APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1994, p. 4456–4460
0099-2240/94/$04.00+0
Copyright © 1994, American Society for Microbiology
Vol. 60, No. 12

Uniparental Mitochondrial Transmission in the Cultivated Button Mushroom, *Agaricus bisporus*

TIANRU JIN AND PAUL A. HORGEN*

Centre for Plant Biotechnology, Department of Botany, University of Toronto, Erindale Campus, Mississauga, Ontario L5L 1C6, Canada

Received 20 July 1994/Accepted 24 September 1994

A uniparental mitochondrial (mt) transmission pattern has been previously observed in laboratory matings of the cultivated mushroom *Agaricus bisporus* on petri dishes. In this study, four sets of specific matings were further examined by taking mycelial plugs from the confluent zone of mated homokaryons and inoculating these plugs into rye grain for laboratory fruiting and for fruiting under industrial conditions. Examination of the mt genotype of each individual fruit body for mt-specific restriction fragment length polymorphisms further confirmed that the mt genome was inherited uniparentally. The vegetative radial growth and the fruiting activity of two pairs of intraspecific heterokaryons, each pair carrying the same combination of nuclear genomes but different mt genotypes, were compared. Our results suggested that the mt genotype did not appreciably affect radial growth or fruiting activity. The failure to recover both heterokaryons, each carrying either parental mt genotype in any given cross, therefore clearly indicated that in matings of *A. bisporus*, the mt genome from one of the parental homokaryons is either selectively excluded in the newly formed heterokaryon or selectively eliminated in the immediate heterokaryotic mitotic progeny of the newly formed heterokaryon.

The patterns of organelle (mitochondria [mt] and chloroplast) transmission are quite variable among eukaryotes (3, 4, 9, 18, 28, 35). In fungi, uniparental inheritance of mt genomes from maternal or protoperitheciating parents has been observed in *Neurospora*, *Aspergillus*, and *Podospora* spp. (2, 20, 21, 24). In interspecific crosses of the aquatic fungus *Allomyces* spp., Borkhardt and Olson (5) provided evidence for the paternal inheritance of mitochondria. In members of the class *Homobasidiomycetes*, mating is usually carried out by anastomosis between mycelia of two compatible homokaryotic cultures. mt inheritance has been examined in laboratory matings for a number of members of the *Homobasidiomycetes*, including *Coprinus cinereus* (22), *Agaricus bitorquis* (11), *Armillaria bulbosa* (31), and *Schizophyllum commune* (32). In these organisms, it was always possible, in a given pairing, to recover two types of heterokaryons (each carrying the mt genotype from either of the parental homokaryons).

We have recently initiated studies on the mt transmission in another basidiomycete, *Agaricus bisporus* (15). Using 15 homokaryons derived from 10 heterokaryons possessing 4 different mt genotypes, we examined the mt transmission of *A. bisporus* in laboratory matings performed in petri dishes. Our observations suggested that the resultant heterokaryons from any given pairing always carried the mt genotype from one of the parental homokaryons. In most crosses, the mt genotype from the faster-growing homokaryon was transmitted. Some slower-growing homokaryons could transmit their mt genotype when they were allowed to grow on the mating plate for a number of days prior to the inoculation of the faster-growing parental homokaryon. In the crosses in which the slower-growing homokaryon transmitted its mt genome, heterokaryons carrying the mt genome of the faster-growing homokaryon were not recovered (15). Our observations suggested that uniparental mt inheritance was related to but not determined by the radial growth of the homokaryons used for the matings (15).

To further examine the uniparental mt inheritance in *A. bisporus*, we compared the growth and the fruiting ability of two pairs of intraspecific heterokaryons (each pair carrying the same combination of nuclear genomes but different mt genomes). Four sets of matings, which had previously exhibited uniparental mt transmission (15), were reexamined by a modified procedure for recovering heterokaryons. The results from this study further confirmed that uniparental mt transmission exists in *A. bisporus*.

MATERIALS AND METHODS

Materials. Unless specified, the chemicals used in this study were of reagent grade. The nutrient components of the media were purchased from Difco Laboratories, Detroit, Mich. Restriction endonucleases were the products of Bio/Can, Mississauga, Canada, or Life Technologies, Bethesda Research Laboratories, Bethesda, Md.

