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Genetic variation in the widespread lichenicolous fungus *Marchandiomyces corallinus*

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Abstract: The lichenicolous basidiomycete *Marchandiomyces corallinus* is widely distributed in North America and Europe, where it commonly is found on a variety of lichens. Theoretically either of these characteristics, a wide geographic range or generalized host ecology, could provide opportunities for genetic differentiation within this species. To determine how genetic variation is partitioned in *M. corallinus,* 12 fungal isolates were obtained from locations in North America and Europe; at two locations, in Washington County, Maine, and on the Isle of Mull in Scotland, fungi also were isolated from different lichen hosts. Vegetative mycelial compatibility tests were used to determine compatibility groupings from among the isolates; in addition, several PCR amplification products (RAPD, nuITS rDNA) were obtained for each isolate. A number of distinct compatibility groups were recognizable based on geography, not host ecology. In addition compatible isolates always were restricted to either North America or Europe. However RAPD markers indicated that compatible isolates are not always genetically identical. The presence of sequence heterozygosity at specific positions indicated that the isolates are heterokaryotic and a number of distinct haplotypes could be identified based on ITS variation at three separate locations. This type of genetic variation in these fungi suggests that sexual recombination is possible and that genetic differentiation has taken place recently as a result of geographic isolation, not host switching.

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INTRODUCTION

Lichenicolous fungi form obligate associations with lichens, either as saprotrophs that colonize dead lichen thalli or parasites that range in virulence from relatively benign to aggressively pathogenic. The most recent survey of these fungi by Lawrey and Diederich (2003) lists more than 1500 species with an estimate of over 3000. More than 95% of these species are ascomycetous, although a number of basidiomycete groups have this habit as well (Sikaroodi et al 2001). The host specificity of these fungi appears to be high (Diederich 2000), with as many as 95% thought to be associated with a single lichen genus. Nevertheless some of the best known lichenicolous fungi have wide geographic distributions and broad host ecologies.

Several authors have discussed the evolution of the lichenicolous habit and speculated on possible evolutionary trends. Hawksworth (1978, 1982a, b, 1988a, b) emphasized the reticulate nature of fungal habits including the lichenicolous habit, an idea supported by phylogenetic reconstructions of transitions among nutritional modes in major fungal clades of ascomycetous (i.e., Gargas et al 1995) and basidiomycetous fungi (i.e., Hibbett et al 2000). Given the tools of molecular biology, investigators now routinely explore microevolutionary patterns in parasite-host (especially plant-fungal) associations. At present lichenicolous fungi have not been studied genetically at the intraspecific level, so the mechanisms responsible for the origin and evolution of lichen-parasite interactions have yet to be documented. Are there obvious differences in the genetic variation of these fungi? How different is the genetic variation of host-specialists and host-generalists? Is there evidence of genetic differentiation among lichenicolous fungi that use different lichen hosts? Is there evidence of geographic differentiation in widely distributed lichenicolous fungi?

The lichenicolous basidiomycete *M. corallinus* (Roberge) Diederich & D.Hawksw is collected commonly throughout eastern North America and Eu-

rope from a variety of host lichens, including species of *Parmelia* s.l., *Physcia* s.l., *Lepraria, Pertusaria, Lecanora* and *Lasallia*. Infected lichens exhibit obvious coral-colored bulbils clustered on the surface of the thallus. A sexual stage has not been observed, and the nuclear condition of the bulbilliferous stage is not known. However basidiomycete teleomorph of another species in the genus, *M. aurantiacus* (Lasch) Diederich & Etayo, was described (Diederich et al 2003) as *Marchandiobasidium aurantiacum* Diederich & Schultheis. The anamorph is common in polluted habitats in Europe, and it also attacks a variety of lichens, especially species of *Physcia*. In addition to *M. corallinus* and *M. aurantiacus* the genus includes one other species, *M. lignicola* Lawrey & Diederich, that appears from molecular data to be most closely related to *M. corallinus* but is lignicolous in habit (DePriest et al 2005), indicating a remarkably flexible nutritional ecology in the members of this genus.

The presumed close relationships among *Marchandiomyces* and other mitosporic genera (*Hobsonia, Illosporium*), discussed at various times in the literature (Lowen et al 1986), were shown by Sikaroodi et al (2001) to be erroneous, as predicted by Etayo and Diederich (1996). It should be noted that the most recent edition of the Dictionary of the Fungi (Kirk et al 2001) classifies *Marchandiomyces* as an anamorphic ascomycete, citing Sikaroodi et al (2001); this is clearly a mistake because the latter study demonstrates an unambiguous basidiomycetous position for *Marchandiomyces*.

The phylogenetic reconstructions of DePriest et al (2005) placed the three species of *Marchandiomyces* in a clade made up of representatives of the types of the genera *Dendrocorticium, Duportella, Laeticorticium* and *Vuilleminia*. Various authors (Hibbett and Thorn 2001, Binder and Hibbett 2002, Hibbett and Binder 2002, Larsson et al 2004) have recognized the distinctiveness of this clade but have referred to it using different names. Hibbett and Binder (2002) refer to it as the *Dendrocorticium* clade, and Larsson et al (2004) call it a corticioid clade; both studies make clear that fungi traditionally considered to be corticioid are widely distributed among the basidiomycetes.

