

# Phylogenetic Evidence for Horizontal Transmission of Group I Introns in the Nuclear Ribosomal DNA of Mushroom-Forming Fungi

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Group I introns were discovered inserted at the same position in the nuclear small-subunit ribosomal DNA (nuc-ssu-rDNA) in several species of homobasidiomycetes (mushroom-forming fungi). Based on conserved intron sequences, a pair of intron-specific primers was designed for PCR amplification and sequencing of intron-containing rDNA repeats. Using the intron-specific primers together with flanking rDNA primers, a PCR assay was conducted to determine presence or absence of introns in 39 species of homobasidiomycetes. Introns were confined to the genera *Panellus*, *Clavicornia*, and *Lentinellus*. Phylogenetic analyses of nuc-ssu-rDNA and mitochondrial ssu-rDNA sequences suggest that *Clavicornia* and *Lentinellus* are closely related, but that *Panellus* is not closely related to these. The simplest explanation for the distribution of the introns is that they have been twice independently gained via horizontal transmission, once on the lineage leading to *Panellus*, and once on the lineage leading to *Lentinellus* and *Clavicornia*. BLAST searches using the introns from *Panellus* and *Lentinellus* as query sequences retrieved 16 other similar group I introns of nuc-ssu-rDNA and nuclear large-subunit rDNA (nuc-lsu-rDNA) from fungal and green algal hosts. Phylogenetic analyses of intron sequences suggest that the mushroom introns are monophyletic, and are nested within a clade that contains four other introns that insert at the same position as the mushroom introns, two from different groups of fungi and two from green algae. The distribution of host lineages and insertion sites among the introns suggests that horizontal and vertical transmission, homing, and transposition have been factors in intron evolution. As distinctive, heritable features of nuclear rDNAs in certain lineages, group I introns have promise as phylogenetic markers. Nevertheless, the possibility of horizontal transmission and homing also suggest that their use poses certain pitfalls.

## Introduction

Molecular phylogenetics leans heavily on the assumption that the phylogenies of genes within organisms reflect the phylogeny of the organisms themselves. This assumption is violated by horizontal transmission, which is the nonsexual transfer of genetic elements between lineages (Lawrence and Hartl 1992; Kidwell 1993; Syvanen 1994). Hypotheses of horizontal transmission are upheld if it can be demonstrated that there is strongly supported conflict between the phylogeny of the element that is thought to have been transmitted horizontally and the "species" phylogeny of the organisms that bear the element (de Queiroz 1993). A diversity of methods now exist for assessing the significance of incongruence between phylogenetic trees and data sets, including the Kishino-Hasegawa maximum-likelihood test (Kishino and Hasegawa 1989; Felsenstein 1993), the T-PTP test (Faith 1991), Rodrigo's method (Rodrigo et al. 1993), incongruence length differences (Farris et al. 1994), and Templeton's nonparametric test (Templeton 1983). These methods are useful not only for detecting horizontal transmission, but also for revealing cases of lineage sorting and hybridization.

Parsimony analysis can also be used to evaluate potential cases of horizontal transmission. This method is especially appropriate when the genetic element in question is rare among the taxa under consideration, and it can be used even if there is no positive conflict be-

tween the topology of the host lineage and that of the putatively horizontally transmitted element. In this approach, the distribution of the putatively mobile element is optimized ("mapped") onto the best estimate of the organismal phylogeny, and the number of independent gains and losses of the element are determined (Maddison and Maddison 1992, pp. 35-36; Clark, Maddison and Kidwell 1994). If only a single gain (and any number of losses) is required to explain the distribution of the element, then the null hypothesis of strictly vertical transmission cannot be rejected. On the other hand, if the optimization suggests multiple gains of the element, then a hypothesis of horizontal transmission is supported. The optimization of the element on the host topology, and hence the support for a hypothesis of horizontal transmission, is influenced by two factors: (1) the host topology and (2) the relative weight assigned to losses vs. gains. If gain of the element is assigned a sufficiently high weight (=low probability) relative to its loss, then any tree topology can be interpreted as suggesting strictly vertical transmission.

Various kinds of genetic elements have been proposed to have been transmitted horizontally, including genomic DNA segments, such as genes involved in nitrogen fixation (cf. Hirsch et al. 1995), mobile elements, such as the mariner-like elements and *P* elements of insects (e.g., Houck et al. 1991; Lohe et al. 1995), and introns of protein-coding or structural RNA genes (see reviews by Kidwell 1993 and Syvanen 1994). The latter include the group I introns, which are a class of genetic elements defined in part by the possession of a set of conserved sequences, termed the P, Q, R, and S sequences (Davies et al. 1982). Base-pairing between the P, Q, R, and S sequences enables the transcribed introns to assume a characteristic secondary structure which func-

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tions in splicing reactions that remove the intron from the RNA transcript (Cech and Bass 1986; Dujon 1989; Cech 1990; Michel and Westhof 1990). Splicing of certain group I introns involves an endonuclease or other maturase that can be encoded by the introns themselves or by independent genes (Dujon 1989). Other group I introns, however, such as that of the *Tetrahymena* nuclear rDNA, have been demonstrated in vitro to encode autocatalytic, self-splicing RNAs (cf. Cech 1990). Because of their semiautonomous nature, group I introns and other catalytic RNAs have fueled speculations about the role of RNAs in the evolution of precellular life (e.g., Sharp 1985; Yarus 1988).

Group I introns are known from bacteriophages, prokaryotes, and nuclear and organellar genomes of diverse eukaryotes (Dujon 1989). They are especially well documented in the nuclear rDNAs (reviewed by Gargas, DePriest, and Taylor 1995), where they have often been found in the course of molecular phylogenetic studies. Group I introns have been called the "ultimate parasites" (Lambowitz 1989). This characterization seems especially fitting for the group I introns of nuclear rDNA (nuc-rDNA) genes, which occur in multiple tandem repeats. In fungi, the number of nuc-rDNA repeats ranges from approximately 60 in the mushroom *Coprinus* (Cassidy et al. 1984) to 220 in the ascomycete mold *Neurospora* (Russell et al. 1984). Group I introns that target nuc-rDNA could achieve a higher density per genome than those that colonize particular single-copy genes. By virtue of their ability to excise from the 40S precursor rRNA, group I introns presumably confer little reduction in fitness to their hosts, even when all copies of the nuc-rDNA tandem repeats are "infected." Conversely, it has been suggested that drugs that inhibit self-splicing of group I introns, and thus prevent the formation of functional ribosomes, might be effective against pathogens that have group I introns in their rDNAs, such as the fungus *Pneumocystis carinii*, which is a major cause of morbidity in AIDS patients (Lin et al. 1992; Liu and Leibowitz 1993).

Horizontal transmission of group I introns of nuc-rDNA has previously been proposed to have occurred within or among fungi, plants, amoebae, *Tetrahymena*, and red algae (Michel and Cummings 1985; Sogin et al. 1986; Nishida, Blanz, and Sugiyama 1993; Gast, Fuerst, and Byers 1994; Oliveira and Ragan 1994; Nishida and Sugiyama 1995). To rigorously support the hypothesis that group I introns of nuc-rDNA have been distributed by horizontal transmission, however, it is necessary to satisfy three conditions: (1) The introns do not reside elsewhere in the genome besides the nuc-rDNA and merely transpose occasionally into the rDNA. (2) In organisms thought to lack introns, the introns are not present in so small a number of copies of the nuc-rDNA tandem repeats that they are simply undetected by normal polymerase chain reaction (PCR) procedures. (3) The organismal phylogeny of the hosts is strongly incongruent with a hypothesis of vertical intron transmission, or a hypothesis of strictly vertical transmission is far less parsimonious than a model involving some degree of horizontal transmission.

This study was derived from ongoing research on systematics of homobasidiomycetes, which include the mushroom-forming fungi (Hibbett and Donoghue 1995a, 1995b). PCR products of nuclear small-subunit rDNAs (nuc-ssu-rDNAs) in several species in these studies exceeded their expected size by about 400 bp. Sequence analysis revealed that the increase in size of the PCR products was due to group I introns that were always found inserted at the same position. The purpose of the present study was to determine, in the context of a broad phylogenetic analysis of homobasidiomycetes, whether horizontal transmission should be invoked to explain the distribution of group I introns in nuclear rDNA of homobasidiomycetes. The taxonomic significance of the results will be addressed elsewhere.

