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Phylogeny, Biogeography, and Processes of Molecular Differentiation in *Quercus* Subgenus *Quercus* (Fagaceae)

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Quercus **is one of the most abundant and economically important genera of woody plants in the Northern Hemisphere. To infer phylogenetic relationships within *Quercus* subgenus *Quercus,* chloroplast DNA (cpDNA) restriction sites and nucleotide sequences of the internal transcribed spacers (ITS) and the 5.8S coding region of the nuclear ribosomal DNA repeat were obtained for 44 individuals, including 25 species, intraspecific samples, and three outgroups. Separate parsimony analyses of each data set showed that individual gene trees were congruent and often complementary in supporting clades that generally corresponded to previously recognized taxonomic groups. Only one instance of strongly supported gene tree incongruence was detected and this anomalous pattern was explained best by ancient introgression of cpDNA across sectional boundaries. Simultaneous parsimony analysis of the pruned data sets supported the recognition of the strictly Eurasian section *Cerris* and resolved a novel hypothesis for the major infrageneric groups (*Cerris*- (*Lobatae*- (*Protobalanus* + *Quercus sensu stricto*))). The biogeographic hypothesis that all major oak lineages evolved locally at middle latitudes within the general distribution of their fossil ancestors was fully supported. This set of relationships also suggested a New World origin for the widespread white oaks of the Northern Hemisphere (section *Quercus s. s.*). For both data sets, inter- and intraspecific sampling within section *Protobalanus* showed little correspondence to morphological species. Greater cladistic structure among the samples was obtained by cpDNA restriction sites and two well-delimited plastomes types comprising a total of 15 distinct haplotypes were resolved. Haplotypes of 2 of the peripheral species in this species complex occupy terminal portions of one of the plastome clades, suggesting a more recent origin relative to those of more widespread species. The phylogeography of the two divergent plastome types suggested a north–south pattern, consistent with a Late Tertiary disjunction in the ancestral distribution of section *Protobalanus.***

INTRODUCTION

The genus *Quercus* comprises approximately 500 species of trees and shrubs distributed throughout much of the Northern Hemisphere (Nixon, 1993). Oaks are conspicuous members of the temperate deciduous forests of North America, Europe, and Asia, in addition to being important evergreen elements of Mediterranean woodlands and subtropical forests. The largely temperate species of subgenus *Quercus* (Fig. 1) are distinguished from the strictly southeast Asian members of subgenus *Cyclobalanopsis* by several characters, notably the presence of expanded stigmatic surfaces on the pistillate flowers and small, inconspicuous bracts which subtend single-staminate flowers (Nixon, 1985, 1993). The Middle and Late Tertiary fossil record of North America and Asia clearly establishes the long-term abundance of subgenus *Quercus* and provides a framework for considering the historical biogeography of the group (for review see Axelrod, 1983; Crepet, 1989; Crepet and Nixon, 1989a; Zhou, 1993). Leaf impressions, some with clear affinities to modern species, establish a minimum age of 40 million years for several of the major oak groups recognized today.

Oak species are well known for their taxonomically perplexing patterns of intraspecific morphological variation which may be due, in part, to hybridization (e.g., Trelease, 1924; Palmer, 1948; Muller, 1952; Tucker, 1961; Hardin, 1975; Rushton, 1993; Spellenberg, 1995; Bacilieri *et al.,* 1996; Howard *et al.,* 1997). Hybridization in the oaks has been used to argue for alternatives to the biological species concept (e.g., Burger, 1975), as well as to suggest a mechanism by which species can adapt genotypically to a changing ecological landscape (van Valen, 1976). The hypothesis of hybridization between oak species is supported by various types of observation (see Bacilieri *et al.,* 1996). DNA-based evidence of gene flow between species was first reported on the basis of shared patterns of chloroplast DNA (cpDNA) haplotypes in sympatric populations of white oak species (Whittemore and Schaal, 1991). Additional studies of cpDNA from a broad sampling of populations

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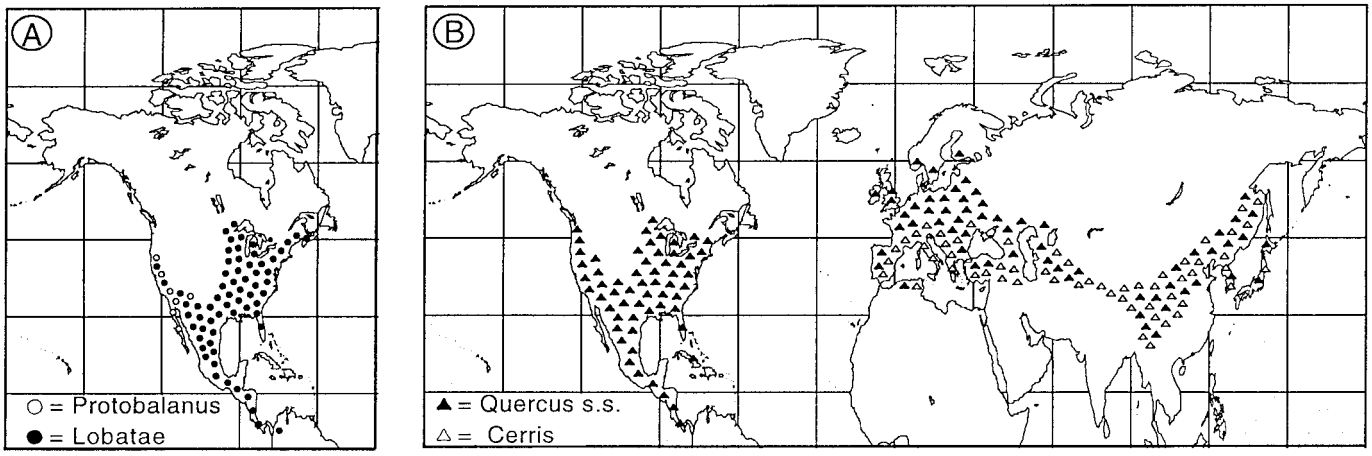

FIG. 1. Generalized distribution of *Quercus* subgenus *Quercus* in the Northern Hemisphere modified from Camus (1936–54), Soepadmo (1972), and Nixon (1985). (A) The distribution of New World sections *Lobatae* and *Protobalanus;* (B) the distribution of section *Quercus sensu* Nixon, emphasizing the distinction between the two groups, *Quercus s.s.* and section *Cerris* (Camus, 1936–54).

of two western European white oak species suggested that geographical patterning and discontinuities among haplotypes also could be explained by geological barriers (Ferris *et al.,* 1993) and postglacial migration via long-distance dispersal (Petit *et al.,* 1993a, 1997). Although it is well known that sterility barriers between oak species are poorly developed, hybridization appears to be limited to species that belong to the same major group or section within the genus (Stebbins, 1950; Grant, 1981; Cottam *et al.,* 1982).

These findings suggest that phylogenetic studies of organellar DNA among interfertile oak species are certain to be compromised by processes acting at the population level, such as introgressive hybridization and lineage sorting, well-documented sources of error in the reconstruction of species relationships (Neigel and Avise, 1996; Whittemore and Schaal, 1991; Doyle, 1992; Rieseberg and Wendel, 1993; Rieseberg *et al.,* 1996). Nonetheless, the broad boundary between apparently hierarchic relationships and interbreeding in the oaks presents an interesting opportunity to compare gene trees derived from both organellar and nuclear DNA at various taxonomic levels across the phylogenetic breadth of subgenus *Quercus.* The major goal of this study is to use independent gene trees to infer organismal relationships or the "species tree" in the oaks.

In the genus *Quercus,* phylogenetic relationships based on morphology are somewhat obscure, largely due to pronounced vegetative variation (Tucker, 1974), which is sharply contrasted by a stabilized, seemingly constant set of floral characters. The primary sources of evolutionary trends and phylogenetic characters in *Quercus* are the pistillate flower and fruit with subtending cupule (acorn and cup; Kaul, 1985; Nixon, 1985, 1993), although previous classifications never employed them explicitly (Trelease, 1924; Camus, 1936–1954; Schwarz, 1936, 1937). The most current classification of *Quercus* (Fig. 2; Table 1; Nixon, 1993), the first to be based on explicit morphological cladistic analysis, recognized fewer infrageneric groups than previous classifications. Within *Quercus,* two subgenera were recognized, *Cyclobalanopsis* and *Quercus,* the latter comprising three sections: *Lobatae* (red oaks), *Protobalanus* (golden cup or intermediate oaks), and *Quercus* (white oaks). In this phylogenetic treatment, section *Quercus* corresponded to the white oaks in the broadest sense, defined by the synapomorphy of the basal position of the abortive ovules on the surface of the developed seed. Included here were the widespread white oaks of North and Central America and Eurasia (section *Quercus s.s.,* e.g., *Q. alba, Q. robur, Q. virginiana*), species with a preponderance of derived characters, and several groups with many plesiomorphic characters limited to Eurasia, some of which have been recognized at the sectional or subgeneric level in previ-

