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Evolution in the Andean Epiphytic Genus *Columnea* (Gesneriaceae). II. Chloroplast DNA Restriction Site Variation

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ABSTRACT. A cladistic analysis of chloroplast DNA (cpDNA) restriction site variation was performed on *Columnea* (Gesneriaceae) sections *Pentadenia* and *Stygnanthe*. Two species of *Alloplectus* were included in the analyses and one of *Drymonia* was used as the outgroup. Restriction site variation was analyzed using Wagner parsimony and character state weighting that favors convergent losses over convergent gains. The large numbers of equally most parsimonious trees prevented examination of each tree independently. Thus, examination of phylogenetic relationships was based on a strict consensus of all most parsimonious trees. The resulting phylogeny is largely congruent with recent classification schemes, although the positions of several species are not in accordance with traditional relationships. As a result of the cladistic analysis of the cpDNA restriction site variation, section *Pentadenia* is at least paraphyletic, and possibly polyphyletic with section *Stygnanthe*. The lack of resolution among the several clades within section *Stygnanthe* does not provide evidence for or against monophyly of this section. Several clades are strongly supported with cpDNA data and provide insight into biogeography and origin of morphological adaptations.

Cladistic analyses, using either molecular or morphological data, have provided insights into the evolutionary relationships of many plant groups. A phylogeny resulting from a cladistic analysis can prove to be a valuable resource for studying the origins of morphological features and biogeographic relationships of the species involved (Olmstead 1989; Sytsma 1990; Sytsma et al. 1991; Albert et al. 1992a; Baldwin 1992, 1993). A priori, phylogenies based on morphological or molecular data are equally valid; however, there may be instances in which one type of data is best examined in light of a phylogeny derived from another. To avoid circularity, hypotheses regarding the origin of morphological features can be interpreted using a phylogeny derived independently from those features (Olmstead 1989; Sytsma et al. 1991). In these instances, phylogenies derived from molecular data alone may prove more desirable. Likewise, patterns of cpDNA variation where hybrids (Spooner et al. 1991; Rieseberg and Brunsfeld 1992) or chloroplast capture (Smith and Sytsma 1990; Soltis et al. 1991; Wendel et al. 1991; Rieseberg and Brunsfeld 1992) are potentially important factors, may best be studied with a phylogeny derived from morphological data.

A useful source of molecular data for cladistic analyses has been cpDNA restriction site variation (Palmer et al. 1988; Crawford 1990; Clegg and Zurawski 1992; Downie and Palmer 1992). The utility of this method relies on the slow rate of evolution of cpDNA (Curtis and Clegg 1984; Palmer et al. 1988), the large number of characters generated, and the relative ease with which homology of characters can be interpreted. In addition, analysis of cpDNA restriction site data provides a phylogeny independent of morphology and biogeography that permits analysis of these characters in reference to the phylogeny (Olmstead 1989; Sytsma 1990; Sytsma et al. 1991). Thus, the use of cpDNA restriction site variation in reconstructing phylogenies has proven highly successful across a wide taxonomic array of plants (Cattolico 1985; Palmer 1985a, 1985b, 1987; Palmer et al. 1988; Crawford 1990).

Epiphytes possess numerous morphological features that can be viewed as adaptive to their environment (Ackerman 1986; Benzing 1987; Gentry and Dodson 1987). Phylogenetic analyses of epiphytes can provide insights into the evolution and origin of the numerous adaptive traits found in epiphytes (Ackerman 1986; Benzing 1987; Gentry and Dodson 1987; Chase and Palmer 1988, 1989). Many morphological characters have been proposed as adaptations to the epiphytic habit. These include brightly colored tubular corollas to attract birds as pollinators (Ackerman 1986) and a reduced, com-

pact, vegetative habit to make better use of the limited available space (Benzing 1987). However, there has been little investigation regarding the evolutionary origin of these traits.

Similarly, the biogeography of Andean plant groups has only recently been examined with respect to cladistic relationships (Funk 1989; Grifo 1989; Sobrevila 1989). These studies have provided intriguing insights into the radiation of high elevation Andean groups; many of the phylogenetic groupings are consistent with biogeographical patterns. However, it is possible that the phylogenies are biased by homoplastic characters that are strongly selected during evolution in the Andean environment. The uplifting of the Andean range created habitats that would have caused similar types of selection pressure, resulting in convergent character states over a wide taxonomic array. If such characters were used in phylogenetic analyses, the resulting trees could be misleading in that they represent processes of selection rather than patterns of speciation. Therefore, an analysis derived independently of these characters could provide better estimates of evolutionary patterns in such cases, and be less dependent on forces of selection (Sytsma et al. 1991).

Gesneriaceae are a plant family well known for its large number of epiphytic taxa (Madison 1977; Kress 1986; Gentry and Dodson 1987). *Columnea* L. is a large genus containing both terrestrial and epiphytic species. Two of its smaller sections, *Pentadenia* (Planch.) Benth. and *Stygnanthe* Hanst., are distributed primarily in the northern and central Andes and contain terrestrial herbs and facultative and obligate epiphytes. These species also display a wide array of diversity in morphological traits, some of which are traditionally associated with epiphytic species, such as pendent habit, ornithophilous flowers, anisophyllous leaves, and brightly colored leaves as additional pollinator signals (Jones and Rich 1972; Ackerman 1986). Many of these characters can also be found within other sections of *Columnea*. The decision to focus on sections *Pentadenia* and *Stygnanthe* was made for several reasons. 1) These two sections were thought to be monophyletic on the basis of several characters each. Section *Stygnanthe* is characterized by small, relatively inconspicuous corollas that are only slightly swollen, many flowers per inflorescence, and short pedicels, whereas section *Pentadenia* is characterized by large, strongly ventricose, showy corollas, few flowers per inflorescence, long pedicels, and a robust herbaceous habit. 2) These two sections will serve to anchor further cladistic analyses within *Columnea*. Both sections are hypothesized to be basal within the genus because of characteristics shared with other neotropical Gesneriaceae. The nectary of sections *Pentadenia* and *Stygnanthe* is a five-parted gland, the same form found in the closely related genus *Alloplectus* Mart. The remaining four sections of *Columnea* are characterized by a two-lobed dorsal gland. Thus, the two-lobed gland is likely to be a synapomorphy that separates the remainder of *Columnea* from sections *Pentadenia* and *Stygnanthe*. 3) Sections *Pentadenia* and *Stygnanthe* are the only sections with a predominantly Andean distribution and would thus provide a model for Andean phytogeography.

This paper examines the phylogeny of sections *Pentadenia* and *Stygnanthe* using cpDNA restriction site variation with the following goals in mind: 1) to determine a phylogeny independent of morphological data; 2) to compare the phylogeny based on cpDNA with a cladistic analysis of morphological data and traditional classification schemes for the species of these sections; 3) to examine the evolution of specific morphological features proposed as adaptations, and 4) to examine the biogeography of a primarily Andean group of species.

Materials and Methods

The taxa studied and sources of leaf material are listed in Table 1. Total DNA was extracted from frozen leaf tissue using a modified CTAB method (Smith et al. 1991). In addition, the β-mercaptoethanol concentration was raised from 0.2 to 2.0% (vol:vol); this tenfold increase led to a greater yield of DNA.

Purified total DNA was digested with the following 42 restriction enzymes; *Afl*II, *Apa*I, *Apa*LI, *Ase*I, *Ava*I, *Bam*HI, *Ban*II, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*BI, *Bst*EII, *Bst*NI, *Cfo*I, *Cla*I, *Dra*I, *Eco*NI, *Eco*O109, *Eco*RI, *Eco*RV, *Hae*III, *Hin*dIII, *Hpa*I, *Kpn*I, *Mlu*I, *Msp*I, *Nci*I, *Nru*I, *Nsi*I, *Pst*I, *Pvu*II, *Rsa*I, *Sal*I, *Sca*I, *Sma*I, *Sph*I, *Sst*I, *Sst*II, *Stu*I, *Xba*I, *Xho*I, *Xmn*I. Chloroplast genome sizes and maps of *Drymonia stenophylla*, *Alloplectus meridensis*, and *Columnea strigosa*, were determined by single and double digests of *Pst*I, *Bgl*I, and *Sst*I.

Fragments of digested DNA were separated by electrophoresis on horizontal agarose gels (0.8% and 1.0% for six and four base recognition site enzymes, respectively). DNA was transferred to nylon membranes (Biotrans) using the bidirectional method (Smith and Summers 1980). The resulting filters were probed a total of 14 times with chloroplast probes derived from *Petunia* Juss. (P18, S8, S6, P16, P3, P6, P8, P10, P19, and P12 and P14 combined as a single probe [IR1], see Sytsma and Gottlieb 1986 for positions and sizes), and *Lactuca* L. (1.8, 3.5, and 6.2 kb fragments from the inverted repeat, combined as a single probe [IR2], Jansen and Palmer 1988). The small single copy region for the first part of the study was probed with *Oncidium* Sw. (6.7, 2.8, and 3.9 kb fragments combined as two equally sized probes [SSC1 and SSC2]; Chase and Palmer 1989). Later filters were probed with *Nicotiana* L. fragments from the small single copy region as these clones became available for use. The nuclear ribosomal DNA (rDNA) was probed with the pGmr-1 clone from *Glycine* Willd. Procedures for nick translations, hybridizations, and autoradiography followed the methods of Sytsma and Schaal (1985).