## Materials and Methods

### Taxa

Species examined in this study are listed in table 1. Except for *Clavicornia pyxidata* and the two isolates of *Lentinellus montanus*, all ingroup isolates and DNA preparations are the same as ones that were used in a previous phylogenetic study of mitochondrial small-subunit rDNA (mt-ssu-rDNA) sequences in homobasidiomycetes (Hibbett and Donoghue 1995a). Methods for culturing, storage, harvesting, and DNA isolation for *C. pyxidata* and *L. montanus* are the same as for the other species in the ingroup (Hibbett and Donoghue 1995a).

In a previous study (Hibbett and Donoghue 1995a), the heterobasidiomycete "jelly fungus" *Auricularia auricula-judae* (Auriculariales) was used to root the homobasidiomycete ingroup. This outgroup choice was based on results of other phylogenetic studies at more inclusive taxonomic levels, especially the study by Swann and Taylor (1993) on higher-level phylogenetic relationships of the basidiomycetes using 18S rDNA sequences. Since then, however, new studies have been published (Gargas et al. 1995; Swann and Taylor 1995a, 1995b), also using 18S rDNA, that cast doubts on the monophyly of the homobasidiomycetes and suggest that the Auriculariales may actually be nested within the homobasidiomycetes. For the present study, exemplars of the Dacrymycetales and Tremellales (Wells 1994) were chosen as outgroups to the homobasidiomycetes plus Auriculariales. Like the Auriculariales, the Dacrymycetales and Tremellales include jelly fungi, and are classified as heterobasidiomycetes (Wells 1994), but phylogenetic studies of nuc-ssu-rDNA data (Gargas et al. 1995; Swann and Taylor 1995a, 1995b) have placed them basal to the clade that includes the homobasidiomycetes and Auriculariales.

### Amplification and Sequencing of rDNA Coding Regions

Mt-ssu-rDNA sequences for ingroup species, except *C. pyxidata*, are from Hibbett and Donoghue (1995a). For *C. pyxidata*, the same protocols for generating mt-ssu-rDNA sequences as in Hibbett and Donoghue (1995a) were followed. Mt-ssu-rDNA sequences were not generated for *L. montanus* or the outgroups, Dacrymycetales and Tremellales.

**Table 1**  
**Taxa Examined**

Classification	Isolate Number <sup>a</sup>
<b>Ingroup</b>	
Auriculariales	
<i>Auricularia auricula-judae</i> <sup>b,c</sup>	FPL 11504 (mt-ssu-rDNA only)
Homobasidiomycetes	
<i>Antrodin carbonica</i> <sup>c,d</sup>	DAOM 19782
<i>Auriscalpium vulgare</i> <sup>c,d</sup>	DAOM 197828
<i>Bjerkandera adusta</i> <sup>c,d</sup>	DAOM 21586
<i>Bondarzewia berkeleyi</i> <sup>c,d</sup>	DSH 93 190
<i>Bondarzewia montana</i> <sup>c,d</sup>	DAOM 415
<i>Ceriporia purpurea</i> <sup>c,d</sup>	DAOM 2 13 16
<i>Clavicornia pyxidata</i> <sup>d,e</sup>	CBS 535.90
<i>Coltricia perennis</i> <sup>c,d</sup>	DSH 93-198
<i>Daedalea quercina</i> <sup>c,d</sup>	DAOM 14247
<i>Echinodontium tinctorium</i> <sup>c,d</sup>	DAOM 16666
<i>Fistulina hepatica</i> <sup>c,d</sup>	DSH 93 183
<i>Fomes fomentarius</i> <sup>c,d</sup>	DAOM 12903
<i>Fomitopsis pinicola</i> <sup>c,d</sup>	DAOM 189 13
<i>Hericium ramosum</i> <sup>c,d</sup>	DSH 93-1 YY
<i>Heterobasidion annosum</i> <sup>c,d</sup>	DAOM 73191
<i>Inonotus hispidus</i> <sup>c,d</sup>	FPL 3597
<i>Laetiporus sulphureus</i> <sup>c,d</sup>	DSH 93- 194
<i>Lentinellus montanus</i> <sup>f,g</sup>	VT 242
<i>Lentinellus montanus</i> <sup>f,g</sup>	VT 246
<i>Lentinellus omphalodes</i> <sup>c,d</sup>	DSH-9
<i>Lentinellus ursinus</i> <sup>c,d</sup>	VT 237
<i>Lentinula lateritia</i> <sup>c,d</sup>	DSH 92-143
<i>Lentinus tigrinus</i> <sup>c,d</sup>	DSH 93 181
<i>Meripilus giganteus</i> <sup>c,d</sup>	DSH 93-193
<i>Panellus serotinus</i> <sup>c,d</sup>	DSH 93-218
<i>Panellus stypticus</i> <sup>c,d</sup>	DSH 93-2 13
<i>Panus rudis</i> <sup>c,d</sup>	DSH 92-139
<i>Peniophora nuda</i> <sup>c,d</sup>	FPL 4756
<i>Phaeolus schweinitzii</i> <sup>c,d</sup>	DSH 93-196
<i>Phnnerochaete chrysosporium</i> <sup>c,d</sup>	FPL 5 175
<i>Pleurotus tuberregium</i> <sup>c,d</sup>	DSH 92-155
<i>Polyporus squamosus</i> <sup>c,d</sup>	FPL 6846
<i>Pulcherricium caeruleum</i> <sup>c,d</sup>	FPL 7658
<i>Russula ballouii</i> <sup>c,d</sup>	DUKE 36
<i>Russula compacta</i> <sup>c,d</sup>	DUKE s.n.
<i>Sparassis spathulata</i> <sup>c,d</sup>	DSH 93-184
<i>Stereum annosum</i> <sup>c,d</sup>	FPL 8562
<i>Stereum hirsutum</i> <sup>c,d</sup>	FPL 8805
<i>Trametes suaveolens</i> <sup>c,d</sup>	DAOM 19632
<i>Trichaptum abietinum</i> <sup>c,d</sup>	FPL 8973
<b>outgroup</b>	
Dacrymycetales	
<i>Calocera cornea</i> <sup>b,f</sup>	
<i>Dacrymyces chrysospermus</i> <sup>b,f</sup>	
Tremellales	
<i>Cryptococcus neoformans</i> <sup>b,f</sup>	
<i>Tremella foliacea</i> <sup>b,f</sup>	

<sup>a</sup> Isolate number prefixes reflect original sources: DAOM isolates from Canadian Collection of Fungus Cultures, DAOM Ottawa, Ontario; FPL isolates from USDA Forest Products Laboratory, Madison, Wis.; VT isolates from Orson K. Miller, Jr., Virginia Polytechnic Institute, Blacksburg, Va.; DUKE isolates from Rytas Vilgalys, Duke University, Durham, N.C.; CBS isolate from Centraalbureau Voor Schimmelcultures, Baarn; DSH isolates in personal collection of the author.

<sup>b</sup> Nuc-ssu-rDNA sequences published by Swann and Taylor (1995a), also GenBank accession M55625 (*C. neoformans*).

<sup>c</sup> Mt-ssu-rDNA sequence from Hibbett and Donoghue (1995a).

<sup>d</sup> Nuc-ssu-rDNA sequences from this study, deposited in GenBank under accession numbers U59059–U59098.

<sup>e</sup> Mt-ssu-rDNA sequence from this study, deposited in GenBank under accession number U59099.

<sup>f</sup> No mt-ssu-rDNA sequence data.

<sup>g</sup> No nuc-ssu-rDNA sequence data, taxon not included in homobasidiomycete phylogenetic analysis.

