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Genetic Evidence for Nonrandom Sorting of Mitochondria in the Basidiomycete *Agrocybe aegerita*

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We studied mitochondrial transmission in the homobasidiomycete *Agrocybe aegerita* during plasmogamy, vegetative growth, and basidiocarp differentiation. Plasmogamy between homokaryons from progeny of three wild-type strains resulted in bidirectional nuclear migration, and the dikaryotization speed was dependent on the nuclear genotype of the recipient homokaryon. Little mitochondrial migration accompanied the nuclear migration. A total of 75% of the dikaryons from the fusion lines had both parental mitochondrial haplotypes (mixed dikaryons), and 25% had only a single haplotype (homoplasmic dikaryons); with some matings, there was a strong bias in favor of one parental haplotype. We demonstrated the heteroplasmic nature of mixed dikaryons by (i) isolating and subculturing apical cells in micromanipulation experiments and (ii) identifying recombinant mitochondrial genomes. This heteroplasmy is consistent with the previously reported suggestion that there is recombination between mitochondrial alleles in *A. aegerita*. Conversion of heteroplasmons into homoplasmons occurred (i) during long-term storage, (ii) in mycelia regenerated from isolated apical cells, and (iii) during basidiocarp differentiation. Homokaryons that readily accepted foreign nuclei were the most efficient homokaryons in maintaining their mitochondrial haplotype during plasmogamy, long-term storage, and basidiocarp differentiation. This suggests that the mechanism responsible for the nonrandom retention or elimination of a given haplotype may be related to the nuclear genotype or the mitochondrial haplotype or both.

In eukaryotic organisms, the extrachromosomal genetic material is inherited in a non-Mendelian manner, which is generally explained by unequal contributions of the parental cytoplasms (3, 4). However, in the last few years, segregation mechanisms that lead to the selection or elimination of one chloroplastic or mitochondrial genome have been described (17, 25).

Unlike higher plants or animals, which generally possess specialized organs or cells for fertilization, sexual matings in homobasidiomycetes rely on the fusion (plasmogamy) of two compatible vegetative homokaryotic mycelia. The resulting dikaryotic mycelium can differentiate basidiocarps in which karyogamy and meiosis take place. Mitochondrial transmission during mating has been studied in the homobasidiomycetes *Coprinus cinereus* (14), *Agaricus bisporus* (8, 9), *Agaricus bitorquis* (7), *Armillaria bulbosa* (22), *Pleurotus ostreatus* (13), *Lentinula edodes* (6), and *Schizophyllum commune* (24). In all of these fungi except *Agaricus bisporus* and *Agaricus bitorquis*, bidirectional nuclear migration occurs along hyphae from the junction line of the two mated homokaryotic mycelia. Since mating involves numerous hyphal fusions between the two parental homokaryons, mitochondrial mixing and recombination could occur. Dikaryons possess one or two mitochondrial haplotypes depending on their location on the mating petri dish (14); dikaryotic mycelia from sites distal to the fusion lines have the mitochondrial haplotype of the nuclear recipient homokaryon, while most of the dikaryotic mycelia from the fusion lines have both parental mitochondrial haplotypes (mixed dikaryons). Except for *P. ostreatus*, in which recombinant mitochondrial genomes appeared in dikaryons (13), there is no direct evidence for the existence of heteroplasmons possessing both mitochondrial haplotypes in the same dikaryotic hyphae. In this context, the mixed dikaryons obtained in *C. cinereus* (14) and *Armillaria bulbosa* (22) have been interpreted as mosaic mycelia formed by two kinds of homoplasmic dikaryotic hyphae.

Previous restriction fragment length polymorphism (RFLP) analyses of dikaryotic mycelia and basidiocarps have led to apparently contradictory results; dikaryotic mycelia possessing both parental mitochondrial haplotypes have been described in most species, but a single mitochondrial haplotype has always been seen in wild or cultivated basidiocarps of *Armillaria* spp. (22), *Agrocybe aegerita* (1), and *Agaricus bisporus* (9). Only one basidiocarp possessing both parental mitochondrial haplotypes has been described in one in vitro subculture of *Agaricus bitorquis* (7). In most studies of mitochondrial transmission, analysis of mitochondrial behavior during basidiocarp differentiation has been lacking or has been hampered by the small numbers of basidiocarps examined.

