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Mitochondrial Genetics in a Natural Population of the Plant Pathogen Armillaria

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ABSTRACT

Transmission and propagation of mitochondrial genotypes in fungi have not been previously investigated in the field. This study examined the distribution of nuclear and mitochondrial genotypes in a natural, local population of the fungal (Basidiomycetes) root-rot pathogen, Armillaria. Six vegetative clones, ranging in size up to 635 m, were identified on the basis of mating-type alleles. Mitochondrial DNA (mtDNA) restriction fragment patterns indicated that each vegetative clone has one, unique mtDNA type. However, as in other basidiomycetous fungi, biparental transmission of mitochondria following laboratory matings of sexually compatible haploid isolates of Armillaria resulted in a uniformly diploid mycelium that was a mosaic for both parental mitochondrial types. Therefore, either matings between monosporous, haploid isolates are uncommon in nature, or when mating does occur, cytoplasmic markers of one partner predominate during subsequent vegetative growth.

As evolutionary studies of fungi rely increasingly on analysis of molecular characters of mtDNA, the question of how miotochondrial genotypes actually disperse in nature becomes important. The clonal pattern evident in mitochondrial lineages of mouse (Fort *et al.* 1984) and pocket gopher (Avise *et al.* 1979) serves as an evolutionary model for organisms that inherit mitochondria from one parent only, where sequence variation gradually accrues over time within a lineage. This model may not apply to organisms that frequently inherit mitochondria from both parents, where the potential for recombination between mitochondrial genomes exists in heteroplasmons (cells that contain more than one mitochondrial genotype).

The Basidiomycetes, a class of higher fungi, may provide an exception to the clonal evolutionary pattern resulting from uniparental organelle inheritance. In matings of basidiomycetes, haploid nuclei from a donor mycelium can migrate extensively throughout a recipient mycelium resulting in a dikaryon, a mycelium in which two haploid nuclei are paired in each cell (Buller 1931; Raper 1966). Nuclear migration is usually bidirectional, where both parents simultaneously act as donor and recipient. Recent studies of the basidiomycetes *Agaricus bitorquis* (Hintz, Anderson and Horgen 1988) and *Coprinus cinereus* (Baptista-Ferreira, Economou and Casselton 1983; May and Taylor 1988) showed that mitochondria do not migrate along with nuclei in sexual crosses. Cytoplasmic mixing in these crosses is limited to the narrow zone of hyphal fusion between parents, where recombination between different mtDNAs may occur (Baptista-Ferreira, Economou and Casselton 1983). The dikaryon resulting from bidirectional nuclear migration is therefore a mosaic of two mitochondrial types. One could hypothesize that there is an extended period during which mitochondrial recombination can occur within such an "individual."

Although patterns of mitochondrial transmission in basidiomycetes have been well described in laboratory studies, the possibility of biparental transmission of mtDNA in fungi has not been investigated in nature where multiple mitochondrial types might confer a selective advantage under constantly changing macro- and microenvironmental conditions. Inheritance and propagation of mitochondrial genotypes in nature can be studied by examining the distribution of mitochondrial and nuclear markers within and between established vegetative clones. The different modes of inheritance of nuclear and mitochondrial genomes can provide useful criteria in studies of the mechanism and frequency of mating events in natural basidiomycete populations, since the detection of mitochondrial mosaics within a single natural clone would provide evidence that the sampled individual arose after a mating event.

The genus *Armillaria* is comprised of several biological species distributed throughout the world as wood saprobes and as increasingly important root-rot pathogens in reforested areas. This group offers an excellent model for studying mitochondrial transmission in the field for several reasons. First, members of the genus are long-lived clonal organisms that can spread vegetatively over several hectares through the soil (Anderson *et al.* 1979; Kile 1983; Korhonen 1978;

SHAW and ROTH 1976). Vegetative clones of Armillaria in forest sites are therefore amenable to long term study. Second, the mating system of Armillaria is well understood. Unlike other basidiomycetes in which the sexually fertile mycelium is dikaryotic, that of Armillaria is diploid (HINTIKKA 1973; ULLRICH and ANDERSON 1978). Like many other basidiomycetes (RAPER 1966), sexual compatibility within biological species is determined by a bifactorial nuclear system (ULLRICH and ANDERSON 1978; KORHONEN 1978). Compatible mating interactions occur when different alleles at two unlinked loci are carried by the paired, haploid mycelia. In basidiomycetes, multiple alleles occur at each mating-type locus and distribution of these alleles among nonclonal isolates is apparently random within local populations but nonrandom within individual substrates (ULLRICH 1977). By determining the distribution of mating-type alleles among fruit-bodies in a given area it has been possible to map the extent of vegetative clones of Armillaria (ULLRICH and ANDERSON 1978; KORHONEN 1978; KILE 1983). Fruit-bodies from the same clone have identical mating-type alleles, while those from different clones have different alleles at one or both loci. Third, it has been shown that mtDNA restriction fragment length polymorphisms (RFLPs) are diagnostic of biological species in the genus Armillaria, and that individual isolates within biological species can be identified by minor variations in restriction fragment patterns (SMITH and ANDERSON 1989). This variation in mtDNA can be used to follow mitochondrial inheritance in the field.