Nuc-ssu-rDNA sequences for *A. auricula-judae*, the Dacrymycetales, and Tremellales were published previously (Swann and Taylor 1993) and retrieved from GenBank (table 1). For the remaining ingroup species (except *L. montanus*) a 1.2-kb fragment from the 3' end of the nuc-ssu-rDNA was amplified, using primers PNS 1 and NS4 I, and sequenced. Standard amplification parameters were 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, 30 s, repeated for 30 cycles. PCR reactions used Geneamp kits (Perkin-Elmer), typically with 25-μl reaction volumes, and were run in a Perkin-Elmer 9600 thermal cycler. Amplification and sequencing of both strands of nuc-ssu-rDNA used 10 primers, listed in table 2. Sequencing protocols were the same as those used in Hibbett and Donoghue (1995).

#### PCR Assay for Presence of Group I Introns

PCR and sequencing of nuc-ssu-rDNA revealed an approximately 400-bp insertion at the same site in *P. stypticus*, *L. omphalodes*, and *C. pyxidutu*. In the intron-labeling system of Gargas, DePriest, and Taylor (1995), which has been adopted here, the insertions are at position 943, which corresponds to the homologous nucleotide position in the *Escherichia coli* ssu-rDNA. The insertion sequences were manually aligned, and PCR primers, called 943a and 943b (table 2), were designed from two conserved sites. Primers 943a and 943b and two additional flanking nuc-ssu-rDNA coding sequence primers were used to perform a PCR assay for presence or absence of the introns (fig. 1). The assay uses a battery of four PCR amplifications: (1) Amplification with nuc-ssu-rDNA primers SR1c and NS6 amplifies an 875-bp product when no intron is present, or an approximately 1.3-kb fragment when an intron is present. This is the "A" fragment. The primers in the A reaction target highly conserved nuc-ssu-rDNA coding regions. Thus, the A reaction serves as a positive control for the PCR process itself for each individual surveyed. (2) Amplification with primers SR1c and 943b amplifies an approximately 930-bp product when an intron is present at site 943, but no product when no intron is present. This is the "B" fragment. (3) Amplification with primers 943a and NS6 amplifies an approximately 660-bp fragment, the "C" fragment, when the intron is present at site 943, but no product when the intron is absent. Introns were known from sequence analysis to be present at site 943 in *L. omphalodes*, *C. pyxidutu*, and *P. stypticus*, and so in these taxa the B and C reactions served as reciprocal positive controls for primers 943a and 943b. (4) Finally, amplification with 943a and 943b amplifies an approximately 300-bp "D" fragment when the intron is located anywhere in the genome. The B, C, and D reactions selectively amplify intron-containing rDNA repeats even if they are present in low copy number. Negative controls, in which the DNA templates were replaced by water blanks, were performed for each reaction. Parameters for this assay were 94°C for 30 s, 60°C for 30 s, 72°C for 30 s, for 30 cycles. This assay was performed for all of the homobasidiomycete taxa in this study.

**Table 2**  
**Primers Used for Amplification and Sequencing of nuc-ssu-rDNA and Introns**

Name	Sequence 5' → 3'	Position in Yeast nuc-ssu-rDNA <sup>a</sup>
<b>rDNA primers<sup>b</sup></b>		
PNS1	CCA AGC TTGAAT TCG TAG TCA TAT GCT TGT CTC	20-38
NS19B.....	CCG GAG AGG GAG CCT GAG AAA C	381-401
NS19Bc	GTT TCT CAG GCT CCC TCT CCG G	381-401
SRI	Al-I- ACC GCG GCT GCT	567-581
SR1c	AGC AGC CGC GGT AAT	567-581
SR4	AAA CCA ACA AAA TAG AA	825-841
SR4c	TTC TAT TTT GTT GGT TT	825-841
NS51	GGG GGA GTA TGG TCG CAA GGC	1095-1116
NS4	CTT CCG TCA ATT CCT TTA AG	1120-1141
NS41	CCC GTG TTG AGT CAA ATT A	1169-1187
NS6..	GCA TCA CAG ACC TGT TAT TGC CTC	1404-1427
<b>Intron-specific primers<sup>c</sup></b>		
943a..	CTC TGC WCT RAA MAC CAG CCC KAA MGG GTG	
943b..	CCT CCA TCT AGT CTC TGA ACC TKT CCC	

<sup>a</sup>Rubstov et al. (1980).

<sup>b</sup>Nuc-ssu-rDNA primer sequences from Tom Bruns (personal communication, NS19), Rytas Vilgalys (personal communication, SR1,SR4), Kerry O'Donnell (personal communication, PNS1,NS41,NS51), and White et al. (1990, NS4, NS6), with some modifications.

<sup>c</sup>Ambiguity code: W = (T/A), M = (A/C), K = G/T

### Intron Sequence Analysis

Intron-bearing taxa were sequenced with primers 943a and 943b, in addition to the other nuc-ssu-rDNA primers. Intron boundaries were determined by alignment to sequences of nuc-ssu-rDNA that lack the intron. The intron sequences from *L. omphalodes* and *P. stypiticus* were used as queries in BLAST searches (Altschul et al. 1990) with normal stringency. The top 20 scoring sequences (eliminating duplication) from the BLAST searches were compared to the mushroom introns using dot-plot analysis in SeqApp 1.9 (Gilbert 1992) with a 25-base window, 15 matches minimum. Conserved regions identified in dot-plots were manually aligned in the data editor of PAUP 3.1 (Swofford 1993).

Parsimony analyses were conducted to estimate the relationships of the introns using PAUP 3.1. Characters were scored only from the regions that could be aligned across all the introns (fig. 2). Parsimony analyses were performed with midpoint rooting, all characters unordered, character state transformations not differentially weighted, and gaps treated as missing data. One hundred heuristic searches were used, each with a random taxon addition sequence, maxtrees unrestricted, and TBR branch swapping. Topological robustness was estimated using the bootstrap. Each bootstrap replicate used a heuristic search, with the same settings as the main analyses, except that maxtrees was limited to one hundred.

Constraint trees were used to force monophyly of several groups of introns from taxonomically related hosts. Groups of introns that were tested for monophyly included: (1) introns of the fungus *Protomyces*, (2) introns of the alga *Dunaliella*, and (3) all fungal introns (vs. all algal introns). No other topological structures were specified in the constraint trees. Parsimony analyses were performed under these constraints, with the

same settings as in the unconstrained analyses, and the resulting trees were evaluated against the unconstrained trees with the Kishino-Hasegawa maximum-likelihood test, using DNAML of PHYLIP (Felsenstein 1993; Kishino and Hasegawa 1989).

### Host Phylogenetic Analyses

To estimate the organismal phylogeny of the homobasidiomycetes, parsimony analyses of the mt-ssu-rDNA and nuc-ssu-rDNA sequences were performed using PAUP. The data matrix is available on request. Settings were the same as in the analyses of the intron sequences, including bootstrapping. Independent parsimony analyses of the mt-ssu-rDNA and nuc-ssu-rDNA were performed, including bootstrapping using the same settings as described above. Results of independent bootstrapped parsimony analyses were compared to determine if there was strongly supported positive incongruence, with "strong support" arbitrarily defined as 80% bootstrap frequency. Because there was no such strong incongruence (trees not shown), data sets were combined to estimate the overall species phylogeny.

