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A Genetic Mosaic in the Fruiting Stage of *Armillaria gallica*

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Peabody, R. B., Peabody, D. C., and Sicard, K. M. 2000. A genetic mosaic in the fruiting stage of *Armillaria gallica. Fungal Genetics and Biology* 29, 72–80. The basidiome stage of *Armillaria gallica* can be a genetic mosaic. Ten cells isolated from a single basidiome in 1986 produced nine different genotypes when analyzed for variation at six nuclear loci. Four additional basidiomes collected in 1986 produced mosaic patterns when analyzed for variation at a single nuclear (PCR-RFLP) locus. One basidiome collected in 1993 was not a genetic mosaic because 15 cells isolated from it produced only one genotype when analyzed for six nuclear loci. Two hundred seventy-four samples collected in the field between 1981 and 1998 were analyzed for variation at the PCR-RFLP locus. Samples collected prior to 1988 produced patterns consistent with the existence of mosaicism, but samples collected after 1988 showed no evidence of mosaicism. Genetic mosaicism represents a novel mechanism for partitioning genotypes among the cells of a basidiomycete and has interesting implications for the biology of *A. gallica.*

Index Descriptors: Armillaria gallica; genetic mosaicism; PCR-RFLPs; isozyme polymorphism; mating type loci.

Armillaria gallica is a basidiomycete fungus that functions as a saprophyte and infects roots of woody plants in many boreal and temperature forests (Kile *et al.*, 1991). Individuals of this species have the ability to survive and grow for long periods (several centuries) and to colonize hosts in large areas (up to 15 hectares) of their forest habitat (Smith *et al.*, 1992; Saville *et al.*, 1996).

The life cycle of *Armillaria* differs from that of typical basidiomycetes. In typical basidiomycetes the initial interaction of compatible mating types involves cell fusion with delayed nuclear fusion. Consequently, a two-nucleus (n + n) or dikaryotic stage is the predominant nuclear condition of most stages in the life cycle (Raper, 1966). In contrast, the dikaryotic condition is not characteristic of most tissues in rhizomorphs and basidiomes of *Armillaria* (Singer, 1962; Motta, 1969; Korhonen and Hintikka, 1974; Tommerup and Broadbent, 1975; Peabody and Peabody, 1985, 1987). Mated cells of *Armillaria* are predominantly monokaryotic and diploid, and it has long been assumed that basidiomes forming on them are also diploid because most basidiome cells are monokaryotic. However, we reported that *A. gallica* basidiomes collected in Bridgewater, Massachusetts, were monokaryotic and haploid, because no quantitative differences were found when the nuclear DNA content of haploid spores and three different basidiome cell types were compared (Peabody and Peabody, 1985). To determine whether this geographic isolate of *A. gallica* was atypical, eight additional isolates were sampled and basidiomes were all found to be monokaryotic and haploid (Peabody and Peabody, 1987).

Haploidy has an important implication for the distribution of genetic information among the cells of a basidiome. Basidiome development in *Armillaria* is preceded by the fusion of compatible and genetically different haploid cells

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(Hintikka, 1973; Korhonen and Hintikka, 1974; Ullrich and Anderson, 1978; Korhonen, 1978; Franklin *et al.*, 1983; Peabody and Peabody, 1984). If basidiomes were to form exclusively from relict haploid portions of the mated mycelium, they would likely include one or the other or a mixture of the two original parental genotypes. Alternatively, if haploid, monokaryotic basidiomes form from diploid portions of the mated mycelium, haploidization must occur prior to basidiome formation. If this haploidization involves independent assortment and/or recombination, basidiome tissues could develop from a collection of cells with many different genotypes. When we first reported that *A. gallica* basidiomes were monokaryotic and haploid, we proposed a genetic mosaic model for basidiome development and suggested that if this mechanism of partitioning genotypes among cells of an individual existed, it might confer a selective advantage on mosaic individuals (Peabody and Peabody, 1985, 1987).

