Mating Type Switching in the Tetrapolar Basidiomycete *Agrocybe aegerita*

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ABSTRACT

The study of fruiting in the basidiomycete *Agrocybe aegerita* has shown that some haploid homokaryotic strains can spontaneously switch their mating specificities at the two unlinked *A* and *B* mating type factors. This event causes the dikaryotisation of primary homokaryons without plasmogamy and leads to the differentiation of sporulating fruit-bodies (pseudo-homokaryotic fruiting). For each mating type factor, the genetic analyses have revealed that: (1) parental and switched mating types segregate meiotically as Mendelian markers, (2) a total of six switched mating type factors (two parental and four nonparental) were obtained from a wild strain, (3) most of the nonparental factors have specificities differing from those of a large series of wild factors, (4) strains with the same expressed mating type can generate different specificities, (5) switching is always restricted to the same mating type in a homokaryon, (6) nonparental types can switch again, and (7) meiosis fixes the specificities to which switching can occur. This suggests, for the first time in filamentous fungi, the existence of a mechanism analogous to the mating type switching in yeasts. We hypothese that both *A* and *B* mating type regions in *A. aegerita* are constituted of three loci, one specialized in expression and two other carrying silent information. Mating type switching in homokaryotic strains would occur by copy transposition of silent *A* and *B* information into the expression loci. Moreover, we propose that during meiosis the silent loci are substituted by copies of the expressed loci.

THE life cycle of *Agrocybe aegerita* is controlled by the tetrapolar mechanism of homogenic incompatibility which involves the two unlinked factors *A* and *B* (MEINHARDT and LESLIE 1982). The dikaryon, which contains two haploid nuclear types, is obtained by the anastomosis (plasmogamy) of two homokaryons heteroallelic for both the *A* and *B* mating type factors. When subjected to favorable conditions, the dikaryon can differentiate fruit-bodies (basidiocarps) in which karyogamy and meiosis occur, whose four haploid products enter basidiospores.

The mating type (or incompatibility) factors *A* and *B* are known to regulate a series of morphogenetic events implied in the establishment and the maintenance of the dikaryotic state (reviewed in RAPER 1966; CASSELTON 1978). They could also play a role in the developmental program of higher fungi, as suggested by the DNA-binding property of the homeodomain-related motif found in the constant regions of four *b* alleles of *Ustilago maydis* (SCHULZ *et al.* 1990). Although several models have been proposed, the molecular mechanism by which the *A* and *B* factors determine the mating competence between two haploid homokaryons has not been resolved thus far (METZENBERG 1990; SCHULZ *et al.* 1990; MAY, LE CHEVANTON and PUKKILA 1991).

The complexity of interactions between two alleles is increased by the large genetic polymorphism of mating type factors in basidiomycetes (RAPER 1966). This multiallelism was partially explained in some basidiomycetes, such as *Schizophyllum commune* (RAPER, BAXTER and MIDDLETON 1958; RAPER, BAXTER and ELLINGBOE 1960) and *Coprinus cinereus* (DAY 1963), by the fact that the *A* and *B* factors are both composed of two linked genes ($A\alpha$, $A\beta$ and $B\alpha$, $B\beta$). Each gene is multiallelic and the combination of one α and one β allele gives a unique specificity for the *A* or the *B* factor (RAPER 1966; KOLTIN, STAMBERG and LEMKE 1972). However, the two-gene model, based on genetic analyses, is now challenged by the molecular cloning of the mating type *A* factor in *C. cinereus*, from which it appears that the *A* factor encompass at least three loci, each of them being functional in the expression of the *A* regulated pathway (MAY, LE CHEVANTON and PUKKILA 1991).

The multiallelism of each subunit composing the *A* and *B* factor poses questions about the origin and the evolution of the mating type alleles. Many attempts to recover new alleles from preexistent ones by mutation have failed, because all primary mutations selected for change in specificity resulted in a loss of self-recognition and in the constitutive expression of the mutated factor (reviewed in KOLTIN, STAMBERG and LEMKE 1972; KOLTIN *et al.* 1979), which could be the consequence of large deletions (MAY, LE CHEVANTON and PUKKILA 1991). Furthermore, a great molecular di-

vergence has been reported between different mating type alleles of *S. commune* (GIASSON *et al.* 1989), *C. cinereus* (MUTASA *et al.* 1990) and *U. maydis* (SCHULZ *et al.* 1990; FROELIGER and LEONG 1991). Lastly, the fact that the *Aα* gene of *S. commune* is encoded by a single locus (GIASSON *et al.* 1989) excludes silent copies of multiple *Aα* alleles, and is consistent with the fact that a mating type switching such as in yeasts (NASMYTH and TATCHELL 1980; BEACH 1983) has not been observed in this species (METZENBERG 1990).

Mating type factors of *A. aegerita* seem to behave differently. The failure to recover evidence of intrafactorial recombination in the *A* and *B* factors leads one to consider *A* and *B* as single genetic units, which could correlate with the restricted allelic series expected for the entire population of *A. aegerita* compared to other tetrapolar species (MEINHARDT, EPP and ESSER 1980; NOËL, HO HUYNH and LABARÈRE 1991). Moreover, we provide here genetic evidence of a mating type switching to new functional factors in some homokaryotic strains of *A. aegerita*.

This study was carried out on the homokaryotic progeny from three wild dikaryotic strains, that presented the well-known phenomenon of homokaryotic fruiting (MEINHARDT and ESSER 1981, 1990). We distinguished three phenotypes in homokaryotic fruiting; one of them, called pseudo-homokaryotic fruiting (PHF), was discerned after the spontaneous dikaryotization of primary homokaryons. This dikaryotization was shown to be the corollary of the emergence of switched mating types at both the *A* and *B* loci. The switched mating types were compared with specificities identified from wild strains collected in distinct geographic regions. With the goal of understanding the mechanism of switching, we studied the changes in specificities in different subcultures of the same homokaryon, and over two generations obtained from PHF and resulting from successive switch events. Further characterization of the mechanism was provided by analysing how mating type change occurred in the progeny from controlled crosses between homokaryons resulting from two successive mating type switchings. These data allow us to propose a model that shares properties with mating type switching in yeasts, and which takes into account intra- and intergenomic transposition events.

MATERIALS AND METHODS

Strains and nomenclature: Thirteen wild dikaryotic strains, collected in nature from different geographic areas (Table 1), were used to derive haploid homokaryotic mating type tester strains carrying the wild factors *A1* to *A18* and *B1* to *B16* (NOËL, HO HUYNH and LABARÈRE 1991). Homokaryotic fruiting and mating type change was studied in the homokaryotic progeny derived from the randomly chosen dikaryotic strains WT-1, WT-2 and WT-3, and in the progeny of WT-4 kindly provided by F. MEINHARDT. Homokaryons are designated with a letter (G for WT-1, R for WT-2, L for WT-3 and M for WT-4) and a number to distinguish sib-homokaryons. Auxotrophic homokaryotic Ura^- and Leu^- strains possess a UV-induced single-gene recessive mutation unlinked to the mating type genes (unpublished results). Homokaryotic mutant strains GM18 *A3 B3 ura-1*, GM27 *A3 B3 ura-1* and GM33 *A2 B3 ura-1* were derived from a cross between G30 *A3 B3* and M358 *A2 B2 ura-1*, the last strain provided by F. MEINHARDT. Homokaryotic strains GG3 *A3 B4 leu-1* and GG12 *A4 B3 leu-1* were obtained from a cross between G6 *A4 B3* and G2 *A3 B4 leu-1*. Figure 1 shows the successive generations analysed and the symbols or acronyms used to designate the different progeny and fruiting events. To avoid confusion, a distinction was made between the homokaryotic progeny obtained from the wild dikaryotic strain and those deriving from pseudo-homokaryotic fruiting, that were called PHF progeny. PHF_1 homokaryons are designated by the name of the homokaryon from which they are derived, followed by the letter h (symbolizing pseudo-homokaryotic fruiting) and a number to distinguish sib-homokaryons. PHF_2 homokaryons are designated by the name of the PHF_1 homokaryon from which they are derived and by an additional number to distinguish sib-homokaryons. For the designation of mating types, the term parental makes reference to the types expressed in the original wild dikaryotic strain, and the term nonparental to the types not expressed in the wild strain.

