Maternal Inheritance and Diversity of Mitochondrial DNA in the Chestnut Blight Fungus, *Cryphonectria parasitica*

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ABSTRACT

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The inheritance of mitochondrial DNA (mtDNA) in the chestnut blight fungus, *Cryphonectria parasitica*, was investigated with laboratory crosses and progeny from perithecia collected from a natural population. In a reciprocal cross, mtDNA haplotypes, determined from restriction fragment length polymorphisms (RFLPs), were inherited only from maternal parents. Another lab cross, in which the maternal strain was not known, showed uniparental inheritance for 43 ascospore progeny. All 87 progeny from eight perithecia collected in the field showed uniparental inheritance of mtDNA haplotypes identical to the maternal (canker) isolates. These data strongly support the hypothesis that mtDNA is maternally inherited in *C. parasitica*. In samples from three populations of *C. parasitica*, mtDNA haplotypes were highly diverse. Estimates of haplotypic diversity were high, ranging from 0.832 to 0.968 (maximum diversity is 1.0 when all individuals have unique haplotypes). The possible causes of hypervariability in mtDNA are discussed.

Additional keywords: *Endothia parasitica*, genetic diversity.

Mitochondrial DNA (mtDNA) is a convenient marker used for many population biology and evolutionary studies (4,32,36). There are two major reasons mtDNA has been useful. First, for many organisms, mtDNA is maternally inherited and therefore, does not recombine during sexual reproduction, as nuclear markers do. Second, in animals, the rate of evolution of mtDNA generally is greater than the rate for nuclear DNA (6), which provides sufficient polymorphisms for studying populations and inferring phylogenies. For fungi, Bruns et al (8) list additional reasons mtDNA is popular for evolutionary studies, including its convenient size, the lack of methylation, ease of isolating purified mtDNA, visualization of high-copy numbers when cut with some restriction endonucleases, and abundant polymorphisms resulting from length mutations. Although mtDNA has been used for a number of evolutionary and systematic studies of fungi (3,7,19,20,34), there have been relatively few population biology studies of fungi that have used mtDNA as a genetic marker (11,17,18,31).

In previous studies on the chestnut blight fungus, *Cryphonectria (Endothia) parasitica* (Murrill) Barr, we used nuclear markers, such as DNA fingerprinting, restriction fragment length polymorphisms (RFLPs), and vegetative compatibility, to address population biology questions (24–27). The goal of these studies was to understand genetic diversity and how it affects biological control of chestnut blight with transmissible hypovirulence, which is associated with a double-stranded RNA virus (10,35). One important factor in determining population structure is the extent to which spores disperse within and between populations. To track the dispersal of sexually produced ascospores, a maternally inherited marker, such as mtDNA, would be ideal, instead of nuclear markers that recombine during sexual reproduction. In addition to maternal inheritance, a marker must be sufficiently polymorphic to identify individuals within fungal populations. The mtDNA of *C. parasitica* is known to be polymorphic (15), with almost every individual having a unique haplotype in some samples (N. K. Van Alfen, *personal communication*). It is not currently known, however, whether mtDNA is inherited maternally in *C. parasitica*, as it is in other Ascomycetes, such as *Neurospora* spp. (21–23,28), or whether all populations of *C. parasitica* are as diverse as those found by Van Alfen (*unpublished data*).

To evaluate mtDNA as a genetic marker for population biology studies of *C. parasitica*, we had two objectives. The first objective was to test the hypothesis that mtDNA in *C. parasitica* is maternally inherited. The second objective was to estimate the diversity of mtDNA in three natural populations of *C. parasitica*.

MATERIALS AND METHODS

Determination of mtDNA haplotypes. All methods for culturing *C. parasitica*, preparation of DNA, agarose gel electrophoresis, Southern blotting, probing, and autoradiography, have been described previously (25). Total genomic DNA from each isolate was digested with the restriction endonuclease *Pst*I and was separated by gel electrophoresis in a 0.5% agarose gel for 68 h at 0.75 V/cm or in a 0.7% agarose gel for 18 h at 1.0 V/cm. The two gels were used for resolution of restriction fragments from approximately 20 to 3 kb in the 0.5% gel and from 7 to 0.5 kb in the 0.7% gel. Purified mtDNA of *C. parasitica* isolate EP67 (ATCC 38753) was prepared from intact mitochondria using the method of Cramer et al (12), as described by Taylor and Natvig (33). A mtDNA haplotype for each isolate was determined by the hybridization pattern that resulted from probing Southern blots of *Pst*I-digested total DNA with ^{32}P-labeled purified mtDNA; we also probed Southern blots of *Eco*RI-digested DNA from isolates electrophoresed in 0.5% agarose gels. No attempts were made to determine whether RFLPs were the result of differences in restriction sites or in deletions and insertions.