With the exception of a few studies of monosporous hyphae or vegetative hyphal tips (Anderson and Ullrich, 1982; Smith *et al.*, 1992; Banik and Burdsall, 1998), most investigations of *Armillaria* have relied on DNA samples taken from multicell "chunks" of tissue. This procedure combines DNA from many different cells within a sample and thereby conceals any genetic differences that might exist between cells. In order to avoid this problem, we developed a method for isolating single stipe tip cells from individual basidiomes. This method makes it possible to compare genotypes of different cells isolated from a single basidiome and thereby test the *genetic mosaic hypothesis.*

MATERIALS AND METHODS

Collection site. All samples were collected in a 72-m^2 plot of residential property (lawn + 1 lilac, *Syringa vulgaris*, + 1 maple, *Acer rubrum*, + 1 spruce, *Picea sp.*, + 1 ash, *Fraxinus americana*) located along the shared boundary of 34 Oak St. and 42 Oak St. in Bridgewater, Massachusetts. Mosaic samples collected prior to 1988 and nonmosaic samples collected after 1988 were distributed evenly over the entire site.

Stipe tip cell isolations. A basidiome cap is cut off and the freshly exposed stipe surface is grasped with two pairs of forceps and pulled apart into two approximately equal, half-cylinder-shape longitudinal sections. A small chunk of central tissue is pulled free and transferred to a malt-extract agar (MEA)[2] plate. After 48–72 h the stipe chunk is removed and the plate is scanned for growing hyphal tips using an inverted microscope. Well-separated individual hyphal tips embedded in cylinders of agar are removed and transferred to fresh MEA using sterile bent Pasteur pipettes. Individual hyphal tips transferred by this method are likely to contain several hyphal compartments growing in tandem. Because hyphal elongation occurs by apical growth, cells within a hyphal tip should be genetically identical to one another. Our genotyping of stipe tip cell cultures supports this assumption.

Single spore isolations. A pie-shape wedge of a sporulating basidiome cap is briefly suspended above MEA. After 48 h, the MEA surface is examined using an inverted microscope. Well-separated germinating spores with a single growing hypha are transferred to fresh MEA by the method described above for stipe tip cells.

Multicell samples. Multicell samples were taken from whole basidiomes, rhizomorphs, and spore deposits. PCR-RFLP analysis worked equally well for basidiome and rhizomorph samples that were preserved by any of the following four methods: drying as voucher specimens, storing in 70% ethanol, freeze-drying, or freezing at −80°C. Spore deposits were collected on sterile empty plates and stored either at room temperature or at 4°C. Some data for the years 1981–1983 were obtained from dried voucher specimens. Ethanol-preserved specimens from 1982 and 1983 were also used. Preservation in ethanol is a standard method that minimizes degradation of DNA in samples that are stored for long periods of time (Dessauer *et al.*, 1996). From 1984 on, all samples were preserved in ethanol with some portions also being preserved either by freeze-drying or by freezing at −80°C. The PCR-RFLP banding patterns produced by dried voucher specimens and spore deposits were identical to those produced by ethanol-preserved specimens; and the PCR-RFLP banding patterns produced by ethanol-preserved specimens were in turn identical to those produced by fresh field-collected or fresh lab-grown specimens.

PCR-RFLP analyses. The PCR-RFLP locus examined in this study is located within the intergenic spacer region (*IGS-1*) of the ribosomal RNA operon between the 26S and 5S ribosomal genes. It was analyzed according to the methods of Harrington and Wingfield (1995), and precautions were taken to avoid contamination of samples with trace amounts of extraneous DNA (Lindahl, 1993).

[2] Abbreviations used: MEA, malt-extract agar; MDH-1, -2, malate dehydrogenase-1, -2; PGM, phosphoglucomutase; IDH, isocitrate dehydrogenase.

