Three Divergent rDNA Clusters Predate the Species Divergence in *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L.

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*Quercus petraea* and *Quercus robur* are two closely related oak species that frequently hybridize. We sequenced 70 clones containing the 5.8S and ITS2 regions of ribosomal DNA (rDNA) from these two species and did not detect a species-specific difference. Surprisingly, three divergent (up to 12.6%) rDNA families were identified in both species, indicating that they predate the speciation event. Despite a large between-rDNA-families divergence, rDNA sequences were very similar within families, suggesting ongoing concerted evolution. Expression analysis, relative-rate tests, and mutation spectrum analyses indicated that only a single rDNA family is functional. We propose that past hybridization events, combined with nucleolar dominance, were the evolutionary processes underlying the contemporary rDNA variability in *Q. petraea* and *Q. robur."

**Introduction**

The evolutionary dynamics of tandemly repeated gene families have been a fascinating research area since their discovery. In contrast to the evolution of single genes, members of a multigene family do not evolve independently, but in a concerted manner. Hence, no, or only very little, sequence variability is observed among individual copies of the multigene family (Arneheim et al. 1980). Despite this high intraspecific homogeneity, interspecific divergence is not affected. This phenomenon has been called concerted evolution (Arneheim et al. 1980; Dover 1982). The underlying molecular processes are unequal crossing over and gene conversion, but the relative contribution of each as homogenizing agents is still debated. Irrespective of what mechanism has greater influence, the degree of homogenization is a result of the interplay between homogenization processes and novel base substitutions (Schlöterer and Tautz 1994).

The classic example of a multigene family is the DNA coding for the RNA components of the ribosome (rDNA). One interesting aspect of rDNA evolution is that in many species, e.g., *Drosophila* (Tartof and Dawid 1976), humans (Worton et al. 1988), and *Allium cepa* (Panzena et al. 1996), more than a single nucleolar organizer region (NOR) has been described. Many studies, covering a wide phylogenetic range, have suggested that concerted evolution is preventing sequence divergence among the rDNA families located at different chromosomal positions (Arneheim et al. 1980). The most convincing demonstrations of the homogenization process among rDNA families located on different chromosomes come from species with a hybrid origin. Despite diverged rDNA copies in the ancestors, the rDNA in those species was converted to a single sequence variant similar to one of the ancestral rDNA sequences (Hillis et al. 1991; Wendel, Schnabel, and Seelanan 1995a).

Regardless of small differences being reported between rDNA families located in different chromosomal positions, in a phylogenetic context the rDNA has been treated as a single-copy gene (Hillis and Dixon 1991). Insights into the functional importance of concerted evolution come from studies on the expression of rDNA genes in hybrids. Allotetraploid species, such as *Brassica napus* (Chen and Pikaard 1997; Frieman et al. 1999) and *Arabidopsis suecica* (Chen, Comai, and Pikaard 1998), still carry rDNA sequences from both progenitors, but only a single rDNA type is expressed. This phenomenon is called nucleolar dominance and has also been observed in hybrid Xenopus and Drosophila individuals (Honjo and Reeder 1973; Durica and Krider 1977). While the molecular basis of this phenomenon is not yet fully resolved, DNA methylation and histone deacetylation seem to be important, along with large chromatin structure based mechanisms (Frieman et al. 1999).

Different selective forces are acting on the rDNA region which result in varying degrees of sequence conservation across single repeat units. Consequently, each part can be employed for specific phylogenetic questions across a broad taxonomic spectrum (Hillis and Dixon 1991). Recently, particularly for plant systematics, the faster-evolving internal transcribed spacer (ITS) sequences have developed into a ubiquitous tool for phylogenetic reconstruction of closely related species. Very rarely, however, have the underlying assumptions associated with rDNA evolution been tested. We used a particularly difficult evolutionary setting—*Quercus petraea* and *Quercus robur*, two species which frequently hybridize but still form two discrete genetic entities (Muir, Fleming, and Schlötterer 2000)—to study the molecular evolution of the rDNA. Based on ITS and 5.8S sequences, we show that both oak species, *Q. petraea* and *Q. robur*, carry three divergent rDNA clusters, of which only a single one has maintained function.