Two species of *Alloplectus* (Table 1) were included in the analyses, but because of past taxonomic confusion with *Columnea* (Morton 1953; Stearn 1969; Gibson 1972; Wiehler 1973, 1983; Morley 1974), *Drymonia stenophylla* alone was used as a global outgroup (Maddison et al. 1984). The genus *Drymonia* Mart. is clearly a close relative of *Columnea* and *Alloplectus* based on its habit, corolla form, and nodal anatomy (Wiehler 1983). In addition, a cladistic analysis of morphological characters of representative genera from the entire family indicates that *Drymonia*, *Alloplectus* and *Columnea* are a monophyletic group with *Alloplectus* and *Columnea* as sister species (unpubl. data). Only *Drymonia* was designated as a global outgroup, thus allowing the data and parsimony to determine the relationships between and among sections *Pentadenia* and *Stygnanthe* and all other taxa in the analysis (Maddison et al. 1984). Nine species of sections *Pentadenia* and *Stygnanthe* were not included in this analysis because leaf material was not available. The lack of tissue for these species is primarily because taxa are very rare or occur in places that are difficult to reach.

It is unlikely, although unknown, whether the omission of these species would have an effect on the topology of the tree. There is congruence between the trees from independent analyses of morphological and molecular data for the species that are in common despite different species used in the two analyses (Smith and Sytsma 1994), thus implying that the elimination of some taxa may not have a major impact on the topology of the tree.

Restriction fragment patterns from autoradiographs were interpreted as site gains and losses with respect to *Drymonia stenophylla* and scored appropriately (0-absent, 1-present) for the species involved. Enzymes that recognize both six and four base-pair sequences were used in this study. Because some pairs of six and four base-pair enzymes have overlapping recognition sequences, only the additional sites obtained from the four base-pair recognition site enzymes not found with the six base-pair recognition site enzymes were scored.

Phylogenetic Analysis. Phylogenetic divergence was reconstructed using Swofford's (1993) computer program PAUP version 3.1.1 to implement Wagner parsimony (Farris 1970; Farris et al. 1970; Swofford and Maddison 1987). This program allows parallelisms and reversals (homoplasy), and provides an option for missing data. In this analysis, trees were generated using the heuristic option with 1000 replicate searches of random taxon addition using tree-bisection reconnection (TBR) branch swapping, and saving minimal trees only, with the collapse zero-length branches and ignore uninformative characters options in effect. Because of the large number of taxa in this analysis, the branch and bound and exhaustive search options would have consumed an excessive amount of computer time. Therefore the trees presented here are best approximations and not exact solutions. The manner in which the program reconstructs phylogenetic sequences may create "islands" of trees (Maddison 1991). Therefore the analysis was repeated 1000 times using the random taxon addition option for each replicate.

For several individuals used in this analysis, leaf material was depleted before completing the restriction site analysis. As a result, there is a considerable amount of missing data (Appendix 1). Another analysis was performed omitting all taxa with less than 90% of the full data set (Table 1). Because of the potential errors produced by missing data, all subsequent anal-

TABLE 1. Species used in chloroplast DNA analysis. Accessions marked with * were eliminated in the reduced data set analyses.

Species	Voucher and locality
Drymonia stenophylla (J. D. Smith) H. E. Moore	*J. F. Smith 2148* (WIS), from Bailey Hortorium greenhouses, Cornell University
Alloplectus meridensis Klotzch	**J. F. Smith 1182* (WIS), Mérida, Venezuela
A. peruvianus (Zahlb.) Kvist & L. Skog	*J. F. Smith 1989* (WIS), Imbabura, Ecuador
Columnea section *Collandra*	
C. densibracteata Kvist & L. Skog	*J. F. Smith 1972* (WIS), Pichincha, Ecuador
Columnea section *Columnea*	
C. schiedeana Schlecht.	*J. F. Smith 288* (WIS), plant collected by *H. H. Iltis s.n.*, Veracruz, Mexico
Columnea section *Ortholoma*	
C. mira B. Morley	**J. F. Smith 2450* (WIS), from Bailey Hortorium greenhouses, Cornell University, probably from Panama
Columnea section *Pentadenia*	
C. isernii Cuatr.	*J. F. Smith 2010* (WIS), Cañar, Ecuador
C. nervosa (Kl. ex Oerst.) Hanst.	**J. F. Smith 1963* (WIS), Loja, Ecuador
C. oblongifolia Rusby	*J. F. Smith 1721* (WIS), Cusco, Peru
	J. F. Smith & S. G. Beck 1725 (WIS), La Paz, Bolivia
C. strigosa Benth.	*J. F. Smith 1849* (WIS), Pichincha, Ecuador
	J. F. Smith & G. Adamo 1201 (WIS), Mérida, Venezuela
	J. F. Smith 1220 (WIS), Táchira, Venezuela
	J. F. Smith 1927 (WIS), Pichincha, Ecuador
C. trollii Mansf.	*J. F. Smith & S. G. Beck 1723* (WIS), La Paz, Bolivia
	J. F. Smith & D. N. Smith 1830 (WIS), La Paz, Bolivia
Columnea section *Stygnanthe*	
C. angustata (Wiehler) L. Skog	*J. F. Smith 2126* (WIS), plant from Marie Selby Botanical Gardens, originally from Coclé, Panama
	**J. F. Smith et al. 1433* (WIS), Valle del Cauca, Colombia
	J. F. Smith 2247 (WIS), plant from Marie Selby Botanical Gardens, origin unknown
	J. F. Smith 2248 (WIS), plant from Marie Selby Botanical Gardens, origin unknown
C. byrsina (Wiehler) Kvist & L. Skog	*Madison et al. 4451* (SEL), plant from Marie Selby Botanical Gardens, originally from Carchi, Ecuador
	J. F. Smith & M. Galeano 1505 (WIS), Nariño, Colombia
C. colombiana (Wiehler) Kvist & L. Skog	*H. Wiehler 72-130* (SEL), plant from Marie Selby Botanical Gardens, originally from Valle del Cauca, Colombia
C. crassicaulis (Wiehler) Kvist & L. Skog	*no voucher, collected in Pichincha, Ecuador by J. F. Smith
C. manabiana (Wiehler) J. F. Smith & L. Skog	*C. H. & H. C. Dodson 6791* (SEL), plant from Marie Selby Botanical Gardens, originally from Manabí, Ecuador
C. inconspicua Kvist & L. Skog	*J. F. Smith 1945* (WIS), Pichincha, Ecuador
C. lavandulacea Kvist & L. Skog	*J. F. Smith 2100* (WIS), Morona-Santiago, Ecuador
C. rileyi (Wiehler) J. F. Smith	*J. F. Smith 1944* (WIS), Pichincha, Ecuador
C. moesta Poepp.	*J. F. Smith 1776* (WIS), Cochabamba, Bolivia
C. orientandina (Wiehler) Kvist & L. Skog	*Madison & Coleman 2537* (SEL), plant from Marie Selby Botanical Gardens, originally from Morona-Santiago, Ecuador
C. ovatifolia Kvist & L. Skog	*J. F. Smith 1921* (WIS), Pichincha, Ecuador
C. spathulata Mansf.	*J. F. Smith 1960* (WIS), Loja, Ecuador
	**J. F. Smith 1221* (WIS), Falcón, Venezuela

TABLE 1. Continued.

Species	Voucher and locality
	J. F. Smith 2229 (WIS), plant from Marie Selby Botanical Gardens, originally from Aragua, Venezuela
	J. F. Smith 1853 (WIS), Pichincha, Ecuador
	Skog & Hodapp 5398 (US), plant from Smithsonian Institution, originally from Ecuador
C. ultraviolacea J. F. Smith & L. Skog	*J. F. Smith & D. N. Smith 1829* (WIS), La Paz, Sud Yungas, Bolivia

yses (differential weighting, decay analysis) were performed on full, and reduced data sets.

In addition, character state weighting of Albert et al. (1992b) that differentially favors convergent losses and gain/losses over convergent gains and loss/gains (Templeton 1983a, 1983b) was used on these data. The data were analyzed by PAUP version 3.1.1 (Swofford 1993) with weights of 1.1, 1.3, 1.8, and 2.0 applied to site gains (Albert et al. 1992b). The analysis was performed identically to the equally weighted analysis except 500 replicates were examined instead of 1000.

A decay analysis was performed to examine trees that were one or more steps longer than the most-parsimonious trees (Bremer 1988; Donoghue et al. 1992). This type of analysis provides an indication of the robustness of the data by determining which clades persist in a consensus tree as parsimony is relaxed.

Character state changes were plotted onto trees based on the acctran option. The deltran option prefers parallelisms over reversals, whereas the acctran option tends to prefer reversals over parallelisms. Because the deltran option can sometimes drastically alter the distribution, character state changes were also plotted with the deltran option and compared.

