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Phylogenetic Relationships of the Santalales and Relatives

Daniel L. Nickrent and Cheryl R. Franchina

Department of Plant Biology, University of Illinois, Urbana, Illinois 61801, USA

Summary. Determining relationships among parasitic angiosperms has often been difficult owing to frequent morphological reductions in floral and vegetative features. We report 18S (small-subunit) rRNA sequences for representative genera of three families within the Santalales (Olacaceae, Santalaceae, and Viscaceae) and six outgroup dicot families (Celastraceae, Cornaceae, Nyssaceae, Buxaceae, Apiaceae, and Araliaceae). Using Wagner parsimony analysis, one most parsimonious tree resulted that shows the Santalales to be a holophyletic taxon most closely related to *Euonymus* (Celastraceae). The santalalean taxa showed approximately 13% more transitional mutations than the group of seven other dicot species. This suggests a higher fixation rate for mutations in these organisms, possibly owing to a relaxation of selection pressures at the molecular level in parasitic vs nonparasitic plants. Outgroup relationships are generally in accord with current taxonomic classifications, such as the grouping of Nyssaceae and Cornaceae together (Cornales) and the grouping of Araliaceae with Apiaceae (Apiales). These data provide the first nucleotide sequences for any parasitic flowering plant and support the contention that rRNA sequence analysis can result in robust phylogenetic comparisons at the family level and above.

Key words: Parasitic angiosperms — Santalales — Rosidae — Small-subunit rRNA — Phylogeny

Introduction

The classification of angiosperms at higher taxonomic levels has remained one of the most difficult undertakings for botanical systematists. Both morphological (Dahlgren and Bremer 1985) and molecular (Syvanen et al. 1989) characters of flowering plants frequently contain large numbers of reversals and convergences (homoplasy) that confound the analysis of phylogenetic relationships. Some of the most enigmatic of all angiosperms are those that have developed parasitic associations with other plants. Parasitism in angiosperms has evolved several times as evidenced by its occurrence in 17 families of the Magnoliidae, Rosidae, and Asteridae. Among these, the orders Santalales and Rafflesiales include approximately 50% of the total 3700 species of parasitic flowering plants (Table 1).

Assessing evolutionary relationships among parasitic plants has proven difficult for several reasons. First, morphological and physiological features may be reduced or lost entirely such as chlorophyll, leaves, perianth parts, and characters of the flower traditionally considered evolutionarily conservative such as ovular integuments. These losses reduce the number of characters available for analysis. For those features that are present, determination of homology is difficult, especially among divergent taxa. Given the difficulty in identifying homologous morphological characters for use in establishing higher taxonomic categories, the need for alternate data is apparent. An efficient and appropriate means for generating phylogenies at the familial level or above is to compare nucleotide sequences of homologous genes. A molecular approach using rRNA sequences was used in this study to determine phylogenetic relationships in these plants.

Placement and Circumscription of the Santalales

There exists little agreement when attempts are made to derive the Santalales and Rafflesiales from non-

Offprint requests to: D.L. Nickrent

Table 1. Documented cases of parasitism in angiosperms[a]

Class Magnoliopsida (Dicots)		
A. Subclass Magnoliidae		
Order 1. Laurales		
Family 1. Lauraceae		1 (20)[b]
B. Subclass Rosidae		
Order 1. Santalales		
Family 1. Olacaceae		22 (200)
2. Opiliaceae		9 (30)
3. Santalaceae		30 (480)
4. Misodendraceae		1 (10)
5. Loranthaceae		74 (700)
6. Viscaceae		7 (350)
7. Eremolepidaceae		3 (11)
8. Balanophoraceae		18 (47)
Order 2. Rafflesiales		
Family 1. Hydnoraceae		2 (10)
2. Mitrastemonaceae		1 (2)
3. Rafflesiaceae		7 (50)
Order 3. Polygalales		
Family 1. Krameriaceae		1 (15)
C. Subclass Asteridae		
Order 1. Solanales		
Family 1. Cuscutaceae		1 (150)
Order 2. Lamiales		
Family 1. Lennoaceae		3 (5)
Order 3. Scrophulariales		
Family 1. Scrophulariaceae (Subfamily Rhinanthoideae only)		30 (1500)
2. Orobanchaceae		17 (150)

[a] Classification of Magnoliophyta according to Cronquist (1981)
[b] Number of genera (number of species)

parasitic groups. Takhtajan (1980) indicates a common ancestor for the Celastrales and Santalales, both being derived from the Saxifragales. In this system the Rafflesiales are placed in the Magnoliidae. Cronquist (1981) shows both the Santalales and Rafflesiales as sharing a common ancestor and derives these orders from the archaic Rosales as was done for 10 other ordinal groups.