The objective of this study was to determine the clonal structure and distribution of mitochondrial genotypes in a local population of Armillaria. Furthermore, the genetic structure of these natural clones was compared to that of clones derived from sexual crosses in laboratory experiments.

MATERIALS AND METHODS

Clone mapping: Distribution of Armillaria clones in a 122 × 53 m plot were determined by standard sampling and diagnostic methods (KORHONEN 1978; ULLRICH and ANDERSON 1978). The study site is located within a clear-cut hardwood stand (Acer-Quercus-Vaccinium habitat type) in northern Michigan (lat. 45° 58′ 28″ N, long. 88° 21′ 46″ W), replanted with red pine seedlings in June 1984. Data based on demarcation lines between diploid cultures (ADAMS 1974; SHAW and ROTH 1976) indicated that several clones of Armillaria occurred at the site (data of J.N.B.). In pairings of diploid mycelia, a demarcation line indicates genetic nonidentity (KORHONEN 1978). In September 1988, 35 Armillaria fruit-bodies (collection numbers 424–458) were collected at approximately regular intervals throughout the study site, and up to ten single-spore isolates (designated 424-1, 424-2, etc.) were obtained from each fruit-body. To determine mating types for each fruit-body, sibling single-spore isolates were paired in all combinations by placing inocula 2–3 mm apart on malt extract (ME) agar in the center of petri plates (ULLRICH and ANDERSON 1978). In

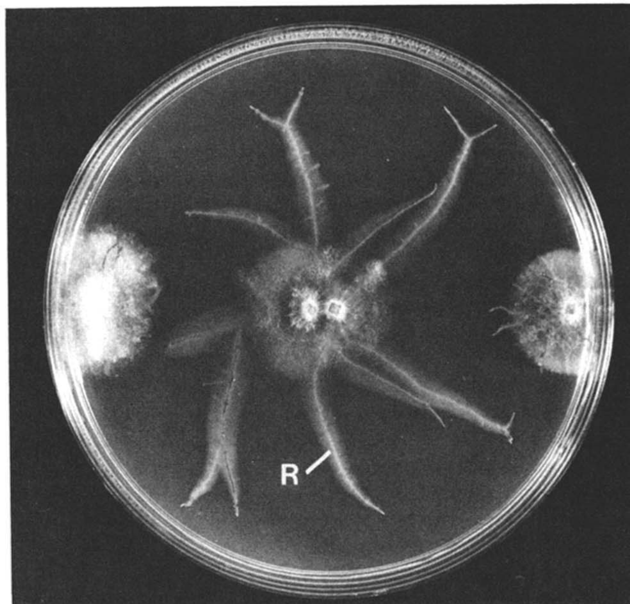

FIGURE 1.—Diploid mycelium (middle) from the compatible mating interaction of single-spore isolates of *A. bulbosa* parents from clone 1 (left), and clone 2 (right). Rhizomorphs (R) are linear, cordlike structures comprised of hyphae and characteristic of the genus *Armillaria*.

addition, isolates were inoculated at the respective opposite sides of each petri plate to aid comparison of mated and unmated colony morphology (Figure 1). Mating type representatives were chosen for each fruit-body and paired with those of two or three of the closest fruit-bodies to establish clonal distribution at the site. Crosses between representatives from widely separated areas within each clone were also performed to verify the clonal structure of Armillaria at the site. To identify biological species, representatives of each clone were mated with two haploid isolates from each of the known North American biological species (NABS) (ANDERSON 1986).