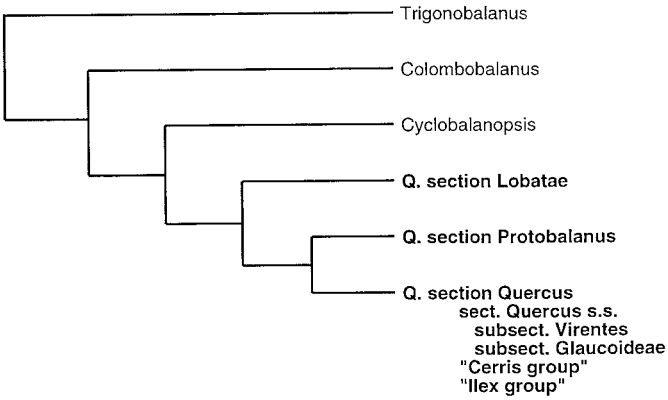

FIG. 2. Phylogenetic relationships within subgenus *Quercus* based on morphological data. (Nixon, 1985, 1989, 1993). Boldface taxa represent *Quercus* subgenus *Quercus.*

ous taxonomic treatments (e.g., section *Cerris* sensu Camus, see Fig. 1; subgenus *Sclerophyllodrys* sensu Schwarz). Phylogenetic character analysis of morphology also suggested that section *Lobatae* had the most plesiomorphic characters within subgenus *Quercus* and thus lacked the synapomorphies which united the sister sections *Quercus* and *Protobalanus* (Nixon, 1985, 1993).

In terms of phylogeny and biogeography, section *Protobalanus* is the most interesting of the American oaks. Trelease's (1924) treatment of American oaks depicted section *Protobalanus* as ancestral to both the North American members of section *Quercus* and section *Lobatae* on the basis of an apparent intermediate morphology. Speculation concerning the relationships of section *Protobalanus* has also included a possible reticulate origin (Nixon, 1985). The distribution of section *Protobalanus* (see Figs. 1 and 4) and phenetic similarity to certain species of Eurasian oaks (e.g. section *Cerris sensu* Camus) also raises the possibility of a relationship to sclerophyllous oaks of the Old World. Alternatively, additional evidence for a relationship to New World oaks would support Axelrod's (1983) hypothesis that the major lineages of *Quercus* evolved *in situ*, with localized radiations and rare instances of monophyletic groups with transcontinental distributions.

Since there are a limited number of morphological characters available from reproductive structures for constructing a phylogenetic hypothesis within subgenus *Quercus*, molecular data provide an independent test of morphological homologies to gain insight into the evolutionary history of this challenging plant genus. The specific objective of this study is to investigate how well phylogenetic trees of chloroplast DNA restriction site data and sequence data from the internal transcribed spacers of nuclear ribosomal DNA (ITS region) correspond to morphologically based hypotheses. Intersterility between many of the previously recognized taxonomic groups of oaks (Cottam *et al.*, 1982) provides additional evidence for comparing morphologically and reproductively defined groups with those revealed by phylogenetic analysis of molecular data. Comprehensive sampling within these groups, with emphasis on the five allopatric species of section *Protobalanus*, will also address the systematic utility of nuclear ITS sequences and cpDNA RFLP variation.

MATERIALS AND METHODS

Taxon Sample

Leaf material for 44 terminal taxa was collected from naturally occurring populations or cultivated plantings. The names, authorities, sources, geographic distribution, and GenBank accession numbers are listed in Table 1. *Quercus* subgenus *Quercus* was represented by 25 species including at least 4 species from each of the currently recognized sections and several of the previously recognized sections of other authors. *Quercus* section *Protobalanus*, the subject of previous systematic studies (Manos, 1992, 1993), was represented by 21 accessions including at least three individuals from each of the 5 morphologically defined species. Outgroup taxa were chosen on the basis of previous phylogenetic studies within Fagaceae (Nixon, 1985, 1989; Nixon and Crepet, 1989; Crepet and Nixon, 1989b; Manos *et al.*, 1993; Manos and Steele, 1997).

Chloroplast DNA

Methods of DNA isolation, restriction digests, electrophoresis, Southern transfers, hybridization, and autoradiography were identical to those outlined by Manos *et al.* (1993). The following 14 endonucleases that recognize six-base pair restriction sites were used: *Ase*I, *Bam*HI, *Bcl*I, *Bgl*II, *Bst*BI, *Cla*I, *Dra*I, *Eco*RI, *Eco*RV, *Hinc*II, *Hind*III, *Kpn*I, *Nde*I, and *Nsi*I. A survey of cpDNA variation of 44 terminal taxa was conducted using 40 cloned fragments of a *Nicotiana tabacum* cpDNA library provided by J. D. Palmer (see Olmstead and Palmer, 1992, for coordinates and sizes of cloned fragments). The size of the chloroplast genome in Fagaceae was calculated by using the approximate additive size of all fragments from restriction site maps constructed using *Eco*RI and *Eco*RV. Inheritance of cpDNA in *Quercus* is known to be maternal (Dumolin *et al.*, 1995).

Restriction site maps of the cpDNA of each species were constructed for each enzyme from single-digest phenotypes. Inferred restriction site mutations were scored as presence and absence character states; there were no missing data.

nrDNA ITS Sequences

Double-stranded amplifications from the same genomic DNAs were performed for the complete ITS 1, 5.8S gene, and ITS 2 (ITS region) using the primer pairs ITS 5/ITS 4 following the methods of Baldwin (1992) with modifications to account for equal volumes of each 10 μol/L primer. For all Fagaceae, single-banded PCR products of approximately 619 bp were recovered. These products were immediately cloned using the TA Cloning Kit available through Invitrogen. Plasmids were purified following modification of standard alkaline lysis methods and screened for presence of the ITS region by PCR using the ITS 5 and ITS 4 primers. A single plasmid stock per taxon was then diluted approximately and sequence reactions were prepared using the two amplification primers following the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit. Sequences of both strands were determined for all taxa using an Applied Biosystems 373 DNA Sequencer. Double-stranded sequences were edited using the program SEQUENCHER 3.0 (Gene Codes Corp.).

TABLE 1

Outgroups and Species of Subgenus *Quercus* Surveyed in this Study

Taxa	Distribution	Source	GenBank Accession No.
Outgroups			
Trigonobalanus verticillata Forman (RBG)	Malaysia	RBG 1967-421	AF098413
Colombobalanus excelsa (Lozano, Hdz-C. J & Henao) Nixon & Crepet	Colombia	K. Nixon 4655	AF098412
Cyclobalanopsis myrsinaefolia Blume. (CRS)	Asia	PSM s.n.	AF098414
Ingroup			
subgenus *Quercus*			
section *Lobatae* Louden.			
Q. agrifolia Nee.	w. N. America	PSM 542	AF098415
Q. kelloggii Newb.	w. N. America	PSM 123	AF098416
Q. palustris Muench. (CUP)	e. N. America	PSM s.n.	AF098417
Q. rubra L.	e. N. America	PSM s.n.	AF098418
section *Quercus*			
Q. alba L.	e. N. America	PSM s.n.	AF098419
Q. lobata Nee.	w. N. America	PSM 999	AF098422
Q. robur L. (CUP)	Europe	PSM s.n.	AF098424
Q. rugosa Nee.	s. w. N. America	PSM 570	AF098425
Q. turbinella Greene. (UCD)			
subsection Glaucoideae	s. w. N. America	J. Tucker s. n.	AF098423
Q. engelmannii Greene.	s. w. N. America	PSM 212, 213	AF098420
Q. laeta Liebm.			
subsection *Virentes*	s. w. N. America	PSM 563	AF098421
Q. geminata Small.	s. e. N. America	L. Robbins s.n.	AF098426
Q. virginiana Mill.	s. e. N. America	T. Engstrom s.n.	AF098427
"*Cerris* group"			
Q. acutissima Carruth. (CUP)	Asia	PSM s.n.	AF098428
Q. cerris L. (UCD)	Eurasia	PSM 935	AF098430
Q. phillyraeoides Gray. (UCD)	Asia	PSM 936	AF098433
Q. suber L. (UCR)	Eurasia	PSM 423	AF098434
"*Ilex* group"			
Q. calliprinos Webb. (UCD)	e. Europe	PSM 933	AF098429
Q. coccifera L. (UCD)	e. Europe	PSM 931	AF098431
Q. ilex L. (UCSB)	w. Europe	PSM 412	AF098432
section *Protobalanus* (Trelease) A. Camus			
Q. cedrosensis Muller.			
A. Sierra San Pedro Martir, Baja	Mexico	PSM 738	AF098449
B. Santo Tomas, Baja	Mexico	PSM 716	AF098450
C. Cerro Colorado, Baja	Mexico	PSM 732	AF098451
Q. chrysolepis Liebm.			
A. La Crescenta, Los Angeles Co.	California	K. Nixon s.n.	AF098438
B. E. of Hamburg, Del Norte, Co.	California	PSM 954	AF098439
C. Oak Creek Canyon, Coconino Co.	California	PSM 771	AF098440
D. Sierra San Pedro Martir, Baja	Mexico	PSM 744	AF098441
E. E. Point Reyes, Marin Co.	California	PSM 965	AF098442
F. Chirachua Mts., Yavapai Co.	Arizona	PSM 766	AF098443
G. Hualapai Mts., Mojave Co.	Arizona	PSM 603	AF098444
H. Mt. St. Helena, Sonoma Co.	California	PSM 906	AF098445
Q. palmeri Engelm.			
A. Peachy Canyon, San Louis Obispo Co.	California	K. Nixon 4590	AF098446
B. Oak Creek Canyon, Coconino Co.	Arizona	PSM 777	AF098447
C. Garner Valley, Riverside Co.	California	PSM 602	AF098448
Q. tomentella Engelm.			
A. San Clemente Island, San Diego Co.	California	PSM 684	AF098435
B. Anacapa Island, Ventura Co.	California	PSM 545	AF098436
C. Santa Cruz Island, Santa Barbara Co.	California	PSM 983	AF098437
Q. vaccinifolia (Kell.) Curran			
A. near Echo Lake, El Dorado Co.	California	PSM 909	AF098452
B. Echo Lake, El Dorado Co.	California	PSM 914	AF098453
C. Scott Mt., Trinity Co.	California	PSM 945	AF098454
D. Gold Lake, Sierra Co.	California	PSM 962	AF098455