Strains. Five homokaryons carrying three different mt types were used in matings (Table 1). Two pairs of heterokaryons generated in the previous study (15), each member of the pair carrying the same combination of nuclear genomes but different mt types, were compared on the basis of radial growth and fruiting ability.

Matings. The mating procedure was described previously (8). In this study, we modified the method for recovering heterokaryons. After two homokaryons had formed a 3-cm confluent zone on a mating plate, a mycelial plug from the confluent zone (about 1.5 cm from each side) was excised and inoculated into 100 g of rye grain in a 500-ml flask for laboratory fruiting. Each individual fruit body was considered an intraspecific heterokaryon. One of the matings, Ag1-$1_{(II)}$ × Ag 89-$65_{(I)}$ (I and II refer to the mt genotype [15]), was also performed directly in rye grain. Homokaryons Ag 1-$1_{(II)}$ and

* Corresponding author. Mailing address: Centre for Plant Biotechnology, Department of Botany, University of Toronto, Erindale Campus, Mississauga, Ontario L5L 1C6, Canada. Phone: (905) 828-5424. Fax: (905) 828-3792.

TABLE 1. mt genotypes of the mushrooms generated by laboratory fruiting

Pairings[a] (trial)[b]	Time (days) for first break[c]	No. of fruit bodies produced/container[d]	No. of fruit bodies examined	mt genotype
Ag1-1(II) × Ag89-65(I) (T1)	30.5	3.5	6	All II
Ag1-1(II) × Ag89-65(I) (T2)	33.0	4.0	8	All II
Ag1-1(II) × Ag89-65(I)[e]	32.0	4.0	8	All II
Ag2-20(II) × Ag89-65(I) (T1)	20.0	>16	10	All II
Ag2-20(II) × Ag89-65(I) (T2)	22.0	>16	25	All II
Ag2-20(II) × Ag50HB(I) (T1)	23.5	6	5	All I
Ag2-20(II) × Ag50HB(I) (T2)	26.0	5.5		
Ag2-20(II) × Ag85-51(IV) (T1)	17.5	5.5	5	All II
Ag2-20(II) × Ag85-51(IV) (T2)	21.0	3.5	5	All II

[a] Ag2-20 and Ag1-1 (both fast growing) were derived from $Ag2_{(II)}$ (ATCC 24558), an old commercial heterokaryon; Ag89-65 (slow growing) was from a wild isolate $Ag89_{(I)}$ (8); Ag50HB (fast growing) was from a commercial heterokaryon, $Ag50_{(I)}$(U3); Ag85-51 (slow growing) was derived from a field-collected isolate, $Ag85_{(IV)}$ (17).
[b] T1, trial 1; T2, trial 2.
[c] After casing.
[d] Plastic cup (hold approximately 60 g [dry weight] of rye grain).
[e] Matings performed directly in rye grain.

Ag 89-$65_{(I)}$ were grown on individual potato dextrose agar plates for 14 days. An agar block measuring about 9 cm^2 was excised from each homokaryotic culture, sliced into small pieces, and inoculated directly into 100 g of sterile rye grain. Fruiting trials were either done by the cased-grain method of San Antonio (29) in our laboratory or fruited under industrial conditions by L. F. Lambert Spawn Company Inc., Coatsville, Pa.

DNA extraction. The DNA extraction procedure used in this study has been described previously (10).

Examination of the mt genotypes of mushrooms. Plasmids p50m1, p50m4b, p50m6, and p50m7 (10), mixed at equal concentrations, have been previously used as mt probes to examine the mt restriction fragment length polymorphisms of intraspecific heterokaryons (15). In this study, in addition to this probe, a recombinant plasmid, p50m1B1E1 (14), was used to examine the mt genotype of mushrooms. p50m1B1E1 contains a 2.7-kb fragment of the large inverted repeat of the Ag50 mt genome. DNA homology and size variation of the *Eco*RI fragments within the large inverted repeat (IR) has been observed in three mt genotypes used in this study (14) (Fig. 1).