In September 1989, additional Armillaria fruit-bodies were obtained from the surrounding hardwood stand. The location of collections around the perimeter was determined by triangulation with mapped points within the clear-cut area. Collections were also made along two transects, extending 500 m east of the collection point of fruit-body 430, and 500 m west of fruit-body 439 collection point (Figure 2). The 1989 fruit-body collection numbers and coordinates, in meters from the study site origin (northeast, northwest) are: 490 (109, 59), 492 (98, 51), 495 (75, 67), 496 (42, 66), 514 (−27, 13), 515 (15, −59), 516 (−131, 224), 517 (−106, 196), 518 (−105, 198), 519 (−50, 168), 521 (17, 67), 522 (4, 61), 523 (12, 85), 524 (34, 110), 525 (92, 99), 526 (336, −207), 528 (203, −198), 530 (133, −125), 531 (113, −64), 532 (−36, 152). Mating-type representatives for each fruit-body were determined as above. The sibling mating interactions suggested that all 20 collections belong to NABS VII. Verification of species identification and clonal associations for each fruit-body was possible after mating the representatives with all four mating types of 445 (clone 1), and 435 (clone 2).

Distribution of mtDNAs in field isolates: Total cellular DNAs and mtDNAs were extracted from single-spore isolates as described by SMITH and ANDERSON (1989). DNAs were digested with restriction enzymes according to the manufacturers specifications (Bio/Can, Mississauga, Ontario), prior to electrophoresis in 0.7% agarose gels. Southern blotting of total cellular DNA was performed (Gene-

screen Plus, Du Pont Canada, Inc., Lachine, Québec) by the alkaline method of REED and MANN (1985). mtDNA *Eco*RI and *Hin*dIII restriction fragment patterns were visualized through autoradiography, using radiolabeled mtDNA from isolate 450-1 as a probe of Southern blots (ANDERSON, PETSCHE and SMITH 1987).

Intraclonal mtDNA RFLPs: To investigate possible variations of the mtDNA within the largest clone, mtDNA RFLPs were also examined in the five isolates 436-2, 437-1, 446-1, 450-1 and 454-1, after digestion with the enzyme *Sau*3AI, which recognizes a 4-bp sequence. mtDNAs were dephosphorylated with calf intestinal phosphatase (Bio/Can) and end-labeled using T4 polynucleotide kinase (Bio/Can) and [γ-^{32}P]dATP. Electrophoresis was in a 40 × 30 cm × 0.4 mm, nondenaturing 3.5% polyacrylamide gel, and restriction fragments were visualized by autoradiography (MANIATIS, JEFFREY and VAN DESANDE 1975). PhiX174 DNA (Bio/Can), digested with *Taq*I and end-labeled as above, provided the molecular size standards.

Inheritance of mitochondrial genotypes in laboratory matings: The inheritance of both mitochondrial and nuclear markers was determined in a laboratory mating between isolate 425-2 of clone 1, and 435-6 of clone 2 (see Figure 2). After 1 month incubation at room temperature, rhizomorphs originating from both parents were observed. Rhizomorph and mycelial explants were taken from 20 areas throughout the mated colony and grown on ME agar for 2 weeks. To avoid sampling a mixture of the two parental mycelia, hyphal tip cells were isolated from each explant. Hyphal tip cells were transferred to fresh ME plates, incubated for two weeks at room temperature, then transferred to liquid complete yeast medium (CYM; RAPER, RAPER and MILLER 1972) for one month before harvest and lyophilization (ANDERSON, BAILEY and PUKKILA 1989). DNA was extracted from 20–40 mg of lyophilized mycelia by the method of ZOLAN and PUKKILA (1986). Distribution of nuclear types in the sampled cells was determined by Southern analysis of *Bam*HI digested DNAs, probed with pUC 9 clones containing a portion of the nuclear rDNA repeat of Armillaria isolate 300-2 (ANDERSON, BAILEY and PUKKILA 1989). Restriction fragment patterns of *Eco*RI digested mtDNAs were analyzed as described above using radiolabeled 450-1 mtDNA as probe.

RESULTS

A total of 1757 sibling pairings were necessary to establish mating type representatives for each fruit-body. After one month, compatible mating interactions (Figure 1) could be recognized by a reduction in aerial mycelium (HINTIKKA 1973) and in some cases (NABS VII) an increase in rhizomorph production. All crosses were scored independently by two investigators. In most cases three or four mating types were recovered from each fruit-body (Table 1). In cases where only three mating-type representatives were recovered, all four mating-type alleles could be identified based on non-sibling matings with four mating-type representatives of another fruit-body.