The wide geographic distribution and host amplitude of *M. corallinus* would appear to present numerous opportunities for genetic differentiation in this fungus. During the past decade a small group of samples has been obtained from various locations in North America and Europe representing the known range of this fungus, and in certain locations samples were taken from different lichen hosts in the same habitat. We since have used pairwise tests of mycelial compatibility (Rayner 1991, Worrall 1997) and presence of various molecular markers (RAPD, nuITS rDNA) to describe the isolates genetically and assess the level of genetic differentiation among the populations they represent.

We expected results of these investigations to shed light on a number of questions, among them: (i) How genetically distinct are these bulbilliferous (presumably asexual) fungi? (ii) Is there evidence, direct or indirect, for a heterokaryotic nuclear condition in these fungi? (iii) How are vegetative compatibility groups distributed geographically? (iv) Is genetic differentiation correlated with geographic distance or host ecology?

METHODS

Fungal specimens.—Isolates from 12 specimens of *Marchandiomyces corallinus* were used. Specimens were collected from North America and Europe from a variety of locations representing the known range of this fungus (TABLE I). In two locations (Maine and Scotland) a sufficient amount of material could be obtained from different lichen hosts growing in the same habitat to test for genetic differentiation possibly caused by host switching in the fungus.

Isolation of fungal cultures.—Fungal cultures were isolated from freshly collected material. Infected thalli were washed briefly in sterile water, and bulbils were removed using a flamed needle. Bulbils were placed on either potato-dextrose agar (PDA) or Sabouraud's medium (Difco Laboratories Inc., 10 g Bacto Neopeptone/L, 20 g Bacto Dextrose/L), and mycelial outgrowths were subcultured monthly (Lawrey 2002). Voucher cultures of new isolates were sent to the American Type Culture Collection for reference. These cultures were used for compatibility/incompatibility tests and for DNA extraction.

Mycelial compatibility groupings.—Pairwise tests of mycelial compatibility were done with 8-mm diam mycelial plugs taken from the margin of a 14 d old colony of each isolate and placed about 25 mm apart on 100 × 15 mm dishes containing PDA. All possible pairwise combinations were tested, including pairs from the same isolate. The dishes were incubated 14 d and examined for the presence of an aversion or barrage reaction in the zone of contact between mycelial outgrowths. Isolates that formed a reaction were assigned to different compatibility groups. All tests were replicated.

DNA extraction.—Total DNA was extracted from each of the cultures using the TES extraction protocol modified from Grube et al (1995). Fungal tissue was ground in TES buffer (100 mM Tris, 10 mM EDTA, 2% SDS, pH 8.0), with 1.4 M NaCl and 10% CTAB, and extracted twice with 1 volume chloroform : isoamyl (24:1). DNA was precipitated in 0.6 volume of isopropanol with sodium acetate and washed with 70–80% ethanol. The DNA pellet was suspended in 20–30 mL of deionized water, and the DNA was quantified by visualizing with ethidium bromide on a 1% agarose gel.

TABLE I. Specimens of *Marchandiomyces corallinus* used in the study with collector, location of collection, lichen substrate and ATCC accession number

GMU isolate No	Collector	Location	Lichen substrate	ATCC number
JL106-95	Lawrey 1619	Bear Is, Maryland (USA)	*Flavoparmelia baltimorensis*	200796
JL108-96	Lawrey 1626	Augusta Co., Virginia (USA)	*Lasallia papulosa*	200797
JL136-00	Cullen & Fox 216	Isle of Mull, Scotland (Europe)	*Pertusaria amara*	MYA-1118
JL160-00	Buck 37514	Howell Co., Missouri (USA)	*Pertusaria neoscotia*	MYA-1119
JL161-00	Buck 37404	Madison Co., Arkansas (USA)	*Pertusaria plittiana*	MYA-1120
JL167-00	Fox (no number)	County Kerry, Ireland (Europe)	*Tephromela atra*	MYA-1377
JL171-00	Fox 322	Isle of Mull, Scotland (Europe)	*Ramalina subfarinacea*	MYA-1378
JL172-00	Fox 322	Isle of Mull, Scotland (Europe)	*Parmelia sulcata*	MYA-1379
JL173.00	Fox 322	Isle of Mull, Scotland (Europe)	*Hypogymnia physodes*	MYA-1380
JL213-01	Cole 9290	Washington Co., Maine (USA)	*Lasallia papulosa*	MYA-2499
JL222-01	Diederich 14869	Moselle, France (Europe)	*Porpidia cinereoatra*	MYA-2501
JL236-02	Hoffman (no number)	Washington Co., Maine (USA)	*Xanthoparmelia sp.*	MYA-2827

When the extractions were carried out using natural (not isolated) material or when the amount of the culture was small, DNA was extracted using the Dneasy Plant Mini Kit (Qiagen) with minor modifications as described elsewhere (Crespo et al 2001).