Growth measurement. Three different solid media were used to measure the radial growth of heterokaryons. The formula for complete yeast medium (CYM) was that of Stevens (33). Potato dextrose agar was purchased from Difco. The composition of malt extract agar was the same as that of CYM, except that yeast extract was replaced by 1% malt extract. A 2-mm inoculum cube was placed in a freshly prepared solid agar plate. Radial growth was determined every 7 days by measuring the distance from the center of the petri dish (marked on the underside when the inoculation was made) to the colony margins (26). For each plate, the average of at least three measurements from different angles were taken. Each isolate was examined in three separate cultures containing the same medium. Mean values from triplicate plates were compared.

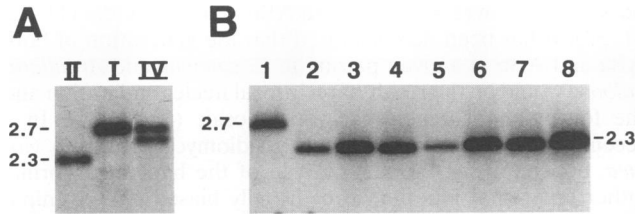

FIG. 1. Determination of mt genotype of *A. bisporus*. (A) Three mt genotypes were identified by hybridizing p50m1B1E1 (15) to *Eco*RI-digested mycelial DNA of $Ag2_{(II)}$, $Ag50_{(I)}$, and $Ag85_{(IV)}$. (B) The mt genotype of mushrooms recovered from a pairing of homokaryons Ag1-$1_{(II)}$ and Ag89-$65_{(I)}$. *Eco*RI-digested mushroom DNA hybridized to p50m1B1E1. Lanes: 1, Ag89-$65_{(I)}$; 2, Ag1-$1_{(II)}$; 3 to 8, six mushrooms recovered from a cross of Ag1-1 × Ag89-65.

RESULTS

Uniparental mt transmission. Pairings were performed in duplicate for four different matings (Table 1). The pairing between Ag1-$1_{(II)}$ and Ag89-$65_{(I)}$ was also performed directly in rye grain. mt genotypes of 5 to 35 fruit bodies were examined for each pairing. As shown in Table 1, for each specific pairing the fruit bodies examined all carried the same mt genotype. Figure 1A shows the hybridization of p50m1B1E1 (14) to *Eco*RI-digested mycelial DNA of Ag50 (type I), Ag2 (type II), and Ag85 (type IV). This probe clearly differentiates the three mt genotypes used in this study. Figure 1B shows the hybridization of p50m1B1E1 to *Eco*RI-digested mycelial DNA of homokaryon Ag1-$1_{(II)}$ and Ag89-$65_{(I)}$ and six fruit bodies of Ag1-$1_{(II)}$ × Ag89-$65_{(I)}$. These mushrooms all carried the mt genotype of Ag1-$1_{(II)}$.

Radial growth measurement. In a previous study (15), heterokaryons carrying either the $Ag2_{(II)}$ or $Ag89_{(I)}$ mt genome were obtained in separate pairings between Ag2-$23_{(II)}$ (slow growing) and Ag89-$65_{(I)}$ (slow growing [34]). Crosses between Ag1-$1_{(II)}$ (fast growing [34]) and Ag89-$65_{(I)}$ were all found to produce heterokaryons carrying the mt genome of Ag1-$1_{(II)}$. However, in three separate crosses, Ag89-$65_{(I)}$ was allowed to grow on the mating plate for 7 days prior to the inoculation of Ag1-$1_{(II)}$. These three pairings all generated heterokaryons carrying the Ag89-$65_{(I)}$ mt genome (15).

Four heterokaryons of Ag1-1 × Ag89-65 (two carrying the type II mt genome, and two carrying the type I mt genome) and four heterokaryons of Ag2-23 × Ag89-65 (two carrying the type II mt genome, and two carrying the type I mt genome) were compared with respect to radial growth (Fig. 2). The results suggested that (i) the heterokaryons of Ag1-1 × Ag89-65 exhibited faster radial growth than the heterokaryons of Ag2-23 × Ag89-65 did, (ii) malt extract agar was the most effective nutrient source used in this study and gave the fastest