To determine the clonal structure at the site (Figure 2), 1616 nonsibling crosses were carried out. Six clones were detected at the study site, belonging to two biological species. Mating-type representatives from clone 1 were compatible in all combinations with those of clone 2, but incompatible with representatives from clones 3, 4, 5 and 6. Interclonal matings among monosporous isolates from clones 3, 4, 5 and 6 were all compatible. Crosses between widely separated isolates from within a given clone confirmed that the assigned mating-types for monosporous isolates were internally consistent within each clone. We could identify only three mating-type alleles in the representatives recovered from fruit-bodies 425, 496 and 528. The possibility that these fruit-bodies represent distinct clones is unlikely given the clone structure at the site since the three alleles that were identified from 425, 496 and 528 were identical to those in clones 1, 1 and 2, respectively. We determined that collection 426 represents a distinct clone (clone 3) when it was observed that 426-8 was compatible with all representatives from clones 4, 5 and 6, however, we cannot rule out the possibility that clone 3 and 5 may share a mating-type allele at either A or B, or both loci (Table 1).

For species identification, one monosporous isolate of each clone was mated with two haploid isolates from the known North American biological species of Armillaria. Monosporous isolates 434-1 (clone 1), 427-4 (clone 2), 426-8 (clone 3), 430-5 (clone 4), 441-6 (clone 5), and 451-1 (clone 6) were paired with 28-4 and 70-1 (NABS I), 35-5 and 160-8 (NABS II), 11-1 and 21-2 (NABS III), 48-6 and 205-4 (NABS V), 49-8 and 97-1 (NABS VI), 90-10 and 137-1 (NABS VII), 121-1 and 139-1 (NABS IX), 140-6 and 206-1 (NABS X) (ANDERSON 1986; SMITH and ANDERSON 1989). Clones 1 and 2 were compatible with haploid testers of *A. bulbosa* (Barla) Romagn. (= *A. lutea* Gillet) (NABS VII). Clones 3, 4, 5 and 6 were compatible with haploid testers of *A. ostoyae* (Romagn.) Herink (NABS I).

Minimum clone size ranged up to 635 m, with *A. bulbosa* clones being larger than those of *A. ostoyae.* Three *A. ostoyae* clones are represented by a single fruit-body collection. Three clones were sampled more than once and could be assayed for intraclonal mtDNA RFLPs, the largest *A. bulbosa* clone was sampled at forty-two locations.

Analysis of *Hin*dIII and *Eco*RI mtDNA restriction fragment patterns for the 35 fruit-bodies collected in 1988, and *Eco*RI fragment patterns from the 20 fruit-bodies collected around the study site in 1989 indicates that each of the six clones has one, unique mtDNA genotype (Figure 3). mtDNA fragment patterns were characteristic of *A. bulbosa* and *A. ostoyae* (SMITH and ANDERSON 1989), and were consistent with species identification of clones based on mating tests with NABS tester strains.

Polymorphisms were not detected within the largest clone by a more sensitive method of restriction fragment analysis in which mtDNA was extracted from five isolates of clone 1 and digested with the enzyme *Sau*3AI. For each isolate, more than 70 end-labeled

TABLE 1

Mating types of monosporous isolates of Armillaria

Species, clone	Mating-types of monosporous isolates			
	A_1B_1	A_2B_2	A_1B_2	A_2B_1
A. bulbosa[a]				
Clone 1				
424	−7	−9	−2	−5
425[b]	−4			−1
428	−8	−3	−9	−1
429	−5	−2	−8	−1
431	−6		−7	−2
432		−1	−3	−10
433		−4	−1	−10
434	−5	−8	−10	−1
436	−8	−4	−2	
437	−4	−3	−2	−9
438	−1	−8	−9	−2
439	−2	−4	−9	−7
440	−9	−7	−1	−4
442	−7	−4	−10	−3
443	−6	−2	−3	−9
444	−2	−8	−4	
445	−3	−10	−6	−4
446		−9	−8	−1
447	−8	−2	−10	−3
448	−1	−8		−5
449	−2	−3	−4	−8
450	−3	−5	−4	−6
453	−2	−10	−7	−9
454	−2	−4	−9	−7
455	−9	−10	−5	−8
457	−1	−2	−10	
458	−9	−3	−7	−5
490	−2	−6	−3	
492		−1	−7	−6
495		−8	−7	−2
496[b]	−3		−1	
516	−6	−5	−2	−3
517	−10	−1	−2	−3
518		−1	−2	−3
519	−3	−7	−10	−5
521	−8	−6	−9	−7

