Inheritance of Mitochondrial DNA and Plasmids in the Ascomycetous Fungus, *Epichloë typhina*

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Manuscript received June 10, 1995

Accepted for publication October 14, 1995

ABSTRACT

We analyzed the inheritance of mitochondrial DNA (mtDNA) species in matings of the grass symbiont *Epichloë typhina*. Eighty progeny were analyzed from a cross in which the maternal (stromal) parent possessed three linear plasmids, designated Callan-a (7.5 kb), Aubonne-a (2.1 kb) and Bergell (2.0 kb), and the paternal parent had one plasmid, Aubonne-b (2.1 kb). Maternal transmission of all plasmids was observed in 76 progeny; two progeny possessed Bergell and Callan-a, but had the maternal Aubonne-a replaced with the related paternal plasmid Aubonne-b; two progeny lacked Callan-a, but had the other two maternal plasmids. A total of 34 progeny were analyzed from four other matings, including a reciprocal pair, and in each progeny the plasmid transmission was maternal. The inheritance of mitochondrial genomes in all progeny was analyzed by profiles of restriction endonuclease-cleaved mtDNA. In most progeny the profiles closely resembled those of the maternal parents, but some progeny had nonparental mtDNA profiles that suggested recombination of mitochondrial genomes. These results indicate that the fertilized stroma of *E. typhina* is initially heteroplasmic, permitting parental mitochondria to fuse and their genomes to recombine.

IN most oogametous eukaryotes inheritance of organellar DNA is maternal, but many exceptions are known. Low levels of paternal transmission (leakage) have been observed in several fungi (HINTZ *et al.* 1988; SMITH *et al.* 1990; YANG and GRIFFITHS 1993a) and animals (KONDO *et al.* 1990; GYLLENSTEN *et al.* 1991; HOEH *et al.* 1991; SKIBINSKI *et al.* 1994). Both uniparental maternal and paternal transmission, as well as leaky paternal transmission, occur in plants (NEALE *et al.* 1986; ERICKSON and KEMBLE 1990; WAGNER *et al.* 1991; FAURÉ *et al.* 1994). A mitochondrion-associated linear plasmid in *Brassica* tends to be paternally transmitted (ERICKSON *et al.* 1989). Recently, occasional male transmission of circular and linear mitochondrial plasmids has been documented in some crosses of *Neurospora* (MAY and TAYLOR 1989; YANG and GRIFFITHS 1993a). These exceptions demonstrate that maternal inheritance of mitochondrial elements in a species should not be assumed without experimental documentation.

Epichloë typhina, an ascomycete of the family Clavicipitaceae, is a grass biotroph and a sexual relative of a group of seedborne mutualists (e-endophytes) (SCHARDL *et al.* 1994; TSAI *et al.* 1994). *E. typhina* systemically and asymptotically infects vegetative tissues of certain cool-season (C3) grass species, then, during host inflorescence development, develops sporogenous stromata encompassing the host flagleaf sheaths and prevents

maturation of the affected inflorescences (BACON and HINTON 1991). At this stage, the sexual cycle of *E. typhina* is initiated when spermatia are deposited on stromata of the opposite mating type (WHITE 1993). For several days following mating a thick mycelial growth, thought to be heterokaryotic, appears on the stroma surface (WHITE 1994). The sexual stage is completed by production of perithecia and ejection of meiotic ascospores, which are presumably able to infect new host plants (BACON and HINTON 1988).

Three linear plasmids in *E. typhina* isolate E8 copurify with mitochondria (MOGEN *et al.* 1991). These plasmids are of sizes 7.5, 2.1 and 2.0 kb, and are designated Callan-a (formerly Et7.5L), Aubonne-a (formerly Et2.1L) and Bergell (formerly Et2.0L), respectively. Various combinations of related plasmids of similar sizes are present in other *E. typhina* isolates (this study). Although linear plasmids have been identified in many fungi (MEINHARDT *et al.* 1990), plasmid inheritance has been investigated for very few, and there have been no such studies of plant biotrophs. The purpose of this investigation was to assess the pattern of transmission of linear plasmids and mitochondrial genomes in *E. typhina*. We analyzed progeny from five different *E. typhina* crosses and found that the mitochondrial plasmids were mainly transmitted from the stromal (maternal) parent; however, there was occasional replacement of a maternal with a related paternal plasmid, or loss of a maternal plasmid, in ascospore progeny. These results, and analysis of polymorphisms of mitochondrial genomic DNA, indicated that heteroplasmons form in *E. typhina* matings.