Note. Classification follows Nixon (1985, 1993) and includes informal designations for certain Eurasian species groups treated within section *Quercus*. Collections from cultivated plantings are coded as follows: CUP = Cornell University Plantations, NY; CRS = USDA Coastal Research Sation, Savannah, GA; AA = The Arnold Arboretum, MA; RBG = Royal Botanic Garden, Edinburgh, UK; UCD = Shields Grove, University of California at Davis Arboretum, CA; UCSB = University of California at Santa Barbara Campus; UCR = University of California at Riverside Campus. All other samples were collected from natural populations. Unless otherwise specified, vouchers are P. S. Manos collection numbers (BH). A general geographic distribution is provided for most species and specific locations are given for *Quercus* section *Protobalanus*.

The boundaries of the internal transcribed spacers (ITS 1, ITS 2) and nrDNA coding regions in the 44 taxa included here were determined by comparison to several published sequences obtained from a range of angiosperms (Yokota *et al.*, 1989; Venkateswarlu and Nazar, 1991; Baldwin, 1992; Wojciechowski *et al.*, 1993; Manos, 1997). The ITS sequences also were evaluated for several standard descriptive parameters, including size, percentage G + C content, percentage pairwise divergence, percentage of aligned sites with gaps, and percentage of phylogenetically informative sites with gaps.

All ITS DNA sequences were aligned visually by first comparing sequences from species groups of subgenus *Quercus* reported to be closely related on the basis of morphological evidence. Once these alignments were determined, more divergent groups of sequences were compared until all sequences of subgenus *Quercus* and outgroup sequences were aligned.

Phylogenetic Analyses

Two data matrices were analyzed phylogenetically using PAUP version 3.1.1 (Swofford, 1993), PAUP* version 4d64 (Swofford, 1998), and NONA version 1.6 (Goloboff, 1997). Maximum parsimony analyses were conducted on both data sets using the following heuristic search strategies: (1) PAUP runs consisted of 1000 replicates of random-order-entry with TBR and MULPARS; (2) NONA tree searches involved 1000 random order sequences to produce Wagner trees followed by SPR and TBR, holding one tree at each replication. All trees from each replicate were swapped to completion and strict consensus trees were obtained.

Restriction site data were explored further for bias of parallel site gain or loss–gain over parallel site loss or gain–loss events (Debry and Slade, 1985; Templeton 1983). The character state weighting procedure outlined by Albert *et al.* (1992) was used to construct most-parsimonious trees based on a stepmatrix of character state transformations within each restriction site character. Differential transformational weights of gain (1.3) versus loss (1.0), as recommended for lower taxonomic levels (Albert *et al.*, 1992; Wendel and Albert, 1992), were implemented in PAUP 3.1.1.

Relative levels of support for individual clades were estimated by bootstrap analysis (Felsenstein, 1985) as implemented in PAUP (100 replicate samples). For each pseudoreplicate, all minimum-length trees were saved. Estimates of support, such as the bootstrap, are used routinely; however, this measure is not independent of the pattern of character state distribution (see Davis, 1995, and Olmstead and Sweere, 1994, for contrasting opinions).

Combined parsimony analysis of the complete 44-taxon sample for cpDNA restriction site and ITS sequence data was performed. Incongruence between data sets was quantified using the Mickevich–Farris incongruence index (I_{MF}; Mickevich and Farris, 1981; Kluge, 1989) and tested using the random partitions test of Farris *et al.* (1994), as implemented in PAUP*. Potentially incongruent relationships derived from separate analyses were also evaluated using relative bootstrap support (Mason-Gamer and Kellogg, 1996). Morphologically based knowledge of taxonomic group membership and the recent morphological cladistic analysis (Nixon, 1985, 1993) were used to represent the putative organismal or species tree.

Alternative topologies were assessed by implementing the TOPOLOGICAL CONSTRAINTS option in PAUP. Constraint trees were used to test our results with the most recent morphological cladistic hypothesis for subgenus *Quercus* (Nixon, 1985, 1993; see Fig. 2). The minimum number of steps required to produce the resulting topologies was recorded.

RESULTS

Chloroplast DNA Restriction Site Data

The chloroplast genome of Fagaceae is about 150 kb in length. Approximately 700 six-base pair restriction recognition sites for 14 enzymes were surveyed in the study of *Quercus*. These 700 sites represented about 3.3% of the chloroplast genome. Single-digest mapping studies using heterologous probes indicated that the cpDNA of *Quercus* roughly corresponded in size, structure, and relative gene order to typical chloroplast genomes (e.g., *Petunia* (Palmer, 1985) and *Nicotiana* (Shinozaki *et al.*, 1986)).

Hypotheses of site homology among the cpDNAs of subgenus *Quercus* were unambiguous, but several complex fragment-length polymorphisms between the ingroup and outgroups, *Colombobalanus* and *Trigonobalanus*, were ambiguous, and these sites were not included in subsequent analyses. For the cpDNAs of 44 taxa surveyed, a total of 150 variable sites were determined, of which 80 sites were useful for phylogenetic analysis, while 70 were autapomorphic. Using PAUP 3.1.1, unweighted parsimony analysis of these 80 phylogenetically informative sites recovered 15 minimum-length trees of 102 steps. In contrast, PAUP*, when set to "amb-," and NONA recovered only 1 unambiguously supported tree (see Nixon and Carpenter, 1996a). This tree identified seven cpDNA lineages within subgenus *Quercus* (Fig. 3), with most of the monophyletic cpDNA lineages corresponding to previously recognized taxonomic groups (Table 1 and Fig. 2). Parsimony analysis with differential character-state weighting (weights 1.3 for sites gains vs losses) identified 2 trees, 1 of which corresponded to the unweighted tree. Relative support, as measured by bootstrap values, for the major clades identified was high (see Fig. 3).

Individuals of section *Protobalanus* were resolved into two distinct plastome clades that differed by 13 restriction sites (Fig. 3; Plastomes 1 and 2). Nested within the Plastome 1 clade was the haplotype of one