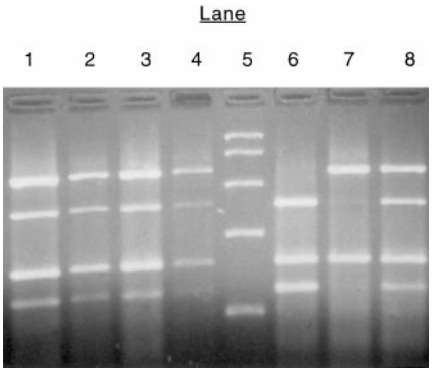

FIG. 1. PCR-RFLP patterns. Lanes 1–4 are four-banded patterns of cross-sections of whole mosaic basidiomes. Lane 5 is a standard molecular weight marker with fragment sizes of 1000, 750, 500, 300, and 150 bp. Lane 6 is the three-banded pattern typical of stipe tip cells such as t-12, t-19, and t-28 (see Table 3). Lane 7 is the two-banded pattern typical of stipe tip cells such as t-15, t-16, t-17, t-24, t-25, t-26, and t-27 (see Table 3). Lane 8 was obtained by combining equal amounts of t-12 and t-15 mycelia prior to DNA extraction and PCR-RFLP analysis. By comparison with lanes 1–4, lane 8 demonstrates that a sample containing two genetically different cells produces the same four-banded pattern that a stipe cross-section of a whole mosaic basidiome produces.

For single haploid cells, *Alu*I digestion of amplified *IGS-1* fragments produces either a two-banded (582 + 240 bp) or a three-banded (399 + 240 + 183 bp) PCR-RFLP pattern (Fig. 1). These patterns differ due to variation at a single *Alu*I restriction site within the 582-bp fragment. If a basidiome is a genetic mosaic at the *IGS-1* locus, it contains both of the above genotypes, and stipe tip cells isolated from it produce either a two-banded or a three-banded pattern. A multicell chunk or cross-section of a mosaic basidiome's stipe produces a four-banded pattern because it contains a mixture of both cell types and yields restriction fragments of all four sizes (i.e., 582 + 399 + 240 + 183 bp). If a basidiome is not a genetic mosaic at the *IGS-1* locus, it contains cells of only one genotype and stipe tip cells isolated from it produce either a two-banded pattern or a three-banded pattern. A multicell chunk or cross-section of a nonmosaic basidiome produces either a two-banded or a three-banded pattern but not a four-banded pattern.

Isozyme loci. Fourteen loci were analyzed using the methods of Brewer (1970), Shaw and Prasad (1970), Selander *et al.* (1971), and Highton and Peabody (2000). Malate dehydrogenase-2 (MDH-2), phosphoglucomutase (PGM), and isocitrate dehydrogenase (IDH) were polymorphic in mosaic basidiomes but monomorphic in nonmosaic basidiomes. Aconitase, esterase, glutamic-oxaloacetic transaminase, malate dehydrogenase-1 (MDH-1), lactate dehydrogenase, glutamate dehydrogenase, β-glucosidase, glucose-6-phosphate isomerase, and 6-phosphogluconate dehydrogenase were monomorphic in both mosaic and nonmosaic basidiomes. All isozyme analyses were carried out on fresh field-collected or fresh lab-grown specimens.

Mating type loci. Mating type loci were determined using the methods of Hintikka (1973) and Ullrich and Anderson (1978) within 3 weeks to 2 months of isolation from basidiomes.

Statistical confirmation of haploidy. The 10 stipe tip cells in Table 3 represent cells originally isolated from the stipe of a single basidiome. If these 10 stipe tip cells were diploid, then heterozygous genotypes should have been observed for at least some of the six polymorphic loci that were assayed. Allele frequencies for the six loci in Table 3 are 0.5 and 0.5 for PGM and mating-type locus B, 0.6 and 0.4 for IDH and mating-type locus A, 0.8 and 0.2 for MDH-2, and 0.7 and 0.3 for the PCR-RFLP locus. For any 1 cell the probability of obtaining only homozygous genotypes for all six loci (assuming equal segregation of alleles at all six loci) would therefore be $(0.5^2 + 0.5^2)(0.5^2 + 0.5^2)(0.6^2 + 0.4^2)(0.6^2 + 0.4^2)(0.8^2 + 0.2^2)(0.7^2 + 0.3^2) = 0.027$. The combined probability that all 10 cells would produce only homozygous genotypes at all six loci would therefore be $(0.027)^{10} = 2 \times 10^{-16}$, which is highly unlikely. Taken together, the genotypes presented in Table 3 and earlier photometric measurements (Peabody and Peabody, 1985, 1987) demonstrate conclusively that haploidy is the predominant nuclear condition in *A. gallica* basidiomes at the Bridgewater, Massachusetts, site.