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MATERIALS AND METHODS

Fungal strains and matings: *E. typhina* isolate E8 was from the host plant, *Lolium perenne* (MOGEN *et al.* 1991). Isolate E470 was from infected *Anthoxanthum odoratum*, and isolates E2466, E468 and E469 from *Dactylis glomerata* plants, all collected in Switzerland by D. SCHMIDT (Federal Agricultural Research Station, Nyon, Switzerland). Methods for fungal isolation, maintenance, and matings were as described previously (AN *et al.* 1993; SCHARDL and AN 1993; LEUCHTMANN *et al.* 1994). For mating tests, grass-*E. typhina* symbiota were vernalized to initiate inflorescences and associated fungal stromata. Conidia from unfertilized stromata served as spermatia in some matings; in others, cultured mycelia undergoing sporulation were suspended in water using an OMNI-mixer homogenizer (Omni International, Waterbury, CT) set at speed 3.5, and the suspension was rubbed onto stromata with a cotton swab. After 3–4 wk, perithecia were squashed in aniline blue solution and examined for mature ascospores. Mature stromata were taped to the lids of overturned water-agar plates to collect ejected ascospores. Viable spores germinated within 48 hr of ejection and were then transferred to nutrient agar medium. Each culture was subsequently streaked three times for single colonies. Each mating is designated by the formula: stromal (maternal) parent $\times$ spermatial (paternal) parent.

DNA isolation and nuclease digestion: Total fungal DNA was prepared as previously described (BYRD *et al.* 1990; SCHARDL and AN 1993). Cell fractionation and mitochondrial DNA (mtDNA) isolation was as described by SCHARDL *et al.* (1994). Following electrophoresis in 0.6% agarose gel, plasmid bands were excised, and DNA was purified with the GeneClean II kit (BIO 101, La Jolla, CA) as per the instructions of the supplier (VOGELSTEIN and GILLESPIE 1979). Restriction endonucleases, DNase I, and lambda exonuclease were from BRL Life Technologies (Gaithersburg, MD). *Bal*31 nuclease and exonuclease III were from New England BioLabs (Beverly, MA). Nuclease activity and specificities were checked by digestion of supercoiled and *EcoRV*-cleaved pBluescriptKS(+) (Stratagene Cloning Systems, La Jolla, CA).

Southern-blot hybridization analysis: Restriction endonuclease digestion of DNA and electrophoresis were as described in SAMBROOK *et al.* (1989) and AUSUBEL *et al.* (1994). Southern blots of DNA onto a Hybond-N+ positively charged nylon membrane (Amersham, Arlington Heights, IL) were by the alkaline method (AUSUBEL *et al.* 1994). Hybridization probes were labeled with digoxigenin-dUTP (Boehringer-Mannheim Corp., Indianapolis, IN) by polymerase chain reaction (PCR) (LION and HAAS 1990), or by random primer extension (GEBEYECHEU *et al.* 1987) using the Genius kit (Boehringer-Mannheim Corp.). Oligonucleotide primers for PCR were synthesized with a model 394A DNA synthesizer (Perkin-Elmer, Applied Biosystems Division, Foster City, CA). They were designed based on known plasmid sequences (MOGEN *et al.* 1991; K.-R. CHUNG, A. LEUCHTMANN and C. L. SCHARDL, unpublished data), such that PCR with primer numbers 1303 (5'-TCTGAGACTTAGGAACTAT-3') and 1307 (5'-ATC-TAAGTTGATCGATTCT-3') amplified a 278-bp fragment from Bergell; and PCR with primers 6012 (5'-CAACATCTGTCGCTCATACG-3') and 6013 (5'-TTACGACTATCGCCGCGATG-3') amplified a 285-bp fragment from Aubonne-a. PCR reactions were in 100 μ l mixtures with 100 ng mtDNA or total DNA template, 5 μ M each primer, 200 μ M each of dGTP, dATP and dCTP, 185 μ M dTTP, 15 μ M digoxigenin-dUTP (Boehringer-Mannheim Corp.), 4 mM MgCl₂, 1 $\times$ PCR buffer [10 mM Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) pH 8.3, 50 mM KCl, 0.01% gelatin], and 1 unit of *Taq* DNA polymerase (Perkin Elmer, Norwalk, CT). The reaction mixtures were placed in a thermal cycler (Perkin-Elmer) for 30 cycles of the following temperature regimes:

94° for 1 min, 50° (for primers 1303 and 1307) or 56° (for primers 6012 and 6013) for 1 min, and 72° for 1 min. Southern blots were prehybridized at 42° for at least 7 hr in 50% formamide, 6 $\times$ SSC, 5 $\times$ Denhardt's solution (AUSUBEL *et al.* 1994), 0.1% sodium lauroylsulfate (SDS), 0.1% Na₄P₂O₇ 10H₂O, 50 mM Tris-HCl pH 7.5, and 100 μ g/ml denatured herring sperm DNA. Probes were denatured by boiling at 95° for 10 min and added to the prehybridization solution. Hybridization was at 42° overnight. Low-stringency washes of membranes in 2 $\times$ SSC, 0.1% SDS were twice for 10 min at room temperature and once at 55° for 1 hr. For high stringency they were then washed 1 hr at 68° in 0.1 $\times$ SSC, 0.1% SDS. Immunological detection of the probe (GEBEYECHEU *et al.* 1987) used the Genius kit and Lumi-Phos 530 (Boehringer Mannheim Corp.) lumigenic substrate for alkaline phosphatase (SCHAAP *et al.* 1989).

RESULTS

Linear plasmids in parental isolates: Plasmid profiles of parental *E. typhina* isolates and their progeny are listed in Table 1. Copurification of these plasmids with mitochondria of each parental isolate was confirmed by Southern-blot hybridization of total DNA to probes from purified mitochondria (data not shown). All of these plasmids were susceptible to digestion with exonuclease III, *Bal* 31 and DNase I, but not with lambda exonuclease, indicating they were linear double-stranded DNA with blocked 5'-termini (data not shown). For clarity, the three linear plasmids in isolate E8 (MOGEN *et al.* 1991) were redesignated Callan-a (7.5 kb), Aubonne-a (2.1 kb) and Bergell (2.0 kb). Plasmids in the other *E. typhina* isolates were designated according to their relationships based on size and Southern-blot hybridization. Related plasmids from different isolates were distinguished by letter suffixes. Aubonne plasmids in different isolates were distinguished by Southern-blot hybridization analysis of *EcoRI*-digested DNA, and the Callan plasmids were distinguishable by *HindIII* digests (data not shown).

Only one linear plasmid, 2.1 kb in size, was detected in isolate E2466, and this was designated Aubonne-b (Table 1) because of its size and its hybridization at low stringency, but not high stringency, to the Aubonne-a PCR probe (data not shown). Aubonne-b was electrophoretically purified and also used as probe (Figure 1A). Aubonne-a hybridized to Aubonne-b at low stringency (Figure 1A, lane 1). Also, plasmids of 2.1 kb from isolates E468 and E470 hybridized at low stringency (Figure 1A, lanes 4 and 3, respectively) and high stringency (data not shown) to Aubonne-b, and were designated Aubonne-c (in E468) and Aubonne-d (in E470). These plasmids hybridized with the PCR-labeled Aubonne-a fragment only under low-stringency conditions (data not shown). Isolate E470 also contained a 7.5-kb plasmid that hybridized with Callan-a probe (Figure 1A, lane 3), and was designated Callan-d. No plasmid was detected in isolate E469 either by visualizing stained electrophoresis gels or by Southern-blot hybridization

TABLE 1
Mitochondrial linear plasmids in *Epichloë typhina* parental isolates and progeny