Analysis of maternal inheritance. Reciprocal crosses were made between *C. parasitica* isolates EP67 and EP155 (ATCC 38755). Crosses were set up as described by Anagnostakis (2), with minor modifications. Two chestnut-stem pieces were placed in each petri

plate, and each stem was colonized by either EP67 or EP155 to control for maternal parents. Fertilization was performed by flooding the plate with sterile distilled water and shaking gently by hand for 1 min, so conidia dislodged from one stem could contact receptive hyphae on the other. Water with suspended conidia was decanted, and after 4–6 wk of incubation at 20 C, random ascospores were sampled from a single perithecium from each stem. Because each stem was colonized by only a single strain prior to fertilization, perithecia from each stem were assumed to have been produced by a known maternal strain (2). *C. parasitica* is homothallic but preferentially outcrosses (24,30); therefore, DNA fingerprinting was performed (25) on the parents and on six ascospore isolates from each perithecium to confirm that progeny were not the result of self-fertilization. DNA fingerprints were determined by probing *Pst*I-digested DNA on Southern blots from 0.5% agarose gels with plasmid pMS5.1, which contains a highly polymorphic, moderately repetitive nuclear sequence (25). Mitochondrial DNA haplotypes were determined, as described above (*Pst*I digests in 0.5% agarose gels only), for both parents and for six progeny from each perithecium.

Mitochondrial inheritance was also investigated in ascospore progeny used in previous studies (24,25). Mitochondrial DNA haplotypes were determined for both parents and for 43 ascospore progeny from a single perithecium from a laboratory cross between isolates EP155 and RH14-2, in which the perithecial (maternal) parent was not controlled (24,25). There was segregation for DNA-fingerprint fragments among the progeny from this cross, confirming that self-fertilization did not occur (25). In addition to this laboratory cross, we determined the mtDNA haplotypes of isolates from eight naturally occurring cankers in the Mountain Lake, VA, population (described below) and from three to 49 ascospore isolates from single perithecia found in each canker (Table 1). All field-collected progeny showed segregation for DNA fingerprints (24).

Population samples. To estimate the diversity of mtDNA in *C. parasitica*, we sampled three populations. First, we sampled 39 isolates from chestnut blight cankers (one isolate from each canker) in a 25-×25-m forest plot at the Mountain Lake Biological Station, Giles County, VA (25). We also sampled 68 isolates from Depot Hill Multiple Use Area, Dutchess County, in southeastern New York, and 67 isolates from Danby State Forest, Tompkins County, in central New York. Isolates from Depot Hill and Danby also were collected from understory forest plots approximately 30 × 30 m in size.

We determined the mtDNA haplotypes in two stages. First, Southern blots with *Pst*I-digested DNA for all isolates were probed with ^{32}P-labeled mtDNA. Additional Southern blots were made (0.5% agarose gels only) with *Eco*RI-digested DNA for all isolates that did not have unique *Pst*I haplotypes within each population; these isolates were probed with mtDNA to determine how much additional information could be gained from a second restriction enzyme. Haplotypic diversity was estimated as $\hat{H} = n(1 - \Sigma p_i^2) / (n - 1)$, in which p_i is the frequency of the ith mtDNA haplotype and n is the sample size (29). Haplotypic diversity can range from zero, when all isolates have the same haplotype, to 1.0, when all isolates have unique haplotypes.

