Int. J. Plant Sci. 162(6):1361–1379. 2001.

1058-5893/2001/16206-0019$03.00

SYSTEMATICS OF FAGACEAE: PHYLOGENETIC TESTS OF REPRODUCTIVE TRAIT EVOLUTION

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The family Fagaceae includes nine currently recognized genera and ca. 1000 species, making it one of the largest and most economically important groups within the order Fagales. In addition to wide variation in cupule and fruit morphology, polymorphism in pollination syndrome (wind vs. generalistic insect) also contributes to the uniqueness of the family. Phylogenetic relationships were examined using 179 accessions spanning the taxonomic breadth of the family, emphasizing tropical, subtropical, and relictual taxa. Nuclear ribosomal DNA sequences encoding the *5.8S* rRNA gene and two flanking internal transcribed spacers (ITS) were used to evaluate phylogenetic hypotheses based on previous morphological cladistic analysis and intuitive schemes. Parsimony analyses rooted with *Fagus* supported two clades within the family, *Trigonobalanus sensu lato* and a large clade comprising *Quercus* and the castaneoid genera (*Castanea* + *Castanopsis*, *Chrysolepis*, *Lithocarpus*). Three DNA sequence data sets, 179-taxon ITS, 60-taxon ITS, and a 14-taxon combined nuclear and chloroplast (*matK*), were used to test a priori hypotheses of reproductive character state evolution. We used Templeton's (1983) test to assess alternative scenarios of single and multiple origins of derived and seemingly irreversible traits such as wind pollination, hypogeal cotyledons, and flower cupules. On the basis of previous exemplar-based and current in-depth analyses of Fagaceae, we suggest that wind pollination evolved at least three times and hypogeal cotyledons once. Although we could not reject the hypothesis that the acorn fruit type of *Quercus* is derived from a dichasium cupule, combined analysis provided some evidence for a relationship of *Quercus* to *Lithocarpus* and *Chrysolepis*, taxa with dichasially arranged pistillate flowers, where each flower is surrounded by cupular tissue. This indicates that a more broadly defined flower cupule, in which individual pistillate flowers seated within a separate cupule, may have a single origin.

Keywords: Fagaceae, ITS, *Lithocarpus*, *matK*, phylogeny, pollination syndrome, *Quercus*, systematics, wind pollination.

Introduction

The family Fagaceae currently includes nine genera: *Fagus* L., *Castanea* L., *Castanopsis* Spach., *Chrysolepis* Hjelmquist, *Colombobalanus* (Lozano, Hdz-C. & Henao) Nixon & Crepet, *Formanodendron* (Camus) Nixon & Crepet, *Lithocarpus* Bl., *Quercus* L., and *Trigonobalanus* Forman. Fagaceae dominate forests in the temperate, seasonally dry regions of the Northern Hemisphere, with a center of diversity found in tropical Southeast Asia, particularly at the generic level. Diversity at the species level is distributed evenly between the seasonal subtropical and evergreen tropical forests of Central America (e.g., *Quercus*) and southern continental Asia and the Malayan Archipelago (subfamily Castaneoideae). As a whole, the Fagaceae offer an exceptional array of evolutionary topics for investigation, including limits to gene flow (Whittemore and Schaal 1991), phylogeographic patterns across the Northern Hemisphere (Dumolin-Lapegue et al. 1997; Petit et al. 1997; Manos et al. 1999), and complex patterns of taxonomy and macroevolution viewed in the context of the rich fossil record for the family (Axelrod 1983; Daghlian and Crepet 1983; Crepet and Nixon 1989*a*, 1989*b*; Nixon and Crepet 1989; Herendeen et al. 1995; Sims et al. 1998). In this article, we present new DNA sequence data to address phylogeny reconstruction and morphological evolution for the entire family.

Taxonomic limits within the Fagaceae are based on a small set of relevant fruit and floral characteristics (Forman 1964, 1966*a*, 1966*b*). Traditionally, the major divisions in the family have focused on pollination syndrome and the relationship between flower and cupule valve number (fig. 1; table 1). In general, floral characteristics related to pollen transmission fall into two tightly correlated suites of features characterized by wind (e.g., *Quercus*) and generalistic insect (subfamily Castaneoideae) pollination syndromes. By virtue of having extant wind- and insect-pollinated species, Fagaceae are unique within the largely wind-pollinated Fagales (but see Endress 1986 on *Platycarya*). Wind pollination has been derived at least once within the family as implied by the recognition of subfamily Fagoideae (fig. 2*A*; Crepet and Nixon 1989*a*; Nixon 1989). With the finding that *Fagus* represents an early branch within the family, the monophyly of wind-pollinated Fagaceae appears less likely (fig. 2*B*; Manos et al. 1993; Manos and Steele 1997).

Fruit morphological variation, related to seed dispersal, is much more complex. The cupule subtending the fruit or nut

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Manuscript received February 2001; revised manuscript received June 2001.

and its relationship to fruit or pistillate flower number provides most of the important characteristics. The evolution and origin of the cupule has generated considerable discussion (Berridge 1914; Hjelmquist 1948; Brett 1964; Forman 1966*a*; Abbe 1974; Endress 1977; MacDonald 1979; Fey and Endress 1983; Kaul and Abbe 1984; Nixon 1989; Nixon and Crepet 1989; Jenkins 1993; Herendeen et al. 1995; Manos and Steele 1997; Sims et al. 1998). The modern consensus is that the cupule is composed of higher-order sterile axes of the pistillate inflorescence. Two major types occur within Fagaceae (fig. 1). The dichasium cupule, in which numerous pistillate flowers and subsequent fruit are subtended by a valvate structure, is the most taxonomically widespread, occurring in both subfamilies and in several genera. In this category, the cupule is composed of triangular valves, which are either open from the earliest stages or enclose the developing fruit to various degrees and later dehisce upon maturity. Cupule valve number is dependent on the number of pistillate flowers in the dichasium in an $N+1$ relationship; for example, a three-flowered dichasium will be subtended by a four-valved cupule (Nixon and Crepet 1989). Reduction in flower number to a single, central flower has occurred in almost all genera. One specific hypothesis of reduction stipulates that the classic acorn cup of *Quercus* has been derived from a dichasium cupule (fig. 1; Forman 1966*b*; Nixon and Crepet 1989). Other apomorphic types (see fig. 1) include the cupule of *Chrysolepis*, with its internal valves (Berridge 1914; Hjelmquist 1948; Forman 1966*b*; Nixon and Crepet 1989; Jenkins 1993), and the two-flowered, four-valved cupule of *Fagus* (MacDonald 1979; Nixon and Crepet 1989; Okamoto 1989*b*).

Table 1

Comparison of the Classification Schemes for Fagaceae

Traditional:[a]	Nixon (1989):
Fagaceae:	Nothofagaceae:
Fagoideae:	*Nothofagus* (35)
Fagus	Fagaceae:
Nothofagus	Fagoideae:
Castaneoideae:	*Fagus* (12)
Chrysolepis	*Quercus* (450)
Castanea	*Trigonobalanus* (1)
Castanopsis	*Colombobalanus* (1)
Lithocarpus	*Formanodendron* (1)
Quercoideae:	Castaneoideae:
Quercus	*Chrysolepis* (2)
Trigonobalanus[b]	*Castanea* (10)
	Castanopsis (120)
	Lithocarpus (300)

Note. The approximate number of species within each genus follows in parentheses.

[a] E.g., Forman (1964), Hutchinson (1967), Abbe (1974).

[b] Also placed in Fagoideae (Melchior 1964) or unassigned (Abbe 1974).

The dichasium cupule is not unique to the family (e.g., Nothofagaceae), but the second category, or the flower cupule, in which each pistillate flower is subtended by a valveless cupule,

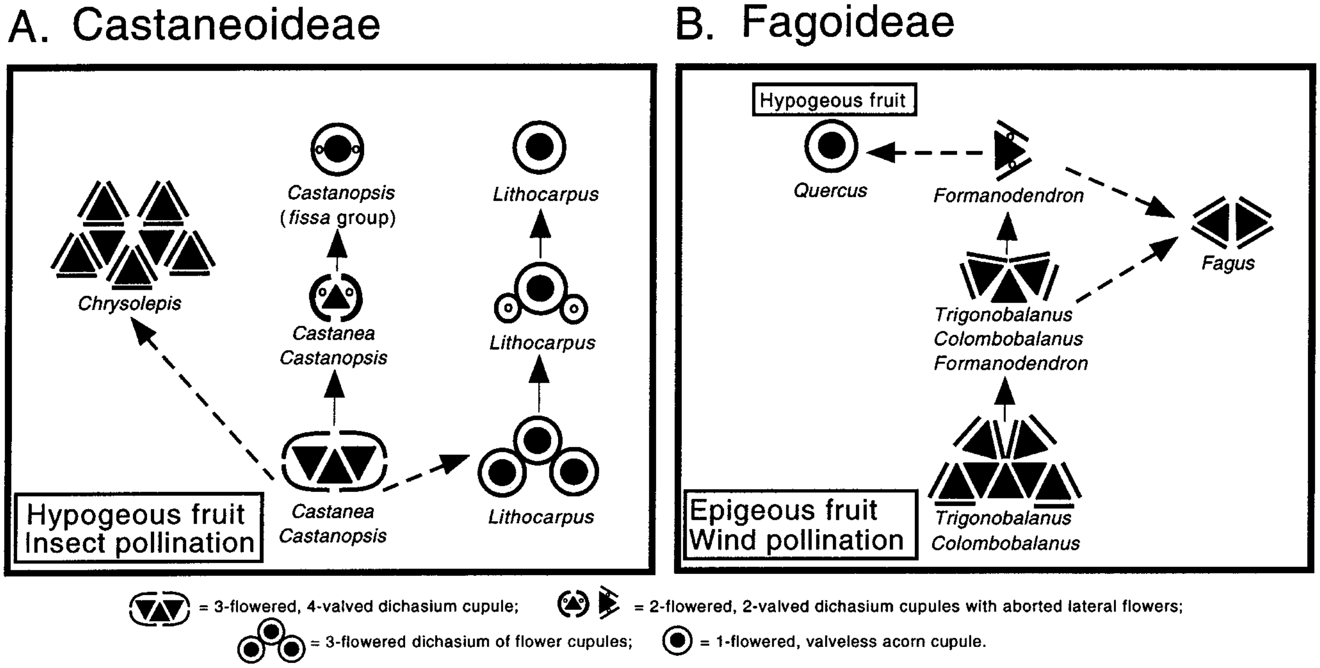


Fig. 1 Reproductive character states and cupule-to-fruit arrangement for the nine genera of Fagaceae. Classification and relationships among cupule types modified from Nixon and Crepet (1989). Cupule valves are indicated with straight or curved lines; fruit are shown with solid circles or triangles; aborted flower position is shown with small open circles; arrows with solid lines indicate likely transformations; arrows with dashed lines indicate hypothetical transformations. *A*, Subfamily Castaneoideae. Four-valved, three-fruited dichasium cupule has given rise to other cupule types. *B*, Subfamily Fagoideae. Complex dichasium cupule has given rise to other cupule types.

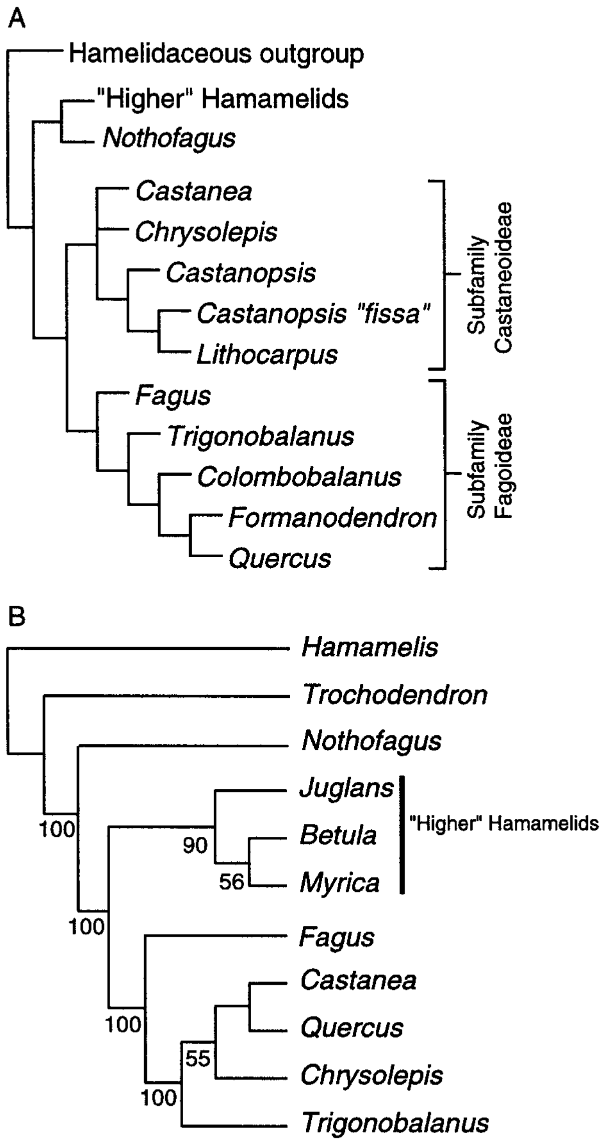


Fig. 2 Phylogenetic hypotheses for Fagaceae. *A*, Strict consensus cladogram based on morphology (Nixon 1985, 1989; Nixon and Crepet 1989). *B*, Single most parsimonious cladogram based on *matK* sequences (Manos and Steele 1997).

appears to be expressed only by the genus *Lithocarpus*. Depending on the species, acorn-like fruit develop from both dichasial and solitary flowers, the latter proving to be the main source of taxonomic confusion with *Quercus*. Ontogenetic studies have shown that valveless cupules of *Quercus* are initiated by two distinct primordia that later fuse (MacDonald 1979; Fey and Endress 1983), whereas cupule development in *Lithocarpus* begins with a primordial ring that rapidly develops from at least two points of inception (Okamoto 1989*a*). The organization of the vascular system in a solitary cupule of *Lithocarpus* is similar to that of *Quercus*, both differing relative to unifloral *Castanopsis* (Soepadmo 1970). Earlier workers suggested flower cupules were the ancestral condition in the family (Hjelmquist 1948; Forman 1966*b*), with fusion between adjacent flower cupules producing the dichasium-cupule type. More recently, cladistic analysis suggested dichasium cupules are plesiomorphic (Nixon and Crepet 1989), in agreement with recent fossil evidence (Herendeen et al. 1995; Sims et al. 1998).