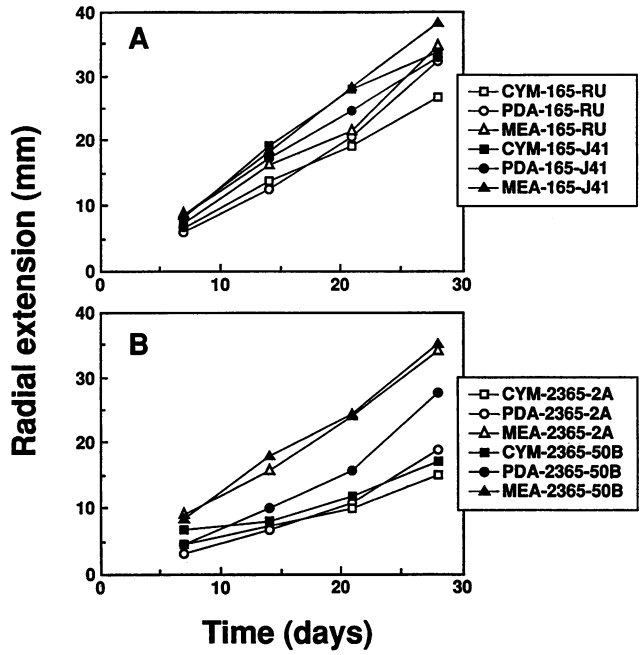

FIG. 2. Radial extension of selected intraspecific heterokaryons. Each point represents the average of measurements taken from three petri dishes. Variations for each point were very small. Standard deviations for all measurements were less than 15%. (A) Heterokaryons obtained from the Ag1-1 × Ag89-65 crosses, i.e., $165RU_{(II)}$ and $165J4I_{(I)}$. (B) Heterokaryons obtained from the Ag2-23 × Ag89-65 crosses, i.e., $2365\text{-}2A_{(II)}$ and $2365\text{-}50B_{(I)}$.

radial extension for all the strains examined, and (iii) there were no appreciable differences in radial extension between the heterokaryons with the same nuclear background but with the different mt genotypes used for this study (Fig. 2).

Fruiting activity. The fruiting activity of selected heterokaryons was examined and is presented in Table 2. Two heterokaryons of $Ag1\text{-}1_{(II)}$ × Ag $89\text{-}65_{(I)}$, $165\text{-}J41_{(I)}$ and $165\text{-}RU_{(II)}$, were demonstrated to have comparable fruiting activity. We were unable to produce fruit bodies from the recovered heterokaryons of $Ag2\text{-}23_{(II)}$ × $Ag89\text{-}65_{(I)}$ in three separate fruiting trials. However, in the successful fruiting trials, no appreciable difference in fruiting was observed between $2365\text{-}2B_{(II)}$ and $2365\text{-}50A_{(I)}$ (Table 2). The radial growth on potato dextrose agar, CYM and compost extract agar and the fruiting ability of $2365\text{-}2B_{(II)}$ and $2365\text{-}50A_{(I)}$ have been independently examined by L. F. Lambert Spawn Company Inc. Their results were consistent with our observation, which suggests that these two strains showed similar growth characteristics and gave similar yields of mushrooms (Table 2).

TABLE 2. Effect of mt genotype on fruiting of selected intraspecific heterokaryons

Heterokaryon[a]	mt genotype	Time (days) for first break	No. of fruit bodies/container	Yield (lb/ft^2)
165-RU	II	23[b]	11.0	
165-J41	I	25[b]	13.0	
2365-2B	II	33[b], 28.4[c]	2.5	0.87[c]
2365-50A	I	35[b], 27.1[c]	3.0	0.91[c]

[a] The intraspecific heterokaryons were generated in the previous study (15). Each pair possesses an identical nuclear background.
[b] Performed in plastic cups for laboratory fruiting.
[c] Performed by H. Fox, L. F. Lambert Spawn Co. The Système International d'Unités equivalents for 2365-2B and 2365-50A are 0.42 and 0.45 g/cm^2, respectively.