RAPD-PCR amplification.—The protocol for PCR amplification is derived from Sikaroodi et al (2001) and modified for RAPD-PCR amplification. Fragments were amplified from the genomic DNA (~10 ng) with 1.25 units of Klentaq 1 (Ab peptides Inc.) in 100 μL PCR reactions in a reaction buffer (10 mM Tris pH 8.3, 50 mM KCl and 2 mM $MgCl_2$), with 200 μM of each of the four dNTPs and 0.5 μM of each primer. The reactions were carried out in a Perkin-Elmer Cetus DNA Thermal Cycler, for 42 cycles with these conditions for most reactions: template denaturation at 94 C for 1 min, primer annealing at 30 C for 1 min, and primer extending at 72 C for 2 min (extended by 5 s in each cycle). The primers used were P102 (5′-GGTGGGGACT-3′), P130 (5′-GGTTATCCTC-3′), P131 (5′-GAAACAGCGT-3′), P128 (5′-GCATATTCCG-3′) and P129 (5′-GCGGTATAGT-3′). Amplification products were separated by electrophoresis, stained with ethidium bromide and visualized under UV light. All clearly visible bands were included in the analysis; faint bands were considered unidentifiable. To assess the reproducibility of our data RAPD amplifications were twice repeated; all replicates produced identical band patterns.

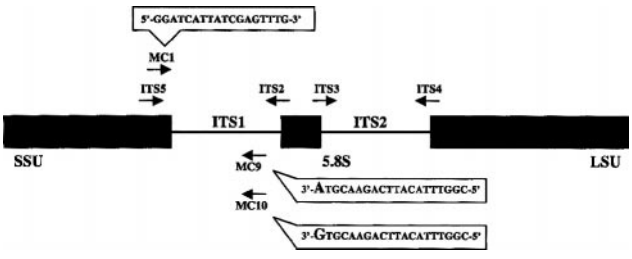

FIG. 1. Position of primers used in this analysis. The nu-SSU-1785-5′ (MC1) primer is *Marchandiomyces corallinus*-specific for the ITS region. The nu-ITS-146A-3′ (MC9) and nu-ITS-146G-3′ (MC10) primers were used to establish the heterokaryotic character of *M. corallinus*.

nu-ITS PCR amplification.—DNA isolates were diluted (10–300-fold) to get approximately 10 ng for amplifications. Amplification reactions were carried out as described in Sikaroodi et al (2001). The nuITS1 region was amplified between primer nu-SSU-1766-5′ (ITS5) and primer ITS2 (White et al 1990). We obtained one sequence from bulbils of a field-collected specimen of *M. corallinus* (JL236) growing on *Xanthoparmelia* sp, using a primer designed especially for this purpose, nu-SSU-1785-5′ MC1 (FIG. 1) and ITS4 (White et al 1990). Two new primers to amplify a fragment of ITS1 also were designed to establish the presence of one or two templates in the isolates: nu-ITS-146A-3′ and nu-ITS-146G-3′ (FIG 1.). Both were paired with ITS5.

PCR products were purified of excess primers with either of two protocols: (i) precipitation with 20% polyethylene glycol and 2.5 mol/L NaCl or (ii) filtration through PCR Wizards (Promega) following the manufacturer instructions. The concentration and size of the PCR amplification products were estimated by comparing to nucleotide weight and size markers after agarose gel electrophoresis and stained with ethidium bromide and exposure to UV light.

DNA sequencing.—Double stranded PCR products were sequenced from each of the amplification primers and a number of internal sequencing primers (ITS2 and ITS3). Approximately 100 ng of cleaned products were sequenced from 3.2 pM of primer with the PRISM Ready Reaction Dye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems). The reaction was carried out in a Perkin Elmer Cetus DNA Thermal Cycler for 25 cycles under these conditions: template denaturation was done at 96 C for 30 s, primer annealing at 50 C for 15 s and primer extension at 60 C for 4 min. The cycle sequencing products were purified of excess dye with filtration through Sephedex G-50 Fine (Pharmacia) columns and were run on a 4% polyacrylamide gel in a 373A and 377 Automatic Sequencer (Applied Biosystems).

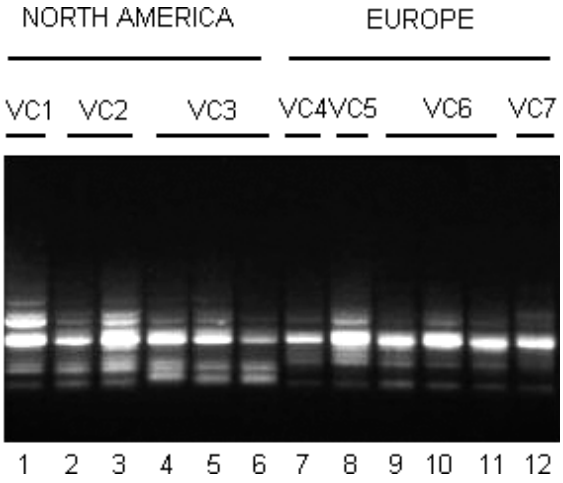

FIG. 2. Example of a gel showing amplification products from DNA of *Marchandiomyces corallinus* isolates using RAPD primer "102". Isolates 1 = JL106(MD), 2 = JL213(ME), 3 = JL236(ME), 4 = JL108(VA), 5 = JL160(MO), 6 = JL161(AR), 7 = JL167(IRE), 8 = JL136(SCO), 9 = JL171(SCO), 10 = JL172(SCO), 11 = JL173(SCO), 12 = JL222(FRA).