Unlike pollination syndrome and floral morphology, the description of fruit-dispersal and germination syndromes does not appear to follow subfamilial classification (fig. 1). Large, animal-dispersed fruit with hypogeous germination in which the cotyledons remain underground are produced by both dichasium and flower-cupule taxa. Species diversity is highest among taxa that consistently express the combination of valveless cupules and hypogeous germination, although *Castanopsis*, with its mostly valvate cupules, possesses moderate diversity in Southeast Asia. Smaller, passively dispersed fruit, with epigeous germination and with the cotyledons appearing aboveground, are solely associated with dichasium-cupule genera, all comprised of relatively few species and often of limited geographic distribution (*Fagus*; *Colombobalanus*, *Formanodendron*, and *Trigonobalanus* = trigonobalanoids).

Taxonomic schemes within Fagaceae have been stable, with most differences restricted to the classification of *Fagus* and the trigonobalanoid taxa (table 1). The placement of *Fagus* together with the trigonobalanoid genera and *Quercus* in the subfamily Fagoideae has defined a diverse wind-pollinated clade (fig. 2*A*; table 1; Crepet 1989; Crepet and Nixon 1989*a*; Nixon 1989). While a few treatments have recognized the trigonobanoid taxa at the subfamilial level (e.g., Lozano et al. 1979), most schemes have implied a relationship with *Quercus* (Forman 1964; Hutchinson 1967; Soepadmo 1972). Nixon and Crepet (1989) attributed these widely varying treatments of the trigonobalanoid taxa to the fact that the characters shared by these taxa are symplesiomorphic within Fagaceae. In contrast, the four insect-pollinated castaneoid genera have been treated as a cohesive taxonomic group, most often recognized at the subfamilial level, and only rarely associated with *Quercus* (see Brett 1964).

Overall, Fagaceae appear to have evolved within a relatively narrow range of morphological possibility. In this striking example of the combined effects of abiotic and biotic selection pressures, transitions to wind pollination and origins of particular fruit types have fostered diversification within several major lineages. The derived condition of large-seeded, animal-dispersed fruits appears to be associated with appreciable levels of diversification (e.g., *Quercus* and *Lithocarpus*), while small seeded, more passively dispersed fruit are found among divergent, often relictual species-poor lineages (e.g., *Fagus* and the trigonobalanoids). As with wind pollination, highly specialized animal-dispersed fruit also are unlikely to show reversal to more plesiomorphic forms (Manos and Stone 2001). Given the current subfamilial classification, cupule morphology and germination type have seemingly undergone convergent evolution while correlated floral syndromes neatly divide the family (fig. 1). Because strong patterns of selection appear to have shaped the distribution of characters states associated with the reproductive biology of Fagaceae, our goal was to apply DNA sequence data to reconstruct phylogeny, assess systematic relationships, and explore alternative patterns of morphological specialization.

A Priori Hypotheses

The following explicit hypotheses about the distribution of reproductive character states for Fagaceae were developed from both analysis-based and intuitive perspectives on the relationships of genera within the family (fig. 3). These hypotheses are based on the assumption that the evolution of wind pollination, hypogeous germination, and flower-cupules in the strict sense are derived and irreversible within Fagaceae.

A. Wind pollination derived a single time, hypogeous germination two times, flower cupules one time, and a paraphyletic grade of trigonobalanoids. In the original presentation of this hypothesis, *Fagus* and a grade of trigonobalanoid genera were shown to form a clade with *Quercus*. Implicit to this arrangement is homology between the acorn cupule and dichasium cupule (fig. 2*A*; Nixon 1985, 1989; Nixon and Crepet 1989). This relationship is supported mostly by floral features (e.g., anther type, pollen exine, stigma type, inflorescence type). Subsequent molecular evidence indicated the position of *Fagus* and its putative synapomorphies with the trigonobalanoids and *Quercus* should be reconsidered. Based on this new evidence, we exclude *Fagus* and present the following modified form of this hypothesis: (*Trigonobalanus* – ((*Colombobalanus* – (((*Formanodendron* + *Quercus*)))))) + (Castaneoideae).

B. Wind pollination derived a single time, hypogeous germination two times, flower cupules one time, and a monophyletic Trigonobalanus. Forman (1964, 1966*a*, 1966*b*) based this hypothesis on comparative morphological study of the two Asian species *Trigonobalanus verticillata* and *Formanodendron doichangensis*. A monophyletic *Trigonobalanus sensu lato* also was implied by Lozano et al. (1979) when they later described *Trigonobalanus excelsa* and treated all three species in subfamily Trigonobalanoideae. This arrangement also tests the specific hypothesis that the acorn cup of *Quercus* was derived from the dichasium cupule of *Trigonobalanus* (see fig. 1): ((*Trigonobalanus sensu lato*) + (*Quercus*)), ((Castaneoideae)).

C. Two derivations of wind pollination, hypogeous germination one time, flower cupules one time, and a monophyletic Trigonobalanus. Previous phylogenetic studies of cpDNA restriction sites and combined analysis of *matK* and *rbcL* sequences suggested *Trigonobalanus* is sister to a clade of *Quercus* and castaneoid genera (fig. 2*B*; Manos et al. 1993; Manos

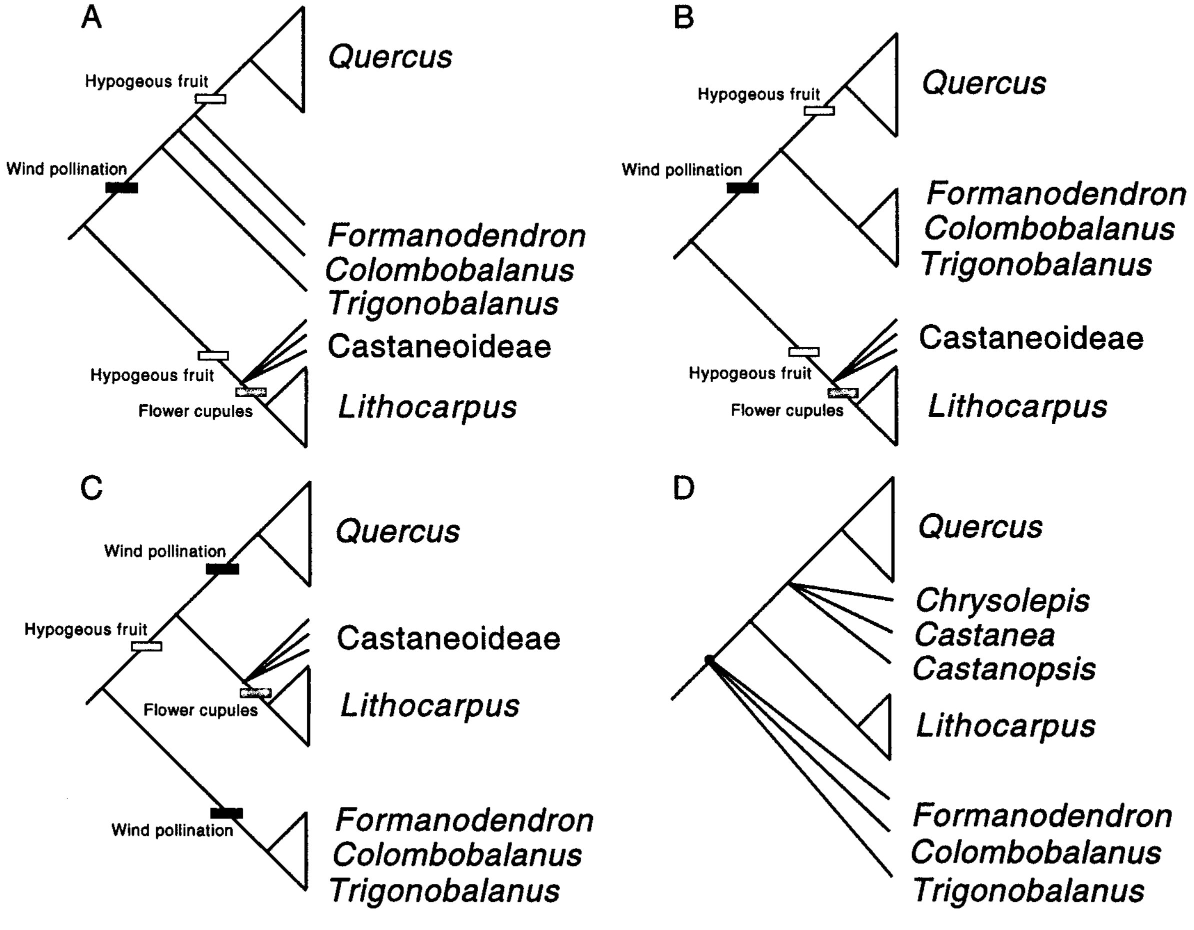


Fig. 3 Phylogenetic hypotheses for Fagaceae and distribution of derived reproductive character states. *A*, Morphological cladistic hypothesis (Nixon 1985, 1989; Nixon and Crepet 1989). *B*, Intuitive morphological hypothesis (Forman 1964, 1966*a*, 1966*b*). *C*, DNA-based cladistic hypothesis (see fig. 2*B*) modified from Manos and Steele (1997). *D*, Modified version of hypothesis C addressing the secondary hypothesis that acorn cupule of *Quercus* is derived from immediate castaneoid ancestors bearing dichasium cupules.

and Steele 1997): ((*Trigonobalanus sensu lato*) + (Castaneoideae + *Quercus*)).

D. Derivation of the acorn cupule of Quercus *from immediate castaneoid ancestors bearing dichasium cupules*. Most authors have recognized that dichasium cupules of the genera *Castanea*, *Castanopsis*, and *Formanodendron* have been transformed independently to variously formed single-fruited types (see fig. 1). In order to extend this hypothesis to *Quercus*, evidence for a dichasium-cupule origin is based on the purported close relationship to *Trigonobalanus* (see figs. 1, 2) and data from cupule development (e.g., MacDonald 1979). Building on hypothesis C, we specifically test whether the acorn cupules of *Quercus* are derived from the dichasium cupules of castaneoid genera: ((*Trigonobalanus sensu lato*) + (*Castanea*, *Castanopsis*, *Chrysolepis*, *Quercus*) + (*Lithocarpus*)))).

Material and Methods

Taxon Sampling

Leaf material for 179 terminal taxa was collected from natural populations or cultivated plantings. The names, authorities, sources, geographic distribution, and GenBank accession number are listed in the appendix. All of the currently recognized genera within Fagaceae were sampled, including each of the monotypic genera *Trigonobalanus*, *Colombobalanus*, and *Formanodendron*. For the intermediate to large genera *Quercus*, *Lithocarpus*, and *Castanopsis*, sampling included species from most infrageneric groups (Camus 1929, 1936–1954; Barnett 1944). Subfamily Castaneoideae is represented by a total of 94 accessions, including 62 from throughout the range of *Lithocarpus*. Sampling within *Quercus* was, in part, based on Manos et al. (1999); however, 38 additional accessions are included here, many of which represent Southeast Asian taxa (appendix).

Molecular Methods

Extraction of DNA was performed in the laboratory and field using the DNeasy Plant Mini Kit (Qiagen, Valencia, Calif.) on fresh and silica gel–dried leaf material. The internal transcribed spacers (ITS) region was amplified using Clontech Advantage-GC cDNA polymerase mix (Palo Alto, Calif.), which contains DMSO to reduce the possibility of obtaining nonfunctional paralogues. All other protocols for obtaining ITS sequences follow Manos et al. (1999). Because several studies have reported nonfunctional, paralogous ITS sequences in Fagaceae (Vazquez et al. 1999; Mayol and Rosselo 2001; Muir et al. 2001), we used three criteria to identify functional ITS copies: (1) minimal-length variation across the spacers and high levels of sequence conservation in the *5.8S* gene, (2) modest amounts of sequence divergence within clades and among the entire sample, and (3) general "taxonomic sense" of preliminary results. Several putative ITS sequences also were subjected to BLAST (Altschul et al. 1997) in GenBank as a check for contaminants. Methods for sequencing the *matK* region follow Manos and Steele (1997).