DISCUSSION

A. bisporus has a secondarily homothallic life cycle (27). The basidiospores are binucleate and more than 90% self fertile (12, 17). Sexual mating is therefore not necessarily part of the life cycle. Our group has used protoplast production, protoplast regeneration, and restriction fragment length polymorphism analysis to generate sexual haplotypes to break down this breeding barrier (7, 8, 12). This ability to somatically generate homokaryons has allowed us to examine the mitochondrial transmission patterns in *A. bisporus* (15). Heterokaryons generated from laboratory matings with regenerated homokaryotic protoplasts are not always able to generate fruit bodies, especially under laboratory conditions (8). We chose four compatible pairs for use in this study. When they were mated, the resultant heterokaryons could be fruited under laboratory conditions (8, 15).

Unlike higher plants and animals, which generally possess specialized sexual structures, members of the *Homobasidiomycetes* rely on vegetative mycelial fusions for sexual reproduction. Since plasmogamy involves numerous hyphal fusions between parental colonies, mitochondrial mixing and recombination between mt genotypes may occur (22, 31). However, laboratory studies with members of the *Basidiomycetes* indicated that mt recombination was not a common event (1, 11, 15, 22, 31). In this study, mt restriction fragment length polymorphisms of 35 fruit bodies from the matings of $Ag2\text{-}20_{(II)}$ × $Ag89\text{-}65_{(I)}$ were examined by a mixture of the recombinant plasmids, representing approximately 30% of $Ag50_{(II)}$ mt genome (10, 14). No detectable mt mixing or mt recombination was observed.

When pairing two homokaryons carrying different nuclear genotypes (A and B) and different mitochondrial genotypes (a and b), it would be possible to generate heterokaryons designated as ABa, ABb, ABab, and ABa/b. ABab, which carries the mt genomes from both parental homokaryons, is classified as a heteroplasmon. There is no direct evidence which shows the formation of a stable heteroplasmon in any member of the *Homobasidiomycetes*. ABa/b would carry the mt genome which is generated by recombination between the mt genomes of the two parental homokaryons. In members of the *Basidiomycetes*, ABa/b was observed in matings between the homokaryons of *Coprinus cinereus*, which carried antibiotic resistance and auxotrophic mutations (1, 6). In a number of members of the *Basidiomycetes* examined (i.e., *C. cinereus*, *A. bitorquis*, *Armillaria bulbosa*, and *Schizophyllum commune*), it was always possible to recover both ABa and ABb in a given cross (11, 22, 31, 32). It has been demonstrated that the generation of both ABa and ABb in a given pairing in *C. cinereus* and *Armillaria bulbosa* would be the result of reciprocal nuclear migration and the formation of a heterokaryotic mosaic (1, 22, 31). In a recent study involving the heterobasidiomycete *Ustilago violaea*, depending on the mating type of the haploid offspring, either biparental inheritance or heavily biased (94%) uniparental inheritance was shown (36). The dikaryon formed after a mating remains a heteroplasmon until haploid formation is induced (36).

The formation of the heterokaryotic mosaic of two mt genotypes by reciprocal nuclear migration has been described as uniparental inheritance by Baptista-Ferreira et al. (1), Casselton and Economou (6), and Specht et al. (32). However,

this phenomenon has been interpreted as biparental inheritance by others (11, 31). In this study, we interpret uniparental inheritance to be the mt transmission, from a given mating, in which all the progeny generated have the same mt genotype (derived from only one of the parents).

In an earlier study, many heterokaryons produced on petri plates would inherit the mitochondrial genome from the faster-growing homokaryon (15). There were, however, some exceptions. In one pairing, we were successful in reversing the mitochondrial transmission pattern by first allowing the slower-growing homokaryon (Ag89-65) to establish itself on the medium before mating it with the faster-growing homokaryon (Ag1-1) (15). Therefore, we had two different heterokaryons, each of which carried the same combination of nuclear genotypes but possessed a different mitochondrial genome (i.e., 165-RU type II and 165-J41 type I). When we used the same strategy in the pairings of Ag2-20 (faster growing) and Ag89-65 (slower growing), we were unable to generate heterokaryons carrying the Ag89-65 mt genotype (15). When two slow-growing homokaryons (Ag89-65 and Ag2-23) were mated, heterokaryons carrying either parental mt genotype were generated (i.e., 2365-2A and 2365-50B used in this study). These two heterokaryons were never recovered from any single mating. Our working hypothesis is that the radial growth of the paired homokaryons is related to mitochondrial transmission but is not the determining factor.

