

# LEAF SHAPE EVOLUTION IN THE SOUTH AFRICAN GENUS *PELARGONIUM* L' HÉR. (GERANIACEAE)

Cynthia S. Jones,<sup>1,2</sup> Freek T. Bakker,<sup>3</sup> Carl D. Schlichting,<sup>1</sup> and Adrienne B. Nicotra<sup>4</sup>

<sup>1</sup>*U-3043, Department of Ecology and Evolutionary Biology, 75 N. Eagleville Road, University of Connecticut, Storrs, Connecticut 06269*

<sup>2</sup>*E-mail: Cynthia.s.jones@uconn.edu*

<sup>3</sup>*National Herbarium Nederland, Wageningen University branch, and Biosystematics Group, Wageningen UR; Generaal Foulkesweg 37, 6703 BL Wageningen, The Netherlands*

<sup>4</sup>*School of Botany and Zoology, The Australian National University, Canberra, ACT 0200 Australia*

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Leaf shapes reflect complex assemblages of shape-determining elements, yet evolutionary studies tend to treat leaf shape as a single attribute, for example cordate or linear. As with all complex structures, individual elements of a leaf could theoretically evolve independently and at different rates to the extent permitted by genetic and functional limitations. We examined relative evolutionary lability of shape-determining elements in the highly diverse South African plant genus *Pelargonium* (Geraniaceae). We used SIMMAP to calculate Bayesian posterior probabilities for ancestral states of leaf-shape characters for major nodes across multiple phylogenetic trees. Trees were derived from a Bayesian analysis of DNA sequence data from four partitions. We found that shape elements differed in rates of character-state transformations across the tree. Leaf base, apex, and overall outline had low rates. Transformations in venation occurred at slightly higher rates and were associated with shifts in venation among major clades. Leaf margin type and overall leaf size showed intermediate rates, whereas high rates were observed in the extent of lamina lobing and functional leaf size. The results indicate that suites of elements characteristic of the recently evolved xerophytic lineage, for example pinnate venation, dissected lamina, and entire margins, were acquired piecemeal over nested levels of the phylogeny.

**KEY WORDS:** Ancestral character-state reconstruction, functional leaf size, leaf venation, leaf evolution, xerophytic plants.

From tiny scales on photosynthetic stems to highly elaborated structures meters long, leaves are morphologically the most diverse vegetative organs produced by plants. Among angiosperms, this diversity arises in part from large differences among lineages in rates of within-taxon morphological evolutionary change. Some taxa bear leaves varying relatively little in shape, such as the genus *Protea*, whereas in others leaf shapes vary extensively, for example the Papaveraceae (e.g., Gleissberg and Kadereit 1999). Given the importance of leaves in general and their diversity of shape, there have been surprisingly few queries into leaf-shape evolution

within clades. This lack of attention may result from underlying assumptions. In lineages with invariant leaf shapes, shape evolution is assumed highly constrained and therefore uninteresting. In highly diverse lineages, leaf shapes are assumed adaptive and therefore potentially homoplasious such that strong selection for functional traits would overwhelm phylogenetic signatures. Influencing these assumptions is the general tendency to consider leaf shape in its totality, as a single trait, for example lobed versus not lobed, or wide versus narrow. Selection, by extension, is assumed to act on this overall shape.

As with all complex modules, overall shape may evolve as a result of changes in individual shape-determining elements (Hulsey et al. 2006; Young et al. 2007). In leaves, for example, lobing occurs within the overall leaf outline, bounded by varying shapes of the apex and base and influenced by margin roughness. Final leaf shape reflects developmental and functional integration of these separate elements, but there is no a priori reason to assume that they cannot evolve independently and at different rates within the limits set by integration; the extent to which this occurs is unknown at present.

Leaf shape is assumed to be adaptive, primarily because it influences functional leaf size ("effective leaf size" [Givnish 1987]). Measured often as leaf width, functional leaf size affects boundary layer thickness, leaf thermal properties, and ultimately water-use efficiency (Parkhurst and Loucks 1972). However, recent studies have shown that the location of edges relative to the midrib and petiole explains variation in leaf temperature that is independent of leaf area or functional leaf size, a result not predicted by boundary layer considerations alone (Ball et al. 2002; Leigh et al. 2006). As well, total leaf size is directly influenced by allometric and architectural relationships that may be independent of functional leaf size because large, highly dissected leaves can have the same functional leaf size as small, undissected leaves. Finally, leaf shape has also been shown to influence herbivore behavior (Brown and Lawton 1991; Rivero-Lynch et al. 1996; Degen and Städler 1997), suggesting biotic interactions as additional important selective factors.

The predominantly South African genus *Pelargonium* presents an excellent opportunity to investigate leaf-shape evolution. With roughly 280 species, *Pelargonium* is one of relatively few monophyletic lineages whose large radiation contributes to the angiosperm species-level hyperdiversity in the Cape Floristic Region (CFR) (Linder 2003). More than 150 species occur in the CFR in Fynbos and Succulent Karoo biomes, mostly in the winter rainfall region, making it the seventh largest clade of flowering plants in this region (Goldblatt and Manning 2002; Linder 2003). In part because CFR has been designated as a biodiversity hotspot, recent attention has focused on factors driving and maintaining species richness in this area. Primary drivers are likely to be a combination of strong climatic gradients over short distances, high variation in soils, frequent fires, pollinator specialization, and short dispersal distances (Linder 2005; van der Niet et al. 2006).

In *Pelargonium*, both leaf shape and growth form vary tremendously. Growth form ranges from herbaceous annuals and perennials to geophytes and woody shrubs and shows a relatively strong phylogenetic signal closely aligned with infrageneric sections (Bakker et al. 1999b). Leaf shapes range from entire to dissected to the midrib (Fig. 1) and do not correlate strongly with growth form: a wide range of leaf dissection can be found within most sections (see "*Pelargoniums of Southern Africa*," Volumes

I–III (van der Walt 1977; van der Walt and Vorster 1981; van der Walt and Vorster 1983)). Perhaps most intriguing is that species with leaves of very different shapes and functional sizes can be found growing within meters of each other.