Media, culture and fruiting conditions: Vegetative mycelial cultures, and single basidiospore isolation and germination were performed on agar solidified CYM medium (RAPER and HOFFMAN 1974). Minimal medium MM (RAPER and HOFFMAN 1974) and MM supplemented with either 100 μg/ml uracil or 100 μg/ml leucine were used for the characterization of auxotrophic phenotypes. For screening pseudo-homokaryotic fruiting, homokaryotic strains were precultured in CYM-containing Petri dishes (90 mm) for 10 days, at 25°, in darkness and then inoculated into Erlenmeyer flasks containing chopped wheat straw enriched with liquid CYM (50% v/w). For fruiting auxotrophic strains, CYM was supplemented with 200 μg/ml uracil or leucine requirement. Incubation was continued at 25° in darkness until the mycelium had grown all over the medium (15–21 days). Flasks were then transferred to 22° with a 12-hr photoperiod for fruiting. Fruit-bodies were obtained under sterile conditions until sporulation in 30 to 90 days depending upon the strain used. In some cases, fruiting was obtained in Petri dishes with CYM agar as substrate. Before each inoculation for fruiting, the homokaryotic state of each strain was checked by appropriate mating type tests, and by examination of mycelia under the microscope (absence of clamp connections).

Isolation and germination of basidiospores: To collect basidiospores, mature basidiocarps were placed above sterile tracing paper under a glass cover. After sporulation, a piece of tracing paper supporting basidiospores was applied on the surface of solid CYM. Single basidiospores were isolated one by one using a thin glass rod with a flame-rounded tip using a stereo-magnifier (40 ×). The spores were deposited on new CYM and incubated at 25° for germination. Mycelia derived from germination constituted the haploid strains, whose homokaryotic state was verified before mating type determination.

Mating type determination: Mating type tests were carried out as previously described (NOËL, HO HUYNH and LABARÈRE 1991). Results of pairings were examined under the 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 specificities for the *A* and *B* loci ($A\neq$, $B\neq$); when

TABLE 1

Mating type, geographic origin and source of the wild dikaryotic strains used to derive homokaryotic progeny (from NOËL, HO HUYNH and LABARÈRE (1991)

Collection No.[a]	Strains[b]	Genotype	Geographic origin	Zone[c]	Source
51	WT-1	*A3, A4 B3, B4*	France, southwest	A	Laboratory collection
48	WT-2	*A5, A6 B5, B6*	France, southwest	A	Laboratory collection
47	WT-3	*A7, A8 B4, B7*	France, southwest	A	Laboratory collection
350	WT-4	*A1, A2 B1, B2*	Czechoslovakia, central	E	F. MEINHARDT
871027	WT-5	*A9, A10 B5, B7*	Spain, eastern	D	Laboratory collection
871012	WT-6	*A11, A12 B8, B9*	France, southwest	A	Laboratory collection
871102	WT-7	*A13, A14 B2, B10*	France, southeast	B	D. MOUSAIN
871103	WT-8	*A14, A13 B2, B10*	France, southeast	B	D. MOUSAIN
871021	WT-9	*A10, A15 B11, B12*	Spain, eastern	D	Laboratory collection
750905	WT-10	*A9, A16 B9, B13*	France, southwest	A	Laboratory collection
751002	WT-11	*A13, A1 B10, B14*	France, southwest	A	Laboratory collection
750904	WT-12	*A1, A9 B1, B5*	France, southwest	A	Laboratory collection
750901	WT-13	*A17, A18 B15, B16*	France, northwest	C	Laboratory collection

[a] The collection number corresponds to the collection nomenclature of the laboratory.
[b] Basidiocarps were obtained from each dikaryon, and the homokaryotic progeny recovered from single spore isolation were used for the determination of mating type alleles.
[c] The five geographic zones are located at least 500 km apart.

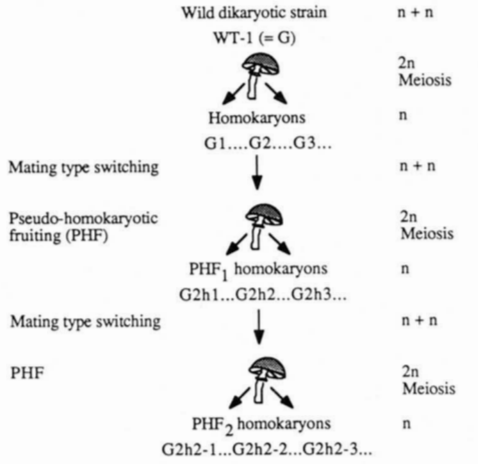

FIGURE 1.—Symbols and acronyms used to designate the generations of this study.

unfused clamp connections were observed, the two mycelia had different *A* and common *B* specificities ($A\neq$, $B=$); when no clamp connections could be seen, the two mycelia had common *A* and different *B* ($A=$, $B\neq$) or common *A* and common *B* specificities ($A=$, $B=$).

Dedikaryotization: The separation of the two nuclear components of a dikaryotic mycelium was carried out by the isolation and the regeneration of protoplasts. For protoplast isolation, strains were grown in liquid CYM in Roux flasks at 25° for 72 hr. The young mycelium obtained was harvested on sterile gauze, abundantly washed with sterile distilled water and then with the incubation buffer consisting of 0.5 M sucrose, 0.01 M $MgCl_2$, 0.1 mM EDTA, 0.05 M maleic acid, pH 6.5. About one gram of wet weight mycelium was incubated for 3 hr at 25° with 10 ml of incubation buffer containing 2.5 mg/ml Novozym 234 (Novo Industri A/S). After incubation, mycelial debris was removed from the lytic mixture by filtration through sterile gauze. Protoplasts harvested in the filtrate were concentrated by centrifugation (10,000 × *g*, 15 min, 20°) and washed twice with 20 ml of incubation buffer. They were finally layered on top of a 1 M sucrose cushion and centrifuged (300 × *g*, 15 min, 20°). For regeneration, 1-ml aliquots of diluted protoplasts (about 10^3 protoplasts/ml) were mixed with 7 ml CYM containing 1% (w/v) Sea Plaque agarose (FMC Corp.) kept at 40°, and overlayered on CYM-sucrose agar plates. About 50 mycelial colonies were picked out randomly and subcultured on CYM. The homokaryotic or dikaryotic state of mycelial colonies was determined under the microscope by checking the presence of clamp connections. Homokaryotic mycelia were then used for mating type determination. From 60% to 90% of homokaryotic isolates were recovered by this method.

RESULTS

Types of fruit-bodies differentiated by homokaryotic strains: *A. aegerita* is well known for homokaryotic fruiting (MEINHARDT and ESSER 1981, 1990), a developmental stage which bypasses the plasmogamy, generally a prerequisite for the completion of differentiation of fruit-bodies. We found that homokaryons derived from four wild strains (WT-1, WT-2, WT-3 and WT-4) could differentiate three types of fruit-bodies: fruit-bodies which never open or sporulate (Abortive Homokaryotic Fruiting, AHF), well-developed fruit-bodies with a reduced frequency of sporulation containing only two-spored basidia (True Homokaryotic Fruiting, THF) and well-developed fruit-bodies with abundant four-spored basidia (Pseudo-Homokaryotic Fruiting, PHF). AHF and THF explants gave rise to mycelia without clamp connection while PHF explants yielded mycelia bearing clamp connections.

