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ALGAL SWITCHING AMONG LICHEN SYMBIOSES[1]

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Lichens are intimate and long-term symbioses of algae and fungi. Such intimate associations are often hypothesized to have undergone long periods of symbiotic interdependence and coevolution. However, coevolution has not been rigorously tested for lichen associations. In the present study we compared the nuclear internal transcribed spacer (ITS) phylogenies of algal and fungal partners from 33 natural lichen associations to test two aspects of coevolution, cospeciation and parallel cladogenesis. Since statistically significant incongruence between symbiont phylogenies rejected parallel cladogenesis and minimized cospeciation events, we conclude that switching of highly selected algal genotypes occurs repeatedly among these symbiotic lichen associations.

Key words: *Cladonia*; cospeciation; host switching; ITS rDNA; lichens; parallel cladogenesis; phylogeny; symbiosis.

Lichens are intimate and long-term symbioses of photosynthetic algae or cyanobacteria and heterotrophic fungi. As intimate symbioses where the photosynthetic partner is inhabiting its heterotrophic partner (Law and Lewis, 1983; however, see Ahmadjian, 1993, p. 5), lichen symbionts are often hypothesized to have undergone long-term coevolution, especially when one or both symbionts appear obligate and specialized (Ahmadjian, 1987). However, coevolution has not been rigorously tested for lichen associations. To demonstrate coevolution directly requires an assessment of increased fitness resulting from reciprocal genetic change (Thompson, 1994), although coevolution could be demonstrated indirectly by showing parallel cladogenesis or cospeciation between symbiont lineages (Page and Hafner, 1996). A hypothesis of parallel cladogenesis would be accepted with highly specific associations between algal and fungal partners, especially if there is strict vertical transfer of inhabiting algal partners throughout a fungal lineage. In contrast, this hypothesis would be rejected in the case of horizontal transfer of algal partners among fungal lineages. This phylogenetic process is here called algal switching (and is distinct from the ''cyanobiont/phycobiont switches'' by an individual lichen-forming fungus of Hawksworth, 1988). When reciprocal evolution leads to cospeciation, coordinated speciation events, equal numbers of species among symbiont partners should evolve—a situation not predicted from morphological studies of these algae (Tschermak-Woess, 1988) and their fungal partners (Hawksworth et al., 1995).

Cospeciation has been predicted for many symbiotic associations: lice with birds (Page et al., 1998; see also, Clayton, Price, and Page, 1996) or pocket gophers (Demastes and Hafner, 1993; Hafner and Page, 1994) and wasps in figs (Herre et al., 1996); chemoautotrophic bacteria in bivalves (Distell, Felbeck, and Cavanaugh, 1994; Peek et al., 1998), bacteria in aphids (Clark et al., 2000) and rhizobia in root nodules (Jeong, Ritchie, and Myrold, 1999); arenaviruses in rodents (Bowen, Peters, and Nichol, 1997); and fungi in plants (Schardl et al., 1997) or with attine ants (Chapela et al., 1994; Hinkle et al., 1994; Mueller, Rehner, and Schultz, 1998) and bark beetles (Six and Paine, 1999). However, cospeciation is often obscured by host switching, resource tracking (Kethley and Johnston, 1975), geographically patchy evolution (Thompson, 1999), taxon sampling (Page, 1993), and invalid assumptions in evolutionary models (Clark et al., 2000). More recently, host switching has been proposed in a number of symbiotic associations, even those where cospeciation was previously reported or partial cospeciation confirmed, as with endosymbiotic bacteria and aphids (Clark et al., 2000) or clams (Peek et al., 1998), respectively.

Among lichen associations, stealing of algal genotypes is known to occur in some lichen-forming fungi that are transiently parasitic on other lichen associations. For example, parasitic *Diploschistes muscorum* (Friedl, 1987) first associates with the algal partner *Trebouxia irregularis* of its lichen host *Cladonia* and later its preferred partner *Trebouxia showmanii.* A recent study of overall algal partners in lichen communities has shown that algal morphospecies and genotypes are shared and, therefore, presumably switched among different species, genera, and families of lichen-forming fungi (Beck, Friedl, and Rambold, 1998; see also, Ahmadjian, 1987, and Rambold, Friedl, and Beck, 1998). However, algal switching and cospeciation among lichen symbionts have not been tested with comparative phylogenetic methods and depend, at least in part, on traditional taxonomic concepts. We present rigorous phylogenetic tests of both relationships within and among species of each of the lichen partners and of the hypotheses of parallel cladogenesis and cospeciation between them. In the present study our goals were: (1) to examine algae in natural lichen associations with worldwide representatives of the diverse fungal family Cladoniaceae for cospeciation, parallel cladogenesis, and algal switching and (2) to identify sets of taxa for further study of lichen coevolution.

MATERIALS AND METHODS

Collections—We used axenic cultures of *Trebouxia* and podetia (lichenized stipes of generative tissue) or squamules (lichenized scale-like tissue) from either freshly collected or herbarium material of natural lichens (Table 1) for extracting total DNA. *Trebouxia* cultures are available through UTEX, and voucher specimens are deposited in the U.S. National

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Museum of Natural History (US), the Finnish Museum of Natural History, Helsinki (H), New York Botanical Garden (NY), and Duke University (DUKE).

DNA isolation and PCR amplification of nuclear internal transcribed spacers (ITS) from fungal and algal partners—Total DNA was extracted from either cultures or lichenized tissues using a CTAB (cetyltrimethylammonium bromide) extraction buffer and a protocol modified from Grube et al. (1995) and Ivanova et al. (1999). DNA, suspended in dH_2O, was amplified by the polymerase chain reaction (PCR). Initial amplification of the ITS1, ITS2, and 5.8S regions was from universal primers annealing in the nuclear small subunit (SSU) ribosomal DNA (rDNA), nu-SSU-1583-5′ (CAACGAGGAATTCCTAGT; SR18R in DePriest, 1993) and in the nuclear large subunit (LSU) rDNA, ITS4–3′ (TCCTC CGCTTATTGATATGC; White et al., 1990). Subsequent amplifications were from newly designed primers that discriminated between algal and fungal sequences. Algal specific primer nr-SSU-1780-5′Algal spanned the 3'SSU rDNA and ITS1 junction, (5′-CTGCGGAAGGATCATTGATTC-3′) and nr-LSU-0012-3′ Algal, a variable region of the LSU rDNA, (5′-AGTTCAGCGGGTGGTCTTG-3′). Fungal specific primers were at the same locations, nr-SSU-1780-5′ Fungal (5′-CTGCGGAAGGATCATTAATGAG-3′), and nr-LSU-0012-3′ Fungal (5′-AGTTCAGCGGGTATCCCT-3′), respectively. Amplifications were performed in 100 μL reactions (10 mmol/L Tris-HCl pH 8.8, 1.5 mmol/L MgC_{l2}, 50 mmol/L KCl, 0.1% Triton X-100), with 1.25 units Klentaq 1 (AB Peptides, St. Louis, Missouri, USA), 200 μmol/L of each dNTP, 0.5 μmol/L of primers, and between 1.0 and 10 ng of DNA. Amplification conditions in Perkin Elmer (Foster City, California, USA) Cetus 480 or 9700 thermal cyclers were: template denaturing at 94°C for 60 s, primer annealing at 50°C for 60 s, and primer extension at 72°C for 2 min (extended by 5 s per cycle) for 30 cycles. PCR products were cleaned with Wizard PCR Preps (Promega, Madison, Wisconsin, USA) and quantified on 1% agarose gel stained with ethidium bromide.