Stromal parent	Plasmids ^a	Spermatial parent	Plasmids	Progeny	Plasmids
E8	Cal.-a, Aub.-a, Ber.	E2466	Aub.-b.	1–34, 36–51, 53–64, 66–79	Cal.-a, Aub.-a, Ber.
E8	Cal.-a, Aub.-a, Ber.	E2466	Aub.-b	35, 52	Aub.-a, Ber.
E8	Cal.-a, Aub.-a, Ber.	E2466	Aub.-b	65, 80	Cal.-a, Aub.-b, Ber.
E470	Cal.-d, Aub.-d	E468	Aub.-c	1–9	Cal.-d, Aub.-d
E470	Cal.-d, Aub.-d	E469	None	1–7	Cal.-d, Aub.-d
E469	None	E470	Cal.-d, Aub.-d	1–17	None
E469	None	E8	Cal.-a, Aub.-a, Ber.	1	None

^a Cal., Callan; Aub., Aubonne; Ber., Bergell.

analysis. No plasmids related to Bergell were detected in any parental isolate except E8 (Figure 1B).

Southern-blot analysis of Aubonne plasmids in undigested DNA revealed additional, fainter bands only when these plasmids were present (Figure 1A, lanes 1–4). These generally included a faint but distinct band at 4.0 kb (lane 2) and various other bands and smears (e.g., the band at 1.8 kb most apparent in lane 4). The nature of these plasmid-related molecules remains unknown.

Inheritance of linear plasmids: To address the inheritance of linear plasmids, stromata produced by E8 on perennial ryegrass were mated with E2466, and 80 viable progeny were collected and analyzed by Southern-blot hybridization to probes from gel-purified plasmids and PCR-generated fragments. Table 1 summarizes the plasmid profiles of the E8 × E2466 progeny. Each had Bergell from the maternal parent, and all except progeny numbers 65 and 80 had Aubonne-a from the mater-

nal parent (data not shown). As demonstrated by experiments described later, progeny 65 and 80 lacked Aubonne-a, but had Aubonne-b from the paternal parent. All except progeny 35 (Figure 2A) and 52 (Figure 2B) had Callan-a from E8. Only high-molecular-size DNA from progeny 35 hybridized to the Callan-a probe (Figure 2A, lane 4), possibly due to contaminating genomic DNA in the probe. Undigested DNA of progeny 52 probed with Callan-a showed bands around the 7.5-kb region, but not a distinct band at 7.5 kb (Figure 2B, lane 3). To determine if progeny 35 or 52 had Callan-a sequences, their DNAs were digested with restriction endonucleases and analyzed by Southern-blot hybridization (Figure 2, A and B). There were no hybridizing *Bam*HI fragments from progeny 35 and 52 that corresponded to those of Callan-a from E8. There was a hybridizing *Eco*RI fragment of 0.9 kb in progeny 52 (Figure 2B, lane 5), the maternal parent (Figure 2A, lane 2), and other progeny tested except progeny 35 (Figure 2A, lane 6). The 6.6-kb *Eco*RI fragment from Callan-a was not observed in either progeny 35 or 52. These results indicated that Callan-a was lost in progeny 35 and 52, but heterogeneous DNA species with sequences from Callan-a remained in progeny 52.

The 80 E8 × E2466 progeny were also examined for Aubonne-a and Aubonne-b by Southern-blot analysis of undigested DNA samples probed with a PCR-amplified Aubonne-a fragment and with gel-purified Aubonne-b DNA (data not shown). All except progeny 65 and 80 had Aubonne-a. Progeny 65 and 80 had a 2.1-kb DNA species that hybridized at high stringency only to labeled Aubonne-b, suggesting that these progeny lacked Aubonne-a and had inherited the related plasmid from their paternal parent. This was confirmed by Southern-blot analysis of DNA digested with *Eco*RI, to distinguish Aubonne-a from Aubonne-b, and probed at low stringency with Aubonne-b (Figure 3A). Only progeny 65 (Figure 3A) and 80 (not shown) gave the same pattern as the paternal parent E2466. All other progeny showed two fainter bands with fragment sizes 1.1 and 0.6 kb, indicative of Aubonne-a from the maternal parent (Figure 3A, data not shown). The Southern blots were also hybridized to PCR-generated probes for Ber-