Each strain produced multiple but restricted classes of fruiting phenotypes (Table 2). There was no preferential appearance order when both AHF and THF were produced by the same homokaryotic culture. In contrast, AHF always appeared before PHF, and it did not further differentiate. The differentiation of

TABLE 2

Fruiting phenotypes in homokaryotic progeny from four wild strains

Wild strain	No. of progeny tested	Fruiting phenotypes[a]	No. of progeny in each phenotypic class
WT-1	37	AHF	9
		PHF	2
		AHF + PHF	9
WT-2	15	AHF	8
		PHF	1
		AHF + PHF	1
WT-3	19	AHF	10
		PHF	0
		AHF + PHF	9
WT-4	7	AHF	1
		THF	3
		AHF + THF	2

[a] AHF, abortive homokaryotic fruiting; PHF, pseudo-homokaryotic fruiting; THF, true homokaryotic fruiting. Some homokaryons could differentiate two types of fruit-bodies sequentially from the same culture (AHF + PHF or AHF + THF).

PHF occurred primarily in association with the AHF phenotype (19 times over the 22 PHF observed). Further characterization of THF and PHF showed that no segregation of the mating type factors occurred in THF progeny (data not shown), while an unexpected Mendelian segregation was observed in PHF progeny. Presented here are the results of genetic analyses of PHF class.

Recovery of changed mating types from PHF_1 progeny: The segregation of the mating type factors was studied in PHF_1 homokaryotic progeny obtained from five homokaryons of WT-1, two of WT-2 and nine of WT-3. Determination of mating types in each progeny was done in two steps. First, sib-homokaryons were themselves paired in all possible combinations and classified into incompatibility mating interaction groups. Second, homokaryons from each group were paired with homokaryons of the wild strain WT-1, WT-2 or WT-3. This series of matings made it possible to place each of the progeny obtained from PHF into four incompatibility groups (see example in Table 3), typical of the Mendelian segregation of two alleles at each of two loci. The mating types originally expressed by the homokaryons prior to PHF, and changed mating types, designated *A* B**, were identified in the progeny. Thus, the G39 PHF_1 line is of *A3*, *A** and *B3*, *B** constitution. The changed mating types at both loci *A* and *B* were always compatible with the types expressed by the fruiting homokaryon and self-incompatible, indicating that they carried an alternate but functional recognition specificity.

Characterization and segregation pattern of the changed mating types in PHF_1 progeny: Among the changed mating type factors recovered in the PHF_1 progeny from 16 homokaryons (Table 4), 14 *A* and 14 *B* were nonparental, that is to say not expressed in the respective wild dikaryotic strains. As the same nonparental type could emerge in different progeny, 10 different specificities (designated by small letters) for both the changed *A* and *B* factors were identified. Surprisingly, for three homokaryons (G30 *A3 B3* from WT-1, L13 *A7 B7* and L1 *A7 B4* from WT-3), the mating types changed at either factor were parental forms expressed in the original wild dikaryotic strains (*B4* in G30, *A8* in L13 and *A8* B7 in L1). In each PHF_1 progeny, the segregation pattern of the four mating types reflect the premeiotic change of the mating specificities.

No correlation could be found between the incompatibility genotype of a homokaryon and the mating type newly expressed. For instance, *B7* from WT-3 in five different homokaryons gave rise to four nonparental types (*Be, Bf, Bg, Bh*). Furthermore, the two different mating type factors of sib-homokaryons could give rise to the same factor (*e.g.*, both *A3* and *A4* from WT-1 can yield *Ab*). On the other hand, the nonparental factors generated from the progeny of a wild strain appear to be specific to this strain. Of particular interest is *B4* which can generate *Bb* from WT-1, and *Be* (twice), *Bf, B7,* but not *Bb* from WT-3. Thus, there is some relationship between the genotype of a wild dikaryotic strain and the specificities of the mating types changed in descendants. The frequency of repeats for some newly changed mating types in the progeny of WT-3 (*A8, Af, Bf* and *Bg* are repeated twice, *Ae* and *Be* three times) suggest that the possibilities to switch are limited: considering our data, no more than six specificities (two parental and four nonparental) for each of the *A* and *B* factor can be obtained from a particular wild strain.

TABLE 3

Mating interactions and genotypes of PHF_1 progeny differentiated from G39 *A3B3*

	Incompatibility groups in PHF_1 progeny				Mating type tester strains from WT-1				
Group	I	II	III	IV	*A3B3*	*A4B4*	*A3B4*	*A4B3*	Deduced genotype
I	−	+	−	ucc	−	+	−	ucc	*A3B3*
II		−	ucc	−	+	+	+	+	*A*B**
III			−	+	−	+	−	+	*A3B**
IV				−	ucc	+	+	ucc	*A*B3*

Homokaryon G39 belongs to the progeny of the wild strain WT-1. Symbols used are: (+) compatible mating ($A\neq$, $B\neq$); (ucc) incompatible mating with presence of unfused clamp connections ($A\neq$, $B=$); (−) incompatible matings ($A=$, $B\neq$ or $A=$, $B=$). Siblings pairing (left part of the table) revealed compatibility (dikaryon formation) only between strains of groups I and II, and between strains of groups III and IV. All other combinations were incompatible. The breakdown of strains within groups III and IV was determined in relation to groups I and II by searching the presence of unfused clamp connections. The changed mating type factors are provisionally designated *A** and *B**.

TABLE 4

Identification and segregation of the mating types in 16 PHF_1 progeny

Parental wild dikaryotic strains	Fruting homokaryons derivative	Genotypes in PHF_1 progeny of incompatibility groups[a]				Segregation
		I	II	III	IV	
WT-1	G39 *A3B3*	*A3B3*	*Aa*Ba**	*A3Ba**	*Aa*B3*	12:14:11:11
A3, A4 B3, B4	G30 *A3B3*	*A3B3*	*Ab*B4**	*A3B4**	*Ab*B3*	9:9:4:4
	G31 *A4B4*	*A4B4*	*Ab*Bb**	*A4Bb**	*Ab*B4*	6:9:3:5
	G23 *A4B3*	*A4B3*	*Ac*Bc**	*A4Bc**	*Ac*B3*	5:8;10:10
	G6 *A4B3*	*A4B3*	*Ad*Bd**	*A4Bd**	*Ad*B3*	6:6:9:5
WT-3	L9 *A7B7*	*A7B7*	*Ae*Be**	*A7Be**	*Ae*B7*	12:10:10:14
A7, A8 B7, B4	L13 *A7B7*	*A7B7*	*A8*Bf**	*A7Bf**	*A8*B7*	5:6:4:10
	L14 *A7B7*	*A7B7*	*Af*Bg**	*A7Bg**	*Af*B7*	4:12:8:12
	L2 *A8B4*	*A8B4*	*Ae*Be**	*A8Be**	*Ae*B4*	5:5:9:5
	L15 *A8B7*	*A8B7*	*Af*Bg**	*A8Bg**	*Af*B7*	7:5:4:7
	L19 *A8B7*	*A8B7*	*Ag*Bh**	*A8Bh**	*Ag*B7*	9:8:4:8
	L1 *A7B4*	*A7B4*	*A8*B7**	*A7B7**	*A8*B4*	3:1:4:7
	L8 *A7B4*	*A7B4*	*Ae*Be**	*A7Be**	*Ae*B4*	6:4:10:8
	L17 *A7B4*	*A7B4*	*Ah*Bf**	*A7Bf**	*Ah*B4*	8:4:4:8
WT-2	R16 *A5B5*	*A5B5*	*Ai*Bi**	*A5Bi**	*Ai*B5*	6:7:4:7
A5, A6 B5, B6	R34 *A6B6*	*A6B6*	*Aj*Bj**	*A6Bj**	*Aj*B6*	16:9:14:12

[a] Changed mating type factors recovered in groups II, III and IV are marked with an asterisk. When their mating specificities were different from the parental types of the relevant dikaryotic strain, they were designated by a small letter (*a* to *j*). When nonparental factors from different PHF_1 progeny were found to be identical, they were designated by the same letter.

Comparison of the nonparental mating types with wild specificities isolated from nature: The nonparental factors derived from change in homokaryons from WT-1, WT-2 and WT-3 were compared to 18 different *A* and 16 different *B* wild factors previously identified from 13 wild strains (NOËL, HO HUYNH and LABARÈRE 1991). One tester strain carrying one or two non-parental factor representative of groups II, III and IV (from Table 4) were selected in each PHF_1 progeny (except that of L1), and paired with tester strains representative of the four mating type groups from each wild strain. The results of the 2,340 mating tests thus performed are summarized in Table 5.