RESULTS AND DISCUSSION

Support for the genetic mosaic hypothesis. We tested the genetic mosaic hypothesis by analyzing variation at the PCR-RFLP locus in 274 multicell (basidiomes, attached rhizomorphs, or spore prints) and single-cell (stipe tip cells and hyphae from single spores) samples collected from 1981 to 1998 (Table 1). In 121 samples collected before 1988 (a dry year in which no samples were collected), four-banded PCR-RFLP patterns were produced by all multicell samples. Four-banded PCR-RFLP patterns indicate the presence of two alleles in a sample (Fig. 1, lanes 1–4 and 8, Materials and Methods/PCR-RFLP analyses). Three alternative hypotheses are

TABLE 1
PCR-RFLPs Provide Evidence of Genetic Mosaicism Prior to 1988

	Year																	
	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998
Multicell samples																		
No. of basidiomes with																		
Two bands	0	0	0	0	0	0	0	[a]	9	1	3	2	4	4	2	2	2	7
Three bands	0	0	0	0	0	0	0	[a]	0	0	0	0	0	0	0	0	0	0
Four bands	9	10	10	20	12	5	9	[a]	0	0	0	0	0	0	0	0	0	0
No. of rhizomorphs with																		
Two bands				0				[a]	1									
Three bands				0				[a]	0									
Four bands				1				[a]	0									
No. of spore prints[b] with																		
Two bands	0	0	0		0		0	[a]			2						1	
Three bands	0	0	0		0		0	[a]			0						0	
Four bands	1	3	1		1		6	[a]			0						0	
Single-cell samples																		
No. of stipe tip cells with																		
Two bands						15		[a]			5	4	15	8	10	10		
Three bands						12		[a]			0	0	0	0	0	0		
Four bands						0		[a]			0	0	0	0	0	0		
No. of single spores with																		
Two bands						4		[a]			3	18	8	12	10	9	1	
Three bands						2		[a]			0	0	0	0	0	0	0	
Four bands						0		[a]			0	0	0	0	0	0	0	

Note. Four-banded PCR-RFLP patterns indicate the presence of two alleles in a sample (see Fig. 1, lanes 1–4 and 8). Four-banded patterns were only found in multicell samples and only in the years 1981–1987. Two-banded PCR-RFLP patterns and three banded PCR-RFLP patterns each indicate the presence of only one allele in a sample (see Fig. 1, lanes 7 and 6, respectively). The five basidiomes sampled in 1986 each contained some stipe tip cells that produced two-banded patterns and some stipe tip cells that produced three-banded patterns (see Table 2). These data are consistent with the interpretation that basidiomes and attached rhizomorphs were genetic mosaics in the years 1981–1987. From 1989 to 1998 only two-banded PCR-RFLP patterns were recovered. These data are consistent with the interpretation that basidiomes and attached rhizomorphs were nonmosaic during this period.

[a] No samples were collected in 1988.