Species, clone	Mating-types of monosporous isolates			
	A_1B_1	A_2B_2	A_1B_2	A_2B_1
524	−2		−5	−3
525	−5	−2	−1	−4
526	−1	−2	−5	−4
530	−7	−4	−1	−2
531	−3	−4	−8	−6
532	−4	−1	−6	−7
	A_3B_3	A_4B_4	A_3B_4	A_4B_3
A. bulbosa[a]				
Clone 2				
427	−5	−2	−4	−1
435	−2	−6	−8	−10
456		−1	−10	−5
514	−4		−3	−7
515	−7		−8	−10
522	−2	−6	−3	−5
523	−5	−2	−8	−7
528[b]		−2	−1	
	A_7B_7	A_xB_x	A_7B_x	A_xB_7
A. ostoyae[a]				
Clone 3[c]				
426	−8			
	A_1B_1	A_2B_2	A_1B_2	A_2B_1
A. ostoyae[a]				
Clone 4				
430	−1	−5	−9	−7
	A_3B_3	A_4B_4	A_3B_4	A_4B_3
A. ostoyae[a]				
Clone 5				
441	−3	−6	−8	
	A_5B_5	A_6B_6	A_5B_6	A_6B_5
A. ostoyae[a]				
Clone 6				
451	−9	−1	−5	−10
452	−10	−1	−6	−7

[a] Mating-type alleles of *A. ostoyae* and *A. bulbosa* cannot be compared because intersterility overrides any possibility of mating. Within each species alleles are assigned arbitrarily, beginning with number one.
[b] Three of the four mating-type alleles were identified for these collections.
[c] Because only one monosporous isolate was obtained from clone 3, only two of the four mating-type alleles could be determined.

mtDNA fragments, ranging in size from 90 to 3,000 bp, could be resolved on polyacrylamide gels. Although the samples originated from as far apart as 112 m in clone 1, fragment patterns were identical in all five mtDNAs (results not shown).

To determine whether isolates at the study site have the potential for bidirectional nuclear migration, the distribution of both mitochondrial and nuclear markers was followed in a laboratory mating between isolate 425-2 of clone 1, and 435-6 of clone 2 (Table 2). Hyphal tip cells originating throughout the mated colony developed the characteristic morphology of diploid mycelium. The heterozygosity of these cells was confirmed by Southern analysis of nuclear rDNA fragments (Figure 4). Isolate 435-6 has a 0.3-kb insertion within the nuclear rDNA repeat that distinguishes it from 425-2. The rDNA repeats of both haploid parents were present throughout the mated colony, indicating that bidirectional nuclear migration between the two parent colonies had occurred. However, Southern transfers probed with radiolabeled 450-1 mtDNA revealed that one of two distinct *Eco*RI fragment patterns was present in each subculture. Hyphal tip isolates from the 435-6 side of the mated colony had the clone 2 mtDNA type, while hyphal tips from the 425-2 side had the clone 1 mtDNA type (mtDNA *Eco*RI fragment patterns as in Figure 3). No demarcation lines were evident when four diploids,

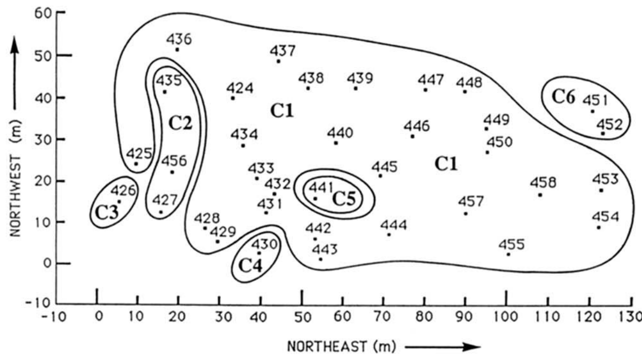

FIGURE 2.—Location of fruit-bodies collected in 1988 within a 122 × 53 m plot at the study site, and the distribution of Armillaria clones determined by mating interactions among single-spore isolates. Clone boundaries are drawn midway between fruit-bodies that differed in mating-type alleles, and between isolates from different biological species. Clones designated as C1 and C2 belong to *A. bulbosa* (NABS VII), C3, C4, C5 and C6 belong to *A. ostoyae* (NABS I). From 1989 collections in the surrounding hardwood forest, it was determined that clone 1 extends at least 229 m west and 316 m east, and that clone 2 extends at least 44 m northwest and 305 m east, of these clone boundaries.

