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Genetic variability of the wild incompatibility alleles of the tetrapolar basidiomycete *Agrocybe aegerita*

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Summary. The variability of the sexual incompatibility genes of *Agrocybe aegerita* was investigated in the homokaryotic progeny of 13 wild dikaryotic strains from five distinct European geographic origins. Results of mating tests allowed identification of 18 *A* alleles and 16 *B* alleles out of a possible 26 different alleles for each in the sample. The determination and the comparison by a contingency χ^2 test of the frequencies of allele replications between intra- and interregional matings showed no departure from a random distribution of incompatibility alleles. The allelic series estimated for the incompatibility genes of the entire population of *A. aegerita*, 30 *A* and 25 *B* aleles, are significantly less extensive than those already hypothesized for other tetrapolar hymenomycetes. However, the low variability of incompatibility genes has little effect on the outbreeding efficiency (92.6%) of this mushroom. The low variability of the incompatibility alleles and the apparent absence of intrafactorial recombination could relate to a single-locus structure of the incompatibility genes in *A. aegerita*.

Key words: *Agrocybe aegerita* – Genetic variability – Incompatibility genes – Allelic series

Introduction

The variability of sexual incompatibility genes has been intensively studied in fungi, particularly in higher basidiomycetes (homobasidiomycetes), where the multiple allele system of heterothallism encourages outbreeding as opposed to inbreeding. This multi-allelism facilitates the study of the origin, the evolution, and the genetic structure of the incompatibility genes, and can be the basis for identifying genetically distinct isolates for genetic improvement or for separating individual mycelia within a natural population.

Agrocybe aegerita is an edible basidiomycete (Agaricales) whose genetic variability is attractive because of its improvement in industrial production (Esser et al. 1974; Marmeisse 1989). Its life cycle is controlled by the tetrapolar mechanism of homogenic incompatibility (Meinhardt and Leslie 1982), which involves the two unlinked genes *A* and *B*. The dikaryon, obtained by the anastomosis (plasmogamy) of two homokaryons that are heteroallelic for both the *A* and *B* genes, is able to differentiate fruit bodies harboring basidia in which karyogamy and meiosis occur. Furthermore, *A. aegerita*, which is able to produce fruit bodies from homokaryotic mycelia (Esser and Meinhardt 1977; Meinhardt and Esser 1981), constitutes a model to study the triggering of differentiation in the presence or absence of the regulatory control of incompatibility genes.

In order to gain information about the genetic structure of the incompatibility genes of *A. aegerita*, we have examined the allelic variability of the incompatibility loci in nature. The multi-allelism of incompatibility loci (homogenic incompatibility) is not encountered in all fungi. In phycomycetes and ascomycetes, unifactorial and biallelic incompatibility systems generally exist to ensure the achievement of sexual cycles. Multi-allelic incompatibility systems, regulated by one or two loci, are a feature of basidiomycetes, of which the hymenomycetes include all the tetrapolar forms. In the latter, an extensive series of natural allelic specificities were recovered for both the *A* and *B* factors in many species. For instance, in the case of *Schizophyllum commune*, 96 and 56 different *A* and *B* alleles, respectively, were isolated from a sample of 114 homokaryons (Raper et al. 1958b). Similar results were

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Table 1. Geographic origin and source of the wild strains of *A. aegerita* studied

Collection no.[a]	Strains[b]	Geographic origin	(zone)[c]	Source
51	WT-1	Bordeaux, France	(A)	Laboratory collection
48	WT-2	Ordonnac, France	(A)	Laboratory collection
47	WT-3	Agen, France	(A)	Laboratory collection
350	(WT-4)	Brünn, Czechoslovakia	(E)	Dr. F. Meinhardt
871027	WT-5	Albaladejito, Spain	(D)	Laboratory collection
871012	WT-6	Tripoteau, France	(A)	Laboratory collection
871102	WT-7	Montpellier, France	(B)	Dr. D. Mousain
871103	WT-8	Montpellier, France	(B)	Dr. D. Mousain
871021	WT-9	Albaladejito, Spain	(D)	Laboratory collection
750905	WT-10	Camarsac, France	(A)	Laboratory collection
751002	WT-11	Bordeaux, France	(A)	Laboratory collection
750904	WT-12	Bordeaux, France	(A)	Laboratory collection
750901	WT-13	Le Mans, France	(C)	Laboratory collection

[a] The collection number corresponds to the collection nomenclature of the Laboratory of Molecular Genetics and Improvement of Cultivated Mushrooms