Cronquist (1981) treats the Rafflesiales as a sister group to the Santalales; however, current evidence suggests it is not a member of this order. Comparative embryology was used by Cocucci (1983) to link the Rafflesiaceae and Hydnoraceae to annonaceous ancestors, thus supporting their placement in the Magnoliidae as proposed by Takhtajan (1980), Dahlgren (1983), and Thorne (1983). The cladistic study of morphological features by Dahlgren and Bremer (1986) also placed the Rafflesiales in the Magnoliidae. Cronquist (1981) also includes the Balanophoraceae in the Santalales, whereas the three other classifications (above) treat this group as distinct at the ordinal level or above. Kuijt (1969) states that the evidence for associating this group with the Santalales is weak because it is based upon severe morphological reduction. Cronquist (1981) admits that the family has no other evident allies but looks

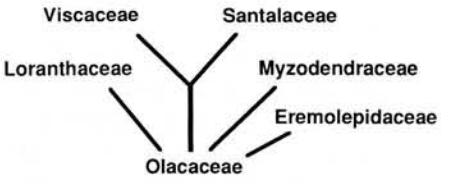

Fig. 1. Relationships among the santalalean families as proposed by Kuijt (1968, 1969)

to the Santalales as the only logical group from which to derive this family.

Relationships within the Santalales

Taxonomic treatments of the Santalales and Rafflesiales date to the work of Engler, Hieronymus, and Solms in "Die Natürlichen Pflanzenfamilien" (Engler and Prantl 1894). Later treatments in this series have dealt with the Olacaceae and Opiliaceae (Sleumer 1935), Myzodendraceae (Scottsberg 1935), Loranthaceae (Engler and Krause 1935), Santalaceae (Pilger 1935), and Rafflesiaceae, Hydnoraceae, and Balanophoraceae (Harms 1935). Phylogenetic relationships among the families of the Santalales have been proposed by Schellenberg (1932), Fagerlind (1948), Johri and Bhatnagar (1960), and Kuijt (1968).

The most recent depiction of relationships among the families of the Santalales was that of Kuijt (1968, 1969), which was based largely upon inflorescence features (Fig. 1). In agreement with Schellenberg (1932), Sleumer (1935), and Fagerlind (1948), Kuijt (1968) considered the Olacaceae the "plexus" from which the other santalalean families were derived. In contrast to earlier systems, the Rafflesiaceae and Hydnoraceae are not considered a part of this order. Many of the features used to delimit the other families of the order are found in the Olacaceae. These include: (1) presence of both sepals and petals, (2) bisexual flowers, (3) both superior and inferior ovaries, and (4) both parasitic and nonparasitic habits. The Erythropalaceae, Aptandraceae, and Opiliaceae were considered members of the Olacaceae by Kuijt (1969), although he commented that their recognition at the family level "merits careful evaluation." Most current treatments consider the Opiliaceae a distinct family but retain the Erythropalaceae and the Aptandraceae within the Olacaceae. The polymorphic nature of the Olacaceae is also indicated by cytological evidence, where a range of chromosome numbers (x = 10, 12, 13, and 19) exist (Raven 1975).

The primary objectives of this study were to use 18S (small-subunit) rRNA sequences to infer phylogenetic relationships among three families in the Santalaceae and test the hypothesis that the Olacaceae are cladistically basal relative to the other families in the order. The sequence data from non-

parasitic angiosperms were used to estimate which group or groups are most closely related to the Santalales. These analyses provide additional data, independent of comparative morphology, relating to the origin and evolution of parasitism. With sequences for outgroups within the Rosidae, relationships within and among the orders and families of this subclass will be ascertained.

