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Nuclear Migration and Mitochondrial Inheritance in the Mushroom *Agaricus bitorquis*

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ABSTRACT

Mitochondrial (mt) DNA restriction fragment length polymorphisms (RFLPs) were used as genetic markers for following mitochondrial inheritance in the mushroom *Agaricus bitorquis*. In many basidiomycetes, bilateral nuclear migration between paired homokaryotic mycelia gives rise to two discrete dikaryons which have identical nuclei but different cytoplasms. Although nuclear migration is rare in *A. bitorquis*, unidirectional nuclear migration occurred when a nuclear donating strain (8-1), was paired with a nuclear recipient strain (34-2). The dikaryon recovered over the nuclear recipient mate (Dik D) contained nuclei from both parents but only mitochondria from the recipient mate; thus nuclei of 8-1, but not mitochondria, migrated through the resident hyphae of 34-2 following hyphal anastomosis. The two mitochondrial types present in a dikaryon recovered at the junction of the two cultures (Dik A) segregated during vegetative growth. Dikaryotic cells having the 34-2 mitochondrial type grew faster than cells with the 8-1 mitochondrial type. Fruitbodies, derived from a mixed population of cells having the same nuclear components but different cytoplasms, were chimeric for mitochondrial type. The transmission of mitochondria was biased in favor of the 8-1 type in the spore progeny of the chimeric fruitbody. Protoplasts of dikaryon (Dik D), which contained both nuclear types but only the 34-2 mitochondrial type, were regenerated and homokaryons containing the 8-1 nuclear type and the 34-2 mitochondrial type were recovered.

THE field mushroom, *Agaricus bitorquis*, is heterothallic with mating types controlled by a single multiallelic incompatibility locus (RAPER 1976). The fruitbodies produce four-spored basidia and single-spore isolates are homokaryotic with haploid nuclei. A compatible mating of two homokaryotic isolates results in the presence of a raised line of dikaryotic mycelium at the confluence of the paired cultures (RAPER and KAYE 1978; ANDERSON *et al.* 1984). Clamp connections, often a feature of dikaryons within the Hymenomycetes, are absent in *A. bitorquis*. Dikaryosis can be confirmed visually with a microscope or by fruitbody formation. Central to any study of breeding biology is the transmission of organellar genomes as well as the transmission of nuclear traits. We were interested in determining the mitochondrial inheritance pattern in crosses of *A. bitorquis* and assessing the mitochondrial contribution to the phenotype of vegetative cultures.

In another basidiomycete, *Coprinus cinereus*, biparental inheritance of mitochondria results from events directly following hyphal anastomosis in a pairing of homokaryons. Donor nuclei migrate bilaterally through the established cells of each recipient mate, resulting in two discrete dikaryons, having identical pairs of nuclei but different cytoplasms (BAPTISTA-FERREIRA, ECONOMOU and CASSELTON 1983). Migration of nuclei occurs independently of any movement of organelles (CASSELTON and CONDIT 1972). Although nuclear migration is rare in *A. bitorquis*, one stock (8-1) has been identified, which when paired with a compatible mate (34-2), donates nuclei which migrate unilaterally through the resident mycelium of the recipient mate (ANDERSON *et al.* 1984).

Extensive mt DNA restriction pattern heterogeneity exists between isolates of *A. bitorquis* (HINTZ *et al.* 1985). The main purpose of this study was to develop RFLP markers to follow the transmission of specific mitochondrial and nuclear types after hyphal anastomosis, nuclear migration, fruitbody formation and sporulation. We have shown that there is no movement of mitochondria associated with the migration of nuclei following hyphal anastomosis. A second purpose of this study was to resolve a dikaryon, of known nuclear and mitochondrial genotypes, into homokaryons, of predicted genotypes, by regenerating protoplasts. This allowed the reassociation of the 34-2 mitochondrial genotype with the 8-1 nuclear genotype which resulted in a new homokaryon, without sexual fusion of the nuclei in the dikaryon.