The distribution of introns was optimized according to parsimony onto trees derived from rDNA coding sequences using MacClade (Maddison and Maddison 1992) under two different models of intron evolution: In the first model, introns are allowed to be independently gained, such as by horizontal transmission, or lost by deletion. In the second model, introns are assumed to be present in the stem species of the ingroup, with strictly vertical transmission and loss accounting for their observed distribution. The number of gains and losses under the first model was compared to the number of losses alone under the second model. Under weighted parsimony, the two models would be considered equally

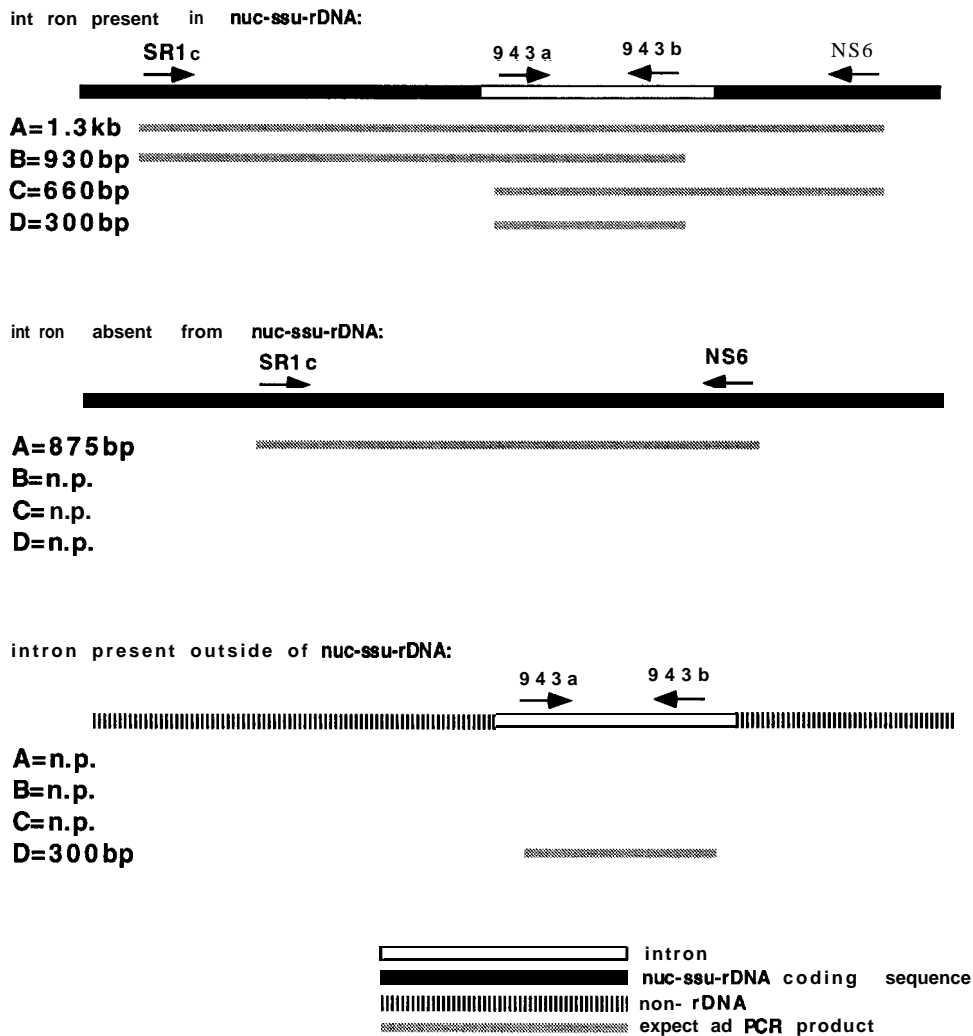


FIG. 1.—Schematic diagram of PCR assay for presence or absence of group I introns in nuc-ssu-rDNA or elsewhere in genome. Arrows indicate primer sites. A, B, C, and D amplification products are described in text. From top to bottom, figure panels show expected results if intron is present at site 943 in nuc-ssu-rDNA, absent from rDNA, or present elsewhere in genome.

likely if the weighted cost of gains plus losses under the first model were equal to the weighted cost of losses alone under the second model. Therefore, the critical loss-gain bias, below which horizontal transmission is not supported, can be estimated algebraically from the two optimizations. Thus, this exercise asks: In the context of the unconstrained rDNA tree, how much more likely must intron loss be relative to intron gain before horizontal transmission is no longer supported?

The exercise described in the preceding paragraph examined support for the hypothesis of horizontal transmission in the context of the most parsimonious, unconstrained tree based on rDNA alone. Other topologies, however, could have different implications for intron mobility. In particular, topologies that showed the intron-containing taxa to be closely related or basal (monophyletic or paraphyletic) could be consistent with a hypothesis of strictly vertical transmission. To generate such topologies, constraint trees were used that forced either: (1) monophyly of all intron-containing taxa, (2) monophyly of intron-lacking taxa (This constraint forces

the intron-containing taxa to be basal, and allows certain paraphyletic configurations of the intron-containing taxa.), or (3) monophyly of *Lentinellus* and *Clavicornia*, which are two of the intron-containing genera. The rDNA coding sequences were analyzed with parsimony under the topological constraints, with the same settings as in the unconstrained analyses, and the resulting trees were evaluated with the Kishino-Hasegawa maximum-likelihood test. These exercises examined support by the rDNA data for certain alternate topologies that could weaken arguments for horizontal transmission.

## Results and Discussion

### PCR Assay for Group I Introns

Results of the PCR assay for the intron are shown in figure 3. Of the 39 species surveyed, only five, *L. omphalodes*, *L. montanus* (both isolates), *L. ursinus*, *C. pyxiduta*, and *P. stypticus*, produced results that suggest the presence of an intron at position 943 in the nuc-ssu-rDNA or anywhere else in the genome. Except for *L.*



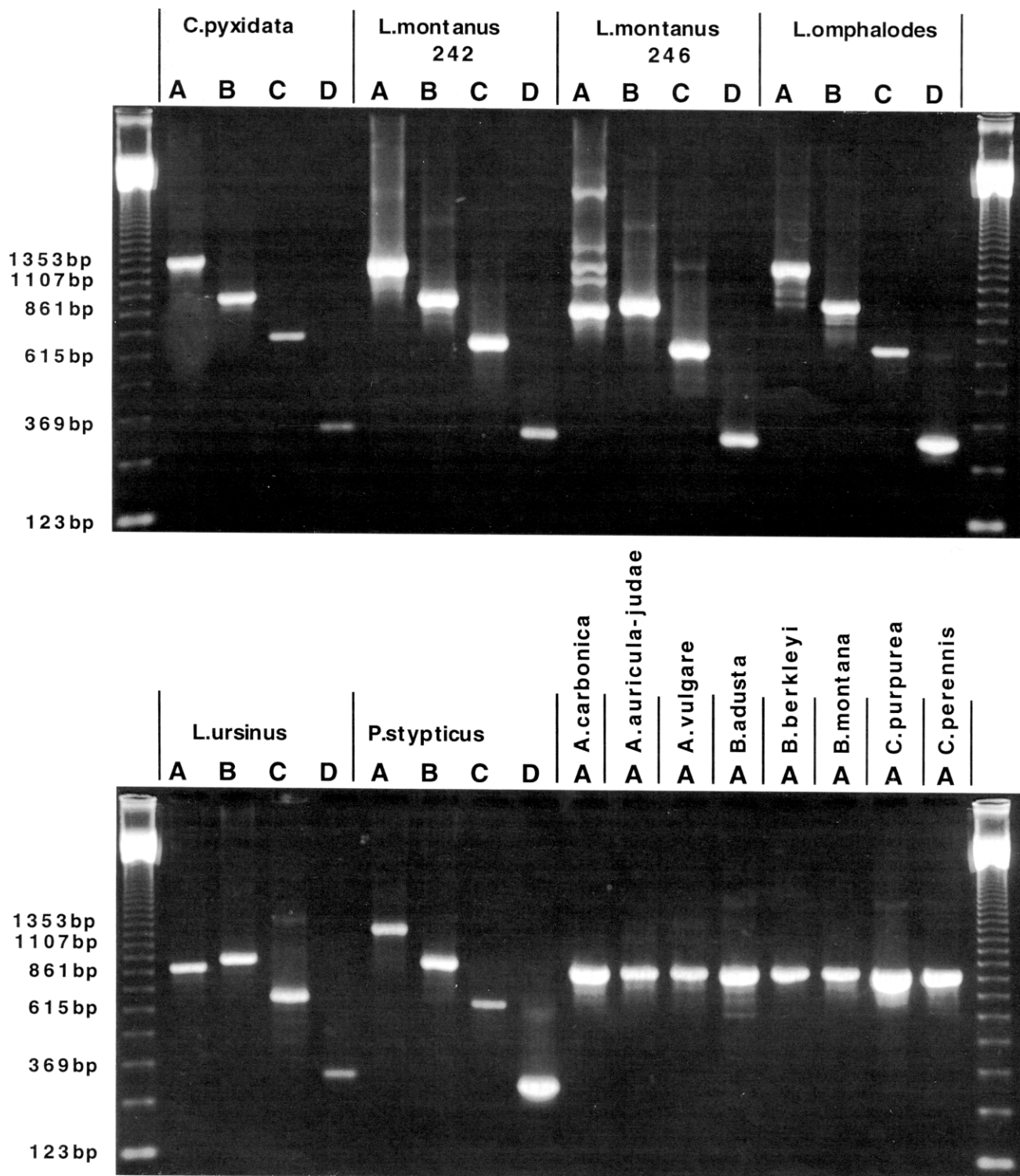


FIG. 3.—Results of PCR assay for presence or absence of introns. For intron-containing taxa, all four reactions are shown (A, B, C, and D; see fig. 1). For representative intron-lacking taxa, only the A reaction product is shown. Unlabeled lanes are 123 base pair ladder (Gibco-BRL) molecular weight standard. See figure 1 for expected product sizes.