FIGURE 1.—Identification of mitochondrial plasmids in *Epichloë typhina* isolates. Total, undigested DNAs from E8 (lane 1), E2466 (lane 2), E470 (lane 3), E468 (lane 4) and E469 (lane 5) were electrophoresed in 1% agarose gel, blotted to membranes, probed, and washed at low stringency. Sizes of the hybridizing DNAs are indicated in kilobasepairs (kb). (A) The blot was hybridized first to Callan-a and then to Aubonne-b without stripping the Callan-a probe. The 7.5-kb Callan-a plasmid hybridized to itself in E8 (lane 1), and to the 7.5-kb Callan-d plasmid in E470 (lane 3). Plasmids of 2.1 kb, which hybridized to Aubonne-b probe, were designated Aubonne-a (lane 1), -b (lane 2), -d (lane 3) and -c (lane 4). Also revealed with the Aubonne-b probe were bands and smears, such as the 4.0 kb species in E2466 (lane 2) and ca. 1.8 kb bands in lanes 1–4, the nature of which is unknown. The signal from high-molecular-size genomic DNA was due to contamination of the gel-purified plasmid probes. (B) The blot was hybridized to PCR-generated probe from Bergell, and only the Bergell plasmid in E8 (lane 1) was detected.

FIGURE 2.—Absence of maternal plasmid Callan-a in progeny 35 (A) and 52 (B) of the E8 × E2466 mating. Total fungal DNAs from E8 and five progeny, undigested (un) or digested with *Bam*HI (B) or *Eco*RI (E), were electrophoresed, blotted, hybridized with gel-purified Callan-a probe, and washed at high stringency. Sizes of the hybridizing bands are indicated in kb. A distinct 7.5-kb band in the maternal parent, E8, is absent in progeny 35 (A, lane 4) and 52 (B, lane 3). Cross-hybridizing species in the undigested DNA lane were due either to probe contamination or to Callan-related species. No bands from *Bam*HI-digested Callan-a in progeny 35 and 52 DNAs corresponded to those from the maternal parent, E8. However, a small (0.9 kb) fragment from *Eco*RI-digested Callan-a is detected in progeny 52 DNA (B, lane 5), but not in DNA from progeny 35 (A, lane 6). The other three progeny 41, 42 and 66 inherited Callan-a.

gell and Aubonne-a. All progeny and the maternal parent had the 0.28-kb *Eco*RI fragment from Bergell (data not shown). This result also demonstrated that the DNA in each sample was successfully digested with *Eco*RI. The maternal parent and all progeny except 65 (Figure 3B) and 80 (data not shown) had the 0.3-kb fragment from Aubonne-a. Thus, of the 80 progeny from the E8 × E2466 mating, all inherited Bergell from E8, all but progeny 35 and 52 inherited Callan-a from E8, and progeny 65 and 80 lacked the maternal Aubonne-a but inherited the paternal Aubonne-b. Coexistence of the four linear plasmids—Callan-a, Aubonne-a, Aubonne-b, and Bergell—was never observed (Table 1).

FIGURE 3.—Replacement of plasmid Aubonne-a with Aubonne-b in progeny 65 of the E8 × E2466 mating. *Eco*RI-digested DNA was probed at low stringency with Aubonne-b (A), and at high stringency with Aubonne-a PCR probe (B). Lanes had DNA from E8 (♀), E2466 (♂), and the progeny numbers indicated. Sizes of the hybridizing bands are indicated in kb. Plasmid Aubonne-a from E8 is cleaved by *Eco*RI to generate fragments of 1.1 kb, 0.6 kb (A), and 0.3 kb (B), whereas plasmid Aubonne-b is not cleaved by *Eco*RI (A, female parent and progeny 65 lanes). In addition to the 2.1-kb plasmids, numerous DNA species of unknown nature were detected with both probes.

As stated above, bands and smears resulted from molecules that hybridized to Aubonne plasmid probes, but were not directly attributable to the 2.1-kb plasmids (Figures 1 and 3). Such molecules seemed most closely related to the particular Aubonne plasmid present. For example, upon high-stringency probing for Aubonne-a in the female parent, E8, there were intense smears above the expected 0.3-kb band in all strains except those (E2466 and progeny 65 and 80) that lacked Aubonne-a (Figure 3B and data not shown). Similarly, when Aubonne-b was used as probe, a smear below the 2.1-kb plasmid band was observed from the male parent (E2466) and progeny 65 (Figure 3A) and 80 (not shown). The nature of these additional molecules remains unknown.