MATERIALS AND METHODS

Growth and maintenance of strains: Working cultures of *A. bitorquis* (8-1, 34-2) were maintained on CYM agar

(STEVENS 1981) at 23°. Mating was done by pairing two 1 mm^3 plugs of homokaryotic cultures at a distance of 1.0 cm on CYM agar plates (ANDERSON *et al.* 1984). Plugs of dikaryotic mycelium were removed from the interface and also distal to the interface of the two homokaryons and transferred to CYM agar plates. For fruiting trials sterile grain (1.0 g $CaCO_3$; 100 g rye seed; 120 ml H_2O) (CHANG and HAYES 1978) was inoculated with dikaryotic mycelium and incubated for 21 days at 23°. The "spawn" was cased with water-saturated calcined earth (Turface, International Minerals and Chemical Corp. Mundelein, Illinois) mixed with, 5.0% (w/w) $CaCO_3$ and 1.0% (w/w) Hydrogel (Viterra Planta-gel, Nepera Chemical Co., Harriman, New York) (SAN ANTONIO 1971) and transferred to an incubator with 85% relative humidity at 21°. Spore prints were prepared from mature fruiting bodies and single germlings were isolated following dilution plating of spores on CYM agar. Protoplasts were isolated from dikaryotic mycelium and homokaryons were recovered as described by CASTLE, HORGEN and ANDERSON (1987). Mycelium for DNA isolations was grown in CYM liquid medium in petri dishes with no agitation.

Source of genetic markers: For the generation of nuclear DNA markers, *A. bitorquis* (8-1) nuclear DNA was separated from mt DNA by centrifugation in bis-benzimide (Hoescht 33258, Sigma Chemical Co.) cesium chloride gradients (HUDSPETH *et al.* 1980). Purified nuclear DNA was digested with the restriction enzyme *Eco*RI (Pharmacia, Uppsala, Sweden) and ligated to similarly digested bacterial plasmid vector pBR 325 (MANIATIS, FRITSCH and SAMBROOK 1982). Several clones, containing 2.0–7.0 kilo-basepair (kbp) inserts, were chosen at random for use as nuclear markers. Mitochondrial DNA markers were generated by cloning *Bam*HI digested *A. brunnescens* mt DNA into the polylinker region of the bacterial plasmid vector pUC 18. The cloned mt DNAs used in this study were between 6.0 and 10.0 kbp in length.

Restriction analysis: Whole-cell DNA was isolated from freeze-dried mycelium according to the procedure of ZOLAN and PUKKILA (1986). DNA samples (*ca.* 5 μg) were digested with *Bam*HI and subjected to electrophoresis on 15 × 18 cm, 0.7% agarose gels in TBE (89 mM Tris-HCl; 89 mM boric acid; 2.0 mM EDTA, pH 8.0) at 30 V for 16 hr. Bacteriophage lambda DNA, digested with both *Eco*RI and *Hin*dIII, was used as a size standard. The digested DNA was transferred from the gel onto Genescreen Plus membrane (Dupont NEN Research Products, Boston, Massachusetts) by capillary blotting according to manufacturer's directions. Plasmid DNAs (*ca.* 1 μg) were labeled with [α-^{32}P]dCTP (3000 Ci/mmol; Dupont NEN) using the Bethesda Research Laboratories (Gaithersburg, Maryland) nick-translation kit. On average, specific activity of 10^8 cpm/μg DNA was obtained. Hybridization and wash conditions were as described by HINTZ *et al.* (1985). Autoradiography was performed using a Dupont NEN intensifying screen and Kodak XAR-5 film.

RESULTS

Two homokaryotic isolates of *A. bitorquis* (8-1, 34-2), having unique mt DNA restriction patterns, were paired on CYM agar at a distance of 1 cm. After 21 days of growth, fluffy mycelium, distinctive of dikaryon formation, appeared at the junction of the two homokaryotic strains. During the next 14 days, the zone of dikaryotization migrated unilaterally over the resident mycelium of strain 34-2 (Figure 1). Plugs of

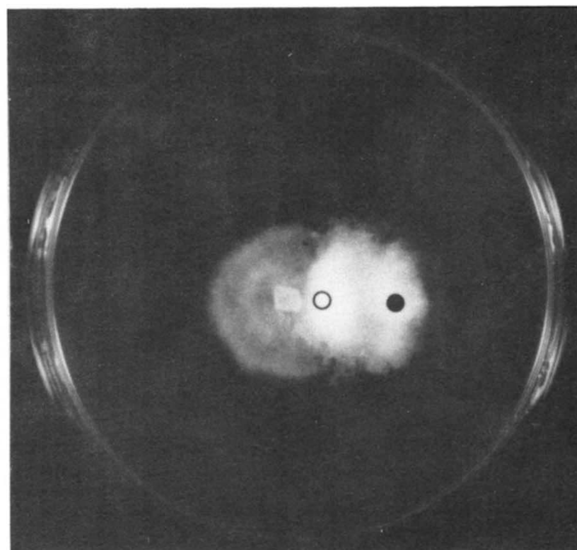

FIGURE 1.—Nuclear migration in Agaricus. An *A. bitorquis* nuclear donating strain (8-1, *left*) was paired with a sexually compatible nuclear accepting strain (34-2, *right*) on CYM agar. Following hyphal anastomosis and nuclear migration, plugs of dikaryotic mycelium were recovered from the junction of the two parental cultures (Dik A, *open circle*) and from the recipient mate (Dik D, *closed circle*).