TABLE 1. Number of ascospores collected from a population of *Cryphonectria parasitica* at Mountain Lake, VA, and tested for mtDNA haplotype (a canker isolate was taken from the same stroma as each perithecium to determine the maternal mtDNA haplotype)

Perithecium[a]	No. of progeny tested	Frequency of canker mtDNA haplotype[b]
V8-1	49	0.03
V11-1	3	0.03
V13-1	7	0.15
V19-1	7	0.15
V23-1	8	0.08
V33-1	5	0.03
V34-1	4	0.13
V36-1	4	0.15

[a]Canker number followed by perithecium number within a canker. DNA fingerprinting was done on progeny from each perithecium to determine that outcrossing had occurred (24,25).

[b]Haplotypes determined by digestion of DNA with *Pst*I only; $n = 39$ for the canker sample.

RESULTS

Maternal inheritance. The reciprocal cross between EP67 and EP155 showed that mtDNA is maternally inherited. Mitochondrial haplotypes of ascospore isolates from the cross between EP67 and EP155 were identical to the maternal isolates (Fig. 1A). Nuclear DNA fingerprint fragments segregated in these same progeny, indicating that cross-fertilization had occurred and that progeny did not have mtDNA haplotypes identical to the maternal isolate because of selfing (Fig. 1B).

All other results were consistent with maternal inheritance of mtDNA in both laboratory crosses and field-collected perithecia. All 43 progeny from the cross between EP155 and RH14-3 had the same mtDNA haplotype as RH14-3; no progeny had the EP155 haplotype. Similarly, all ascospore isolates from the field-collected perithecia had haplotypes identical to the isolates from the cankers in which the perithecia were found. No mutations were detected in mtDNA haplotypes among any of the progeny relative to canker (maternal) strains.

Population diversity. Mitochondrial DNA was highly diverse in all three natural populations of *C. parasitica*. At Mountain

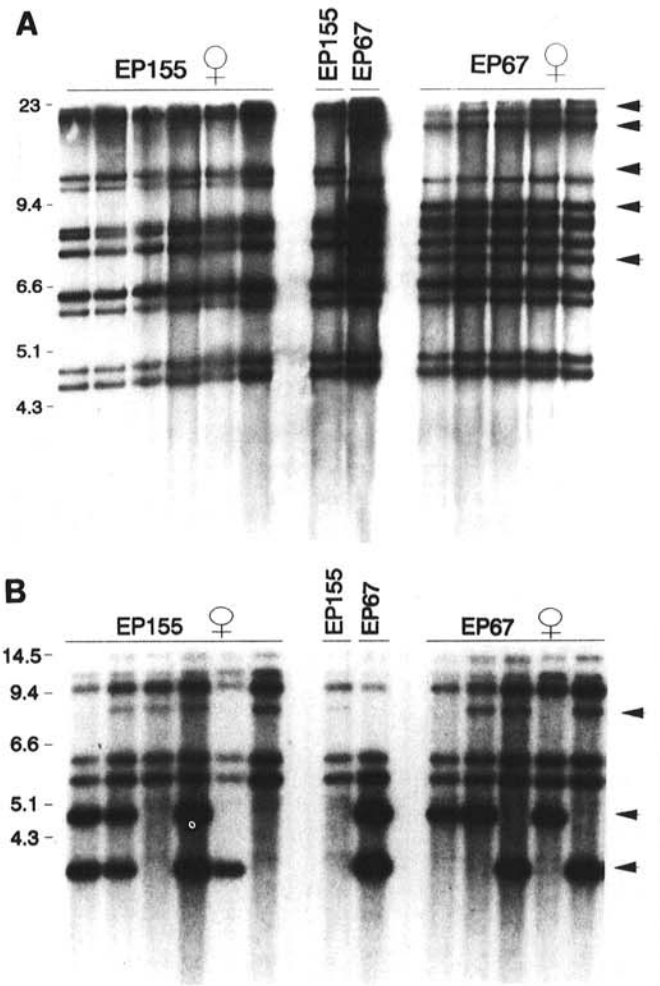

Fig. 1. A, Hybridization patterns of mtDNA to *Pst*I-digested DNA of *Cryphonectria parasitica* isolates EP155 and EP67 and their progeny from a reciprocal cross. Arrows indicate bands present in only one parental strain. **B,** Hybridization of DNA fingerprinting probe pMS5.1 (25) to the same isolates as in **A** to demonstrate crossing between the parental strains. Arrows indicate segregating bands.