DNA sequencing and sequence alignment—Double-stranded products were sequenced using the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Applied Bio-Systems, Foster City, California, USA), following the manufacturer's instructions, in a Perkin Elmer Cetus 9700 thermal cycler with detection on a 373 Automatic Sequencer (Applied Bio-Systems) from primers described above as well as ITS3–5′ and ITS2–3′ (White et al., 1990). Excess dyedeoxy terminators were removed by filtration through Sephadex G-50 Fine (Pharmacia). Sequences were assembled into full-length sequences using Sequence Navigator 1.0 (Applied Bio-Systems); both strands were sequenced from multiple primers. Sequences representing the SSU rDNA, SSU rDNA insertions or introns, and LSU were removed and only the ITS sequences used for the analysis. ITS sequences from 73 algal partners or *Trebouxia* cultures and from 33 fungal partners were aligned manually in PAUP 4.0d65 (Swofford, 1998).

Phylogenetic analyses—Aligned sequences from each of the algal and fungal data sets were subjected to two methods of phylogenetic analysis, maximum likelihood and maximum parsimony, using PAUP 4.0d65. All nucleotide positions of ITS1 and ITS2 were included in the analyses and alignment gaps were treated as missing data; nucleotide positions representing the 5.8S were excluded from the analyses. Maximum parsimony was performed using the options tree bisection and reconnection (TBR) branch swapping, collapse zero length branches, and acctran character-state optimization. Heuristic searches were conducted using 100 random addition replicates with a limit of 1000 trees per search and bootstrap searches of 500 resamplings (Felsenstein, 1985). All trees were unrooted. Maximum likelihood analysis used HKY85 model (Hasagawa, Kishino, and Yano, 1985) with two substitution types and an estimated ti/tv (transition/transversion) ratio. Maximum likelihood (ML) trees were found by random addition of taxa in 100 replications. The fungal trees were obtained using the same search parameters except there was no limit applied to the number of trees saved per search. Because it is more likely that two trees will be congruent if one or both contain little phylogenetic signal, the skew (g1) in the distributions of tree length was measured using the ''evaluate random trees'' option in PAUP 4.0 and compared with critical values in Hillis and Huelsenbeck (1992).

Incongruence tests were performed for tree topologies and subsets of the data. Tree topologies were compared using the Kashino-Hasagawa and Templeton tests performed in PAUP (phylogenetic analysis using parsimony) using parsimony treescore options (Kashino-Hasagawa and nonparametric tests, respectively), and Rodrigo's second test (Rodrigo et al., 1993) using the filter tree option. The partition homogeneity test was also implemented in PAUP and was used for the Incongruence Length Difference (ILD) test. Cospeciation events in algal and fungal phylogenies were estimated using TreeMap 1.0 (Page, 1995). The algal topology was mapped onto the fungal topology using an exact search to find all the best reconstructions. The algal and fungal topologies were taken from the fully resolved maximum likelihood trees. The topologies each contained 22 taxa because some of the algal taxa that were identical had to be excluded to produce fully dichotomized topologies. To test whether reconstructed cospeciation events could be due to chance alone, 1000 random parasite (algal) trees were generated using the Markovian model (Harding, 1971) and the maximum number of cospeciation events was estimated between each pair of random host (fungal) and parasite (algal) trees. A $P > 0.05$ does not reject the null hypothesis that the cospeciation events were due to chance alone.

RESULTS AND DISCUSSION

In the course of producing sequences of nuclear rDNA ITS for a phylogenetic analysis of the lichen-forming fungi in the family Cladoniaceae, we took the opportunity to produce sequences for many of their algal partners. By designing specific primers we selectively amplified algal ITS sequences from 73 samples, including 14 cultures from previous morphological studies (Table 1). Of the remaining samples, 57 were associated with Cladoniaceae fungi and two with non-Cladoniaceae fungi including *Anzina carnionivea* from which the algal partner *Asterochloris phycobiontica* was described (Table 1). Rambold, Friedl, and Beck (1998) recently proposed that Cladoniaceae symbionts may represent the genus *Asterochloris* sensu Friedl.

Algal sequences from the 57 natural lichen associations were highly similar, even though they were from dispersed geographic locations and with different representatives of Cladoniaceae. Pairwise ITS sequence similarities among algal symbionts of the natural lichen associations were >95%. Pairwise ITS sequence similarities among the symbionts from natural lichen associations and 11 of the cultures were >93%; similarity among the 11 putative ''Asterochloris'' cultures was >97%. They were almost identical to the ITS sequence reported earlier for lichen symbiont *Trebouxia erici* (DePriest, 1992). However, ITS sequences from three remaining cultures (*T. impressa*, UTEX culture 893 GenBank accession # AF345890, UTEX culture 892 accession # AF345891; and *T. asymmetrica*, UTEX culture 2507 accession # AF345889) and some other available sequences from lichen-forming algae (*T. impressa*, accession number AJ007388; *T. jamesii*, Z68700 and Z68699; and *T. arboricola* Z68703) could not be aligned to the ''Asterochloris'' sequences. Therefore, the ''Asterochloris'' algal symbionts are apparently distinct from those of *Trebouxia* sensu stricto as proposed by Rambold, Friedl, and Beck (1998).

When sequences were compared among paired algal and fungal partners from 33 Cladoniaceae associations, there were 24 distinct algal genotypes, and similarities among ge-

TABLE 1. List of fungal taxa and collections or cultures used for sequencing of algal and fungal[a] nuclear rDNA ITS, with GenBank accession numbers.