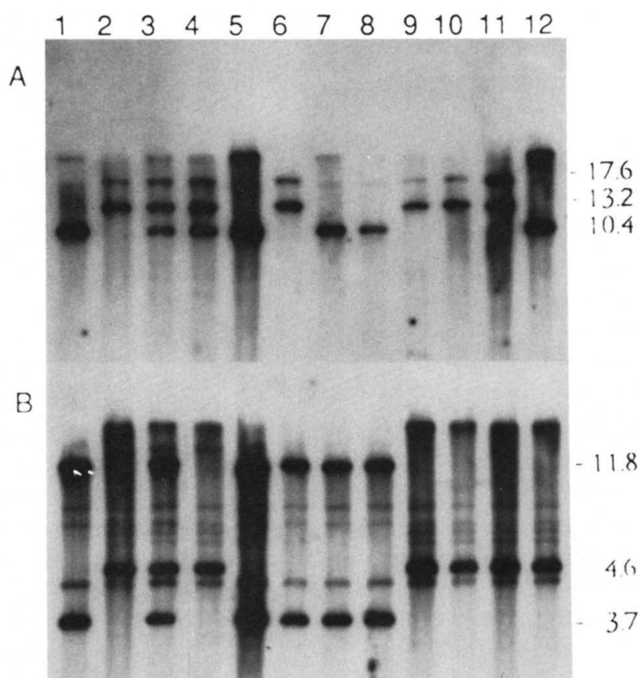

FIGURE 2.—Segregation of nuclear and mitochondrial markers during fruiting. *Bam*HI digested DNAs from *A. bitorquis* parental homokaryotic strains 8-1, 34-2 (lanes 1 and 2 respectively), the dikaryon recovered at the junction of the homokaryons (Dik A, lane 3), the dikaryon recovered over the nuclear recipient mate (Dik D, lane 4), homokaryotic spore progeny of Dik A (A1S1, A1S6, A2S5, A2S6; lanes 5–8), and homokaryotic spore progeny of Dik D (D2S6, D2S7, D3S6, D3S7; lanes 9–12) were electrophoresed on a 0.7% agarose gel, transferred to a Genescreen Plus filter, and hybridized with radiolabeled nuclear DNA marker p8-1n3 (A) or mt DNA marker p50m9 (B). The sizes of strongly hybridizing fragments are indicated at the right of the figure (kbp). Weakly hybridizing fragments or partial digestion products were not included in the analysis.

TABLE 1

Genotypes of *A. bitorquis* strains

Strain	Mating type	Nuclear probes			Mitochondrial probes		
		p8-1n3	p8-1n7	p8-1n33	p50m9	p50m10	p50m4b
8-1	A1	a(10.4)[a]	a(5.0/4.4/4.1)	a(17.1)	a(11.8/3.7)	a(15.6)	a(13.5)
34-2	A2	b(17.6/13.2)	b(5.5/4.7/3.8)	b(8.4/1.7)	b(4.6)	b(19.2)	b(18.2)
Dik A	A1/A2	ab	ab	ab	ab	ab	ab
A1S1	A2	b	a	a	a	a	a
A1S6	A1	b	b	b	a	a	a
A2S5	A2	a	a	a	a	a	a
A2S6	A1	a	b	b	a	a	a
Dik D	A1/A2	ab	ab	ab	b	b	b
D2S6	A2	b	b	b	b	b	b
D2S7	A1	b	b	a	b	b	b
D3S6	A2	b	b	b	b	b	b
D3S7	A1	a	a	b	b	b	b

[a] Sizes of restriction fragment(s) (kbp) hybridizing to the probe in parentheses.