In our previous study, after mating, heterokaryons were isolated by subculturing mycelial blocks taken from the confluent zone (15). It could be argued that heterokaryons carrying either of the mt genomes were generated in a given mating but that one of the heterokaryons would overgrow the other. In this study we have shown that two heterokaryons carrying the same combination of nuclear genomes but unique mt genotypes exhibited similar radial growth on three different solid media (including CYM [15] and potato dextrose agar). If heterokaryons carrying either of the two mt genotypes were generated, we would predict that we should be able to recover both heterokaryons with one or the other of the mt genotype. This was not observed.

Heterokaryons carrying the same combination of nuclear genomes but with two different mt genomes (ABa and ABb) exhibited similar fruiting activity (Table 2). Therefore, if two unique heterokaryons were generated, both heterokaryotic types (ABa or ABb) would have a similar chance of producing mushrooms. In this study, 5 to 25 mushrooms were examined for each pairing. In every case, all the fruit bodies examined carried the same mt genotype (Table 1). The uniparental mt transmission pattern observed in this study was consistent with our previous observations (15). These observations clearly indicate that in *A. bisporus*, when two homokaryons fuse, heterokaryons carrying one of the two possible mt genotypes were not (or rarely) generated.

In a number of experiments on fruiting, we attempted to inoculate the slower-growing Ag89-65 into the rye grain 7 days before we inoculated the faster-growing Ag1-1. We were unable to produce any mushrooms in these attempts.

The uniparental (usually maternal) inheritance of mt genomes in higher plants and animals is mediated by a number of physical exclusion processes (3, 4, 25). We agree with Casselton and Economou (6) that physical exclusion may also occur in higher fungi. Uniparental mt inheritance in the genera *Neurospora*, *Podospora*, and *Aspergillus* (20, 21, 24) is believed to be controlled mainly by the substantially unequal contribution of cytoplasm from one of the parents. In a recent report, Lee and Taylor (19) show that if somatic fusions of *N. tetrasperma* are generated, followed by mutually exclusive nuclear migration, 3 days after the hyphal fusions, acceptor mtDNA completely replaces donor mtDNA. In members of the *Basidiomycetes*, nuclear migration may also be considered a physical exclusion process. Reciprocal nuclear migration, which occurs in a number of members of the *Basidiomycetes*, prevents the organelle genomes of both parents from being present in a single newly formed heterokaryon (1).

In *A. bisporus*, heterokaryons are generated by anastomosis between the vegetative mycelia of two compatible homokaryons. It seems likely that each parental homokaryon involved in the anastomosis would possess a defined amount of mtDNA. Therefore, the failure to recover mushrooms carrying one or the other type of mt genome in any given mating may indicate that one of the parental mt genomes was either physically excluded during the formation of the heterokaryon or selectively eliminated in the immediate mitotic progeny of the newly formed heteroplasmon. The elimination of one of the parental organelle genomes is a common feature in a number of isogamous organisms (16, 23, 18, 28, 30).

In an attempt to design experiments to distinguish whether physical exclusion or selective elimination was involved in the uniparental mt transmission in the button mushroom, we attempted the following. We sampled mycelial plugs at different distances from the confluent zone in the direction of the slower-growing homokaryon to determine if we could recover a heteroplasmon carrying both mt genotypes. We have tried this strategy for the matings between Ag2-20 (faster growing) and Ag89-65 (slower growing). Analysis of samples recovered up to 3 mm away from Ag2-20 in the direction of the Ag89-65 homokaryon revealed either heterokaryons carrying Ag2-20 mt genotype or unmated Ag89-65 (13). The difficulty with these experiments was that the samples had to be subcultured twice before enough total DNA could be extracted for restriction fragment length polymorphism analysis to determine both nuclear and mt status. This technical difficulty makes the interpretation of these results problematic. The slow growth of *A. bisporus*, the lack of a reliable mutational marker(s) for homokaryons, and the multinuclear status of the mycelia limit the approaches available to investigate this phenomenon. One possible molecular strategy would be to use PCR to determine the nuclear and mt status of the mycelium samples taken initially from the mating plates, which would bypass the subculturing step described above.