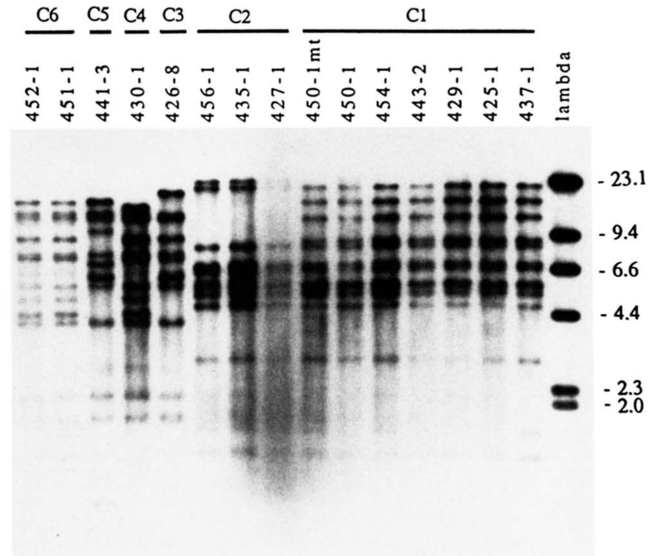

FIGURE 3.—Autoradiograph of *Eco*RI restriction fragment patterns of mtDNAs from six representative isolates of clone 1, three from clone 2, and all isolates from clones 3, 4, 5 and 6. mtDNA from isolate 450-1 (450-1mt) was included to monitor non-specific binding of mtDNA probe to nuclear DNA. Molecular size standard is bacteriophage lambda DNA digested with *Hin*dIII.

two of each mitochondrial type from this mating, were paired in all combinations.

DISCUSSION

In this study, the clonal structure of a natural population of Armillaria was determined through an analysis of nuclear encoded mating-type alleles. By examining the distribution of mtDNA RFLPs within this population, propagation and inheritance of mitochondria in field clones was investigated and compared to mitochondrial genetics following a mating event of monosporous isolates in the laboratory.

Clonal identity of mtDNA: Within the local population at the Michigan forest study site there was perfect alignment of mtDNA types with clone maps based on mating-type alleles. Each of the six clones sampled had unique mtDNA restriction fragment patterns. The variation among mtDNAs within this local population corroborates the observation that two non-clonal isolates are unlikely to carry exactly the same mtDNA types (SMITH and ANDERSON 1989), and indicates that mtDNA RFLPs may be extremely useful markers for population studies of this plant pathogen. Based on restriction fragment analysis of Armillaria isolates from both Europe (JAHNKE, BAHNWEG and WORRALL 1987) and North America (SMITH and ANDERSON 1989), there appears to be substantially more variation in mtDNAs between species than within species. Mitochondrial RFLPs in higher fungi are often the result of length mutations (reviewed in TAYLOR 1986; BRUNS *et al.* 1988), similar to other lower eukaryotes (WOLF and DEL GIUDICE 1988) and to flowering plants (NEWTON 1988). How and when length mutations occur in fungi is largely unknown. It is clear from analysis of Armillaria RFLPs in our 1988 sample that mtDNA variation, evident at the population level in the Michigan site, did not arise during the vegetative propagation of the clones. The overall *Eco*RI, *Hin*dIII and *Sau*3AI restriction fragment pattern from mtDNA is conserved throughout the 1988 samples of clone 1.

The 1989 survey in the surrounding hardwood forest revealed that clone 1 (as defined by mating-type alleles) extends at least 229 m west and 316 m east of the boundaries shown in Figure 2, and clone 2 extends at least 44 m northwest and 305 m east of the clone boundary as presented. These are among the largest described fungal clones (KILE 1983; ANDERSON *et al.* 1979; DICKMAN and COOK 1989). The age of clones 1 and 2 can be approximated from empirical field growth rate estimates of 0.8–1.4 m per year (reviewed in: SHAW and ROTH 1976). By assuming that vegetative growth initiated at a point midway between the most distant points sampled, we estimate that the minimum age of clone 1 is between 227 and 397 years (distance between outlying collections 516 and 526 is 635 m), and that clone 2 is at least 122 to 213 years old (distance of 341 m between collections 523 and 528). Evidently during this time, mtDNA has been clonally propagated, with no apparent length mutations, since *Eco*RI fragment patterns of mtDNAs from the outlying 1989 collections are identical to those shown for clones 1 or 2, respectively, in Figure 3. The mtDNA homogeneity within each clone is striking in contrast to the variability observed in this local population, and within Armillaria species. We expect that selectively neutral length mutations in the mtDNA would be maintained by clonal expansion of sectors within an individual. Therefore homogeneity within extended individuals suggests a low mutation