RFLP analysis of mitochondrial genomes of a population of *Agrocybe aegerita* wild-type strains suggested that recombination between mitochondrial alleles is likely (1), and this recombination requires mitochondrial mixing and a sorting out of one mitochondrial haplotype. Recently, Jin and Horgen (9) demonstrated that the strong bias leading to uniparental transmission of mitochondria in *Agaricus bisporus* was not determined by the radial growth of the paired homokaryons and suggested that it could be due to the selective exclusion of one parental haplotype from the newly formed heterokaryons.

Numerous details of the mitochondrial transmission process in homobasidiomycetes remain to be described, including (i) the heteroplasmic nature of the mixed dikaryotic mycelia recovered from the fusion lines, (ii) the main characteristics of the sorting out mechanism leading to the homoplasmy observed in dikaryotic wild-type strains, (iii) the correlation of mitochondrial segregation with a mitochondrial or nuclear

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component, and (iv) the step(s) of the fungal life cycle in which this segregation occurs.

Agrocybe aegerita is a cultivated basidiomycete that can serve as a model biological system. Its life cycle can be completed in vitro, and molecular markers for the mitochondrial genome are available (1). We used molecular markers to distinguish and identify mitochondrial haplotypes during the major stages of the *Agrocybe aegerita* life cycle, i.e., during plasmogamy, vegetative growth, long-term storage, and basidiocarp differentiation. Mixing was investigated by isolating and subculturing apical cells and searching recombinant genomes.

MATERIALS AND METHODS

Wild-type strains, homokaryotic progeny, and dikaryons. The three wild-type strains used, SM 47, SM 751002, and SM 750901 (designated WT-3, WT-11, and WT-13, respectively, in this paper), were obtained from subcultures of pieces of wild basidiocarps (Table 1) on complete solid CYM medium (20). The polymorphic nature of the mitochondrial DNA (mtDNA) of these organisms was reported previously (1). Dikaryotic mycelia were cultivated in vitro to obtain basidiocarps from which basidiospores, which germinated to produce homokaryotic progeny, were isolated (10). Mating type alleles were identified as previously described (19).

The lack of common mating type alleles in the three wild-type strains allowed universal matings between the homokaryons. Plasmogamy was obtained by placing homokaryotic samples 50 mm apart on complete solid CYM medium in petri dishes (diameter 90 mm) and incubating the preparations at 26°C in the dark. Dikaryons were recognized by the appearance of clamp connections. To determine the mitochondrial content of the dikaryons constructed, five mycelial samples were taken and subcultured for each mating, as soon as the dikaryotic state was confirmed. Three samples were obtained from the fusion line, and one was obtained from the regions most distant from this line on both nuclear recipient homokaryon sides.

Dikaryons were maintained for long-term storage (24 months) at 8°C in slants of CYM medium. To investigate their mitochondrial stability, mycelial samples from the strains stored for a long time were subcultured once on solid CYM medium in a petri dish at 28°C before the total DNA was extracted by the miniprep method.

Isolation of apical cells by micromanipulation. Mycelial fragments were placed in drops of liquid CYM medium on microscope cover glasses and incubated, under sterile conditions, for 24 h at 28°C in the dark. Apical cells were isolated from the growing hyphae with a De Fonbrune type micromanipulator (2) and placed in drops of liquid CYM medium for an additional 24 h before subculturing in petri dishes containing solid CYM medium.

Molecular probes. The cloned probes (designated mtH4 and mtH7) used as molecular markers of the mitochondrial genome have been described previously (1, 16). Recombinant plasmids carrying mitochondrial inserts mtH4 and mtH7 were purified from *Escherichia coli* JM83 clones by a conventional miniprep method (12). The polymorphism revealed by hybridization of the mtH4 and mtH7 probes made it possible to define, for each probe, allelic forms, which were designated H4-1 and H4-2 (mtH4 probe) and H7-1, H7-2, and H7-3 (mtH7 probe) (1). For each strain, the haplotype was characterized by a combination of the various allelic forms (Table 1).