Genetic distances (p) were calculated for pairwise comparisons between *Drymonia stenophylla, Alloplectus peruvianus, Columnea strigosa, C. oblongifolia,* and *C. spathulata* according to Nei and Li (1979).

RESULTS

Over 800 restriction sites recognized by 42 different enzymes were surveyed, of which 296 were found to be variable (Appendix 1). The chloroplast genome was mapped (Fig. 1) for *Drymonia stenophylla, Alloplectus meridensis,* and *Columnea strigosa* using both single and double digests of *Sst*I, *Bgl*I, and *Pst*I. The size was determined to be ~162 kb. A small deletion of 0.3 kb was found in three taxa, *C. lavandulacea, C. rileyi* and *C. ovatifolia.* Because of problems in determining exact homology of insertions and deletions between taxa (Palmer et al. 1985; Sytsma and Gottlieb 1986), this deletion was not used in the phylogenetic analyses.

Only 21 variable sites resulting from 12 enzymes (Appendix 1) were interpretable from the rDNA data. The repeat length was estimated to be 10.8 kb based on digestions with *Nru*I that cut the rDNA repeat unit once. Because of the minimal amount of data from rDNA, these data were not included in the analysis, however none of the rDNA data contradicts the results from the cpDNA analysis (Appendix 1). A list of restriction fragment sizes for each of the cpDNA and rDNA mutations is available from the first author upon request.

The cladistic analysis of all accessions resulted in 4316 equally most parsimonious trees of 193 steps each. The strict consensus of these trees is presented in Fig. 2. The consistency index of each was 0.84, retention index 0.93. This consistency index is high for the number of taxa involved regardless of the use of morphological or molecular characters (Sanderson and Donoghue 1989). Character state weighting (Albert et al. 1992b) resulted in 156 most parsimonious trees for weights of both 1.1 and 1.3, 18 most parsimonious trees for a weight of 1.8, and 102 most parsimonious trees with a weight of 2.0. The strict consensus trees of each of these four analyses were identical to the strict consensus tree with equal weighting (Fig. 2).

The large number of trees is mainly due to the lack of data for some taxa. This lack of data can be attributed to the fact that for some taxon/enzyme combinations, the DNA was poorly cut

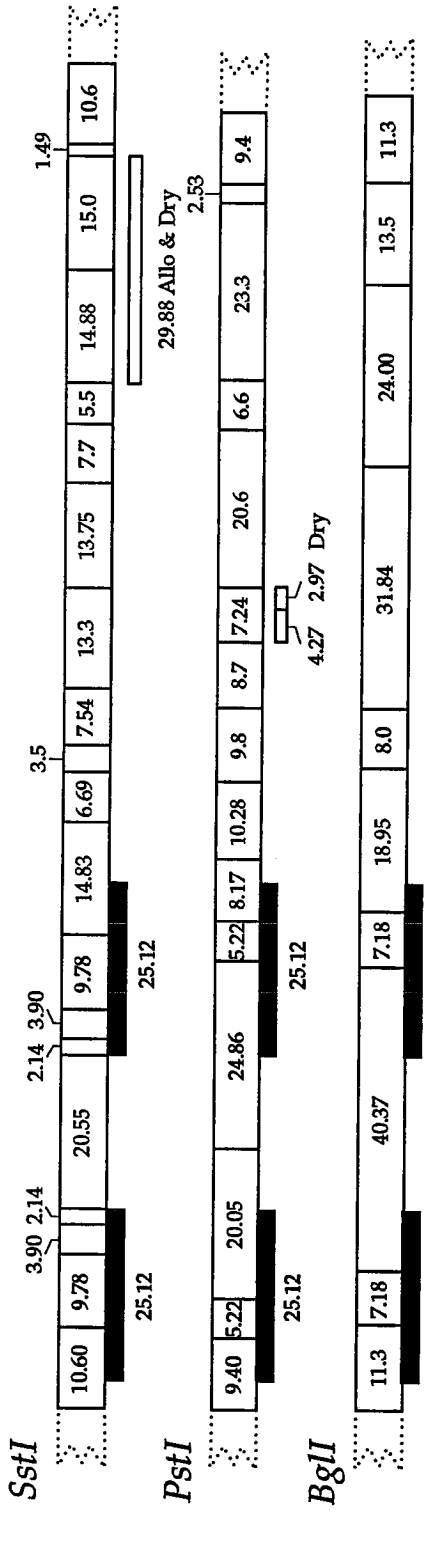

FIG. 1. Chloroplast DNA map for *Columnea strigosa*, *Drymonia stenophylla*, and *Alloplectus meridensis* of *SstI*, *PstI*, and *BglI* restriction sites. Total chloroplast genome length is ~162 kb. The dark bar represents the position and size of the inverted repeat.

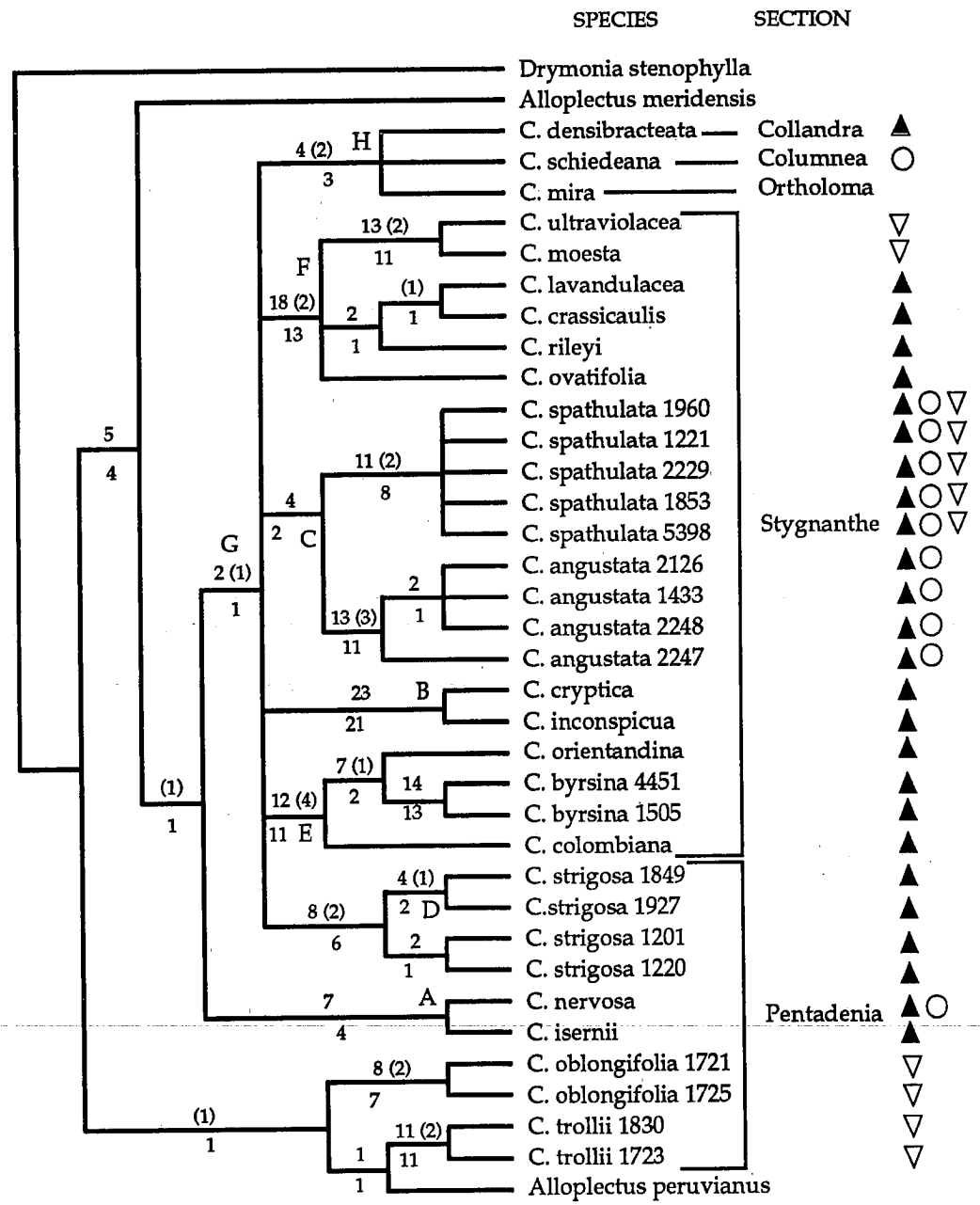

FIG. 2. Strict consensus of 4316 most parsimonious Wagner trees (length 193, consistency index 0.84, retention index 0.93 each) derived from cpDNA restriction site data for *Columnea* sects. *Stygnanthe* and *Pentadenia*. Restriction site mutations are plotted onto the cladogram using the acctran option of PAUP. Numbers above clades indicate the number of mutations defining that clade. Numbers in parentheses indicate the number of mutations that are homoplastic. Numbers below the clades refer to the number of additional steps in which the clade is lost in the decay analysis. In addition, there are five other mutations not shown, including two convergences, that define clades not present in the strict consensus tree. This strict consensus Wagner tree is topologically congruent with the strict consensus tree using character state weighting. Geographic distribution is denoted by symbols: ▲ = Northern South America; ▽ = Central/Southern South America; ○ = Caribbean/Central America. Note that distributions are indicated for species and not necessarily for the individuals used in the analysis. Clades A–H are discussed in the text.