TABLE 2

Biparental inheritance of mtDNAs in a mating of Armillaria strains

Mycelial sample No.	Origin of sample	Mitochondrial DNA type	Nuclear rDNA type
1	425 (m)	425	425 ± 435
2	435 (m)	435	425 ± 435
3	425 (r)	425	425 ± 435
4	425 (r)	425	425 ± 435
5	425 (r)	425	425 ± 435
6	435 (r)	435	425 ± 435
7	435 (r)	435	425 ± 435
8	435 (r)	435	425 ± 435
9	435 (r)	435	425 ± 435
10	435 (r)	435	425 ± 435

Note: monosporous isolates 425-2 (clone 1) and 435-6 (clone 2) were paired as described in MATERIALS AND METHODS. After the compatible mating interaction, samples were removed from the 425-2 or 435-6 side of the pairing as mycelium (m), or rhizomorph (r). Mitochondrial DNA types are shown in Figure 3; nuclear rDNA types are shown in Figure 4.

rate (BIRKEY, MARUYAMA and FUERST 1983) and variation within local populations may reflect dispersal within a large panmictic population. This situation differs from that of crickets where length mutation in mtDNAs may occur relatively frequently to produce variation within local populations (RAND and HARRISON 1989). It is not surprising that site mutations were not detected given that relatively few restriction enzymes were utilized in this study, and that base substitution rates for mtDNA of animals are estimated to be approx. 10^{-8} sites per year (BROWN, GEORGE and WILSON 1979).

Large fungal clones also present a unique opportunity for direct study of somatic mutation and mitotic recombination in the nuclear genome. Recently, genotypic variation was observed in parthenogenetic offspring from two lineages of the dandelion plant, Taraxacum (KING and SCHAAL 1990). This indicates that mutation can occur at detectable rates in obligately asexual organisms. We are currently investigating the stability of the nuclear genome in Armillaria clones by RFLP analysis of dispersed, repetitive DNA elements that are associated with a high frequency of RFLPs among individual isolates. We expect to find little genetic variation within these clones since, unlike Taraxacum, Armillaria is believed to propagate both asexually, through vegetative growth and sexually, by basidiospores.

Inheritance of mtDNA: A distribution pattern of one, unique mtDNA type for each clone at the study site, suggests that mitochondria are uniparentally inherited in Armillaria. In a mating between *A. bulbosa* isolates from the study site, we were able to determine the mode of mitochondrial inheritance in the laboratory. Hyphal tip cells originating throughout the mated colony carried nuclear markers of both parents in approximately equal stoichiometry. Depending upon their point of origin, these same cells carried

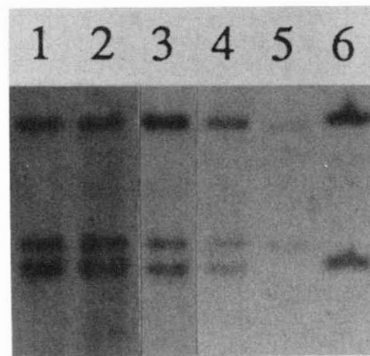

FIGURE 4.—Autoradiograph of *Bam*HI digested nuclear rDNAs from hyphal tip cells from laboratory mating between 435-6 and 425-2. In lanes 1 and 2 hyphal tips were derived from rhizomorphs initiating at the 435-6 inoculum (clone 2). In lanes 3 and 4 rhizomorphs initiated at the 425-2 inoculum (clone 1). Parental rDNA fragment patterns are shown in lane 5 (6.8 and 3.7 kb for 435-6) and lane 6 (6.8 and 3.4 kb for 425-2).

only one of the two parent mtDNA types. That vegetative demarcation lines (antagonistic interactions) were not observed when diploid explants carrying identical nuclear markers, but different mtDNA types, were confronted may be interpreted to mean that mitochondrial mosaics behave as "individuals" (MAY 1988), that is, genetically distinct, physiological units. The indication is that after mating, bidirectional migration of nuclei occurs and a stable, diploid individual is formed. This individual is a mosaic for mitochondria inherited from both parents, and presumably contains heteroplasmic cells in the zone of hyphal fusion. The data from laboratory crosses support the hypothesis that heteroplasmons could potentially arise after a mating event in the field, yet no evidence of multiple mitochondrial genotypes was found within clones at the Michigan field site.