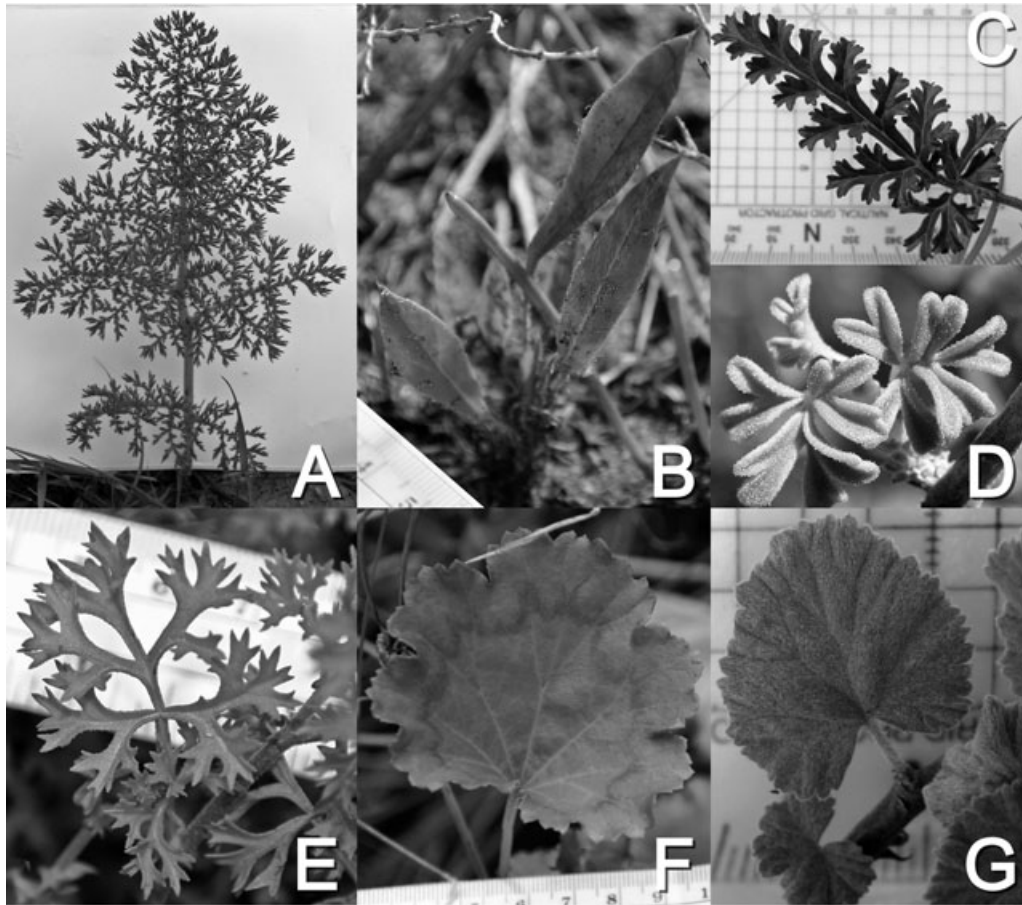
In this study, we examine leaf-shape evolution in *Pelargonium*. We describe leaves according to shape-determining elements typically described by systematists, for example leaf base, apex, outline, venation, etc., to ask to what extent leaves evolve as entire modules versus independent elements. We hypothesize that if leaves evolve as integrated modules, all characters (shape elements) will show similar patterns of ancestral state reconstructions with similar rates of transformations among states. In this case, even multiple independent evolutionary origins would result in high correlations among specific character states. An alternative hypothesis is that all characters evolve independently. In the most extreme scenario, all states of each character are independent, highly labile and potentially homoplasious, resulting in large numbers of evolutionary transitions, low probabilities that any given character state will be reconstructed as ancestral, and little correlation between pairs of states for any two characters. Between these extremes lies mosaic evolution (de Beer 1954), in which traits show differing degrees of lability and thus vary in their rates of evolutionary transition across the tree. In this case, only some traits would change in a coordinated fashion due to genetic correlation or simultaneous selection on them.

To evaluate relative evolutionary lability and independence of different leaf shape elements in *Pelargonium*, we first confirm previously recognized major clades (Bakker et al. 2005) with a Bayesian analysis. We then reconstruct ancestral states of leaf-shape elements for these clades using both parsimony and stochastic models and examine correlated character evolution in key shape elements. Our empirical Bayesian approach to ancestral state reconstructions of shape-determining elements reveals leaves of *Pelargonium* evolve neither as indivisible modules nor as highly labile and independent assemblages of shape-determining elements. Instead, different shape elements evolve at different rates. Some trait associations consistent with functional adaptations to the abiotic environment appear to have been acquired incrementally over nested levels of the phylogeny.

## Materials and Methods

### STUDY SYSTEM

*Pelargonium* is sister to the clade containing the remaining clades in the Geraniaceae, characterized by zygomorphic corollas and nectar spurs adnate to the pedicels. The genus comprises two main clades distinguished by molecular sequences, differences in chromosome size (see Bakker et al. 2004) and potential for hybrid formation (Gibby et al. 1996), but not by gross morphology. Branching from this deep bifurcation are five major clades (C1,



**Figure 1.** Representative leaf diversity among *Pelargonium* species. (A) *P. triste*. (B) *P. pilosellifolium* (C) *P. myrrhidifolium*. (D) *P. praemorsum* (E) *P. fruticosum*. (F) *P. zonale*. (G) *P. magenteum*. All photos were taken by the authors.

C2, B, A1, and A2) well-supported as monophyletic by molecular sequence data. These clades show some divergence in distributions, dispersal capacity, pollination, and karyotypes (Bakker et al. 2004); their origin appears to be associated with changes in paleo-environments (discussed below) (Bakker et al. 2005). These major clades are congruent with current sectional classification; species within sections have similar growth forms (Bakker et al. 1999b). Here we focus on major clades (and two well-supported subclades within the speciose Clade A) to emphasize major evolutionary transitions; ancestral states of higher splits are not discussed in detail, but they can be seen (using parsimony) on the figures showing character-state reconstructions.

Using molecular clock dating, Bakker et al. (2005) proposed that the main subdivision within *Pelargonium* between the Large Chromosome Clade C and the Small Chromosome Clades A + B occurred approximately 30 Mya, during a 10 million year drier phase between warmer, mesic tropical periods (Cowling and Richardson 1995; Zachos et al. 2001). Twenty percent of *Pelargonium* species are in Clade C, relatively broadly distributed from southwestern to eastern Africa, with a few species in Asia Minor, Madagascar, and the Arabian Peninsula. Of the two subclades, C1

includes sections *Jenkinsonia*, *Myrrhidium*, and *Chorisma*, whose species are mostly subshrubs with woody stems, although there are also a few short-lived perennials or annuals. They are distributed from Namaqualand and the western Cape, to the summer rainfall regions of the eastern Cape and further north into eastern Africa. Clade C2 contains sections *Ciconium* and *Subsucculentia*, both with subsucculent subshrub growth forms, and although predominantly in eastern Africa, there are several large geographic disjunctions (Bakker et al. 2004).