Fungal taxa	Collection or culture	GenBank accession no.[b] for algal symbiont
Cladina stellaris (Opiz) Brodo (MN-102)	Finland, Tavastia austr., 1997, Haikonen 18505 (H)	GBAN-AF345410
Cladina stellaris (Opiz) Brodo (2904)	USA, NC, 1998, DePriest 2904 (US)	GBAN-AF345391
Cladina arbuscula (Wallr.) Hale (1334)	USA, NC, 1998, DePriest 1334 (US)	GBAN-AF345384
Cladina arbuscula (Wallr.) Hale (1264)	USA, NC, 1998, DePriest 1264 (US)	GBAN-AF345390
Cladonia atlantica Evans (MN-173)	USA, MA, 1993, Hammer 5735 (US)	GBAN-AF345392
Cladonia atlantica Evans (MN-174)	USA, AL, 1992, Harris 28528 (US)	GBAN-AF345393
Cladonia bellidiflora (Ach.) Schaer. (MN-097)	Norway, 1998, Shaw 9689b (DUKE)	GBAN-AF345413
Cladonia botrytes (Hag.) Willd. (MN-137)	Canada, NS, 1999, Normore 257. (US)	GBAN-AF345377
Cladonia capitellata (Hook. F. & Taylor) C. Bab. (7212)	Australia, 1998, Hammer 7212 (US)	GBAN-AF345421
Cladonia caroliniana s.str. (Schwein.) Tuck. (MN-094)	USA, AL, 1998, Normore 178 (US)	GBAN-AF345395
Cladonia cenotea (Ach.) Schaer. (MN-106)	Russia, 1998, Oksanen 502 (H)	GBAN-AF345408
Cladonia cervicornis subsp. *verticillata* (Hoffm.) Schaer. (MN-104)	USA, NC, 1998, DePriest 1219 (US)	GBAN-AF345394
Cladonia chlorophaea (Flörke ex Sommerf.) Spreng. (MN-161)	Canada, NS, 1999, Normore 342. (US)	GBAN-AF345431
Cladonia crispata (Ach.) Flot. (MN-103)	Canada, NF, 1998, Normore 222 (US)	GBAN-AF345378
Cladonia coccifera (L.) Willd. (MN-101)	Russia, Karelia, 1997, Oksanen 191 (H)	GBAN-AF345409
Cladonia cristatella Tuck.	*Trebouxia erici* Ahmadjian UTEX 910, MA, USA (UTEX Culture)	GBAN-AF345439
Cladonia cristatella Tuck.	*Trebouxia erici* Ahmadjian UTEX 911, MA, USA (UTEX Culture)	GBAN-AF345440
Cladonia cristatella Tuck.	*Trebouxia erici* Ahmadjian UTEX 912, MA, USA (UTEX Culture)	GBAN-AF345441
Cladonia cristatella Tuck. (MN-057)	USA, AL, 1998, Normore 173 (US)	GBAN-AF345425
Cladonia didyma (Fée) Vain. (MN-082)	USA, AL, 1998, Normore 150 (US)	GBAN-AF345415
Cladonia farinacea (Vain.) Evans (IO-38)	USA, NY, 1997 Harris 40872 (NY)	GBAN-AF345432
Cladonia fimbriata (L.) Fr. (MN-107)	Sweden, Gothenburg, 1999, Gustavsson & Tholleson s.n. (US)	GBAN-AF345434
Cladonia floridana Vain. (MN-171)	USA; MA, 1994, Hammer 5807, Exsicc. No. 1196.1924. (US)	GBAN-AF345396
Cladonia furcata (Huds.) Schrad. (IO-24)	Russia, Karelian Republic, 1998 Oksanen 505 (H+US)	GBAN-AF345429
Cladonia glauca Flörke (MN-188)	Denmark, 1996, Hansen and Christensen, Exciss. No. 94. (US)	GBAN-AF345420
Cladonia gracilis subsp. *gracilis* (L.) Willd. (IO-50)	Russia, 1997, Oksanen 187 (H)	GBAN-AF345414
Cladonia grayi Merr. (MN-156)	Canada, NS, 1999, Normore 246. (US)	GBAN-AF345376
Cladonia grayi Merr. (MN-158)	Canada, NS, 1999, Normore 280. (US)	GBAN-AF345397
Cladonia grayi Merr. (MN-159)	Canada, NS, 1999, Normore 300. (US)	GBAN-AF345379
Cladonia grayi Merr. (MN-163)	Canada, NS, 1999, Normore 366. (US)	GBAN-AF345380
Cladonia grayi Merr. (MN-165)	Canada, NS, 1999, Normore 380. (US)	GBAN-AF345385
Cladonia ochrochlora Flk. (7103)	Australia, 1998, Hammer 7103. (US)	GBAN-AF345438
Cladonia parasitica (Hoffm.) Hoffm. (MN-191)	Canada, NS, 1999, Normore 289. (US)	GBAN-AF345426
Cladonia peltastica (Nyl.) Muell. Arg. (MN-070)	Guyana, 1996, DePriest 10056 (US)	GBAN-AF345416
Cladonia pleurota (Flk.) Schaer. (MN-105)	USA, NC, 1998, DePriest 1338 (US)	GBAN-AF345398
Cladonia pulviniformis Ahti (MN-068)	Guyana, 1996, DePriest 10014 (US)	GBAN-AF345443
Cladonia pyxidata (L.) Hoffm. (MN-063)	Italy, Prov. Biella, 1998, Isocrono 1 (US)	GBAN-AF345436
Cladonia rangiformis Hoffm. (IO-3)	Jugoslavia, 1983, Frost-Olsen 5501 (US)	GBAN-AF345435
Cladonia rappii Evans (MN-098)	USA, NC, 1998, Normore 136 (US)	GBAN-AF345417
Cladonia robbinsii Evans (KK-145)	USA, NC, 1998, Karkkainen 314 (H)	GBAN-AF345386
Cladonia scabriuscula (Del. In Duby) Nyl. (7090)	Australia, 1998, Hammer 7090. (US)	GBAN-AF345424
Cladonia sp. (MN-155)	Canada, NS, 1999, Normore 241. (US)	GBAN-AF345403
Cladonia sp.	*Trebouxia magna* Archibald UTEX 67 (UTEX Culture)	GBAN-AF345423
Cladonia spinea Ahti (MN-069)	Guyana, 1996, DePriest 10016 (US)	GBAN-AF345418
Cladonia squamosa (Scop.) Hoffm. (MN-193)	Canada, NS, 1999, Normore 276. (US)	GBAN-AF345381
Cladonia squamosa (Scop.) Hoffm.	*Trebouxia pyriformis* Archibald UTEX 1712, MA, USA (UTEX Culture)	GBAN-AF345406
Cladonia staufferi Abbayes Bot. Jahrb. (7051)	Australia, 1998, Hammer 7051 (US)	GBAN-AF345422
Cladonia strepsilis (Ach.) Vain. (MN-005)	Sweden, 1976, Hale *s.n.* (US)	GBAN-AF345383
Cladonia strepsilis (Ach.) Vain. (MN-007)	Japan, 1979, Kashiwadani 14972 (US)	GBAN-AF345399
Cladonia strepsilis (Ach.) Vain. (MN-096)	USA, NC, 1992, DePriest 3600 (US)	GBAN-AF345387
Cladonia strepsilis (Ach.) Vain. (MN-150a)	Canada, NS, 1999, Normore 372—podetium. (US)	GBAN-AF345388
Cladonia strepsilis (Ach.) Vain. (MN-150b)	Canada, NS, 1999, Normore 372—squamules. (US)	GBAN-AF345400
Cladonia strepsilis (Ach.) Vain. (MN-151a)	Canada, NS, 1999, Normore 308—podetium. (US)	GBAN-AF345401
Cladonia strepsilis (Ach.) Vain. (MN-153)	Canada, NS, 1999, M. Maxfield. s.n.—squamules. (US)	GBAN-AF345402
Cladonia strepsilis (Ach.) Vain. (MN-154)	Canada, NS, 1999, M. Maxfield. s.n.—podetium. (US)	GBAN-AF345375

TABLE 1. Continued.

Fungal taxa	Collection or culture	GenBank accession no.[b] for algal symbiont
	Germany, 1994, Pittoni *s.n.* (US)	GBAN-AF345427
Cladonia subulata (L.) Wigg. (PD-2709)		
Cladonia symphycarpa (Ach.) Fr. (KK-321)	Finland, 1991, Pykala 9550 (H)	GBAN-AF345430
Cladonia turgida (Ehrh.) Hoffm. (IO-18)	Russia, 1997, Oksanen 186 (H)	GBAN-AF345428
Cladonia uncialis (L.) Wigg. (IO-49)	Findland, 1997, Haikonen 18571 (H)	GBAN-AF345412
Cladonia variegata Ahti (MN-075)	Guyana, 1996, DePriest 10015 (US)	GBAN-AF345419
Stereocaulon pileatum Ach.	*Trebouxia pyriformis* Archibald UTEX 1713, MA, USA (UTEX Culture)	GBAN-AF345407
Stereocaulon pileatum Ach.	*Trebouxia glomerata* (Waren) Ahmadjian UTEX 897, MA, USA (UTEX Culture)	GBAN-AF345405
Stereocaulon pileatum Ach.	*Trebouxia glomerata* (Waren) Ahmadjian UTEX 896, MA, USA (UTEX Culture)	GBAN-AF345404
Stereocaulon evolutoides	*Trebouxia glomerata* (Waren) Ahmadjian UTEX 895, MA, USA (UTEX Culture)	GBAN-AF345382
Stereocaulon dactylophyllum Flk.	*Trebouxia excentrica* Archibald UTEX 1714 (UTEX Culture)	GBAN-AF345433
Stereocaulon sp.	*Trebouxia irregularis* Hildr. and Ahmadjian UTEX 2236 (UTEX Culture)	GBAN-AF345411
Stereocaulon dactylophyllum Flk (MN-203)	Canada, NS, 1999, Normore 375 (US)	GBAN-AF345442
Anzina carninivea (Anzi) Scheidegger (MN-149)	Austria, 1981, Poelt and Schiedegger s.n. (US)	GBAN-AF345374
Cladia aggregata (7000)	Australia, 1998, Hammer 7000. (US)	GBAN-AF345437
Pycnothelia papillaria (Ehrh.) Duf. (PD-2903)	USA, 1999, DePriest 2903. (US)	GBAN-AF345389