[b] Each spore print sample was collected from a single basidiome.

consistent with the presence of two alleles in a multicell sample: (1) the multicell sample could be a heterozygous diploid, (2) the multicell sample could contain two conjugate nuclei that tend to remain unpaired and in separate cells until karyogamy in the basidium, or (3) the multicell sample could be a haploid genetic mosaic. To distinguish among these alternatives, we analyzed a series of stipe tip cells and spores that were isolated from five basidiomes collected in 1986 (basidiome Nos. I–V in Table 2 and basidiome No. I in Table 3). All of the stipe tip cells and spores were haploid and produced either two-banded or three-banded PCR-RFLP patterns, but never four-banded PCR-RFLP patterns. Two-banded or three-banded PCR-RFLP patterns are produced when a sample contains only a single allele (Fig. 1, lanes 6 and 7, Materials and Methods/PCR-RFLP analyses). Multicell samples from basidiome Nos. I–V produced four-banded PCR-RFLP patterns. These data allowed us to eliminate the heterozygous diploid hypothesis because single-cell samples always contained one or the other of two possible alleles, but never two alleles. The data in Table 3 (for basidiome No. I) allow us to eliminate the separate conjugate nuclei hypothesis. Ten stipe tip cells from basidiome No. I were analyzed for six loci (three isozyme loci, two mating-type loci, and one PCR-RFLP locus) and produced a total of nine different genotypes. If the separate conjugate nuclei hypothesis were correct, then only two "parental" genotypes would have been recovered. The presence of more than two (i.e., nine) haploid genotypes provides evidence that genetic recombination has occurred. The presence of multiple, haploid, recombinant genotypes within a single basidiome is consistent with the haploid genetic mosaic hypothesis and eliminates both the heterozygous diploid and the separate conjugate nuclei

TABLE 2
PCR-RFLPs of 1986 Basidiomes I–V Provide Evidence of Mosaicism

	Basidiome				
	I	II	III	IV	V
Single-cell samples					
No. of stipe tip cells[a] with					
Two bands	7	4	1	2	1
Three bands	3	2	2	2	3
Four bands	0	0	0	0	0
No. of single spores with					
Two bands	0	1	1	2	0
Three bands	0	0	0	0	2
Four bands	0	0	0	0	0
Multicell samples					
No. of basidiomes[b] with					
Two bands	0	0	0	0	0
Three bands	0	0	0	0	0
Four bands	1	1	1	1	1

[a] All five basidiomes are genetic mosaics because each contains some stipe tip cells with two-banded patterns and some stipe tip cells with three-banded patterns.

[b] All five whole basidiome samples produced four-banded patterns because multicell basidiome samples include stipe tip cells of both genotypes.

hypotheses. The four-banded PCR-RFLP patterns obtained for basidiome Nos. I–V (Table 2) and all other multicell samples prior to 1988 (Table 1) are also consistent with the genetic mosaic hypothesis.

The genotypes in Table 3 provide independent confirmation of earlier photometric studies that demonstrated haploidy in *A. gallica* basidiomes. If basidiome No. I contained diploid cells then at least some of the genotypes listed in Table 3 should exhibit patterns typical of a heterozygous diploid, but none do (see Materials and Methods/Statistical confirmation of haploidy).

Possible changes of nuclear DNA in culture. PCR-RFLP assays for single-cell samples from 1986 mosaic basidiome Nos. I–V ($N = 33$, Tables 2 and 3) and single-cell samples from the 1993 nonmosaic basidiome ($N = 15$) were performed on samples that were grown in culture and then preserved immediately in ethanol. Although all of these cultures have been maintained in the lab since the time of their collection, samples of each were preserved in ethanol within 2 months of the time when their source basidiome was collected in the field. All 48 of these samples produced identical PCR-RFLP genotypes regardless of whether the sample had been maintained in culture for 2 months, 12 years, or an intermediate period of time. All six whole basidiome samples (five from 1986 and one from 1993) were preserved in ethanol at the time of collection. Mating-type determinations were made within 3 weeks to 2 months of the time when samples were collected in the field. Although isozyme analyses cannot be repeated on ethanol-preserved samples, results from the one PCR-RFLP locus and the two mating-type loci give us confidence that genetic mosaicism prior to 1988 is not an artifact produced by mutation or other form of genetic change under cultural conditions.