Until relatively recently, most molecular data for angiosperms involved sequences of proteins such as cytochrome-c (Boulter 1973), plastocyanin (Boulter et al. 1979), and ribulose biphosphate carboxylase (Martin and Dowd 1984). With the advent of rapid and efficient sequencing methods, the number of plant nucleotide sequences continues to grow. Martin et al. (1989) examined nonsynonymous substitutions of nucleotides in the glyceraldehyde-3-phosphate dehydrogenase genes of nine flowering plants. The time of divergence for the monocots vs dicots was estimated at 319 million years (Myr) before present (Carboniferous). This date is 180 Myr before the earliest indisputable angiosperm fossil. Because these dates depend upon a constant rate of nucleotide substitution over time, such results require further testing with other molecules.

Ribosomal RNA sequences are ideal for addressing phylogenetic relationships above the level of genus and family. These RNA molecules are present in and homologous among even the most divergent of the parasitic taxa considered here. The large number of tandem repeats of the ribosomal DNA (rDNA) genes [hundreds to thousands of times within the genome (Appels and Honeycutt 1986)] result in high concentrations of rRNA in a cell. This is fortunate as it allows extraction of milligram amounts of rRNA from only a few grams of fresh plant material. The molecule contains highly conserved as well as variable regions. The conserved regions permit the use of direct sequencing via construction of complementary oligonucleotide primers. These areas also facilitate manual alignments of sequences derived from many taxa.

The 5 and 5.8S rRNA molecules have been sequenced for the largest array of organisms. Very deep phylogenetic branches among the prokaryotes and eukaryotes have been examined using the 5S molecule (Kuntzel et al. 1983; Pace et al. 1986; Wolters and Erdmann 1986; Hori and Osawa 1987). Sequences of 16 and 18S rRNAs have also been extensively used to address deep branches such as relationships between prokaryotic and eukaryotic lineages (Sogin et al. 1986). Others have combined the 5S and 16–18S sequences to conduct phylogenetic analyses of these groups (Wolters and Erdmann 1986; Lake 1988). Among the available 5 and 5.8S sequences, a large number have been obtained from higher plants (Erdmann et al. 1984). The phenetic analysis of 5S rRNA sequences by Hori et al. (1985) focused on the major lineages of green plants. Their results supported several traditional taxonomic relationships, for example, the placement of *Nitella* as the closest living relative of the land plants, as well as some nontraditional relationships, such as the evolution of the Bryophyta from the Pteridophyta by degeneration. For contrasting views on the use of 5S rRNA data in phylogeny construction see Steele et al. (1988) and Mishler et al. (1988).

Given that the large (18 and 26S) rRNAs have a lower level of sequence homology than the small (5S and 5.8S) rRNAs, more variation exists that can be used for phylogenetic comparisons at higher taxonomic levels, such as within the angiosperms. In a study by Nairn and Ferl (1988), the monophyletic origin of angiosperms was supported by 18S sequence comparisons using *Zamia* (a cycad) and published sequences of maize (Messing et al. 1984), rice (Takaiwa et al. 1984), and soybean (Eckenrode et al. 1985). Rice is currently the only angiosperm with a published sequence available for the entire nuclear 26S molecule (Takaiwa et al. 1985). A combination of portions of the 18S and 26S rRNA molecules was used in the study by Hamby and Zimmer (1988) to examine intrafamilial relationships within the Poaceae. Their results support the subfamilial placement of *Zea, Tripsacum, Saccharum,* and *Sorghum* within the Panicoideae and *Hordeum, Avena,* and *Triticum* within the Pooideae.

Materials and Methods

Plant Collections. The plants used for RNA extractions, their location, and origin are listed in Table 2. Voucher specimens are deposited in the University of Illinois Herbarium (ILL).

RNA Extractions. All rRNA extractions were made using the hot borate method of Hamby and Zimmer (1988). Approximately 5.0 g of fresh tissue was used for each extraction. It was discovered that frozen tissue (−100°C) resulted in poor RNA yields, hence freezing was avoided. Unsuccessful attempts to extract rRNA from such plants as *Comandra* (Santalaceae), *Dendropemon* (Loranthaceae), *Ximenia* (Olacaceae), and *Rhizophora* (Rhizophoraceae) led us to try alternate methods. Extraction protocols that utilize guanidinium isothiocyanate (e.g., Chirgwin et al. 1979; Maniatis et al. 1982; Chomczynski and Sacchi 1987) were attempted with these plants, as well as isolation by centrifugation through CsCl (Glisin et al. 1974; Maniatis et al. 1982), but without success.