*montanus* 246 and *L. ursinus*, all of the isolates with the insertion had the expected results when an intron was uniformly present at position 943, namely a 1.3-kb A fragment, 900-bp B fragment, 660-bp C fragment, and

300-bp D fragment. *Lentinellus montanus* 246 had the same B, C, and D products as *L. montanus* 242, *Panelus*, and *Clavicornia*, but the A amplification produced an 875-bp major fragment as well as weaker fragments

of approximately 1.3 kb (a doublet is present, which may be due to production of some single-stranded DNA, or possibly heterogeneity among the introns). *Lentinellus ursinus* had B, C, and D products like those of *L. montanus* 246 and the other intron-containing species, but the A reaction produced only the 875-bp product. These results suggest that both *L. montanus* 246 and *L. ursinus* have some copies of the rDNA that have an intron, and some that lack the intron. If the relative frequency of intron-containing and intron-lacking rDNA types in the population of PCR products reflects that in the genomic rDNA repeats, then these results suggest that the intron-lacking repeat type is the major repeat type in *L. montanus* 246 and *L. ursinus*. This interpretation is confounded, however, by the fact that PCR amplification may be biased in favor of the intron-lacking repeats because they are shorter than the intron-containing repeats. Quantitative densitometry of Southern blots of restriction-enzyme-digested genomic DNA probed with rDNA sequences that flank the intron could be used to determine the relative frequency of intron-containing and intron-lacking rDNA repeats.

Thirty-three species had an 875-bp A fragment, and no products in the B, C, or D reactions, which suggests the absence of an intron at position 943 or elsewhere in the genome. The only anomalous results came from *Pleurotus tuberregium*, which had an 875-bp A product and a 1.0-kb B product. Both the C and D reactions were negative, however, and so the 1.0-kb B product of *P. tuberregium* was interpreted as a false positive that is not indicative of a group I intron homologous to those found at position 943 in *Lentinellus*, *Panellus*, and *Clavicornora*.

The PCR assay used here includes a positive control for the PCR process itself (reaction A), and three separate reactions using two different intron-specific primers and two flanking rDNA primers for the detection of introns (reactions B, C, and D). Because the assay relies on PCR, it is sensitive to mutations in the primer sites. Still, there would have to be mutations in both the 943a and 943b sites to obtain a false negative result (intron present but not detected by PCR). Nevertheless, it would be useful to probe Southern blots of digested genomic DNAs with cloned intron sequences to confirm the results obtained with PCR.

The PCR assay results indicate that there is intra-genomic as well as interlineage heterogeneity for the presence or absence of group I introns at position 943 in homobasidiomycetes. The results from *Lentinellus*, and prior observations in the lichen-forming fungus *Cladonia* (DePriest and Been 1992) and the pathogenic fungus *Pneumocystis carinii* (Liu and Leibowitz 1993), demonstrate that there can be considerable heterogeneity in the distribution and frequency of group I introns in rDNA among closely related taxa. The results from *L. ursinus* suggest that PCR amplification with conserved rDNA primers may fail to reveal intron-containing rDNA repeats when they are present at low copy number. Consequently, for studies that focus on the distribution of the introns, it is critical to use intron-specific PCR primers to assess presence or absence of introns,

especially for investigations of closely related taxa of which some are known to contain the intron. Furthermore, as discussed by Gargas, DePriest, and Taylor (1995), certain rDNA primers that are commonly used in phylogenetic studies anneal across intron insertion sites, and therefore will not amplify intron-containing rDNA repeats. Studies that use such primers will be blind to the occurrence of certain introns. Indeed, one of the rDNA primers used in this study, primer NS6, anneals across an intron insertion site that was described by Gargas, DePriest, and Taylor (1995). Thus, the results of this study do not address the presence or absence of group I introns in the NS6 primer site in homobasidiomycetes.

### Sequence Analyses of Introns

The *Lentinellus*, *Clavicornora*, and *Panellus* intron sequences were excised from the flanking rDNA sequences and manually aligned in the PAUP data editor (fig. 2). Identity of the insertion sequences as group I introns is suggested by their possession of characteristic P, Q, R, and S sequences, based on comparison to other published sequences of group I introns in nuc-ssu-rDNA of fungi (DePriest and Been 1992; Nishida and Sugiyama 1995). The P sequence of *P. stypticus* differs from the *Clavicornora* and *Lentinellus* P sequences (which are identical) by one single-base indel and three single-nucleotide differences. The Q sequence of *Clavicornora* differs from that of the other homobasidiomycetes by a single nucleotide. The R and S sequences are identical in all the homobasidiomycetes. The introns range from 374 bp in *P. stypticus*, to 400 bp in *L. montanus* and *L. ursinus*, to 414 bp in *C. pyxidata* and *L. omphalodes*. There is one region of approximately 60 bp beginning about 80 bp from the 5' end of the intron sequence that is only alignable within *Lentinellus*. Outside of this region, the *Lentinellus*, *Clavicornora*, and *Panellus* introns are alignable over approximately 270 bp, which is about 70% of the length of the sequences. Eleven indels of 1–7 bases and 85 single-nucleotide differences separate the *Panellus* intron from the *Lentinellus* and *Clavicornora* introns. The *Clavicornora* and *Lentinellus* introns differ by approximately 6 indels of 1–4 bases and 11 single-nucleotide differences. Except for several undetermined bases, the sequences of the *L. omphalodes* and *L. ursinus* introns are identical, as are those of the two isolates of *L. montanus*. There are three indels of 1, 3, and 10 bases and one single-nucleotide difference that separate the *L. omphalodes*/*L. ursinus* introns from the *L. montanus* introns.

BLAST searches under normal stringency using the *L. omphalodes* and *Panellus* introns as query sequences retrieved 20 matches (eliminating duplication, table 3), all of which are group I introns. Eight are from the nuclear large-subunit rDNA (nuc-ssu-rDNA) of ascomycete or basidiomycete fungi, three are from the nuc-lsu-rDNA of ascomycetes, eight are from the nuc-ssu-rDNA of green algae, and one is from the mitochondrial  $\epsilon$  ssDNA of the ascomycete fungus *Podospira anserina*. Four of the intron sequences retrieved by the BLAST search occur at position 943, as did the homobasidi-