Lack of mosaicism after 1988. All 153 multicell and single-cell samples collected after 1988 produced two-banded PCR-RFLP patterns (Table 1), suggesting that variation no longer existed at this locus. In addition, when 15 stipe tip cells isolated from a single basidiome collected in 1993 were analyzed for the six loci that were polymorphic in basidiome No. I from 1986, no genetic variation was found. All 15 stipe tip cells had the S allele for MDH-2, the F allele for PGM, the S allele for IDH, and a two-banded PCR-RFLP pattern. These genotypes are identical to those found in stipe tip cells t-16 and t-17 from 1986 basidiome No. I. Pairings of the 15 stipe tip cells in all possible combinations failed to produce any compatible mating reactions. Samples collected after 1988 are therefore not genetic mosaics.

All data in this report were collected in Bridgewater,

TABLE 3
Genotypes of 10 Stipe Tip Cells From 1986 Basidiome No. I Provide Evidence of Mosaicism,[a] Recombination,[b] and Haploidy[c]

	Isozyme loci[d]			Mating-type loci		PCR-RFLP locus
Stipe tip cell	MDH-2	PGM	IDH	Locus A	Locus B	Number of bands
t-12	S	F	S	A1	B1	3
t-15	S	S	S	A2	B2	2
t-16	S	F	S	A1	B2	2
t-17	S	F	S	A2	B2	2
t-19	S	S	F	A1	B1	3
t-24	S	S	F	A2	B1	2
t-25	F	F	F	A2	B1	2
t-26	F	F	F	A2	B2	2
t-27	S	S	F	A2	B2	2
t-28	S	S	F	A1	B1	3

[a] Basidiome No. I was genotyped for six loci. Eight of 10 stipe tip cells have unique genotypes. Only t-19 and t-28 share common alleles for all six loci.

[b] See Results and Discussion, Life cycle implications.

[c] See Materials and Methods, Statistical confirmation of haploidy.

[d] Electrophoretic mobility notations are S for slow migrating bands and F for fast migrating bands.

Massachusetts, so the geographic distribution of genetic mosaicism in southeastern Massachusetts remains to be studied. Preliminary data suggest that mosaicism exists at a second site in the adjoining town of Raynham, Massachusetts. The next step will be to sample multiple geographic sites and to isolate sets of spores, stipe tip cells, and rhizomorph tip cells from several basidiomes within each of these sites.

Life cycle implications. The six loci assayed in basidiome No. I (Table 3) are useful in determining whether mosaic basidiomes developed from haploid or diploid portions of the mated mycelium. Even if two of the nine genotypes recovered from basidiome No. I were parental, the remaining seven must have been recombinant. The existence of recombination suggests that this basidiome most likely developed from a diploid mated mycelium that was heterozygous for all six loci. In order to produce stipe tip cells with multiple haploid genotypes, the diploid mated mycelium must have undergone an extrabasidial haploidization that resulted in recombination.

Although diploidization followed by an extrabasidial haploidization is the most parsimonious explanation for the recombination pattern exhibited in Table 3, there are other scenarios that could account for it. For example, somatic recombination between dikaryotic nuclei has been reported for *Schizophyllum commune* (Crowe, 1960). Recombination has also been reported following laboratory diploid–monokaryon pairings for *A. gallica* (Carvalho *et al.,* 1995), *A. ostoyae* (Rizzo and May, 1994), and *Coprinus radiatus* (Prud'homme, 1963), and mtDNA recombination has been demonstrated in natural populations of *A. gallica* (Saville *et al.,* 1998).

Rizzo and May's work (1994) with diploid–monokaryon pairings of *A. ostoyae* has an interesting implication for our work. Some of the 2N + N mycelia produced in laboratory pairings were stable and the authors suggested that if 2N + N mycelia were stable in natural populations, they might lead to the production of basidiomes with many different combinations of alleles. If natural populations of *A. gallica* produce stable 2N + N mycelia which undergo recombination between 2N and N nuclei, then the production of genetically variable haploid cells in basidiomes might be enhanced beyond the level produced by our model of diploidization followed by an extrabasidial haploidization.