**Materials and Methods**

**DNA Extraction**

Total genomic DNA was extracted from leaves using the Nucleon Phytopure Kit (Amersham) according
to the manufacturer's instructions. The internal transcribed spacer, ITS2, and 5.8S of the rDNA repeat array were sequenced from five Q. petraea individuals and two Q. robur individuals. The species were identified using morphological characters (Clapham, Tutin, and Warburg 1981).

PCR Amplification

Plant-specific primers were designed by hand from a published alignment of 18S and 28S sequences (Schlüterter 1998). To account for polymorphisms in the alignment, wobbles were incorporated to recognize all plant ribosomal targets. The primer sequences were CCT TMT CAT YTA GAG GAA GGA G for 18S and CCG CTT ATT KAT ATG CTT AAA for 28S. A 40-μl reaction was prepared with 100 ng of genomic DNA, 1.5 mM MgCl₂, 200 μM dNTPs, 1 μM of each primer, and 1 U Taq polymerase. The cycling profile consisted of an initial denaturation step of 3 min, followed by 40 cycles of 60 s at 94°C, 60 s at 56°C, and 90 s at 72°C. PCR products were blunt-ended using Klenow polymerase I (GibcoBRL) and subsequently cloned into M13mp19 (Yanisch-Perron, Vieira, and Messing 1985). Cloning and Sequencing

The standard 20-μl ligation mix contained 20–100 ng of phosphorylated PCR product, 100 ng M13mp19, 1 U T4 ligase (Promega), and 1 × T4 ligation buffer (Promega). Ligation was carried out at 18°C overnight. Clones carrying inserts were identified with blue/white selection. Sequencing templates were prepared from overnight cultures of positive clones using standard protocols. Clones were sequenced using ABI dye terminator chemistry (Perkin Elmer) according to the manufacturer's instructions and run on an ABI 377 automated sequencer.

RT-PCR

Total genomic RNA was extracted from a single Q. robur individual. Fifty milligrams of fresh leaf material was homogenized using liquid nitrogen with a mortar and pestle. The homogenate was subsequently treated with TRIzol (GibcoBRL) according to the manufacturer’s instructions. The RNA was treated with 20 U DNase I (Boehringer Mannheim) before first-strand cDNA synthesis of the 5.8S using 200 U Superscript II (Gibco-BRL). Primers were designed to amplify the full range of genomic 5.8S rDNA sequences in Q. petraea/Q. robur. Their sequences were CRA CTC TCR GCA ACG GAT A for 5.8Sf and YRT GAC ACC CAG GCA RAC for 5.8Sr. The cDNA was amplified using the same reaction conditions and cycling profile as that given for the 18S and 28S primers. The cDNA was subsequently cloned into M13mp19. To verify the absence of DNA in the RNA extraction, four templates (total genomic DNA, DNase-treated RNA, the cDNA, and a negative control) were amplified with the 5.8Sr primers using the same PCR mix. The RNA extraction was considered DNA-free, provided that PCR products were present in the correct size range for DNA and cDNA templates, but were absent in the DNase-treated RNA and negative control reactions. As an additional control, the ITS2-spanning primers 5.8f and 28S (mentioned above) were used with the same templates. DNA was considered absent only if the ITS fragment was absent in the cDNA control.

Data Analyses

Phylogeny Reconstruction

Sequences were edited in Sequence Navigator (Perkin Elmer), aligned using CLUSTAL W (Thompson, Higgins, and Gibson 1994), and manually adjusted. Phylogenetic reconstruction was carried out with the software package PUZZLE, version 4.0 (Strimmer and von Haeseler 1996), using the HKY (Hasegawa, Kishino, and Yano 1985) model of sequence evolution with eight categories of rate heterogeneity. The phylogenetic tree was graphically displayed using TREEVIEW (Page 1996). Two Fagaceae sequences from GenBank (Colombobalanus excelsa and Trigonobalanus verticillata; accession numbers AF098412 and AF098413) were used as an outgroup. Tables of polymorphic sites, including singletons, were produced with the aid of the SITES program (Hey and Wakeley 1997).