**Table 3**  
**Group I Intron Sequences Retrieved by BLAST Search**

Abbreviation	Host Classification	Position <sup>a</sup>	Length (bp)	GenBank No. <sup>b</sup>
Sequences aligned to homobasidiomycete introns				
A.stip1046	<i>Ankistrodesmus stipitatus</i> Chlorophyta, Chlorococcales	1046	398	X56100
C.lute1052	<i>Chlorella luteoviridis</i> Chlorophyta, Chlorococcales	1052	618	X73998
C.sacc1512.....	<i>Characium saccatum</i> Chlorophyta, Chlorococcales	1512	444	M84319
D.salina943	<i>Dunaliella salina</i> Chlorophyta, Chlorococcales	943	381	M84320
D.parva943	<i>Dunaliella parva</i> Chlorophyta, Chlorococcales	943	397	M62998
D.parva1512	<i>Dunaliella parva</i> Chlorophyta, Chlorococcales	1512	419	M62998
H.eric1506.	<i>Hymenoscyphus ericae</i> Fungi, Ascomycota	1506	336	U06868
H.eric25S	<i>Hymenoscyphus ericae</i> Fungi, Ascomycota	nuc-lsu-rDNA	351	U06869
M.cald1506	<i>Mougeotia caldarium</i> Chlorophyta, Zygnematales	1506	409	X15763
M.scal1506.	<i>Mougeotia scalaris</i> Chlorophyta, Zygnematales	1506	535	x70705
P.cari1506.	<i>Pneumocystis carinii</i> Fungi, Ascomycota	1506	390	M86534
P.cari25Sa.	<i>Pneumocystis carinii</i> Fungi, Ascomycota	nuc-lsu-rDNA	340	L13614
P.cari25Sb.	<i>Pneumocystis carinii</i> Fungi, Ascomycota	nuc-lsu-rDNA	359	L13615
P.inou1506.....	<i>Protomyces inouyei</i> Fungi, Ascomycota	1506	393	D11377
P.inou943	<i>Protomyces inouyei</i> Fungi, Ascomycota	943	340	D11377
U.may943.	<i>Ustilago maydis</i> Fungi, Basidiomycota, Teliomycetes	943	411	X62396
Sequences retrieved but not alignable to homobasidiomycete introns				
	<i>Chlorella sorokiniana</i> Chlorophyta, Chlorococcales	323	463	x73993
	<i>Chlorella sorokiniana</i> Chlorophyta, Chlorococcales	1046	431	x73993
	<i>Chlorella mirabilis</i> Chlorophyta, Chlorococcales	1506	486	x74000
	<i>Podospora anserina</i> Fungi, Ascomycota	mitochondrial $\epsilon$ senDNA intron 1	2221	X03127 (and X55026)

<sup>a</sup> Numbers represent positions of introns of nuc-ssu-rDNA labeled according to Gargas, DePriest, and Taylor (1995), unless otherwise noted.

<sup>b</sup> Consult GenBank accessions for full reference information.

omycete introns. These include introns from the basidiomycete “corn smut” fungus *Ustilago maydis*, the ascomycete plant pathogen *Protomyces inouyei*, and two unicellular green algae in the genus *Dunaliella*. Distribution and lengths of the introns are summarized in table 3 and figure 4.

Despite the presence of several conserved regions, the mushroom introns and those retrieved by the BLAST search are too divergent to be aligned across their entire length with Clustal V. Instead, dot-plot comparisons were used to delimit the conserved regions which were excised and aligned manually and with Clustal V (fig. 2). Three of the algal introns and the *Podospora* mitochondrial  $\epsilon$  senDNA intron were so far diverged from the homobasidiomycete introns that there were no extensive regions that could be aligned, and so they were

excluded from further comparison (table 3). For the remaining introns, five blocks of sequence (blocks 2, 4, 6, 8, 10) were deemed alignable, which alternate with six other blocks that are too divergent to align (blocks 1, 3, 5, 7, 9, 11). The nuc-ssu-rDNA introns of the green algae *Characium saccatum* and *Chlorella luteoviridis* lack sequences alignable to block 6, but all other introns were alignable for all of the even blocks. The sum of the length of the five even blocks in each intron is from 127–156 bp, with an aligned length of 167 bp. The P, Q, R, and S sequences occur in blocks 2, 6, 8, and 10, respectively (fig. 2). Primer sites 943a and 943b occur in blocks 1 and 8, respectively (fig. 2).

Parsimony analysis of the sequences in the alignable even blocks resulted in 50 equally most-parsimonious trees, length = 326 steps, CI = 0.451, RI = 0.540

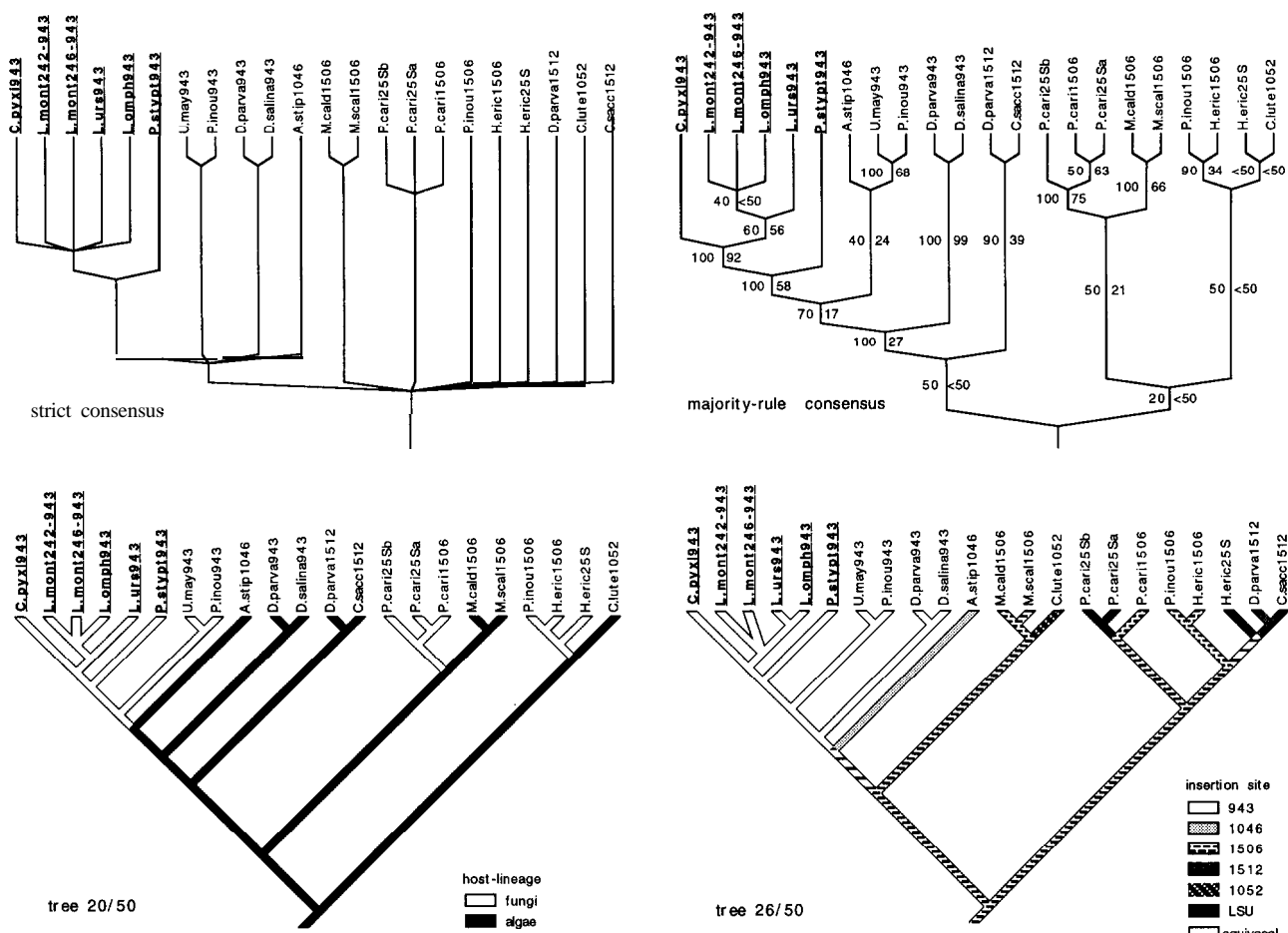


FIG. 4.—Phylogenetic hypotheses for introns based on parsimony analysis of sequence blocks 2, 4, 6, 8, and 10. Strict, majority-rule consensus, and two exemplar trees. Majority-rule consensus tree shows frequency (%) of clades in equally parsimonious trees to left of branches and bootstrap frequency (%) to right of branches. The homobasidiomycete introns are in boldface type and underlined. Trees 20/50 and 26/50 show optimizations of host-lineage or insertion position. Other equally parsimonious trees would result in optimizations that require the same or a greater number of changes in both host lineage and insertion position.