dikaryotic mycelium were excised from the zone of initial culture contact (Dik A) and also from distal to the zone of contact over the recipient mate (Dik D) (Figure 1). These dikaryons were allowed to fruit and single-spore isolates were recovered from several fruitbodies of each dikaryon. For the RFLP analysis, DNA was isolated from the parental homokaryons (8-1, 34-2), the two dikaryons described in Figure 1 (Dik A, Dik D), four single-spore progeny of Dik A (A1S1, A1S6, A2S5, A2S6), and four single-spore progeny of Dik D (D2S6, D2S7, D3S6, D3S7). Segregation of nuclear markers in the spore progeny was shown by hybridization experiments with cloned nuclear DNA probes. The hybridization pattern of the nuclear marker p8-1n3 is shown in Figure 2A. The two parental nuclear types were distinguished by p8-1n3 hybridizing to a single 10.4-kbp fragment of 8-1 DNA and two fragments (13.2 and 17.6 kbp) of 34-2 DNA (Figure 2A, lanes 1 and 2). The dikaryons, Dik A and Dik D, contained nuclei from both parents as was clearly demonstrated by the presence of both parental hybridization patterns (Figure 2A, lanes 3 and 4). The single-spore progeny were homokaryotic and had either the 8-1 pattern or the 34-2 pattern in approximately a 1:1 ratio (Figure 2A, lanes 5–12). The blot was probed with two additional nuclear markers (p8-1n7, p8-1n33) and the sizes of homologous restriction fragments determined (Table 1). The mating types A1 and A2 served as an additional marker locus for the spore progeny (Table 1). Each of the four markers (p8-1n3, p8-1n7, p8-1n33, mating type) segregated in a pattern typical of Mendelian inheritance. Recombinant nuclear genotypes were found among the progeny of the dikaryons, but the number of progeny examined was too low to detect linkage.

When the DNAs were hybridized with the cloned mt DNA markers, a non-Mendelian pattern of inheritance was observed. Markers, which distinguished the parental mitochondrial types, were selected from the *A. brunnescens* mt DNA library. The mt DNA marker p50m9, hybridized to two 8-1 mt DNA fragments, 11.8 kbp and 3.7 kbp in length, and a single 34-2 mt DNA fragment, 4.6 kbp in length (Figure 2B, lanes 1 and 2). The dikaryon (Dik D), collected from a site distal to the junction of the two cultures, carried only the 34-2 mitochondrial type (Figure 2B, lane 4). This indicated that nuclei, but no mitochondria, migrated through the resident hyphae of the nuclear recipient mate (34-2) following hyphal anastomosis. All of the spore progeny of Dik D contained the 34-2 mitochondrial type (Figure 2B, lanes 9–12). The dikaryon (Dik A), from the interface of the paired homokaryons, initially contained both the 8-1 and 34-2 mt types (Figure 2B, lane 3). During the routine subculturing of Dik A, in which plugs of mycelium were removed from the leading edge of a mature colony and transplanted to a fresh CYM agar plate, only one mitochondrial type (34-2) could be detected in the culture after a few transfers. The 8-1 mitochondrial type was lost as a result of serial transfer. To determine how quickly the two mitochondrial types segregated in culture, a junction dikaryon was transferred to CYM agar and grown for 14 days. Seven plugs of mycelium were removed randomly from the center of the colony outward, and the mitochondrial types determined (Figure 3). Certain sectors of the colony contained dikaryotic cells containing the 8-1 mitochondrial type (Figure 3, lanes 3 and 4) while other sectors of the colony contained the 34-2 mitochondrial type. Mycelium at the leading edge of the colony (Figure 3, lanes 5–7) consistently carried the 34-2 mitochondrial type while both mitochondrial types were present in the center of the colony (Figure 3, lanes 1 and 2). Cells containing the 34-2 mitochondrial type had outcompeted