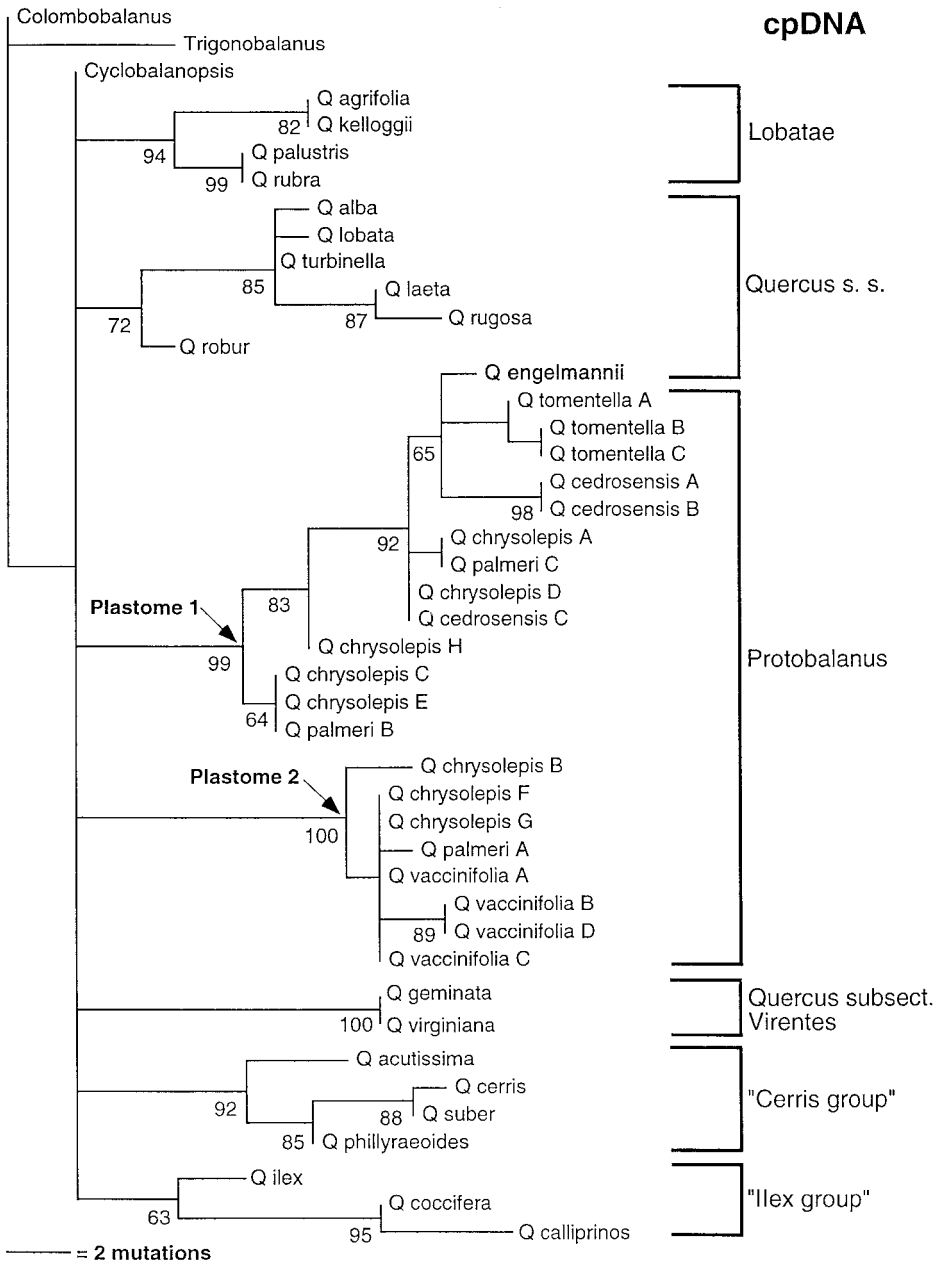

FIG. 3. The single most-parsimonious tree based on chloroplast DNA restriction sites of subgenus *Quercus* and outgroups. Length = 102 steps; consistency index = 0.78; retention index = 0.92; branch lengths are drawn according to scale. Percentage of 100 bootstrap replications is given for nodes with bootstrap values >50%. Classification of subgenus *Quercus* follows Nixon (1993). The position of the boldfaced taxon is discussed in the text.

individual of a Los Angeles County population of *Q. engelmannii* (section *Quercus* subsection *Glaucoideae*). This haplotype formed a subclade with haplotypes of individuals of *Q. tomentella* and *Q. cedrosensis* (section *Protobalanus*), but differed from them by several restriction sites. To verify this result, an additional individual of *Q. engelmannii* from this population was screened and found to possess the identical haplotype. Individuals of two species, *Q. chrysolepis* and *Q. palmeri*, appeared in both plastome clades. Only the haplotypes of *Q. tomentella* formed a monophyletic group. The distribution of the two major plastome types corresponded loosely to a north–south discontinuity, though with several exceptions (Fig. 4A). Within these two clades a total of 16 distinct haplotypes were identified. Figure 4B shows the unrooted tree obtained by unweighted parsimony analysis of the 22 haplotypes. This single most parsimonious tree of 44 steps is drawn according to scale for branch lengths and indicates the cladistic distribution of haplotypes within the sample.

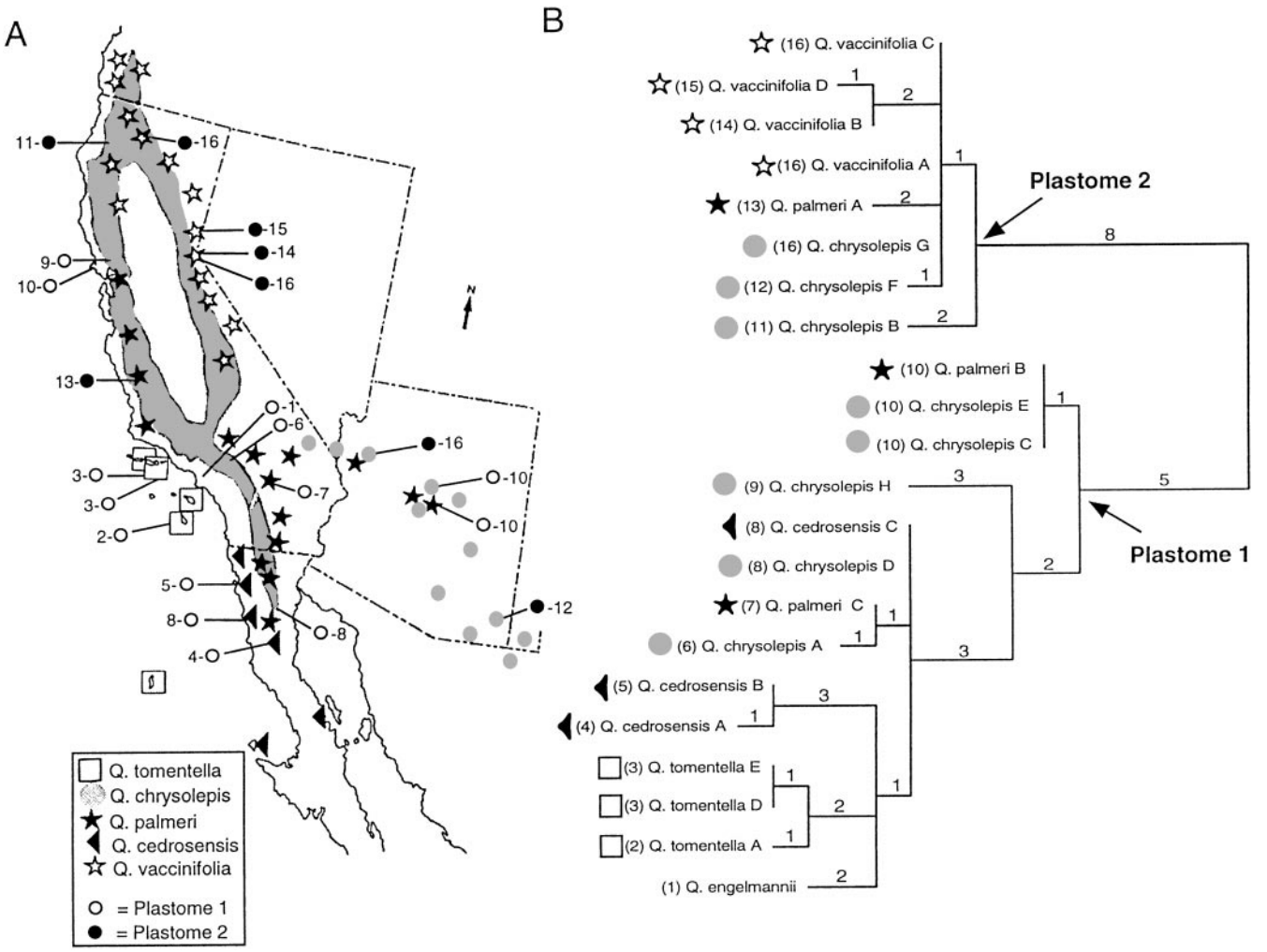

FIG. 4. (A) The distribution of the five species of *Quercus* section *Protobalanus* is indicated by symbols and shading. Lines show the general location of population samples for chloroplast DNA studies. From this sample, the distribution of the two major plastome types is indicated with circles followed by a number corresponding to a distinct cpDNA haplotype. (B) The single most-parsimonious tree for the 22 haplotypes identified with branch lengths indicated. Length = 44 steps; consistency index = 0.93 (autapomorphies included); retention index = 0.98. Locality data is given for intraspecific samples (see Table 1) and haplotype number is shown in parentheses.

Nuclear Ribosomal DNA Sequence Data

Comparative descriptions and analyses of sequences for all taxa are based on 614 bp of the ITS/5.8S region. The length of ITS 1 varied from 223 to 234 bp, whereas that of ITS 2 varied from 206 to 214 bp. The 5.8S coding region was determined to be 164 bp long. Average percentage G + C content across the entire set of sequences was 64.1%. The ITS lengths and general pattern of nucleotide composition in the sequences obtained from *Quercus* and outgroup taxa are similar to those reported for a broad sample of angiosperms in general (Baldwin *et al.*, 1995) and to related "higher" hamamelid genera *Nothofagus*, *Betula*, and *Corylus* in particular (Manos, 1997). Exemplar sequences of ITS and 5.8S were subjected to BLAST (Altschul *et al.*, 1997) and showed strongest homology with other angiosperms.