Only the maternal plasmid profiles were observed among all 34 progeny of four addition matings: E470 × E468, the single ascospore from E469 × E8 (most matings with E8 spermatia failed), and the reciprocal matings E470 × E469 and E469 × E470 (Table 1).

Inheritance of mitochondrion genomic DNA: Total DNAs of E8, E2466 and 25 progeny from E8 $\times$ E2466 were digested with *Bam*HI and *Pst*I, and Southern blots were hybridized to E2466 mtDNA probe (Figure 4A). Numerous restriction fragment-length polymorphisms were observed between E8 and E2466 mtDNAs, and the profile from each progeny was nearly identical to that of E8. However, some progeny (e.g., numbers 4, 52, 65 and 66 in Figure 4A) showed a nonparental profile with a 3.5-kb fragment possibly derived from their paternal parent, and lacking a 3.4-kb mtDNA fragment from the maternal parent. The mtDNA profiles were also analyzed for a total of 34 progeny from four other matings, including the reciprocal crosses E470 $\times$ E469 and E469 $\times$ E470. The mtDNA profiles of E469 $\times$ E470 progeny resembled the E469 profile, but many had additional bands such as the 3.8-kb *Bam*HI/*Pst*I fragment evident in progeny 1, 4, 7, 10, 11 and 12, faint in progeny 2 and 3, and absent from the maternal parent and progeny 5, 6, 8 and 9 (Figure 4B). However, all progeny from the reciprocal cross, E470 $\times$ E469, had the maternal mtDNA profiles (data not shown). The single progeny from E469 $\times$ E8 (Figure 4C) had fragments of sizes 3.4 kb (strong) and 2.5 kb (faint) that comigrated with fragments from the paternal parent, E8, and lacked 6.1- and 5.4-kb fragments from the maternal parent. Each progeny from E470 $\times$ E468 had the same profile as E470 (data not shown).

DISCUSSION

Transmission of linear mitochondrial plasmids in *E. typhina* was mainly, but not exclusively, maternal. Among 80 E8 $\times$ E2466 progeny, 76 had maternal plasmid profiles. Of the remaining four, two inherited the paternal plasmid, Aubonne-b, and lacked Aubonne-a, but had the other two maternal plasmids, Callan-a and Bergell; another two progeny lacked Callan-a, but had Aubonne-a and Bergell. These results, and mitochondrial genome profiles suggestive of recombination in several matings, indicated that heteroplasmons formed during the *E. typhina* sexual cycle.

The distributions of plasmids among *E. typhina* strains and progeny suggest that none is required for maintenance of any other. Plasmids related to Aubonne-a were present in isolates that lacked Callan, Bergell, or any related sequences. Bergell was present with only Callan-a in the mutant E98 (MOGEN *et al.* 1991), but two progeny with Bergell and Aubonne-a lacked Callan-a. Finally, Callan-d was present with only Aubonne-d in E470.

Aubonne and Bergell plasmids are too small to encode a 120–140-kD DNA polymerase, typically needed for replication of other linear plasmids in fungi and plants, adenoviruses in animals, and *Bacillus subtilis* phage ϕ 29 (CHAN *et al.* 1991). This suggests that plasmid maintenance functions are encoded in the *E. typh-*

FIGURE 4.—Inheritance of mitochondrial genomic DNA in progeny from matings E8 $\times$ E2466 (A), E469 $\times$ E470 (B), and E469 $\times$ E8 (C). Total DNAs from the stromal (♀) and spermatial (♂) parents, and progeny as indicated, were cleaved with *Pst*I and *Bam*HI, electrophoresed, blotted, and probed with DNA from mitochondria of E2466 (A) or E469 (B and C). Arrows at right indicate differences between some progeny and their stromal parents. Progeny from E470 $\times$ E469, the reciprocal cross to that in B, had similar profiles to the maternal parent (data not shown). Size markers were DNA from bacteriophage lambda cleaved with *Hind*III (A), or with *Hind*III and *Eco*RI (B and C), and their positions are indicated in kb.

ina mitochondrial or nuclear genome, or that *E. typhina* plasmids are replicated by unusual polymerases. Also of unknown significance are the various DNA species related to, and associated with, Aubonne plasmids (e.g., the 4.0-kb species in Figure 1A, lane 2). These might represent other forms of linear plasmids, but it is more likely that the larger species are dimers and those migrating faster on gels may be single-stranded forms, both of which may be replication intermediates as suggested for other linear plasmids (MIYASHITA *et al.* 1990).