Among the 10 nonparental *A* and 10 nonparental *B* factors, eight *A* and six *B* exhibited specificities different from that of the 18 *A* and 16 *B* wild factors tested. Identity with wild factors was found for six changed specificities: *Ag* (=*A2*) and *Ah* (=*A5*) from WT-3, for *Ba* (=*B15*) and *Bd* (=*B1*) from WT-1 and for *Bg* (=*B5*) and *Bh* (=*B2*) from WT-3. These specificities, which have emerged from mating type change in strains from the geographic zone A, display in the wild sample different geographic origins and repeat frequencies: *A2, A5* and *B15* were identified once in geographic zones E, A and C, respectively; *B1* was repeated twice in zones E and A; *B5* and *B2* were repeated three times in zones A and D, and E and B, respectively (Table 1). Overall, this demonstrates that some of the nonparental mating type factors are similar or identical to the wild factors of strains derived from various geographic regions.

Segregation of auxotrophic markers during PHF: To verify that the newly expressed mating type factors actually were derived from mating type changes in homokaryotic strains, the segregation of auxotrophic markers, unlinked to the mating type genes, was analysed during PHF. Five progeny obtained from PHF of three Ura^- (GM18, GM27 and GM33) and two Leu^- (GG3 and GG12) auxotrophic homokaryons were studied. In each of the five PHF progeny, four mating types were recovered, and all the descendants exhibited the same auxotrophy as the parental fruiting homokaryon (data not shown). Thus the change of mating type had occurred in the original strains used and was not due to contamination of the culture.

Repeatability of mating type change: To address the question whether one homokaryon can repeatedly change its mating type factors, and towards how many different specificities, four separate subcultures of the homokaryon G39 *A3 B3*, three of G30 *A3 B3* and two of each auxotroph GM18 *A3 B3 ura-1*, GM27 *A3 B3 ura-1*, GM33 *A2 B3 ura-1*, were fruited in independent conditions and at different time intervals from the first inoculation for fruiting (from two to 31 month according to the strains, data not shown). The mating type factors *A3* and *B3* of G39 always changed to the same specificities *Aa* and *Ba*, and *A3* and *B3* of G30 to *Ab* and *B4*. Likewise, change always occurred towards *Ax Bx* in GM18, *Ay By* in GM27 and *Az Bz* in GM33 (denomination *x*, *y* and *z* is used because these specificities have not been compared with each other, nor with the nonparental ones characterized in Table

TABLE 5

Results of pairings between the changed nonparental mating types and the wild mating type specificities *A1* to *A18* and *B1* to *B16*

	Nonparental mating type factors from PHF_1 homokaryons									
Locus *A*	*Aa*	*Ab*	*Ac*	*Ad*	*Ae*	*Af*	*Ag*	*Ah*	*Ai*	*Aj*
Origin[a]	WT-1	WT-1	WT-1	WT-1	WT-3	WT-3	WT-3	WT-3	WT-2	WT-2
Specificity[b]	New	New	New	New	New	New	*A2*	*A5*	New	New
Found in	—	—	—	—	—	—	WT-4	WT-2	—	—
Geographic zone[c]	—	—	—	—	—	—	E	A	—	—
Locus *B*	*Ba*	*Bb*	*Bc*	*Bd*	*Be*	*Bf*	*Bg*	*Bh*	*Bi*	*Bj*
Origin[a]	WT-1	WT-1	WT-1	WT-1	WT-3	WT-3	WT-3	WT-3	WT-2	WT-2
Specificity[b]	*B15*	New	New	*B1*	New	New	*B5*	*B2*	New	New
Found in	WT-13	—	—	WT-4 WT-12	—	—	WT-2 WT-5 WT-12	WT-4 WT-7 WT-8	—	—
Geographic zone[c]	C	—	—	E, A	—	—	A, D	E, B	—	—

[a] Wild strains WT-1 *A3, A4 B3, B4*, WT-2 *A5, A6 B5, B6* and WT-3 *A7, A8 B4, B7* were collected in the geographic zone A.

[b] The term new was used to designate the specificities of the nonparental factors that did not show identity with those of the wild factors tested.

[c] Geographic zones A, B, C, D, E are defined in Table 1.

4). This clearly shows that each homokaryon is restricted to change to only one mating type.

Hypothesis for mating type change: Specific changes of the mating types can be selected when homokaryons are subjected to fruiting conditions through the PHF differentiation. The Mendelian segregation of changed mating types in PHF progeny is thus the consequence of premeiotic changes in mating type specificities. What is the mechanism for change? A puzzling contradiction arises between the number of different factors potentially expressible in the whole progeny of a wild dikaryotic strain (six for each *A* and *B*), and the appearance of a unique form from multiple independent switch events in a specific homokaryon (example G39). This suggests the existence of several potentially expressible *A* and *B* factors stored as silent copies in the genome. The following model can be proposed (Figure 2): considering that collectively six specificities (two parental and four nonparental) for each of the *A* and *B* genes are present within the progeny of a wild strain, the existence of three loci for each mating type factor *per* haploid genome can be inferred, one specialized in expression (locus shaded) and the two others carrying silent information (Figure 2A). During meiosis in the wild strain, any diploid nucleus should carry originally two different expressed forms at the expression loci and four different nonexpressed forms at the silent loci. In some homokaryons of the progeny, the possible switching between the two factors originally expressed in the dikaryotic strain (*B4* from *B3* of G30, *A8* from *A7* of L1 and L13, and *B7* from *B4* of L1, Table 4) requires the existence of these two factors in the same haploid nucleus but in a cryptic state (Figure 2B). Therefore, with respect to the three-locus structure, we must assume that during meiosis of a dikaryotic

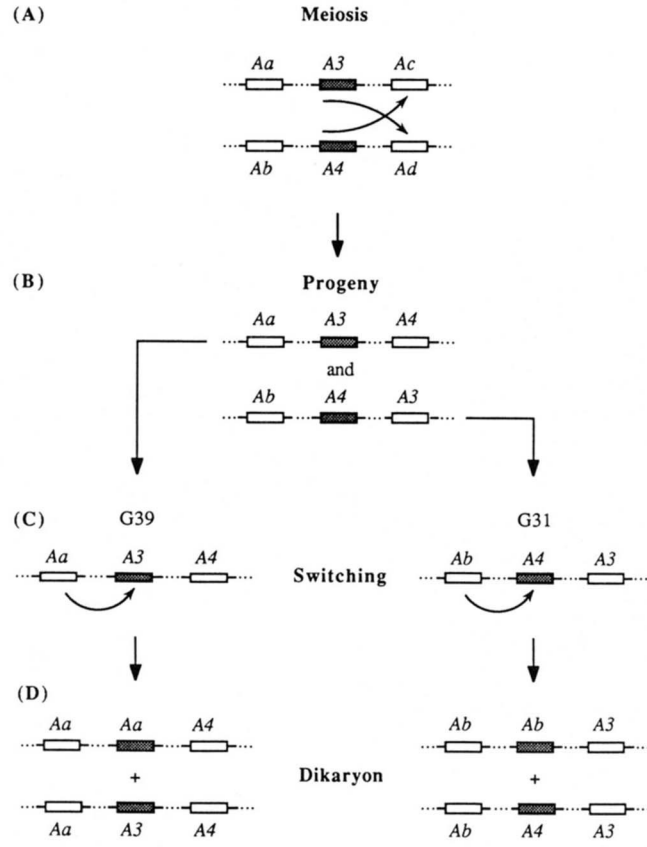

FIGURE 2.—The three-locus structure hypothesis for mating type in *A. aegerita* (example for the *A* factor). (A) Diploid nucleus *A3/A4* of WT-1 during meiosis: intergenomic exchanges of copies of the loci expressed (*A3* and *A4*) and their storage in silent loci (*Ad* and *Ac*). (B) Two haploid nuclei (*A3* and *A4*) obtained after meiosis. (C) Mating type switching in descendants G39 and G31 by copy transposition of the silent loci *Aa* and *Ab* into the expression locus. (D) Genotypes of the dikaryons resulting from mating type switching. The loci expressed are represented by shaded boxes; transposition events are indicated by arrows.