Sequence Variation, Outgroups, and Rooting

Although the broader relationships of Fagaceae within the eudicots are well established by single and multigene phylogenetic analysis (e.g., Qiu et al. 1998; Savolainen et al. 2000*a*, 2000*b*), phylogenetic hypotheses within the family are based on relatively few morphological and molecular data sets (Nixon 1985; Nixon and Crepet 1989; Manos et al. 1993; Li 1996; Manos and Steele 1997). Phylogenetic studies based on the plastid genes *rbc*L and *matK* suggested limited variation within most Fagaceae, especially among castaneoids and *Quercus* (Manos and Steele 1997), consistent with the slow rate of cpDNA variation reported for Fagaceae (Frascaria et al. 1993; Manos et al. 1999). Fortunately, additional sequencing of the ITS region across Fagaceae, in combination with previously published data (Manos et al. 1999), suggested resolution within Fagaceae could be obtained.

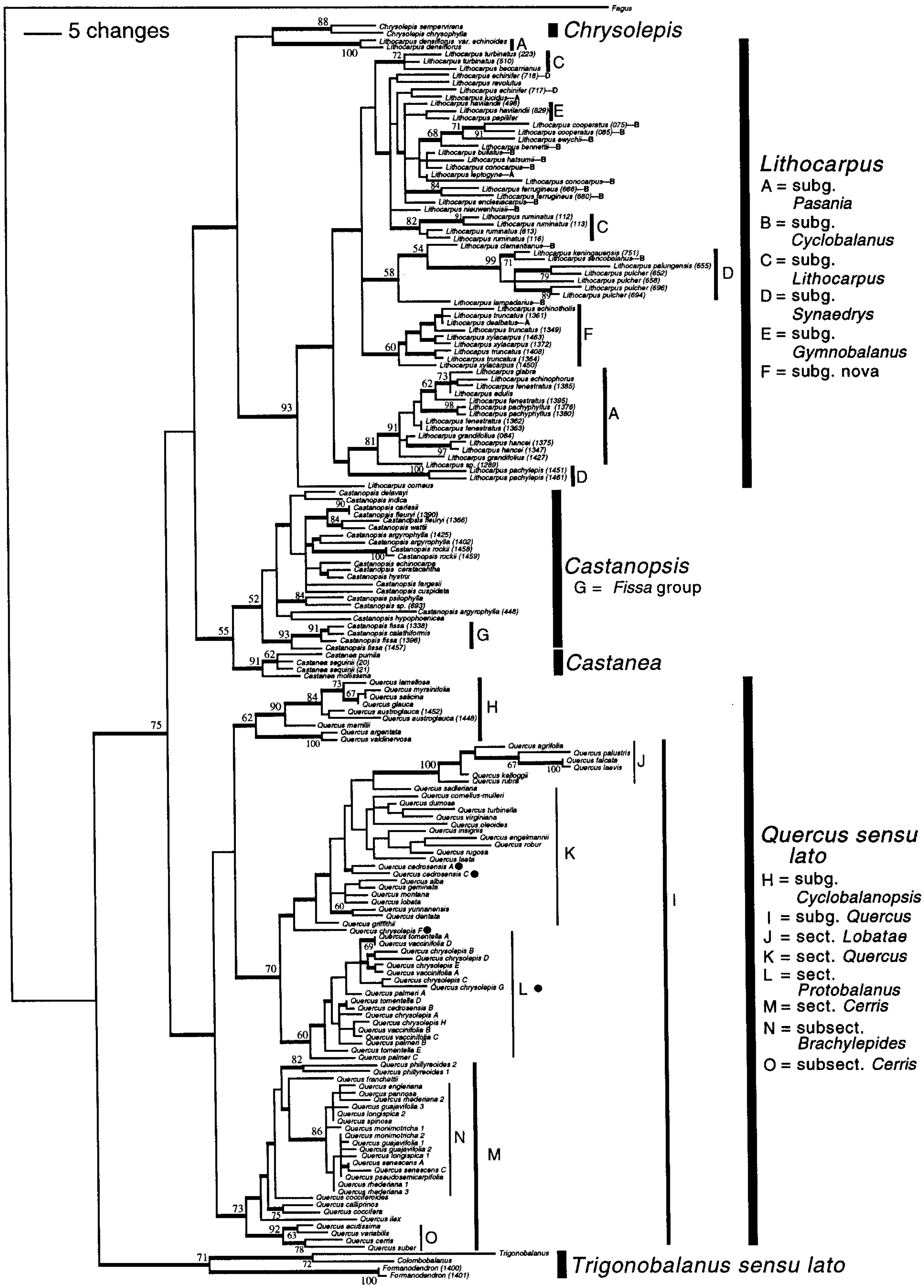
Because Fagaceae is somewhat isolated among Fagales, the use of rapidly evolving, noncoding sequence data compromised our selection of outgroups. Preliminary alignments of the ITS region using Fagaceae and a broad sample of sister or related Fagales (Betulaceae, Juglandaceae, and Nothofagaceae) revealed alignment ambiguities throughout ITS 1 and ITS 2 (P. S. Manos, unpublished data). The ITS sequence of *Fagus*, though divergent, proved much easier to align with those of other Fagaceae than with those of presumably more distant taxa from Fagales (fig. 2*B*). Therefore, we used *Fagus* as the outgroup for rooting the ITS trees, in agreement with its phylogenetic position based on plastid sequences (fig. 2*B*). The position of the root was explored further using constrained trees to test the morphological cladistic hypothesis (figs. 2, 3). Unrooted ITS trees also were rooted with *Fagus* using the Lundberg (1972) method which parsimoniously positions the outgroup sequence as the ancestral states to one of the nodes of the unrooted tree without performing simultaneous analysis.

Alignment

The boundaries of the internal transcribed spacers (ITS 1, ITS 2) and nrDNA coding regions for all sequences included here were determined following the procedure outlined in Manos et al. (1999). With the exception of the ITS sequence of *Fagus*, all sequences were aligned visually by first comparing sequences obtained from species belonging to the same genus on the basis of classical morphological evidence. Once these alignments were determined, sequences representing groups of genera were compared until all sequences were aligned. The genus *Fagus* was added to this alignment using the program CLUSTAL W version 1.8 (Thompson et al. 1994) followed by manual adjustment. Within this final alignment, sequence gaps were noted and, if phylogenetically informative, were added to the matrix as single binary characters. In regions where demonstrably different gaps showed partial overlap, the character was scored as missing in the appropriate cells of the supplemental binary matrix.

Phylogenetic Reconstruction

A complete data matrix (available from the authors) for 179 sequences of the ITS region was analyzed with equally weighted maximum parsimony (MP) with gaps treated as miss-


5 changes
Fagus
Chrysolepis sempervirens
Chrysolepis chrysophylla
Chrysolepis
Lithocarpus densiflorus var. echinoides
Lithocarpus densiflorus
A
Lithocarpus turbinatus (223)
Lithocarpus turbinatus (510)
Lithocarpus beccarianus
C
Lithocarpus echinifer (718)—D
Lithocarpus revolutus
Lithocarpus echinifer (717)—D
Lithocarpus lucidus—A
Lithocarpus havilandii (498)
Lithocarpus havilandii (829)
Lithocarpus papilifer
E
Lithocarpus cooperatus (075)—B
Lithocarpus cooperatus (085)—B
Lithocarpus ewychii—B
Lithocarpus bennettii—B
Lithocarpus bullatus—B
Lithocarpus hatsumii—B
Lithocarpus conocarpus—B
Lithocarpus leptogyne—A
Lithocarpus conocarpus—B
Lithocarpus ferrugineus (666)—B
Lithocarpus ferrugineus (680)—B
Lithocarpus enclesiacarpus—B
Lithocarpus nieuwenhuisii—B
Lithocarpus ruminatus (112)
Lithocarpus ruminatus (113)
Lithocarpus ruminatus (613)
Lithocarpus ruminatus (116)
C
Lithocarpus clementianus—B
Lithocarpus keningauensis (751)
Lithocarpus sericobalanus—B
Lithocarpus palungensis (655)
Lithocarpus pulcher (652)
Lithocarpus pulcher (658)
Lithocarpus pulcher (696)
Lithocarpus pulcher (694)
D
Lithocarpus lampadarius—B
Lithocarpus echinotholis
Lithocarpus truncatus (1361)
Lithocarpus dealbatus—A
Lithocarpus truncatus (1349)
Lithocarpus xylacarpus (1463)
Lithocarpus xylacarpus (1372)
Lithocapus truncatus (1408)
Lithocarpus truncatus (1364)
Lithocarpus xylacarpus (1450)
F
Lithocarpus glabra
Lithocarpus echinophorus
Lithocarpus fenestratus (1385)
Lithocarpus edulis
Lithocarpus fenestratus (1395)
Lithocarpus pachyphyllus (1376)
Lithocarpus pachyphyllus (1380)
Lithocarpus fenestratus (1362)
Lithocarpus fenestratus (1363)
Lithocarpus grandifolius (084)
Lithocarpus hancei (1375)
Lithocarpus hancei (1347)
Lithocarpus grandifolius (1427)
A
Lithocarpus sp. (1289)
Lithocarpus pachylepis (1451)
Lithocarpus pachylepis (1461)
D
Lithocarpus corneus
Lithocarpus
A = subg. Pasania
B = subg. Cyclobalanus
C = subg. Lithocarpus
D = subg. Synaedrys
E = subg. Gymnobalanus
F = subg. nova
Castanopsis delavayi
Castanopsis indica
Castanopsis carlesii
Castanopsis fleuryi (1390)
Castanopsis fleuryi (1366)
Castanopsis wattii
Castanopsis argyrophylla (1425)
Castanopsis argyrophylla (1402)
Castanopsis rockii (1458)
Castanopsis rockii (1459)
Castanopsis echinocarpa
Castanopsis ceratacantha
Castanopsis hystrix
Castanopsis fargesii
Castanopsis cuspidata
Castanopsis psilophylla
Castanopsis sp. (693)
Castanopsis argyrophylla (448)
Castanopsis hypophoenicea
Castanopsis fissa (1338)
Castanopsis calathiformis
Castanopsis fissa (1396)
Castanopsis fissa (1457)
G
Castanopsis
G = Fissa group
Castanea pumila
Castanea seguinii (20)
Castanea seguinii (21)
Castanea mollissima
Castanea
Quercus lamellosa
Quercus myrsinifolia
Quercus salicina
Quercus glauca
Quercus austroglauca (1452)
Quercus austroglauca (1448)
Quercus merrillii
Quercus argentata
Quercus valdinervosa
H
Quercus agrifolia
Quercus palustris
Quercus falcata
Quercus laevis
Quercus kelloggii
Quercus rubra
J
Quercus sadleriana
Quercus cornelius-mulleri
Quercus dumosa
Quercus turbinella
Quercus virginiana
Quercus oleoides
Quercus insignis
Quercus engelmannii
Quercus robur
Quercus rugosa
Quercus laeta
Quercus cedrosensis A ●
Quercus cedrosensis C ●
Quercus alba
Quercus geminata
Quercus montana
Quercus lobata
Quercus yunnanensis
Quercus dentata
Quercus griffithii
Quercus chrysolepis F ●
K
Quercus tomentella A
Quercus vaccinifolia D
Quercus chrysolepis B
Quercus chrysolepis D
Quercus chrysolepis E
Quercus vaccinifolia A
Quercus chrysolepis C
Quercus chrysolepis G
Quercus palmeri A
Quercus tomentella D
Quercus cedrosensis B
Quercus chrysolepis A
Quercus chrysolepis H
Quercus vaccinifolia B
Quercus vaccinifolia C
Quercus palmeri B
Quercus tomentella E
Quercus palmer C
L ●
I
Quercus phillyreoides 2
Quercus phillyreoides 1
Quercus franchettii
Quercus engleriana
Quercus pannosa
Quercus rhederiana 2
Quercus guajavifolia 3
Quercus longispica 2
Quercus spinosa
Quercus monimotricha 1
Quercus monimotricha 2
Quercus guajavifolia 1
Quercus guajavifolia 2
Quercus longispica 1
Quercus senescens A
Quercus senescens C
Quercus pseudosemicarpifolia
Quercus rhederiana 1
Quercus rhederiana 3
N
Quercus cocciferoides
Quercus calliprinos
Quercus coccifera
Quercus ilex
Quercus acutissima
Quercus variabilis
Quercus cerris
Quercus suber
O
M
Quercus sensu lato
H = subg. Cyclobalanopsis
I = subg. Quercus
J = sect. Lobatae
K = sect. Quercus
L = sect. Protobalanus
M = sect. Cerris
N = subsect. Brachylepides
O = subsect. Cerris
Trigonobalanus
Colombobalanus
Formanodendron (1400)
Formanodendron (1401)
Trigonobalanus sensu lato

ing data using Macintosh versions of PAUP 3.1.1 (Swofford 1993) and PAUP* version 4.0a3b (Swofford 2000). The search strategy used by Moncalvo et al. (2000) was adopted to effectively find sets of minimum-length trees. Heuristic searches started with 10,000 rounds of random taxon-entry sequences in conjunction with TBR with one, 10, and 100 trees saved per round. Sets of shortest trees were then used to initiate additional searches using MULPARS, TBR, AMB options. At least 1000 random addition sequences were used to search smaller data sets for tree islands. Consistency index (CI; Kluge and Farris 1969) and retention index (RI; Farris 1989) also were calculated. Consensus trees were constructed to evaluate branches common to sets of equally parsimonious trees. Bootstrap analysis (Felsenstein 1985) was used to determine the relative support for individual clades and, unless noted otherwise, all minimum-length trees were saved for each pseudoreplicate.

