH_3−H_2O), 85 (adjacent to OH, allylic to two double bonds, base peak). The infrared spectrum (film, μ) showed: 3.02 (OH), 6.26 (conjugated C=C), 9.80 (C–OH), 10.08 and 11.10 (CH=CH_2). The NMR spectrum ($CDCl_3$, τ) was 3.63 (=C–CH=CH_2, two pairs), 4.6 to 5.1 (multiplet, five olefinic protons), 5.52 (multiplet, CHOH), 7.61 (apparent doublet, CH_2), 8.30 and 8.34 (each split by small allylic coupling, two CH_3 groups), 8.40 (OH). In acetone (K_2CO_3 added), the OH peak was found as a sharp doublet (*J*, 5 cy/sec) at 6.75, and the apparent doublet of the CH_2 group became a multiplet. Decoupling (in $CDCl_3$) the proton at 5.52 collapsed the doublet at 7.61 to a singlet and altered the five-proton olefinic pattern. The ultraviolet spectrum (hexane) was: λ 226 mμ, ϵ 15,000, and the optical rotation (1.0 percent in methanol): $[\alpha]_D^{20} = +10 \pm 0.9°$.

Compound I was synthesized by the sequence shown in Fig. 2. Compound II was synthesized by $NaBH_4$ reduction of (−)-verbenone, and compound III through the sequence depicted for compound I, with the use of blocked β,β-dimethylacrolein.

The mass, infrared, NMR, and ultraviolet spectra of the synthesized samples were congruent with those of the respective isolated compounds.

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3. Infrared spectra were run on a Perkin-Elmer 221 and 137, NMR spectra on a Varian HR 100, ultraviolet spectra on a Cary 14 M, mass spectrum of compound II on a CEC 21-103C at 70 ev, and optical rotation on a Perkin-Elmer 141. The mass spectrum of compound III was run under nonstandard conditions at a microgram level on a modified CEC 21-103C instrument. Gas chromatography was carried out on an Aerograph A90P3. Analytical runs and separation on the 0.3 cm column were done on an Aerograph 204 (stream splitter and flame ionization detector).
4. Supported by Forest Service, U.S. Department of Agriculture, under contract No. 13-125 to Stanford Research Institute and grant No. 1 to University of California, Berkeley; supplementary support of work at U.C. from California Division of Forestry and from T. B. Walker and Surdna foundations.
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Schizophyllum commune: New Mutations in the B Incompatibility Factor

Abstract. *Two new mutations in the* B *factor of* Schizophyllum commune *provide additional indications regarding control by the incompatibility factors of mating and sexual morphogenesis in this and other tetrapolar fungi.*

Events leading to sexual fertility in the higher Basidiomycetes include reciprocal nuclear exchange and nuclear migration, nuclear pairing, conjugate division, and hook-cell formation and fusion (*1*). In tetrapolar forms, typified by *Schizophyllum commune*, this sequence of events is regulated by an incompatibility system composed of two series of factors, *A* and *B*, each of which is constituted of two linked loci, $A\alpha$-$A\beta$ and $B\alpha$-$B\beta$, with multiple alleles (*2*). In either series of factors, *A* or *B*, each unique combination of specific alleles at the α and β loci determines a distinct factor that is compatible with all others of the series. A homokaryotic strain carries an *A* factor and a *B* factor; mating to initiate the entire sequence in a fertile heterokaryon, the dikaryon, occurs only between strains having neither factor in common ($A \neq B \neq$). Matings between strains having one factor ($A \neq B =$ and $A = B \neq$) or both factors in common ($A = B =$) lead to the establishment of three distinct infertile heterokaryons. The major characteristics of the four types of heterokaryons, as related to the incompatibility factors, are listed in Table 1.

Mutations in the incompatibility loci were first reported in 1960 in *S. commune (3, 4, 5)* and *Coprinus lagopus* (*6*); 20 such mutations have now been characterized. All are located in the $A\beta$ and $B\beta$ loci, and each results in loss of the factor's normal discriminatory functions of self-recognition and self-sterility. This loss simulates the presence of two different normal factors of the same series and makes the homokaryon a mimic of the corresponding common-factor heterokaryon. In 1965 another mutation was detected (*5*), a secondary mutation, $B\beta 2(1\text{-}1)$, derived from a primary mutation, $B\beta 2(1)$, found earlier in the $B\beta 2$ allele of *S. commune* (*4*). (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.) This secondary mutation was morphologically normal, was capable of self-recognition, and had a new allelic specificity; it differed, however, from wild-type factors in that

reciprocity of nuclear migration in the mating response was dependent on the $B\alpha$ allele of the *B* factor in the mate.

Recently two new mutations induced by x-ray were recovered by selective methods (*4*, *5*). The mutations were derived from: (i) a homokaryon carrying *B2*, a nonrecombining *B* factor, (*7*) and thus of unknown allelic constitution; and (ii) a homokaryon carrying the primary mutation of $B\beta2(1)$ (*4*). Both mutations were mapped in the *B* factor, although only the mutation derived from the primary mutation, hereafter designated $B\beta2(1\text{-}2)$, could be mapped in a specific locus; for their characteristics and progenitors, see Table 2.