TABLE 2. *p* values (Nei and Li 1979) computed pairwise for *Drymonia stenophylla* (DRY), *Alloplectus peruvianus* (ALLO), *Columnea oblongifolia* accession 1721 (COBL), *C. strigosa* accession 1849 (CSTR), and *C. spathulata* accession 1853 (CSPA).

	DRY	ALLO	COBL	CSTR
DRY	—	—	—	—
ALLO	0.011	—	—	—
COBL	0.011	0.004	—	—
CSTR	0.013	0.005	0.006	—
CSPA	0.013	0.006	0.007	0.006

or DNA was unavailable because of limited leaf material. The missing data prevented the computer program from collapsing essentially zero length branches. Six individuals had over 10% missing data (Table 1). When these six were eliminated from the analysis, the consensus trees based on this reduced data set were identical to the consensus trees based on the full data set with the exception that the excluded individuals were not present.

In the decay analysis, strict consensus of all trees 194 steps or fewer is much less resolved. Only a few clades are retained in the consensus of all trees 194 steps or fewer (A–E completely resolved, F with only the sister group relationship of *C. ultraviolacea* and *C. moesta* resolved, Fig. 2). A strict consensus of all trees 195 steps or fewer loses the resolution of clade C (Fig. 2), as well as resolution within clade E (Fig. 2). The number of extra steps necessary to lose the remaining resolved clades are indicated on Fig. 2.

The majority of mutations in the cpDNA consensus tree are found either as autapomorphies or as synapomorphies supporting terminal clades and range from four to 23 mutations. Only a few synapomorphies support clades located in the basal parts of the tree. Comparison of character state change distributions between the acctran (Fig. 2) and deltran options showed only minor differences. In only two clades is there a difference between the options that changes a strongly supported clade into a weakly supported one. The first is the clade containing species representing sections *Columnea, Collandra* (Lem.) Hanst., and *Ortholoma* Benth. With the acctran option in effect, four mutations, including two convergent mutations, support this clade (Fig. 2), whereas only one mutation supports this clade with the deltran option. One other such rearrangement of characters is in the basal nodes of the tree. In the acctran tree, five mutations support all the nodes above the first node of the tree and one mutation supports the clade above this node (Fig. 2). With the deltran option, these mutations are reversed, and only one mutation supports the clade containing *Columnea oblongifolia* as the sister group of all other species in the analysis, and five mutations support *Alloplectus meridensis* as the sister group of all remaining species of *Columnea* (Fig. 2). In no instance, other than those mentioned above, is there a difference between acctran and deltran that results in a different degree of support for a clade.

Genetic distance estimates are presented in Table 2 and range from 0.004 to 0.013 (Nei and Li 1979).

DISCUSSION

Chloroplast DNA Variation. PHYLOGENETIC ANALYSIS. Despite the large number of trees generated by parsimony analysis of the cpDNA restriction site data, the resulting strict consensus tree is resolved with the exception of three major polytomies, excluding intraspecific polytomies (Fig. 2). Many clades are not well supported as evidenced by the few synapomorphic mutations in some clades and the loss of resolution seen in the decay analysis. Weakly supported clades are especially frequent at the base of the phylogeny. The position of many of these clades, however, is reinforced by examining the most parsimonious trees derived from morphological data (Smith and Sytsma 1994) and trees derived from the combination of morphology and cpDNA restriction site variation (Smith and Sytsma, in manuscript).

RATE OF CPDNA DIVERGENCE. Although the exact age of the Gesneriaceae is unknown, it is thought to be one of the most recent plant families (Wiehler 1983). To date, no fossil Gesneriaceae pollen has been discovered, probably because of the low amount of pollen produced and the habitats occupied by the Gesneriaceae. Species radiation in neotropical Gesneriaceae is hypothesized to have occurred in conjunction with Andean orogenies, and would place the date of origin for many of the species at ~6 mya or later (van der Hammen 1974; Simpson 1975, 1979). The amount of cpDNA variation in such a recent group is likely to be low because of the

slow rate of change in cpDNA (Curtis and Clegg 1984; Palmer et al. 1988). The low amount of cpDNA divergence seen in the species of *Columnea* sections *Pentadenia* and *Stygnanthe* might reflect the recent divergence of these species. The amount of cpDNA divergence seen between congeneric species in members of the Asteraceae, another recently derived plant family dating from the Oligocene (~30 mya) (Cronquist 1977; Müller 1981), has proven to be quite low, and at times has limited the utility of cpDNA at the species level (Crawford et al. 1990; Jansen 1990; Suh and Simpson 1990; but see Schilling and Jansen 1989).

Zurawski et al. (1984) and Zurawski and Clegg (1987) estimated substitution rates for several chloroplast genes to be 0.12 and 0.16% per million years. A crude estimate of divergence time can be made using the estimated substitution rates of Zurawski et al. (1984) and Zurawski and Clegg (1987) with the genetic distance values calculated for the species in this analysis. Based on the *p* values (Table 2) of Nei and Li (1979) and the average estimated substitution rate of 0.14% per million years, the estimated time of separation for *Drymonia stenophylla* and *Columnea spathulata* is 18.6 million years. Because this value represents the amount of time for divergence along both lineages, the actual time of divergence is estimated at 9.3 mya. This figure is plausible because the origin of many of the genera in neotropical Gesneriaceae may have preceded most of the species radiation in the Andes. Other divergence times range from 2.85 mya for *Alloplectus peruvianus* and *Columnea oblongifolia* to 4.3 mya for *C. spathulata* and *C. strigosa*.

Comparison to Morphological Analysis. The relationship of *Columnea colombiana* to *C. byrsina* and *C. orientandina* is not apparent based on morphological data. There are no known morphological features that unite *C. colombiana* with *C. byrsina* and *C. orientandina*. *Columnea colombiana* is a pendent, thin-stemmed herb with small ovate leaves, and deeply dissected calyx lobes. The corolla is superficially similar to that of *C. byrsina* in that it is red with a yellow limb, but it lacks exserted stamens and stigmas and is more densely pubescent. However, the placement of *C. colombiana* as the sister species of *C. byrsina* and *C. orientandina* is well supported with 12 cpDNA restriction site mutations (Fig. 2).

Although there is some lack of resolution within the clade, the relationship of the species in the *C. lavandulacea* clade is strongly supported by 18 synapomorphies. A superficial examination of the species would fail to find any morphological feature that would unite them as a clade. However, with a cladistic analysis of morphology, the majority of these species are united in a single clade by the following synapomorphies: glabrous styles, sericeous corolla tube exteriors with the trichome density greater on the limb, pedicel glands present, and leaves glabrous abaxially (Smith and Sytsma 1994). The only species of this clade (as based on molecular data) that are not united based on morphological data are *C. rileyi* and *C. moesta* (Smith and Sytsma 1994). The possibility that *C. moesta* is of hybrid origin is discussed below, but its placement at the base of the morphological tree is probably because of reversals. *Columnea rileyi* is morphologically unique within section *Stygnanthe* for many characters, but is allied with the *C. lavandulacea* clade because of the presence of darkened spots on the corolla lobes, a character not used in the morphological analysis of Smith and Sytsma (1994).

Species Status. Chloroplast DNA provides evidence for unity of the *Columnea strigosa* complex. Populations of *C. strigosa* have been described as six different species (*C. aurantiaca* Dcne. ex Planch., *C. campanulata* Benth., *C. macrantha* Benth., *C. pichinchensis* Hanst., *C. kucyniakii* Raymond, *C. strigosa*). Each description has been based on material with a slightly different morphology, but all of these "species" have been combined into *C. strigosa* (Kvist and Skog 1993; Smith 1994). The morphological variant named as *C. kucyniakii*, included in this analysis as *C. strigosa* accession 1927, is the most distinctive with large, almost ovate leaves, terrestrial habit, and several to many, narrow corolla tubes per axil. The maintenance of *C. kucyniakii* as a species is not supported by cpDNA restriction site data. The accession is imbedded within *C. strigosa* and is the sister of Ecuadorian populations of *C. strigosa*, represented in this study by accession 1849 (Table 1; Fig. 2).

Biogeography. Once the phylogenetic history of a group is known, inferences to its biogeography can also be made by constructing area cladograms (Rosen 1975; Platnick and Nelson 1978; Humphries and Parenti 1986). Although there is a lack of resolution in some

parts of the cpDNA consensus tree, useful clues to the biogeography of the species involved are still available. Species at the base of the tree, such as *Columnea oblongifolia* and *C. trollii* are found only in Bolivia or southern Peru, indicating a southern origin for *Columnea* (Fig. 2). Species in more recently derived clades are from northern Andean countries and indicate a movement northward and subsequent radiation in Ecuador and Colombia. Radiations of other sections occurred in more northernly areas such as section *Collandra* in Colombia and southern Panama, and section *Columnea* in Central America.