We also tested a series of likelihood models using the program Modeltest 3.0 (Posada and Crandall 1998), with a subset of 60 taxa selected by the following criteria: (1) taxa were chosen to represent subclades resolved in the strict consensus of parsimony analysis based on the complete data set, (2) taxa were excluded if their sequences were similar to others based on visual inspection of branch-length variation across 50 randomly chosen trees, and (3) the number of taxa representing the genus *Quercus* was reduced because infrageneric relationships have been addressed previously (Manos et al. 1999). We performed hierarchical likelihood ratio tests (see Huelsenbeck and Crandall 1997) starting with a neighbor-joining tree and determined that the TIM + G model (Posada and Crandall 1998), a submodel of the general time reversible model (e.g., Yang 1994), was appropriate for tree estimation. Models with additional parameters, such as estimation of invariable sites, were not significantly more likely. TIM is a transitional model with six rates ([A-C] = 1.000, [A-G] = 2.8742, [A-T] = 0.4388, [C-G] = 0.4388, [C-T] = 7.0880, [G-T] = 1.000) assumed to vary following a γ distribution (shape parameter = 0.4006) as applied to a matrix based on the following estimated nucleotide frequencies: A = 0.1935, C = 0.3301, G = 0.3087, T = 0.1677. Maximum likelihood (ML) analyses were conducted with PAUP* using stepwise addition to generate starting trees followed by two heuristic searches with TBR. We also analyzed these data using MP as described above, but separately analyzed the data with and without binary, gap-derived characters. MP trees were tested against ML trees by mapping parsimony informative sites onto the topologies derived from each analysis using the Templeton's test (1983) as implemented in PAUP*.

A combined data set also was assembled for 14 phylogenetically critical taxa within Fagaceae based on ITS sequences and 889 base pairs of the *matK* gene and its 3′-spacer region. Incongruence between data sets was tested using the incongruence-length difference (ILD) test of Farris et al. (1995) using PAUP*.

Parsimony-based analyses using constraints enforced to match a priori hypotheses (fig. 3) were conducted using the same heuristic MP search protocols as above. Differences in tree lengths between constrained searches and sets of MP trees were tested using Templeton's test. When numerous MP trees were recovered, a total of 100 trees chosen at random were evaluated. A priori hypotheses were tested using MP-based trees derived from the 179-, 60-, and 14-taxon data sets, respectively.

Results

Sequences of the ITS region for 179 taxa produced an alignment of 635 bp. Average percentage G + C content and length variation within individual spacers and the *5.8S* coding region was within the range reported by Manos et al. (1999) based on a smaller sample of Fagaceae. Several new sequences of ITS/5.8S were subjected to BLAST and showed strongest homology with angiosperms, specifically other Fagaceae and related taxa. We considered the pattern of minimal to no site substitution within conserved regions of the *5.8S* gene as primary evidence in support of comparing functional copies of ITS across the study group (see Muir et al. 2001).

The ITS region in *Fagus* was on average 40 bp longer than all other Fagaceae. This size difference was confined to a single indel within ITS 1 and reconciled by excluding the region while aligning *Fagus* to other taxa. Alignment of the final matrix also required the introduction of several 1- or 2-bp indels (insertion or deletion mutations) distributed throughout ITS 1 and ITS 2, eight of which were unique to species of particular taxonomic groupings. We coded these as binary characters and combined them with sequence data. Twenty-three sites were excluded from all subsequent analyses because of ambiguous alignment. On the basis of the final alignment, values of pairwise percentage sequence divergence among the ingroup were below 12.2%, whereas values between the outgroup *Fagus* and ingroup ranged from 17.8% to 20.8%.

The 889 bp sequenced from the *matK* gene and 3′ spacer for 14 representative taxa of Fagaceae provided only 13 phylogenetically informative sites. Sequence divergence among the ingroup was low and generally less than 1.0% in comparisons among castaneoids and *Quercus*, roughly 2.0% between trigonobalanoids and other ingroup taxa, and ca. 7.0% between *Fagus* and the ingroup.

Phylogenetic Analyses

From the complete data set of 179 ITS sequences, a total of 237 phylogenetically informative characters (including indels) formed the basis for MP analyses. Numerous heuristic searches

Fig. 4 One of thousands of most parsimonious phylograms based on the 179-taxon ITS data including indel characters (length = 1038, CI = 0.34, RI = 0.82). Thickened branches indicate the node occurs in the strict consensus tree. Bootstrap values (above and below branches) are based on saving 100 trees for each pseudoreplicate. Thickened vertical lines indicate traditionally recognized genera, subgenera, sections, and subsections.

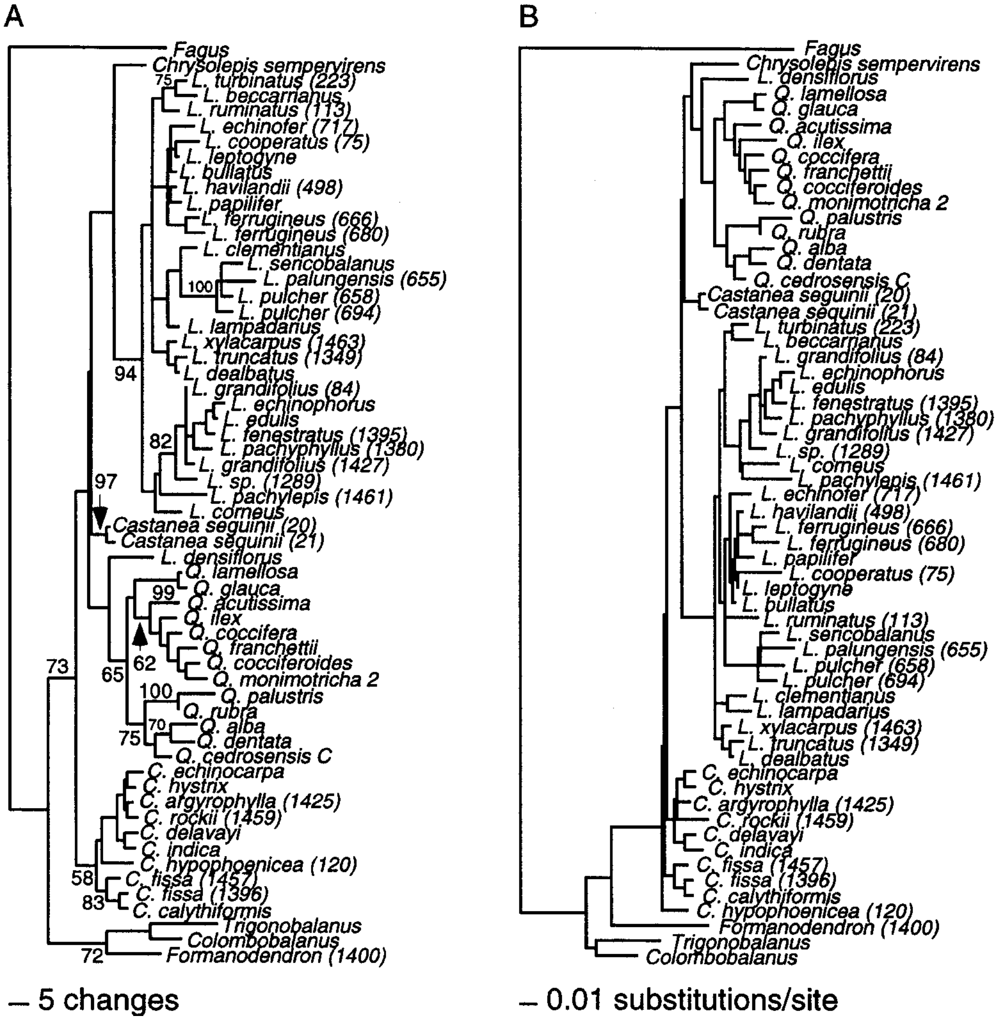


Fig. 5 Phylogenetic hypotheses for Fagaceae based on 60-taxon ITS data excluding indel characters. *A*, One of 36 most parsimonious phylograms (length = 537, CI = 0.44, RI = 0.70). Bootstrap values (above and below branches) are based on 1000 pseudoreplicates. *B*, One of two most likely phylograms recovered in maximum likelihood searches.

consistently identified at least 40,000 minimum-length trees of 1038 steps (fig. 4). Outgroup rooting suggested four major clades: (1) Trigonobalanoids, (2) *Quercus*, (3) *Castanea* + *Castanopsis*, and (4) *Chrysolepis* and *Lithocarpus*. Subclades within *Quercus* generally correspond to previously delimited taxonomic groups. Within *Castanopsis*, only the *fissa* group formed a well-supported subclade. *Lithocarpus densiflorus* was not found among Asian *Lithocarpus* species and remained unresolved at the base of the clade that also included species of *Chrysolepis*. Within Asian *Lithocarpus*, several subclades corresponded to previously delimited groups, while others indicated paraphyletic to unresolved groupings. Percentage values based on heuristic bootstrap analysis, saving 1000 trees per pseudoreplicate, supported the basal division between the trigonobalanoid genera and remaining Fagaceae and numerous subclades resolved in the consensus received moderate (>50%) to strong support including, *Trigonobalanus sensu lato*, *Castanea*, *Catanopsis*, *Castanopsis fissa* group, Asian *Lithocarpus*, part of *Lithocarpus* subg. *Pasania*, and three groups of *Quercus*.

The use of the Lundberg (1972) method for rooting trees also suggested a root along the branch leading to trigonobalanoid clade, in agreement with the results of outgroup rooting. Alternative positions for the root include the arrangement depicted in figure 2*A* and hypothesis A of figure 3. This alternative is discussed below.

Heuristic MP searches of the 60-taxon data set based on 161 informative characters including indel-based binary characters produced a single island of 210 equally parsimonious trees. The consensus (not shown) was similar to that based on 179 taxa, except for the position of *Chrysolepis* and *Lithocarpus densiflorus*, which were unresolved relative to the same four major clades described above (see fig. 4). Bootstrap values were generally similar to those obtained for the 179-taxon data set, but with less than 50% support for the clade including *Castanea* + *Castanopsis* and increased support for the *Quercus* clade (62%). Searches excluding indel characters recovered a single island of 36 minimum-length trees and more resolution within the consensus, but with similar levels of overall branch support (fig. 5*A*). Heuristic analyses of this same data set using ML-generated trees with a similar overall topology (fig. 5*B*). Because of the computational difficulties associated with ML analyses and data sets of this size, branch support was not calculated. Mapping of the parsimony informative data set onto ML trees required an additional seven steps; however, this difference was not significant according to the Templeton test.

Data sets for cpDNA and ITS sequences (including indels) based on 14 taxa representing all major groups were found to be combinable according to the ILD test ($P = 0.20$). A combined data set consisting of 87 phylogenetically informative characters was analyzed using MP with BRANCH AND BOUND producing a single tree (fig. 6) that was largely congruent with those derived from separate analyses (see figs. 4, 5). As before, there was moderate to strong support for two clades, one composed of the trigonobalanoid genera and the other including the remaining genera. Within the larger and well-supported clade, there was weak support for the paraphyly of subfamily Castaneoideae relative to *Quercus* and notable increase in bootstrap support for the *Castanea* + *Castanopsis* clade (76%).

Hypothesis Testing

The results of performing the Templeton test (1983) on the a priori hypotheses presented in figure 3 using optimal trees–based MP analyses are summarized in table 2.

A. Relationships based on morphological cladistic analysis; paraphyletic trigonobalanoids form a clade with Quercus*—rejected.* All MP analyses support a clade of trigonobalanoid genera. Trees conforming to the alternative hypothesis are significantly longer and bootstrap support for the clade is moderate: 71% in 179-taxon MP, 72% in 60-taxon MP, and 73% in 14-taxon MP analyses, respectively.

B. Traditional taxonomic concept of Trigonobalanus sensu lato *as monophyletic and closely related to* Quercus*—equivocal.* Although this hypothesis consistently requires four extra steps in each constrained analysis, only the 14-taxon combined MP analysis indicated a significant difference, and thus rejection of the hypothesis.

C. Relationships suggested by cpDNA—not rejected. Support for two basic clades within Fagaceae is found in each unconstrained MP analysis. The position of *Formanodendron* and *Colombobalanus* based on ITS and *matK* sequences confirms the hypothesis of two ingroup clades resolved in previous analyses.

D. Acorn cupules of Quercus *are derived from castaneoids with dichasial cupules—not rejected.* This hypothesis requires extra steps in the all analyses, but these differences are not significant. Considering the lack of support among the *Quercus* lineage and castaneoid genera, there is no basis to choose among equally likely scenarios.