Alignment of the final matrix required the introduction of 13 one- or two-base pair indels (insertion or deletion mutations) distributed throughout ITS 1 and 2, with two larger indels (nine and three base pairs) that were unique to species of section *Lobatae.* There were no instances of small indels overlapping with larger indels. Sixteen of 234 positions (6.8%) within ITS 1 had at least one sample with a gap, and only one of these was required to align *Quercus* and outgroup sequences. Similarly, 18 of 214 positions within ITS 2 (8.4%) had at least one gap, six of which were required to align *Quercus* and outgroup sequences. Using this final alignment, values of pairwise percentage sequence divergence ranged from 1.1 to 6.7% among *Quercus* species and from 7.2 to 11.3% between *Quercus* and the outgroups *Colombobalanus* and *Trigonobalanus.*

For the 44 sequences, a total of 94 nucleotide positions were phylogenetically informative: 43 in ITS 1, 6 in 5.8S, and 45 in ITS 2. For ITS 1, 6 of the 43 phylogenetically informative sites included gaps; for ITS 2, 2 of 45 sites included gaps. Therefore, 8 (8.3%) of the informative positions of the final matrix included at least 1 sequence with gaps.

When sequences from all taxa were considered and gaps treated as missing data, parsimony analysis using PAUP 3.1.1. recovered 96 shortest trees of 235 steps, whereas PAUP* set to "amb-" and NONA recovered a subset of only 12 unambiguously supported trees. The strict consensus for both sets of the trees was identical and well resolved (Fig. 5). In all trees, subgenus *Cyclobalanopsis* was sister to a well-supported clade of subgenus *Quercus*. Within subgenus *Quercus*, Eurasian evergreen and deciduous species (section *Cerris sensu* Camus) formed a basal, well-supported clade,

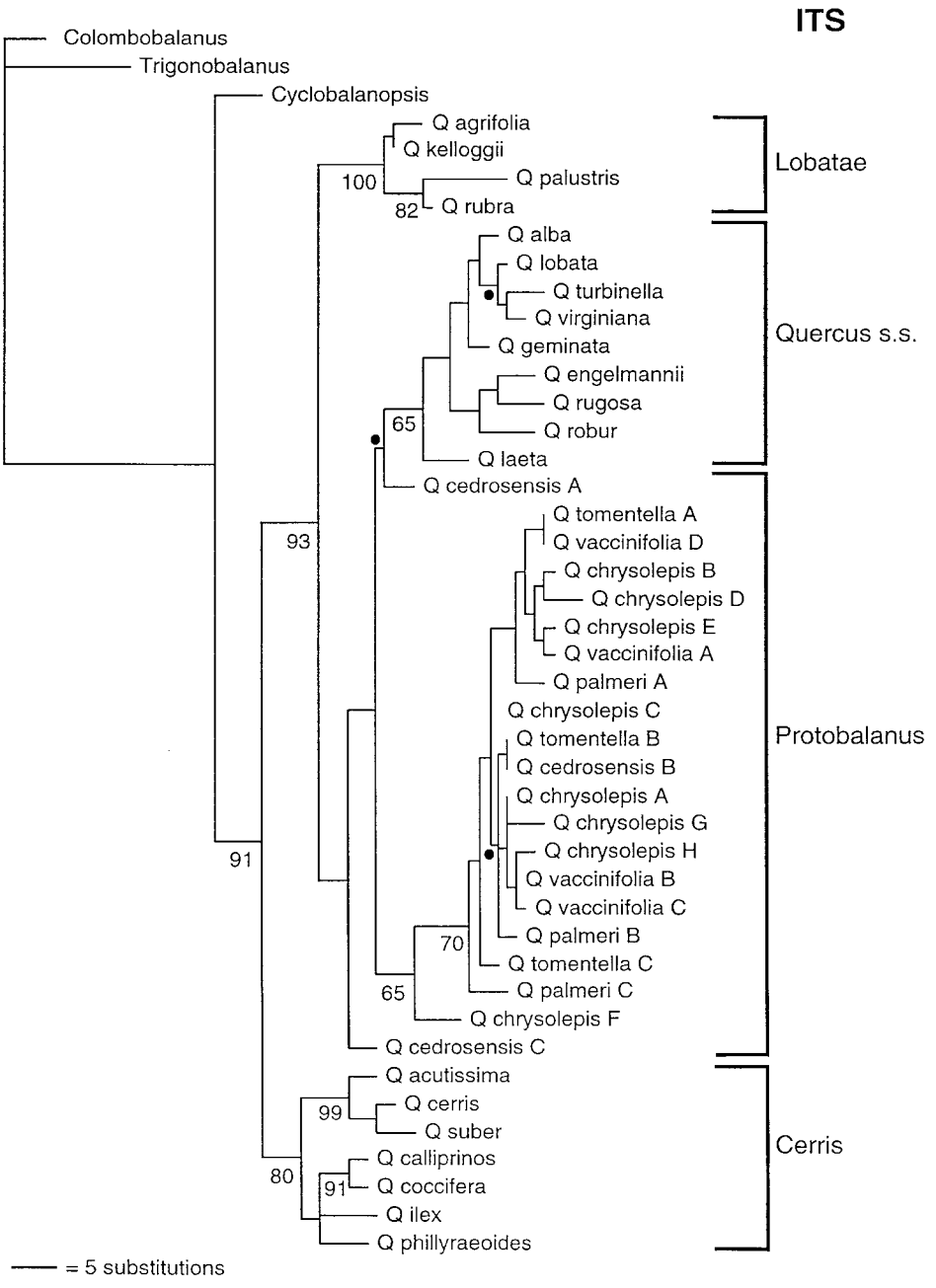

FIG. 5. One of 12 most-parsimonious trees for subgenus *Quercus* based on nrDNA ITS sequences. Length = 235 steps; consistency index = 0.47; retention index = 0.76; branch lengths are drawn according to scale. Solid circles indicate branches that collapse in the strict consensus. Percentage of 100 bootstrap replications is given for nodes with bootstrap values >50%. Clade designations follow Nixon (1993) with modifications discussed in the text.

which was sister to a larger, strongly supported clade formed by members of the sections *Lobatae*, *Protobalanus*, and *Quercus sensu stricto*. Within this clade, section *Lobatae* was sister to a clade consisting of a paraphyletic section *Protobalanus* in which section *Quercus s.s.* was nested, although the support for the major branches was generally low.

These results contradicted recent ITS sequencing efforts based on a small sample of subgenus *Quercus* (Samuel *et al.*, 1998) in which two highly divergent sets of sequences apparently have produced spurious, unsubstantiated phylogenetic patterns. All ITS sequences presented here showed high sequence similarity to only one set of the sequences reported by Samuel *et al.* (1998). The reasons for this discrepancy perhaps are related to technical differences between laboratories; however, it should be noted that the ITS region has been sequenced, either directly from PCR or by cloning, using our protocols in eight genera and well over 100 species of Fagaceae without revealing sequence divergence values greater than 12.0%.

Inter- and intraspecific variation among the individuals of *Quercus* section *Protobalanus* produced groupings that did not correspond to morphological species or to geographical location. In order to examine the effects of the larger sampling effort within section *Protobalanus* on cladistic resolution, additional parsimony analyses including random subsets of *Protobalanus* sequences were performed. These analyses (not shown) also produced trees supporting either a sister group relationship with section *Quercus s.s.* or a paraphyletic *Protobalanus* with *Quercus s.s.* weakly nested within it, as in Fig. 5.

In the set of 12 trees, the lack of monophyly of section *Protobalanus* was due to two sequences: *Q. cedrosensis*-A sometimes formed a monophyletic group with sequences of section *Quercus s.s.* and *Q. cedrosensis*-C always formed a basal lineage with respect to the entire clade of remaining individual sequences sampled from sections *Protobalanus* + *Quercus s.s.* For both of these samples the ITS region showed no evidence of increased sequence evolution; thus, their phylogenetic position relative to other sequences obtained from section *Protobalanus* is most likely not a result of sequencing nonfunctional ITS paralogues (see Buckler *et al.*, 1997).

Branch support, as measured by the bootstrap was variable among and within sections (Fig. 5). For example, sequences within section *Lobatae* and the Eurasian *Cerris* group formed strongly supported subclades, whereas few well-supported subclades were detected within sections *Quercus s.s.* and *Protobalanus*.