Sequence compiling.—Base calling software (Sequencing Analysis, ABI Prism, 2.1.1) was used to produce a preliminary nucleotide sequence. The nucleotide sequence fragments were compiled with Sequence Navigator 1.0 (Applied Biosystems). The sequences were confirmed by comparison to sequences produced from the opposite strand and altered by manual base calling where appropriate.

RESULTS

Mycelial compatibility.—Pairwise tests of mycelial compatibility were used to obtain a general idea of genetic similarity. Genetically identical (or sufficiently similar) mycelia will anastomose readily when grown together on agar, but genetically incompatible mycelia will form interaction zones. Several mycelial compatibility (MC) groups were discovered for *M. corallinus* (TABLE III), and they appear to be correlated with geographic location because compatibility was never observed between any North American sample and any European sample. Samples from North America form three compatibility groups: MC1 is represented by a single culture JL106 (Maryland), MC2 is represented by two cultures from different lichens in the same location, JL213 and JL236 (Maine), and MC3 is represented by JL108 (Virginia), JL160 (Missouri) and JL161 (Arkansas). Samples from Europe form four compatibility groups. Three are represented by single isolates, MC4 from JL167 (Ireland), MC5 from JL136 (Scotland), MC7 from JL222 (France), and a fourth (MC6) is represented by three isolates from different lichen hosts in the same habitat, JL171, JL172 and JL173 from Scotland. In both North America and Europe samples from different lichen substrates in the same location are always from the same compatibility group (TABLE III). European samples collected from Lochbuie Stone Circle, Isle of Mull (Scotland), included those growing on *Ramalina subfarinacea* (Ramalinaceae), *Parmelia sulcata* and *Hypogymnia physodes* (Parmeliaceae), and all are of the MC6 compatibility group. North American samples collected at the Eagle Hill Field Station in Maine from two lichen substrates, *Xanthoparmelia* sp. and *Lasallia papulosa,* were of the MC2 compatibility group.

RAPD analyses.—RAPD PCR band patterns were distinct for most isolates, even those from the same mycelial compatibility group (FIG. 2, TABLE II); only JL160 and JL161 had the same band pattern. RAPD bands cannot distinguish heterokaryotic from haploid nuclear conditions, nor can they distinguish homozygotes from heterozygotes in heterokaryotic individuals. Given the possibility that *M. corallinus* bulbilliferous stages are heterokaryotic, we were hesitant to assign much meaning to these data beyond the observation that isolates are rarely identical, which indicates a much higher level of genetic variation among both North American and European populations than we expected for asexual fungi.

ITS1 results.—Sequences of certain of the cultures (e.g., JL106, JL213, JL236) exhibited a double peak

TABLE II. RAPD band patterns in each fungal isolate using five different primers. ? = unidentifiable, D′ = D pattern except in one band

	North America						Europe					
RAPD Primers	JL106 MA	JL213 ME	JL236 ME	JL108 VA	JL160 MO	JL161 AR	JL167 IRE	JL136 SCO	JL171 SCO	JL172 SCO	JL173 SCO	JL222 FRA
"102"	A	A	A	A′	A′	A′	B	B	B	B	B	B
"128"	A	B	B	C	C	C	D	D	D′	D′	D′	F
"129"	A	B	B	C	B	B	D	D	D	D	D	D
"130"	A	B	C	?	B′	B′	E	F	G	E	?	?
"131"	A	A	A	A	A	A	B	B	C	C′	C′	F