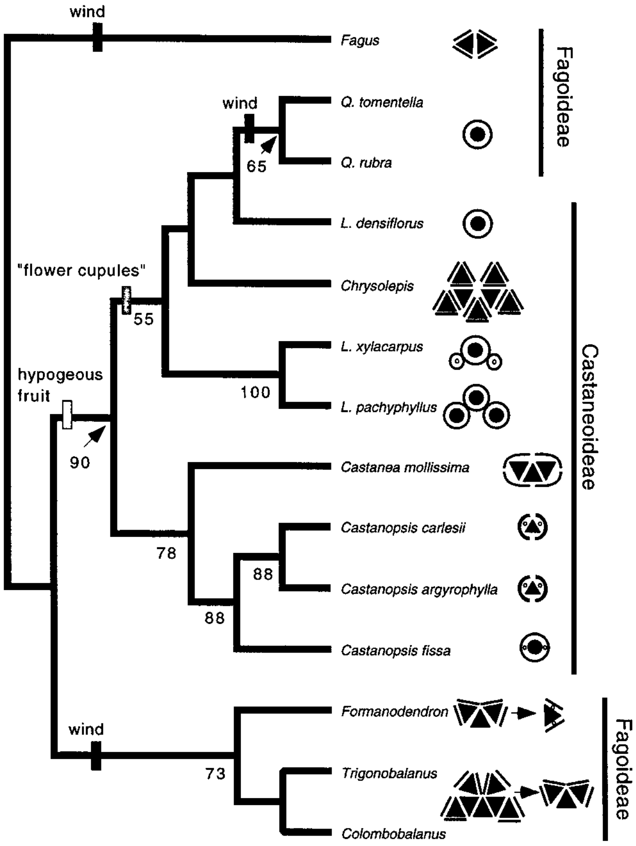


Fig. 6 Single most parsimonious tree based on combined analysis of ITS and *matK* (length = 219, CI = 0.58, RI = 0.54). Bootstrap values (below branches) are based on 1000 pseudoreplicates. Derived reproductive traits are mapped, and cupule type follows exemplar taxa. Cupule valves are indicated with straight or curved lines; fruit are shown with solid circles or triangles; aborted flower position is shown with small open circles. Classification is based on the results of morphological cladistic analysis (Nixon and Crepet 1989).

Discussion

Systematic and Phylogenetic Inferences

Our analyses of DNA sequences from a broad sample of Fagaceae have revealed phylogenetic patterns to further evaluate relationships and the evolution of reproductive traits within the family. Specifically, the molecular data presented here reject the most recent classification of the family based on morphological cladistic analysis (fig. 2*A*; table 1). We instead find support for a monophyletic *Trigonobalanus sensu lato* as sister group to a large clade comprised of the four castaneoid genera and *Quercus* (figs. 4–6). Several previously published analyses suggested that this clade of fagoid and castaneoid genera is sister to the genus *Fagus* (Manos et al. 1993; Manos and Steele 1997; Qiu et al. 1998; Savolainen et al. 2000*a*). Taken together, resolution of three major clades of Fagaceae and support for a close relationship of *Quercus* to the castaneoid genera raises several important issues that specifically address the origin of the genus *Quercus* and evolution of morphological specialization in general.

Table 2

Results of the Templeton (1983) Test of Alternative Hypotheses

Hypothesis	179-taxon ITS (TL = 1038)	60-taxon ITS (TL = 548)	14-taxon ITS + *matK* (TL = 219)
A	1059 (0.004–0.0112)	566 (0.0009–0.0083)	244 (<0.0001)
B	1042 (ns)	552 (ns)	228 (0.0201)
C	1038 (ns)	548 (ns)	219 (ns)
D	1041 (ns)	552 (ns)	225 (ns)

Note. Hypothesis letters refer to those outlined in the text and figure 3. Tree lengths (TL) are provided for the optimal trees based on unconstrained and constrained analyses. *P* values (in parentheses) are for the constrained trees based on each hypothesis and data set, respectively; ns, not significant.

Castaneoideae

The monophyly of subfamily Castaneoideae is suggested by the uniform expression of staminate flowers bearing 12 stamens with a nectariferous pistillode, pistillate flowers with punctate styles, and hypogeal cotyledons. However, the isolated position of these insect-pollinated Fagaceae among potential sister taxa, with clearly derived floral features associated with wind pollination, raises the possibility that various aspects of castaneoid flowers are retained plesiomorphies. While some features of this pollination syndrome could be derived, no other extrafloral morphological character state, except for hypogeal cotyledons shared with *Quercus*, is unique to the four genera of the subfamily. Thus, only floral attributes of Castaneoideae consistently serve to distinguish this previously recognized taxon from other Fagaceae. We note that molecular support for either monophyly or paraphyly is lacking, but the latter, as suggested by combined data (fig. 6), remains an intriguing possibility.

Regardless of the phylogenetic status of Castaneoideae, phylogenetic resolution among the various genera contradicts the notion that *Castanopsis* and *Lithocarpus* are closely related (see fig. 2*A*). Analyses based on the 179-taxon and 14-taxon combined data sets support *Castanea* and the strictly southeast Asian genus *Castanopsis* as sister taxa (figs. 4, 6), in agreement with traditional taxonomic treatments (Camus 1929). Both genera are delimited consistently by morphological apomorphies and represent the only clear example of a temperate-subtropical genus pair within the family. The inflorescences of *Castanopsis* are unisexual, a condition that appears to be constant on further herbarium study (P. S. Manos, personal observation). The uniqueness of *Castanea* lies in the pistillate flowers, which always have six or more styles (Camus 1929), although the constant expression of annual fruit maturation represents another potentially derived feature.

The presence of spiny cupule appendages largely defines this clade, but it is clear that spines have been lost in several species and species groups of *Castanopsis*. One example is the *Castanopsis fissa* group (fig. 4, group G) which is well supported by sequence data, unique ruminate cotyledons (Okamoto 1980), and derived fruit type in which a circular nut is subtended by a valveless to irregularly dehiscent cupule (see fig. 1). Our analysis provides the first independent evidence to corroborate the taxonomic transfer of this group of species from *Lithocarpus* (subg. *Pseudocastanopsis sensu* Camus 1936–1954) to *Castanopsis* (Barnett 1944). Within *Castanopsis*, distinction between *fissa* species and other sampled taxa appears to form a significant infrageneric division within the genus, while many of the other traditional species groups are scattered. Species of the *fissa* group fruit annually (Camus 1929; P. S. Manos, personal observation), producing a single fruit within a valveless cupule, whereas most other single-fruited taxa within *Castanopsis* have valvate cupules.

The unique morphological arrangement of cupule valves to fruit serves to segregate the two currently recognized species within the genus *Chrysolepis* from their former placement within *Castanopsis* (Hjelmquist 1948; see fig. 1). The occurrence of *Chrysolepis* in montane western North America provides an element of distinctiveness as well. Our data support the current taxonomic treatment and morphological cladistic position that *Chrysolepis* and *Castanopsis* are not sister taxa, the former more likely related to *Quercus* and *Lithocarpus* (figs. 4, 6). Combined analysis weakly supports a clade consisting of *Chrysolepis*, a paraphyletic *Lithocarpus*, and *Quercus* (fig. 6).

Lithocarpus

All sampled species of *Lithocarpus*, except for *Lithocarpus densiflorus*, formed the most strongly supported group within Fagaceae (figs. 4, 5*A*, 6). This genus is morphologically unique within Fagaceae based on the production of flower cupules in the strict sense, such that each pistillate flower within dichasia is seated within its own distinct, valveless cupule. This synapomorphy becomes less clear with the loss of lateral flowers (e.g., *L. densiflorus*, *C. fissa* group and *Quercus*). The finding of little to no molecular phylogenetic signal to unite *L. densiflorus* with other *Lithocarpus* species has interesting implications. The traditional characters used to define *Lithocarpus* (castaneoid flowers, flower cupules, and evergreen habit) are consistently present in *L. densiflorus*; however, this species is distinct on the basis of trichome type, an important vegetative trait that defines *Lithocarpus* (Jones 1986). *Lithocarpus densiflorus* possesses multiradiate leaf trichomes, whereas all Asian species with leaf vestiture have the more typical appressed two- to four-rayed trichomes, not found among other Fagaceae (Jones 1986; Cannon and Manos 2000). Biogeographically, both *L. densiflorus* and *Chrysolepis* occupy an area of high endemism, incidental supporting evidence for the relictual nature of the castaneoids occurring in western North America (Manos and Stanford 2001).

Phylogenetic structure within Asian *Lithocarpus* is both appreciable and striking considering this initial assessment of Camus's (1936–1954) infrageneric taxonomy (fig. 4). In this light, we present a brief appraisal of the phylogenetic results emphasizing several of the most distinctive morphological groups within the genus. Because widespread species are rare within the genus, many of the groupings reflect the regional geographical distribution of related species. An exception is the widespread *Lithocarpus grandifolius* that we sampled from southwest China and Borneo that group within in the same clade (fig. 4, group A). Species placed within this clade are classified within subg. *Pasania* (*sensu* Camus) and our sample includes taxa from Japan and China as well. However, other Bornean species and *L. densiflorus*, also classified within subg. *Pasania*, show no relationship to this group.

One overriding pattern is that the least specialized taxa within *Lithocarpus*, generally those species classified within the subgenera *Pasania* and *Cyclobalanus*, form paraphyletic assemblages within which more specialized groups are nested (fig. 4, groups A and C). Subgenus *Pasania* contains at least 40% of the classified species (Camus 1936–1954) and essentially occurs throughout the range of the genus, whereas subg. *Cyclobalanus* is restricted to West Malaysia and the Malayan Archipelago. Both groups show some variation in cupule enclosure and cupule-appendage type, but neither group exhibits derived-fruit features observed in subg. *Lithocarpus* and *Synaedrys*, such as cupule-to-fruit fusion or proliferation of receptacular tissue around the cotyledons (Cannon and Manos 2000, 2001). For the taxa classified within these subgenera (fig. 4, groups C and D), ITS data suggest convergent evolution in fruit type. Morphometric data on fruit shape analyzed in combination with ITS further support the hypothesis of convergence in subgenus *Lithocarpus* (Cannon and Manos 2001). Additional studies of subgenus *Synaedrys* are needed to address the initial hypothesis of convergence presented here.

We also find support for a novel group of continental species localized in at least southwest China, Myanmar, and North Vietnam classified within at least three of Camus's subgenera (fig. 4, group F). Preliminary data from fruit morphology support this grouping based on the convex shape of the fruit scar. The scar itself may be small as in *Lithocarpus dealbatus*, or quite large as in *Lithocarpus xylacarpus* and *Lithocarpus truncatus*. The full range of fruit variation and its relationship to infrageneric diversity within *Lithocarpus* will be considered elsewhere (P. S. Manos and C. H. Cannon, unpublished data).

Quercus sensu lato

The monophyly of the genus *Quercus* is supported in separate and combined analyses (figs. 4–6). Morphological synapomorphies include constant expression of a single pistillate flower, valveless cupules, decurrent styles, expanded stigmatic surfaces, unisexual inflorescences, lax staminate inflorescences, and scabrate pollen exine structure (Nixon 1985; Nixon and Crepet 1989). Neither the current subgeneric classification nor purported relationship to a paraphyletic grade of trigonobalanoids is supported by sequence data. Our data instead suggest that *Quercus* is closely related to castaneoid genera, and possibly derived among them.

ITS data resolve three clades of *Quercus* with moderate support: (1) subtropical to tropical Southeast Asian species classified within *Quercus* subg. *Cyclobalanopsis*; (2) mostly New World species classified within subg. *Quercus* representing sections *Quercus* s.s. as defined by Manos et al. (1999), *Protobalanus*, and *Lobatae* (*sensu* Nixon 1993, 1997); and (3) Old World species previously classified within subg. *Quercus* section *Cerris* (Camus 1936–1954). Section *Cerris* appears to contain several morphologically distinct groups resolved by ITS data, in particular, an expanded group mainly comprising species of subsection *Brachylepides* (fig. 4, group N), which are also defined by fused cotyledons (Zhou et al. 1995). In contrast, resolution among the New World oaks is particularly weak, and increased sampling among section *Quercus* s.s. appears to have promoted instability throughout this part of the tree. The inability of ITS to distinguish fully between species representing sections *Protobalanus* and *Quercus* s.s. is most likely indicative of incomplete lineage sorting (Manos et al. 1999).

Trigonobalanus sensu lato

The monophyly of continentally disjunct *Trigonobalanus sensu lato* is supported in most analyses, except for ML analysis of the 60-taxon ITS data set (fig. *5B*) and MP analysis of the 14-taxon *matK* data set (not shown). Interestingly, the paraphyletic arrangements suggested by these two analyses place different trigonobalanoid species as sister to the remaining members of Fagaceae. Each molecular data set shows considerable sequence divergence among *Trigonobalanus* species and between them and other Fagaceae. The great antiquity and relictual nature of these species also is evident from a variety of perspectives, including apomorphies detected in pollen ultrastructure (Nixon and Crepet 1989), widespread distribution of fossil equivalents (Mai 1970; Crepet 1989; Crepet and Nixon 1989*a*, 1989*b*; Kvaček and Walther 1989), and presence of anomalous features in certain species, such the whorled leaf arrangement and polyploid chromosome number ($2x=44$) observed in *Trigonobalanus verticillata* (Hou 1971).

Previous morphocladistic study of the three species placed in the genus *Trigonobalanus* supported their segregation into monotypic genera based on the lack of synapomorphies (Nixon and Crepet 1989). Our results suggest that *Trigonobalanus sensu lato* also could be defined by many of the floral and inflorescence features that have convergently evolved in *Quercus* (see above). Despite unique apomorphies discovered for each species and a wealth of shared plesiomorphies, such as branched inflorescences, valved cupules, and epigeous germination, our recommendation is to recognize a single genus on the basis of combined phylogenetic analysis.