Purification of DNA from *Agrocybe aegerita* mycelia. Total DNA was extracted from vegetative mycelia by using the *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide (CTAB) method (18) adapted to small quantities of mycelium and to the simultaneous analysis of a large number of samples. Mycelium (0.2 g) from an 8-day culture on solid complete CYM medium was collected with a scalpel and then crushed frozen in liquid nitrogen in a mortar. The crushed mycelium was resuspended in 0.7 ml of extraction buffer (100 mM Tris-HCl [pH 8], 2% [wt/vol] CTAB, 20 mM EDTA, 1.4 M NaCl, 2% [vol/vol] β-mercaptoethanol) and incubated for 20 min at 56°C. To this preparation 0.7 ml of chloroform-isoamyl alcohol (24:1 vol/vol) was added, and the two phases were mixed. The resulting emulsion was disrupted by centrifugation (9,000 × *g*, 20°C, 15 min). The upper aqueous phase was removed and then subjected to a second extraction with 0.7 ml of chloroform-isoamyl alcohol, as described above. Nucleic acids were precipitated with 0.7 ml of precipitation buffer (50 mM Tris-HCl [pH 8] 1% [wt/vol], CTAB, 10 mM EDTA) for 30 min at room temperature. A precipitate was recovered by centrifugation (9,000 × *g*, 15 min, 20°C) and then resuspended in 0.5 ml of 1 M NaCl and incubated at 56°C for 15 min. Nucleic acids were precipitated at room temperature by adding 2 volumes of absolute ethanol and letting the preparation sit for 30 min and then were pelleted by centrifugation (11,000 × *g*, 20°C, 15 min), and the pellet was washed three times in 1 ml of 70% (vol/vol) ethanol to completely eliminate the CTAB. The pellet was dried and then resuspended in sterile distilled water. At that time, nucleic acids could be used for immediate restriction analyses or stored at 4°C.

In vitro manipulation of DNAs. Digestion of DNA with restriction endonuclease *Hin*dIII or *Hae*IIII was performed according to the enzyme supplier's instructions (Promega).

TABLE 1. Origins, mating types, and mitochondrial haplotypes of *Agrocybe aegerita* dikaryotic wild-type strains WT-3, WT-11, and WT-13 and their homokaryotic progeny

Strain	Origin	Mating type	Mitochondrial haplotype	Restriction fragments used to distinguish mitochondrial alleles in[a]:	
				mtH4 region	mtH7 region
WT-3 (= SM 47)[b]	Agen (France)	*A7A8 B4B7*	[A] (H4-2, H7-1)[c]	4.2-kbp *Hin*dIII, 11-kbp *Hae*III	3.7-kbp *Hin*dIII, 5-kbp *Hae*III
H3-4	Homokaryotic progeny of WT-3	*A8B4*	[A]		
H3-13	Homokaryotic progeny of WT-3	*A7B7*	[A]		
WT-11 (= SM 751002)[b]	Pont-de-la Maye (France)	*A1A13 B10B14*	[P] (H4-1, H7-3)[c]	3.5-kbp *Hin*dIII, 5.3-kbp *Hae*III	1.4- and 2.3-kbp *Hin*dIII, 1.7- and 3.1-kbp *Hae*III
H11-1	Homokaryotic progeny of WT-11	*A13B10*	[P]		
H11-3	Homokaryotic progeny of WT-11	*A1B14*	[P]		
WT-13 (= SM 750901)[b]	Le Mans (France)	*A17A18 B15B16*	[S] (H4-1, H7-2)[c]	3.5-kbp *Hin*dIII, 5.3-kbp *Hae*III	3.7-kbp *Hin*dIII, 1.7- and 3.1-kbp *Hae*III
H13-2	Homokaryotic progeny of WT-13	*A18B16*	[S]		
H13-4	Homokaryotic progeny of WT-13	*A18B15*	[S]		

[a] mtH7 and mtH4 probe-hybridizing *Hin*dIII and *Hae*III restriction fragments used to distinguish the alleles.
[b] The designation in parentheses is the Laboratory of Molecular Genetics and Improvement of Cultivated Mushrooms designation.
[c] The alleles corresponding to the haplotype are given in parentheses.