Lake, VA, 17 mtDNA haplotypes were identified among 39 isolates, based on *Pst*I digests (Fig. 2); digestion with *Eco*RI revealed an additional seven mtDNA haplotypes, for a total of 24 haplotypes among 39 isolates (Table 2). Results for the Depot Hill, NY, population were similar to Mountain Lake: There were 25 *Pst*I haplotypes, with an additional five haplotypes revealed by digestion with *Eco*RI (Table 2). The Danby, NY, population was less diverse: There were only 11 haplotypes with *Pst*I, but the haplotypes increased to a total of 20 with *Eco*RI (Table 2). Observed frequencies of the haplotypes from each population are shown in Figure 3.

Estimates of haplotypic diversity for mtDNA in *C. parasitica* were very high, ranging from 0.968 in Mountain Lake to 0.832 in Danby; haplotypic diversity was 0.944 at Depot Hill (Table 2). Isolates from Mountain Lake that previously were determined to be clones, based on nuclear DNA (25), always had identical mtDNA haplotypes. If duplicate clones were eliminated, the estimated haplotypic diversity for 33 nonclonal isolates at Mountain Lake would be 0.991.

DISCUSSION

Analysis of ascospore isolates from lab crosses and field collections strongly supports the hypothesis of maternal inheritance of mtDNA in *C. parasitica*. The strongest evidence for maternal inheritance is that progeny from the reciprocal cross had mtDNA haplotypes identical to the maternal (perithecial) isolates. An additional laboratory cross with 43 progeny supported uniparental inheritance of mtDNA (the maternal parent was not known in this cross). Similarly, we found that all progeny in perithecia collected from cankers in a natural population, including one from which 49 ascospores were analyzed, had mtDNA haplotypes identical to the canker isolates. These results are consistent with uniparental maternal inheritance of mtDNA in *C. parasitica*, as

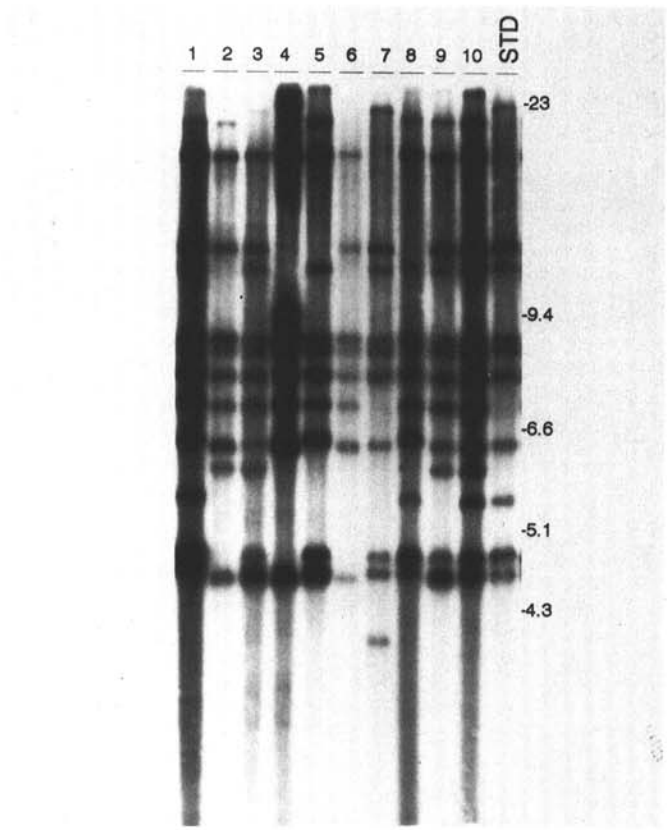

Fig. 2. Hybridization patterns of mtDNA to *Pst*I-digested DNA of *Cryphonectria parasitica* isolates collected from a natural population in Mountain Lake, VA. The lane labeled STD contains DNA from a standard isolate used on each gel.

TABLE 2. Diversity of mtDNA in *Cryphonectria parasitica* in three populations

Population	*n*	No. of *Pst*I haplotypes	Total no. of haplotypes[a]	Haplotypic diversity[b]
Mountain Lake, VA	39	17	24	0.968
Depot Hill, NY	68	25	30	0.944
Danby, NY	67	11	20	0.823

[a]Number of haplotypes determined after digestion with *Pst*I and *Eco*RI separately.