Reproductive Trait Evolution

Our analysis of gene sequences across the taxonomic breadth of Fagaceae provides an objective means of assessing the distribution of morphological character states associated with key reproductive traits. Interpretations of trait evolution require specific hypotheses of character state polarity, and these have been formulated during the course of previous cladistic and evolutionary investigations of Fagales and Fagaceae (Kaul 1985; Nixon 1985; Nixon and Crepet 1989). Our data generally support the basic framework of hypothesis C (see fig. 3), thus providing a useful platform to consider hypotheses of independent origin. We present our working hypotheses in the context of the 14-taxon combined analysis assuming insect pollination, dichasium cupules, and epigeous germination are ancestral states for Fagaceae and that once transformed, reversals are highly unlikely (fig. 6).

Evolution of Wind Pollination

Under the assumption that the ancestors of modern Fagaceae were insect pollinated, our analyses suggest three separate origins of wind pollination within the family (fig. 6). Recent topology-based correlation analyses of trait evolution among angiosperm families found that transition from biotic to abiotic pollination is strongly asymmetric and correlated with a net decrease in speciation rate (Dodd et al. 1999). Cox (1991) had previously noted that this seemingly irreversible transition (*sensu* Bull and Charnov 1985) is tightly linked to the physical separation of male and female reproductive functions and that when viewed in the absence of ecological context, the correlates and consequences of abiotic pollination could be oversimplified. Fagaceae are nested within a clade of wind-pollinated families that largely share monoecious flowers, similar patterns of floral reduction, and generally low levels of species diversity (Manos and Steele 1997; fig. 2*B*). However, the family is unique based on the presence of two pollination syndromes and significant levels of species diversification within several genera, including the wind-pollinated genus *Quercus* (table 1).

Although parsimony favors a single origin of wind pollination for the Fagales (see Dodd et al. 1999), the disparate nature of floral morphology among Fagalean families provides some evidence to consider multiple origins from a diverse assemblage of extinct insect-pollinated lineages. Macrofossil evidence minimally dates the origin of modern fagalean families back to the earliest Tertiary (Manchester 1999 for review), and the recovery of fossil flowers bearing *Normapolles* pollen suggests their precursors diverged at least by the Upper Cretaceous (Friis 1983; Sims et al. 1999; Schonenberger 2001). Comparative morphological and anatomical evidence also supports deep divergence among modern families, as synapomorphic character states are few (Abbe 1974; Hufford 1992; Manos and Steele 1997) and not surprisingly, include similarities in inflorescence structure (e.g., catkins) and features of the pollen, such as aperture and exine morphology (Nixon 1985, 1989). Cupulate fossils of proto-Nothofagaceae–Fagaceae-bearing, castaneoid-like staminate flowers, some with nectaries, from the Late Cretaceous provide evidence for an insect-pollinated ancestry for at least modern cupule-bearing descendant lineages (Herendeen et al. 1995; Sims et al. 1998).

Based on the position of Fagaceae within Fagales (see fig. 2*B*) and under the assumption that the condition of wind pollination is reversible, parsimony suggests that insect pollination in the castaneoid taxa is secondarily derived (see fig. 2*B*). We argue that ancestral character state reconstruction with equally weighted character state transition is biologically unrealistic in this case. Although hypotheses of irreversible evolution are difficult to test by using ancestral character state reconstruction (Cunningham 1999), application of a subjectively chosen weighting scheme to achieve the desired result, i.e., that all ancestors within Fagaceae are insect pollinated, emphasizes the limitations of this approach (Omland 1997, 1999). Admittedly, weak support within the largest clade of Fagaceae and the limited number of independent contrasting states prohibits adequate testing of the evolution of pollination syndromes. However, widespread asymmetry of the insect-to-wind transition (biotic to abiotic) within angiosperms provides a measure of external support on the general irreversibility of abiotic pollination (Cox 1991; Dodd et al. 1999).

While only one clear-cut reversal to insect pollination was observed by Dodd et al. (1999) (e.g., Joinvilleaceae within Poales, but see Bayer and Appel [1998] for a contrasting view of pollination in *Joinvillea*), other examples of secondary derivations of insect pollination probably exist at the intrafamilial level, such as those noted in the largely anemophilous Cyperaceae (Goetghebeur 1998). Indeed, a potential exception also occurs within Fagales, as an entomophilous pollination syndrome reported for the genus *Platycarya* (Juglandaceae) was characterized as a secondary derivation from wind-pollinated ancestors (Endress 1986). We merely emphasize the difficulties in considering reversal to entomophily within Fagaceae because it would involve the evolution of the castaneoid floral syndrome (smaller anthers, versatile filament attachment, fragrance, and small and smooth pollen grains) from ancestors bearing the features of highly derived oaklike flowers. Thus, the maintenance of pollination syndrome polymorphism in modern Fagaceae, coupled with the fossil floral evidence for castaneoid-like precursors and potential paraphyly of the castaneoid genera, suggests an entomophilous ancestry and likely tropical origin for the family.

Assuming that the modern castaneoids have retained plesiomorphic floral character states, the derived character states associated with wind pollination observed in *Fagus*, *Trigonobalanus sensu lato*, and *Quercus* could have originated independently. Previous phylogenetic analyses within Fagaceae robustly support independent derivation of the traits associated with wind pollination in *Fagus* (e.g., Manos et al. 1993; Manos and Steele 1997), and this is reconciled easily by the superficial nature of floral similarities between this genus and other wind-pollinated Fagaceae. In contrast, similarities of the staminate and pistillate flowers and pollen of *Trigonobalanus sensu lato* and *Quercus* provide compelling evidence for a close relationship on the basis of intuitive and morphocladistic perspectives (Forman 1964, 1966*a*, 1966*b*; Nixon 1985; Nixon and Crepet 1989). While clear rejection of the hypothesis for a monophyletic *Trigonobalanus* as sister to *Quercus* is possible only with additional sequence data from the chloroplast genome, we suggest that constraints on morphological form under similar conditions favoring the evolution of anemophily may be one source of floral and pollen convergences. Our hypothesis suggests that *Trigonobalanus sensu lato* may be viewed as an independent, early trial of wind pollination from castaneoid-like ancestors. The fossil history of the trigonobalanoids is compatible with this assertion as it is generally contemporaneous with the earliest castaneoid records (Crepet and Nixon 1989), indicating wind pollination had evolved by the end of the Paleocene, minimally 15 million years before the appearance of fossils unequivocally assigned to *Quercus* (Crepet 1989; Crepet and Nixon 1989*a*, 1989*b*).

Evolution of the Cupule and Fruit

The results of our phylogenetic analysis are in agreement with morphocladistics and the stratigraphic record in suggesting that dichasial cupules are plesiomorphic within Fagaceae (Nixon and Crepet 1989; Herendeen et al. 1995; Sims et al. 1998). Dichasial cupules and a variety of their transformed states occur in each of the three clades resolved within Fagaceae, and similar types of modification also occur in Nothofagaceae (Hill and Read 1991). Hallmarks of this transformation include reduction in pistillate flower number and apparent cupule valve fusion (fig. 6). Overall, we find support for the independent dichasial origins of the single-flowered cupules in *Castanea*, *Castanopsis*, and *Formanodendron*.

While the acorn cup of *Quercus* is most likely not derived from an ancestral trigonobalanoid species (see fig. 1), our data can neither accept nor reject the traditional hypothesis of an origin from ancestors bearing dichasium cupules. Combined analysis provides some evidence for a relationship with *Lithocarpus* and *Chrysolepis*, taxa that basically express dichasially arranged pistillate flowers (fig. 6) but with each flower surrounded by cupular tissue. This hypothesis suggests that a more broadly defined flower cupule in which individual pistillate flowers are seated within a separate cupule may have a single origin, thus defining the clade composed of *Lithocarpus*, *Chrysolepis*, and *Quercus* (Soepadmo 1968). Therefore, the loss of lateral flowers bearing their own cupule, rather than loss of lateral flowers within dichasial cupules, could also explain the origin of the classical acorn fruit in *Quercus*. This is consistent with Soepadmo's (1970) interpretation of the cupule vasculature similarity in *Quercus* and *Lithocarpus*. The placement of *Chrysolepis* among these taxa is generally compatible with Forman's (1966*b*) transformation series linking its unique dichasium cupule to the flower cupules of *Lithocarpus*. More sequence data and explicit developmental studies are needed to address the possibility that a more broadly defined "flower cupule" is homologous in these taxa.

Our analyses also suggest that the evolution of hypogeous germination occurred once in the evolutionary history of Fagaceae. Given the novel arrangement of castaneoids + *Quercus*, only hypogeous germination and fruit wall anatomy (Soepadmo 1968) appear to unite this otherwise heterogeneous group of taxa. The fossil record for Fagaceae shows that large, presumably hypogeous fruit evolved by the Middle Eocene suggesting a relatively early transition to more specialized forms of animal dispersal (Crepet and Daghlian 1980; Manchester 1994). The animal-dispersal syndrome, characterized by large nut size and lack of wings, also appears in the related Juglandaceae within the same time frame. The evolution of animal-dispersed fruit seems to reflect generalized coevolution between these families and rodents (Manchester 1987). Within the Rodentia, the first fossil member of the family Douglassia is known from Late Eocene of North America, while the first "sciuromorph" squirrel fossil, those with jaw muscles arranged in typical modern-squirrel fashion, giving it good mechanical advantage, is *Palaeosciurus goti* from the Lower Oligocene of Europe (Vianey-Liaud 1985).

Diversification within Fagaceae may have been spurred by particular combinations of morphological innovation including transitions to animal-dispersed fruit (including hypogeous germination), evolution of wind pollination, and evolution of single-fruited, valveless cupules. Our analysis suggests that the evolution of animal-dispersed fruit could represent a key innovation in the generic diversification of Fagaceae. With the exception of *Castanea*, all other genera show appreciable species diversity relative to *Fagus* and *Trigonobalanus*. It is of particular interest that the two most species-rich genera, *Lithocarpus* and *Quercus*, taxa with contrasting pollination syndromes, are potentially characterized by flower cupules in the broad sense. The evolution of a valveless cupule subtending the basal portion of the circular nut is constant in *Quercus* and widespread in *Lithocarpus*, and the fruit of both genera are clearly associated with active dispersal by rodents (Payne et al. 1985). However, further specialization within certain Asian *Lithocarpus* involving cupule-to-nut fusion and increased lignification suggests strong selection toward protection, perhaps in response to the more diverse array of seed-eating vertebrates in the paleotropics (Cannon and Manos 2001).

Acknowledgments

We thank M. E. Jones for providing expert technical assistance throughout this study. Special thanks to the Kunming Institute of Botany (China) for assistance with travel, collection permits, and the handling of plant specimens. Thanks to the Institute of Biodiversity and Environmental Conservation at the University of Malaysia, Sarawak, for sponsoring C. H. Cannon and the Indonesian Institute of Sciences for permission to collect in Kalimantan. Phylogenetic analyses were improved by the helpful comments of J. Mercer, J.-M. Moncalvo, and D. L. Swofford. Three anonymous reviewers also provided constructive criticism. Support for this work was provided by a National Science Foundation grant (DEB 9707945) to P. S. Manos, a National Natural Science Foundation of China grant (39930020) to Z.-K. Zhou, and an Agency for Educational Development fellowship to C. H. Cannon.

Appendix

DNA Vouchers

Each entry includes species, locality, voucher specimen, and GenBank accession number (^{1}ITS/*5.8S* accession, ^{2}*matK* accession).

Castanea mollissima Blume; U.S.A.: New York. Tompkins Co. Cornell University Plantations; *Manos 1038* (BH); ^{1}AY040396, ^{2}U92862.

Castanea pumila (L.) Miller; U.S.A.: Connecticut. Connecticut Agricultural Research Station; *Stanford 17-R2T2* (UNC-CH); ^{1}AY040394.

Castanea seguinii Dode; U.S.A.: Connecticut. Connecticut Agricultural Research Station; *Stanford 20-R2T16*; (UNC-CH); ^{1}AY040395.

Castanea sequinii Dode; U.S.A.: Connecticut. Connecticut Agricultural Research Station; *Stanford 21-R3T8*; (UNC-CH); [1]AY040397.

Castanopsis argyrophylla King ex Hook.f.; China: Yunnan. Menglian; *Manos & Zhou 1402* (DUKE); [1]AY040376, [2]AY040497.

Castanopsis argyrophylla King ex Hook.f.; China: Yunnan. Menglun; *Manos & Zhou 1425* (DUKE); [1]AY040374.

Castanopsis argyrophylla King ex Hook.f.; Myanmar: Yezin District; *Cannon 448* (DUKE); [1]AY040385.

Castanopsis calathiformis (Skan) Rehder; China: Yunnan. Wuliang Mt.; *Manos & Zhou 1371* (DUKE); [1]AY040393.

Castanopsis carlesii (Helmsley) Hayata; China: Yunnan. Jingu; *Manos & Zhou 1382* (DUKE); [1]AY040372, [2]AY040496.

Castanopsis ceratacantha Rehder & E. H. Wilson; China: Yunnan. Wuliang Mt.; *Manos & Zhou 1359* (DUKE); [1]AY040382.

Castanopsis cuspidata (Thunb.) Schottky; U.S.A.: North Carolina. Orange Co. Parks Nursery; *Manos s.n.* (DUKE); [1]AY040387.