(fig. 4). The introns of the homobasidiomycetes form a monophyletic group in all of the shortest trees, but it is only weakly supported by bootstrapping (bootstrap = 58%). Nevertheless, these results suggest that the introns of the nuc-ssu-rDNA of homobasidiomycetes are more closely related to each other than to any other intron sequences. The *Panellus* intron is basal in this clade, with the monophyly of the *Clavicornia-Lentinellus* introns moderately strongly supported at 92%. The *Lentinellus* introns are monophyletic, with the *Clavicornia* intron as the sister taxon, in 60% of the equally most-parsimonious trees and 56% of the bootstrap replicates.

The distribution of insertion sites and host lineages among introns on the equally parsimonious trees may reflect the history of transposition and horizontal and vertical transmission in intron evolution. In all of the equally most-parsimonious trees, the homobasidiomycete introns are nested in a clade that includes all of the introns of position 943, eight from fungi and two from green algae, as well as an intron from position 1046 in the green alga *Ankistrodesmus stipitatus* (fig. 4). Although this clade is weakly supported (bootstrap = 27%), it suggests that the homobasidiomycete introns

are part of an intron lineage that has invaded both fungal and algal hosts and that is characterized by insertion (almost always) at position 943. Neither the algal introns or the fungal introns were supported as monophyletic in any of the equally parsimonious trees, which suggests that host lineage switching via horizontal transmission between algal and fungal hosts has occurred repeatedly in the evolution of these introns (fig. 4). The diversity of insertion positions among the introns suggests that transposition has also been a factor in intron evolution. The intron trees suggest that introns that occur at the same position are often closely related, even if they occur in different hosts. For example, all most-parsimonious intron trees agree that the *Protomyces* intron that occurs at position 943 is more closely related to other introns at position 943 than it is to the *Protomyces* intron at position 1506 (fig. 4). Similarly, the *Dunaliella parva* intron at position 943 is more closely related to other introns at position 943 (including that of *D. salina*) than it is to the *D. parva* intron at position 1512 (fig. 4). This suggests that in certain intron lineages there has been conservation of insertion position that has persisted through horizontal and vertical transmission events.

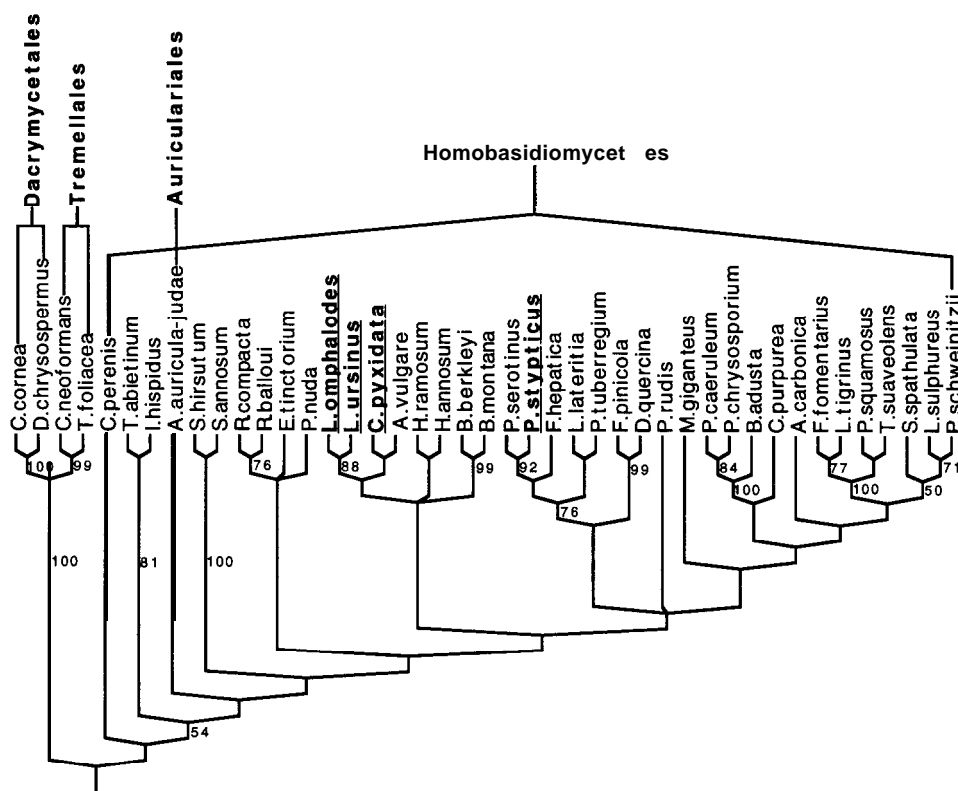


FIG. 5.—Phylogenetic hypothesis for homobasidiomycetes based on parsimony analysis of nuc-ssu-rDNA and mt-ssu-rDNA sequences. Strict consensus of six equally parsimonious trees. Intron-containing taxa are in boldface type and underlined. Bootstrap frequencies shown to right of branches. Unlabeled branches supported by fewer than 50% of bootstrap replicates.

Models of group I intron splicing involve site-specific recombination mediated by an internal guide sequence that transiently base-pairs with flanking rDNA sequences (Cech 1990). This mechanism may account for the apparent conservation of insertion sites. It should not be construed, however, that all introns that occur at a particular site are necessarily closely related, or that introns at different sites in the same organism cannot be homologous. For example, the group I intron of *Acanthamoeba lenticulata* (GenBank accession U02539) occurs at position 943 (Gargas, DePriest, and Taylor 1995), but it was not retrieved by the BLAST search and could not be aligned to the homobasidiomycete introns. In addition, all the equally parsimonious intron trees support monophyly of the *Pneumocystis* introns (bootstrap = 75%), even though they occur in both the nuc-ssu-rDNA and nuc-lsu-rDNA, which suggests that there has been a relatively recent transposition event in the *Pneumocystis* intron lineage.

Unfortunately, support for the intron phylogeny is tenuous. There is conflict among the equally parsimonious intron trees, and most bootstrap values are low. Constrained analyses that forced the monophyly of either the *Protomyces* introns, *Dunaliella* introns, or fungal (vs. algal) introns resulted in trees that could not be rejected by the Kishino-Hasegawa maximum-likelihood test, even though they are from four to six steps longer than the trees from unconstrained analyses (results not shown). Potential sources of error in the estimate of the intron phylogeny include saturation of variable sites by

multiple substitutions and incorrect hypotheses of homology due to alignment ambiguities. Convergent evolution of conserved regions of intron sequences may also be a significant source of error. If intron sequences reflect and are constrained by flanking rDNA sequences, then it is possible that unrelated introns at the same rDNA site could become similar in certain parts of their sequences through convergence. For these reasons, the estimate of the intron phylogeny, and the conclusions about intron mobility that are derived from the phylogeny, should be viewed as tentative working hypotheses to be tested through additional phylogenetic analyses as well as empirical studies of intron behavior. In the meantime, the best estimate of the phylogeny of the introns at hand suggests that the introns of position 943 of homobasidiomycetes, other fungi, and green algae are homologous. If so, then for the homobasidiomycete introns to have been transmitted strictly vertically, they must have been present in the stem species of the homobasidiomycetes; the introns could not have originated de novo within the homobasidiomycetes.

#### Phylogeny and Distribution of Introns Within Homobasidiomycetes

Unconstrained parsimony analyses of the mt-ssu-rDNA and nuc-ssu-rDNA coding sequences resulted in six equally-most-parsimonious trees of 1,515 steps (CI = 0.372, RI = 0.473; fig. 5). As observed previously in analyses of the mt-ssu-rDNA data alone (Hibbett and Donoghue 1995a), there are certain strongly supported



FIG. 6.—Two hypotheses to explain distribution of introns in homobasidiomycetes. Both trees are topologically identical to that in fig. 5. Tree A shows hypothesis under a model that permits introns to be horizontally transmitted (ACCTRAN optimization). Tree B shows hypothesis under a model that forbids horizontal transmission and postulates that intron was present in stem species of homobasidiomycetes. Gains and losses suggested under the two models are indicated along branches by G and L, respectively.

terminal clades, but many internal nodes are weakly supported, according to bootstrapping. *Lentinellus*, *Clavicornia*, and *Auriscalpium* (which lacks an intron) make up a weakly supported monophyletic group (bootstrap = 41%), with *Panellus* distantly related. The phylogeny of the intron-bearing homobasidiomycetes is, strictly speaking, congruent with the phylogeny of the introns themselves.