[b]Haplotypic diversity is defined as $\hat{H} = n(1 - \Sigma p_i^2) / (n - 1)$, in which p_i is the frequency of the ith mtDNA haplotype and n is the sample size (29).

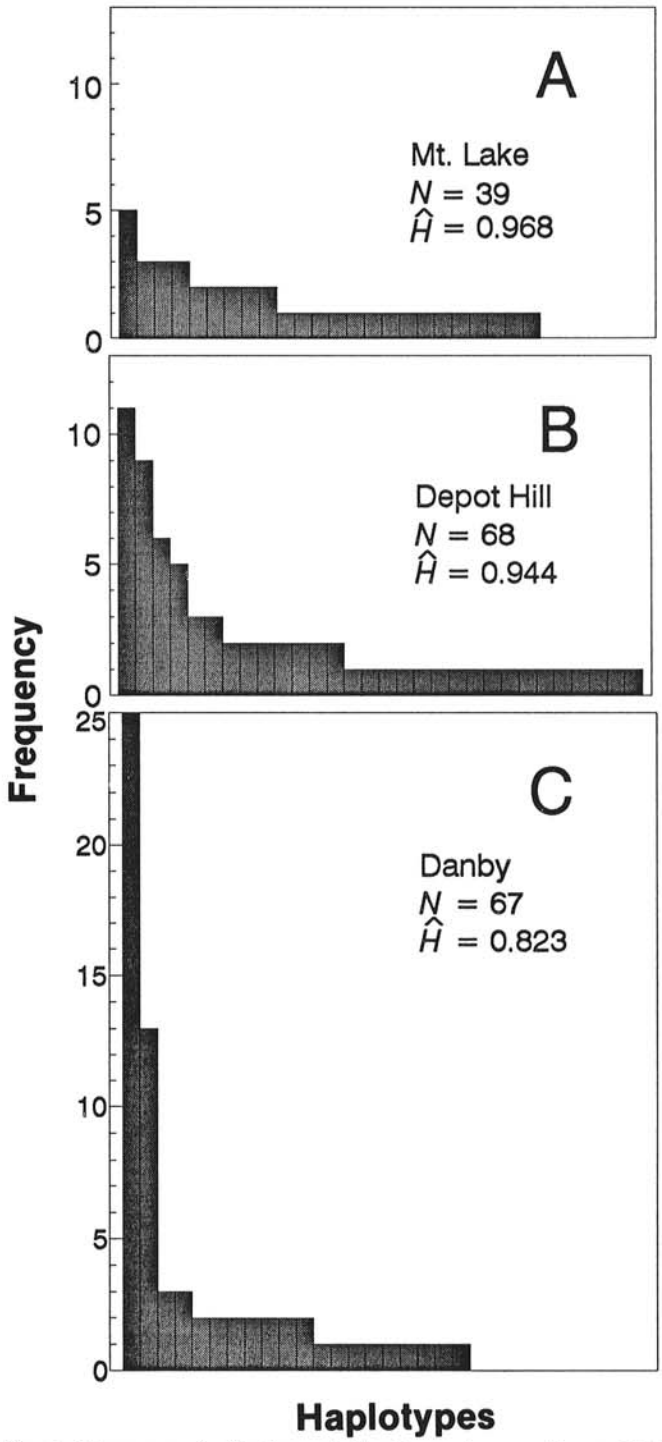

Fig. 3. Frequency distributions of *Cryphonectria parasitica* mtDNA haplotypes in the three populations studied: **A,** Mountain Lake, VA; **B,** Depot Hill, NY; and **C,** Danby, NY. Haplotypes were determined from restriction digests with endonucleases *Pst*I and *Eco*RI.

previously shown for mitochondrial inheritance in *Neurospora* spp. (21–23,28).

Interpretation of mtDNA inheritance of field-collected progeny is based on the assumption that progeny resulted from cross-fertilization with a paternal strain that had a different mtDNA haplotype than the maternal parent. We know from segregation of DNA fingerprint fragments that outcrossing occurred in all progeny tested (24). Furthermore, given the high diversity of mtDNA haplotypes in the population and the low frequency of each maternal mtDNA haplotype in particular (Table 1), it is probable that ascospore progeny resulted from outcrossing with strains that had different mtDNA haplotypes than the canker (maternal) strains. The result that all ascospores from the field had the same mtDNA haplotype as their maternal parents supports the use of mtDNA haplotypes as markers for identifying maternal genotypes in dispersal studies in local populations.