[b] For all the wild strains studied we disposed of the dikaryotic phase, except for strain WT-4, for which we possessed only the homokaryotic progeny. Basidiocarps were obtained from each dikaryon, and the homokaryotic progeny recovered from monosporous isolates were used for the determination of incompatibility alleles

[c] Geographic zones are: southernwest France (A), southerneast France (B), northernwest France (C), eastern Spain (D), central Czechoslovakia (E)

obtained in *Coprinus cinereus* (Day 1963), for which 31 *A* and 27 *B* alleles were recovered from 33 homokaryons, and in *Pleurotus ostreatus* (Eugenio and Anderson 1968), where 17 *A* and 20 *B* alleles were identified from 22 homokaryons. The observation of such variability gave rise to many questions about the origin of the incompatibility alleles, which was later partially explained by the genetic structure of the loci.

In the tetrapolar basidiomycetes, the genetic structure of incompatibility loci has been extensively studied in *S. commune* (Raper 1966). The separation by recombination of two closely linked genes, designated α and β, was first reported for the *A* factor (Papazian 1951) and later confirmed for both the *A* and *B* factors (Raper et al. 1958a; Raper et al. 1960). The same genetic structure was then shown for the *A* factor of *C. cinereus* (Day 1963) and for the *A* and *B* factors of *Collybia velutipes* (Takemaru 1961) and *P. ostreatus* (Eugenio and Anderson 1968). The two-gene structure model was assumed for all tetrapolar fungi, even though it could not be demonstrated for all of them, and was used to explain, to some extent, the high degree of allelic variability that affects the *A* and *B* factors. For *S. commune*, in respect to the α and β subunits of each gene, 9 $A\alpha$ and 32 $A\beta$ alleles (Raper et al. 1960) and 9 $B\alpha$ and 9 $B\beta$ alleles (Parag and Koltin 1971) have been identified thus far. Assuming that the combination of one α with one β subunit gave a unique allelic specificity, the occurrence of at least 288 different *A* alleles and 81 different *B* alleles could be inferred in the worldwide population of *S. commune*.

Thus, it became of interest to determine the degree of variability affecting the *A* and *B* alleles in *A. aegerita*, knowing that a great number of possible alleles in *S. commune* can be explained by the two-gene structure of the incompatibility factors and that, on the other hand, no genetic recombination within both the *A* and *B* factors of *A. aegerita* was found up to a frequency of 8.10^{-4} (Meinhardt et al. 1980).

In this paper, we report the identification of the *A* and *B* incompatibility alleles from 13 wild dikaryotic strains of *A. aegerita* from various geographic origins. The natural distribution of the alleles was examined within intraregional and interregional matings by determining and comparing the frequencies of allele repeats for the *A* and *B* genes. These frequencies of repeated alleles will be discussed in terms of the number of specific *A* and *B* alleles that can be calculated for the natural population of *A. aegerita*, but will also be compared to the data reported for other heterothallic basidiomycetes, in particular, those for which a two-locus structure has been shown for the *A* and *B* factors.

Materials and methods

Strains

Thirteen wild dikaryotic strains of *A. aegerita* were used in this study. The source and the geographic origin of each strain are indicated in Table 1: 10 wild dikaryotic strains were collected in France (7 in zone A, 2 in zone B, and 1 in zone C), 2 in Spain (zone D), and 1 in Czechoslovakia (zone E). A piece of the wild basidiocarps was grown on nutritive solid medium to generate vegetative dikaryotic mycelia. Each dikaryotic strain was then cultivated in vitro to obtain basidiocarps from which homokaryotic progeny were isolated. The homokaryotic progeny were used for the identification of the incompatibility alleles.

Media and culture conditions

Dikaryotic and homokaryotic mycelia were cultured vegetatively on complete solid CYM medium (Raper et al. 1972) at 25 °C in the dark. Differentiation of basidiocarps and isolation and germination of basidiospores were carried out as previously described (Labarère et al. 1989).

Determination of the A and B incompatibility alleles

To determine the incompatibility genotypes of homokaryons, two different homokaryotic mycelia were inoculated about 5 cm apart in the center of a petri dish (90 mm diameter) containing CYM and incubated at 25 °C in the dark until the two mycelia formed a large zone of contact (15–20 days, according to the strains). As the reaction type was not obvious macroscopically in *A. aegerita*, a piece of mycelium was cut off from the contact zone and examined under a microscope (630 ×). The presence of clamp connections on hyphae resulting from mating meant that a dikaryon was formed and, therefore, that the two mycelia tested had different alleles for the *A* and *B* loci ($A \neq$, $B \neq$): when false clamp connections were observed, the two mycelia had different *A* and common *B* alleles ($A \neq$, $B =$); when no clamp connections could be seen, the two mycelia had common *A* and different *B* alleles ($A =$, $B \neq$) or common *A* and common *B* alleles ($A =$, $B =$).