Why are field clones observed to be uniform for mtDNA types? Mechanisms believed to limit heteroplasmy are uniparental inheritance and rapid segregation of mitochondria, with intracellular drift causing fixation or elimination of neutral mutations (BIRKEY, MARUYAMA and FUERST 1983). Uniparental organelle inheritance, common in animals (DAWID and BLACKLER 1972; HUTCHINSON *et al.* 1974; GILES *et al.* 1980; LANSMAN, AVISE and HUETTEL 1983), plants (SCHMITZ 1988; NEALE, MARSHALL and SEDEROFF 1989) and some fungi (MANNELLA, PITTENGER and LAMBOWITZ 1979; MARTIN 1989), reduces the possibility of heteroallelism at mitochondrial gene loci, since progeny inherit only one parent mitochondrial type. While naturally occurring length heteroplasmy has recently been identified from several animal taxa [reviewed in RAND and HARRISON (1989) and BUROKER *et al.* (1990)], it is believed to arise in the female germline through mutational events, such as intramolecular misalignment in the D-loop region prior to mtDNA replication in sturgeon fish (BUROKER *et al.* 1990). In such cases, length heteroplasmy does

not necessarily correlate with heteroallelism of mitochondrial genes. Mitochondrial heteroallelism resulting from biparental inheritance in isogamous organisms such as Saccharomyces (BIRKY 1983), and Chlamydamonas (BOYNTON *et al.* 1987), or through microinjection of mitochondria into Drosophila eggs (HAYASHI and MURAKAMI 1988; NIKI, CHIGUSA and MATSUURA 1989), is noted as a transient state due to rapid segregation of mitochondrial types in relatively few generations. Rapid segregation of mitochondrial polymorphisms has also been observed after neutral point mutations in Holstein cow lineages (ASHLEY, LAIPIS and HAUSWIRTH 1989), but has not been evident where length mutations are involved, possibly due to high rates of replication error, that re-introduce length variants to the mtDNA population within individuals, thus offsetting segregation (RAND and HARRISON 1989; BUROKER *et al.* 1990).

That heteroplasmy has not, to our knowledge, been directly observed in higher fungi where there is good indication that it occurs following laboratory mating in basidiomycetes (BAPTISTA-FERREIRA, ECONOMOU and CASSELTON 1983) suggests that segregation of mitochondrial types is rapid in fungal cell lines as well. From our observations, however, it is difficult to envision rapid segregation of different mitochondrial types at the level of an entire basidiomycete clone. In laboratory matings between haploid Armillaria isolates we rarely observe unidirectional nuclear migration (evident when only one parent converts to the diploid morphology). Equally rare in the crosses we carried out for this experiment, was evidence of one parent outcompeting, or growing over, the other. The vast majority of compatible matings resulted in prolific rhizomorph production from both parental inocula as shown in Figure 1. In the field, however, it is possible that one mitochondrial type may provide a selective advantage to the diploid mycelium, and thereby "outcompete" the other mitochondrial type.

Other hypotheses can be suggested to explain why mtDNA inheritance patterns in field and laboratory experiments apparently differ. Since the actual extent of each vegetative clone at the site is uncertain, our samples may be from single mtDNA sectors within extremely large clones. In addition, the two clones we sampled most thoroughly (clones 1 and 2) are likely very old and any remnants of a mtDNA mosaic established after mating may be lost for some reason. We are now analyzing nuclear and mitochondrial DNA markers in vegetative samples from multiple sites within young, well defined *A. ostoyae* clones, a situation where we will be more likely to detect mtDNA mosaics, if they exist. An additional possibility is that Armillaria clones arise through asymetric matings, in which an established haploid mycelium is essentially fertilized by a germinating, compatible basidiospore. In this hypothetical case, one partner would contribute little or no cytoplasm and mitochondria would be inherited uniparentally. If this regularly occurs in the field, evidence for a mating event would be difficult to obtain. Alternatively, mating among haploid mycelia originating from basidiospores may be less common in nature than previously believed. Clearly, there is no evidence of a mating event within the scope of our field sample, despite the laboratory experiment showing that our methods were capable of detecting such an event. In nature, Armillaria may commonly disperse via fragments of diploid vegetative inoculum, or by binucleate or diploid basidiospores, containing only one mtDNA type, since variation in basidiospore number, as well as the inclusion of more than one postmeiotic nucleus in basidiospores at a variable frequency, has been documented in Agaricus (KERRIGAN and ROSS 1987) and Coprinus (LANGE 1952). The apparent absence of mitochondrial mosaics and heteroplasmons of Armillaria in nature may be explained by these or other mechanisms, but these data, along with the apparent absence of heteroallelism at mitochondrial genes in diverse organisms, suggest that there is no benefit, or perhaps a cost, to maintaining multiple mitochondrial genotypes within individuals in nature.

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