Sequencing Protocol. The methods for sequencing RNA using reverse transcriptase are well known (Sanger et al. 1977; Peattie 1979; Qu et al. 1983; Lane et al. 1985). The method of Lane et al. (1985) was successfully employed with all plants from which rRNA samples were obtained. The reaction mixtures used here contained ^{35}S-dATP as the label. Oligonucleotide priming sites and primer sequences used in this study are shown in Table 3. Sequences were read from long and short runs from each primer. For some primers, this resulted in overlap, which was used to

Table 2. Plants used for RNA extractions

Order	Family	Species (abbreviation)	Source of material	Native to
Santalales	Viscaceae	*Phoradendron serotinum* (Raf.) M.C. Johnson (Phos)	Field collected, Lousiana	Eastern U.S.
		Dendrophthora domingensis (Spreng.) Eichler (Dend)	Field collected, Puerto Rico	Caribbean
	Santalaceae	*Buckleya distichophylla* (Nutt.) Torr. (Bucd)	Field collected, Virginia	Eastern U.S.
	Olacaceae	*Schoepfia arenaria* Britton (Scha)	Field collected, Puerto Rico	Caribbean
Celastrales	Celastraceae	*Euonymus alatus* (Thunb.) Regel (Euoa)	Cultivated, University of Illinois campus	Asia
Cornales	Nyssaceae	*Nyssa sylvatica* Marsh. (Nyss)	Cultivated, University of Illinois campus	Eastern U.S.
	Cornaceae	*Cornus florida* L. (Corf)	Cultivated, University of Illinois campus	Eastern U.S.
		Cornus racemosa Lam. (Corr)	Cultivated, University of Illinois campus	Eastern U.S.
Euphorbiales	Buxaceae	*Buxus sempervirens* L. (Buxs)	Cultivated, University of Illinois campus	Eurasia
Apiales	Apiaceae	*Hydrocotyle sibthorpioides* Lam. (Hyds)	Cultivated, University of Illinois Greenhouse	Asia
	Araliaceae	*Hedera helix* L. (Hedh)	Cultivated, University of Illinois campus	Eurasia

Table 3. Oligonucleotide primers used for direct sequencing of angiosperm rRNA

RNA sequence (5′ → 3′)	Primer length	Position on *Glycine*	Primer name
1) GGAUAACUGUGGYAAUUCUAG	20	141–161	145hs
2) CUGCCCUAUCAACUUUCGAUGG	22	309–330	18Ecmp
3) CCGGAGAGGGAGCCUGA	17	385–401	309e
4) CCGGAGAGGGAGCCUGAGAAA	21	385–405	428hs
5) GCCAGCMGCCGCGGU	15	568–582	517
6) AAGCUCGUAGUUGGA	15	626–640	626zm
7) CGGGAUCGGAGUAAUGAU	18	847–864	854
8) GGUGAAAUUCUUGGA	15	905–919	690e
9) AAACUUAAAGGAAU	14	1135–1148	906
10) AAACUYAAAKGAAUUGACGG	20	1135–1154	907
11) GGUGGUGCAUGGCCG	15	1269–1283	1324
12) GUCUGUGAUGCCCUUAGA	18	1433–1450	18Jcmp
13) UUGUUGGUCUUCAACGAG	18	1562–1579	1575
14) GYACACACCGCCCGUC	16	1634–1649	1392
15) AACAAGGUUUCCGUAGGUG	19	1769–1787	1830hs
16) GUAGGUGAACCUGCRG	16	1781–1796	1511e

double check the sequence. The 18S rRNA sequences from the 11 taxa listed in Table 2 were submitted to the EMBL data library (via GENBANK) and received the following accession numbers (order as in Table 2): X16607, X16601, X16598, X16606, X16600, X16603, X17370, X16602, X16599, X16605, and X16604.

Sequence Analysis and Phylogeny Reconstruction. Sequences read from autoradiograms were aligned manually with the published sequence of soybean (Eckenrode et al. 1985) as the standard. The published sequences of two monocots, maize (Messing et al. 1984) and rice (Takaiwa et al. 1984), were included as outgroups. Bases present in the penultimate helix of the 18S rRNA molecule (39 bases, from positions 1786 through 1824) were excluded, as they were upstream from the most 3′ primers used (1830hs and 1511e). For the three published angiosperm 18S rRNA sequences, no mutations exist in this region. Sequence information for approximately 98% of the total 1824 positions was determined.