Castanopsis delavayi Franchet; China: Yunnan. Simao; *Manos & Zhou 1393* (DUKE); [1]AY040371.

Castanopsis echinocarpa Hook.f. & Thompson ex Miq.; China: Yunnan. Wuliang Mt.; *Manos & Zhou 1352* (DUKE); [1]AY040375.

Castanopsis fargesii Franchet; China: Yunnan. Da Wei Shan; *Manos & Zhou 1455* (DUKE); [1]AY040383.

Castanopsis fissa (Champ. ex Benth.) Rehder & E. H. Wilson; China: Yunnan. Kunming Botanical Garden; *Manos & Zhou 1338* (DUKE); [1]AY040390.

Castanopsis fissa (Champ. ex Benth.) Rehder & E. H. Wilson; China: Yunnan. Simao; *Manos & Zhou 1396* (DUKE); [1]AY040392, [2]AY040498.

Castanopsis fissa (Champ. ex Benth.) Rehder & E. H. Wilson; China: Yunnan. Da Wei Shan; *Manos & Zhou 1457* (DUKE); [1]AY040391.

Castanopsis fleuryi Hickel & A. Camus; China: Yunnan. Simao; *Manos & Zhou 1390* (DUKE); [1]AY040373.

Castanopsis fleuryi Hickel & A. Camus; China: Yunnan. Wuliang Mt.; *Manos & Zhou 1366* (DUKE); [1]AY040381.

Castanopsis hypophoenicea (Seemen) Soepadmo; Borneo: Malaysia. Sarawak; *Cannon 120* (DUKE); [1]AY040386.

Castanopsis hystrix Hook.f. & Thomson ex A. DC.; China: Yunnan. Yuanyan; *Manos & Zhou 1441* (DUKE); [1]AY040384.

Castanopsis indica (Roxb. ex Lindley) A. DC.; China: Yunnan. Menglun; *Manos & Zhou 1426* (DUKE); [1]AY040377.

Castanopsis psilophylla Soepadmo; Borneo: Indonesia. West Kalimantan; *Cannon 648* (DUKE); [1]AY040380.

Castanopsis rockii A. Camus; China: Yunnan. Da Wei Shan; *Manos & Zhou 1458* (DUKE); [1]AY040378.

Castanopsis rockii A. Camus; China: Yunnan. Da Wei Shan; *Manos & Zhou 1459* (DUKE); [1]AY040379.

Castanopsis sp. Borneo: Indonesia. West Kalimantan; *Cannon 693* (DUKE) [1]AY040388.

Castanopsis wattii (King ex Hook.f.) A. Camus; Myanmar: Mandalay district; *Cannon 464* (DUKE); [1]AY040389.

Chrysolepis chrysophylla (Douglas ex Hooker) Hjelmq.; U.S.A.: Oregon: Benton Co. St. Mary's Peak; *Manos s.n.* (DUKE); [1]AF389087.

Chrysolepis sempervirens (Kell.) Hjelmq.; U.S.A.: California. San Bernardino Co. Black Mt.; *Manos 160* (BH); [1]AY040369, [2]U92863.

Colombobalanus excelsa (Lozano, Hdz-C. & Henao) Nixon & Crepet; Colombia: Virolin; *Nixon 4655* (BH); [1]AF098412, [2]AY040492.

Fagus grandifolia Ehrh; U.S.A.: New York. Tompkins Co.; *Manos 114* (BH); [1]AY040509, [2]U92861.

Formanodendron doichangensis (A. Camus) Nixon & Crepet; China: Yunnan. Menglian; *Manos & Zhou 1400* (DUKE); [1]AY040452, [2]AY040499.

Formanodendron doichangensis (A. Camus) Nixon & Crepet; China: Yunnan. Menglian; *Manos & Zhou 1401* (DUKE); [1]AY040453.

Lithocarpus beccarrianus (Benth.) A. Camus; Borneo: Indonesia. West Kalimantan; *Cannon 682* (DUKE); [1]AF389101.

Lithocarpus bennettii (Miq.) Rehder; Borneo: Indonesia. West Kalimantan; *Cannon 632* (DUKE); [1] AY040412.

Lithocarpus bullatus Hatus. ex Soepadmo; Borneo: Malaysia; *Cannon 485* (DUKE); [1]AY040409.

Lithocarpus clementianus (King ex Hook.f.) A. Camus; Borneo: Malaysia; *Cannon 638* (DUKE); [1]AF389107.

Lithocarpus conocarpus (Oudem.) Rehder; Borneo: Malaysia. Sarawak; *Cannon 110* (DUKE); [1]AF389095.

Lithocarpus conocarpus (Oudem.) Rehder; Borneo: Malaysia. Sarawak; *Cannon 135* (DUKE); [1]AY040417.

Lithocarpus cooperatus (Blanco) Rehder; Borneo: Malaysia. Sarawak; *Cannon 075* (DUKE); [1] AY040406.

Lithocarpus cooperatus (Blanco) Rehder; Borneo: Malaysia. Sarawak; *Cannon 085* (DUKE); [1] AY040407.

Lithocarpus corneus (Loureiro) Rehder; U.S.A.: Georgia. USDA Coastal Research Station. Savannah; *Manos s.n.* (BH); [1]AY040440.

Lithocarpus dealbatus (Hook.f. & Thomson ex Miq.) Rehder; China: Sichuan. Yong-Jia; *Manos 1292* (DUKE); [1]AY040430.

Lithocarpus densiflorus (Hooker & Arnott) Rehder var. *echinoides* (R. Brown ter) Abrams; U.S.A.: California. Nevada Co. Washington; *Manos & Tucker 922* (BH); [1]AY040370.

Lithocarpus densiflorus (Hooker & Arnott) Rehder; U.S.A.: California; *Nixon 4585* (BH); [1]AF389086, [2]AY040495.

Lithocarpus echinifer (Merr.) A. Camus; Borneo: Malaysia. Sarawak; *Cannon 718* (DUKE); [1]AY040399.

Lithocarpus echinifer (Merr.) A. Camus; Borneo: Malaysia. Sarawak; *Cannon 717* (DUKE); [1]AF389089.

Lithocarpus echinophorus (Hickel & A. Camus) A. Camus; China: Yunnan. Kunming Botanical Garden; *Manos & Zhou 1335* (DUKE); [1]AY040437.

Lithocarpus echinotholis (Hu) Chun & C. C. Huang ex Y. C. Hsu & H. W. Jen; China: Yunnan. Wuliang Mt.; *Manos & Zhou 1370* (DUKE); [1]AY040424.

Lithocarpus edulis (Makino) Nakai; U.S.A.: North Carolina. Orange Co. Parks Nursery; *Manos s.n.* (DUKE); [1]AY040439.

Lithocarpus enclesiacarpus (Korth.) A. Camus; Borneo: Malaysia. West Kalimantan; *Cannon 621* (DUKE); [1]AY040415.

Lithocarpus ewychii (Korth.) Rehder; Borneo: Malaysia. Sabah; *Cannon 556* (DUKE); ^{1}AY040413.

Lithocarpus fenestratus (Roxb.) Rehder; China: Yunnan. Jingu; *Manos & Zhou 1385* (DUKE); ^{1}AY040443.

Lithocarpus fenestratus (Roxb.) Rehder; China: Yunnan. Simao; *Manos & Zhou 1395* (DUKE); ^{1}AY040449.

Lithocarpus fenestratus (Roxb.) Rehder; China: Yunnan. Wuliang Mt.; *Manos & Zhou 1362* (DUKE); ^{1}AY040444.

Lithocarpus fenestratus (Roxb.) Rehder; China: Yunnan. Wuliang Mt.; *Manos & Zhou 1363* (DUKE); ^{1}AY040445.

Lithocarpus ferrugineus Soepadmo; Borneo: Malaysia. West Kalimantan; *Cannon 666* (DUKE); ^{1}AY040411.

Lithocarpus ferrugineus Soepadmo; Borneo: Malaysia. West Kalimantan; *Cannon 680* (DUKE0; ^{1}AY040414.

Lithocarpus glaber (Thunb.) Nakai; U.S.A.: North Carolina. Orange Co.; Parks Nursery. *Manos s.n.* (DUKE); ^{1}AY040435.

Lithocarpus grandifolius (D. Don) S.N. Biswas; Borneo: Malaysia. Sabah; *Cannon 084* (DUKE); ^{1}AY040436.

Lithocarpus grandifolius (D. Don) S.N. Biswas; China: Yunnan. Menglun; *Manos & Zhou 1427* (DUKE); ^{1}AY040450.

Lithocarpus hancei (Benth.) Rehder; China: Yunnan. Mt. Ailao; *Manos & Zhou 1375* (DUKE); ^{1}AY040448.

Lithocarpus hancei (Benth.) Rehder; China: Yunnan. Wuliang Mt.; *Manos & Zhou 1347* (DUKE); ^{1}AY040451.

Lithocarpus hatsumii Soepadmo; Borneo: Malaysia. Sabah; *Cannon 484* (DUKE); ^{1}AY040410.

Lithocarpus havilandii (Stapf) Barnett; Borneo: Malaysia. Sabah; *Cannon 498* (DUKE); ^{1}AY040404.

Lithocarpus havilandii (Stapf) Barnett; Borneo: Malaysia; *Cannon 829* (DUKE); ^{1}AY040405.

Lithocarpus keningauensis S. Julia & Soepadmo; Borneo: Malaysia. Sarawak; *Cannon 751* (DUKE); ^{1}AF389106.

Lithocarpus lampadarius (Gamble) A. Camus; Borneo: Malaysia. Sabah; *Cannon 483* (DUKE); ^{1}AY040433.

Lithocarpus leptogyne (Korth.) Soepadmo; Borneo: Malaysia. Sabah; *Cannon 061* (DUKE); ^{1}AY040416.

Lithocarpus lucidus (Roxb.) Rehder; Borneo: Indonesia. West Kalimantan; *Cannon 33* (DUKE); ^{1}AY040408.

Lithocarpus nieuwenhuisii (von Seeman) A. Camus; Borneo: Malaysia. Sarawak; *Cannon 111* (DUKE); ^{1}AY040400.

Lithocarpus pachylepis A. Camus; China: Yunnan. Da Wei Shan; *Manos & Zhou 1451* (DUKE); ^{1}AY040441, ^{2}AY040494.

Lithocarpus pachylepis A. Camus; China: Yunnan. Da Wei Shan; *Manos & Zhou 1461* (DUKE); ^{1}AY040442.

Lithocarpus pachyphyllus (Kurz) Rehder; China: Yunnan. Mt. Ailao; *Manos & Zhou 1376* (DUKE); ^{1}AY040446.

Lithocarpus pachyphyllus (Kurz) Rehder; China: Yunnan. Mt. Ailao; *Manos & Zhou 1380* (DUKE); ^{1}AY040447.

Lithocarpus palungensis Cannon & Manos; Borneo: Indonesia. West Kalimantan; *Cannon 655* (DUKE); ^{1}AY040420.

Lithocarpus papilifer Hatus. ex Soepadmo; Borneo: Malaysia. Sabah; *Cannon 42* (DUKE); ^{1}AY040418.

Lithocarpus pulcher (King) Markgr.; Borneo: Indonesia. West Kalimantan; *Cannon 652* (DUKE); ^{1}AF389104.

Lithocarpus pulcher (King) Markgr.; Borneo: Indonesia. West Kalimantan; *Cannon 658* (DUKE); ^{1}AY040421.

Lithocarpus pulcher (King) Markgr.; Borneo: Malaysia. Sarawak; *Cannon 694* (DUKE); ^{1}AY040423.

Lithocarpus pulcher (King) Markgr.; Borneo: Malaysia. Sarawak; *Cannon 696* (DUKE); ^{1}AY040422.

Lithocarpus revolutus Hatus. ex Soepadmo; Borneo: Malaysia. Sabah; *Cannon 491* (DUKE); ^{1}AY040434.

Lithocarpus ruminatus Soepadmo; Borneo: Indonesia. West Kalimantan; *Cannon 613* (DUKE); ^{1}AY040402.

Lithocarpus ruminatus Soepadmo; Borneo: Malaysia. Sarawak; *Cannon 112* (DUKE); ^{1}AY040401.

Lithocarpus ruminatus Soepadmo; Borneo: Malaysia. Sarawak; *Cannon 113* (DUKE); ^{1}AF389097.

Lithocarpus ruminatus Soepadmo; Borneo: Malaysia. Sarawak; *Cannon 116* (DUKE); ^{1}AY040403.

Lithocarpus sericobalanus E. F. Warb.; Borneo: Indonesia. West Kalimantan; *Cannon 634* (DUKE); ^{1}AY040419.

Lithocarpus sp. China: Sichuan. Cold Water Valley; *Manos 1289* (DUKE); ^{1}AY040438.

Lithocarpus truncatus (King ex Hook.f.) Rehder & E. H. Wilson; China: Yunnan. Wuliang Mt.; *Manos & Zhou 1349* (DUKE); ^{1}AY040428.

Lithocarpus truncatus (King ex Hook.f.) Rehder & E. H. Wilson; China: Yunnan. Wuliang Mt.; *Manos & Zhou 1361* (DUKE); ^{1}AY040429.