[a] The fungal sequences will be described in another manuscript and submitted to GenBank at that time.
[b] The prefix has been added to link the online version of *American Journal of Botany* to GenBank but is not part of the actual accession number.

notypes were very high, 95–100%. Similarities among fungal genotypes were by comparison relatively low, 81–98%. Therefore, there is substantially less variation among the algal symbionts relative to that of their fungal partners. This difference could indicate a longer time of divergence or higher mutation rate in the fungi, strong fungal selection of algal genotypes, or population processes leading to genotypic fixation in the algae (Nuismer, Thompson, and Gomulkiewicz, 1999). Because random amplified polymorphic DNA (RAPD) analysis with 23 primers showed little polymorphism among ''Asterochloris'' cultures of algae with similar ITS sequences (data not shown), the lack of ITS variation in this study may reflect a low level of variation across the entire algal genome.

Maximum likelihood analysis of the 70 aligned sequences found a single most likely topology (Fig. 1), identical to one of the trees found in maximum parsimony (MP) analysis. Both the unrooted ML network and the MP consensus showed two clades separated by sequences from cultured *Trebouxia erici* and the algal symbiont of *Stereocaulon dactylophyllum.* One of the clades, Clade I (bootstrap support 100%) encompassed ''Asterochloris'' morphospecies *Trebouxia glomerata, T. irregularis,* and *T. pyriformis,* along with 33 natural lichen-forming algae. The lack of resolution within Clade I, the near identity of these ITS sequences (>98.5%), and the similarity of *T. glomerata* and *T. pyriformis* RAPD amplification patterns support the assertion that they may represent genotypes of a single species, *T. irregularis,* as previously reported by Friedl (1989). Preliminary data predict comparable sequence homogeneity among their mitochondrial large and small subunit rDNA (>99%). The Clade I genotypes associate with fungi of different genera, families, and even orders because four cultures were isolated from associations with *Stereocaulon* (Stereocaulaceae, Lecanorales) and two algal sequences were amplified from *Pycnothelia papillaria* (Cladoniaceae, Lecanorales) and *Anzina carnionivea* (Trapeliaceae, possibly Agyriales). This is in agreement with Hildreth and Ahmadjian's (1981) earlier report that a single species, or in this case even genotype, of alga may form associations with taxonomically unrelated lichens, even those differing in growth form (fruiticose *Cladonia* vs. crustose *Anzina*) and geographic location (Table 1).

For 33 of the natural lichen associations we compared the phylogenies of their algal and fungal partners and found no compelling evidence of parallel cladogenesis (Fig. 2). When the same regions, ITS1 and ITS2, were examined, the algal data set had 25 informative characters and the fungal data set 172 (Table 2), although both data sets contained significant phylogenetic signal based on the skew in the distributions of tree lengths ($g1 = -0.43$ for fungus, -0.34 for alga). Despite the low level of variation, both MP and ML analyses of sequences from 33 algal partners produced stable backbone topologies, if unresolved in their terminal nodes, that were in agreement with Fig. 1. We compared this algal data set and topologies to those of their fungal symbionts using incongruence and randomization tests (Table 3). Rodrigo et al.'s second test (1993) showed that there are no MP topologies in common between the two data sets, indicating that fungal topologies were not estimates of the algal phylogeny. The Kashino-Hasagawa (Kashino and Hasagawa, 1989) and Templeton's tests (Templeton, 1983) produced P values <0.05, indicating that topologies from the two data sets were significantly different. Since the P value for the partition homogeneity test (incongruence length difference, ILD; Farris et al., 1994) was <0.05, the characters in the algal and fungal data sets were incongruent. However, Clark et al. (2000) suggested that these tests may be confounded by different rates of evolution between symbiotic partners or even gene regions, as shown in our rejection of the combinability of even the linked ITS1 and 2 regions (Table 3). Using a randomization test implemented in