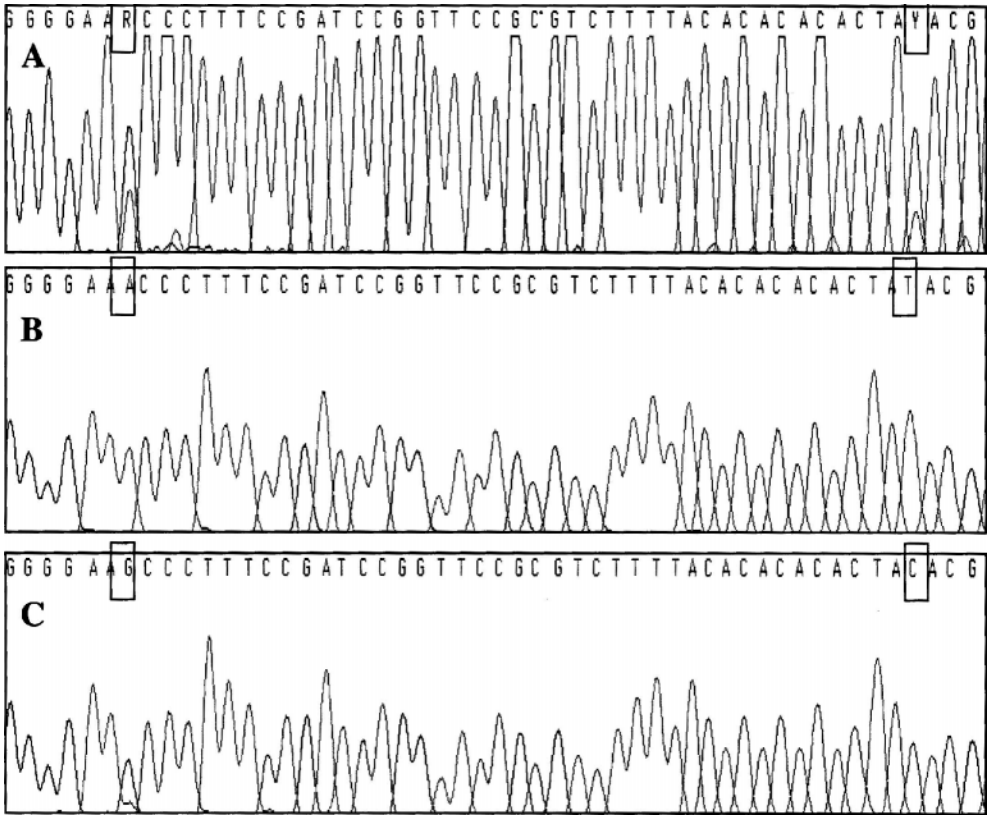

FIG. 3. Electropherograms obtained from DNA using several primers. A. Sequence from culture JL106 (MD) that is presumably heterozygous at the 146 position of ITS1 using the ITS2 primer (the same electropherogram was obtained using ITS5), R = double peak A and G, Y = double peak C and T. B. Sequence of culture JL106 (MD) using M9 primer (nu-ITS-146A-3′, 5′-CGGTTTACATTCAGAACGTA-3′). C. Sequence of culture JL106 (MD) using M10 primer (nu-ITS-146G-3′, 5′-CGGTTTACATTCAGAACGTG-3′).

on positions 103 (A/G) and 146 (T/C) on the ITS1 segment (FIG. 3A) when they were obtained using ITS5 or ITS2. When this fragment was sequenced using the primer MC9 (nu-ITS-146A-3′, 5′-CGGTTTACATTCAGAACGTA-3′), designed to match one of the possible sequences for this region, a clear electropherogram was found with an ''A'' at the downstream 103 position (FIG. 3B). When this same fragment was sequenced using the primer MC10 (nu-ITS-146G-3′, 5′-CGGTTTACATTCAGAACGTG-3′), designed to match the other possible sequence for this position, a uniform electropherogram without double peaks was found with a ''G'' at the downstream 103 position (FIG. 3C). These molecular markers indicated the presence of two distinct DNA haplotypes in the extract obtained from these isolates, suggesting a heterozygous state for these particular sequence positions. One haplotype has ''A'' at position 103 and ''T'' at position 146, and the other has ''G'' at position 103 and ''C'' and position 146. This is not true of all isolates, however, because several cultures (e.g., JL136 from Scotland) exhibited a uniform electropherogram when ITS2 (FIG. 4A) and ITS5 (FIG. 4B) were used to sequence the ITS1 fragment. If these are also heterokaryotic, this pattern suggests a homozygous state for this particular sequence position.

Certain isolates (e.g., JL171, JL172 and JL173 from Scotland) also exhibited double peaks downstream from the 141 ITS1 position when the electropherogram was obtained with ITS2 (FIG. 4C) and downstream from the 132 ITS1 position when the ITS5 was used (FIG. 4D). This alteration is also apparently a consequence of overlap of two different templates. One of them was constituted by the sequence 5′-ACACACACAC-3′ (5AC repeated) and the other one by 5′-ACACACACACAC-3′ (6AC repeated). A uniform electropherogram was obtained when both templates had the same number of ''ACs'' at the position (FIG. 4A and B) and double peaks were detected when the templates had a different number of ''ACs'' repeated (FIG. 4C and D). Four haplotypes were obtained for this microsatellite, two of which we are interpreting as homozygous (5AC/5AC and 6AC/6AC) and two heterozygous (5AC/4AC and 6AC/5AC). One group of isolates (e.g., JL108, JL160, JL161) had double peaks on position 103 (A/C) and on the position