The remaining 80% of *Pelargonium* species reside in the small chromosome subgenus with three major clades—A1, A2, and B. Divergence time estimates of Clades A and B range from approximately 22 Mya (plastid DNA) to 16 Mya (rDNA). Clade B comprises two recognized sections. The paraphyletic section *Peristera* is widespread in southern and eastern Africa with several additional species in Australia. Plants tend to be small, weedy herbaceous annuals, slightly woody at the base with highly branched growth, low pollinator specialization, and small, abundant seeds. Section *Reniformia* arises within *Peristera* and contains evergreen, perennial subshrubs found predominantly in the eastern Cape (Bakker et al. 1998).

Clade A, the “winter rainfall clade” (Bakker et al. 1999a), includes over 170 species in eight sections that exhibit a wide range of perennial growth forms. This clade diversified during a drying trend that began 18–20 Mya, becoming well-established 12–15 Mya (Cowling and Richardson 1995; Linder and Hardy 2004). Most species have small, restricted distributions and high pollinator specificity. Clade A1 includes section *Pelargonium*—woody, evergreen shrubs found throughout the winter rainfall region, and section *Campylia*—partially evergreen, small herbaceous subshrubs found principally on sandy flats of the southern Western Cape Province. The origin of Clade A2, the “xerophytic clade,” has been estimated at 8–12 Mya, consistent with a period of increasing aridification in the Western Cape (Bakker et al. 2005). Distributed predominantly in the arid regions of Western Cape, it includes stem succulents (sections *Otidia* and *Cortusina*), the semi-succulent subshrubs of section *Ligularia*, and the geophytes of sections *Hoarea*, *Polyactium*, and *Magnistipulacea* (Bakker et al. 1999a, b).

### THE PHYLOGENETIC ANALYSIS

The DNA sequence data matrix from Bakker et al. (2004) included four partitions: (1) cpDNA trnL-F (1116 /284 total/informative sites), (2) nrDNA ITS (590/125), (3) mtDNA nad 1b/c (51/33), and (4) 30 recoded indels inferred from the plastid partition. Here we added sequences for *P. boranense*, making the total number of terminals 154. Outgroups were as in Bakker et al. (2004), that is *Hypseocharis biloba*, *Geranium pusillum*, and *Sarcocaulon vanderhaetiae* (only *Hypseocharis* was designated as outgroup in the phylogenetic analysis). A Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) analysis was performed in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) using a temperature of  $T = 0.1$  and tree sampling every 1000<sup>th</sup> generation. Best-fitting models for the three nucleotide sequence-containing partitions were estimated using ModelTest version 3.7 (Posada and Crandall 1998). Parameter values estimated by MrBayes were analyzed in Tracer version 3.1.2 to check convergence as well as effective sample sizes of parameter estimates. The Phylogenetic Network approach (Holland et al. 2006) implemented in SplitsTree version 4 (Huson 1998) was used to inspect the Bayesian trees for topological conflict (which could indicate “non-tree like behavior” of the underlying data).

### CHARACTER SCORING

To score as many of the species used in the phylogenetic analyses as possible, a variety of sources were used; many species were observed from more than one source. We scored species from the field in South Africa (46 species, 10+ individuals, several from multiple sites), as well as the living *Pelargonium* collection at the University of Connecticut (26 additional species, 2–3 plants), herbarium specimens from the Gray Herbarium (GH) at

Harvard and the New York Botanical Garden (NYBG) (14 more species, 1–3 plants), photographic images or drawings from (1) the three-volume series *Pelargoniums of Southern Africa* (van der Walt 1977; van der Walt and Vorster 1981, 1988), (2) *Geophytic Pelargoniums* (Craib 2001), and (3) *Pelargoniums* (Miller 1996) (55 species from all printed sources). Of these 55, we verified venation patterns of 24 species from specimens in the Compton Herbarium and South African Museum Herbarium (NBG and SAM) at the Kirstenbosch Research Center.

We evaluated leaf shape using traditional systematic descriptors of leaf outline, apex, base, and margin (Judd et al. 2002). Overall leaf outline was scored as one of five states: ovate, obovate, elliptic, oblong with parallel sides, or “modified oblong” when the distal and basal lateral lobes extended the same distance from the midrib, even if leaves were not elongate. Overall outline was determined directly in leaves with entire margins, or by an imaginary outline that extended between tips of the major lobes (Supporting Fig. S1A). Lamina base and apex were scored using categories in Judd et al. (2002). Lamina margin was scored as one of five categories: entire, toothed (dentate or serrate), toothed along only the lobe apices, crenate, or other (erose or revolute).

Among species of *Pelargonium*, the degree of dissection between the major lobes of the lamina varied continuously from not dissected (entire) to dissected-to-midrib. We categorized the degree of dissection in five states (Supporting Fig. S1B) that described the depths of sinuses between the major lobes. Categories were applied without regard to overall lamina outline, the pattern of major veins, or the degree of secondary lobing on the major lobes.

In general, categories for different states within characters were broad enough that intraspecific variation did not present frequent problems (e.g., “toothed” margins includes dentate, serrate and all other sharp tooth shapes). Note also that most species of *Pelargonium* could be recognized by the combination of leaf and growth form in the field. Increased sampling might have revealed greater intraspecific variation, but unless this variation was extensive, changing the states of a few taxa would not have affected the ancestral state reconstructions for major nodes given the large number of taxa in the tree. An exception was some intraspecific variation in the degree of dissection of the lamina, so we scored 29 species as polymorphic for this trait. Because the software we used, SIMMAP (described below), could not resolve polymorphic characters, we created two datasets, one in which the degree of lobing was resolved as biased toward the “most lobed” state, and a second in which the degree of lobing was resolved toward the “least lobed” state.

We scored leaf venation patterns only from living or pressed leaves except in a few cases when the leaf venation category (e.g., pinnate) could be determined with 100% certainty from photos or drawings. Our sample included 82% of the taxa represented in

the phylogenetic analysis (Bakker et al. 2004), distributed more or less evenly across the tree. Major vein classification considered position, the number diverging at the base of the lamina, and the relative sizes of all veins diverging from the midvein to recognize pinnate and palmate venation, that is the categories of major vein patterns frequently recognized by botanists (Supporting Fig. S1C).