Statistical analyses

The distribution of incompatibility alleles found in nature was examined by contingency χ^2 analyses (Ullrich 1977) of the frequency with which isolations of identical alleles are recovered within one geographic region and between different geographic regions.

Results

Distribution of the homokaryotic progeny into incompatibility groups

Homokaryotic progeny from each of the 13 wild strains were recovered from random single-spore isolation. To determine the incompatibility groups in the progeny, all the homokaryons issuing from the same dikaryotic strain were paired with one another in all possible combinations. The homokaryotic progeny of each basidiocarp were divided into four incompatibility groups: Group I *AxBx*, Group II *AyBy*, Group III *AxBy*, and Group IV *AyBx* (Table 2). The breakdown of homokaryons within Group III or IV was determined in relation to Groups I and II by checking the presence of false clamp connections (*A* different, *B* common). The number of homokaryons studied in each offspring varied from 19 to 38 (except for the progeny of the strain WT-4). It should be noted that minimum samples of 19 or 20 homokaryons deriving from each basidiocarp were found to be sufficient to recover all four incompatibility groups. Accordingly, the results showed that all the strains studied were tetrapolar and that intrafactorial recombination events (leading to the occurrence of more than four incompatibility groups in the progeny) could never be detected.

Table 2. Distribution of the homokaryotic progeny from 13 wild strains of *A. aegerita* in incompatibility groups

Wild dikaryotic strains	No. of homokaryotic descendants studied	Number of segregants in each incompatibility group			
		I *AxBx*	II *AyBy*	III *AxBy*	IV *AyBx*
WT-1	37	11	12	5	9
WT-2	38	11	12	10	5
WT-3	19	8	4	5	2
WT-4[a]	4	1	1	1	1
WT-5	20	2	4	5	9
WT-6	20	4	8	2	6
WT-7	20	4	4	8	4
WT-8	20	5	6	6	3
WT-9	20	9	3	7	1
WT-10	19	5	2	2	10
WT-11	20	4	4	9	3
WT-12	27	6	7	5	3
WT-13	20	6	4	5	5

[a] For strain WT-4, the progeny were obtained and classified by Dr. F. Meinhardt, who provided us with one tester strain from each incompatibility group

Table 3. Alleles of the *A* and *B* incompatibility genes identified from the 13 wild strains of *A. aegerita* of distinct geographic origins

Wild strains	Geographic zones[a]	Incompatibility alleles	
		Ax, Ay	*Bx, By*
WT-1	A	*A3, A4*	*B3, B4*
WT-2	A	*A5, A6*	*B5, B6*
WT-3	A	*A7, A8*	*B4, B7*
WT-4[b]	E	*A1, A2*	*B1, B2*
WT-5	D	*A9, A10*	*B5, B7*
WT-6	A	*A11, A12*	*B8, B9*
WT-7	B	*A13, A14*	*B2, B10*
WT-8	B	*A14, A13*	*B2, B10*
WT-9	D	*A10, A15*	*B11, B12*
WT-10	A	*A9, A16*	*B9, B13*
WT-11	A	*A13, A1*	*B10, B14*
WT-12	A	*A1, A9*	*B1, B5*
WT-13	C	*A17, A18*	*B15, B16*

[a] See footnote of Table 1

[b] The progeny of WT-4 were used as reference strains for the incompatibility alleles according to Meinhardt and Leslie (1982)

Identification of the incompatibility alleles

To determine the number of alleles present in the 13 wild strains for both the *A* and *B* genes, one representative homokaryotic strain from each incompatibility group was selected from the 13 offspring, resulting in the creation of a pool of 52 tester homokaryons. These tester homokaryons were then paired with one another in all possible combinations, leading to the realization of 1,326 mating tests.