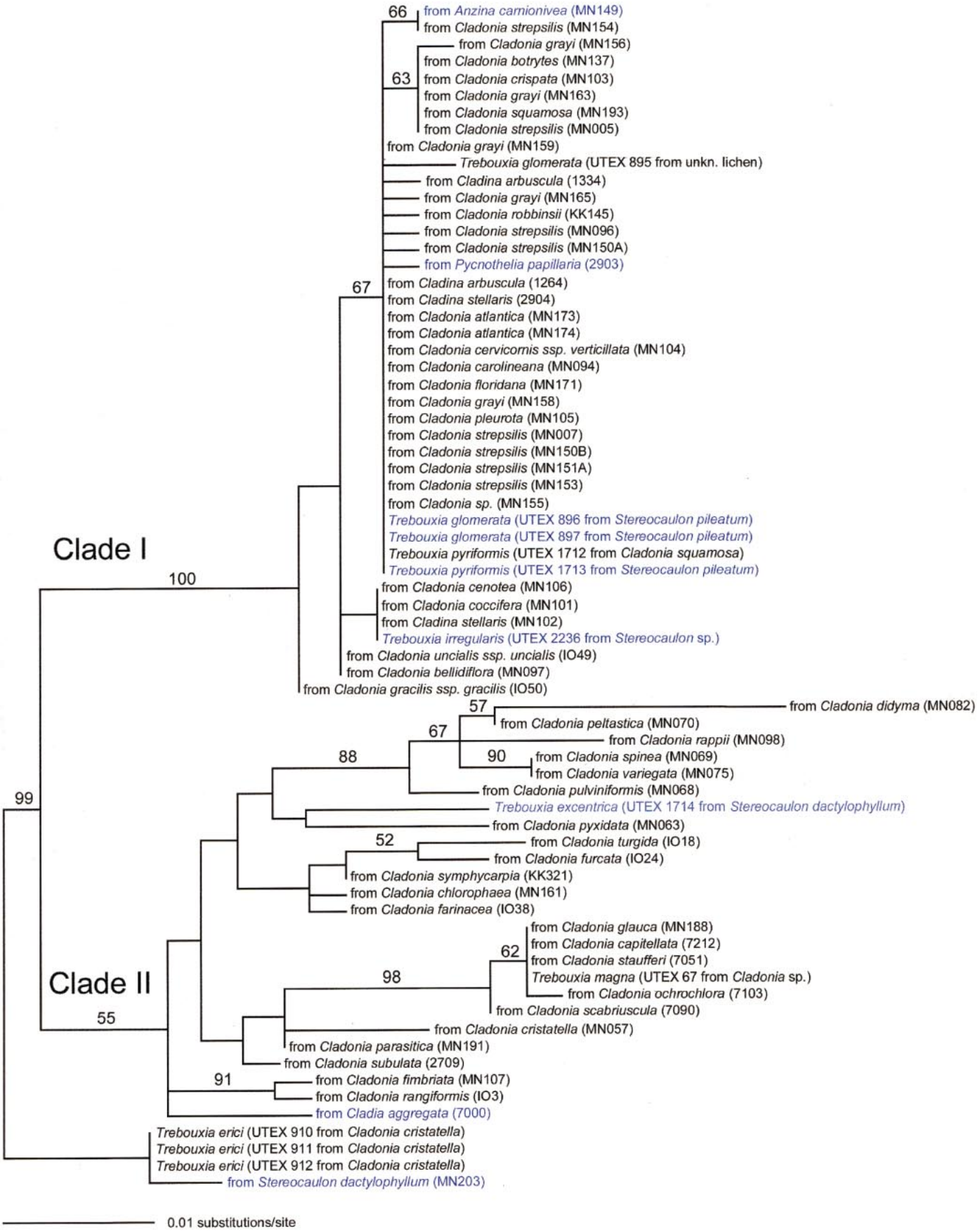
66
from Anzina carnionivea (MN149)
from Cladonia strepsilis (MN154)
from Cladonia grayi (MN156)
from Cladonia botrytes (MN137)
63
from Cladonia crispata (MN103)
from Cladonia grayi (MN163)
from Cladonia squamosa (MN193)
from Cladonia strepsilis (MN005)
from Cladonia grayi (MN159)
Trebouxia glomerata (UTEX 895 from unkn. lichen)
from Cladina arbuscula (1334)
from Cladonia grayi (MN165)
from Cladonia robbinsii (KK145)
from Cladonia strepsilis (MN096)
from Cladonia strepsilis (MN150A)
from Pycnothelia papillaria (2903)
67
from Cladina arbuscula (1264)
from Cladina stellaris (2904)
from Cladonia atlantica (MN173)
from Cladonia atlantica (MN174)
from Cladonia cervicornis ssp. verticillata (MN104)
from Cladonia carolineana (MN094)
from Cladonia floridana (MN171)
from Cladonia grayi (MN158)
from Cladonia pleurota (MN105)
from Cladonia strepsilis (MN007)
from Cladonia strepsilis (MN150B)
from Cladonia strepsilis (MN151A)
from Cladonia strepsilis (MN153)
from Cladonia sp. (MN155)
Trebouxia glomerata (UTEX 896 from Stereocaulon pileatum)
Trebouxia glomerata (UTEX 897 from Stereocaulon pileatum)
Trebouxia pyriformis (UTEX 1712 from Cladonia squamosa)
Trebouxia pyriformis (UTEX 1713 from Stereocaulon pileatum)
Clade I
100
from Cladonia cenotea (MN106)
from Cladonia coccifera (MN101)
from Cladina stellaris (MN102)
Trebouxia irregularis (UTEX 2236 from Stereocaulon sp.)
from Cladonia uncialis ssp. uncialis (IO49)
from Cladonia bellidiflora (MN097)
from Cladonia gracilis ssp. gracilis (IO50)
57
from Cladonia didyma (MN082)
from Cladonia peltastica (MN070)
67
from Cladonia rappii (MN098)
88
90
from Cladonia spinea (MN069)
from Cladonia variegata (MN075)
from Cladonia pulviniformis (MN068)
99
Trebouxia excentrica (UTEX 1714 from Stereocaulon dactylophyllum)
from Cladonia pyxidata (MN063)
52
from Cladonia turgida (IO18)
from Cladonia furcata (IO24)
from Cladonia symphycarpia (KK321)
from Cladonia chlorophaea (MN161)
from Cladonia farinacea (IO38)
from Cladonia glauca (MN188)
62
from Cladonia capitellata (7212)
from Cladonia staufferi (7051)
98
Trebouxia magna (UTEX 67 from Cladonia sp.)
from Cladonia ochrochlora (7103)
Clade II
from Cladonia scabriuscula (7090)
55
from Cladonia cristatella (MN057)
from Cladonia parasitica (MN191)
from Cladonia subulata (2709)
91
from Cladonia fimbriata (MN107)
from Cladonia rangiformis (IO3)
from Cladia aggregata (7000)
Trebouxia erici (UTEX 910 from Cladonia cristatella)
Trebouxia erici (UTEX 911 from Cladonia cristatella)
Trebouxia erici (UTEX 912 from Cladonia cristatella)
from Stereocaulon dactylophyllum (MN203)
0.01 substitutions/site

TABLE 2. Results from maximum parsimony analyses of rDNA ITS1 and ITS2 sequence data (excluding 5.8S) for full algal taxa set (as in Fig. 1) and for pruned algal and fungal taxa sets (as in Fig. 2).

	Fungal symbiont			Algal symbiont			
Phylogenetic data[a]	ITS1 and ITS2	ITS1 only	ITS2 only	Pruned taxa	Full taxa	ITS1 only full taxa	ITS2 only full taxa
No. taxa	33	33	33	33	70	70	70
Length of sequence (bp)	375–445	212–273	149–173	381–400	381–400	179–197	201–203
Length of alignment (bp)	583	371	212	403	403	197	206
No. variable characters	293	186	107	61	78	39	39
No. informative characters	172	120	52	25	40	19	21
No. most parsimonious trees	4	4	489	>20 000	>20 000	>20 000	2160
No. islands	4	4	32	40	40	96	65
Tree length (steps)	684	450	219	81	104	46	55
CI	0.617	0.607	0.662	0.852	0.856	0.957	0.818
HI	0.383	0.393	0.338	0.148	0.144	0.044	0.182
RI	0.679	0.684	0.717	0.939	0.964	0.989	0.956

[a] CI = consistency index, HI = homoplasy index, and RI = retention index.

TreeMap, the null hypothesis that ''the parasite [algal] phylogeny is independent of the host [fungal] phylogeny'' (Page, 1994) was not rejected since the *P* value was >0.05, which excluded the possibility of comparing rates of symbiont evolution (Huelsenbeck, Rannala, and Yang, 1997). The rejection of parallel cladogenesis in these five tests suggests that vertical transmission alone cannot account for the current symbiont associations. Therefore, horizontal transfer or algal switching must be ongoing even within the Cladoniaceae.

Since rejection of the cospeciation hypothesis for the entire data sets does not exclude the possibility that a subset of algae have cospeciated with their fungal partners, the algal topology was mapped onto the fungal topology in TreeMap. From an unresolved algal topology produced from 24 distinct genotypes, we constructed representative, fully dichotomous trees required for cospeciation analysis. When each of the trees was mapped onto a fully dichotomous fungal topology to optimize the number of cospeciation events, TreeMap found 9–94 optimal reconstructions each with 10–11 cospeciations. However, this number of cospeciation events required 7–9 duplications (independent speciations of the algae), 3–5 algal switches (horizontal transfers, dispersals, or host switches) and 65–81 sorting events (algal genotypes missing due to extinction or sampling error). Therefore, cospeciation in the more distant past has been largely obscured by these other evolutionary events.