(A) **Cassette associations (meiosis)**

1	2	3	4
Aa <u>*A3*</u> *Ac*	*Aa* <u>*A4*</u> *Ad*	*Aa* <u>*A3*</u> *Ad*	*Aa* <u>*A4*</u> *Ac*
Ab <u>*A4*</u> *Ad*	*Ab* <u>*A3*</u> *Ac*	*Ab* <u>*A4*</u> *Ac*	*Ab* <u>*A3*</u> *Ad*

(B) **Intergenomic transpositions (meiosis)**

Replacement of *Aa* and *Ab*

1'	2'	3'	4'
A4 <u>*A3*</u> *Ac*	*A3* <u>*A4*</u> *Ad*	*A4* <u>*A3*</u> *Ad*	*A3* <u>*A4*</u> *Ac*
A3 <u>*A4*</u> *Ad*	*A4* <u>*A3*</u> *Ac*	*A3* <u>*A4*</u> *Ac*	*A4* <u>*A3*</u> *Ad*

Replacement of *Ac* and *Ad*

1"	2"	3"	4"
Aa <u>*A3*</u> *A4*	*Aa* <u>*A4*</u> *A3*	*Aa* <u>*A3*</u> *A4*	*Aa* <u>*A4*</u> *A3*
Ab <u>*A4*</u> *A3*	*Ab* <u>*A3*</u> *A4*	*Ab* <u>*A4*</u> *A3*	*Ab* <u>*A3*</u> *A4*

(C) **Genotypes of progeny analysed**

G39	*Aa* <u>*A3*</u> *A4*	[Cassette association 1 or 3; Replacement 1" or 3"]
G30	*Ab* <u>*A3*</u> *A4*	[Cassette association 2 or 4; Replacement 2" or 4"]
G31	*Ab* <u>*A4*</u> *A3*	[Cassette association 1 or 3; Replacement 1" or 3"]
G23	*A3* <u>*A4*</u> *Ac*	[Cassette association 3 or 4; Replacement 3' or 4']
G6	*A3* <u>*A4*</u> *Ad*	[Cassette association 1 or 2; Replacement 1' or 2']

FIGURE 3.—Cassette structures for the *A* factor obtained during meiosis and in the progeny of WT-1. (A) The reciprocal cassette associations obtained from recombination. (B) All possible genotypes expected in the progeny according to the intergenomic transposition events during meiosis. (C) Genotypes of progeny of WT-1 analyzed in this study, with the original cassette associations and the transposition event from which they could be derived.

strain, each locus expressed in one haploid genome may be copied and stored in a silent locus of the other genome (Figure 2A), a transposition event which also results in the elimination of the sequence carried by the silent receptor locus. In homokaryons, mating type switching would occur by the copy transposition of a silent information into the expression locus (Figure 2C), which results in the formation of dikaryotic mycelia (Figure 2D).

Under this simplified form, the model suggests that, whatever the silent loci replaced from transposition at meiosis, at most two nonparental factors can only emerge from the switching of one parental factor (*Aa* or *Ac* from *A3* and *Ab* or *Ad* from *A4*). Accordingly, recombination and transposition at meiosis should result in different silent cassette associations in the progeny, that makes it possible for one parental factor to switch towards four nonparental types in different descendants (see example of B7 which gives *Be, Bf, Bg, Bh* in different progeny of WT-2, Table 4). Figure 3 shows the four cassette associations obtained from recombination and the possible transposition events during meiosis in WT-1. The four cassette associations and two possibilities for transposition are both requisite to deduce the genotypes of the five homokaryons from WT-1 analyzed in this study.

Mating type switching in these progeny should yield either one specific nonparental factor or the alternate parental factor. The mating types recovered in PHF_1 progeny (Table 4) revealed that switching at both *A* and *B* occurred preferentially to the nonparental factors (28 times over a total of 32 *A* and *B* changes), and to only 4 parental factors in three homokaryons (*B3* to *B4* in G30, *A7* to *A8* in L13 and L1, and *B4* to *B7* in L1). This suggests that transposition for switching mobilizes in most cases the locus that has not received a copy during meiosis.

Subsequent mating type switching in PHF_1 homokaryons: The model predicts that, if subsequent switching occurs in PHF_1 homokaryons, only the two parental factors and the nonparental factor specific to each homokaryotic lineage should be observed in the progeny. To test this, 11 PHF_2 progeny from 11 PHF_1 homokaryons of WT-1 were obtained and analyzed (Table 6). Subsequent switching occurred mainly to the expressed mating type factors of the original wild dikaryotic strain WT-1 *A3, A4 B3, B4*. In one case only, *A3* of G39h9 switched to the nonparental configuration *Aa*, specific to the G39 homokaryotic lineage. Switching thus occurs as expected from the model: additional unknown nonparental forms do not appear during the second PHF cycle. As in the first PHF cycle, switching in homokaryons having the same incompatibility genotype can give rise to different factors (G39h7, G39h8 and G39h9 which are *A3 Ba* yield *Aa* or *A4* and *B3* or *B4*), as well as to the same mating type factors (for example G23h4 and G23h6 which are both *A4 Bc* lead to *A3* and *B3*). On the other hand, homokaryons having different genotypes can generate the same factors (for example, G39h7 and G31h3 which are *A3 Ba* and *Ab Bb*, respectively, give *A4 B3*). Thus, there is apparently no correlation between the mating type expressed in one PHF_1 homokaryon and the mating specificities that emerge from a second mating type switching.

The successive switchings occurring from the parental mating type factors of the progeny of WT-1 are summarized in Figure 4. The sequences observed for changes in mating specificities may be generalized as follows: a first mating type switching in homokaryons derived from the wild strain WT-1 leads in most cases to the emergence of factors different from the forms expressed in the parental wild strain (Table 4); the second mating type switching taking place in PHF_1 homokaryons allows mainly the recovery of either of the two parental factors of WT-1. Thus, for both *A* and *B*, the two parental factors of WT-1 and only one

TABLE 6

Identification and segregation of the mating types in PHF_2 progeny

Original fruiting homokaryons	PHF_1 fruiting homokaryons	Genotypes in PHF_2 progeny of incompatibility groups[a]				Segregation
		I	II	III	IV	
G39 *A3B3*	G39h4 *AaBa*	*AaBa*	*A4*B4**	*AaB4**	*A4*Ba*	6:5:7:9
	G39h7 *A3Ba*	*A3Ba*	*A4*B3**	*A3B3**	*A4*Ba*	13:4:6:2
	G39h8 *A3Ba*	*A3Ba*	*A4*B4**	*A3B4**	*A4*Ba*	5:8:9:7
	G39h9 *A3Ba*	*A3Ba*	*Aa*B3**	*A3B3**	*Aa*Ba*	11:11:6:6
	G39h12 *AaB3*	*AaB3*	*A4*B4**	*AaB4**	*A4*B3*	7:9:6:8
G31 *A4B4*	G31h3 *AbBb*	*AbBb*	*A4*B3**	*AbB3**	*A4*Bb*	13:2:4:5
	G31h9 *AbB4*	*AbB4*	*A3*B3**	*AbB3**	*A3*B4*	6:2:4:8
G23 *A4B3*	G23h4 *A4Bc*	*A4Bc*	*A3*B3**	*A4B3**	*A3*Bc*	3:3:9:9
G23 *A4B3*	G23h6 *A4Bc*	*A4Bc*	*A3*B3**	*A4B3**	*A3*Bc*	5:2:5:6
	G23h7 *AcB3*	*AcB3*	*A3*B4**	*AcB4**	*A3*B3*	13:9:6:10
G6 *A4B3*	G6h3 *A4Bd*	*A4Bd*	*A3*B4**	*A4B4**	*A3*Bd*	5:6:5:5

[a] Mating type factors switched on in PHF_2 progeny are marked with an asterisk (groups II, III, IV).