For cladistic analysis, maximum parsimony using the Wagner algorithm available through PAUP version 3.0d (Swofford 1990) was employed. In this analysis, invariant sequences were excluded and all characters were treated as unordered. Transitions and transversions were either given equal weighting or were weighted based upon the observed transition and transversion frequencies when comparing the two most divergent dicots (*Glycine* and *Phoradendron*). The branch and bound method, which identifies all optimal trees, was used. Aligned sequences were also used to form a proximity (distance) matrix, which was then used to construct an additive tree using the least-squares algorithm of DeSoete (1983) employing the Jukes–Cantor correction (Jukes and Cantor 1969) for unobserved substitutions.

Table 4. Summary of mutations[a] in 18S rRNA from 13 angiosperms

Abbreviation[b]	Transitions	Transversions	Insertions	Deletions	Total mutations
Phos	75 (64)[c]	28 (24)	7 (6)	8 (7)	118
Dend	62 (61)	27 (26)	6 (6)	7 (7)	102
Bucd	47 (63)	18 (24)	4 (5)	6 (8)	75
Scha	29 (47)	18 (29)	4 (6)	11 (18)	62
Mean Santalales	53.2 (60)	22.7 (25)	5.2 (6)	8.0 (9)	89.2
Euoa	28 (38)	29 (40)	2 (3)	14 (19)	73
Nyss	30 (43)	15 (22)	3 (4)	21 (30)	69
Corf	30 (55)	11 (20)	1 (2)	13 (24)	55
Corr	28 (54)	11 (21)	1 (2)	12 (23)	52
Buxs	31 (41)	19 (25)	2 (3)	24 (32)	76
Hyds	37 (51)	20 (27)	1 (1)	15 (21)	73
Hedh	36 (54)	15 (22)	3 (4)	13 (19)	67
Mean other dicots	31.4 (47)	17.1 (26)	1.8 (3)	16.0 (24)	66.4
Zeam	95 (63)	35 (23)	11 (7)	10 (7)	151
Orys	53 (58)	29 (32)	6 (7)	3 (3)	91
Mean monocots	74.0 (61)	32.0 (26)	8.5 (7)	6.5 (5)	121

[a] Mutations based upon comparison with soybean (Glym) sequence (see Fig. 2). Ambiguous nucleotides were not included in this tabulation

[b] Taxon abbreviations as in Table 2; Zeam = *Zea mays*; Orys = *Oryza sativa*

[c] Number of mutations (percent of total for that taxon)

Results

Alignment of the 18S rRNA sequences of the four representatives of the Santalales, eight dicot outgroups, and two monocot outgroups was straightforward, as relatively few length variations were seen. This alignment can be obtained by request from the first author. Bands across all four lanes (indicated as N) were often encountered, presumably as a result of modified bases and secondary structural features that interfere with reverse transcriptase activity. Depending upon the taxon, these ambiguous nucleotides comprised from 2 to 5% of the entire sequence.

Using soybean as the standard and discounting insertion/deletion mutations, 14% of the 1769 bases (1807 minus the 18 bases at the 3′ end of the molecule) showed variation among the 13 angiosperms used in this study. Approximately 50 of the total 257 variable positions involved changes unique to some member of the Santalales, primarily within *Phoradendron serotinum* or *Dendrophthora domingensis*. Only one mutation appears in all four members of the Santalales and not in any other plant sampled (A vs G at position 1367). The change at that position occurs on a helix where the compensating mutation (C to U) has also taken place at position 1384.

A comparison of the number of transitions, transversions, insertions, and deletions for all taxa is shown in Table 4. The greatest number of mutational differences occur between soybean and the monocots (mean of 121). The Santalales showed the next largest number of mutations with a mean of 89.2 followed by the other dicots with a mean of 66.4. Among the dicots, *Phoradendron* shows the greatest number of mutational differences (118) followed by *Dendrophthora, Buckleya,* and *Schoepfia.* The relative percent of transversional changes is essentially the same across all groups as are percent insertions. A striking difference between the Santalales and the other dicot group is the amount of transitional change. The seven other dicot species averaged 47% transitions, whereas the Santalales averaged 60%, comparable to the mean value when the two monocots are compared to soybean. As above, the trend in transitions is from a high percentage in *Phoradendron* to the lowest percentage in *Schoepfia.* The other dicot group shows higher percentages of deletions than either the Santalales or monocots.