The ongoing debate in lichenology over whether the lichen-forming algae live independently or are obligately associated with their fungal partners (citations in Honegger, 1998, and Ahmadjian, 1988, 1993, respectively) is not resolved by this evidence for algal switching. As noted by Honegger (1998), both the algal and fungal partners can be cultured axenically and, therefore, are not physiologically dependent on the symbiotic association, although they may be ecologically dependent. The 3–5 algal switching events required for our reconstructions are consistent with lichen-forming algae living independent of their fungal symbiont and with different fungal species selecting the same genotype from a ''pool of locally available algae'' (Beck, Friedl, and Rambold, 1998). Most Cladoniaceae fungi reproduce sexually, and their spores, discharged from the nurturing symbiosis, in theory must quickly recruit new algal partners from a local ''pool.'' However, even if algae are obligated to lichen associations, then algal switching could occur when fungi steal their algae from other intact associations. Friedl (1987) proposed that in some cases a secondary fungus such as *Diploschistes muscorum* may invade a lichen thallus and take its algal symbiont; such inter-Cladonian associations have been reported in a few situations (see Rambold and Triebel, 1992, p. 106). Robinson (1975) and Ott (1987) proposed that some of these fungi take algae from soredia, special packets of algae and fungi that are easily dispersed. Long-distance dispersal of Cladoniaceae soredia would present these algal genotypes for switching to other lichens. Our analysis cannot distinguish among methods of horizontal transfer that may occur among lichen associations (Friedl, 1987).

We provide statistical evidence to reject overall cospeciation and to support horizontal switching of algal genotypes among their fungal symbionts. Although parallel cladogenesis could not be supported across these paired data sets, cospeciation events may yet be detected in isolated clades of *Cladonia* fungi (Fig. 2). Some of the 10–11 putative cospeciation events mapped onto the algal phylogeny were common among different reconstructions. These cospeciation events were located within the clade containing *C. peltastica, C. spinea* and *C. pulviniformis*, all collected from the Guiana Shield of South America, as well as the clade containing *C. furcata, C. farinacea*, and *C. turgida* (Fig. 2). However, this result may be confounded by the geographic distribution of some algal genotypes.

←

Fig. 1. Phylogenetic relationships among closely related algae, as derived from maximum likelihood analysis of aligned ITS 1 and 2 sequences from 59 natural lichen associations and 11 cultures (morphospecies of *Trebouxia*). The most likely phylogram with a −Ln likelihood of 1263.3428, a kappa of 3.334387, and an estimated transition/transversion (ti/tv) ratio of 1.668521 is shown. This unrooted topology is one of the >20 000 topologies produced in maximum parsimony analysis. Bootstrap percentages from 100 maximum parsimony replications are shown on each branch with support >50%. Two major clades, Clade I and Clade II, supported in all topologies are labeled adjacent to their nodes. Each of the clades comprises multiple genotypes that may represent three or fewer species. Algae from associations with species of *Cladonia* and *Cladina* are shown in black, and those from associations with other fungi in blue.

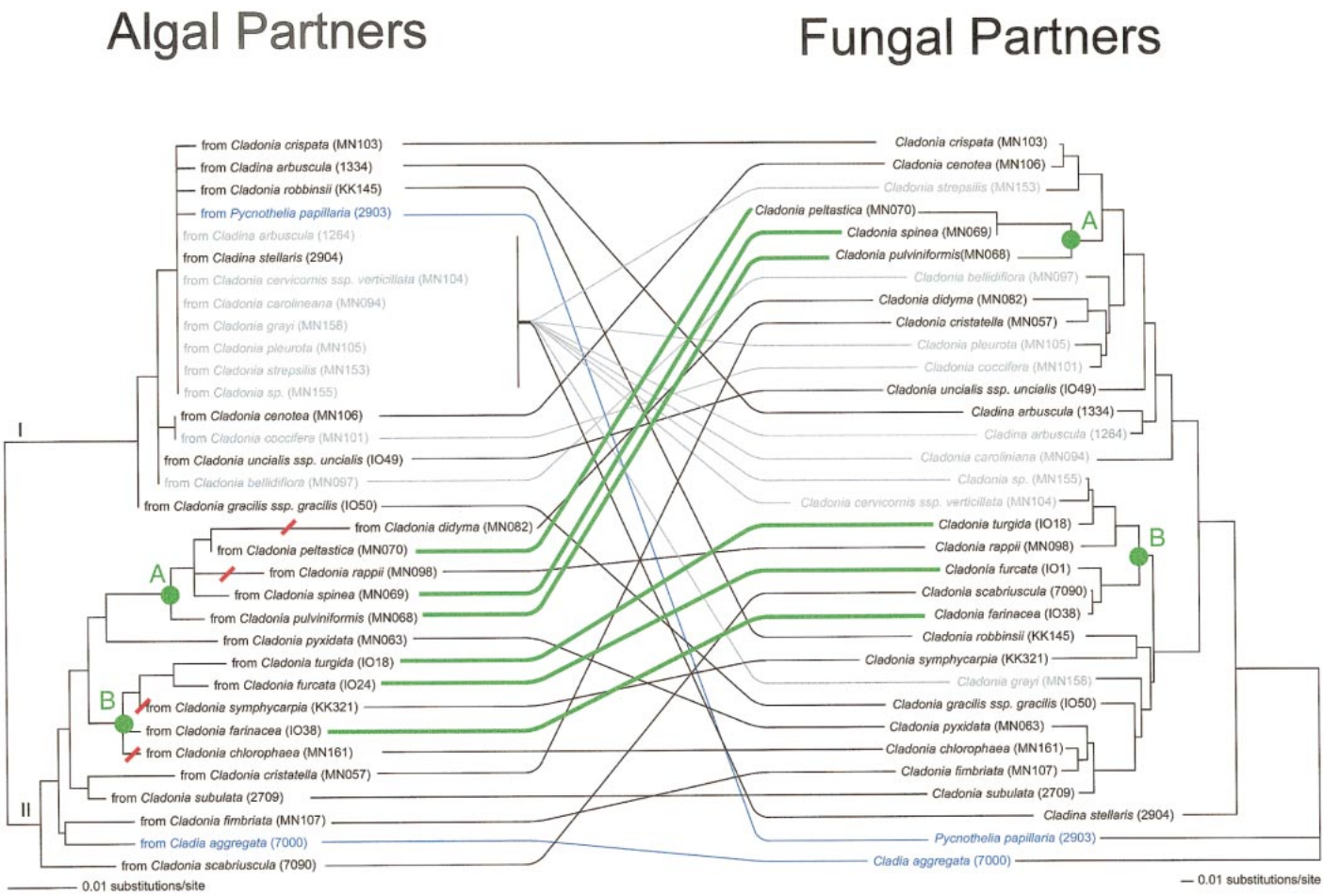

Fig. 2. Incongruence between maximum likelihood phylogenies of algal and fungal partners from 33 natural lichen associations. The most likely phylograms of the algal partners (left) and the fungal partners (right) are shown. Partners from the same lichen association are connected by lines between the phylograms; their crossing shows no overall parallel cladogenesis. Examples of phylogenetic cospeciation, common to all TreeMap reconstructions as described in Table 2, are indicated on both trees with green dots marked A and B, and their partners are connected by green lines. Within these lineages required algal switching events are marked with red slashes on the algal lineage. The two major clades, Clade I and Clade II, are labeled adjacent to their nodes. Algal and fungal partners from *Cladonia* and *Cladina* associations are shown in black or gray with those in gray excluded from TreeMap analyses. Partners from other fungal associations are shown in blue.