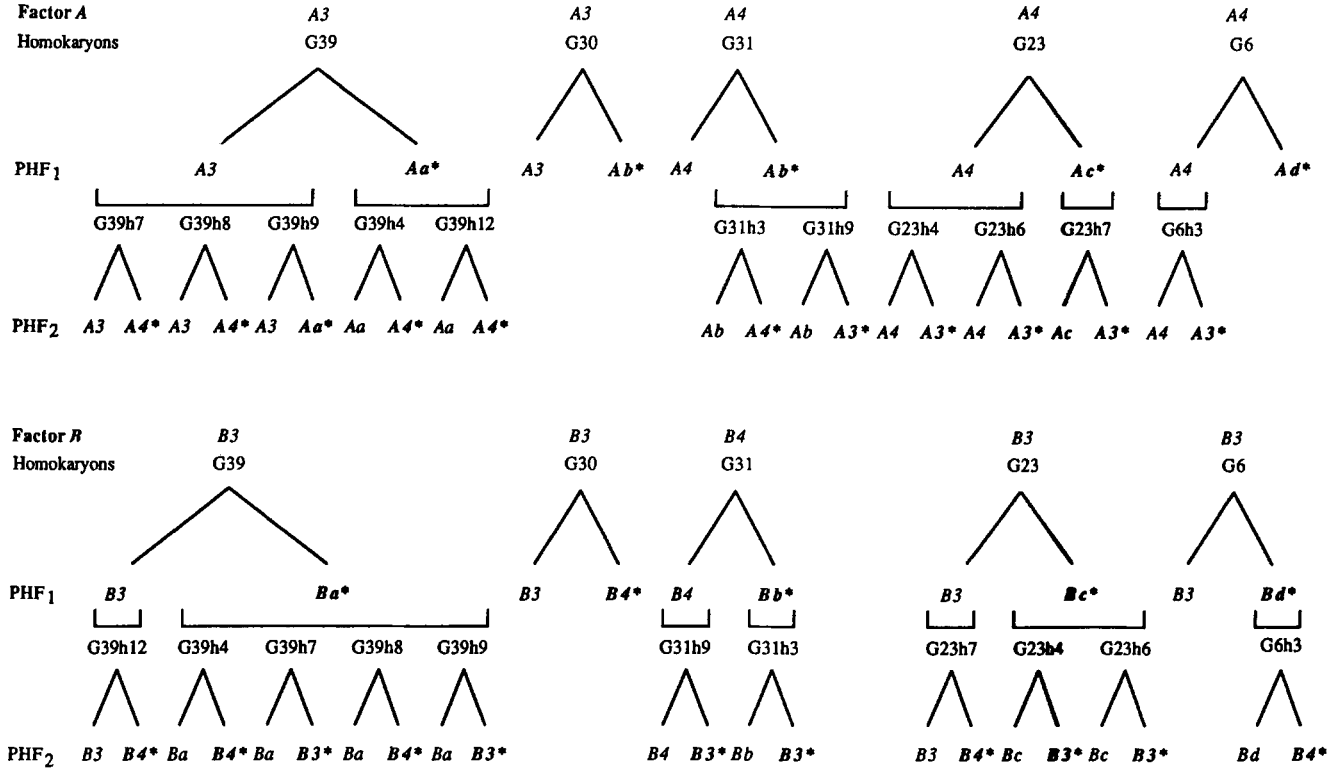

FIGURE 4.—Lineages from sib-homokaryons of WT-1 *A3, A4 B3, B4* showing the successive factors segregating after meiosis of PHF_1 and PHF_2 strains. Factors arising from switching are indicated in bold and marked with an asterisk.

nonparental are generated by multiple cycles of switching from a single homokaryon. In other words, only three forms for each of the *A* and *B* mating type factors are present in any homokaryotic strain, which is fully consistent with the model represented in Figure 2.

Figure 5 shows the successive genotype modifications for the *A* factor of the homokaryons G39 and G31. Consider G39 (Figure 5A): the initial genotype of this homokaryon is *Aa* $\underline{A3}$ *A4*. Mating type switching in G39 results in the formation and the selection of the dikaryon *Aa* $\underline{A3}$ *A4* + *Aa* $\underline{Aa}$ *A4*. The two nuclei will undergo karyogamy and meiosis, during which one copy of the expressed loci will be exchanged. Because the silent loci are homoallelic, meiotic recombination does not change the cassette constitution. As in the wild dikaryotic strain, there are two possibilities for transposition: transposition 1 replaces the silent nonparental forms, and transposition 2 the silent parental forms. However, when analysing similarly all of

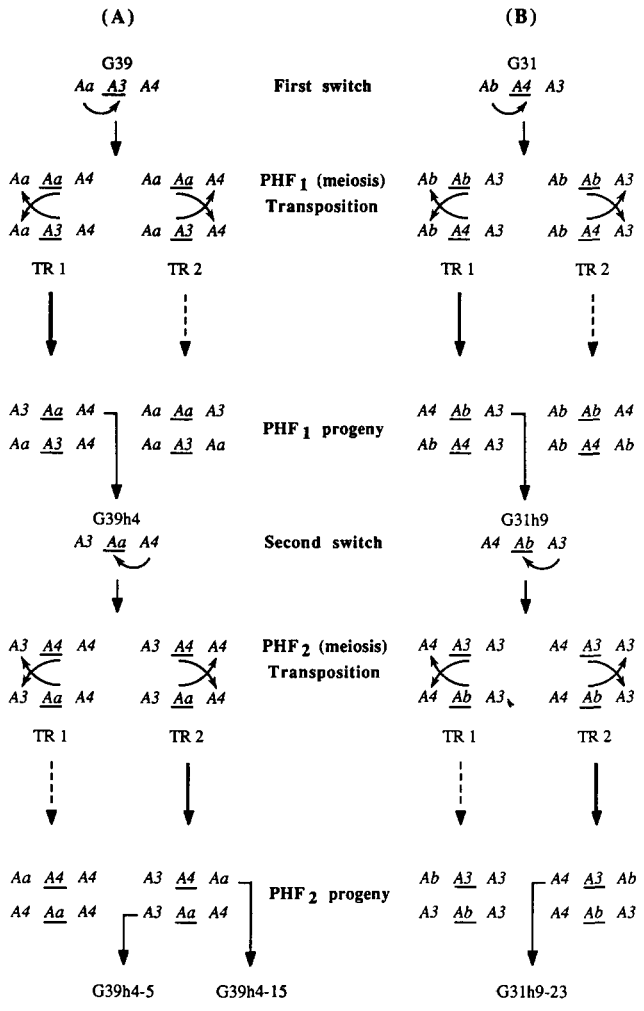

FIGURE 5.—Genetic modifications of the *A* factor from the homokaryons G39 (A) and G31 (B) during two successive switch events. Among the two possible transposition events during meiosis (called TR 1 and TR 2), bold arrows indicate the more probable according to the experimental results. This scheme allows to deduce the genotypes of the three PHF_2 homokaryons further used in crosses (G39h4-5, G39h4-15 and G31h9-23). The loci expressed are underlined; transposition events are indicated by arrows.

the mating types switched on in the 11 PHF_2 progeny (Table 6), it appears that, among the 22 *A* and *B* switched factors, 17 can only survive from transposition 1, and five either from transposition 1 or 2 (*Aa* from *A3* of G39h9, *B3* from *Ba* of G39h7 and G39h9, *B3* from *Bc* of G23h4 and G23h6). Furthermore, to be expressed in the respective progeny, these five factors (and also *A4* from *Ab* of G31h3, deriving from transposition 1) should be mobilized from the locus that has just received a copy during meiosis. These are examples where the receptor locus at meiosis is then transposed for switching, like in G30, L1 and L13 (Table 4). Overall, these observations suggest that transposition during meiosis of the dikaryotized homokaryon is preferentially directed to the silent locus that has been previously transposed for mating type switching. In the case of G39, this is thus probably the silent loci carrying *Aa* that are replaced by the copies of the expressed loci (*A3* and *Aa*, Figure 5A). The genotypes of the resulting PHF_1 progeny can be *A3 Aa A4* (example: homokaryon G39h4, Table 6) and *Aa A3 A4*.