The fact that mosaicism has not been detected after 1988 is intriguing. All samples were collected within a small area (72 m^2), so it is possible that nonmosaic basidiomes collected from 1989 to 1998 were derived from the same mycelium that produced mosaic basidiomes from 1981 to 1987. If this interpretation is correct, then either the original mated mycelium lost some of its alleles in 1988 or it stopped incorporating them into basidiomes after that time. A model that could account for the loss of mosaicism in basidiomes after 1988 is presented in Fig. 2. Figure 2A represents basidiome development as it might occur soon after the putative haploidization, in which a haploid genetically mosaic mycelium has been produced. Because mosaic cells (suggested by the numbers t-12 through t-28) have been recently produced in this model, they have only had enough time to grow into small mycelia and are therefore positioned close together within a small space. If genetically different mycelia within this small space all contribute to the formation of a rhizomorph that produces a single basidiome, the basidiome would include many different haploid cell types and thus be a genetic mosaic. Figure 2B represents basidiome development as it might occur several years after the putative haploidization took place. By this time some of the original haploid mycelia may have died or failed to increase in size. Other haploid mycelia may have prospered and grown to colonize large areas. If the area covered by a single haploid mycelium (e.g., mycelium t-16 in this diagram) were large enough to produce an entire rhizomorph that will form a basidiome, then this basidiome would contain genetically identical cells and would not be a genetic mosaic. The two multicell rhizomorph samples that were analyzed in this study produced PCR-RFLP patterns consistent with this model. The rhizomorph produced in 1984 (when basidiomes were still mosaic) produced a four-banded PCR-RFLP pattern and might therefore have been a genetic mosaic itself (as Fig. 2A suggests). The rhizomorph produced in 1989 (when basidiomes were no longer mosaics) produced a two-banded PCR-RFLP pattern and might therefore have been non-mosaic (as Fig. 2B suggests).

Another possible explanation for the development of nonmosaic basidiomes after 1988 is that these basidiomes simply developed from mycelia other than the one that produced mosaic basidiomes before 1988. For example, if hyphae from a primary mycelium were self-fertile, plasmogamy followed by karyogamy would produce a diploid "mated" mycelium homozygous for all loci. A basidiome developing from this mycelium could only be nonmosaic. Alternatively, two hyphae from different primary mycelia might have identical alleles at the loci listed in Table 3 (assuming that they could be interfertile), but different alleles at other loci. If these hyphae mated to form a secondary mycelium, a basidiome developing from it would appear to be nonmosaic (for the loci we assayed), but might actually prove to be mosaic if other loci were