The placement of some species in this cpDNA analysis are not in agreement with this model of biogeographic evolution. For example, *C. ultraviolacea*, *C. moesta*, and the widespread *C. spathulata* all have populations in Bolivia, but are phylogenetically more recently derived clades. Long distance dispersal by birds may explain these discrepancies. The fruits of *Columnea* are white or pale pink berries, frequently displayed against brightly colored sepals, and are filled with up to hundreds of seeds. These fruits are clearly adapted to bird dispersal, and the fruits of the South American species are likely to be consumed by migratory birds as are other fruits of the Gesneriaceae (Blake and Loiselle 1992). Therefore, the anomalous distributions of primarily Ecuadorian species in Bolivia might be explained in this manner (Berry 1982; Smith 1991). Bird dispersal is also the most likely explanation for the distribution of a single species, *C. nervosa*, in southern Mexico, Guatemala, and Panama, that is allied with *C. isernii* from Ecuador based on a cladistic analysis of morphological data (Smith and Sytsma 1994).

Character State Evolution. While it would be unadvisable to make any inferences on character state evolution of taxa in the poorly supported clades, there are several well supported clades (7–23 synapomorphic mutations each). Because of the larger number of mutations supporting these clades, more confidence can be placed on inferences regarding character state evolution in these clades.

EPIPHYTIC HABIT. Vegetative reduction in epiphytic groups such as Bromeliaceae and Orchidaceae is well documented (Benzing 1986, 1987). Epiphytic *Columnea* species are also smaller than terrestrial species and have a greater tendency to be creeping or vining herbs. In contrast, the terrestrial or facultatively epiphytic species have a tendency to be shrubby with larger internodes and thick stems. These plants almost always grow upright and in only one species (some populations of *C. strigosa*) do they ever become viny. Although the more basally located clades on the cpDNA tree are only weakly supported, the cladogram suggests that obligate epiphytism and subsequent vegetative reduction occurred only once within *Columnea* (Fig. 3). The obligate epiphytes are found in clades above clade G in the strict consensus (Fig. 3). Below this polytomy the species are facultative epiphytes that are generally terrestrial (excluding species of *Alloplectus* and *Drymonia*).

It is possible that epiphytism arose more than once, because the separation of obligately and facultatively epiphytic clades becomes obscured in the strict consensus of trees one or fewer steps longer than the most parsimonious trees. However, cladistic analysis of the morphological data (Smith and Sytsma 1994), and combined analysis of morphology and cpDNA restriction site variation (Smith and Sytsma, in manuscript), support the topology seen in the cpDNA tree in this region. It could be argued that the characters used in the morphological analysis are adaptations to the epiphytic habit. These adaptations would therefore not be additional independent support for the single origin of obligate epiphytism in *Columnea*. However, the characters used in the morphological analysis are not obviously related to epiphytic habit (Smith and Sytsma 1994).

Two species appear to have regained the shrubby habit, *Columnea strigosa*, and *C. moesta* (Fig. 3). However, if *C. strigosa* were not part of clade G, and instead were placed at the base, it would be consistent with a single origin of the epiphytic habit.

The placement of the Bolivian/southern Peruvian *C. moesta* as the sister of another Bolivian species, *C. ultraviolacea*, is well supported (12 mutations). *Columnea moesta* possesses a shrubby habit and large ventricose corolla with a constricted opening found in other species at the base of the cpDNA tree. A cladistic analysis of morphological characters places *C. moesta* as the sister species of *C. trollii* (Smith and Sytsma 1994). Because of the uniparental inheritance of cpDNA in most plant species including Ges-

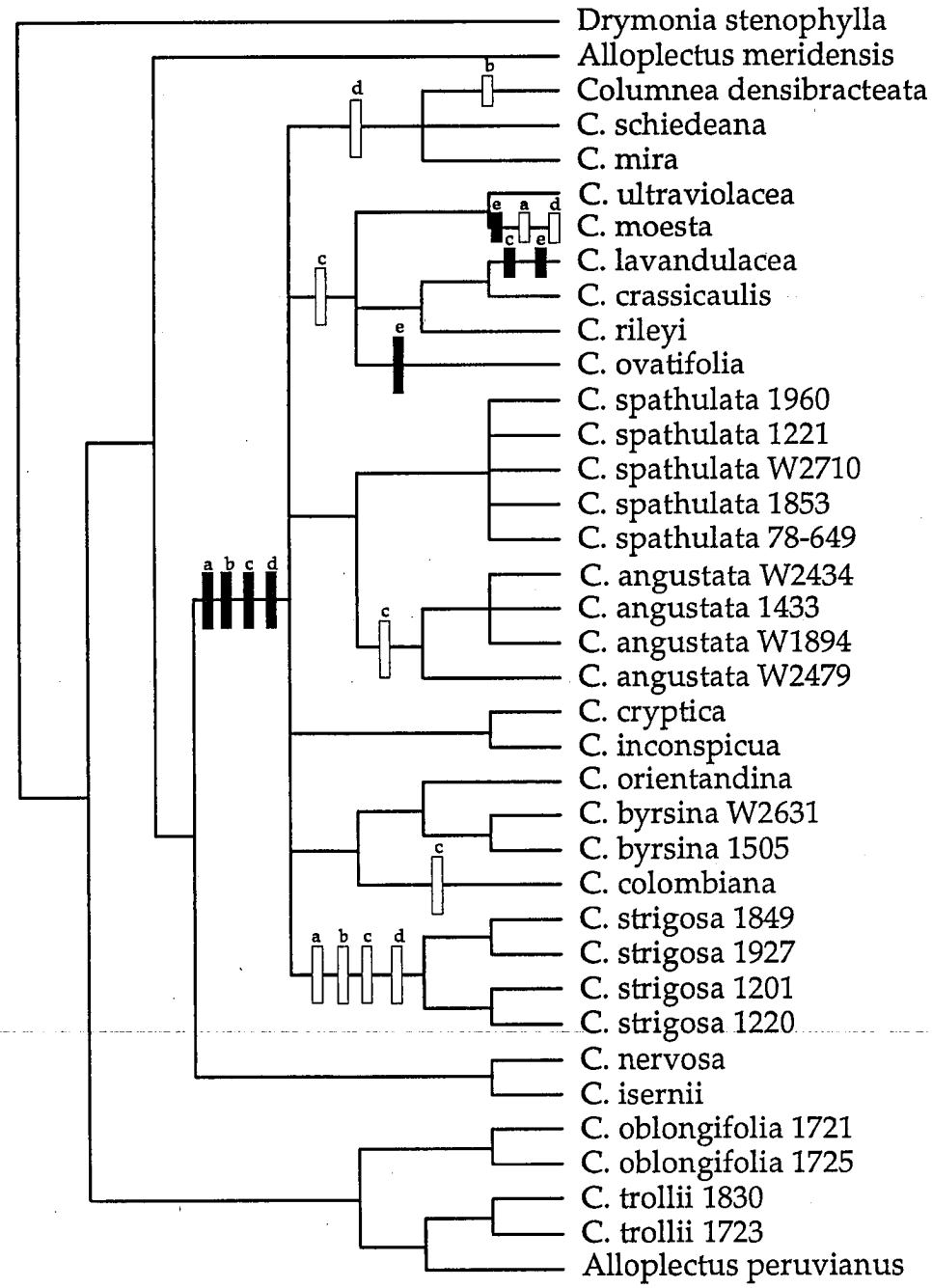

FIG. 3. Strict consensus tree indicating the loss (white bar) and gain (black bar) of morphological traits for *Columnea* sects. *Stygnanthe* and *Pentadenia* a = obligate epiphytic habit, b = adventitious roots, c = anisophylly, d = symmetrical corolla (vs. ventricose), e = violet corolla. Character state changes are plotted based on parsimony, however see text for potential alternative distributions.

neriaceae (Corriveau and Coleman 1988), discrepancies between morphology and cpDNA may indicate hybridity. In such cases examination of a third data set, such as nuclear ribosomal DNA, can resolve the issue (Doyle et al. 1985; Doyle and Doyle 1988; Rieseberg et al. 1988, 1990; Smith and Sytsma 1990; Spooner et al. 1991; Rieseberg and Brunsfeld 1992). Analysis of nuclear ribosomal DNA and morphology do not indicate a hybrid origin for *C. moesta* (Smith 1991; Smith and Sytsma 1994). A more likely explanation for the unusual distribution of character states found in *C. moesta* is strong selection for the character states in the more basally located groups. Several of the other species that possess shrubby habit and ventricose corollas are found in southern Peru and Bolivia, perhaps indicating selection in this geographic region for those character states.