Table 4. Distribution and frequency of repeated A and B incompatibility alleles in intra- and interregional matings

Geographic combinations [a]	No. of mating tests [b]	$A=$		$A\neq$		$B=$		$B\neq$	
		No.	Frequency	No.	Frequency	No.	Frequency	No.	Frequency
Intraregional	105	5	0.0476	100	0.9523	5	0.0476	100	0.9523
Interregional	220	6	0.0272	214	0.9727	8	0.0363	212	0.9636
Total	325	11	0.0338	314	0.9661	13	0.0400	312	0.9600
Contingency χ^2 test		χ^2 A 1 $df=0.898$; $P=0.25-0.50$				χ^2 B 1 $df=0.234$; $P=0.50-0.75$			

[a] Intraregional matings represent the sum of the mating tests realized within each geographic zone (AA+BB+CC+DD+EE). Interregional matings represent the sum of the mating tests performed between each geographic zone (AB+AC+AD+AE+BC+BD+BE+CD+CE+DE)

[b] The number of mating tests was calculated using two homokaryotic incompatibility tester strains from each of wild dikaryotic strain

The mating interactions between the 13 wild strains were determined through the analysis of the 16 mating tests performed by the pairings of the four incompatibility tester strains from two wild strains. When all 16 mating tests resulted in compatible reactions, this was an indication that the two wild strains involved had different alleles for both the A and B genes. When some mating tests resulted in incompatible reactions (4, 7, 10, or 12 incompatible mating tests out of 16), it indicated that the corresponding strains had common alleles (one, two, three, or four alleles in common, respectively). The results of the mating tests allowed identification of 18 different alleles for the A locus and 16 different alleles for the B locus among 26 possible alleles (Table 3). The data also reveal that strains WT-7 and WT-8, which were collected in the same area (zone B), possess the same alleles at the A and B loci (*A13/A14, B2/B10*). Alleles *A10* and *A14* were found twice (*A10* in zone D and *A14* in zone B), and alleles *A1, A9,* and *A13* three times (*A1* in zones A and E, *A9* in zones A and D, *A13* in zones A and B). Alleles *B1, B4, B7,* and *B9* occurred twice (*B1* in zones A and E, *B4* and *B9* in zone A, *B7* in zones A and D), and alleles *B2, B5,* and *B10* were found three times (*B2* in zones B and E, *B5* in zones A and D, and *B10* in zones A and B). The geographic dispersal of the same incompatibility allele can be easily illustrated, e.g., by allele *A9*, which was recovered from strain WT-5 from Spain (zone D) and from strains WT-10 and WT-12 from France (zone A), or by allele *B1* identified in strain WT-4 from Czechoslovakia (zone E) and in strain WT-12 from France (zone A). The distribution of incompatibility alleles was further characterized by examining the frequencies of repeated alleles within and between the geographic zones.

Distribution of the incompatibility alleles

The 13 wild dikaryotic strains were assigned to five geographic regions (Table 1), each at least 500 km apart. To study the distribution of incompatibility alleles, the frequency of repeated alleles for the A and the B genes was determined by scoring the number of incompatible pairings found within intra- and interregional mating tests, considering only two incompatibility tester strains in the progeny of each of the wild strain (representative of Groups I and II). The observed frequencies were then compared by a contingency χ^2 test (Table 4). The frequencies of repeated alleles calculated from the total number of mating tests (325, using two tester strains per offspring) was 0.0338 for A and 0.04 for B. The frequencies of allele repeats in intraregional matings were the same for A and B (0.0476) and were greater than those obtained in interregional matings (0.0272 for A and 0.0363 for B). However, this difference is explained by the fact that strains WT-7 and WT-8, which have the same incompatibility alleles and originate from the same region, enhance the intraregional frequency of allele repeats. Nevertheless, no significant differences in the frequencies of repeated alleles for each of the A and B genes between intra- and interregional pairings could be demonstrated by the application of the statistical test. This result provided no evidence of a significant departure from the random distribution of incompatibility alleles.

Estimate of the number of incompatibility alleles throughout the entire population of A. aegerita

Having determined the frequency of repeated A and B alleles in the sample, it was possible to predict the number of alleles occurring in the natural population of *A. aegerita* by applying the formula of Dobzhansky and Wright (1941). Assuming that the alleles for both the A and B genes really occur with the same frequency in the population, the formula could be simplified to $p=1/n$, where p is the frequency of repeated alleles and n is the number of alleles in the population. This leads to an estimate of 30 alleles for A and 25 alleles for B with 5% limits of 17 and 62 for A, and 15 and 49 for B, respectively (Bateman 1947). The outbreeding efficiency could then

Table 5. Number of wild alleles isolated for the incompatibility genes in tetrapolar and bipolar heterothallic hymenomycetes