Combined Data

Each of the seven lineages identified by cpDNA restriction site data (Fig. 3) was generally consistent with the groupings provided by ITS sequences, except for section *Protobalanus*, which was not monophyletic according to both data sets, section *Quercus* subsection *Virentes*, which was not monophyletic for ITS, and *Q. engelmannii*, which was resolved in a clade of related oaks of section *Quercus s.s.* (Fig. 5). Simultaneous parsimony analysis of the 44 taxa using PAUP* (set to "amb-") and NONA recovered 12 trees (L = 397; CI = 0.48; RI = 0.75) that were similar to the ITS trees in basal structure, but differed in supporting a paraphyletic section *Quercus s.s.* within which a nonmonophyletic section *Protobalanus* is weakly nested (trees not shown). The 44 taxon combined trees (L = 397) were a total of 60 steps longer than the additive lengths of the cpDNA and ITS trees (L = 337). The amount of incongruence between the two data sets, as measured by the Mickevich-Farris incongruence index (I_{MF}) was 0.1511. Random partitions drawn from both data sets were significantly different at the level of $P < 0.01$. Topologically, the results of direct combination in the upper portion of the trees were highly unsatisfactory and most likely due to one strongly supported incongruent relationship resolved by separate analyses. In the cpDNA trees (Fig. 3), the haplotype of *Q. engelmannii* was nested within one of the plastome clades of section *Protobalanus* (Fig. 3; Plastome 1). On the basis of nuclear ITS sequences and morphological evidence, *Q. engelmannii* is related to members of section *Quercus s.s.*, suggesting that the cpDNA gene tree misrepresents the species tree.

Several other topological differences between the two gene trees were observed, for example, *Q. phillyraeoides*, whose cpDNA and ITS relationships differed with respect to subclade membership. In this case, the weak support for placement within the ITS trees (Fig. 5), relative to the strongly supported position in cpDNA trees (Fig. 3), was considered equivocal evidence for different gene histories (Type 3 incongruence *sensu* Brower *et al.*, 1996). Furthermore, the fact that *Q. phillyraeoides* has been placed traditionally with other oak species treated within section *Cerris* (*sensu* Camus 1936–1954) argues for maintaining it in the combined analysis, despite the weakly supported contradictory signal in the ITS data.

A third and more problematic gene tree conflict involved the phylogenetic resolution obtained for the individuals of section *Protobalanus*. In this example, each data set not only failed to establish the monophyly of the section, but also resolved groupings which were incongruent with morphological species concepts. In addition, the resolution obtained from ITS had little in common with cpDNA relationships. Combining data here was the most objective way to include this information as it pertains to the broader relationships of section *Protobalanus*.

Q. engelmannii was pruned from each data set because its position based on cpDNA data was strongly incongruent with ITS and morphological data (Type 4

incongruence *sensu* Brower *et al.*, 1996), and a second combined analysis was conducted. Simultaneous parsimony analysis of the 43 taxa using PAUP*, set "amb-," and NONA produced two minimum-length trees of 383 steps. The well-resolved combined strict consensus (Fig. 6) has a topology similar to that obtained for the ITS data alone, but with more resolution and branch support. Combined analysis supported a monophyletic group of section *Cerris sensu* Camus as sister to a large clade of the remaining sections of subgenus *Quercus*. Within this larger clade, there also was support for the following arrangement of sections: (*Lobatae*-(*Protobalanus* + *Quercus s.s.*)). All trees for the combined data also strongly supported the monophyly of section *Protobalanus*, a result that was robust to random removal of various combinations of *Protobalanus* individuals.

The 43-taxon combined tree was a total of 54 steps longer than the additive lengths of the trees derived

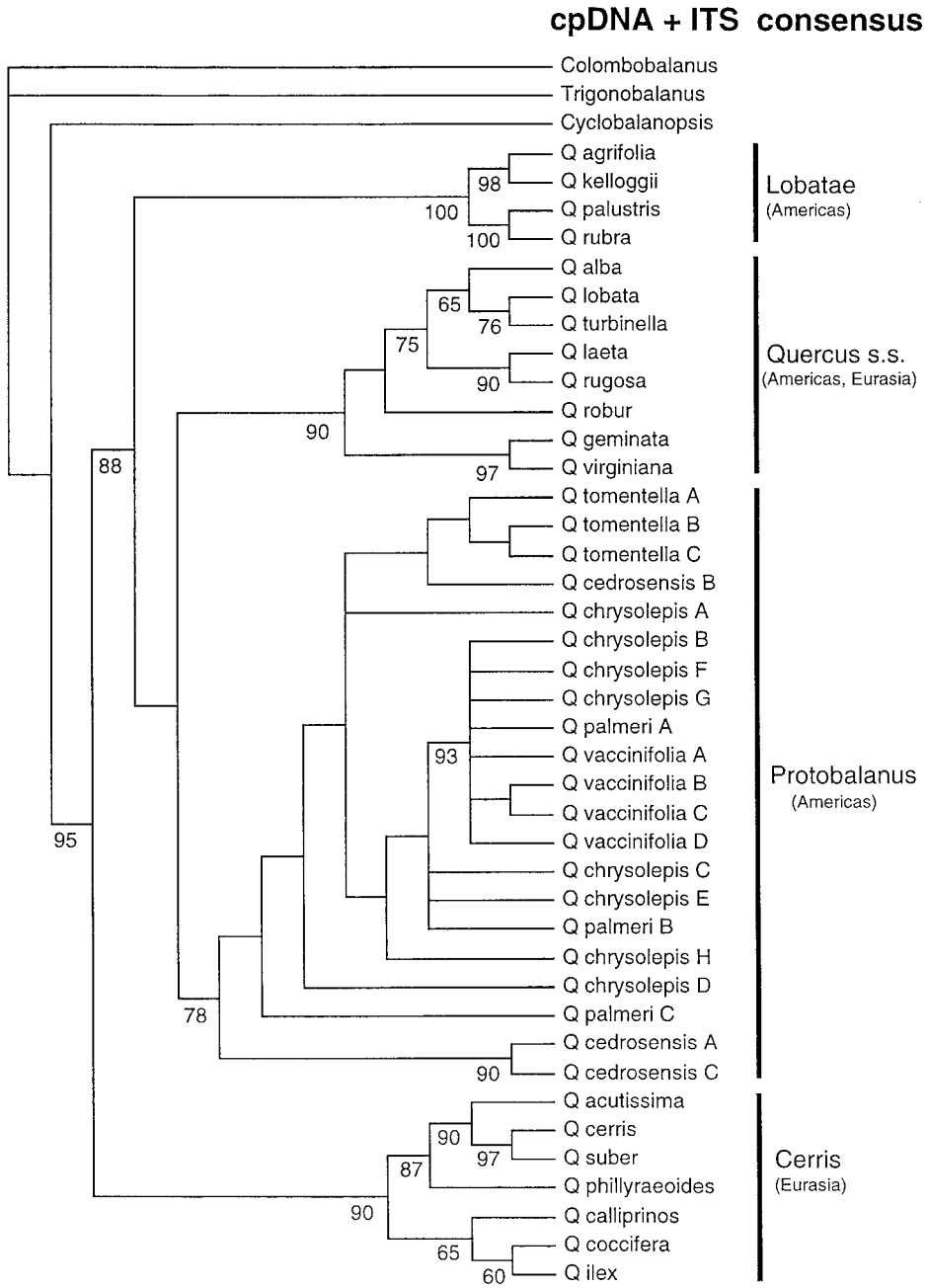

FIG. 6. Strict consensus of the two most-parsimonious trees for subgenus *Quercus* based on simultaneous analysis of chloroplast DNA restriction sites and nrDNA ITS sequences. Length = 383 steps; consistency index = 0.50; retention index = 0.76. Percentage of 100 bootstrap replications is given for nodes with bootstrap values >50%. Clade designations follow Nixon (1993) with modifications discussed in the text. The generalized geographic distribution of each clade follows in parentheses.

from each data set, resulting in a lower I_{MF} value (0.1409). Random partitions remained significantly different at the level of $P < 0.01$. When all individuals of section *Protobalanus* were removed, the difference between the sum of the tree length and the combined tree length decreased to only 12 steps ($I_{MF} = 0.0515$); however, random partitions remained significant at the level of $P < 0.01$. Although the topologies of these trees (not shown) supported no conflicting groups, phylogenetic incongruence between these truncated data sets, as indicated by random partitions, may be due to the higher amount of homoplasy in the ITS data set.

Using the 43-taxon combined molecular data set, one specific alternative hypothesis proposed by Nixon (1985, 1993; see Fig. 2) was tested using constrained topologies. Although the hypothesis for the monophyly of section *Quercus sensu lato* is based mostly on the single character of basal abortive ovules, it remains a viable evolutionary hypothesis for the relationships within subgenus *Quercus*. Using the 43-taxon data set, the alternative hypothesis resulted in trees seven steps or approximately 1.8% longer than the most parsimonious.