The diversity of mtDNA haplotypes in populations of *C. parasitica* is very high (Table 2). The estimates of haplotypic diversity for mtDNA in *C. parasitica* are as large as those found for some scallop species, 0.796–0.990 (14), many vertebrate species (5), and almost as large as for menhaden fish and chuckwalla lizards (0.998 and 0.983, respectively), which have the highest mtDNA haplotype diversity of any species surveyed (5). In another study of mtDNA variability in *C. parasitica*, there were seven haplotypes among eight isolates from a population in Maryland, and 12 haplotypes among 13 isolates in a population in Italy (N. K. Van Alfen, *personal communication*); haplotypic diversity estimates for these populations are 0.964 and 0.987, respectively, comparable to the populations studied here.

Although mtDNA diversity has been described for other fungi, none have been as variable as *C. parasitica*. Gordon and Okamoto (17) found only 11 haplotypes among 40 isolates of *Fusarium oxysporum* Schlechtend.:Fr. in 39 vegetative compatibility groups from two populations; they have subsequently found 23 haplotypes among 120 isolates collected from native and cultivated soils, providing mtDNA haplotype diversity estimates of 0.78 and 0.84, respectively (18). Mitochondrial DNA diversity in *Armillaria ostoyae* (Romagnesi) Herink is also high: Nine mtDNA haplotypes were found among 21 clones in a local population and 13 haplotypes among 13 isolates collected from more diverse geographic locations (M. L. Smith and J. B. Anderson, *personal communication*). In contrast, there were only four mtDNA haplotypes in *Phytophthora infestans* (Mont.) de Bary isolates collected from many parts of the world (9,16); no mtDNA variability in restriction fragments has been found for *Aspergillus nidulans* (Eidam) G. Wint. (13).

The variability in mtDNA found in our populations of *C. parasitica* may be underestimated. Using cloned mtDNA fragments as probes (similar to the approach used by Gordon and Okamoto [17]), Van Alfen and colleagues (N. K. Van Alfen, *personal communication*) detected greater differences in haplotypes than when probing with the entire mtDNA genome, as we did in this study. The cloned-probe method provides greater resolution, and as a result, greater diversity may be apparent, because comigrating fragments are not scored as identical unless they are in fact homologous. Our method, with less resolution, nonetheless, revealed high levels of polymorphism and is simpler to use when studying large population samples, because only one or two probings are needed. We do not know whether using more restriction enzymes would have revealed greater diversity in mtDNA in this population. Because almost all the polymorphisms detected in mtDNA of *C. parasitica* are the result of length mutations (15; N. K. Van Alfen, *personal communication*), we suspect that use of additional restriction enzymes would have limited value, because most length differences would be detected with one or two enzymes.

In the absence of more data, we can only speculate on the causes of hypervariability of mtDNA in *C. parasitica*. Large, effective population size, population subdivision, and enhanced mutation rates have been proposed as causes of mtDNA hypervariability in some vertebrate species (5). The factors affecting mtDNA variability in *C. parasitica* are probably similar. A population subdivided into many small, noninteracting units can buffer against the extinction of haplotypes (5), but this is probably not an important factor in preserving mtDNA variability in *C. parasitica*, because the subdivision would have to occur within extremely small areas (30 × 30 m). Populations recently founded by small numbers of individuals are expected to have small, effective population sizes and low levels of mtDNA diversity (36). The size of the founder population of *C. parasitica* brought into North America 90 years ago is not known precisely. Evidence from the low degree of genetic diversity in *C. parasitica* for nuclear RFLPs in the United States compared to China, where this fungus is native, suggests that the founder population was relatively small (26). In contrast, the rapid population expansion that occurred after *C. parasitica* was introduced into North America (1) may have prevented the random extinction of mtDNA haplotypes in the founder population or of new haplotypes arising from mutation.