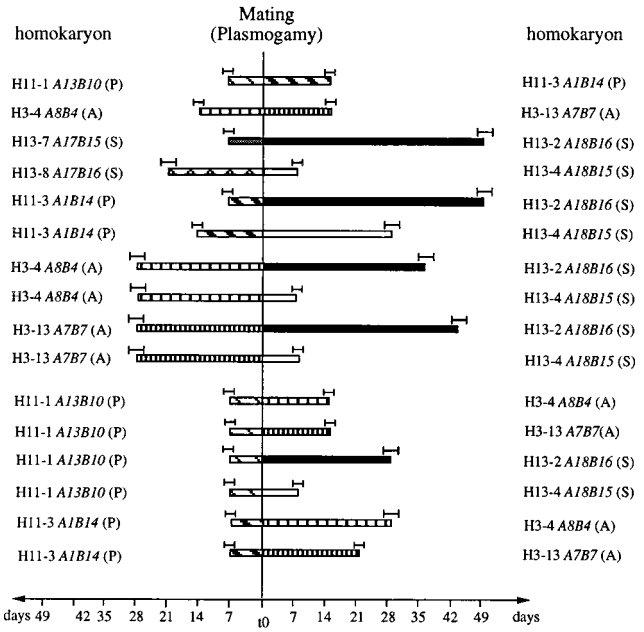

FIG. 1. Delay in dikaryotization expressed by the appearance of clamp connections in zones distal to the fusion lines as a function of the mated homokaryons. t0 represents the day of establishment of the dikaryotic state on the fusion lines (7 ± 2 days after contact between the two mated homokaryons). Each mating was performed in duplicate; the variations in the delay observed between two replicates of the same mating (2 or 3 days) are indicated above each bar. Symbols for recipient homokaryons: bars with widely spaced vertical lines, H3-4; bars with narrowly spaced vertical lines, H3-13; bars with thin diagonal lines, H11-1; bars with thick diagonal lines, H11-3; solid bars, H13-2; open bars, H13-4; shaded bar, H13-7; bar with triangles, H13-8.

DNA labeling and DNA-DNA hybridizations. To determine the mitochondrial haplotypes of the various strains, hybridization of total digested DNA (10 μg) with the probes was carried out by the Southern method (23), as previously described (1).

RESULTS

Dikaryotization kinetics. Dikaryotic strains were constructed by mating (plasmogamy) two homokaryons from the progeny of each of the three wild strains (Table 1 and Fig. 1). After each plasmogamy, dikaryotization kinetics were studied by determining the delay in the appearance of clamp connections (i) in the fusion lines and (ii) in the regions most distant from these lines in both nuclear recipient homokaryons (Fig. 1).

On the fusion lines, the length of the delay in the appearance of clamp connections was 7 ± 2 days after the contact between the two mated homokaryons; this process did not show any variation over 2 days. In contrast, the length of the delay for the dikaryotic state to appear in regions distal to the fusion lines was highly variable between dikaryons, ranging from 7 ± 2 days (when H11-1 was mated with H3-4) to 49 ± 3 days (when H13-2 was mated with H11-3) after the dikaryotization of the fusion line (Fig. 1). There was never a difference of more than 3 days between the two replicates of each mating.

Two homokaryons from the same progeny could have very different rates of distal dikaryotization. The greatest differences observed were the differences for homokaryons from strain WT-13 (H13-2 and H13-4); H13-4 became dikaryotic rapidly (range, 7 ± 2 days for most of the matings to 28 ± 3 days in one mating), while H13-2 dikaryotization always took longer (range, 28 ± 3 to 49 ± 3 days after dikaryotization on the fusion line). Both homokaryons from strain WT-11 (H11-1 and H11-3) became dikaryotic in distal regions 7 ± 2 to 14 ± 3 days after dikaryotization of the fusion line. On the other hand, nuclear migration along the hyphae of the nuclear recipient homokaryons from the WT-3 progeny (H3-4 and H3-13) occurred more slowly than nuclear migration along the hyphae of the nuclear recipient recipient homokaryons from the WT-11 strain; this migration occurred from 14 ± 3 to 28 ± 3 days after dikaryotization on the fusion lines (Fig. 1).

Mitochondrial haplotypes of the constructed dikaryons. Each homokaryon had the same mitochondrial haplotype as the wild-type dikaryotic strain from which it was derived, which showed that a single haplotype was present in each dikaryotic wild-type strain.

For the constructed dikaryons, all 48 dikaryons subcultured from the regions distal to the fusion line possessed only a single mitochondrial haplotype. This haplotype was always that of the nuclear recipient homokaryon. Moreover, when dikaryons were subcultured from both apical distal zones (mating H3-4 [A] × H11-1 [P]) 2 and 5 weeks after the establishment of dikaryotization in these zones, their mitochondrial haplotype was still the haplotype of the nuclear recipient homokaryon, indicating that there was no delayed mitochondrial migration along the nuclear recipient hyphae.