VEGETATIVE REPRODUCTION. An additional factor that is linked to habit is the capacity for vegetative reproduction. Plants that are smaller and tend to be epiphytic also have a greater ability for vegetative reproduction. Field collections of small epiphytic plants tend to have more adventitious roots than shrubby terrestrial species. Vegetative propagation, both from the field and in cultivation, is also more successful with the smaller, epiphytic plants (pers. obs.). Adventitious roots allow for continued maintenance of the individual through vegetative reproduction in a difficult to attain site and aid in mineral and water uptake. As with epiphytism, adventitious roots appear to have had a single origin, and because of the link to habit, the presence of adventitious roots probably represents an adaptive trait for the epiphytic habit (Fig. 3).

ANISOPHYLLY. Anisophylly, the condition where one leaf of a pair of opposite leaves grows much larger than the other, occurs in many species of *Columnea* and other genera in the Gesneriaceae. This character is believed to be under strong selective pressure (Morley 1973; Givnish 1984) and has potentially arisen multiple times within the family. Two arguments exist for the origin of anisophylly and suggest that it is the result of selection. Morley (1973) proposed that the anisophyllous condition would result in greater light capture and would thus be beneficial for plants growing in the understory of an evergreen forest. Givnish (1984) modified this hypothesis by adding the factor of leaf packing. By being anisophyllous, more leaves of a larger size can be put onto a stem without self-shading, thus saving energy and increasing light capture simultaneously. In either case, the potential selection for this character, as well as its presence in other genera and families (Givnish 1984), argues for multiple origins. It is not possible to place this character unambiguously on the cladogram based on this cpDNA analysis (Fig. 3). Anisophylly is found in five of the clades within clade G including the clade representing the remaining sections of *Columnea*. Given this distribution, it is possible that the trait arose independently in the individual clades or, as shown in Fig. 3, arose once, and was lost in the other clades. Multiple origins of anisophylly would require five steps rather than the six steps shown in Fig. 3. However, because of the lack of resolution in clade G, it is possible that the *C. strigosa* clade is basally located with respect to the clades in the polytomy and would therefore make a single origin of anisophylly equally parsimonious.

COROLLAS. Corolla form and color are other characters that have potential adaptive significance because of pollinator selection (Wiehler 1983). The smaller, symmetrical corolla (Fig. 4A) is found only within section *Stygnanthe*. This section is a potentially monophyletic group based on cpDNA (Figs. 2, 3) indicating that the symmetrical corolla originated once. The proposed single origin of the symmetrical corolla is complicated by the presence of a larger strongly ventricose corolla, typical of section *Pentadenia*, in five species placed in clade G; *C. strigosa*, *C. moesta*, and the clade representing three other sections of *Columnea* (clade H of Fig. 2). Although the strict consensus requires three reversals to the ventricose corolla for a single origin of the symmetrical corolla, it is possible that a more resolved tree would place the *C. strigosa* clade and the clade representing other sections of *Columnea* basal to the section *Stygnanthe* clades. In this more resolved tree only one additional step to account for the reversal to the ventricose corolla in *C. moesta* is required for a single origin of the symmetrical corolla. Such a tree is possible because trees based on a combination of morphology and molecules place *C. strigosa* sister to section *Stygnanthe* (Smith and Sytsma, in manuscript). This topology suggests that the symmetrical corolla is derived from the more ventricose corolla (Fig. 4B) and probably

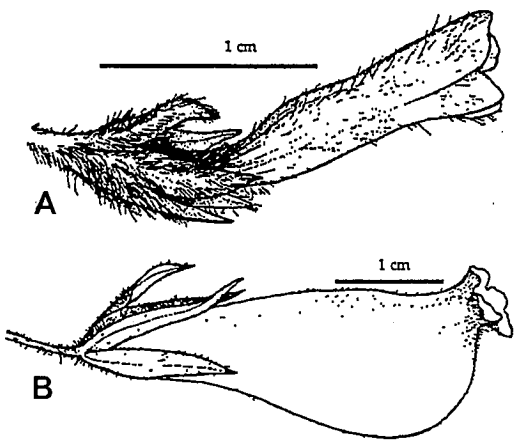

FIG. 4. Corolla forms in *Columnea* sects. *Stygnanthe* and *Pentadenia*. A. The symmetrical corolla from *C. angustata* is typical of section *Stygnanthe*. B. The ventricose corolla from *C. trollii* is more frequent in section *Pentadenia*.

arose only once, with a reversal to the ventricose corolla in *C. moesta*. Another possibility is that the symmetrical corolla may have arisen independently in the several clades of clade G. This hypothesis cannot be eliminated based on the evidence presented here because of uncertainty of relationships of lineages within the unresolved clade, although it is a less parsimonious explanation.

The ventricose corolla form (Fig. 4B), in contrast, appears to have originated at least twice within *Columnea*, once in the basally located species of section *Pentadenia* and again in *C. moesta*. The ventricose corolla forms seen in sections *Columnea*, *Ortholoma*, and *Collandra* clearly arose independently. The corollas in these sections never possess the narrowly constricted opening of species in section *Pentadenia* and thus represent at least one additional origin of ventricose corollas in *Columnea*.

Corolla color is another character that has multiple origins of its several character states. Corollas that are either yellow or red are dispersed throughout the clades, and only violet potentially could have arisen once (Fig. 3). In this example, the violet color may be symplesiomorphic for this clade, and the color changes to yellow in *Columnea crassicaulis* and *C. ultraviolacea*, and to orange in *C. rileyi* may represent the derived condition. However, it is possible the violet color arose independently in all three taxa because this hypothesis is one step shorter than the preceding (Fig. 3).

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APPENDIX 1.

Data matrix used in chloroplast DNA restriction site analysis for *Columnea* sections *Pentadenia* and *Stygnanthe*. Character names are listed first, separated by semicolons. Each species is listed next with all character states in the same order as the character names. Characters are named by using the restriction enzyme/probe combination as in Materials and Methods. More than one mutation per enzyme/probe combination are indicated in numerical order after a dash. Ribosomal DNA (rDNA) characters follow the cpDNA characters and are indicated by the probe designation pGmr. The rDNA character states are separated from the cpDNA character states by a slash (/).