We used lamina size classes proposed by the Leaf Architecture Working Group (Ash et al. 1999), for example leptophyll, nanophyll, etc. Leaf size classes were estimated using the upper limit from the range of lamina lengths measured by us or presented for each species in *Pelargoniums of Southern Africa* (van der Walt 1977; van der Walt and Vorster 1981, 1988). Category assignments were verified by comparing the estimated size class with actual average leaf areas for 40 species collected in South Africa (C. S. Jones, C. D. Schlichting, and A. B. Nicotra, unpubl. data). Estimated size classes based on overall lamina outline generally overestimated the actual leaf area of highly dissected leaves by one size class category, so those leaves were assigned to the next smaller size class category.

Functional lamina size was estimated as the diameter of the largest circle that could be inscribed in the leaf outline. Species were assigned to one of four functional leaf size categories: 1–5, 5–20, 20–40, and > 40 mm.

### CHARACTER ANALYSIS

Character optimization was performed using parsimony with the “Trace over trees” option in Mesquite (Madison and Madison 2006) for 101 trees selected from the stabilized part of the MCMCMC. This option provided the percentage of trees that showed the uniquely best reconstruction of particular character states over the tree at each major node. Character-state changes were modeled as unordered for all characters. Three characters, the degree of lobing, leaf size, and functional leaf size were also modeled with ordered states.

In contrast to the discrete, alternative ancestral character states provided by parsimony optimizations, Bayesian approaches based on stochastic models accommodate uncertainty in ancestral character-state reconstructions as well as in the phylogeny (Ronquist 2004). The program SIMMAP (Bollback 2006) uses a Bayesian mutational mapping approach (Huelsenbeck et al. 2003). Random realizations of character histories (called mappings) consistent with the character states observed at the tips of the tree are used to estimate posterior probabilities for specific ancestral state reconstructions over multiple trees. In addition, various statistics of interest, such as the relative amount of time spent in a particular character state can be obtained from the mappings. Information about tree length is discarded, and only information about branch length proportionality is retained. For each simulated character history, SIMMAP draws a random deviate from a

gamma distribution having shape parameter  $\alpha$  and scale parameter  $\beta$  that is then used to scale the tree accordingly. Values for  $\alpha$  and  $\beta$  must be provided by the user for each character; there are various approaches for choosing appropriate values (Huelsenbeck et al. 2003). We took an empirical Bayesian approach. To determine the mean of the gamma tree length distribution [ $E(T)$ ], we multiplied the maximum-likelihood rate parameter for ancestral state reconstructions of an individual character using the Mk-1 Model in Mesquite by the average estimated tree length of the trees from the Bayesian phylogenetic analysis (i.e., 3.36). We set  $\alpha$  such that the coefficient of variation (CV) was one of a set of predetermined values (i.e., 30%). The formulas used for  $\alpha$  and  $\beta$  parameters given the mean [ $E(T)$ ] and CV (expressed as a percentage) CV were:  $\alpha = (100/\text{CV})^2$ ,  $\beta = \alpha/E(T)$ .

In all ancestral state reconstructions, the priors were resampled 100 times. Every major clade (“constraint”) occurred in each of the 101 trees except subclade A2b, which occurred as defined in only 50% of the trees. Before ancestral state reconstruction, all trees were rooted using *Hypseocharis*, *Geranium*, and *Sarco-caulon* as a monophyletic outgroup. In these analyses, all characters were modeled with unordered character states. Estimated numbers of transformations between states were examined using the “Simulate Histories” option in SIMMAP. Each estimated number of transformations was based on 100 realizations sampled from priors for 10 realizations of each of 101 trees, for a total of 101,000 histories per character.

SIMMAP calculates correlations between any two states, for example s1 of character 1 and s2 of character 2, using the fraction of time that s1 and s2 are simultaneously present in a single lineage for a single realization (mapping). This approach can quantify the extent of overall association between any two characters as well as the extent of association between any two specific states of multistate characters. Assuming complete independence of the characters, the probability of finding any two character states in association is the product of the marginal probabilities of finding each character in a particular state. The  $D$  statistic (overall character association) and  $d_{ij}$  (association among specific states) represent the divergence between the observed association between states and the expected association if the characters are evolving independently given all the trees, branch lengths, and character mappings. This value is compared against the posterior predictive distribution that results in a  $P$  value that represents the probability of observing  $D$  or  $d_{ij}$  as extreme as that obtained when simulated histories of two characters are modeled as independent of each other (Huelsenbeck et al. 2003). In our study,  $D$  was calculated from five realizations sampled from priors for each of 101 trees; the posterior predictive  $P$  value was based on five null realizations for each observed realization, resulting in a null distribution of 505 random samples. Larger samples from the null distribution did not influence the values of  $P$ .

## Results

### PHYLOGENETIC ANALYSIS

The MCMCMC was run for 16,000,000 generations, using the following models for each data partition: (1) cpDNA *trnL-F* and nrDNA ITS, GTR + invgamma; (2) mtDNA *nad1 b/c*, JK; and (3) the recoded indel partition used the “standard” one-parameter morphology model with “coding” set to “informative,” because only informative indel characters were included. Estimation of all parameters for all partitions was unlinked, yielding a total of 16 “free” parameters to be optimized. Rate distribution shape parameters  $\alpha$ , as well base composition parameters, were estimated to be significantly different for each partition (see Table 1). Two 4-chain MCMCMC’s were set up for 150,000,000 generations but stopped after the convergence diagnostic (SD of split frequencies) fell below 1% at generation 13,716,000. Temperature T was set to 0.1 and trees were sampled every 1000th generation. However, as the standard deviation of split frequencies in the tree topologies found within either MCMCMC did not get below 3.2%, we concluded that neither search had converged topologically. Therefore we analyzed the last 1000 trees from the stationary phase of the MCMCMC in SplitsTree using the Phylogenetic Supernetwork approach. SplitsTree detected topological conflict among the trees (at threshold value 0.1) only within the winter rainfall clade with the position of one taxon, *P. nanum*. The MCMCMC search conditions were repeated excluding this taxon and the SD of split frequencies in each decreased to 1.2%, indicating topological convergence. The “burn-out” proportion of trees from these MCMCMCs was summarized in MrBayes using the 50% majority-rule consensus option (depicted as the phylogram in Fig. 2). Subsequently we took 101 trees from these as input for the SIMMAP analyses, sampled from the final stationary phase of the MCMCMC at 1000-generation intervals.