Intimate symbioses such as lichen associations are hypothesized to promote loss of sexual reproduction and lower speciation rates of symbionts enclosed by their partners (as mutualisms; Law and Lewis, 1983). This is in agreement with some observations that sexual reproduction by *Trebouxia* is suppressed in lichen associations (Friedl and Büdel, 1996). However, others have reported algal zoospore production that, when released from a thallus, could form sexually reproducing free-living microcolonies of *Trebouxia.* Sexual products of these microcolonies could then reenter into lichen associations (Slocum, Ahmadjian, and Hildreth, 1980). In our observations, 46 fungal species from five continents are associated with only 36 genotypes, representing perhaps four or fewer species of algae. Although lack of algal specificity could be explained by relatively recent algal switches (horizontal transfer), this would require efficient and recent dispersal of algal genotypes over continental distances independent of that of their fungal partners. Perhaps long-distance dispersal of the algae and accompanying high selectivity by the fungus (Tschermak-Woess, 1988; Beck, Friedl, and Rambold, 1998), can provide an explanation for the broad geographic homogeneity of algal ITS genotypes.

This study demonstrates that there are very few algal genotypes shared among variously related taxa of the family Cladoniaceae, implying that selectivity is not equal between lichen-forming fungi and algae. The fungi may be selecting very specific algal genotypes, while the algae are tolerant of many fungal partners. If fungi can select free-living algae, or even those from other lichen associations, then this horizontal transfer (algal switching) can explain the symbiotic association of algae and fungi with incongruent phylogenetic histories. This would support the common model of the lichen symbiosis as a ''domestication'' of photosynthetic algae by the heterotrophic fungi (see Ahmadjain, 1993), analogous to human agriculture in the selection and distribution of crops across cultural lineages or the evolution of agriculture in ants (Mueller, Rehner, and Schultz, 1998). We propose that horizontal transfer of algae, long-distance dispersal, and high selectivity by the fungus of algal genotypes allow a superior genotype to sweep through populations of taxonomically and geographi-

TABLE 3. Tests of congruence and cospeciation for ITS 1 and 2 data sets and algal and fungal taxa sets. Congruence and cospeciation of pruned algal and fungal data sets and topologies (as in Table 2) were rejected ($P < 0.05$) by four congruence tests implemented in PAUP4.0d65 and a cospeciation test implemented in TreeMap1.0. In the latter, random algal trees were generated using the Markovian model, and the frequencies of trees with different numbers of cospeciation events were used to determine the probability of any reconstruction being explained by random chance. Incongruence between ITS1 and ITS2 topologies, although not between all (ITS1 and 2) and either ITS1 or ITS2, was suggested by statistical rejection of their combinability ($P < 0.05$) in the Kashino-Hasagawa and Templeton's tests. This apparent incompatibility may reflect the low number of informative characters in the ITS1 and/or ITS2 data sets. Therefore, these tests may be limited in their ability to accept congruence between algal and fungal phylogenies with this type of data.

Test	Fungal				Algal			
	All vs. ITS1	ITS1 vs. 2	All vs. ITS2	Fungal vs. algal	All vs. ITS1	ITS1 vs. 2	All vs. ITS2	
Congruence tests (PAUP4.0d65)								
Rodrigo's second test	None	None	None	None	None	None	None	
Templeton's test	$P = 0.1083–0.2386$	$P < 0.05$	$P = 0.0003–0.0540$	$P < 0.05$	$P = 0.0258–0.3173$	$P < 0.05$	$P = 0.0833–0.6547$	
Kashino-Hasagawa test	$P = 0.1084–0.2370$	$P < 0.05$	$P = 0.0002–0.050$	$P < 0.05$	$P = 0.0276–0.3186$	$P < 0.05$	$P = 0.0833–0.6558$	
Partition homogeneity (ILD)	—	—	—	$P < 0.05$	—	—	—	
Cospeciation test (TreeMap)								
Randomization test	—	—	—	$P = 0.23–0.50$	—	—	—	

cally diverse lichens. This would support Law's (1985) hypothesis that through natural selection genotypes of symbionts are produced that are so accommodating they could be transferred even among unrelated hosts.

LITERATURE CITED

AHMADJIAN, V. 1987. Coevolution in lichens. *Annals of the New York Academy of Sciences* 503: 307–315.

———. 1988. The lichen alga *Trebouxia*: does it occur free-living? *Plant Systematics and Evolution* 158: 243–247.

———. 1993. The lichen symbiosis. John Wiley and Sons, New York, New York, USA.

BECK, A., T. FRIEDL, AND G. RAMBOLD. 1998. Selectivity of photobiont choice in a defined lichen community: inferences from cultural and molecular studies. *New Phytologist* 139: 709–720.

BOWEN, M. D., C. J. PETERS, AND S. T. NICHOL. 1997. Phylogenetic analysis of the Arenaviridae: patterns of virus evolution and evidence for cospeciation between areanaviruses and their rodent hosts. *Molecular Phylogenetics and Evolution* 8: 301–316.

CHAPELA, I. H., S. A. REHNER, T. R. SCHULTZ, AND U. G. MUELLER. 1994. Evolutionary history of the symbiosis between fungus-growing ants and their fungi. *Science* 266: 1691–1694.

CLARK, M. A., N. A. MOREN, P. BAUMAN, AND J. J. WERNEGREEN. 2000. Cospeciation between bacterial endosymbionts (*Buchnera*) and recent radiation of aphids (*Uroleucon*) and pitfalls of testing for phylogenetic congruence. *Evolution* 54: 517–525.

CLAYTON, D. H., R. D. PRICE, AND R. D. M. PAGE. 1996. Revision of *Dennyus* (*Collodennyus*) lice (Phthiraptera: Menoponidae) from swiftlets, with descriptions of new taxa and a comparison of host–parasite relationships. *Systematic Entomology* 21: 179–204.

DEMASTES, J. W., AND M. S. HAFNER. 1993. Cospeciation of pocket gophers (*Geomys*) and their chewing lice (*Geomydoecus*). *Journal of Mammalogy* 74: 521–530.

DEPRIEST, P. T. 1992. Molecular-genetic analysis of ribosomal DNA polymorphism in the *Cladonia chlorophaea* complex. Duke University, Durham, North Carolina, USA and University Microfilms International, Michigan, USA.

———. 1993. Small subunit rDNA variation in a population of lichen fungi due to optional group-I introns. *Gene* 134: 67–74.

DISTELL, D. L., H. FELBECK, AND C. M. CAVANAUGH. 1994. Evidence for phylogenetic congruence among sulfur-oxidizing chemoautotropic bacterial endosymbionts and their bivalve hosts. *Journal of Molecular Evolution* 38: 533–542.

FARRIS, J. S., M. KALLERSJO, A. G. KLUGE, AND C. BULT. 1994. Permutations. *Cladistics* 10: 65–76.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

FRIEDL, T. 1987. Thallus development and phycobionts of the parasitic lichen *Diploschistes muscorum*. *Lichenologist* 19: 183–191.

———. 1989. Systematik und Biologie von *Trebouxia* (Microthamniales, Chlorophyta) als Phycobiont der Parmeliaceae (lichenisierte Ascomyceten). Universität Bayreuth.

———, AND B. BÜDEL. 1996. Photobionts. *In* T. H. Nash III [ed.], Lichen biology, 8–23. Cambridge University Press, Cambridge, UK.

GRUBE, M., P. T. DEPRIEST, A. GARGAS, AND J. HAFELLNER. 1995. DNA isolation from lichen ascomata. *Mycological Research* 99: 1321–1324.