The observation that the only two E8 × E2466 progeny with the paternal plasmid, Aubonne-b, also lacked the related maternal plasmid, Aubonne-a, suggested plasmid incompatibility. Possibly, the two different Aubonne plasmids compete for replication or partition functions. If they compete for replication, Aubonne-a and Aubonne-b should have similar sequences near their termini. Furthermore, such sequences should be recognized by factors specifically involved in Aubonne plasmid replication because these plasmids are highly compatible with Bergell and Callan-a. Any incompatibility between Aubonne-a and Aubonne-b cannot be due to competition for replication or partition functions common to all of the *E. typhina* linear plasmids.

The inheritance of mitochondrial genomes and plasmids in oogametes fungi is often assumed to be strictly maternal (SAMAC and LEONG 1989). However, mitochondrial plasmids may be paternally transmitted or fail to transmit maternally, in some progeny, and fungal genotype can affect plasmid inheritance patterns (MAY and TAYLOR 1989; YANG and GRIFFITHS 1993b). Likewise, inheritance of mitochondrial genomes is often not strictly maternal, and biparental inheritance as mixed or recombinant genomes has been described (TAYLOR 1986; ECONOMOU *et al.* 1987; HINTZ *et al.* 1988; MAY and TAYLOR 1988; KAWANO and KUROIWA 1989; MIRFAKHRAI *et al.* 1990; SMITH *et al.* 1990; LEE and TAYLOR 1993; YANG and GRIFFITHS 1993a). Recombination of parental genomes is a likely explanation of the nonparental profiles we observed among many of the *E. typhina* progeny.

Although heteroplasmons probably formed in *E. typhina* matings, the most frequent profiles of mtDNAs among progeny were similar or identical to the maternal parents. Likewise, maternal inheritance of mtDNA profiles has been observed in the related fungus, *Atkinsonella hypoxylon* (VAN HORN and CLAY 1995). Possible mechanisms that have been suggested for predominantly uniparental transmission of mtDNA include destruction or exclusion of paternal elements (KUROIWA and HORI 1986; MOGENSEN 1988), or a stochastic effect due to the initial predominance of maternal cytoplasm (BIRKY *et al.* 1978). Exclusion appears unlikely in *E. typhina* matings. In another oogametes ascomycete, *N. crassa*, exclusion of paternal cytoplasm might result from vegetative incompatibility caused by the interacting mating type idiomorphs (GLASS *et al.* 1988; METZENBERG 1990). In contrast, vegetative incompatibility is not observed in

Epichloë mating, in which fertilization of a stroma is followed by growth of a thick, apparently heterokaryotic mycelium (WHITE 1994; K.-R. CHUNG, A. LEUCHTMANN and C. L. SCHARDL, unpublished data). Furthermore, the observed inheritance patterns of mitochondrial DNA in *E. typhina* did not suggest exclusion by vegetative incompatibility or other means. Rather, the nonparental profiles of plasmids and mitochondrial genomes in several *E. typhina* progeny were evidence that heteroplasmons formed during sexual development, paternal mitochondria persisted together with maternal mitochondria, and maternal and paternal mitochondria often fused. Thus, the more likely explanation for predominantly maternal transmission in *E. typhina* is that the predominance of maternal mitochondrial types in the cytoplasm of the heterokaryon biases inheritance patterns in the progeny. However, it is unknown whether fixation of parental or recombinant mitochondrial genotypes is strictly stochastic, or is the result of selection on a subset of DNA molecules that function in mtDNA replication (PIŠKUR 1994).

Although there appears to be little barrier to proliferation of extranuclear molecular parasites in *E. typhina*, formation of mitochondrial heteroplasmons in matings of this fungus can enhance diversity of mitochondrial genotypes, and might aid adaptation to variable environmental conditions.

We thank A. D. BYRD, W. HOLLIN and K. GARRETT for their able assistance, and M. R. SIEGEL for helpful discussion. This work was supported by National Science Foundation grant DEB-9408018. This is publication number 95-12-166 of the Kentucky Agricultural Experiment Station published with the approval of the director.

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Communicating editor: R. H. DAVIS