**Table 1.** Models and base composition parameters used for each data partition used in the phylogenetic analysis (see text for details);  $\kappa$ , transition/transversion rate ratio,  $\alpha$ , gamma shape parameter.

	cpDNA <i>trnL-F</i>	nrDNA ITS	mtDNA <i>nad1b/c</i>
Model	GTR+ gamma	GTR+ invgamma	K2P+ gamma
$\alpha$	0.494	0.986	0.175
% AT	64.2	43.8	60.3
Relative rates			
(AC-AG-AT- CG-CT-GT)	0.178–0.175– 0.075–0.124– 0.262–0.185	0.092–0.197– 0.143–0.041– 0.442–0.086	$\kappa=3.854$

### PARSIMONY VERSUS STOCHASTIC CHARACTER MAPPING

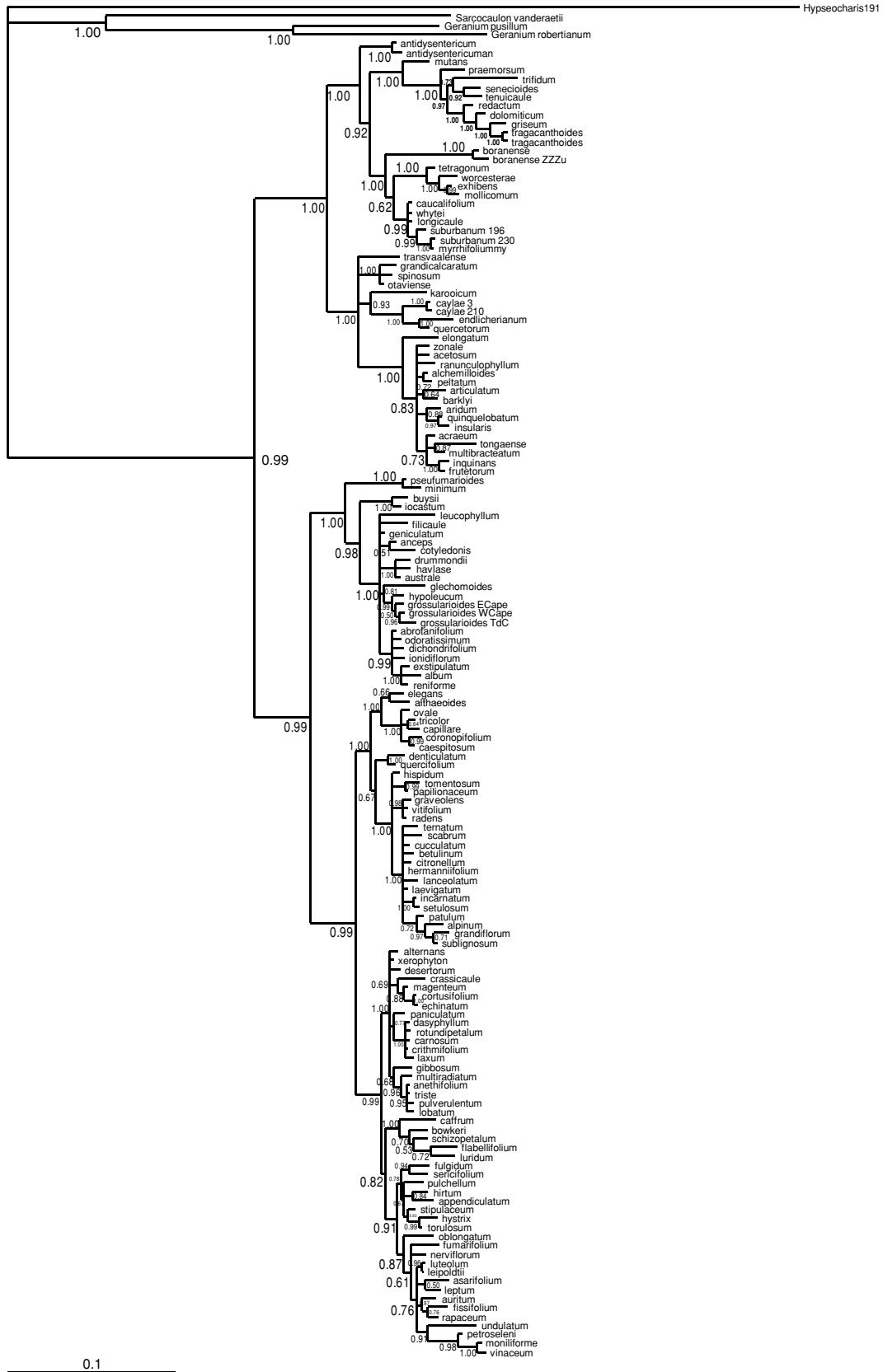
In general, character-state reconstructions based on Mesquite’s parsimony-based “Trace over trees” option were similar to reconstructions based on SIMMAP’s Bayesian approach: character states with high posterior probabilities in the Bayesian analysis were usually reconstructed as the uniquely best state in most or all trees. For illustration, we show results from both parsimony and stochastic analyses in Figures 4–6. Pie diagrams showing posterior probabilities from the stochastic analysis of ancestral state reconstructions (excluding *P. nanum*) were placed at major nodes on the 50% majority rule consensus tree from the original Bayesian analysis, but *P. nanum* has been pruned. This tree was used to optimize ancestral character states using parsimony to illustrate character states in terminal branches.

### ANCESTRAL STATE RECONSTRUCTIONS

*Leaf outline, apex and base*—Two leaf-shape features, overall outline and lamina apex, were reconstructed with high posterior probabilities for ancestral nodes of major clades. The overall outline of the lamina was reconstructed as ovate with posterior probabilities exceeding 0.99 at all nodes of major clades, and the lamina apex was reconstructed as rounded with probabilities exceeding 0.95 (reconstructions not shown; see Supporting Table S1 for posterior probabilities for each character state at each major node). Likewise, all trees were reconstructed with these same states as uniquely best for each node of interest using parsimony. The single exception was the leaf apex for Clade A1, which was reconstructed as rounded with a posterior probability of only 0.30; the alternative reconstruction was for an acute leaf apex (posterior probability of 0.69). Using parsimony, the leaf apex for Clade A1 was reconstructed as rounded in 39 trees, as acute in 19 trees, and as equivocal in 43 trees.