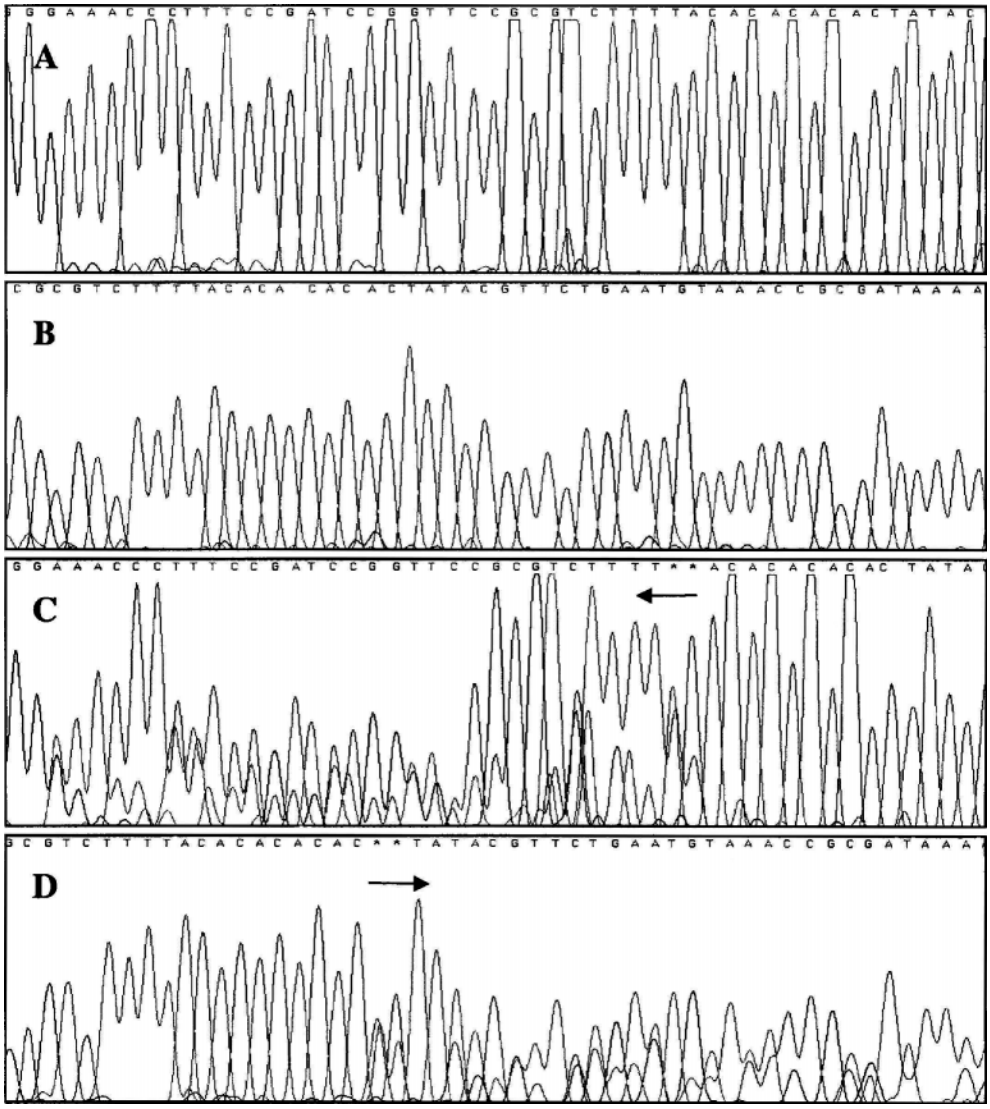

FIG. 4. Electropherograms obtained from DNA using two primers, ITS1 and ITS2. A. Sequence from culture JL136(SCO) that is presumably homozygous for five ''AC'' repeats (molecular marker ''132'' in TABLE IV) in the ITS1, using the ITS2 primer. B. Sequence from culture JL136 (SCO) using the ITS5 primer. C. Sequence from culture JL173 (SCO) that is presumably heterozygous for the number of ''AC'' repeats in the ITS1, using the ITS2 primer. D. Sequence from culture JL173 (SCO) using the ITS5 primer. Asterisks (* *) show the positions where double peaks indicate the overlap of templates with different numbers of ''AC'' repeats. The arrows show the downstream sequence direction.

region 133 to 138 (5AC/4AC). However given the lack of variation on position 146 (T/T) we could not fully resolve the DNA haplotypes.

Altogether our investigations identified three molecular markers from ITS1 at the 103, 132 and 146 positions. The inferred pattern of arrangement of the various haplotypes in each of the sequences is provided (TABLE IV). Patterns observed for these markers generally match those obtained in the mycelial compatibility tests (TABLE III). It is interesting, however, that certain isolates (the North American isolates JL106, JL213 and JL236 and the European isolates JL136 and JL167) share one ITS haplotype (Haplotype 1) but belong to different compatibility groups. However RAPD markers for these isolates are also clearly different, indicating that they are not clonally derived.