Results were different for the 72 dikaryons collected on the fusion lines; most dikaryons (54 of the 72 dikaryons) possessed both parental haplotypes (Table 2). In all of the mixed dikaryons, the quantities of the two parental mitochondrial haplotypes, as estimated by visual comparison of the hybridization signals specific to each haplotype, were similar. The percentage of mixed dikaryons varied from 62.5% (15 of 24 dikaryons) in [A] × [P] crosses to 87.5% (21 of 24 dikaryons) in the [P] × [S] crosses (Table 2).

Some dikaryons from the fusion lines had a single mitochondrial haplotype. In matings involving homokaryons with the highest speed of nuclear acceptance ([P] haplotype), all of the dikaryons with a single mitochondrial haplotype had this haplotype. Mitochondrial segregation from the [A] × [P] matings was not random and favored the [P] haplotype. For matings involving the [A] and [S] haplotypes, dikaryons with a single mitochondrial haplotype had the [A] haplotype (2 of 24 dikaryons [8%]) and the [S] haplotype (4 of 24 dikaryons [17%]) (Table 2).

Mitochondrial haplotypes of mixed dikaryons after vegetative growth and long-term storage. When the mitochondrial haplotypes of 22 mixed dikaryons were determined after three subcultures (7 days each) on solid medium (Table 3), all of the dikaryons had both mitochondrial haplotypes. Thus, no sorting out of any mitochondrial haplotype occurred during this vegetative growth of *Agrocybe aegerita* mixed dikaryons.

To investigate the stability of mitochondrial mixing after

TABLE 2. Mitochondrial haplotypes recovered in the dikaryons from the fusion lines

Mating	No. of dikaryons with the following mitochondrial haplotypes/no. of dikaryons examined				*P* value[a]
	Mixed	[A]	[P]	[S]	
H3 [A] × H11 [P]	15/24	0/24	9/24		$0.001 < P < 0.01$
H3 [A] × H13 [S]	18/24	2/24		4/24	$0.3 < P < 0.5$
H11 [P] × H13 [S]	21/24		3/24	0/24	$0.05 < P < 0.01$
Total	54/72	2/72	12/72	4/72	

[a] *P* value as determined by a chi-square test of random segregation in homoplasmic strains, with one degree of freedom.

TABLE 3. Behavior of the mitochondrial haplotypes of mixed dikaryons during vegetative growth, long-term storage, and basidiocarp differentiation

Mating[a]	Expt	Mitochondrial haplotype(s) of the dikaryon[b]			Mitochondrial haplotype of the basidiocarp[c]	
		Initial	After three subcultures	After long-term storage	First flush	Second flush
H3-4 [A] × H11-1 [P]	1	[A+P]	[A+P]	ND[d]	2[R][e]	
	2	[A+P]	[A+P]	ND	1[P] (2)	1[P]
H3-4 [A] × H11-3 [P]	1	[A+P]	[A+P]	[P]	1[P]	1[P]
	2	[A+P]	[A+P]	ND	1[P]	
H3-13 [A] × H11-1 [P]	1	[A+P]	[A+P]	[A+P]	1[P]	1[P]
	2	[A+P]	[A+P]	[A+P]	1[P]	1[P]
H3-13 [A] × H11-3 [P]	1	[A+P]	[A+P]	[P]	1[P]	1[P]
	2	[A+P]	[A+P]	[R]	1[P]	
H11-1 [P] × H13-2 [S]	1	[P+S]	[P+S]	[P]	1[P] (2)	
	2	[P+S]	[P+S]	ND	1[P] (1)	
H11-1 [P] × H13-4 [S]	1	[P+S]	[P+S]	[P]	1[P] (1)	
	2	[P+S]	[P+S]	ND	1[P]	
H11-3 [P] × H13-2 [S]	1	[P+S]	[P+S]	[P+S]	1[P]	
	2	[P+S]	[P+S]	[P]	1[P]	
H11-3 [P] × H13-4 [S]	1	[P+S]	[P+S]	[P+S]	1[P]	
	2	[P+S]	[P+S]	[S]	1[P]	
H3-4 [A] × H13-2 [S]	1	[A+S]	[A+S]	[A+S]	1[S]	1[S] (1)
	2	[A+S]	[A+S]	[A]	3[A] (1)	2[A]
H3-4 [A] × H13-4 [S]	1	[A+S]	[A+S]	[A+S]	1[S] (3)	1[S] (1)
	2	[A+S]	[A+S]	ND	1[S]	
H3-13 [A] × H13-2 [S]	1	[A+S]	[A+S]	ND	1[S]	
	2	[A+S]	[A+S]	[S]	4[S] (2)	
H3-13 [A] × H13-4 [S]	1	[A+S]	[A+S]	ND	1[A]	