Because the phylogeny of the introns and their hosts do not conflict, parsimony must be used to evaluate the likelihood of horizontal transmission. Scenarios that explain the distribution of the introns on the most parsimonious trees were generated using MacClade under the two alternate models of intron transmission, which either allow or prohibit independent gain of the introns (fig. 6). Under the strictly vertical transmission

model, thirteen independent losses of the intron are required (fig. 6). Under the model that allows horizontal transmission, with ACCTRAN character state optimization, two independent gains via horizontal transmission must be inferred, one on the branch leading to *Panellus stypticus*, and another on the branch leading to *Lentinellus-Clavicornia*, as well as one loss, on the branch leading to *Auriscalpium* (fig. 6). Under DELTRAN optimization, however, the mixed horizontal-vertical transmission model requires three independent gains. Therefore, for the two models of intron transmission to be considered equally likely, 13 losses must have the same cost as 2 gains plus 1 loss, or 3 gains. In other words, to support a hypothesis of strictly vertical intron transmission in the context of the unconstrained rDNA tree, intron loss must be assumed to be at least 6-4.33 times more likely than intron gain.

The intron loss-gain bias used in the tree-based optimizations is critical for conclusions about intron mobility. Unfortunately, there is no way to estimate the actual loss-gain bias from the data at hand. In the absence of evidence for a bias toward either losses or gains, introns have been optimized onto the host topologies using equal weights. Under this weighting regime, the unconstrained tree strongly favors the horizontal transmission model (fig. 6). Empirical studies of intron transmission and heritability could provide insight into the relative probability of intron loss and gain. In addition, more detailed phylogenetic studies of the intron hosts could refine the critical value of the loss-gain bias that would be required to support either a strictly vertical or mixed horizontal-vertical model of intron transmission.

Analyses under topological constraints permitted evaluation of alternate topologies that would be more consistent with a model of strictly vertical transmission. The constrained analysis that forced monophyly of the intron-bearing taxa resulted in four equally parsimonious trees of 1,546 steps (CI = 0.364, RI = 0.456). These are 2% longer than the unconstrained trees and are considered significantly worse explanations of the data than the unconstrained trees according to the Kishino-Hasegawa maximum-likelihood test (results not shown).

It was argued above that the introns of the homobasidiomycetes, which insert at position 943, are homologous to those also found at position 943 in the fungi *Ustilago* and *Protomyces*, as well as certain green algae. If so, and if introns are transmitted only vertically, then possession of the introns must be a plesiomorphic character for the homobasidiomycetes, and the intron-bearing homobasidiomycetes could be expected to form a basal group. The constrained analysis that forced monophyly of the intron-lacking taxa resulted in six trees in which *Lentinellus*, *Clavicornia*, and *Panellus* are a basal, paraphyletic group. These trees are 1,557 steps long (CI = 0.362, RI = 0.450), which is 2.8% longer than the unconstrained trees, and are rejected by the Kishino-Hasegawa maximum-likelihood test.

Constraint trees were also used to examine the fine branching of the *Lentinellus-Clavicornia-Auriscalpium* clade. The unconstrained rDNA tree suggests that *Au-*

*riscalpium*, which has no intron, is nested within the *Lentinellus-Clavicornia* clade. This would suggest that introns were lost in the lineage leading to *Auriscalpium*. Under the topological constraint that forced *Lentinellus* and *Clavicornia* to be monophyletic, to the exclusion of *Auriscalpium*, 10 trees of 1,516 steps were found (CI = 0.371, RI = 0.473). These trees are just one step longer than the unconstrained trees, and could not be rejected according to the maximum-likelihood test. If *Lentinellus* and *Clavicornia* are monophyletic, which cannot be rejected by the rDNA data, then an intron loss on the lineage leading to *Auriscalpium* does not need to be invoked. This conclusion has significance for the interpretation of group I introns as effective "parasites"; if *Lentinellus* and *Clavicornia* are assumed to make up a monophyletic group, then there is as yet no evidence that a lineage of homobasidiomycetes, once infected, has ever been able to shed its rDNA introns.

Although the overall homobasidiomycete topology is weakly supported, maximum likelihood permitted rejection of certain alternate topologies that would have been most consistent with a hypothesis of strictly vertical transmission. The simplest explanation for the distribution of the introns is that they have been twice independently gained via horizontal transmission, once on the lineage leading to *Panellus stypticus*, and again on the lineage leading to *Lentinellus* and *Clavicornia*. It remains unclear whether an intron loss must be invoked to explain the absence of an intron in *Auriscalpium*. More detailed phylogenetic studies to assess the relationship between *Auriscalpium*, *Lentinellus*, and *Clavicornia* could help improve understanding of intron heritability on a fine scale.

The mechanism for horizontal transmission of group I introns of nuc-ssu-rDNA is not known. Many fungi, including certain homobasidiomycetes, are known to harbor viruses (van Zaayen 1979), which are perhaps the most obvious potential vectors. *Lentinellus*, *Clavicornia*, and *Panellus* are all wood-decaying fungi and they have overlapping substrate ranges (Miller and Stewart 1970; Miller 1971; Dodd 1972). It is likely that members of these genera occasionally come into intimate contact in nature as their mycelial thalli compete for wood substrates. Perhaps such close contact would facilitate horizontal transmission.

#### Significance of Group I Introns of rDNA for Phylogenetic Studies

Group I introns of rDNA will probably continue to be serendipitously discovered during molecular systematics studies. For the purpose of inferring phylogenetic relationships, group I introns of rDNA could be viewed as either a nuisance that necessitates extra effort to obtain complete rDNA sequences or a potential source of informative characters. The first interpretation suggests that PCR methods that preferentially amplify intron-lacking rDNA repeats should be pursued. Primers that anneal across intron insertion sites could expedite systematics studies that target only the rDNA coding regions, and would allow the introns to be harmlessly ignored. In contrast, if introns are sought as potential

characters, then primers that anneal across insertion sites should be strictly avoided. Furthermore, as demonstrated by our results in *L. ursinus*, if it is critical to rigorously determine intron presence or absence, then it is necessary to screen for introns using intron-specific primers.

The occurrence of both vertical and horizontal transmission of introns, and their homing properties, have implications for the use of introns as phylogenetic markers. It appears that introns can be transmitted vertically, as in the *Lentinellus-Clavicornia* clade, and so possession of a particular intron can be a property of a lineage, and thus a phylogenetically informative character. The possibility, however, that introns can move horizontally as well as vertically among lineages suggests that shared possession of introns can also be due to convergence. Because of their homing abilities, convergently acquired introns will not be distinguishable simply by virtue of their location. For example, in this study, the *Panellus* and *Clavicornia-Lentinellus* introns are inserted at precisely the same position, but the rDNA sequences suggest that they have been independently acquired. Fortunately, our observations suggest that intron sequences evolve rapidly, and so sequence analyses of introns will make it possible to identify closely related groups of introns that can be interpreted as characters of lineages. The *Lentinellus-Clavicornia* introns were easily distinguished from the *Panellus* intron by phylogenetic analysis of their sequences. The close relationship of *Clavicornia* and *Lentinellus*, which is suggested by phylogenetic analysis of rDNA coding regions, is supported by their possession of closely related rDNA group I introns. The utility of group I introns of rDNA as phylogenetic characters rests on the assumption that sequence evolution of introns significantly exceeds the rate of horizontal transmission. If this assumption is valid, then group I introns could be a useful source of independent characters to corroborate or refute phylogenetic hypotheses based on flanking rDNA coding sequences.

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