Lithocarpus truncatus (King ex Hook.f.) Rehder & E. Wilson; China: Yunnan Wuliang Mt.; *Manos & Zhou 1364* (DUKE); ^{1}AY040431.

Lithocarpus truncatus (King ex Hook.f.) Rehder & E. Wilson; China: Yunnan. Menglian; *Manos & Zhou 1408* (DUKE); ^{1}AY040425.

Lithocarpus turbinatus (Stapf) Forman; Borneo: Malaysia. Sabah; *Cannon 223* (DUKE); ^{1}AY040398.

Lithocarpus turbinatus (Stapf) Forman; Borneo: Malaysia. Sabah; *Cannon 510* (DUKE); ^{1}AF389100.

Lithocarpus xylacarpus (Kurz) Markgr.; China: Yunnan. Da Wei Shan; *Manos & Zhou 1450* (DUKE); ^{1}AY040427.

Lithocarpus xylacarpus (Kurz) Markgr.; China: Yunnan. Da Wei Shan; *Manos & Zhou 1463* (DUKE); ^{1}AY040426, ^{2}AY040493.

Lithocarpus xylacarpus (Kurz) Markgr.; China: Yunnan. Mt. Ailao; *Manos & Zhou 1372* (DUKE); ^{1}AY040432.

Quercus acutissima Carruth.; U.S.A.: New York. Tompkins Co. Cornell University Plantations; *Manos s.n.* (BH); ^{1}AF098428.

Quercus agrifolia Nee.; U.S.A.: California. Santa Barbara Co.; *Manos 542* (BH); ^{1}AF098415.

Quercus alba L.; U.S.A.: New York. Tompkins Co.; *Manos s.n.* (BH); ^{1}AF098419.

Quercus argentata Korth.; Borneo: Malaysia. Sarawak; *Cannon 128* (DUKE); ^{1}AY040459.

Quercus austoglauca (Y. T. Chang ex Y. C. Hsu & H. W Jen) Y. T. Chang; China: Yunnan. Da Wei Shan; *Manos & Zhou 1452* (DUKE); ^{1}AY040455.

Quercus austoglauca (Y. T. Chang ex Y. C. Hsu & H. W Jen) Y. T. Chang; China: Yunnan. Da Wei Shan; *Manos & Zhou 1448* (DUKE); ^{1}AY040461.

Quercus calliprinos Webb.; U.S.A.: California. Yolo Co. Shields Grove Arboretum; *Manos 933*; ^{1}AF098429.

Quercus cedrosensis Muller; A. Mexico: Baja California. San Pedro Martir; *Manos 738* (BH); ^{1}AF098449.

Quercus cedrosensis Muller; B. Mexico: Baja California. Santo Tomas; *Manos 716* (BH); ^{1}AF098450.

Quercus cedrosensis Muller; C. Mexico: Baja California. Cerro Colorado; *Manos 732* (BH); [1]AF098451.

Quercus cerris L.; U.S.A.: California. Yolo Co. Shields Grove Arboretum; *Manos 935* (BH); [1]AF098430.

Quercus chrysolepis Liebm.; A. U.S.A.: California. Los Angeles Co. La Crescenta; *Nixon s.n.* (BH) [1]AF098438.

Quercus chrysolepis Liebm.; B. U.S.A.: California. Del Norte, Co. E. of Hamburg; *Manos 954* (BH); [1]AF098439.

Quercus chrysolepis Liebm.; C. U.S.A.: Arizona. Coconino Co. Oak Creek Canyon; *Manos 771* (BH); [1]AF098440.

Quercus chrysolepis Liebm.; D. Mexico: Baja California. Sierra San Pedro Martir; *Manos 744* (BH); [1]AF098441.

Quercus chrysolepis Liebm.; E. U.S.A.: California. Marin Co. Point Reyes; *Manos 965* (BH); [1]AF098442.

Quercus chrysolepis Liebm.; F. U.S.A.: Arizona. Yavapai Co. Chirachua Mts.; *Manos 766* (BH); [1]AF098443.

Quercus chrysolepis Liebm.; G. U.S.A.: Arizona. Mojave Co. Hualapai Mts.; *Manos 603* (BH); [1]AF098444.

Quercus chrysolepis Liebm.; H. U.S.A.: California. Sonoma Co. Mt. St. Helena; *Manos 906* (BH); [1]AF098445.

Quercus coccifera L.; U.S.A.: California. Yolo Co. Shields Grove Arboretum; *Manos 931* (BH); [1]AF098431.

Quercus cocciferoides Hand.-Mazz.; China: Yunnan; *Zhou. s.n.* (KUN); [1]AY040466.

Quercus cornelius-mulleri Nixon & Steele; U.S.A.: California. San Diego Co. Borrego Springs; *Manos & Steele 1258* (DUKE); [1]AY040485.

Quercus dentata Thunb.; China: Yunnan. Western Hills; *Manos 1304* (DUKE); [1]AY042935.

Quercus dumosa Nutt.; U.S.A. California. San Diego Co. Torrey Pines; *Manos & Kelly 1178* (DUKE); [1]AY040486.

Quercus engelmannii Greene; U.S.A.: California. Los Angeles Co.; *Manos 212* (BH); [1]AF098420.

Quercus engleriana Seemen; China: Yunnan; *Zhou. s.n.* (KUN); [1]AY040465.

Quercus falcata Michx; U.S.A.: Florida. Alachua Co.; *Cavender-Bares FA-19*; no voucher; [1]AY040482.

Quercus franchettii Skan.; China: Yunnan. Kunming Botanical Garden; *Manos 1286* (DUKE); [1]AY040464.

Quercus geminata Small; U.S.A.: Florida. *L. Robbins s.n.* (BH); [1]AF098426.

Quercus glauca Thunb.; China: Yunnan. Kunming Botanical Garden; *Manos 1340* (DUKE); [1]AY040458.

Quercus griffithii Hook.f. & Thompson ex Miq.; China: Yunnan. Bei-Shui; *Manos 1321* (DUKE); [1] AY040490.

Quercus guajavifolia H. Leveille; 1. China: Yunnan. Gang-Ha-Ba; *Manos 1316* (DUKE); [1]AY040470.

Quercus guajavifolia H. Leveille; 2. China: Yunnan. Gang-Ha-Ba; *Manos 1317* (DUKE); [1]AY040471.

Quercus guajavifolia H. Leveille; 3. China: Yunnan. Gang-Ha-Ba; *Manos 1318* (DUKE); [1]AY040472.

Quercus ilex L.; U.S.A.: California. Santa Barbara Co. UCSB Campus; *Manos 412* (BH); [1]AF098432.

Quercus insignis Liebm.; Costa Rica: Puntarenas. San Vito; *Manos & Stone 1268* (DUKE); [1]AY040487.

Quercus kelloggii Newb.; U.S.A.: California. Riverside Co. Banning; *Manos 123* (BH); [1]AF098416.

Quercus laeta Liebm.; Mexico: Morelos. Taxco; *Manos et al. 563* (BH); [1]AF098421.

Quercus laevis Walter; U.S.A.; Florida. Alachua Co.; *Cavender-Bares LV41*; no voucher; [1]AY040483.

Quercus lamellosa (Smith) Oersted; China: Yunnan. Kunming Botanical Garden; *Manos 1283* (DUKE); [1]AY040454.

Quercus lobata Nee.; U.S.A.: California. Santa Barbara Co.; *Manos 999* (BH); [1]AF098422

Quercus longispica A. Camus; 1. China: Yunnan. Lijiang; *Manos 1329* (DUKE); [1]AY040473.

Quercus longispica A. Camus; 2. China: Yunnan. Lijiang; *Manos 1331* (DUKE); [1]AY040474.

Quercus merrillii von Seemen; Borneo: Malaysia. Sarawak; *Cannon 126* (DUKE); [1]AY040456.

Quercus monimotricha Hand.-Mazz.; 1. China: Yunnan. Gang-Ha-Ba; *Manos 1313* (DUKE); [1]AY040467.

Quercus monimotricha Hand.-Mazz.; 2. China: Yunnan. Gang-Ha-Ba; *Manos 1314* (DUKE); [1]AY040468.

Quercus montana Willd.; U.S.A.: North Carolina. *Manos s.n.* (DUKE); [1]AY040484.

Quercus myrsinifolia Blume.; U.S.A.: Georgia. USDA Coastal Research Station. Savannah; *Manos s.n.* (BH); [1]AF098414.

Quercus oleoides Schlect. & Cham.; Costa Rica: Liberia; *Manos & Stone 1261* (DUKE); [1]AY040488.

Quercus palmeri Engelm.; A. U.S.A.: California. San Louis Obispo Co. Peachy Canyon Rd; *Nixon 4590* (BH); [1]AF098446.

Quercus palmeri Engelm.; B. U.S.A.: Arizona. Coconino Co. Oak Creek Canyon; *Manos 777* (BH); [1]AF098447.

Quercus palmeri Engelm.; C. U.S.A.: California. Riverside Co. Garner Valley; *Manos 602* (BH); [1]AF098448.

Quercus palustris Muench.; U.S.A.: New York. Tompkins Co.; *Manos s.n.* (BH); [1]AF098417.

Quercus pannosa Hand.-Mazz.; China: Yunnan. Lijiang; *Zhou 0064* (KUN); [1]AY040469.

Quercus phillyreoides Gray; 1. U.S.A.: California. Yolo Co. Shields Grove Arboretum; *Manos 936* (BH); [1]AF098433.

Quercus phillyreoides Gray; 2. China: Yunnan. Babao; *Ming 0036* (KUN); [1]AY040462.

Quercus pseudosemicarpifolia A. Camus; China: Yunnan. Lijiang; *Zhou 0057* (KUN); [1]AY040480.

Quercus rhederiana Hand.-Mazz.; 1. China: Yunnan. Lijiang; *Manos 1302* (DUKE); [1]AY040475.

Quercus rhederiana Hand.-Mazz.; 2. China: Yunnan. North of Dali; *Manos 1309* (DUKE); [1]AY040476.

Quercus rhederiana Hand.-Mazz; 3. China: Yunnan. Lijiang; *Manos 1328* (DUKE); [1]AY040477.

Quercus robur L.; U.S.A.: New York. Tompkins Co. Cornell Univ. Campus; *Manos s.n.* (BH); [1]AF098424.

Quercus rubra L.; U.S.A.: New York. Tompkins Co. Cornell Univ. Campus; *Manos s.n.* (BH); [1]AF098418, [2]U92864.

Quercus rugosa Nee.; Mexico: Morelos. *Manos et al. 570* (BH); [1]AF098425.

Quercus sadleriana R. Brown; U.S.A.: California. Del Norte Co. *Manos s.n.* (DUKE); [1]AY040489.

Quercus salicina Blume; U.S.A.: North Carolina. Orange Co. Parks Nursery. *Manos s.n.* (DUKE); [1]AY040457.

Quercus senescens Hand.-Mazz.; A. China: Yunnan. Western Hills; Manos *1305A* (DUKE); [1]AY040478.

Quercus senescens Hand.-Mazz.; C. China: Yunnan. Western Hills; Manos *1305C* (DUKE); ^{1}AY040479.

Quercus spinosa Franchet; China: Yunnan. Zhongdian; *Zhou 0058* (DUKE); ^{1}AY040481.

Quercus suber L.; U.S.A.: California. Santa Barbara Co. Orella St.; *Manos 423* (BH) ^{1}AF098434.

Quercus tomentella Engelm.; A. U.S.A.: California. San Diego Co. San Clemente Island; *Manos 684* (BH); ^{1}AF098435.

Quercus tomentella Engelm.; D. U.S.A.: California. Ventura Co. Anacapa Island; Manos 545 (BH); ^{1}AF098436.

Quercus tomentella Engelm.; E. U.S.A.: California. Santa Barbara Co. Santa Cruz Island; *Manos 983* (BH); ^{1}AF098437, ^{2}U92865.

Quercus turbinella Greene; U.S.A.: California. Yolo Co. Shields Grove Arboretum; J. *Tucker s.n.* (BH); ^{1}AF098423.

Quercus vaccinifolia (Kell.) Curran; A. U.S.A.: California. El Dorado Co. Echo Lake; *Manos 909* (BH); ^{1}AF098452.

Quercus vaccinifolia (Kell.) Curran; B. U.S.A.: California. El Dorado Co. Echo Lake; *Manos 914* (BH); ^{1}AF098453.

Quercus vaccinifolia (Kell.) Curran; C. U.S.A.: California. Trinity Co. Scott Mt.; *Manos 945* (BH); ^{1}AF098454.

Quercus vaccinifolia (Kell.) Curran; D. U.S.A.: California. Sierra Co. Gold Lake; *Manos 962* (BH); ^{1}AF098455.

Quercus valdinervosa Soepadmo; Borneo: Malaysia. Sarawak; *Cannon 104* (DUKE); ^{1}AY040460.

Quercus variabilis Blume; China: Yunnan. Kunming Botanical Garden; *Zhou 0072* (KUN); ^{1}AY040463.

Quercus virginiana Miller; U.S.A.: Florida. *T. Engstrom s.n.* (BH); ^{1}AF098427.

Quercus yunnanensis Franchet; China: Yunnan. Lijiang; *Zhou 0050* (KUN); ^{1}AY040491.

Trigonobalanus verticillata Forman; U.K.: Royal Botanical Gardens, Edinburgh, UK RBG 1967-421; ^{1}AF098413, ^{2}U92866.

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