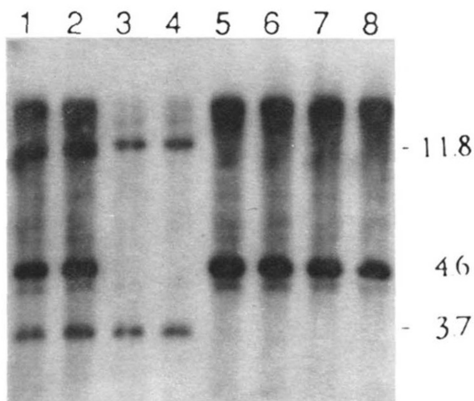

FIGURE 3.—Segregation of mitochondrial types during vegetative growth. A plug of dikaryotic mycelium recovered from the junction of the two cultures was transferred to a fresh CYM agar plate. After 14 days of growth, seven plugs of mycelium were randomly sampled from the center of the colony (lane 1) to the edge of the colony (lane 7) and DNA was extracted. Hybridization with the mt DNA marker p50m9 demonstrated that the two most central samples (lanes 1 and 2) contained both 8-1 and 34-2 mt DNA, two sectors contained 8-1 mt DNA (lanes 3 and 4) while the three outermost samples (lanes 5–7) contained only 34-2 mt DNA. An explant of mycelium was taken from a junction fruitbody, and serially transferred twice before DNA was extracted. The transferred explant contained 34-2 mt DNA (lane 8).

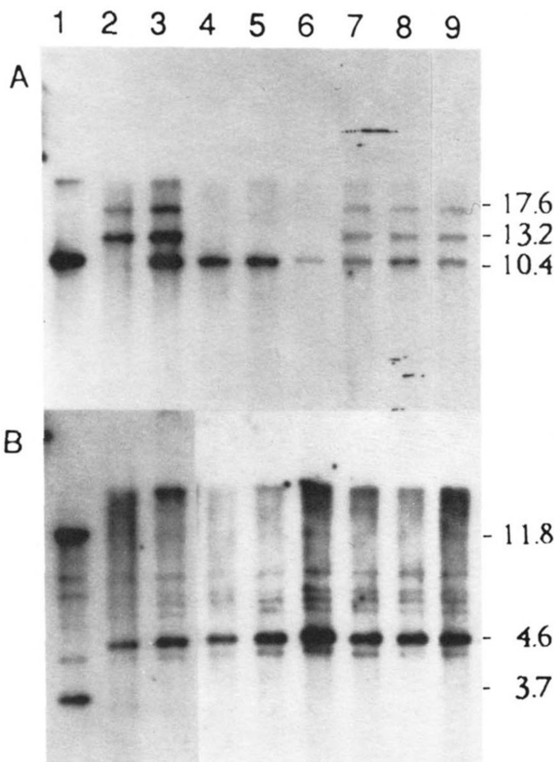

FIGURE 4.—Dedikaryotization of *A. bitorquis*. *Bam*HI digested DNAs from parental homokaryons 8-1, 34-2 (lanes 1 and 2, respectively), the dikaryon Dik D (lane 3) and six derived protoplast cultures; pp21, pp29, pp15, pp23, pp2, pp26 (lanes 4–9 respectively) were electrophoresed on a 0.7% agarose gel, transferred to a Genescreen Plus filter and hybridized with radiolabeled nuclear DNA marker p8-1n3 (A) or mt DNA marker p50m9 (B). The sizes of the hybridizing fragments are indicated at the right of the figure (kbp). Protoplasts pp21, pp29, and pp15 have the same nuclear type as 8-1 but carry the mitochondrial type of 34-2.

the 8-1 mitochondrial type after 2.0 cm of radial growth.

The junction dikaryon (Dik A), composed of a mixture of dikaryotic cells having either the 8-1 or 34-2 mitochondrial types, was fruited and spore progeny recovered. All of the Dik A spore progeny contained the 8-1 mitochondrial type (Figure 2B, lanes 5–8; Table 1). This was confirmed by analyzing DNA from five single-spore cultures, from each of two additional fruiting bodies. These ten Dik A spore progeny also contained only the 8-1 mitochondrial type. The 34-2 mitochondrial type was not found in any of the Dik A spore progeny and recombinant mitochondrial genomes were not detected. It could be argued that the junction fruitbody was composed exclusively of dikaryotic cells having the 8-1 mitochondrial type. An explant was therefore taken from the cap tissue of the Dik A fruitbody and transferred onto CYM agar. This explant was subcultured twice before DNA was extracted so that dikaryotic cells, containing the 34-2 mitochondrial type, could dominate the culture. Only the 34-2 mitochondrial type was detected in the serially transferred cap mycelium (Figure 3, lane 8). From a single fruitbody, both mitochondrial types were recovered, indicating that the fruitbody derived from the junction of 8-1 and 34-2 was chimeric for mitochondria.