[a] The letters in brackets are the mitochondrial haplotypes of the original nuclear recipient homokaryons involved.
[b] The mixed dikaryons were selected after plasmogamy 1 (experiment 1) or 2 (experiment 2).
[c] The number of independent basidiocarps analyzed and the mitochondrial haplotype are indicated; the numbers in parentheses are the numbers of basidiocarps attached by their stipes.
[d] ND, not determined.
[e] The R haplotype resulted from a new combination of alleles H4-2 and H7-3.

long-term storage, we determined the mitochondrial haplotypes of 15 mixed dikaryons. Six of these mixed dikaryons had both mitochondrial haplotypes, and nine had lost one mitochondrial haplotype (Table 3). Mixed dikaryons including the [P] haplotype usually retained the [P] haplotype (five of seven dikaryons); however, due to the small number of dikaryons, no definitive conclusions can be made concerning a relationship between the original haplotypes of the mixed dikaryons and the haplotype conserved after long-term storage.

Mitochondrial haplotypes of mycelia regenerated from apical cells of mixed dikaryons. To determine the homoplasmic or heteroplasmic nature of the mixed dikaryons, apical cells were isolated by micromanipulation from six dikaryons which remained mixed following vegetative growth and long-term storage. Eight apical cells were isolated from each of these six mixed dikaryons (Table 4). Of the 48 apical cells, 14 retained both mitochondrial haplotypes. These results clearly indicate that real heteroplasmic hyphae occur in a homobasidiomycete.

Mitochondrial sorting out during basidiocarp differentiation. Twenty-two mixed dikaryons that were stable following three subcultures on solid medium were placed under fruiting conditions. Of the 52 basidiocarps obtained, 41 were from first flushes and 11 were from second flushes. Total DNA was extracted from each complete basidiocarp and from the dikaryotic mycelia obtained by subculturing a piece of each basidiocarp (Table 3).

All 52 basidiocarps had a single mitochondrial haplotype, and all of the basidiocarps differentiated from a given mixed dikaryon always had the same mitochondrial haplotype, irrespective of the flush and of their physical relationship (i.e., basidiocarps attached by their stipes or not).

Basidiocarps from mixed dikaryons from two independent matings involving the same homokaryons could have the haplotype of either parental homokaryon. For example, the mixed dikaryons from the mating H3-4 [A] × H13-2 [S] differentiated two basidiocarps with the [S] haplotype from the first mating and five basidiocarps with the [A] haplotype from the second mating (Table 3).

In the 28 basidiocarps differentiated by mixed dikaryons carrying the [P] haplotype, neither the [A] nor the [S] haplotype was ever recovered by itself. Twenty-six basidiocarps had the [P] haplotype, and the other 2 basidiocarps had a new haplotype designated [R], which was different from both of the parents. Basidiocarps from the seven [A+S] mixed dikaryons could have either the [A] haplotype (two of seven mixed dikaryons) or the [S] haplotype (five of seven mixed dikaryons). The number of mixed dikaryons was too small for us to be able to distinguish random and biased transmission of mitochondria to the basidiocarps.

Evidence for mitochondrial recombination in mixed dikaryons. RFLP analyses in which the mtH4 and mtH7 probes were used provided evidence that three dikaryons had a new haplotype, designated [R], that resulted from a new combination of alleles H4-2 (mtH4 region) and H7-3 (mtH7 region). One dikaryon was obtained after long-term storage of a mixed [A+P] dikaryon (mating H3-13 × H11-3), and the two other dikaryons were obtained from subcultures of two basidiocarps differentiated by a mixed [A+P] dikaryon (mating H3-4 × H11-1). The presence of all of the *Hin*dIII and *Hae*III restriction fragments characterizing the H4-2 and H7-3 alleles clearly showed that the [R] haplotype resulted from recombination.