Mating type switching in G39h4 *A3 Aa A4*, which leads to the *A4* specificity, results in the dikaryon A3 *Aa A4* + *A3 A4 A4* (Figure 5). As in most cases the locus transposed in the homokaryotic strain then receives a copy during meiosis, *A4* is probably eliminated (transposition 2) and replaced during meiosis by a copy of the expressed loci in each haploid genome (*Aa* and *A4*), yielding for example the progeny G39h4–15 *A3 A4 Aa* and G39h4–5 *A3 Aa A4* that were further used in cross. On the same principle, one may deduce the genotype of the homokaryon G31h9–23 *A4 A3 Ab* (Figure 5B) that was crossed with G39h4–15 (see below and Table 7). However, there is no way to demonstrate that transposition 1 does not occur during PHF_2. G39h4–5 is *A3 Aa A4* because a third switching cycle makes it express *A3* (data not shown). In the lack of such information for G39h4–15 and G31h9–23, we must consider for each strain the two possible genotypes that give the specificities *A4* and *A3*, respectively.

Mating type switching in progeny derived from crosses between PHF_2 homokaryons: In an attempt to verify that silent copies are replaced by copies of expressed loci during meiosis, two crosses were performed between PHF_2 homokaryons (G39h4–5 × G39h4–15 and G39h4–15 × G31h9–23) derived from two successive mating type switching (see Figure 5 for their lineages and genotypes for the *A* factor). The first cross involved *A4 B4* and *A3 B3* strains of different homokaryotic lineage: G39h4–15 *A3 A4 Aa* (or *Aa A4 A4*) × G31h9–23 *A4 A3 Ab* (or *Ab A3 A3*). The second cross concerned *A4 B4* and *Aa Ba* strains of the same homokaryotic lineage: G39h4–15 *A3 A4 Aa* (or *Aa A4 A4*) × G39h4–5 *A3 Aa A4*. In the progeny from these crosses, the factors expressed by the homokaryons segregated 1:1 at both *A* and *B* expressed loci as expected.

The homokaryotic progeny obtained from the two crosses were then placed under fruiting conditions for screening of subsequent switch events. Thirteen PHF progeny were derived and were subjected to genetic analysis (Table 7). In the 10 homokaryons derived from the cross *A4 B4* × *A3 B3*, mating type switching is restricted to *A3* and *A4*, and to *B3* and *B4*. Switching in homokaryons obtained from the cross *Aa Ba* × *A4 B4* was restricted to the forms *Aa*, *A4* and *Ba*, *B4*. These results show that descendants of a cross between PHF_2 homokaryons switch only to the expressed mating type factors of the strains that have been crossed. Accordingly, this suggests that silent copies (for example *Aa* and *Ab* in the first cross) are

TABLE 7

Identification and segregation of the mating types in PHF progeny derived from descendants of PHF_2 crosses

Origin of homokaryons (cross)	Genotypes of fruiting homokaryons	Genotypes in PHF progeny of incompatibility groups[a]				Segregation
		I	II	III	IV	
G39h4-15 *A4B4*	*A3B3*	*A3B3*	*A4*B4**	*A3B4**	*A4*B3*	7:4:7:6
×	*A3B3*	*A3B3*	*A4*B4**	*A3B4**	*A4*B3*	10:8:4:4
G31h9-23 *A3B3*	*A3B3*	*A3B3*	*A4*B4**	*A3B4**	*A4*B3*	11:9:4:8
	A3B3	*A3B3*	*A4*B4**	*A3B4**	*A4*B3*	5:3:2:10
	A3B3	*A3B3*	*A4*B4**	*A3B4**	*A4*B3*	6:6:9:5
	A4B4	*A4B4*	*A3*B3**	*A4B3**	*A3*B4*	8:12:5:9
	A4B4	*A4B4*	*A3*B3**	*A4B3**	*A3*B4*	12:5:8:5
	A4B4	*A4B4*	*A3*B3**	*A4B3**	*A3*B4*	6:10:7:7
	A3B4	*A3B4*	*A4*B3**	*A3B3**	*A4*B4*	4:6:11:9
	A4B3	*A4B3*	*A3*B4**	*A4B4**	*A3*B3*	5:7:13:3
G39h4-15 *A4B4*	*A4B4*	*A4B4*	*Aa*Ba**	*A4Ba**	*Aa*B4*	4:4:4:3
×	*AaBa*	*AaBa*	*A4*B4**	*AaB4**	*A4*Ba*	9:8:5:6
G39h4-5 *AaBa*	*AaBa*	*AaBa*	*A4*B4**	*AaB4**	*A4*Ba*	4:6:5:2

[a] Mating type factors switched on in PHF progeny are marked with an asterisk (groups II, III, IV).

TABLE 8

Genotype determination of individual nuclei of four mycelia subcultured from PHF_2 basidiocarp explants

Dikaryotised strains	Genotypes before dikaryotization	Genotypes of the dikaryotic strains	Genotypes of individual nuclei
G31h3	*AbBb*	*Ab, A4* Bb, B3**	*AbBb* (14) *A4*B3** (1)
G31h9	*AbB4*	*Ab, A3* B4, B3**	*AbB4* (2) *A3*B3** (13)
G23h4	*A4Bc*	*A4, A3* Bc, B3**	*A4Bc* (1) *A3*B3** (14)
G6h3	*A4Bd*	*A4, A3* Bd, B4**	*A4Bd* (2) *A3*B4** (13)

Mating type factors switched on in the original PHF_1 homokaryons are marked with an asterisk. The number of mycelial colonies obtained for each genotype is given in parentheses.

replaced during meiosis by copies of the expressed loci (*A3* and *A4*, respectively).

Mating types of the nuclear components in the dikaryotized strains: It was interesting to determine whether the newly switched factors at both loci could appear within the same nucleus or in separate nuclei. For instance, when a homokaryotic strain *A3 B3* switches to *Aa Ba*, the expressed mating types in the resulting dikaryon can be constituted by *A3 B3* + *Aa Ba* or by *A3 Ba* + *Aa B3*. Four dikaryotic strains, obtained by subculturing PHF basidiocarp explants differentiated from G31h3 *Ab Bb*, G31h9 *Ab B4*, G23h4 *A4 Bc* and G6h3 *A4 Bd*, were converted homokaryotic by isolation and regeneration of protoplasts. Homokaryotic mycelial colonies regenerated from protoplasts were analysed genetically. The results (Table 8) show that both switched factors are present in a single nucleus.

DISCUSSION

Homokaryotic strains of the basidiomycete *A. aegerita* in pure culture conditions are able to differentiate into three distinct types of fruit-body that were called Abortive Homokaryotic Fruiting (AHF), True Homokaryotic Fruiting (THF) and Pseudo-Homokaryotic Fruiting (PHF). AHF and THF were differentiated from typical homokaryotic mycelium and should therefore be related to the homokaryotic fruiting types already described in *A. aegerita* (ESSER and MEINHARDT 1977; MEINHARDT and ESSER 1981), *Pholiota nameko* (ARITA 1978), *Polyporus ciliatus* (STAHL and ESSER 1976) and *S. commune* (ESSER, SALEH and MEINHARDT 1979). The new third fruiting type, PHF, results in mycelium bearing clamp connections and four-spored basidia, a feature similar to homokaryotic fruiting of *C. cinereus* (MIYAKE, TANAKA and ISHIKAWA 1980; VERRINDER GIBBINS and LU 1984). This study has focused on the characterization of the genetic events occurring during differentiation to the PHF type.