A more extensive examination was made of the mutational differences between the sequences of *Glycine* and *Phoradendron.* Discounting unsequenced regions and ambigous nucleotides, 118 mutations were observed among the 1731 bases (6.8%). Secondary structures (available from author by request) for *Glycine* and *Phoradendron* were constructed using features shown on the *Zea* diagram proposed by Gutell et al. (1985) as a starting point. Approximately 54% of the mutations occurred in helical regions, whereas 46% were found in nonhelical (single-stranded) regions or at unpaired positions. For the helical region mutations, 10 involved compensating changes on both sides of the helix, thus retaining canonical (Watson–Crick) pairing. Changes that retained canonical or noncanon-

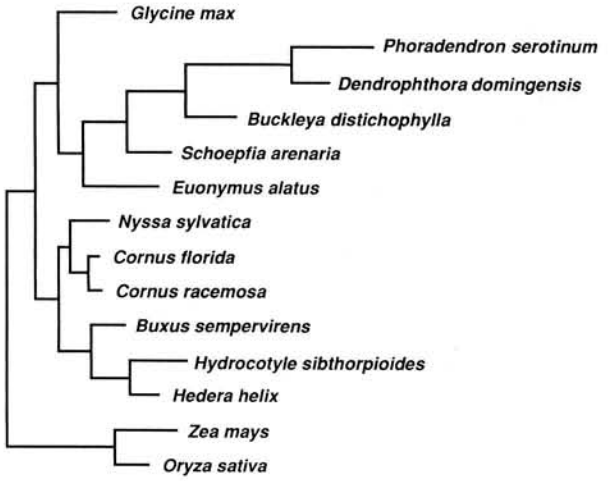

Fig. 2. Most parsimonious phylogram of 446 steps (C.I. = 0.738) resulting from Wagner parsimony analysis of 14 angiosperm 18S rRNA sequences

ical (G·U, G·A) pairing occurred 57 times with transitional changes outnumbering transversions 53 to 4. The most frequent type of transitional change was from C to U. Mutations in *Phoradendron* that resulted in unpaired bases that were paired in *Glycine* occurred seven times.

The cladistic analysis using the branch and bound method resulted in one most parsimonious phylogram (Fig. 2) of 446 steps. The overall consistency index (C.I.) was 0.738; including only informative characters gave a $C.I._i = 0.659$. The phylogram differs from a cladogram in that branch lengths are drawn proportional to the amount of anagenetic change. The phylogram shows the Santalales to be a holophyletic taxon with *Schoepfia* of the Olacaceae at the base of the clade. *Buckleya* of the Santalaceae diverges next, followed by the two members of the Viscaceae (*Phoradendron* and *Dendrophthora*). The phylogram and Table 4 indicate that the Viscaceae have undergone significantly more molecular divergence for this ribosomal gene than other parasitic and nonparasitic dicots. Among the outgroups, *Euonymus* appears closest to the Santalales. The two families of the Cornales, Nyssaceae and Cornaceae, are clustered together as are the two families of the Apiales (Apiaceae and Araliaceae). The two species of *Cornus* (*C. florida* and *C. racemosa*) are grouped very closely. The number of mutations differentiating these species is very low, probably within the range of sequencing error. *Buxus* (Buxaceae) appears as the sister taxon to the Apiales. Soybean (Fabaceae) is positioned as the sister taxon to the *Euonymus*/Santalales clade. Differential weighting of transitions and transversions resulted in no change in the length or topology or the phylogram shown in Fig. 2.

To test the stability of the above results, less parsimonious trees were also examined. Five trees of 447 steps exist (C.I. = 0.736, $C.I._i = 0.657$). All retain the Viscaceae/Santalaceae clade, which includes *Phoradendron, Dendrophthora,* and *Buckleya.* In one of the five trees, *Schoepfia* is grouped with *Euonymus.* Twelve trees of 448 steps exist (C.I. = 0.734, $C.I._i = 0.655$). Again, all retain the Viscaceae/Santalaceae clade and 5 of the 12 trees show *Schoepfia* grouped with *Euonymus* as opposed to with the other members of the Santalales. Features that are retained when the 31 trees of 449 steps are examined include the Viscaceae/Santalaceae clade, the clade comprising the two species of *Cornus,* and the *Hydrocotyle/Hedera* clade. The distance method resulted in nearly the same tree topology as the phylogram shown in Fig. 2. It differed only in the placement of *Glycine,* which clustered as the most distant member of the dicots instead of the sister taxon to *Euonymus* and the Santalalean species.