In the Bayesian analyses, the estimated numbers of transformations for leaf apex and outline were relatively low (Table 2). Transformations from ovate to other states were most common (Fig. 3A), which was to be expected because across all simulations, the character leaf outline was found in the state ovate 62% of the time. For leaf apex, transformations from rounded to acute or obtuse were most common, again because the total time spent in the state “rounded” exceeded 50% (Fig. 3B).

The leaf base shape was reconstructed as cordate with probabilities greater than 0.95 for major nodes of all clades except in the xerophytic Clade A2 (posterior probability of 0.89) and its sub-Clade A2a (reconstructions not shown); the same pattern occurred in the parsimony analysis. In Clade A2a, comprising the stem succulent sections *Otidia* and *Cortusina* and the geophytes in section *Polyactium*, the leaf base was reconstructed as cuneate/decurent with posterior probabilities exceeding



**Figure 2.** Phylogram of 50%-majority rule consensus of Bayesian inference analysis with *P. nanum* omitted (see text for details). Numbers indicate posterior probabilities for clades.

**Table 2.** Summary statistics for simulated character histories. Number of replications in the simulations was based on 100 realizations sampled from priors for 10 realizations of each tree for 101 trees. Rate is the posterior expectation of the overall gamma rate multiplier of branch lengths; transformations are the expected number of state changes for all states. Character-states transitions are assumed unordered.

Character	No. char. states	Replications	Rate	Expected no. of transformations
Leaf apex	7	101,000	31.84	61.57
Outline	5	101,000	41.39	64.12
Leaf base	7	101,000	49.80	86.55
Venation	2	101,000	88.67	93.66
Margin	5	101,000	109.55	136.80
Total leaf size	5	101,000	110.92	134.27
Lobing	5	101,000	203.05	223.68
Funct. leaf size	4	101,000	455.03	461.56

0.98, indicating an evolutionary shift away from cordate leaf bases. The number of transformations across the tree was slightly higher than for leaf apex and outline, but still low compared to other characters (Table 2). Because cordate leaf bases were most common, transformations from cordate to other states prevailed (Fig. 3C). Among these transformations, changes to cuneate or to lobed were more common than changes to other states.

#### Leaf venation

Leaf venation varied more extensively among terminal taxa, both within and among lineages. As expected, there was less certainty in reconstructions of the more basal nodes. In the large chromosome Clade C, leaf venation was reconstructed as palmate with relatively high posterior probabilities (0.72); the posterior probabilities for palmate venation were higher in both subclades C1 and C2 (Fig. 4). In contrast, reconstructions were uncertain in both the Bayesian and parsimony analyses for the nodes supporting the Small Chromosome Clade and Clade B. In Clade A, the winter rainfall clade, venation was reconstructed as pinnate with more certainty: posterior probabilities increased from 0.78 for Clade A and Clade A1 to greater than 0.92 in Clade A2 and its subclades (Fig. 4). The numbers of transformations were intermediate with respect to other leaf traits (Table 2) and transformations from palmate to pinnate and vice versa occurred with roughly equal frequency (Fig. 3D).

#### Leaf margins

Reconstructions of leaf margin type varied across the major clades. Reconstructions of crenate margins had slightly higher posterior probabilities in Clade C, with very high probabilities

in Clade C1 (0.94; Fig. 5). Interestingly, for Clade C2, posterior probabilities were higher for dentate/serrate margins, suggesting that a change in margin type from rounder teeth to sharper teeth accompanied subclade divergence (Fig. 5). Parsimony optimization mapped on the majority rule consensus tree reconstructed the branch subtending Clade C2 as crenate (Fig. 5), but parsimonious reconstructions using “Trace over trees” returned 76 trees with equivocal reconstructions for this node.

Margin reconstructions for the Small Chromosome Clade and Clade B were largely equivocal. At the base of Clade A, margin reconstructions were also uncertain, favoring dentate/serrate over other states (Fig. 5). The ancestral state reconstruction in Clade A1 was dentate/serrate (posterior probability 0.96) whereas Clade A2, the xerophytic clade, showed the highest probabilities associated with entire leaf margins (0.76). Interestingly, reconstructions for Clade A2a were uncertain among three categories, but did not include high probabilities for entire margins. Clade A2b, in contrast, had posterior probabilities for ancestral reconstructions of entire margins greater than 0.99 (Fig. 5).