Emergence of switched functional mating type factors in homokaryotic strains: Genetic analysis of mating types in progeny derived from PHF revealed the occurrence of additional mating type specificities at both the *A* and *B* factors. The switched and the original wild factors showed a typical 1:1 Mendelian segregation. The spontaneous switching in haploid homokaryotic lines generates dikaryotic cells that are

selected under fruiting conditions and PHF differentiation. According to the wild strain, up to 50% of the progeny are able to switch their mating type factors. PHF is the result of two changes at two unlinked mating type factors and should arise at a low frequency which remains difficult to evaluate since the number of nuclei screened during PHF is not known. The existence of mating type switching was demonstrated in the homokaryotic progeny from three of four wild dikaryotic strains collected in nature from distinct geographic locations. Switching appears to be therefore a widespread phenomenon in *A. aegerita*. The genetic variability inherent to the mating type switching may be summarized in five points: (1) With respect to the genotype of a given wild dikaryotic strain, mating type switching of the derived homokaryotic progeny can give rise to either the mating type factors of the parental wild dikaryotic strain, or to nonparental factors. (2) Independent mating type switch events within the same homokaryon always give rise to the same factor. It follows that the formation and the specificities of the newly expressed mating types strictly depends on the genetic background of the homokaryon. (3) In different progeny of a single dikaryotic strain, it seems that, in addition to the possible switching between the two regular wild mating type factors, the mating type switching does not occur to more than four *A* and four *B* specific, nonparental factors. (4) These four nonparental factors can be switched on from the same wild factor but only in different homokaryotic strains. Furthermore, the same nonparental factor can emerge from the switching of the two different parental factors of a wild strain. (5) Switched factors are further switchable. The progeny derived from a cross between newly switched factors are able to switch subsequently. The subsequent switchings are restricted to the factors expressed by the strains used for the cross.

A and B structure and mating type switching model: The nonparental factors behave as known factors in that they discriminate against self, and they can exhibit specificities identical to wild factors identified in unrelated wild strains. Accordingly, it is very unlikely that they derive from random mutations at the mating type loci or in the switching process heterothallism to homothallism, as it was interpreted in the case of *C. cinereus* (MIYAKE, TANAKA and ISHIKAWA 1980; MEINHARDT and ESSER 1990). We suggest that stable, reversible, nonrandom changes at both *A* and *B* loci result from the expression of factors previously stored in silent loci. The model proposed for *A. aegerita* (Figures 2, 3 and 5) presents some analogies with mating type interconversion in the yeast *Saccharomyces cerevisiae* (HICKS, STRATHERN and HERSKOWITZ 1977), whose mechanism involves the transposition of DNA sequences (NASMYTH and TATCHELL 1980). In both organisms, the mating type regions would be constituted of three loci, two of them carrying silent information which are expressed only when moved to the expression locus. However, yeast exhibits only two alternative alleles of *MAT*. The recovery of multiple nonparental forms in *A. aegerita* compels us to consider a more complex mechanism. First, we propose that the six loci for each *A* and *B* present in the parental wild dikaryotic strain carry different mating specificities. Second, different new cassette associations are obtained from recombination during meiosis in the dikaryotic strain. Third, intergenomic exchanges of copies of expressed mating types should take place at meiosis to explain the possible emergence of parental factors from the first mating type switching. This hypothesis is strengthened by the fact that only one nonparental type and the two original parental types are seen during successive mating type switchings in one homokaryotic lineage (Figure 4). Both meiosis and transposition determine the genotypes of homokaryons and therefore their potential to switch. For optimal genotype diversity in progeny, transposition should take place after recombination. Otherwise, mating type switching in some homokaryotic lineages (the half in frequency) would be restricted to only two forms: one nonparental and the parental factor originally expressed in the homokaryon.

The critical role of transposition at meiosis led us to investigate a possible genetic demonstration of its existence. Two haploid genomes, whose possibilities for switching had already been identified, were crossed in order to analyze how mating type switching occurred in the progeny, and thus to deduce the genotype modifications at meiosis. We analyzed mating type switching in the homokaryotic progeny from the cross G39h4–15 *A3* $\underline{A4}$ *Aa* (or *Aa* $\underline{A4}$ *A4*) × G31h9–23 *A4* $\underline{A3}$ *Ab* (or *Ab* $\underline{A3}$ *A3*). Whatever the genotypes of G39h4–15 and G31h9–23 and the cassette associations obtained from recombination, if intergenomic transposition does not take place during meiosis, progeny *A4* should be able to switch to *A3*, *Aa* or *Ab*, and progeny *A3* to *A4*, *Aa* or *Ab* (similar prediction can be made for the *B* factor). Determination of mating types in the progeny revealed that *A4* could only switch to *A3*, and *A3* only to *A4*. This suggested that the silent copies *Aa* and *Ab* were eliminated during meiosis and replaced by copies of the expressed $\underline{A3}$ and $\underline{A4}$, respectively. Although a lesser number of homokaryons were studied from the cross G39h4–15 *A3* $\underline{A4}$ *Aa* (or *Aa* $\underline{A4}$ *A4*) × G39h4–5 *A3* $\underline{Aa}$ *A4*, similar results were obtained. Though these data could confirm the model, one cannot completely eliminate the possibility that the copies which no longer appear during switch events (for example *Aa* and *Ab*) are in fact blocked from transposing.

Regulation and timing of mating type switching: It is hypothetized that each of the three loci constituting the *A* and *B* mating type is a potentially transposable unit. According to the model, the copies carried by the expression loci are transposable between the two haploid genomes paired during meiosis in a dikaryon, whereas the silent copies are transposable within the haploid genome of a homokaryon. During meiotic intergenomic transposition, it seems that the replacement of a silent locus among the two possible candidates occurs randomly. The available data indicates that there is then, within each homokaryotic lineage, an alternate choice of the donor loci for switching as well as for changes of the receptor loci during meiosis. From the crosses between PHF_2 homokaryons (for example G39h4–15 *A3* <u>*A4*</u> *Aa* × G39h4–5 *A3* <u>*Aa*</u> *A4*), the model suggests that during meiosis, the choice of the receptor locus can result in the loss of an optimal genetic variability and can lead to a partial isogenisation (for example genotype *Aa* <u>*A4*</u> *Aa*). Mating type switching in such progeny results in a complete isogenisation (*Aa* <u>*Aa*</u> *Aa*) that would be blocked from further mating switching unless intergenomic transposition takes place. This prediction allows to consider a possible test for the model, that could consist in analysing crosses between isogenitized strains (for example between strains *Aa* <u>*Aa*</u> *Aa* × *A4* <u>*A4*</u> *A4*), whose homokaryotic descendants should switch to the respective expressed mating types. Although complex, and in spite of exceptions for alternation of donor loci during switching (see for example G30, L1 and L13 which lead directly to the wild factors during the first switching cycle, Table 4), these data suggest that meiotic transposition events and mating type switching are under the control of a regulatory system. This assumption is strengthened by the fact that only one silent copy among the two candidates seems to be mobilizable for transposition in a given homokaryon (see example of independent switchings in G39).

Genetic data support the idea that mating type switching occurs before meiosis in PHF, and is selected during the pre-fruiting vegetative phase when mycelium is subjected to fruiting conditions. Nothing is known about the simultaneity of changes at both expression loci *A* and *B*. Our experiments have shown that the switched *A* and *B* factors could appear within a single nucleus. But if a single *A* or *B* switch takes place randomly in the vegetative mycelium, there would be little chance for two compatible nuclei to be paired in the same cell, and to further trigger the formation of a dikaryotic mycelium. Accordingly, single switch events affecting either *A* or *B* cannot be screened through the PHF procedure.

This is the first time that new mating type alleles have been derived from preexistent ones in the multiallelic homogenic incompatibility system of basidiomycetes. The mating type switching model emphasizes the possible existence of a mechanism of duplication and exchange of genetic information during meiosis, allowing this information to be present in each of the progeny. The underlying bases of mating type switching in *A. aegerita* and its regulation could be better understood by the molecular analyses of the mating type factor.

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