DISCUSSION

It is becoming clear from recent molecular studies that fungi assumed to be exclusively clonal actually are capable of recombination in nature (Taylor et al 2000), and this appears to be the case with *Marchandiomyces corallinus* as well. The presence of larger-than-expected ITS and RAPD variation in isolates of

TABLE III. Molecular markers at the 103, 132 and 146 ITS1 positions, mycelial compatibility group (MC), and RAPD band patterns for each isolate

Fungal isolates	ITS1 Positions			MC	RAPD patterns
	"103"	"132"	"146"		
JL106 MD	A/G	5AC/5AC	T/C	MC1	AAAAA
JL213 ME	A/G	5AC/5AC	T/C	MC2	ABBBA
JL236 ME	A/G	5AC/5AC	T/C	MC2	ABBCA
JL108 VA	A/C	5AC/4AC	T/T	MC3	A′CC?A
JL160 MO	A/C	5AC/4AC	T/T	MC3	A′CBB′A
JL161 AR	A/C	5AC/4AC	T/T	MC3	A′CBB′A
JL167 IRE	A/A	5AC/5AC	T/T	MC4	BDDEB
JL136 SCO	A/A	5AC/5AC	T/T	MC5	BDDFB
JL171 SCO	A/A	6AC/5AC	T/T	MC6	BD′DGC
JL172 SCO	A/A	6AC/5AC	T/T	MC6	BD′DEC′
JL173 SCO	A/A	6AC/5AC	T/T	MC6	BD′D?C′
JL222 FRA	A/A	6AC/6AC	T/T	MC7	BFD?F

M. corallinus and the presence of more than one distinct haplotype in some of these isolates suggests that genetic recombination (or less likely hybridization) is at least possible in this fungus and that geographic differentiation has taken place. Even isolates obtained from the same habitat have different RAPD patterns, indicating that many populations of this fungus are made up of more than one genet and that few are derived clonally. The only isolates with identical RAPD patterns were from specimens collected

TABLE IV. Inferred ITS haplotype combinations for each of the fungal isolates used in the study. Five distinct haplotypes (1–5) were inferred from the 12 isolates. Haplotype sequences from cultures JL108 VA, JL160 MO, and JL161 AR could not be fully resolved. Molecular markers at three ITS1 positions, as in Table III are shown in **bold type**. Complete haplotype 1 ITS sequences (ITS1, ITS2, and 5.8SS) for isolates JL106 MD and JL136 SCO were previously deposited as GenBank AY583324 and AY583325, respectively

Fungal isolates	Inferred ITS haplotype combinations	Haplotypes
	"103" "132" "146"	
JL106 MD	**A**CCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACAC**--TA**T**	1
	GCCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACAC**--TA**C**	2
JL213 ME	**A**CCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACAC**--TA**T**	1
	GCCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACAC**--TA**C**	2
JL236 ME	**A**CCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACAC**--TA**T**	1
	GCCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACAC**--TA**C**	2
JL108 VA	**M**CC--TTCSGATYTGGTCCYACGTSTTTA**TCACACACAC**--TA**T**	3
	MCC--TTCSGATYTGGTCCYACGTSTTTA**TCACACAC**----TA**T**	4
JL160 MO	**M**CC--TTCSGATYTGGTCCYACGTSTTTA**TCACACACAC**--TA**T**	3
	MCC--TTCSGATYTGGTCCYACGTSTTTA**TCACACAC**----TA**T**	4
JL161 AR	**M**CC--TTCSGATYTGGTCCYACGTSTTTA**TCACACACAC**--TA**T**	3
	MCC--TTCSGATYTGGTCCYACGTSTTTA**TCACACAC**----TA**T**	4
JL167 IRE	**A**CCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACAC**--TA**T**	1
	ACCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACAC**--TA**T**	1
JL136 SCO	**A**CCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACAC**--TA**T**	1
	ACCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACAC**--TA**T**	1
JL171 SCO	**A**CCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACAC**--TA**T**	1
	ACCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACACAC**TA**T**	5
JL172 SCO	**A**CCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACAC**--TA**T**	1
	ACCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACACAC**TA**T**	5
JL173 SCO	**A**CCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACAC**--TA**T**	1
	ACCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACACAC**TA**T**	5
JL222 FRA	**A**CCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACACAC**TA**T**	5
	ACCCTTTCCGATCCGGTTCCGCGTCTTTT**ACACACACACAC**TA**T**	5

in Missouri and Arkansas, indicating in this case the existence of a single large, geographically widespread clone. No sexual state has been described for *M. corallinus,* but the finding that it is heterokaryotic indicates that mating has taken place in the past, an unexpected result given the absence of clamps in isolated mycelia. Because most isolates are distinct genetically and probably not clonally derived, we would expect that there are a number of different mating types in the species.

Mycelial compatibility groups in *M. corallinus* exhibited some structure assignable to geography, with distinctly North American and European groups and no compatibility between the two continents. This has been observed before for fungal plant pathogens. For example a recent gene genealogical study (O'Donnell et al 2000) of *Fusarium graminearum,* a widespread virulent plant pathogen that causes *Fusarium* head blight of wheat and barley, demonstrated that this species is not panmictic but comprises seven phylogenetically distinct groups with limited gene flow among them. The origin of this structure appeared to be mainly geographic separation, but host-mediated isolation also might have played a role.