To determine the effect of mitochondrial genotype on culture morphology, a new strain, which had nuclei from 8-1 but mitochondria from 34-2, was constructed. Protoplasts of Dik D were allowed to regenerate and colonies were transferred to CYM agar. The recovery rate for viable protoplasts was on the order of 0.01%. Homokaryons were initially identified by their slower growth rate on CYM agar. Of the 36 protoplasts regenerated, 15 were sexually compatible with 34-2 and none with 8-1. The other 17 protoplast derived cultures were dikaryotic. Hybridization with the nuclear DNA probe p8-1n3 confirmed the nuclear status of six homokaryotic and dikaryotic protoplast regenerates. Protoplasts 21, 29, and 15 were homokaryotic, having the 8-1 nuclear type, while protoplasts 23, 2, and 26 were dikaryotic having both the 8-1 and 34-2 nuclear types (Figure 4A). Probing with the other two nuclear DNA probes (p8-1n7, p8-1n33) gave the same result. No recombinant nuclear genotypes were detected. All of the protoplast derived cultures carried only the 34-2 mitochondrial type (Figure 4B). Thus a new homokaryotic strain (8n34m), carrying the 8-1 nuclear type and the 34-2 mitochondrial type, was constructed. When 8n34m was paired with 34-2, nuclear migration towards 34-2 was observed. Hyphae of the parental strain 8-1 grew closely appressed to the surface of the agar and the colony appeared flat while hyphae of 8n34m grew aerially and the colony appeared fluffier (Figure 5). The growth rate of 8n34m was the same as that of 8-1.

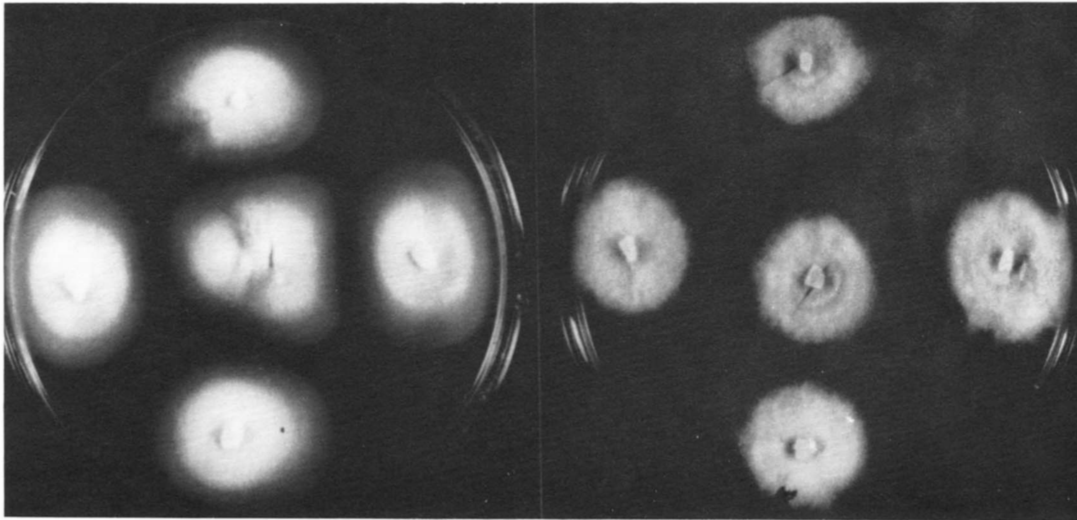

FIGURE 5.—Effect of mitochondrial type on homokaryotic growth. Strain 8-1 (*left*) grew closely appressed to the CYM agar surface while 8n34m (*right*) had a more aerial growth pattern and the colony appeared fluffier.