DISCUSSION

Nuclear migration and dikaryotization kinetics. Dikaryotization kinetics, measured by determining the delay in the appearance of clamp connections, confirmed that *Agrocybe ae-*

TABLE 4. Mitochondrial haplotypes in dikaryons regenerated from isolated apical cells from mixed dikaryons stored for a long time

Mating[a]	Expt	Mitochondrial haplotypes of the mixed dikaryon[b]		No. of dikaryons regenerated from apical cell/no. of dikaryons examined	
		Initial	After long-term storage	Heteroplasmic	Homoplasmic[c]
H3-13 [A] × H11-1 [P]	1	[A+P]	[A+P]	4/8 ([A+P])	4/8 ([P])
	2	[A+P]	[A+P]		8/8 ([P])
H11-3 [P] × H13-2 [S]	1	[P+S]	[P+S]	4/8 ([P+S])	4/8 ([P])
H11-3 [P] × H13-4 [S]	1	[P+S]	[P+S]	6/8 ([P+S])	2/8 ([P])
H3-4 [A] × H13-2 [S]	1	[A+S]	[A+S]		8/8 ([S])
H3-4 [A] × H13-4 [S]	1	[A+S]	[A+S]		8/8 ([S])

[a] The letters in brackets are mitochondrial haplotypes.
[b] The mixed dikaryons were selected after plasmogamy.
[c] A chi-square test was used to test the hypothesis that there was random segregation of the mitochondrial haplotypes in the homoplasmic strains regenerated from an apical cell; one degree of freedom was used. The *P* values were $P < 0.001$, $0.01 < P < 0.02$, and $0.001 < P < 0.01$ for [A+P], [P+S], and [A+S] mixed dikaryons, respectively.

gerita has reciprocal bidirectional nuclear migration like that of other homobasidiomycetes, such as *Armillaria bulbosa* (22), *L. edodes* (6), *Coprinus congregatus* (21), and *S. commune* (24). However, in some species, such as *C. cinereus*, a mating asymmetry caused by nonreciprocal nuclear migration has been described in one-third of the matings (14); in *Agaricus bitorquis*, although nuclear migration was rare, unidirectional nuclear migration was described (7), while in the closely related species *Agaricus bisporus* no significant nuclear migration was observed (8). We found large variations in the delay of dikaryotization in the distal regions of the nuclear recipient homokaryons. This delay was particularly clear for the homokaryons from the WT-11 progeny which readily accepted foreign nuclei. The rates of distal dikaryotization appeared to depend primarily on the genotype of the nuclear recipient homokaryon. The genotype of the migrant homokaryon had little effect on the delay, and this effect was noticeable only when the nuclear recipient homokaryon had a low rate of distal dikaryotization. For example, the short dikaryotization delay (7 ± 2 days) of the homokaryon H11-1 was independent of the migrant homokaryon, while for the homokaryon H13-2, whose dikaryotization delay was long, some of the delay seemed to depend on the nuclear donor; the delay was 28 ± 3 days when H13-2 was mated with H11-1 and 49 ± 3 days when it was mated with H11-3. Our results are similar to previous results obtained with *C. congregatus*, in which nuclear migration varies with the age of the nuclear recipient mycelium (21).

We found that homokaryons from the same progeny could have quite different dikaryotization delays (range, 7 to 49 days). A wide range in dikaryotization speed, leading to recognition of homokaryons with high or low speeds of nuclear acceptance, might be relevant to the mating asymmetry results in *C. cinereus* (14) or to unilateral migration in *Agaricus bitorquis* (7). In the latter species, unilateral nuclear migration was defined by a fluffy mycelial zone 14 days after dikaryotization of the fusion line (obtained in 21 days) and led to the definition of nuclear-donating and nuclear-accepting strains.

Mitochondrial behavior during dikaryotization. All of the dikaryons isolated from the regions distal to the fusion lines confirmed that there was little or no mitochondrial migration, even delayed, along *Agrocybe aegerita* hyphae. This lack of mitochondrial migration in the recipient homokaryons is consistent with previous reports of mitochondrial inheritance in other homobasidiomycetes (6, 7, 8, 14, 22, 24). Thus, in *Agrocybe aegerita*, as in other species, the single mitochondrial haplotype of the dikaryons from the regions distal to fusion lines can be explained either (i) by a mechanism that actively excludes incoming mitochondria or mtDNA (11, 15) or (ii) by a cellular mechanism for nuclear migration that does not act on other organelles and consequently leaves the mitochondria behind.

Mitochondrial genotypes of the dikaryons from the fusion lines. In most homobasidiomycetes studied mitochondrial mixing was found in dikaryons obtained from subcultures of mycelia from the fusion lines of mated homokaryons (6–8, 14, 22, 24). There is no direct evidence for the existence of a stable heteroplasmon in any homobasidiomycete, so the question arose whether the mixed mitochondrial dikaryons resulted from true heteroplasmons or a mixture of homoplasmons.