HAFNER, M. S., AND R. D. M. PAGE. 1994. Molecular phylogenies and host-parasite cospeciation: gophers and lice as a model system. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 349: 77–83.

HARDING, E. F. 1971. The probabilities of rooted tree-shapes generated by random bifurcation. *Advances in Applied Probability* 3: 44–77.

HASAGAWA, M., H. KISHINO, AND T. YANO. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22: 160–174.

HAWKSWORTH, D. L. 1988. Coevolution of fungi with algae and cyanobacteria in lichen symbioses. *In* K. A. Pirozynski and D. L. Hawksworth [eds.], Coevolution of fungi with plants and animals, 125–148. Academic Press, London, UK.

———, P. M. KIRK, B. C. SUTTON, AND D. N. PEGLER. 1995. Dictionary of

the fungi, 8th ed. International Mycological Institute, Cambridge University Press, Cambridge, UK.

HERRE, E. A., C. A. MACHADO, E. BERMINGHAM, J. D. NASON, D. M. WINDSOR, S. S. MCCAFFERTY, W. VAN HOUTEN, AND K. BACHMANN. 1996. Molecular phylogenies of figs and their pollinator wasps. *Journal of Biogeography* 23: 521–530.

HILDRETH, K. C., AND V. AHMADJIAN. 1981. A study of *Trebouxia* and *Pseudotrebouxia* isolates from different lichens. *Lichenologist* 13: 65–86.

HILLIS, D. M., AND J. P. HUELSENBECK. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity* 83: 189–195.

HINKLE, G., J. K. WETTER, T. R. SCHULTZ, AND M. L. SOGIN. 1994. Phylogeny or the attine ant fungi based on analysis of small subunit ribosomal RNA gene sequences. *Science* 266: 1695–1697.

HONEGGER, R. 1998. The lichen symbiosis—what is so spectacular about it? *Lichenologist* 30: 193–212.

HUELSENBECK, J. P., B. RANNALA, AND Z. YANG. 1997. Statistical tests of host–parasite cospeciation. *Evolution.* 51: 410–419.

IVANOVA, N. V., P. T. DEPRIEST, V. K. BOBROVA, AND A. V. TROITSKY. 1999. Phylogenetic analysis of the lichen family Umbilicariaceae on the basis of nuclear SSU, ITS1, 5.8S and ITS2 rDNA sequences. *Lichenologist* 31: 477–489.

JEONG, S. C., N. J. RITCHIE, AND D. D. MYROLD. 1999. Molecular phylogenies of plants and *Frankia* support multiple origins of actinorhizal symbioses. *Molecular Phylogenetics and Evolution* 13: 493–503.

KASHINO, H., AND M. HASAGAWA. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *Journal of Molecular Evolution.* 29: 170–179.

KETHLEY, J. B., AND D. E. JOHNSTON. 1975. Resource tracking patterns in bird and mammal ectoparasites. *Miscellaneous Publications of the Entomological Society of America* 9: 231–236.

LAW, R. 1985. Evolution in a mutualistic environment. *In* D. H. Boucher [ed.], The biology of mutualism: ecology and evolution, 145–170. Oxford University Press, New York, New York, USA.

———, AND D. H. LEWIS. 1983. Biotic environments and the maintenance of sex—some evidence from mutualistic symbioses. *Biological Journal of the Linnean Society* 20: 249–276.

MUELLER, U. G., S. A. REHNER, AND T. R. SCHULTZ. 1998. The evolution of agriculture in ants. *Science* 281: 2034–2038.

NUISMER, S. L., J. N. THOMPSON, AND R. GOMULKIEWICZ. 1999. Gene flow and geographically structured coevolution. *Proceedings of the Royal Society London Series B* 266: 605–609.

OTT, S. 1987. Sexual reproduction and developmental adaptations in *Xanthoria parietina. Nordic Journal of Botany* 7: 219–228.

PAGE, R. D. M. 1993. Genes, organisms and areas: the problem of multiple lineages. *Systematic Biology* 42: 77–84.

———. 1994. Parallel phylogenies: reconstructing the history of host–parasite assemblages. *Cladistics* 10: 155–173.

———. 1995. TreeMap 1.0. Division of Environmental and Evolutionary Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK.

———, AND M. S. HAFNER. 1996. Molecular phylogenies and host–parasite cospeciation: gophers and lice as a model system. *In* P. H. Harvey, A. J. Leigh Brown, J. Maynard Smith, and S. Nee [eds.], New uses for new phylogenies, 255–272. Oxford University Press, New York, New York, USA.

———, P. L. M. LEE, S. A BECHER, R. GRIFFITHS, AND D. H. CLAYTON. 1998. A different tempo of mitochondrial DNA evolution in birds and their parasitic lice. *Molecular Phylogenetics and Evolution* 9: 276–293.

PEEK, A. S., R. A. FELDMAN, R. A. LUTZ, AND R. C. VRIJENHOEK. 1998. Cospeciation of chemoautotrophic bacteria and deep sea clams. *Proceedings of the National Academy of Sciences, USA* 95: 9962–9966.

RAMBOLD, G., T. FRIEDL, AND A. BECK. 1998. Photobionts in lichens: possible indicators of phylogenetic relationships? *Bryologist* 101: 392–397.

———, AND D. TRIEBEL. 1992. The inter-Lecanoralean associations—Bibliotheca Lichenologica, 48. J. Cramer, Berlin, Germany.

ROBINSON, H. 1975. Considerations on the evolution of lichens. *Phytologia* 32: 407–413.

RODRIGO, A. G., K. KELLY-BORGES, P. R. BERGQUIST, AND P. L. BERGQUIST. 1993. A randomization test of the null hypothesis that two cladograms are sample estimates of a phylogenetic tree. *New Zealand Journal of Botany* 31: 257–268.

SCHARDL, C. L., A. LEUCHTMANN, K. R. CHUNG, D. PENNY, AND M. R. SIEGEL. 1997. Coevolution by common descent of fungal symbionts (*Epichloe* spp.) and grass hosts. *Molecular Biology and Evolution* 14: 133–143.

SIX, D. L., AND T. D. PAINE. 1999. Phylogenetic comparison of ascomycete mycangial fungi and Dendroctonus bark beetles. *Annals of the Entomological Society of America* 92: 159–166.

SLOCUM, R. D., V. AHMADJIAN, AND K. C. HILDRETH. 1980. Zoosporogenesis in *Trebouxia gelatinosa*: ultrastructure potential for zoospore release and implications for the lichen association. *Lichenologist* 12: 173–187.

SWOFFORD, D. 1998. Phylogenetic analysis using parsimony (PAUP) version 4.0. Sinauer, Sunderland, Massachusetts, USA.

TEMPLETON, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the humans and the apes. *Evolution* 37: 221–244.

THOMPSON, J. N. 1994. The coevolutionary process. University of Chicago Press, Chicago, Illinois, USA.

———. 1999. Specific hypotheses on the geographic mosaic of coevolution. *American Naturalist* 153: S1–S14.

TSCHERMAK-WOESS, E. 1988. The algal partner. *In* M. Galun [ed.], CRC handbook of lichenology, vol. I, 39–92. CRC Press, Boca Raton, Florida, USA.

WHITE, T. J., T. D. BRUNS, S. B. LEE, AND J. W. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols: a guide to methods and applications, 315–322. Academic Press, New York, New York, USA.