DISCUSSION

Within the basidiomycetes, mitochondrial inheritance has been most closely studied in *C. cinereus*. Reciprocal nuclear migration occurs at the junction of two mating colonies as donor nuclei migrate through the resident cells of each recipient monokaryon (CASSELTON and CONDIT 1972). Because mitochondria remain immobile, one consequence of a reciprocal exchange of migrating nuclei is the establishment of two distinct dikaryons, each carrying a different mitochondrial type. Following nuclear migration, the vast majority of dikaryotic cells contain both parental nuclei but either one or the other parental mitochondrial type. There is little opportunity for recombination of mitochondrial genomes as this can occur only in the anastomosed cells at the junction of the two mates (BAPTISTA-FERREIRA, ECONOMOU and CASSELTON 1983). The normal pattern for basidiomycetes appears to be the equal biparental transmission of mitochondria to the spore progeny. We have observed a variation of this pattern in crosses of sexually compatible strains of *A. bitorquis*. Cloned nuclear and mt DNA markers were used to determine the fate of the parental nuclear and mitochondrial types following hyphal anastomosis, nuclear migration, and sporulation. When homokaryotic strains 8-1 and 34-2 were paired, a dikaryon was formed at the interface of the two cultures, which then migrated unidirectionally over the resident mycelium of 34-2. This was either due to the intrusive growth of 8-1 hyphae through the resident 34-2 hyphae, or the migration of 8-1 nuclei through the resident cells of 34-2. When inoculum of strain 8-1 is transferred to a lawn of macerated mycelium of a nuclear accepting strain, the spread of dikaryotized mycelium is rapid; much more so than the growth of the dikaryon. When the reciprocal expertiment is performed (transfer of a nuclear accepting strain to a lawn of macerated 8-1 mycelium), the dikaryotic mycelium does not spread (ANDERSON *et al.* 1984). The rate of nuclear migration is greater than can be accounted for by simple out-growth of the dikaryon. A dikaryon (Dik D), collected over the recipient mate away from the junction of the two cultures, contained both nuclear types but only the 34-2 mt type. This clearly demonstrated that 8-1 nuclei, but not mitochondria, migrated through the resident hyphae of 34-2 following hyphal anastomosis. As a direct consequence of unidirectional nuclear migration, a single mitochondrial type (34-2) was present in the majority of the dikaryotic cells resulting from this cross. The other mitochondrial type (8-1) was found in dikaryons only from the junction of the cultures. This could ensure that more fruiting bodies are derived from the dikaryon having the 34-2 mitochondrial type. Hypothetically, spores with the mitochondrial type of the nuclear recipient would then be preferentially dispersed within an interfertile population of *A. bitorquis* in the wild. This assumes that there is no bias in mitochondrial transmission during fruitbody formation and sporulation. A second consequence of unidirectional nuclear migration is that nuclei of the donor strain (8-1) are brought into combination with a wide variety of mitochondrial genomes whenever a compatible mating occurs. Nuclear migration, therefore, promotes mixing of nuclear and mitochondrial genotypes.

Unidirectional migration of 8-1 nuclei accounts for the clonal propogation of the 34-2 mt type, but how is the transmission of the 8-1 mt type affected by the lack of reciprocal migration by 34-2 nuclei? At the junction of homokaryotic hyphae of 8-1 and 34-2, anastomosis resulted in the mixing of nuclei and

mitochondria of both mating types in a single fused cell. It is only in these fused cells that mitochondrial genome recombination is possible. In most fungi, persistent heteroplasmons are rare, and organelles segregate rapidly during mitotic divisions subsequent to fusion. This probably invokes an element of randomness in the physical partitioning of organelles (BIRKY 1978; TREAT-CLEMMONS and BIRKY 1983). Segregation of mitochondria from the anastomosed *A. bitorquis* cells gave rise to two dikaryons, having the same nuclear components but different mitochondria. Recombinant mitochondrial genomes were not detected by RFLPs. Since dikaryons having either the 8-1 or 34-2 mitochondrial types were recovered mitochondrial inheritance was biparental. The two cell types segregated upon vegetative growth, and cells having the 34-2 mitochondrial type eventually outcompeted cells having the 8-1 mitochondrial type.

During sporocarp development one, or a very few, germ cells proliferate to give rise to the gills, basidia, and eventually the spores. At the junction of two cultures, one would expect to occasionally recover chimeric fruitbodies, composed of dikaryotic cells having different cytoplasms. This is aptly illustrated by the pale-gill variant of *Coprinus lagopus* described by DAY (1959). This is a cytoplasmically inheritable condition where the density of tetrads on the gill surface is greatly reduced, hence the fruit bodies are pale in color. Pairing of the pale-gill homokaryon with a normal stock results in certain fruitbodies having sectored, rather than uniformly pale or dark, gills. This probably indicates segregation of the two cytoplasmic types even during growth and differentiation of the fruitbody (FINCHAM, DAY and RADFORD 1979). A similar result was expected for the *A. bitorquis* dikaryon collected at the junction of the two cultures. Certain of the germ line cells should contain the 8-1 cytoplasm whereas others should contain the 34-2 cytoplasm with both contributing to the fruitbody and spores. Fruiting of the junction dikaryon (Dik A) revealed a bias which favored the transmission the 8-1 mitochondrial type to the basidiospore progeny despite the junction fruitbody being chimeric for mitochondrial type. We expected that spores with either parental mitochondrial type would be equally recovered from the chimeric fruitbody; however, the transmission of mitochondria to the spore progeny was biased in favor of the nuclear donating strain (8-1). Whether this is a general feature of nuclear donating strains, or is unique to 8-1, remains to be determined. Spore inviability, conferred by the 34-2 mitochondrial genotype, could explain this bias in mitochondrial transmission. This is not the case, however, as spore progeny of the dikaryon collected distal to the junction zone (Dik D), carried the 34-2 mitochondrial type. There was no physiological barrier preventing the transmission of either mitochondrial type to the spores. The bias in mitochondrial transmission may depend on the timing of spore maturation and release. Late release of spores with the 34-2 mitochondrial type would result in an apparent bias in mitochondrial transmission. A third possibility is that the presence of the 8-1 mitochondrial type in a chimeric fruitbody suppresses the development of basidia and basidiospores having the 34-2 mitochondrial type. Microdissection and culture of immature spores and gill tissues in conjunction with mt DNA analysis will resolve this question.