The new primary mutation, *B2mut*, resembles $B\beta2(1)$ in causing abnormal nuclear distribution in the homokaryon to mimic that of the $A{=}B{\neq}$ heterokaryon; it differs, however, in three important characteristics: (i) It has lost the ability to regulate nuclear migration and hook-cell fusion; (ii) it elicits the formation of pseudoclamps, a function normally controlled by the *A* factor; and (iii) it retains the allelic specificity of its progenitor. The uninduced operation of a system normally requiring two different *B* factors (nuclear migration), and the induction of a step usually elicited by two different *A* factors, that is, hook-cell formation (*1*), imply that close similarity exists between the products of the *A* and *B* incompatibility factors. Furthermore, it appears that specificity of the *B* factor is determined by a minimum of two active sites.

This type of mutation supports the currently preferred model for the mode of action of the system (*1*); the model postulates repression in the homokaryon of the genes, implicated in the sexual progression, by a complex formed by the products of the α and β loci of the incompatibility factors. Derepression is achieved by a competitive interaction between the products of unlike alleles, of α or β loci of the interacting strains, to form α--α or β--β complexes. According to this model, the site for self-recognition was not affected in this mutation, whereas an alteration at a different site derepressed certain functions that are normally repressed in the homokaryon.

The secondary mutation $B\beta2(1\text{-}2)$, derived from the primary $B\beta2(1)$, has regained normal morphology and self-sterility but has lost ability to interact with any $B\beta$ allele; consequently it is intersterile with all *B* factors having a common $B\alpha$. The difference between the mating interactions of this secondary mutation and those of one reported (*6*) is shown in Table 3: whereas the latter is assumed to exclude nuclei containing a common $B\alpha$ due to a preexisting state of the cytoplasm (*5*) that leads to superrepression of its system, the new mutation appears nonfunctional at the $B\beta$ site implicated in the $B\beta$--$B\beta$ interaction during mating.

Table 1. Morphogenetic effects as related to the incompatibility factors in mycelial interactions leading to heterokaryosis.

Heterokaryon	Nuclear		Conjugate division	Hook-cell	
	Migration	Pairing		Formation	Fusion
$A{=}B{=}$	No	No	No	No	
$A{=}B{\neq}$	Yes	No	Yes	No	
$A{\neq}B{=}$	No	Yes	Yes*	Yes	No
$A{\neq}B{\neq}$	Yes	Yes	Yes	Yes	Yes

* Only in the apical cells.

Table 2. Morphological and mating characteristics of mutants and their progenitors. SS, simple septa; PC, pseudoclamps; TC, true clamps.

Genotype	Homokaryotic morphology	Septal type (%)			Nuclei per cell (%)				Mating competence
		SS	PC	TC	0	1	2	3–25	
$B\beta2$*	Normal	100	0	0	10	81	7	2	Self-sterile; accepts nuclei from $B\beta2(1)$.
$B\beta2(1)$*	$A{=}B{\neq}$	98	2	0	53	23	10	14	Self-fertile; compatible with all factors, including its progenitor, but accepts nuclei from no strain.
$B\beta2(1\text{-}1)$	Normal	100	0	0	7	88	3	2	Self-sterile; accepts nuclei only from strains heteroallelic for $B\alpha$; donates nuclei to any strain with a different *B*.
$B\beta2(1\text{-}2)$	Normal	100	0	0	10	85	5	0	Self-sterile; neither accepts nor donates nuclei to strains carrying common-$B\alpha$ with any $B\beta$.
B2†	Normal	100	0	0	9	86	4	1	Self-sterile; does not accept nuclei from *B2mut*.
B2mut	$A{\neq}B{=}$	45	40	15	42	22	8	28	Self-fertile; accepts nuclei from its progenitor; does not dikaryotize its progenitor.

* From (*8*). † Allelic constitution unknown (see text).

Table 3. Mating interactions between primary and secondary mutations and wild-type *B* factors. Signs + and − denote compatibility and no reaction, respectively; *com-B*, common-*B* reaction. Symbols left and right of a diagonal specify responses of strains at left and above, respectively.

Genotype	Allelic constitution of wild-type *B* factors		
	$B\ \alpha3\text{-}\beta2$	$B\ \alpha3\text{-}\beta1$	$B\ \alpha2\text{-}\beta2$
$B\ \alpha3\text{-}\beta2$	*com-B*	+/+	+/+
$B\ \alpha3\text{-}\beta2(1)$	−/+	−/+	−/+
$B\ \alpha3\text{-}\beta2(1\text{-}1)$	−/+	−/+	+/+
$B\ \alpha3\text{-}\beta2(1\text{-}2)$	*com-B*	*com-B*	+/+

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