Characters: *Hind*III/P3; *Hind*III/P8-1; *Cla*I/P3-1; *Cla*I/P6; *Cla*I/P8; *Cla*I/P10; *Cla*I/SSC1; *Cla*I/SSC2; *Ava*I/P3-1; *Ava*I/P3-2; *Ava*I/P8-1; *Ava*I/P8-2; *Ava*I/SSC1-1; *Ava*I/SSC1-2; *Ava*I/SSC1-3; *Bgl*II/P3-1; *Bgl*II/P8-1; *Bgl*II/SSC1-1; *Bgl*II/SSC1-2; *Bgl*II/SSC1-3; *Eco*RV/S6-1; *Eco*RV/P3; *Eco*RV/P6; *Eco*RV/P8; *Eco*RV/P10-1; *Eco*O109/P3-1; *Eco*O109/P6-1; *Eco*O109/P6-2; *Eco*O109/P8; *Eco*O109/P18; *Eco*O109/SSC1; *Eco*O109/SSC2; *Bam*HI/S6-1; *Bam*HI/S6-2; *Bam*HI/S8-1; *Bam*HI/S8-2; *Bam*HI/P3-1; *Bam*HI/P3-2; *Bam*HI/P6; *Bam*HI/SSC1; *Afl*II/S8; *Afl*II/S6-1; *Eco*RI/S6; *Eco*RI/P3; *Eco*RI/P8; *Eco*RI/SSC2; *Apa*I/P10; *Apa*I/SSC1-1; *Apa*I/SSC1-2; *Apa*I/SSC2-1; *Apa*I/SSC2-2; *Apa*LI/P8; *Bgl*I/SSC2-1; *Bst*BI/S8; *Bst*BI/P8-1; *Bst*BI/P8-2; *Bst*BI/P10-1; *Bst*BI/P10-2; *Bcl*I/P3-1; *Bcl*I/P3-2; *Bcl*I/P6-1; *Bcl*I/P6-2; *Bcl*I/SSC1-1; *Bcl*I/SSC1-2; *Bcl*I/SSC1-3; *Bcl*I/SSC1-4; *Bcl*I/SSC1-5; *Bcl*I/SSC1-6; *Bcl*I/SSC2-1; *Bcl*I/SSC2-2; *Hind*III/S8; *Cla*I/P3-2; *Cla*I/P3-3; *Cla*I/P3-4; *Cla*I/P3-5; *Cla*I/P8; *Cla*I/SSC1; *Ava*I/P3-3; *Ava*I/P6; *Ava*I/P8-3; *Bgl*II/S6; *Bgl*II/P6-1; *Bgl*II/P6-2; *Eco*RV/S6-2; *Eco*RV/S8-1; *Eco*RV/S8-2; *Eco*RV/P10-2; *Eco*O109/S6; *Eco*O109/S8; *Eco*O109/P6-3; *Eco*O109/P10; *Afl*II/S6-2; *Afl*II/P3; *Afl*II/P6; *Apa*I/P8-1; *Apa*I/P8-2; *Bgl*I/P6; *Bgl*I/P10; *Bgl*I/IR-1; *Bgl*I/IR-2; *Bst*BI/S6-1; *Bst*BI/S6-2; *Bst*BI/SSC1; *Hind*III/P8-2; *Hind*III/P8-3; *Dra*I/S6-1; *Dra*I/S6-2; *Dra*I/P6-1; *Dra*I/P6-2; *Dra*I/P10-1; *Dra*I/P10-2; *Dra*I/P10-3; *Dra*I/P10-4; *Dra*I/IR; *Dra*I/SSC1; *Xba*I/S6-1; *Xba*I/S6-2; *Xba*I/S8; *Xba*I/P6; *Eco*NI/S6; *Eco*NI/S8-1; *Eco*NI/S8-2; *Eco*NI/P3; *Bst*EII/S6-1; *Bst*EII/S6-2; *Bst*EII/P6; *Bst*EII/SSC1-1; *Bst*EII/SSC1-2; *Hpa*I/S6-1; *Hpa*I/S6-2; *Hpa*I/P3-1; *Hpa*I/P3-2; *Hpa*I/P3-3; *Hpa*I/SSC1; *Kpn*I/P8; *Nru*I/S8; *Nru*I/P8-1; *Nru*I/P8-2; *Nru*I/SSC1; *Nsi*I/P3; *Nsi*I/P10; *Nsi*I/IR; *Nsi*I/SSC2; *Stu*I/P3-1; *Stu*I/P3-2; *Stu*I/P3-3; *Stu*I/P8; *Stu*I/SSC1-1; *Stu*I/SSC1-2; *Stu*I/SSC1-3; *Sma*I/S6; *Sma*I/S8; *Sma*I/P3; *Sma*I/P6; *Sma*I/IR; *Sma*I/SSC1; *Sph*I/P3; *Sph*I/P6; *Sph*I/IR; *Sst*I/S6-1; *Sst*I/S6-2; *Sst*I/S8; *Sst*I/P3; *Sst*I/P6; *Sst*I/P8-1; *Sst*I/P8-2; *Sst*I/P10; *Sst*I/SSC1; *Sst*I/SSC2-1; *Sst*I/SSC2-2; *Sst*I/SSC2-3; *Sst*II/S8; *Sst*II/P8-1; *Sst*II/P8-2; *Pst*I/S6; *Pst*I/P3-1; *Pst*I/P3-2; *Pst*I/P6; *Pst*I/P19; *Pst*I/IR; *Pvu*II/S6-1; *Pvu*II/S6-2; *Pvu*II/S6-3; *Pvu*II/S6-4; *Pvu*II/P3; *Pvu*II/P8-1; *Pvu*II/P8-2; *Sal*I/P3-1; *Sal*I/P3-2; *Sal*I/P10; *Sal*I/SSC1-1; *Sal*I/SSC1-2; *Xho*I/P10; *Xho*I/SSC1-1; *Xho*I/SSC1-2; *Xmn*I/S6; *Xmn*I/S8-1; *Xmn*I/S8-2; *Xmn*I/P3; *Xmn*I/P6; *Xmn*I/P10-1; *Xmn*I/P10-2; *Xmn*I/P18; *Xmn*I/SSC1; *Xmn*I/SSC2-1; *Xmn*I/SSC2-2; *Cfo*I/P3-1; *Cfo*I/P3-2; *Cfo*I/P6-1; *Cfo*I/P6-2; *Cfo*I/P6-3; *Cfo*I/P8; *Cfo*I/P10-1; *Cfo*I/P10-2; *Cfo*I/P10-3; *Cfo*I/P10-4; *Cfo*I/SSC1-1; *Cfo*I/SSC1-2; *Cfo*I/SSC1-3; *Msp*I/S6-1; *Msp*I/S6-2; *Msp*I/S6-3; *Hae*III/S6; *Hae*III/S8;

*Hae*III/SSC1; *Hae*III/SSC1; *Rsa*I/SSC1-1; *Rsa*I/SSC1-2; *Bst*NI/S6; *Bst*NI/S8-1; *Bst*NI/S8-2; *Bst*NI/S8-3; *Bst*NI/P3-1; *Bst*NI/P3-2; *Bst*NI/P3-3; *Bst*NI/P6-1; *Bst*NI/P6-2; *Bst*NI/P8-1; *Bst*NI/P8-2; *Bst*NI/SSC1; *Bst*NI/SSC2; *Ase*I/S6-1; *Ase*I/S6-2; *Ase*I/S6-3; *Ase*I/S6-4; *Ase*I/S8-1; *Ase*I/S8-2; *Ase*I/P3-1; *Ase*I/P3-2; *Ase*I/P3-3; *Ase*I/P3-4; *Ase*I/P6; *Ase*I/P8-1; *Ase*I/P8-2; *Ase*I/P8-3; *Ase*I/P10-1; *Ase*I/P10-2; *Ase*I/P10-3; *Ase*I/P10-4; *Ase*I/SSC1-1; *Ase*I/SSC1-2; *Ase*I/SSC2; *Ban*II/S6; *Ban*II/S8; *Ban*II/P3; *Ban*II/P6-1; *Ban*II/P6-2; *Ban*II/P6-3; *Ban*II/P6-4; *Ban*II/P10-1; *Ban*II/P10-2; *Ban*II/P10-3; *Ban*II/SSC1; *Ban*II/SSC2; *Nci*I/S6-1; *Nci*I/S6-2; *Nci*I/S8; *Nci*I/P3-1; *Nci*I/P3-2; *Nci*I/P3-3; *Nci*I/P6; *Nci*I/P8-1; *Nci*I/P8-2; *Nci*I/P19; *Nci*I/SSC1; *Nci*I/SSC2-1; *Nci*I/SSC2-2; *Eco*O109/P3-2; *Bgl*II/P8-2; *Bgl*II/P3-2; *Bst*EII/SSC1-3; *Eco*O109/P3-3; *Dra*I/P6-3; *Hin*dIII/P8-4; *Bgl*I/SSC2-2; *Pvu*II/S6-5; *Xmn*I/S8-3; *Bst*NI/P3-4; *Eco*RI/pGmr-1; *Eco*RI/pGmr-2; *Eco*RI/pGmr-3; *Dra*I/pGmr; *Eco*RV/pGmr-1; *Eco*RV/pGmr-2; *Eco*RV/pGmr-3; *Bam*HI/pGmr; *Eco*NI/pGmr; *Eco*O109/pGmr-1; *Eco*O109/pGmr-2; *Apa*LI/pGmr; *Bgl*I/pGmr; *Nsi*I/pGmr; *Sph*I/pGmr; *Xmn*I/pGmr-1; *Xmn*I/pGmr-2; *Xmn*I/pGmr-3; *Ase*I/pGmr-1; *Ase*I/pGmr-2; *Ase*I/pGmr-3.

Drymonia stenophylla:
0100010011111011011100001101100000100010011001000000001101110100101000011001011010101100010100010000110100000000100110000110100001100100001010000111110100000101000100000000100010011010000000000000011111011100?10011110010010110010010010101001011110000101100110011000110100011110101010010100011110000001 1/101011000001101111000

Alloplectus meridensis:
0001010010111010101100100111100011000010010100100000001101111110101000111011011011101000010001110100110000010001001000001100000000????00001100100111111000???1????????????????????????????010000????????????????????1?????????????????????????0?0100001001111100000???/111010010001111111000

A. peruvianus:
0001010010111011101100100111110000010010010000000000001101111100101000111011111011101000010001110000110000010001000000000110000000110010000111010011111010010010100100000000100000001001001000000000000111010010001001101001001010001001001010100001110000010010001001100011010000111010101000010011111000001 11/1110100101011011111000

Columnea densibracteata:
0101110010011011000110100111000000000001001000000010001011011111001010011110110110111010000100011100001100110100010010000011000000110010100111010011110010000010100000000100010000000100100100001000010001001011001100110000100110100100000101000001101000001001000000110001101000100101000110011001101100010011/???010010001101111000

C. schiedeana:
0001010010101010100010100111101000000001011000000110110110111110010100011101101101110100001000111000011000101010100100000110001101100100101110100110110100000101000000000000100000001001011000000000000010010110011001101001001001001000001110101001010000010010001001110011010001111010001000010011111100000 11/???010010001101111000

C. mira:
00010100101110111001?????????????????????01?????????00011011111011010001100110110111????????0????000011000??????????????????00000110010000111010011111010000010100000000000100000001001 0??????000000001001011001100110100100100100100000101010000101000001001000100110001101000111101000110001001111100?00?10/11101001000110111 1000

C. isernii:
00010100101110111001001001111000000000010??0000000000000100111110010100011101101100100100001 0???1100001100000010001101000001100000001100100001110100111110100000 10???00000000011000001001001000 00???
??001100101100110011010010010010010000010101000011101000100100010111000111100011110100010000100 1111100000011/1111100100011011 10000

C. nervosa:
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C. oblongifolia 1721:
0001010010111011101100100111100000000011010000000000000110111110010100011111101101110100101000111100010000101000100100000110000000110010000111010011111010001010100000000000010000000100100100000 0