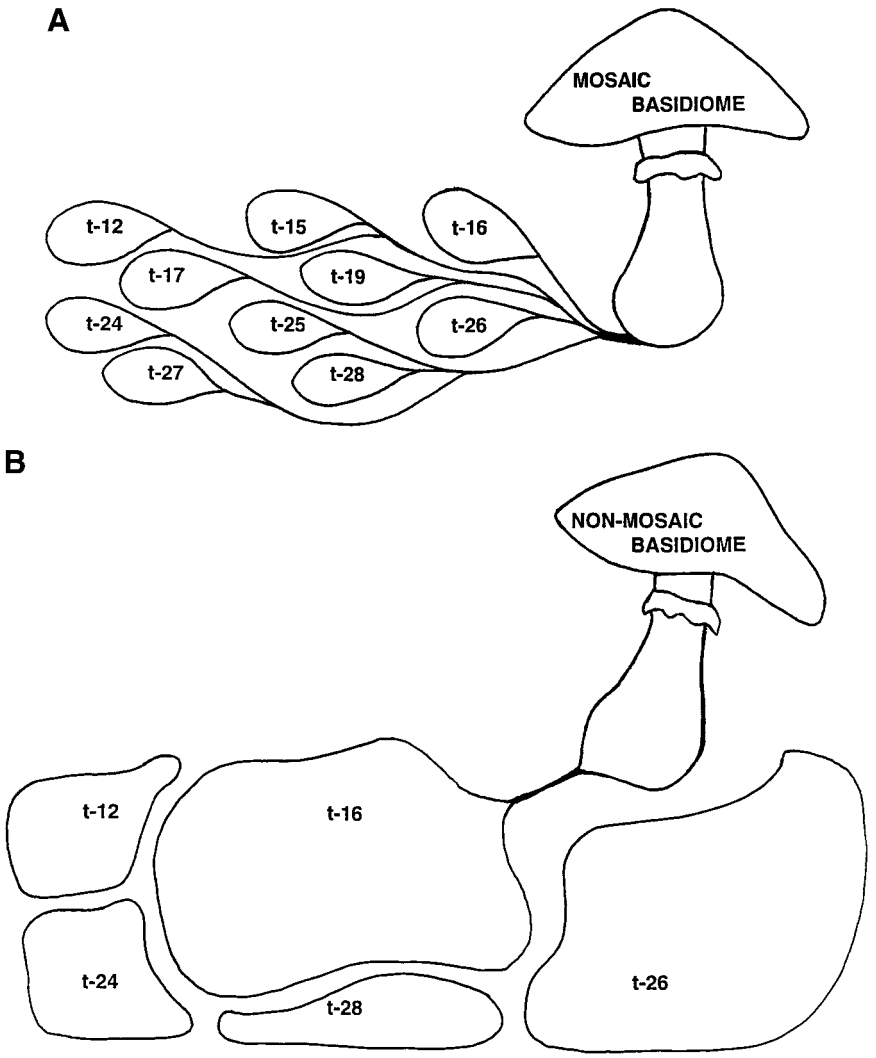

FIG. 2. A model for the loss of mosaicism in basidiomes over time. A. Soon after haploidization produces a haploid genetically mosaic mycelium, a mosaic basidiome might form on a rhizomorph that includes haploid cells of many different genotypes. In this example cells of all genotypes listed in Table 3 are included. B. As the haploid genetically mosaic mycelium ages, some portions might die or fail to grow while others might become quite large. If a haploid mycelium that grew from a single haploid cell such as cell t-16 grew to be large enough, it might produce the entire rhizomorph from which a basidiome develops. This basidiome would contain only one cell type. Mycelia from cells t-15, t-17, t-19, t-25, and t-27 might die.

examined. A third scenario is that nonmosaic basidiomes might develop directly from relict haploid portions of a mated mycelium that never diploidized. Another plausible explanation is that nonmosaic basidiomes formed directly from a haploid, unmated mycelium. It is also possible that more intense sampling in the future will reveal the presence of mosaic basidiomes in our study area even though we did not happen to pick any mosaic basidiomes when we collected samples in the years 1989–1998. At the present we have no evidence to suggest which, if any, of these alternatives accurately explains the origin of nonmosaic basidiomes in nature.

Biological significance of mosaicism. Our data show that the basidiome stage of *A. gallica* has the capacity to partition genetic information in a manner that is fundamentally different from that known in most haploid, diploid, or dikaryotic organisms. Other examples of genetic mosaicism as "normal" stages of naturally occurring life cycles have been reported in plants, animals (Gill *et al.*, 1995), and fungi (Sanders, 1999), but documented exam-

ples are rare. For most species genetic variation exists among individuals that constitute populations, but not among cells that constitute individuals. As individuals live and die, populations persist and presumably benefit from among-individual genetic variation that helps in the process of adaptation. In *A. gallica*, individual mosaic mycelia possess built-in, among-cell genetic variation.

It has been shown that *A. gallica* individuals have the potential to live for unusually long periods of time and to utilize resources in unusually large areas of their forest habitat (Smith *et al.*, 1992; Saville *et al.*, 1996). Could this extraordinary longevity and capacity for growth be related to among-cell variation that *A. gallica* individuals possess? Perhaps in the natural environment one genotype might be more effective in meeting environmental challenges presented by one combination of factors while another genotype might be more effective at meeting challenges presented by a different combination of factors. The possibility that genetic mosaicism might contribute to these abilities and thereby increase the fitness of individuals should be investigated in order to better understand *A. gallica*'s biology and its ecological roles as a saprophyte and plant pathogen.

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