Our results to date suggest that *A. bisporus*, under a number of different conditions of mating, possesses a strong bias to transmit mt genomes in a uniparental fashion. We believe that investigation of the mechanisms involved in this phenomenon would add to our understanding of organelle inheritance, especially in organisms in which undifferentiated sexual organs are involved in the process of plasmogamy.

ACKNOWLEDGMENTS

We thank H. Fox, L. F. Lambert Spawn, for her experiments under industrial conditions.

This work was supported by a grant from the University Research Incentive Fund of the Province of Ontario.

REFERENCES

1. **Baptista-Ferreira, J. L. C., A. Economou, and L. A. Casselton.** 1983. Mitochondrial genetics of *Coprinus*: recombination of mitochondrial genomes. Curr. Genet. **7**:405–407.
2. **Belcour, L.** 1975. Cytoplasmic mutations isolated from protoplasts in *Podospora anserina*. Genet. Res. **25**:155–161.
3. **Birky, C. W., Jr.** 1978. Transmission genetics of mitochondria and chloroplasts. Annu. Rev. Genet. **12**:471–512.
4. **Birky, C. W., Jr.** 1983. Relaxed cellular controls and organelle heredity. Science **222**:468–475.

5. **Borkhardt, B., and L. W. Olson.** 1983. Paternal inheritance of the mitochondrial DNA in interspecific crosses of the aquatic fungus *Allomyces*. Curr. Genet. **7:**403–404.
6. **Casselton, L. A., and A. Economou.** 1985. Dikaryon formation, p. 213–229. *In* D. Moore, L. A. Casselton, D. A. Wood, and J. C. Frankland (ed.), Developmental biology of higher fungi. Cambridge University Press, Cambridge, Mass.
7. **Castle, A. J., P. A. Horgen, and J. B. Anderson.** 1987. Restriction fragment length polymorphisms in the mushrooms *Agaricus brunnescens* and *Agaricus bitorquis*. Appl. Environ. Microbiol. **53:**816–822.
8. **Castle, A. J., P. A. Horgen, and J. B. Anderson.** 1988. Crosses among homokaryons from commercial and wild-collected strains of the mushroom *Agaricus brunnescens* (=*A. bisporus*). Appl. Environ. Microbiol. **54:**1643–1648.
9. **Gillham, N. W.** 1978. Organelle/Heredity, p. 602. Raven Press, New York.
10. **Hintz, W. E. A., J. B. Anderson, and P. A. Horgen.** 1988. Physical mapping of the mitochondrial genome of the cultivated mushroom *Agaricus brunnescens* (=*A. bisporus*). Curr. Genet. **14:**43–49.
11. **Hintz, W. E. A., J. B. Anderson, and P. A. Horgen.** 1988. Nuclear migration and mitochondrial inheritance in the mushroom *Agaricus bitorquis*. Genetics **119:**35–41.
12. **Horgen, P. A., T. Jin, and J. B. Anderson.** 1991. The use of protoplast production, protoplast regeneration and restriction fragment length polymorphisms in developing a systematic and highly reproducible breeding strategy for *Agaricus bisporus*, p. 62–72. *In* L. J. L. D. van Griensven (ed.), Genetics and breeding of *Agaricus*. Proceedings of the First International Seminar on Mushroom Science, Mushroom, Horst. Centre for Agricultural Publishing and Documentation (Pudoc), Wageningen, The Netherlands.
13. **Jin, T.** 1993. Mitochondrial inheritance and further studies on the mitochondrial genome of the cultivated mushroom *Agaricus bisporus* (=*A. brunnescens*). Ph.D. thesis. University of Toronto, Toronto, Canada.
14. **Jin, T., and P. A. Horgen.** 1993. Further characterization of a large inverted repeat in the mitochondrial genomes of *Agaricus bisporus* (=*A. brunnescens*) and related species. Curr. Genet. **23:**228–233.