Species	Homokaryotic strains tested	No. of *A* alleles (fraction[a])	No. of *B* alleles (fraction[a])	References
Heterothallic bifactorial				
Agrocybe aegerita	34[b]	14 (0.41)	17 (0.50)	Meinhardt and Leslie 1982
	16	9 (0.56)	10 (0.63)	Marmeisse 1989
	26	18 (0.69)	16 (0.62)	This work
Coprinus lagopus[c]	33	31 (0.94)	27 (0.82)	Day 1963
Coprinus radiatus	44	40 (0.91)	36 (0.82)	Prévost 1982
Laccaria bicolor	10	10 (1.00)	8 (0.80)	Doudrick and Anderson 1989
Laccaria laccata	16	10 (0.63)	11 (0.69)	Doudrick and Anderson 1989
Pleurotus ostreatus[c]	22	17 (0.77)	20 (0.91)	Eugenio and Anderson 1968
Polyporus abietinus	28	26 (0.93)	23 (0.82)	Fries and Jonasson 1941
Polyporus brumalis	30	23 (0.77)	21 (0.70)	Hoffmann and Esser 1978
Polyporus ciliatus	18	14 (0.78)	16 (0.89)	Hoffmann and Esser 1978
Schizophyllum commune[c]	114	96 (0.84)	56 (0.49)	Raper et al. 1958 b
Heterothallic unifactorial				
Agaricus species[d]	20	13 (0.65)		Raper and Kaye 1978
Coprinus comatus	11	9 (0.82)		Brunswik 1924
Fomes cajanderi	36	25 (0.69)		Neuhauser and Gilbertson 1971
Fomes roseus	10	9 (0.90)		Mounce and Macrae 1937
Fomes subroseus	20	20 (1.00)		Mounce and Macrae 1937
Heterobasidion annosum[e]	106	40 (0.38)		Chase and Ullrich 1983
Polyporus palustris	50	19 (0.40)		Flexer 1963

[a] Fractions given in parentheses represent the number of alleles recovered versus the number of possible alleles in the samples
[b] The precise geographic origin of the dikaryotic strains studied in this report was not mentioned; some of the strains would have been collected from the same substrate within the same location
[c] The two-locus structure was demonstrated for the *A* factor and/or the *B* factor in these species
[d] *Agaricus* species comprised *A. edulis*, *A. bitorquis*, *A. campestris*, *A. rodmani*, and *A. bernardii*, which all showed mating interaction
[e] Several isolates from the same host within the same location were included in this study

be deduced from the predicted number of natural alleles (Raper et al. 1958 b) and resulted in 92.6% for *A. aegerita.*

Discussion

The homokaryotic progeny of the 13 wild dikaryotic strains of *A. aegerita* were always distributed into four incompatibility groups, showing no exception to the tetrapolar mechanism of homogenic incompatibility. From a total sample of 282 homokaryons analyzed, no occurrence of nonparental alleles arising by intrafactorial recombination could be observed. This is in accordance with previous works on *A. aegerita,* from which it transpires that the *A* and *B* loci must be considered, thus far, to be single genetic units in this basidiomycete (Meinhardt et al. 1980).

We identified 18 alleles for *A* and 16 alleles for *B*, among 26 possible alleles in the sample. The fractions representing the number of alleles recovered versus the number of possible alleles in the sample were 0.69 for *A* and 0.62 for *B*. Comparing these ratios with those obtained from previous works on *A. aegerita* (Table 5) reveals a heterogeneity in the fractions of alleles recovered (0.41–0.69 for *A* and 0.50–0.63 for *B*). Although these variations reflect how critical the size of the sample and the sampling techniques are, the number of alleles characterized from a finite number of *A. aegerita* strains is often significantly lower than those reported for other tetrapolar species. However, the limitation of this type of comparison is that the greater the number of homokaryons studied, the more the incompatibility alleles recovered tend to a finite number, thus decreasing the ratio of the number of alleles identified versus the number of possible alleles in the sample.