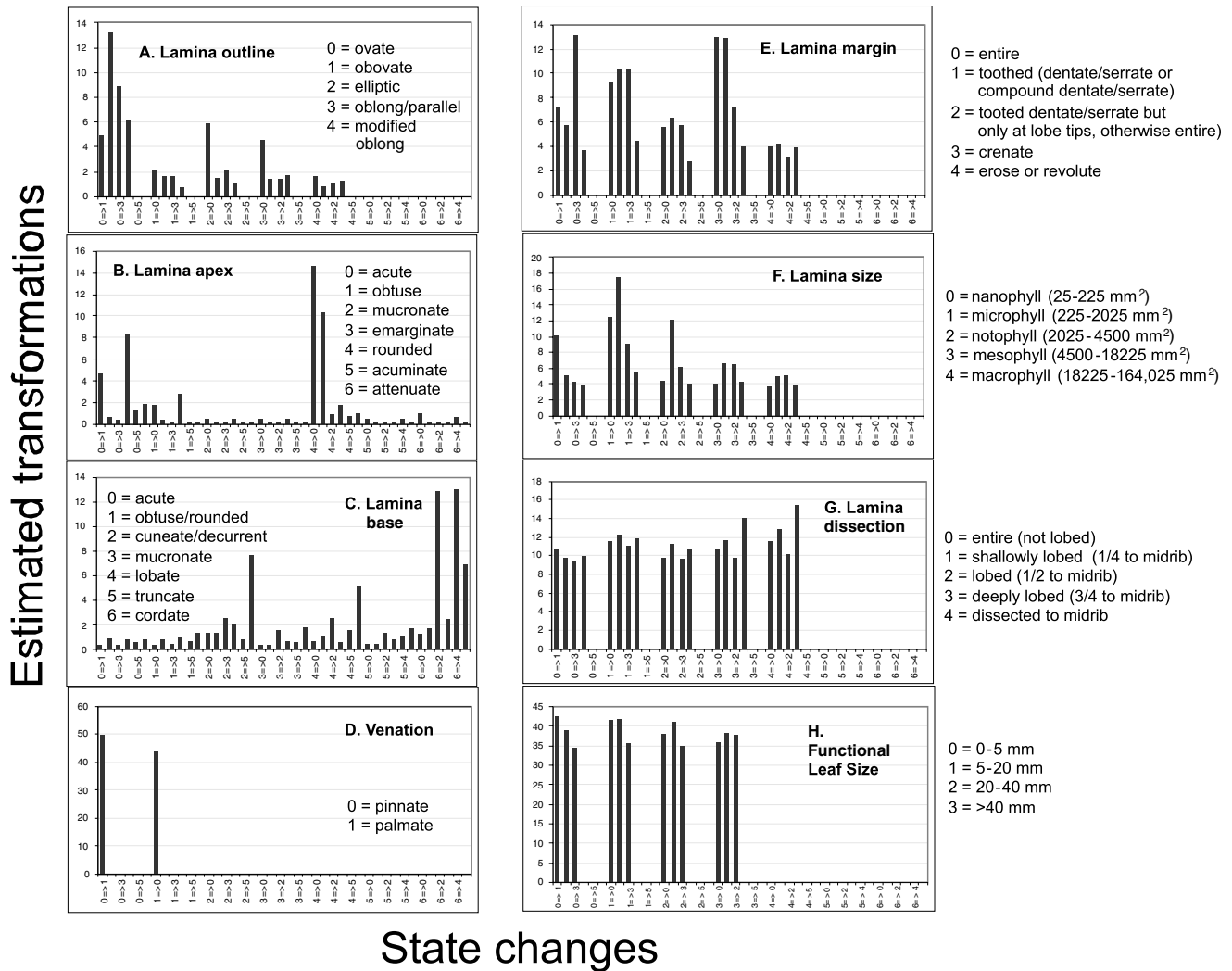
Labiality of the margin type was intermediate as indicated by the transformation rate (Table 2). Transformations from entire to crenate and the reverse, as well as from crenate to toothed, were more common than other types (Fig. 3E).

#### Total lamina size

Lamina size categories based on the Manual of Leaf Architecture were admittedly quite broad. Nevertheless, this method of size class determination allowed repeatable assignment of discrete states that could be used to examine broad-scale patterns. Reconstructions of microphyll (225–2025mm<sup>2</sup>) characterized the majority of clades with posterior probabilities generally greater than 0.75 (reconstruction not shown). Exceptions were the node for the Small Chromosome Clade (Clades A + B), where posterior probabilities for reconstructions of microphyll were uncertain, and Clades A2 and A2a. Clade A2a showed increased support for an ancestral state of nanophyll and in general exhibited a larger diversity of leaf sizes. The parsimony analysis “Trace over trees” gave roughly the same result. Microphyll was reconstructed for the ancestral state for all major nodes over most trees, except for Clade A2a in which 63 trees were reconstructed as equivocal when character states were assumed unordered. If character states were assumed ordered, most nodes were reconstructed as microphyllous in all trees; in Clade A2a, 88 trees were reconstructed as microphyllous, 13 as equivocal.

The number of estimated state changes in total leaf size across the tree was intermediate with respect to other traits, similar to the number of transformations in leaf margin type (Table 2). Because microphyll was the most common state, the highest number of transformations occurred from microphyll to the next smallest leaf size category (nanophyll), and to the next largest category





**Figure 3.** Estimated numbers of state transformations for each character based on simulations of character histories using SIMMAP (see Methods). States for each character are listed.

(notophyll), but transformations in the opposite directions were also common (Fig. 3F).

#### *Degree of dissection between major lobes in the lamina*

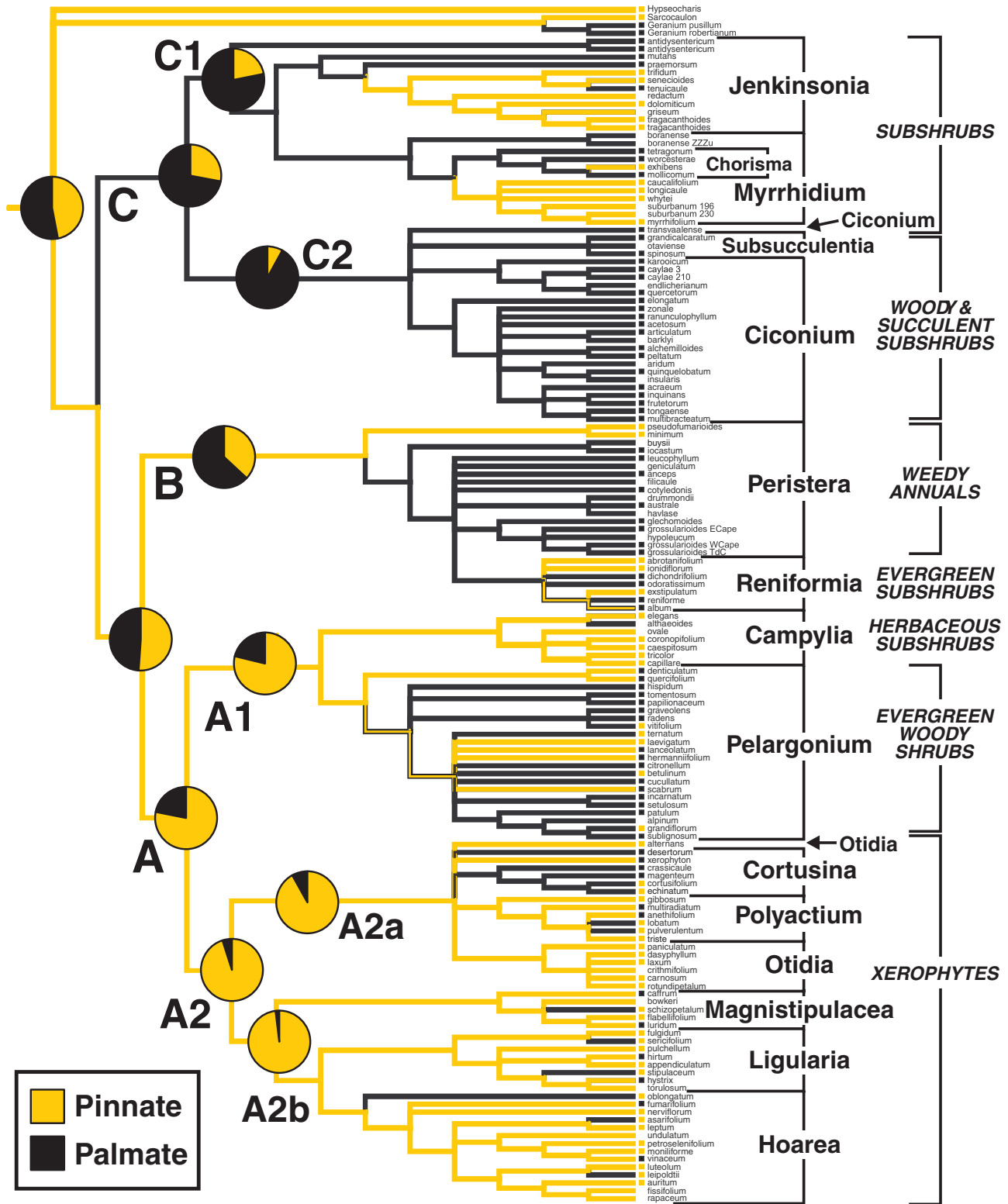
The degree of dissection of the lamina was highly variable across the genus, thus no single ancestral state was reconstructed with high posterior probabilities for any clade except Clade A2 (Fig. 6). Setting the bias toward highly dissected or less dissected for the 29 taxa originally scored as polymorphic did not influence the uncertainty in most clades.