Relative-Rate Test

A two-cluster relative-rate test (Takezaki, Rzhetsky, and Nei 1995) implemented in the software package PHYLETREE, version 2.0 (Kumar 1996), was used to examine the evolutionary rate constancy of rDNA genes among the three divergent clusters. A representative of each haplotype was included in the analysis. The haplotypes were grouped on the basis of the phylogenetic analysis and the test was conducted separately for the 5.8S and ITS2 regions. Rate constancy was examined for pairs of linearized trees (Takezaki, Rzhetsky, and Nei 1995) using Kimura’s (1980) distance with rate heterogeneity. The Gamma distribution shape parameter, alpha (1.41), was estimated from the data set using PUZZLE, version 4.0.

Methylation-Related Substitutions

Deamination-like substitutions (C→T and G→A) were examined at cytosine sites on both strands of the 5.8S and ITS2 sequences. Possible sites of methylation (CpG or CpNpG; Gardiner-Garden, Sved, and Frommer 1992) were determined for the reconstructed ancestral sequence at internal node I (fig. 1). The ancestral character states were inferred using maximum likelihood in PAUP, version 4.0b3a (Swoford 1999), using the Fagaceae sequences as the outgroup. Putative methylation sites present in the ancestral sequence were considered for the analysis. To test whether the putative pseudogenes (clades P1 and P2) showed an elevated rate of deamination-like substitutions, we counted for each clade separately the number of sites showing at least one deamination-like substitution. These observations were compared with the remainder of the sequence, which did
not contain methylation sites. We counted for each group separately those sites that had a mutation in at least one haplotype. Significance levels were determined using a $G$-test with Yates’ correction in a $2 \times 2$ contingency table.

Base Substitutions at Conserved Sites

The 5.8S region of the *Q. petraea/Q. robur* PCR clones was compared with 5.8S sequences derived from a diverse range of species and biological classes. Based on the alignment of 50 5.8S sequences, including vertebrates, invertebrates, fungi, and plants, we determined those sites that were absolutely conserved in this alignment. For those invariant sites, we determined the number of mutations that were observed in oaks for all three clades.

Results

PCR

On an agarose gel, the PCR product obtained from genomic DNA yielded a single band of approximately 720 bp. To verify that the plant-specific primers were amplifying ribosomal sequences from oaks, we conducted a GenBank search with the sequence of a cloned PCR product and detected significant homology with other Quercus sequences. Two adjacent runs of cytosines in the ITS1 prevented this spacer from being sequenced reliably. Therefore, the ITS1 was subsequently excluded from the analysis. Despite the C-rich region, the PCR primers amplified both ITS regions.

Phylogeny Reconstruction

Phylogenetic analysis of the combined 5.8S and ITS2 regions indicated the presence of three highly diverged clades. The clades were supported by quartet
ITS Evolution in Oaks

Fig. 2.—Polymorphic sites between the surveyed Quercus petraea and Quercus robur clones. Haplotypes are shown for (a) 5.8S (161 bp) and (b) ITS2 (228 bp). The letters A±L correspond to those in figure 1. Invariant sites are marked with dashes, and stars indicate indels.

Polymorphism

Seventy PCR clones, originating from five Q. petraea individuals and two Q. robur individuals, were sequenced for the 5.8S and ITS2 regions. The average pairwise differences (π) between the sequences were 0.054 for the 5.8S region and 0.092 for the ITS2 region. The higher variability of the ITS region was also reflected in the number of observed haplotypes. Eight distinct haplotypes were observed in the 5.8S region, and 12 were observed in the ITS2 region (fig. 2a and b).

Within clades, very little sequence variability was detected among the clones, with many identical sequences. This observation strongly suggests high rates of concerted evolution within each clade, despite the large divergence between the three clades.

Recombination

Two sequences obtained from the same PCR reaction showed evidence for recombination between two clades. As both sequences had the recombination break at the same position, we regarded them as the outcome of jumping PCR (PaÈab, Irwin, and Wilson 1990) and excluded them from the analysis. Visual inspection indicated that haplotypes (Q. petraea J±L) share some sites with the two other clades, which may indicate recombination (fig. 2b). In contrast to the excluded sequences, the haplotypes were obtained from two different oak trees and carried specific mutations. Thus, we regard them as true recombinants.

Functionality Tests

The presence of three divergent rDNA genes in oaks raised the question of whether all three sequences are functional. To address this question, we conducted four different types of analyses (relative-rate tests, meth-
ylation-mutation analysis, evolutionary comparisons, and cDNA analysis) to determine the functional status of these genes.