Subcultures of *Agrocybe aegerita* mixed dikaryons were stable for at least 21 days of vegetative growth, although such dikaryons in other species (e.g., *C. cinereus* and *Agaricus bisporus*) sort out quickly (9, 14). In *Agrocybe aegerita*, long-term storage of the mixed dikaryons (2 years at 8°C) led in 60% of the cases to the selection or elimination of one mitochondrial haplotype.

We demonstrated the existence of true heteroplasmic hyphae on the fusion lines in two ways: (i) the retrieval of heteroplasmic mycelia after subculturing of apical cells isolated by micromanipulation, and (ii) the identification of recombinant mitochondrial genomes in mycelia from some mixed dikaryons, confirming previous observations made in RFLP studies of a wild population (1) and results obtained with *P. ostreatus* matings (13).

It is interesting that all of the heteroplasmons recovered after regeneration from apical cells were from mixed dikaryons involving a homokaryon from the WT-11 progeny ([P] haplotype). Moreover, when heteroplasmy was not recovered, the homoplasmons possessed the [P] haplotype when they were regenerated from the initially [A+P] or [P+S] mixed dikaryons and possessed the [S] haplotype when they came from [A+S] mixed dikaryons. For the three types of mixed dikaryons, the χ^2 statistical tests (Table 4) indicate that the eliminated or selected haplotype was not randomly determined.

Mitochondrial sorting out during basidiocarp differentiation. When fruiting was initiated in mixed dikaryons, all of the basidiocarps had only a single mitochondrial haplotype. We do not know whether fixation of one mtDNA occurs prior to fructification. Indeed, it was very difficult to determine the limit of the differentiated organ and to know if a subcultured mycelium was from the vegetative mycelium located at the base of the stipe or from a fragment of the differentiated organ.

For most matings, two replicate dikaryons were fruited. In some cases, these replicates differentiated basidiocarps with different parental haplotypes. However, the mitochondrial haplotype recovered in the basidiocarp was not randomly selected, since (i) all of the basidiocarps differentiated by a given mixed dikaryon always had the same mitochondrial haplotype and (ii) the basidiocarps from a mixed dikaryon possessing the [P] haplotype plus any other haplotype had the [P] haplotype or, in two cases, a recombined genome. Results showed that the selected haplotype in the differentiated basidiocarps seemed to depend on the mixed dikaryon and that a particular plasmogamy could create a bias in mitochondrial selection.

Preferential transmission and maintenance of mitochondrial haplotypes. Homokaryons with the [P] haplotype always transmitted their haplotype after basidiocarp differentiation from mixed dikaryons. When homoplasmic dikaryons were regenerated from apical cells isolated from such mixed dikaryons (i.e., [A+P] or [P+S] dikaryons), all possessed the [P] haplotype. Moreover, on the fusion lines of these two kinds of matings, the recovered homoplasmic strains always had the [P] haplotype. In summary, every time the haplotype [P] was involved in a heteroplasmic or mixed structure, the evolution to homoplasmy led to the maintenance of this haplotype.

The strong bias in favor of the maintenance of the [P] haplotype corresponded to the mitochondrial haplotype of the homokaryons with the highest speed of nuclear acceptance. The hypothesis that the speed of nuclear acceptance and the sorting out of mitochondria are correlated needs additional testing. In a similar manner, in the green alga *Chlamydomonas* sp., uniparental inheritance of the chloroplast genome has been imputed to a mechanism determined by alleles at or near the nuclear mating locus (5). Similarly, nuclear gene products, such as MGT1 in yeast, have been shown to play a role in the transmission of mtDNA (26). A similar system of mitochondrial elimination has been described in the filamentous ascomycete *Neurospora tetrasperma*, in which the mtDNA haplotype of the nuclear recipient strain completely replaced the mtDNA of the nuclear migrant strain 3 days after hyphal fusion, thus suggesting that there is an active mechanism of mitochondrial elimination genetically regulated by either nuclear or cytoplasmic determinants (11).

Our present report of homokaryons that rapidly accept nuclei and that always transmit their mitochondrial type suggests that important further studies should include (i) determination of the role(s) of the nuclear genome or the mitochondrial genome or both in the mechanism involved in the nonrandom sorting out of mitochondria and (ii) the identification and characterization of the relationship between this mechanism and nuclear migration along the hyphae.

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