The growth rate of the dikaryon, and the transmission of mitochondria to the spore progeny, were both influenced by mitochondrial genotype. Does mitochondrial genotype influence the phenotype of the vegetative homokaryons? In order to answer this question we selected homokaryotic strains having identical nuclei but different cytoplasms. This is possible for fungi with an extended dikaryotic stage prior to sexual fusion of the nuclei provided that: (1) the two mitochondrial types segregate in a predictable way and (2) the hyphae can be dedikaryotized and homokaryons recovered. Nuclear migration provided a mechanism for the prediction of mitochondrial type, hence a dikaryon, (Dik D), carrying both parental nuclei (8-1, 34-2) but only one mitochondrial type (34-2), was chosen for dedikaryotization. Protoplasts containing single nuclei, or multiple nuclei of the same type, gave rise to homokaryotic cultures upon regeneration. Almost 50% of the protoplast regenerates were homokaryotic and contained nuclei from 8-1 and mitochondria from 34-2. This strain was called 8n34m to indicate the source of its genetic components. No homokaryons containing nuclei from 34-2 were recovered. Asymmetry in the recovery of homokaryons has been observed in other basidiomycetes and has been correlated to mating type in Schizophyllum (RAPER 1985). For this reason the reciprocal experiment (the recovery of homokaryons containing 34-2 nuclei and 8-1 mitochondria from Dik A) was not possible. When 8n34m was paired with 34-2, nuclear migration toward 34-2 was again observed. Nuclear migration was unaffected by mitochondrial genotype and must therefore be dependent on the 8-1 nucleus. Back-crossing the spore progeny to the parental stocks, gave inconclusive results for the transmission of the nuclear migration trait (not shown). This was in part due to the difficulty in effectively scoring this trait. Nuclear migration is probably an elaborate process requiring the interaction of many nuclear gene products. We have shown, however, that nuclear migration was not dependent on the mitochondrial genome. As in the dikaryon, the mitochondrial genotype of the homokaryons influenced vegetative growth. Unmodified strain 8-1 grew closely appressed to the agar surface while the hyphae of 8n34m grew more aerially. The

two cultures were easily distinguished by colony morphology.

Mitochondrial genotype had an obvious effect on the phenotypic expression of both the dikaryotic and the homokaryotic stages in the life history of *A. bitorquis*. Two strategies can be taken by basidiomycetes for the transmission of mitochondria. Bidirectional nuclear migration results in the equal clonal propagation of the mitochondrial genotypes of both mates. Specific mitochondrial types can be traced back through the pedigree of an individual as mitochondrial inheritance is clonal and biparental (TAYLOR 1986). When there is no nuclear migration, the parental mitochondrial genotypes segregate from the anastomosed cells and there is a much greater opportunity for mitochondrial genome recombination. Mitochondrial inheritance could be either biparental or uniparental, depending on the bias in the transmission of mitochondria via the basidiospore progeny. Recombination of different mt DNAs prevents the tracing of specific mitochondrial types through a pedigree; however, mitochondrial mutations can still be traced. Unilateral nuclear migration combined elements of both of these strategies. Transmission of the parental mitochondrial types to the dikaryon was biparental but unequal, favoring that of the nuclear recipient mate. Alternatively, the bias in mitochondrial transmission via the basidiospore progeny favored the mitochondrial type of the nuclear donor. This compensatory factor could ensure that both mitochondrial types are maintained within a breeding population of *A. bitorquis*.

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