Relative-Rate Test

Assuming that all three rDNA lineages have maintained their function, they should be exposed to similar evolutionary constraints and thus show similar rates of evolution. If some of them have lost function, then these lineages are expected to show an elevated rate of evolution. To discriminate between these two alternative hypotheses, we conducted a relative-rate test. The results in table 1 show a significantly lower substitution rate for the F lineage (denoted as functional) than for both the P1 and P2 lineages (denoted as pseudogenes). Rate constancy can be rejected at the 1% level for both the P1 and the P2 lineages (denoted as pseudogenes). Rate constancy cannot be rejected between the two pseudogenes. However, rate constancy cannot be rejected between the two pseudogenes in the spacer region.

Mutation Spectrum Analyses

The observed elevated mutation rate in clades P1 and P2 is suggestive of a pseudogene status, but we cannot rule out that those clades are located in a genomic region which is exposed to an elevated mutation rate. To support the hypothesis that P1 and P2 have lost function, we compared the mutation spectra in all three clades.

Methylation-mutation analysis.—Pseudogenes are often characterized by cytosine mutations at methylation sites (Li, Wu, and Luo 1984). If P1 and P2 are pseudogenes but F remains active, then the pseudogenes should show an elevated frequency of cytosine mutations at methylation sites. A G-test with Yates’ correction was used to investigate whether deamination-like base substitutions were equally distributed among all clades. Table 2 shows a significantly higher frequency of deamination-like base substitutions at methylation sites in the P1 lineage compared with F, but not in the P2 lineage. Most likely, the P2 lineage has also lost function because the same elevated pattern of deamination-like substitutions present in P1 is clearly visible despite not being statistically significant.

Analysis of conserved sites.—We used a set of 50 5.8S sequences covering a wide phylogenetic range (including vertebrates, invertebrates, fungi, and plants) to determine those sites that were highly constrained, i.e., did not show a single base substitution in the alignment. In total, we detected 21 sites that were completely conserved in all 50 species. If all three rDNA clades in Q. petraea/Q. robur were functional, they should also have followed this pattern. Indeed, sequences from the F clade possessed only one mutation in the highly constrained sites. Interestingly, this substitution was detected in only a single clone and may be a PCR artifact. In contrast, both P1 and P2 had several (5–10) base substitutions in the evolutionarily conserved sites. Thus, the high number of substitutions (up to 10) at sites that were found to be invariant over a wide phylogenetic range suggest that P1 and P2 have lost function.

Additional evidence for the expression of clade F and for the loss of function in clades P1 and P2 comes from cDNA analysis. Sequence variation in the 5.8S sequences allowed us to discriminate between the three clades, even if only a short fragment of the 5.8S region was amplified. Twelve cDNA clones were sequenced, and all showed 100% sequence identity with the most frequent clone present in clade F. No clones were detected from clade P1 or P2.

Assuming that all three rDNA genes are functional, equally likely to be cloned, and represented in the genome according to the frequencies cloned, the probability of obtaining 12 clones from clade F is (20/70)12, or 2 × 10−7. In other words, the chances of yielding cDNA clones from the same clade are extremely small. Hence, our cDNA analysis also suggests that P1 and P2 are not expressed.

Table 1

<table>
<thead>
<tr>
<th>NODE</th>
<th>A</th>
<th>B</th>
<th>bA</th>
<th>bB</th>
<th>δ</th>
<th>Z SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.8S</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I . . . F (P1, P2)</td>
<td>0.0013</td>
<td>0.0795</td>
<td>−0.0782</td>
<td>4.0003**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0.0014</td>
<td>0.0385</td>
<td>−0.0371</td>
<td>2.2603*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0.0012</td>
<td>0.1041</td>
<td>−0.1029</td>
<td>3.5544**</td>
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<td></td>
</tr>
<tr>
<td>II . . . P1 P2</td>
<td>0.0351</td>
<td>0.1010</td>
<td>−0.0658</td>
<td>2.0472*</td>
<td></td>
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</table>

Table 2

<table>
<thead>
<tr>
<th>Clade</th>
<th>Obtained in Comparison with the F Clade</th>
<th>G Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 . . . .</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>P2 . . . .</td>
<td>37</td>
<td>17</td>
</tr>
</tbody>
</table>