The occurrence of common alleles in strains of very different geographic origin as well as the isolation of different alleles in strains collected less than 2 km apart (e.g., strains WT-1 and WT-11 from France) strongly suggest a random distribution of incompatibility alleles in *A. aegerita,* as previously reported (Meinhardt and Leslie 1982; Marmeisse 1989). This distribution was better characterized by comparing the frequency of allele repeats in intra- and interregional matings with a contingency χ^2 test, which provided no evidence for a nonrandom distribution of incompatibility alleles. The same conclusions were drawn with the same type of analysis in *S. commune* (Raper et al. 1958 b), *Polyporus betulinus* (Ullrich 1977), and *Fomes cajanderi* (Neuhauser and Gilbertson 1971). However, the importance of the sampling techniques must be emphasized, since it was shown (Ullrich 1977) that the frequency of repeated alleles is increased by multiple isolations of fruit bodies from a small geographic area or from a single substrate. In our sample, it is not certain that the two dikaryotic strains WT-7 and WT-8 were collected from individual substrates, because they have identical alleles for both the *A* and *B* genes. Reexamination of the data omitting one of these strains resulted in a slight decrease of the frequencies of repeated alleles (0.029 for *A* repeats and 0.032 for *B* repeats, instead of 0.0338 and 0.04, respectively), and strongly increased the probability of random distribution of incompatibility alleles. Nevertheless, these frequencies are about ten times and two to three times greater, respectively, than those observed for *S. commune* (0.0029 for *A* repeats and 0.0155 for *B* repeats; Raper et al. 1958 b), but are comparable to those described in the bipolar species *P. betulinus* (0.055; Ullrich 1977) and *F. cajanderi* (0.028; Neuhauser and Gilbertson 1971). It seems, therefore, that the variability of incompatibility alleles in *A. aegerita* is significantly less extensive than that for *S. commune*.

Accordingly, the numbers of possible alleles for the incompatibility genes in the natural population of *A. aegerita* (estimated at 30 for *A* and 25 for *B*) are considerably lower than those calculated for *S. commune* (288 *A* and 81 *B;* Koltin 1978), *C. lagopus* (164 *A* and 75 *B;* Raper 1966 from Day 1963), or *P. ostreatus* (64 *A* and 190 *B,* Eugenio and Anderson 1968). A two-locus structure was demonstrated for the *A* and/or the *B* genes in these species, and it was used to argue that the extensive variability in allelic specificities could derive from the recombination of the two subunits of each factor. It thus becomes conceivable to consider that a single-locus structure for the incompatibility genes in *A. aegerita* would be an explanation for the low variability and the small number of alleles expected throughout the entire population of this basidiomycete. Despite its less extensive series of incompatibility alleles, the outbreeding efficiency of *A. aegerita* (92.6%) is not much less when compared to that of *S. commune* (98.2%; Raper et al. 1958 b) and does not represent a limiting barrier to genetic improvement programs.

It is generally accepted that multi-allelic homogenic incompatibility favors outbreeding, i.e., genetic variability and heterokaryosis. Considering that a relatively short series of alleles in *A. aegerita* manages to achieve 92.6% of outbreeding efficiency, the heterogeneity in the sizes of allelic series between the various tetrapolar basidiomycetes cannot be regarded as the only factor in outbreeding success. Based on the idea that multiple allelic incompatibility is an evolutionary step in fungal sexuality (Koltin et al. 1972), one may assume that the degree of variability of incompatibility alleles is an indicator of the genetic diversity within a single species and, to some extent, a parameter of the evolutionary outcome. In this case, *A. aegerita* would have a genetic diversity and a genomic organization simpler than those of *S. commune,* which would not justify the occurrence of numerous incompatibility alleles to optimize its heterokaryosis potential. Furthermore, the fact that the same allele was identified from two distant isolates in *A. aegerita* can reflect either a very efficient dispersal and outbreeding rate, or the independent emergence of two identical alleles in two different regions. The latter hypothesis would mean that the mechanism leading to the formation of new alleles is limited to a restricted number of allelic specificities.

In contrast to flowering plants where the incompatibility system makes inbreeding completely impossible, the homogenic incompatibility in fungi is such that the multi-allelism encourages outbreeding, while the occurrence of two factors in tetrapolar species results in only 75% of incompatible matings in the progeny and does not prevent 25% of inbreeding. The heterothallic basidiomycetes thus maintain a balance in their outbreeding-inbreeding competence. The fact that the outbreeding efficiency in *A. aegerita* is reduced to 92.6% from 98.2% in *S. commune* could be interpreted as protecting a series of genes well adapted to the environmental conditions, i.e., which would result in limiting heterokaryosis.

Although this study was undertaken to establish whether a relationship could exist between the variability of incompatibility alleles and their genetic structure, it has also resulted in the recovery of genetic markers that can be used to individualize homokaryotic strains and, in some cases, to determine their inbreeding rate.

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