0000???1101001000100010100100101100100100100010000111000001001000100110001101000111100010100001 0
01111100001011/1110100?0101101011010

C. *oblongifolia* 1725:
00010100101110111011001001111000000000110100000000000001101111100101000111111011011101000010001 1
11000100000101000100100000110000001100100001110100111110100010101000000000000100000010010010000 00
00000011101001000100110100100101100100100101010000111000001001010100110001101000111100010100001
001111100001011/1110100?0001101011010

C. *strigosa* 1849:
0001010010111011100101100111100000000010010000000000001101111100101010111011011011101000010001 1
10000110000011001001000001100001011001000111101001111101000001010000100010010000000100100100000 0
00000001001001001100110100100100100000101010000111000001000000100110000101000111101000000001
001111100000011/1110000?1001111111000

C. *strigosa* 1201:
0001010010111011100100100111100000000010010000000000001101111100101010111011011011101000010001 1
10000110000011011001000001100001011001000111101001111101000001010000100000110000001001001000000
00000001001011001100110100100100100000101010000111000001000000100110000101000111101000000001
001111100000011/1110000?1001111111000

C. *strigosa* 1220:
0001010010111011100100100111100000000010010000000000001101111100101010111011011011101000010001 1
1000011000001101100100000110000101100100011110100111110100000101000010000011000000??????0100000???
??00010010110011001101001001001001000001010100001110000010000001001101001010001111010000000001001
111100000011/1110000?1001111111000

C. *strigosa* 1927:
0001010010111011100101100111100000000010010000000000001101111100101010111010011011101000010001 1
10000110000011001001000001100001011001000111101001111101000001010000100010010000000100100100000 0
00000001001001001100110100100100100000101010000111000001000000100110000101000111101000000001
001111100000011/1110000?1001111111000

C. *trollii* 1830:
0001010010111011101100100111100000000010010000000000001101111100101000111011010011101000010001 1
100001100000100010 01????0100000100110100001110100111111000001010000000000000000000100100101000 00
0000010101001000100110100101101100100100101010000111000001001000100110001101000011101010100001 0
01111100100011/11?01001?001101011000

C. *trollii* 1723:
0001010010111011101100100111100000000010010000000000001101111100101000111011010011101000010001 1
1000011000001000100100000100000100110100001110100111111000001010000000000000000000100100101000 0
0000010101001000100110100101101 10????1001010100001110000010010001001100011010000111010101000010
01111100100011/1100100100011101011000

C. *angustata* 2126:
0001011010111011100010110111100000000010010000000000001101111000101000111011011011101000000001 1
10000110000010001001000011100000011001000011101001111101000001010000001000010100001001001000000
00000001001010001100110101100000101100000000101000011100001100100010011001110100111110100110100 1
001111100000011/11100001001110111100 0

C. *angustata* 1433:
000101101011101110001011011110 00????010??0000000000001101111000101000111011011011101000000??? 110
0001100000100010 01???1110000001100100001 11??????????0000010??000010000101????????????????????00010
010100011001101011000001011000000001010000111000011001000100110011101001111101001101001001111100
000?11/1110000100111011 11000

C. *angustata* 2248:
0001011010111011100010110111100000000 0100?00000000000011011110001010001110110110111010000 00??11
0000110000010001001000011100000011001000011101001111101000001010000001000010100001001001000000 0
00000010010100011001101011000001011000000001010000111000011001000100110011101001111101001101001 0
01111100000011/1110000100111011 11000

C. angustata 2247:
0001011010111011100010110111100000000010010000000000001101111000101000111011011011101000000011
1000011000001000100100001110000001100100001110100111110100000107??000000000101????????????????????
0001001010001100110101100000101100000000101000011100001100100010011001110100011110100110100100 11
11100000011/1110000100111011111000

C. byrsina 4451:
0001010100110111100110100110100000000010000000010000000101111100101000111011011011100000110001 1
1000011000001000100100000001000001110011000011010101110011100111110000000000101000000001001000 00?
????00010010110010001101001101001001000101010100000100001010010001001100001011001111110001000000
11111100000011/1110000100110011111 00

C. byrsina 1505:
00010101001101111001101001101000000000100000000100000001011111001010001110110110111000001100011
10000110000010001001000000010000011100110000110101011100101001111100000000001010000000010010000 0?
????00010010110010001101001101001001000101010100000100001010010001001100001011001111110001000000
11111100000011/1110000100111011111 00

C. colombiana:
00010100101100011000101001111000000000100100000100000011011111001011001110110110111010001100011
10000110000010001000000000110000011100100010110100111100101000101100000000000101000010010010010 00
00000001001011001100110100100100100100000101010000010000001001000100110000101000111111000100000
001111100000011/1110000100?1101111000

C. crassicaulis:
?0?10100?011101110011010 1?111000000001110010000000000000110001110010100011101101101110100001001110
000011000001000100100000110000011100?0001111???1011101000001 0??000?00000101????????????????????000
10000110011?01101001001001001000011011100001110000010010001001100011000101111010001000010011110
00000?11/1110101100?1101111001

C. inconspicua:
00010100101110111001101001111000000000100100000000100011111011001000001010110110111111000011001 1
100001100000100010110010011000000110010001111010011111010000000101000000001010100001000101000000
0010100100101111110111010000010010001000010101100011100100100100010001000110100011100110010000 1
101111101000001/1110100100011011111000

C. lavandulacea:
1011010010111011100110100011100000001110010000000000000110101110010100011101101101110101001001 11
000000100000100010010000011000001110000000111111001011101000001000000001000001010000101100100000 0
000000010001110011101001001001001001000011011100001110000010010001001000011000101111010001000 01
001111000000011/1110100100101011111000

C. rileyi:
1011010010111011100110100011100000001110010000000000000110101110010100011101101101110100001001 11
?00001100000100010 01???01100000011000000111 1????1?11101000001 0??0001000001010000101100100000000 0
000010000110011101101001001001001000011011100001110000010010001101100011000101111010001000010 01
110000000011/1110100100111011 01000

C. manabiana:
00010100101110111001101001111001000000110100100000000001111101100100000101011011011111000011001 1
100011100000100010110010011000000110010001111011011101010000000101000000001010000001001101000 00?
??1?10010010111111001101001001001001100001010110001110010010010001000100011010001110011001000010
01111101000011/11101001000110111 1000

C. moesta:
1011000010111011100100100011100000001110010000000000000110101110010100011101100101110100001001 11
0000011100011000000100010101010001100000001110100101110100000100000000100000100000001011001100000
1000000100001100111011010010010010010000010111000011100000100110010011000110000011110100010000 1
001111000000011/1111100100011001 01000

C. orientandina:
?0010100?011???11001101001101000000000100100000100000011011111001110001110110110111010001100011 1

000011000001000100100000011000000???0?0000011????1111001010001011000000000001011000100100100010000
00101001011001100110100100100100100000101010000010000001101000100110000101100111111000100000011
111100000011/11101001000110111100

C. ovatifolia:
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0001011000001000100100000110000001100000011110100101110100000100000000?000001010000101100100000
000000001000011001110110100100100100100000101110000111000000001000100110001100010111101000100001
001111100000011/1110101100011011010

C. spathulata 1960:
0001010010111011101110110111100000000010010000000000000110111110000100011101101101110100001?011
1000011000001000100101000110000001101100011110100111110100000101000000000000100001110010010000000
0100000100101000110011010010010011010100010101000011100001100100010011000100100011110100010100
001111100000011/11100001001110111100

C. spathulata 1221:
?0010100101110111011101101111000??????10??000000000000011011111000010001110110110111010000100??110
00011000001000100l????????00000?????0001111????????????0??????????????????????????????????0100000????????100101
000110011010010010011010100010101000011??????????????????????????????????????0001010000011111000000011/111
00001001110111100

C. spathulata 2229:
0001010010111011101110110111100000000010010000000000000110111110000100011101101101110100001?011
1000011000001000100101000110000001101100011110100111110100000101000000000000100001110010010000000
0100000100101000110011010010010011010100010101000011100001100100010011000100100011110100010100
001011100000011/01100001001110111100

C. spathulata 1853:
00010100101110111011101101111000000000100100000000000001101111100001000111011011011101000010011
1000011000001000100l0?0001100000011011000111101001111101000001010000000000001000011100100100000000
0100000100101000110011010010010011010100010101000011100001100100010011000100100011110100010100
001111100000011/11100001001110111100

C. spathulata 5398:
?0010100101110111011101101111000000000100100000000000001101111100001000111011011011101000010011
1000011000001000100l0?00011000000?????0001111???????????00000101000000000000010?00?11001001000000000100
000100101000110011010010010011010100010101000011100001100100010011000100100011110100010100000011
11100000011/11100001001110111100

C. ultraviolacea:
1011000010111011100100100011100000001110010000000000000110101110010100011101100101110100001001111
?000011100011000000100010111010001000000001110100101110100000100000000100000100000001011001100000
1000000100001100111011010010010010010000010111000011100000100110010011000110000011110100010000l
001111000000011/1111100101011001010000