* Significant at the 5% level.
Discussion

Quercus petraea and Q. robur are two closely related oak species which frequently hybridize. Nevertheless, informative markers, such as microsatellites, clearly demonstrate that these two species are discrete taxonomic units (Muir, Fleming, and Schlötterer 2000). In contrast to these observations, we could not detect a difference between ITS sequences from Q. petraea and Q. robur. The two species share an identical rDNA composition, most likely because the split between the two species is too recent to be detected with ITS sequences. This result is in sharp contrast to a study of white oaks, where rDNA sequences differentiated among species despite ongoing hybridization (Whittemore and Schaal 1991).

The lack of a significant difference between species is surprising, as we detected three divergent rDNA clades in both species. This observation clearly violates the general rule of concerted evolution in rDNA families located on different chromosomes. The observed average divergence between the three rDNA families is too high to be explained by different homogenization rates between homologous and nonhomologous rDNA copies, as previously suggested (Schlöterer and Tautz 1994; Sang, Crawford, and Stuessy 1995). Assuming a mutation rate of $5 \times 10^{-9}$ (Wolfe, Li, and Sharp 1987) in the ITS2, we estimate that the three rDNA families in Q. petraea and Q. robur have been separated for about 13–17 Myr (for clades P1 and P2, respectively).

Some evidence for the presence of more than a single NOR in Q. petraea and Q. robur stems from fluorescence in situ hybridization. Zoldos et al. (1999) detected two NORs located on different chromosomes. Interestingly, most of the hybridization signal was detected at one NOR. The other NOR hybridized only weakly and could not be detected in some chromosome preparations. This observation contrasts markedly with our cloning of genomic rDNA sequences, as we detected three rDNA families, which were represented at similar frequencies (fig. 1). This discrepancy may be explained by a strong amplification and cloning bias in our experiments or by difficulties with chromosomal preparations from oaks. An alternative explanation of how the in situ hybridization data could be reconciled with our sequence analysis is provided by a recent study of Arabidopsis thaliana. Cloix et al. (2000) demonstrated that functional and nonfunctional 5S sequences are interspersed in an array and concerted evolution continues despite this arrangement. Accordingly, functional, and nonfunctional rDNA sequences may be interspersed in oaks. Further studies are required to resolve this question.

Intensive studies of the evolutionary dynamics of rDNA gene families in hybrids have revealed three different outcomes regarding the presence of diverged NORs. (1) The rDNA families are converted to a single sequence type. The rDNA evolution in allopolyploid cotton species is a particularly good example of this mechanism (Wendel, Schnabel, and Seelanan 1995a). (2) Rather than converting the rDNA family to one type, gene conversion and recombination could generate chimeric molecules, which will most likely be homogenized to a single rDNA molecule, with a sequence intermediate between the two ancestral sequences (van Houten, Scarlett, and Bachmann 1993; Wendel, Schnabel, and Seelanan 1995b). (3) One of the two rDNA families present in hybrids is silenced, and only one copy remains functional (Chen, Comai, and Pikaard 1998; Frieman et al. 1999).

In Q. petraea and Q. robur, we demonstrated a very high level of sequence homogeneity within each rDNA cluster, suggesting ongoing concerted evolution. Nevertheless, between the three rDNA families, extensive sequence divergence was detected, indicative of genetic isolation of those three rDNA families. Reverse transcription implied that only one of the three clades was transcribed, and so the situation resembles the phenomenon of nucleolar dominance, which is well documented for allopolyploid species and hybrids. Previous studies on A. cepa also detected a lack of expression of some of the rDNA clusters, together with some sequence divergence between the rDNA clusters (Panzera et al. 1996). Therefore, even in a species that is not described as allopolyploid, nucleolar dominance occurs. While the situation for oaks resembles that for A. cepa, such a large sequence divergence between rDNA clusters is unusual (but see Hugall, Stanton, and Moritz 1999) and raises the question of whether all rDNA copies have retained functionality.