15. **Jin, T., A. S. M. Sonnenberg, L. J. L. D. van Griensven, and P. A. Horgen.** 1992. Investigations of mitochondrial transmission in selected matings between homokaryons from commercial and wild-collected isolates of *Agaricus bisporus* (=*A. brunnescens*). Appl. Environ. Microbiol. **58:**3553–3560.
16. **Kawano, S., and T. Kuroiwa.** 1989. Transmission pattern of mitochondrial DNA during plasmodium formation in *Physarum polycephalum*. J. Gen. Microbiol. **135:**1559–1566.
17. **Kerrigan, R. W., L. Baller, P. A. Horgen, and J. B. Anderson.** 1992. Strategies for the efficient recovery of *Agaricus bisporus* homokaryons. Mycologia **84:**575–579.
18. **Kuroiwa, T.** 1985. Mechanisms of maternal inheritance of chloroplast DNA: an active digestion hypothesis. Microbiol. Sci. **2:**267–270.
19. **Lee, S., and J. Taylor.** 1993. Uniparental inheritance and replacement of mitochondrial DNA in *Neurospora tetrasperma*. Genetics **134:**1063–1075.
20. **Mannella, C. A., T. H. Pittenger, and A. M. Lambowitz.** 1979. Transmission of mitochondrial deoxyribonucleic acid in *Neurospora crassa* sexual crosses. J. Bacteriol. **137:**1449–1451.
21. **Mason, J. R., and G. Turner.** 1975. Transmission and recombination of extranuclear genes during sexual crosses in *Aspergillus nidulans*. Mol. Gen. Genet. **143:**93–99.
22. **May, G., and J. W. Taylor.** 1988. Patterns of mating and mitochondrial DNA inheritance in the Agaric Basidiomycete *Coprinus cinereus*. Genetics **118:**213–220.
23. **Meland, S., S. Johansen, T. Johansen, K. Haugli, and F. Haugli.** 1991. Rapid disappearance of one parental mitochondrial genotype after isogamous mating in the myxomycete *Physarum polycephalum*. Curr. Genet. **19:**55–60.
24. **Mitchell, M. B., and H. K. Mitchell.** 1952. A case of "maternal" inheritance in *Neurospora crassa*. Proc. Natl. Acad. Sci. USA **38:** 442–449.
25. **Mogensen, H. L.** 1988. Exclusion of male mitochondria and plastids during syngamy in barley as a basis for maternal inheritance. Proc. Natl. Acad. Sci. USA **85:**2594–2597.
26. **Rainey, P. B.** 1991. Effect of *Pseudomonas putida* on hyphal growth of *Agaricus bisporus*. Mycol. Res. **95:**699–704.
27. **Raper, C. A.** 1976. Sexuality and life-cycle of the edible, wild *Agaricus bitorquis*. J. Gen. Microbiol. **95:**54–66.
28. **Sager, R., H. Sano, and C. T. Grabowy.** 1984. Control of maternal inheritance by DNA methylation in Chlamydomonas. Curr. Top. Microbiol. Immunol. **108:**157–173.
29. **San Antonio, J. P.** 1971. A laboratory method to obtain fruit body from cased grain spawn of the cultivated mushroom, *Agaricus bisporus*. Mycologia **63:**16–21.
30. **Silliker, M. E., and O. R. Collins.** 1988. Non-mendelian inheritance of mitochondrial DNA and ribosomal DNA in the myxomycete, *Didymium iridis*. Mol. Gen. Genet. **213:**370–378.
31. **Smith, M. L., L. C. Duchesne, J. N. Bruhn, and J. B. Anderson.** 1990. Mitochondrial genetics in a natural population of the plant pathogen *Armillaria*. Genetics **126:**575–582.
32. **Specht, C. A., C. P. Novotny, and R. C. Ullruch.** 1992. Mitochondrial DNA of *Schizophyllum commune*: restriction map, genetic map, and mode of inheritance. Curr. Genet. **22:**129–134.
33. **Stevens, R. B. (ed.).** 1981. Mycology guidebook. University of Washington Press, Seattle.
34. **Stockton, M., and P. Horgen.** 1993. Analysis of the radial growth in selected pedigrees of *Agaricus bisporus*. Cultiv. Mushroom Res. Newsl **1:**38–43.
35. **Taylor, J. W.** 1986. Topical review: fungal evolutionary biology and mitochondrial DNA. Exp. Mycol. **10:**259–269.
36. **Wilch, G. S., and A. Castle.** 1992. Transmission of mitochondrial DNA in *Ustilago violacea*. Curr. Genet. **22:**135–140.