DISCUSSION

Phylogeny and Biogeography

Comparison of the gene trees derived from each analysis (Figs. 3 and 5) indicates that the two data sets are, with few exceptions (see below), congruent and often complementary. In this study, combining independent molecular data sets after removing one potentially confounding taxon ("conditional combination" *sensu* Bull *et al.,* 1993; Huelsenbeck *et al.*, 1996) yields fewer most-parsimonious trees, well-supported resolution, and general agreement with the taxonomic groups recognized by morphology. In one noteworthy example, separate analyses show different patterns of nonmonophyly (e.g., section *Protobalanus*), whereas combined data analysis provides robust support for monophyly, a result clearly attributable to secondary phylogenetic signal emerging through simultaneous parsimony analysis (e.g., Kluge, 1989; Kluge and Wolfe, 1993; Olmstead and Sweere, 1994; Allard and Carpenter, 1995; Nixon and Carpenter, 1996b; DeSalle and Brower, 1997; Soltis *et al.*, 1998). Incongruence between the data sets presented here and decisions regarding their combination were best evaluated using topology, bootstrap support, and, to a lesser extent, the I_{MF} (Mickevich and Farris, 1981). The results of the random partitions test, however, appear to be largely influenced by the considerable amount of homoplasy within the ITS data set (see Doyle, 1996). Overall, it appears that quantitative approaches using these data cannot explicitly determine when to conduct combined or separate analyses, in agreement with the conclusions reached by Johnson and Soltis (1998).

Although cpDNA data fail to provide information at higher hierarchical levels (Fig. 3), analysis of combined data suggests the following set of infrageneric relationships among the four monophyletic groups: (*Cerris*- (*Lobatae*- (*Protobalanus* + *Quercus s.s.*))) (Fig. 6). In contrast to Nixon's (1985, 1993; Fig. 2) results based on morphological cladistic analysis, molecular data support the monophyly of the strictly Eurasian white oaks (*Cerris* and *Ilex* groups *sensu* Nixon) and suggest their basal placement within subgenus *Quercus* (Figs. 5 and 6). Previous classifications of the species forming this group have varied. For example, Camus (1936–1954) placed all of the species sampled here within section *Cerris*, except for *Q. ilex*, which she considered more closely related to species of section *Quercus s.s.* However, Schwarz (1936, 1937) separated at the subgeneric level *Q. coccifera* (including *Q. calliprinos*) and *Q. ilex* (subgenus *Sclerophyllodrys* = "*Ilex* group" *sensu* Nixon). Within the clade resolved here (Fig. 6), two subclades are supported, each generally consistent with the provisional groups identified by Nixon (1993; e.g., *Ilex* and *Cerris*), the subsectional classification of section *Cerris* (Camus, 1936–1954), pollen and leaf anatomy (Smit, 1973; Zhou *et al.*, 1995), and RFLP analysis of the intergenic spacer of rDNA (Bellarosa *et al.*, 1990). These results support the recognition of section *Cerris sensu* Camus (see Fig. 1), but the circumscription of this clade and resolution of intrasectional relationships among several unsampled Eurasian species groups awaits additional phylogenetic study (Manos and Zhou, in progress). According to the ITS and combined data, the placement of these Eurasian taxa suggests that section *Quercus sensu* Nixon is not monophyletic. The alternative placement presented here hypothesizes two origins of basal abortive ovules, the derived condition within subgenus *Quercus*. Basal abortive ovules in combination with glabrous endocarp unambiguously define the most species-rich group within section *Quercus*, the widespread white oaks of the Northern Hemisphere (e.g., *Q. alba, Q. robur,* and *Q. virginiana*). A second derivation of basal abortive ovules (with tomentose endocarp) supports the monophyly of the otherwise plesiomorphic section *Cerris;* however, developmental studies are needed to determine whether this apparent convergence is morphologically distinguishable from the condition found in section *Quercus s.s.* (Borgardt and Pigg, 1999; Borgardt and Nixon, in progress).

Apart from the position of section *Cerris*, the remaining set of phylogenetic relationships suggested by the combined analysis of molecular data (Fig. 6) is consistent with Nixon's morphological hypothesis (1985, 1993; Fig. 2), including the placement of the North and Central American subsection *Virentes* ("live oaks") within section *Quercus s.s.* The clade (*Lobatae*- (*Protobalanus* + *Quercus s.s.*)) as suggested by ITS and combined data (Figs. 5 and 6) apparently has, at

present, no known morphological character support; however, this hypothesis has significant phylogenetic and biogeographic implications. The combined data support, albeit weakly, a sister group relationship for sections *Quercus s.s.* and *Protobalanus*. Trelease (1924) considered *Protobalanus* to be the evolutionary link between the red oaks (section *Lobatae*) and the white oaks (section *Quercus s.s.*). Molecular data support the idea of a transitional phylogenetic position for *Protobalanus*, in agreement with its unique combination of shared character states, several of which are symplesiomorphic. On the basis of morphological cladistic analysis (Nixon, 1985, 1993), section *Protobalanus* was placed below the branch leading to section *Quercus* on the basis of the lateral position of the abortive ovules on the mature seed. In section *Lobatae* and all outgroups, the position of the abortive ovules is apical relative to the derived, basal condition observed in sections *Quercus s.s.* and *Cerris*. The lateral abortive ovules of section *Protobalanus* oaks either could be interpreted as an intermediate state of an ordered transformation series, a polymorphism, or perhaps is best included as part of a more generalized state of the derived condition. Developmental studies may provide evidence to discriminate between these possibilities. If the narrow definition of section *Quercus* is accepted, the distribution of morphological characters in the (*Lobatae*- (*Protobalanus* + *Quercus s.s.*)) clade needs to be reconsidered. Characters of the pistillate flowers and shared presence of a pronounced glandular structure or tubercle on the cupule scales are likely synapomorphies for sections *Protobalanus* + *Quercus s. s.* (Nixon, 1985; Manos, 1993). Pollen similarities may also indicate a close relationship (Solomon, 1983a,b).

Although the morphology of section *Protobalanus* appears to parallel several of the evergreen *Cerris* oaks sampled here, there is no phylogenetic support (Fig. 6) for a monophyletic group spanning the subhumid and semiarid vegetation zones of the Northern Hemisphere (Axelrod, 1975). Phylogenetic data from other sclerophyllous plant genera with similar disjunct distributions also cast doubt on the number of genera that support the Madrean–Tethyan hypothesis (for review see Fritsch, 1996). In considering the biogeography of the oaks represented in the (*Lobatae*- (*Protobalanus* + *Quercus s.s.*)) clade (Fig. 1), only section *Quercus s. s.* is widespread in the Northern Hemisphere. The phylogeny based on molecular data clearly supports a New World origin for section *Quercus s. s.* (Fig. 6). Trelease (1924), however, used this widespread distribution pattern to postulate a more recent origin for the strictly New World red oaks (section *Lobatae*) relative to section *Quercus s. s.* on the basis that red oaks evolved too late to cross a land bridge between the northern continents. Phylogenetic and paleobotanical evidence, however, suggests that section *Quercus s. s.* evolved at middle latitudes in the Americas and subsequently migrated to the Old World prior to the break up of land bridges linking the northern continents, events that occurred in the general time frame between the Late Eocene (ca. 40 Mya) and the Middle Miocene (ca. 15 Mya) (Tiffney, 1985). This is consistent with paleobotanical data supporting a minimum Oligocene age for the divergence of red oaks and white oaks of section *Quercus s. s.* in North America (Daghlian and Crepet, 1983) and the later (Miocene–Pliocene) fossil appearance of section *Quercus s. s.* in Asia (Zhou, 1993). Oligocene macrofossil evidence for section *Protobalanus* is more equivocal (MacGinitie, 1953); however, the group is clearly present throughout the Miocene of western North America (Axelrod, 1939, 1944; Wolfe, 1969). Other explanations would require the extinction of either section *Protobalanus* or section *Lobatae* in Eurasia, but this is unlikely given that fossils of these groups have not been found there (Zhou, 1993). These findings fully support Axelrod's (1983) hypothesis, that the major oak lineages evolved locally at middle latitudes in the Tertiary and now occupy areas within the distribution of macrofossil equivalents.

Phylogenetic and paleobotanical data suggest two initial centers of diversification for subgenus *Quercus* in the Tertiary. Three major monophyletic groups, sections *Lobatae*, *Protobalanus*, and *Cerris*, evolved at middle latitudes of the Americas and diversified into at least 300 species, with notable secondary radiations occurring in Mexico, eastern North America, and into Eurasia (Nixon, 1993). In contrast, it appears that a single monophyletic group, section *Cerris*, comprising several subgroups totaling no more than 70 species, evolved at similar latitudes in Asia. Within this strictly Eurasian clade, a modest species radiation occurred in eastern Europe and southwestern China. In comparing these two major groups within subgenus *Quercus*, strikingly similar patterns of morphological evolution exist, most of which can be explained by convergence (see Tucker, 1974 for examples within New World groups) or by shared plesiomorphies. In addition to multiple origins of particular types of leaf morphology and habit, probable convergences between taxa of the Eurasian *Cerris* clade and section *Quercus s. s.* in characters derived from reproductive structures include basal abortive ovules and fused cotyledons, important diagnostic features in *Quercus* (Nixon, 1985, 1993; Zhou *et al.*, 1995). This implies that tracking organismal relationships in the oaks with morphology may be seriously compromised by a set of convergently evolving fruit characters.