Because population subdivision or large effective population size are unlikely to have caused mtDNA diversity in *C. parasitica*, we speculate that high mutation rates are the most probable explanation for mtDNA hypervariability in this fungus. Although mtDNA haplotypes were stable from one generation to the next in our sample of ascospores, data from a population of *C. parasitica* in Italy suggests that mtDNA mutation rates may be high. Twelve haplotypes found in an Italian population constitute two mtDNA lineages that correlate with clonal lineages determined from nuclear DNA fingerprinting (N. K. Van Alfen and M. G. Milgroom, *unpublished data*). Because *C. parasitica* was recently introduced into Europe (first observed in 1938 [1]), it is probable that these mtDNA lineages evolved in situ through the accumulation of mutations. Thus, we speculate that the variability in mtDNA haplotypes we currently observe in U. S. populations also may have arisen because of high mutation rates.

The mtDNA diversity observed in *C. parasitica* populations is ideal for studies of spore dispersal when it is necessary to assign maternity to new individuals in a population. An obvious requirement for this type of study is the demonstration of maternal inheritance of mtDNA. Given maternal inheritance and high levels of polymorphism in mtDNA haplotypes, this approach can be used to estimate the distances ascospores disperse within populations.

LITERATURE CITED

1. Anagnostakis, S. L. 1987. Chestnut blight: The classical problem of an introduced pathogen. Mycologia 79:23-37.
2. Anagnostakis, S. L. 1988. *Cryphonectria parasitica*: Cause of chestnut blight. Adv. Plant Pathol. 6:123-136.
3. Anderson, J. B., Petsche, D. M., and Smith, M. L. 1987. Restriction fragment polymorphisms in biological species of *Armillaria mellea*. Mycologia 79:69-76.
4. Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A., Saunders, N. C. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. 18:489-522.
5. Avise, J. C., Bowen, B. W., and Lamb, T. 1989. DNA fingerprints from hypervariable mitochondrial genotypes. Mol. Biol. Evol. 6:258-269.
6. Brown, W. M., George, M., Jr., and Wilson, A. C. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci., USA 76:1967-1971.
7. Bruns, T. D., Fogel, R., White, T. J., and Palmer, J. D. 1989. Accelerated evolution of a false-truffle from a mushroom ancestor. Nature 339:140-142.
8. Bruns, T. D., White, T. J., and Taylor, J. W. 1991. Fungal molecular systematics. Annu. Rev. Ecol. Syst. 22:525-564.
9. Carter, D. A., Archer, S. A., Buck, K. W., Shaw, D. S., and Shattock, R. C. 1990. Restriction fragment length polymorphisms of mitochondrial DNA of *Phytophthora infestans*. Mycol. Res. 94:1123-1128.
10. Choi, G. H., and Nuss, D. L. 1992. Hypovirulence of chestnut blight fungus conferred by an infectious viral cDNA. Science 257:800-803.
11. Correll, J. C., Gordon, T. R., and McCain, A. H. 1992. Genetic diversity in California and Florida populations of the pitch canker fungus *Fusarium subglutinans* f. sp. *pini*. Phytopathology 82:415-420.