Discussion

Parasitic angiosperms are an enigmatic and diverse assemblage of plants that exhibit some of the most striking evolutionary adaptations seen among the flowering plants. Above the level of family, very little progress has been made in determining angiosperm parasite phylogenies. The statement by Kuijt (1969, p. 211) that "evolutionary concepts pertaining to all vascular parasites are in an embryonic state" is just as pertinent today as it was two decades ago. The molecular data presented here, though limited in the number of families sampled, promise to add new information sorely needed in resolving the phylogenetic history of these plants.

The relationships of the Santalalean genera shown in Fig. 2 are in good agreement with the relationships among families of the order proposed by Kuijt (1968, 1969). The Viscaceae have long been considered the most evolutionarily advanced family within the order based upon a large number of unique morphological features, such as isophasic endophytic haustoria, loss of nonessential perianth parts, loss of ovular integuments, and reduction in the amount of chlorophyll (e.g., in *Arceuthobium*). It is of interest that among the 11 dicot sequences analyzed in this study, the members of the Santalales show the largest percentage of transitions. Although what defines a mutation is strongly dependent upon the sequences being compared, these results suggest that the 18S rRNA molecule does not behave in a perfectly clocklike fashion. Given the buffered environment of a parasitic plant, it is tempting to speculate about relaxation of selectional constraints at the molecular level. Absence of the biosynthetic form of L-threonine dehydratase was noted in *Lathraea*

(Scrophulariaceae) and *Orobanche* (Orobanchaceae) by Kagan et al. (1968) and for both L-threonine and L-serine dehydratase by Nandakumar et al. (1976). Recent work by DePamphlis and Palmer (1989) demonstrates that *Epifagus virginiana* (Orobanchaceae) has the smallest chloroplast DNA known to date (71 kb). These results indicate that the chloroplast genome can evolve very rapidly in an achlorophyllous obligate parasite.

The concept that the Olacaceae are cladistically basal within the Santalales is supported by rRNA sequence analysis. Examination of other members representing the range of subfamilies and segregate families (e.g., Opiliaceae) is required. Preliminary results using sequences of *Heisteria,* an apparently autotrophic member of the Olacaceae, indicate it may not be closely related to *Schoepfia.* This provides preliminary evidence that the Olacaceae is at least paraphyletic and probably polyphyletic. Further molecular analyses within the Olacaceae are needed to determine whether the nonparasitic forms are a holophyletic group and how closely related they are to the parasitic forms.

In addition to sequences from the parasitic ingroup taxa, several families representing orders of the Rosidae have been sequenced. Our data indicate that the amount of variation in the 18S rRNA molecule is appropriate to address phylogenetic relationships at this level. The Rosidae are the largest dicot subclass in total number of families and approximately equal to the Asteridae in numbers of species (ca. 58,000). The relationships among the 18 orders and 114 families (Cronquist 1981) are also very poorly known. In this study, 10 dicot families were examined; however, their relationships are interpreted differently as shown by the classification systems of Cronquist (1981), Takhtajan (1980), Dahlgren (1983), and Thorne (1983). All four systems include the Viscaceae, Santalaceae, and Olacaceae in the Santalales. Likewise, all place the Cornaceae and Nyssaceae within one order (Cornales) and the Apiaceae and Araliaceae within another (Apiales or Araliales). In agreement with Takhtajan (1980), the RNA data support a close relationship between the Celastrales and Santalales. The Apiales do not appear to be derived from the Cornales as indicated by Takhtajan (1980) but are shown here as the sister group to that order. Using morphological evidence, considerable disagreement exists concerning the placement of the Buxaceae, even at the subclass level. Although we did not include sequences of plants from other subclasses, the results of this analysis indicate that when the sequence of *Buxus* is compared with those from several other orders in the Rosidae, this genus is nested within the clade composed of the Cornales and Apiales, not in a basal position near or outside of the Fabales.

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