In the case of *M. corallinus,* there is no indication of a significant genetic separation caused by host switching, as is sometimes observed in fungal plant pathogens (e.g., Brem and Leuchtmann 2003, Harvey et al 2001). In our study, isolates from different lichen hosts in the same habitat were always identical in ITS sequence and intercompatible. However they were never identical in RAPD pattern, suggesting that different genotypes might exhibit minor differences in host preference. A more detailed test of this will compare within- and between-host genetic variation in the same compatibility groups using RAPD markers. Given the intercompatibility of host groups, we expect within- and between-group genetic differences to be no different.

Recent genetic studies of other widely distributed fungal species demonstrate that genetic differentiation may take place without either host switching or geographic separation. For example, a study of cryptic species in *Stachybotrys chartarum* (Cruse et al 2002), which has been implicated as a possible cause of sick-building syndrome, indicated little differentiation caused by geographic separation. Steenkamp et al (2002) similarly found evidence for genetic differentiation, even speciation, in various groups within *Fusarium subglutinans,* but the cause of this differentiation could not be attributed to either host-switching or geographic distance.

It has been suggested that mycelial incompatibility maintains the genetic identity of genotypes, although genetic exchange between certain genotypes appears to be permitted. In the case of *M. corallinus,* even this small sample of isolates represents many different compatibility groups and some are known only from a single location at this point. A marked exception is the group formed by isolates from VA, AR and MO, two of which (those from MO and AR) have identical RAPD banding patterns. This particular compatibility group is the most widely distributed one that we studied and might represent a single clone (MO and AR are apparently identical) that has undergone subsequent genetic differentiation in parts of its range.

Isolates from different lichen hosts in the same habitat were always intercompatible and exhibited the same ITS haplotypes, but the RAPD banding patterns were different. Population genetic studies of other bulbilliferous or sclerotial basidiomycetes generally show similar results. For example Punja and Sun (2001) studied mycelial compatibility groups of the widespread soilborne sclerotial basidiomycete *Sclerotium rolfsii* (teleomorph *Athelia rolfsii*) that causes diseases on a wide range of plant species. Isolates were generally unique, single-member compatibility groups structured geographically, but no clear relationship was found between compatibility and host plant of origin. In addition RAPD patterns were usually distinct for each isolate, even those from the same compatibility group, indicating few were clonally derived. High levels of RAPD polymorphism also are observed commonly both within and among compatibility groups in the sclerotial basidiomycete *Rhizoctonia solani* (Duncan et al 1993, Bounou et al 1999).

Genetic polymorphism within compatibility groups of asexual basidiomycetes can be detected using markers other than RAPD markers. For example isolates from the same compatibility group can have different ITS sequences in *Sclerotium rolfsii* (Harlton et al 1995), *Armillaria* spp. (Guillaumin et al 1996) and the *Rhizoctonia solani* species complex (Boysen et al 1996). In *R. solani* genetic differences among and within compatibility groups also are detectable using PCR amplification of SSU nrDNA (Liu et al 1995), RFLPs (Vilgalys 1988, Jabaji-Hare et al 1990, Vilgalys and Gonzales 1990) and isozymes (Laroche et al 1992), suggesting the existence of far greater genetic polymorphism in these groups than we observed in groups of *M. corallinus.*

It was not possible to determine reliably the phylogenetic structure within *M. corallinus* using the limited number of genetic markers we were able to identify in this study. North American isolates were distinctly different from European isolates in the ITS sequence position 103, with all North American isolates being A/G or A/C at this position and all Eu-

ropean isolates A/A. In addition North American and European isolates always were incompatible. However, at the ITS positions 132 and 146, no obvious continental geographic patterns can be seen and there were only minor within-continent differences among either North American or European isolates. We reasoned that the RAPD data could not be used to infer phylogenetic relationships inasmuch as they may represent various combinations of heterokaryotic banding patterns. There may be many of these and no clear indication that they group isolates by location or host type. Because these patterns might have arisen as a consequence of mating events regulated by as yet unknown mating compatibility relationships, we hesitated to make much of them. It is interesting that many of the ITS and RAPD markers found in *M. corallinus* also are found in its close relatives, *M. aurantiacus,* which like *M. corallinus* is lichenicolous, and *M. lignicola,* which is lignicolous. The latter species is also undoubtedly heterokaryotic (it has obvious clamps), but it has not been collected from enough localities to know much about its range (it has not yet been collected in Europe for example). It might be possible in the future to identify enough genetic markers in these species to use in a phylogenetic analysis that addresses both the origin of the nutritionally distinct species and the subsequent divergence of the geographically widespread *M. corallinus*.

As Hawksworth and Rossman (1997) have noted, lichenicolous fungi probably represent an important source of new fungal species, especially because they only recently have been collected extensively outside Europe. Because nearly 95% of described lichenicolous fungi are narrowly host specific the rare but ubiquitous host-generalized species are exceptional and interesting for this reason alone. In addition, however, they might represent heterogeneous assemblages of cryptic species, as has been suggested (Lawrey and Diederich 2003). In the case of *M. corallinus,* among the most geographically widespread of lichenicolous fungi, cryptic speciation probably has not taken place. However there appears to have been some genetic differentiation within this species, generated by geographic, not host, separation.

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