12. Cramer, C. L., Ristow, J. L., Paulus, T. J., and Davis, R. H. 1983. Methods for mycelial breakage and isolation of mitochondria and vacuoles of *Neurospora*. Anal. Biochem. 128:384-392.
13. Croft, A. M. 1987. Genetic variation and evolution in *Aspergillus*. Pages 311-324 in: Evolutionary Biology of the Fungi. A. D. M. Rayner, C. M. Brasier, and D. Moore, eds. Cambridge University Press, Cambridge, England.
14. Gjetvaj, B., Cook, D. I., and Zouros, E. 1992. Repeated sequences and large-scale size variation of mitochondrial DNA: A common feature among scallops (Bivalvia: Pectinidae). Mol. Biol. Evol. 9:106-124.
15. Gobbi, E., Wang, Y., Martin, R. M., Powell, W. A., and Van Alfen, N. K. 1990. Mitochondrial DNA of *Cryphonectria parasitica*: Lack of migration between vegetatively compatible strains. Mol. Plant-Microbe Interact. 3:66-71.
16. Goodwin, S. B. 1991. DNA polymorphism in *Phytophthora infestans*: The Cornell experience. Pages 256-271 in: *Phytophthora*. J. A. Lucas, R. C. Shattock, D. S. Shaw, and L. R. Cooke, eds. Cambridge University Press, Cambridge, England.
17. Gordon, T. R., and Okamoto, D. 1992. Variation within and between populations of *Fusarium oxysporum* based on vegetative compatibility and mitochondrial DNA. Can. J. Bot. 70:1211-1217.
18. Gordon, T. R., Okamoto, D., and Milgroom, M. G. 1992. The structure and interrelationship of fungal populations in native and cultivated soils. Mol. Ecol. 1:241-249.
19. Jacobson, D. J., and Gordon, T. R. 1990. Variability of mitochondrial DNA as an indicator of relationships between populations of *Fusarium oxysporum* f. sp. *melonis*. Mycol. Res. 94:734-744.
20. Kohn, L. M., Petsche, D. M., Bailey, S. R., Novak, L. A., and Anderson, J. B. 1988. Restriction fragment length polymorphisms in nuclear and mitochondrial DNA of *Sclerotinia* species. Phytopathology 78:1047-1051.
21. Lee, S. B., and Taylor, J. W. Uniparental inheritance and replacement of mitochondrial DNA in *Neurospora tetrasperma*. Genetics. In press.
22. Manella, C. A., Pittenger, T. H., and Lambowitz, A. M. 1979. Transmission of mitochondrial deoxyribonucleic acid in *Neurospora crassa* sexual crosses. J. Bacteriol. 137:1449-1451.
23. May, G., and Taylor, J. W. 1989. Independent transfer of mitochondrial plasmids in *Neurospora crassa*. Nature 339:320-322.
24. Milgroom, M. G., Lipari, S. E., Ennos, R. A., and Liu, Y.-C. 1993. Estimation of the outcrossing rate in the chestnut blight fungus, *Cryphonectria parasitica*. Heredity. 70:385-392.
25. Milgroom, M. G., Lipari, S. E., and Powell, W. A. 1992. DNA fingerprinting and analysis of population structure in the chestnut blight fungus, *Cryphonectria parasitica*. Genetics 131:297-306.
26. Milgroom, M. G., Lipari, S. E., and Wang, K. 1992. Comparison of genetic diversity in the chestnut blight fungus, *Cryphonectria (Endothia) parasitica* from China and the US. Mycol. Res. 96:1114-1120.
27. Milgroom, M. G., MacDonald, W. L., and Double, M. L. 1991. Spatial analysis of vegetative compatibility groups in the chestnut blight fungus, *Cryphonectria parasitica*. Can. J. Bot. 69:1407-1413.
28. Mitchell, M. B., and Mitchell, H. K. 1952. A case of "maternal" inheritance in *Neurospora crassa*. Proc. Natl. Acad. Sci., USA 38:442-449.
29. Nei, M., and Tajima, F. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97:145-163.
30. Puhalla, J. E., and Anagnostakis, S. L. 1971. Genetics and nutritional requirements of *Endothia parasitica*. Phytopathology 61:169-173.
31. Smith, M. L., Duchesne, L. C., Bruhn, J. N., and Anderson, J. B. 1990. Mitochondrial genetics in a natural population of the plant pathogen *Armillaria*. Genetics 126:575-582.
32. Taylor, J. W. 1986. Fungal evolutionary biology and mitochondrial DNA. Exp. Mycol. 10:259-269.
33. Taylor, J. W., and Natvig, D. 1987. Isolation of fungal DNA. Pages 252-258 in: Zoosporic Fungi in Teaching and Research. M. S. Fuller and A. Jaworski, eds. Southeastern Publishing Corp., Athens, GA.
34. Taylor, J. W., Smolich, B. D., and May, G. 1986. Evolution and mitochondrial DNA in *Neurospora crassa*. Evolution 40:716-739.
35. Van Alfen, N. K. 1982. Biology and potential for disease control of hypovirulence of *Endothia parasitica*. Annu. Rev. Phytopathol. 20:349-362.
36. Wilson, A. C., Cann, R. L., Carr, S. M., George, M., Gyllensten, U. B., Helm-Bychowski, K. M., and Higuchi, R. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linn. Soc. 26:375-400.