Patterns and Processes of Molecular Differentiation

The reproductive biology of species in the genus *Quercus* presents a significant challenge to the use of molecular data to infer organismal relationships. Considering that published accounts of inter- and intraspecific molecular variation indicate that closely related,

interfertile oak species are not well differentiated (e.g., Whittemore and Schaal, 1991; Ferris *et al.*, 1993; Petit *et al.*, 1993a), this study, in part, examines the nature of the resultant patterns of variation across several taxonomic levels. Comparisons of the gene trees derived from the two data sets provide broad support for the higher level groups distinguished by morphology. There was only one instance of clear-cut incongruence between gene trees and species trees, and other such conflicts were not strongly supported.

Chloroplast DNA data from a wide geographic and taxonomic sampling of species representing section *Quercus s. s.* showed that two individuals of *Q. engelmannii* from the same population possessed a cpDNA plastome type otherwise found only among individuals of section *Protobalanus* (Fig. 3; Plastome 1). Sequences from ITS show unequivocally that the nuclear genome of one of these plants (Fig. 5), as estimated by the nrDNA locus, groups with other oak species of section *Quercus s. s.* Two population-level processes, introgression and lineage sorting, are typically invoked to explain phylogenetic incongruence of this sort (Niegel and Avise, 1986; Doyle, 1992; Rieseberg and Wendel, 1993; Rieseberg *et al.*, 1996; Wendel and Doyle, 1998), and numerous examples of misplaced cpDNA, via the process of introgressive hybridization, have been documented (Rieseberg and Soltis, 1991; Rieseberg and Brunsfeld, 1992). In many cases, a clear donor–recipient relationship establishes the likely source and direction of cytoplasmic gene flow. Following the introgression scenario, the aberrant haplotypes recovered from this population of *Q. engelmannii* would have resulted from ancient hybridization with individuals of section *Protobalanus* bearing Plastome 1 serving as the maternal parent. Phenotypically, the *Q. engelmannii* population in question displays several distinctive morphological synapomorphies placing it within section *Quercus s. s.* subsection *Glaucoideae* (Nixon, 1993). Thus, if this result is an example of misplaced cytoplasm via ancient hybridization, subsequent rounds of backcrossing to paternal-like individuals must have occurred to restore the nuclear background typical of the other oaks sampled within section *Quercus s.s.*

Alternatively, phylogenetic incongruence may have been produced by differential sorting of ancestral polymorphisms (i.e., cpDNA Plastome 1; see Fig. 3) instead of from introgressive hybridization. There are no reports of hybridization between section *Quercus s. s.* and *Protobalanus* in the wild, and artificial crosses between the two sections were unsuccessful (Cottam *et al.*, 1982). In the lineage sorting scenario, some populations of section *Quercus s. s.* from southwestern North America may have retained Plastome 1; thus, the coalescence time for their cpDNA must be more ancient than the divergence of the two groups. Since this particular polymorphism is shared by likely sister taxa (Figs. 2 and 6), it suggests that cpDNA polymorphisms have persisted through the evolution of higher taxa and multiple speciation events. On the basis of the current sampling, ancestral populations of New World *Quercus* possessed at least four major chloroplast haplotypes, with lineage extinctions leading to the unambiguous monophyly of only section *Lobatae*, whereas both sections *Protobalanus* and *Quercus s. s.* still harbor polymorphisms. The weakness of the lineage sorting argument lies in the well-nested position of the misplaced haplotype relative to other haplotypes within the Plastome 1 clade (Figs. 3 and 4). This position is indicative of a recently derived haplotype, whereas a more basal placement would have suggested an ancestral haplotype and thus increased support for the lineage sorting hypothesis (Templeton, 1994). Until other related species from southwestern North America and Central America are sampled, it is not possible to conclusively determine the cause of this incongruent relationship.

Section *Protobalanus*, the most narrowly distributed and least speciose of all oak groups, also provides an unparalleled example in the oaks to examine patterns of molecular variation at lower taxonomic levels. The biogeography of section *Protobalanus* is highlighted by a single widespread species, *Q. chrysolepis*, and four species with much narrower, largely allopatric ranges (Fig. 4). *Q. chrysolepis* also is the only species to form narrow zones of secondary contact with the four species, and hybrids have been reported between it and three of the four species, the exception being *Q. cedrosensis* (Myatt, 1975; Tucker and Haskell, 1960; Thorne, 1967). It is therefore of particular interest to see how gene trees derived from slowly evolving chloroplast DNA and rapidly evolving ITS region compare with morphological species concepts, biogeography, and biological processes which determine cytoplasmic and nuclear gene flow.

The cpDNA haplotypes of *Q. tomentella* form a monophyletic group within the Plastome 1 clade, whereas those of *Q. vaccinifolia* (Plastome 2) and *Q. cedrosensis* (Plastome 1) remain unresolved, but also occur in separate plastome clades (Figs. 3 and 4). In contrast, haplotypes sampled from *Q. palmeri* and *Q. chrysolepis* are distributed within each of the two major plastome clades. The lack of congruence between the distribution of cpDNA haplotypes and the morphological species is consistent with previous haplotype surveys in *Quercus* (Whittemore and Schaal, 1991; Petit *et al.*, 1993). If it is assumed that species in section *Protobalanus* were never fixed for different haplotypes, then the current pattern of differentiation may have phylogeographic implications (Avise *et al.*, 1987; Avise, 1989; Soltis *et al.*, 1989, 1991, 1992a,b; Sewell *et al.*, 1996). While an overall north–south discontinuity is evident for the distribution of the two major plastome types, the haplotypes of the two most geographically peripheral species, *Q. cedrosensis* and *Q. tomentella*, generally appear in terminal positions within the Plastome 2

clade, suggesting a more recent derivation of these haplotypes (Templeton, 1994).

Although the sampling here is far too limited to determine the extent of haplotype diversity within section *Protobalanus,* several comparisons can be made to previous studies of cpDNA variation in *Quercus.* With secondary zones of contact restricted to *Q. chrysolepis* and each of the peripheral species, hybridization and subsequent introgression might be expected to produce localized patterns of shared haplotypes between species. As shown in Fig. 4, two cases (haplotypes 8 and 10) were found in which *Q. chrysolepis* and a geographically adjacent population of another species shared identical cpDNA haplotypes. These two examples are similar to the findings of Whittemore and Schaal (1991), who examined the distribution of haplotypes in populations of sympatric white oak species. According to that study, sympatric individuals from different species typically share a common haplotype, suggesting appreciable localized cytoplasmic gene flow and high levels of cpDNA fixation within populations. Petit *et al.* (1993a) sampled cpDNA broadly throughout most of the range of several interfertile European white oak species and reported lower levels of cytoplasmic gene flow, with particular haplotypes spanning entire parts of the species range, whereas a smaller proportion were geographically more restricted. The widespread distribution of the two major plastomes and several of the nested haplotypes (Fig. 4; 10 and 16) identified within section *Protobalanus* is consistent with the findings of Petit *et al.* (1993a). Recent empirical studies of maternal markers in European beech (Demesure *et al.,* 1996) and oaks (Ferris *et al.,* 1993; Petit *et al.,* 1993a, 1997) and simulations studies (Corre *et al.,* 1997) have suggested geographic structure indicative of postglacial migration and long-distance dispersal from southern refugia. All of these data were viewed within the context of postglacial migration into relatively barren habitats. Unlike the wholesale changes brought about by glaciation in northeastern America and western Europe, the Holocene of western North America is generally believed to have affected the distribution of tree species differentially (Thompson, 1988); therefore, these recent cooling periods may have had limited influence on the current pattern of cpDNA variation with section *Protobalanus.*

The significant cpDNA discontinuity (i.e., Plastomes 1 and 2; Fig. 4B) within section *Protobalanus* in a predominantly north–south pattern is, however, consistent with an extrinsic barrier to gene flow by means of limiting seed dispersal. The substantial divergence between plastome types suggests that the origin of this discontinuity may be related to an earlier disjunction within the ancestral distribution caused by progressive cooling and drying conditions of western North America during the late Tertiary (Axelrod, 1983; Thompson, 1988). Although an explicit historical basis for this disjunction may be unclear, it is worth noting that several modern genera, such as *Juglans, Abies,* and *Pseudotsuga* exhibit similar allopatric distributions. One possible scenario is that an ancestral *Protobalanus* complex in southern California became separated from proto-*Q. vaccinifolia* several hundred kilometers to the north. Subsequent expansion from refugia via seed dispersal would explain the few exceptions to this phylogeographic pattern. In these cases, cpDNA introgression through *Q. chrysolepis* or rare long-distance dispersal events are likely explanations. This overall pattern agrees with lower amounts of gene flow observed for maternally inherited markers in *Quercus* (Petit *et al.,* 1993b) and follows the predictions of theoretical studies as well (Ennos, 1994). In contrast, the groupings provided by ITS sequences generally show no relationship to species or geography, a result probably related to continuous pollen-mediated gene flow among the interfertile species of section *Protobalanus* through time.

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