Further insight comes from a relative-rate test performed on the rDNA sequences from the two oak species. Compared with the functional gene family, we detected a significantly elevated rate of sequence change in the two rDNA gene families which are not expressed. More importantly, 5.8S sequences are affected by this increased rate of evolutionary change, suggesting that these 5.8S genes have lost their functional constraints and evolve as pseudogenes. While it is possible that a local increase in mutation rate has caused this effect, three lines of evidence argue in favor of the pseudogene hypothesis. First, the less constrained ITS sequences do not show the effect of rate heterogeneity as strongly as the 5.8S sequences, indicating that the mutational dynamics changed more dramatically for the 5.8S region. Second, the ratio of ITS2 and 5.8S within-clade variability is higher in the functional clade, suggesting higher sequence conservation in the 5.8S region of the functional clade (table 3). Finally, sites in the 5.8S sequence that are highly conserved over a wide phylogenetic range are more conserved in the functional clade than in the clades containing the putative pseudogenes. Hence, combining all lines of evidence, we conclude that nucleolar dominance is not suppressing the expression of two of the three rDNA clades in Q. petraea and Q. robur. Rather, these sequences have lost functionality and should be regarded as pseudogenes.

It remains speculative, however, why the diverged rDNA copies were not homogenized. Two different scenarios for the presence of more than one rDNA type in oaks are conceivable. First, gene duplications created supernumerary rDNA copies and a lack of genetic ex-
change between old and new rDNA loci would, in the long run, result in diverged rDNA clades. The difficulty with this scenario is to envisage which process(es) may prevent genetic exchange between different rDNA loci but still maintain concerted evolution within the rDNA loci. The second scenario to explain the presence of three highly diverged rDNA families in oaks could be ancient hybridization events which predate the emergence of *Q. petraea* and *Q. robur* bringing diverged rDNA families together. While concerted evolution may be very effective if the rDNA families are not highly diverged, it has been found for mammals that less than 1% sequence divergence is enough to reduce the rate of genetic exchange by a factor of 20 (Modrich and Lahue 1996). Since the mismatch repair machinery, which is responsible for this phenomenon, seems to be sensitive to remote mismatches (several hundred bases), high divergence in the spacer regions would be sufficient to suppress recombination across the entire rDNA array.

Support for such a scenario being realistic comes from suppress recombination across the entire rDNA array. Convergence in the spacer regions would be sufficient to prevent mispairing (several hundred bases), high divergence responsible for this phenomenon, seems to be sensitive to remote mismatches (several hundred bases), high divergence in the spacer regions would be sufficient to suppress recombination across the entire rDNA array.

In this study, we demonstrated that within a single individual, multiple rDNA copies exist which are quite divergent. Direct sequencing of PCR products would probably have resulted in an unreadable sequence. Assuming that the PCR products are cloned and a single clone is sequenced from each species, several different phylogenetic reconstructions could have been obtained (depending on which of the three clades was included in the analysis). Consequently, phylogenetic reconstruction based on ITS sequences may bear some intrinsic risks, but if the evolutionary dynamics of this multigene family are included in the interpretation and acquisition of the data, ITS sequences could still provide a useful tool for phylogenetic reconstruction.

### Supplementary Material

In addition to the outgroup, all seventy sequences have been deposited in GenBank as an alignment (accession numbers AF248354–AF248425). The alignment of 50 5.8S sequences and *Q. petraea*/*Q. robur* PCR clones can be accessed at http://i122server.vu-wien.ac.at.

### Acknowledgments

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### Literature Cited


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**Table 3**

<table>
<thead>
<tr>
<th>Region</th>
<th>Clade</th>
<th>Average Pairwise Distance Between Clones</th>
<th>Within-Clade Average Pairwise Distance Ratio (ITS2/5.8S)</th>
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</thead>
<tbody>
<tr>
<td>5.8S</td>
<td>F</td>
<td>0.000624</td>
<td>2.91</td>
</tr>
<tr>
<td></td>
<td>P1</td>
<td>0.002568</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>0.007794</td>
<td>1.69</td>
</tr>
<tr>
<td>ITS2</td>
<td>F</td>
<td>0.001817</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P1</td>
<td>0.003166</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>0.013168</td>
<td></td>
</tr>
</tbody>
</table>

*Note:* Distances were calculated using maximum likelihood with the HKY model of sequence evolution.


Kumar, S. 1996. PHYLTEST: phylogenetic hypothesis testing, version 2.0. Pennsylvania State University, University Park.


Elizabeth Kellogg, reviewing editor

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