The exception occurred in the xerophytic Clade A2 and its subclades. When the dataset was biased toward lobing, Clade A2 and its subclades were reconstructed with an ancestral state of dissected to midrib (posterior probabilities greater than 0.95; Fig. 6). The parsimony analysis “Trace over Trees” produced a similar result if characters were assumed unordered: Clade A2 and subclades were reconstructed with dissected to midrib as the

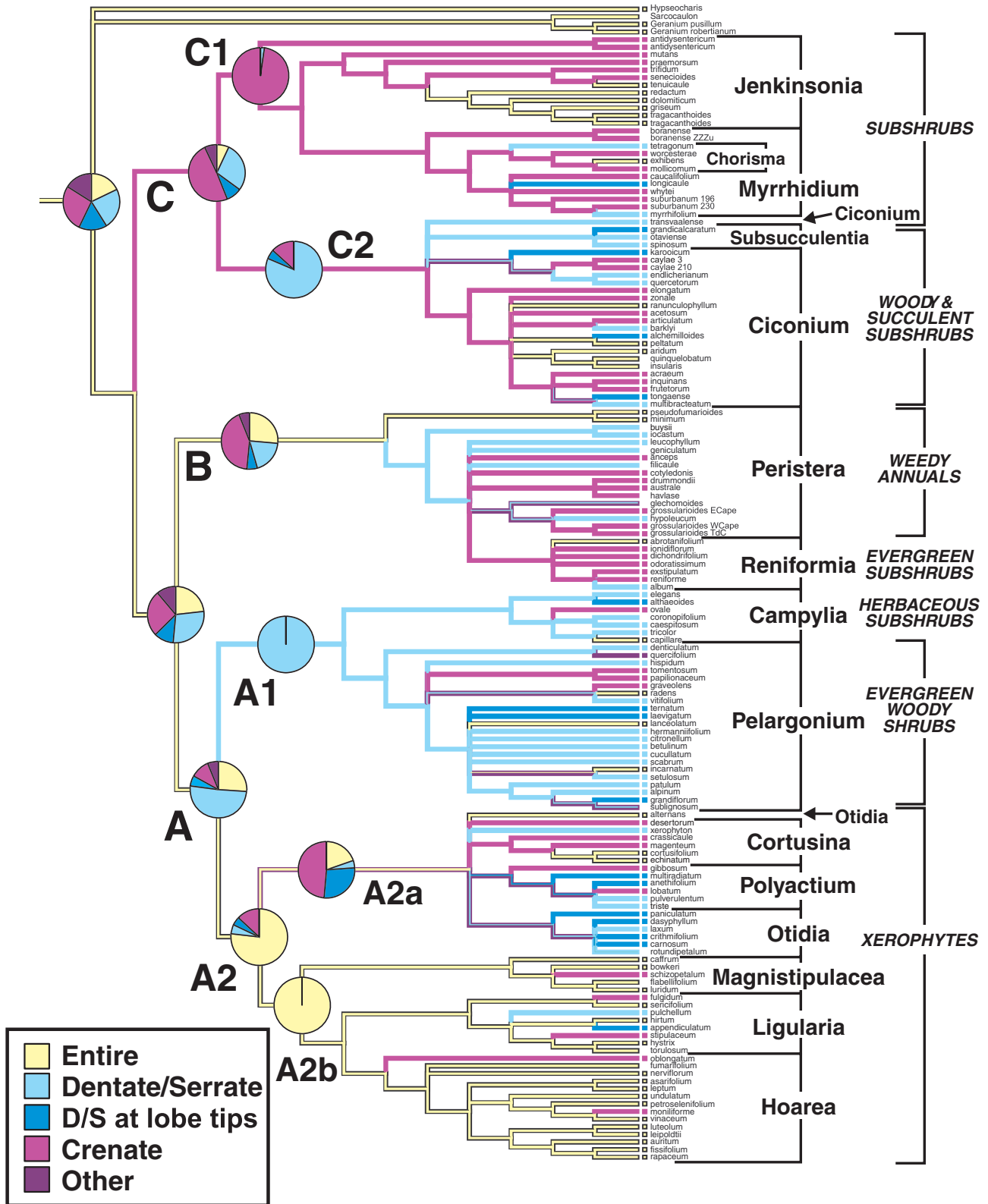
ancestral state in 98 trees. If characters were assumed ordered, this number dropped to 80, with most additional trees reconstructed as equivocal at that node.

When the dataset was biased toward less lobing with unordered character states, posterior probabilities for Clade A2 changed to 0.46 for a reconstruction of dissected to midrib and to 0.30 for reconstructions of highly lobed, indicating that the ancestral leaf state at this node was highly lobed or dissected to midrib (reconstructions not shown). However, parsimony revealed a different result. If states were assumed unordered, only 54 of 101 were reconstructed with ancestral states as dissected to midrib for Clade A2. In 17 trees, undissected leaves were reconstructed as ancestral, and 30 were equivocal. If states were assumed ordered for this dataset, this node was reconstructed as equivocal in the majority of trees.

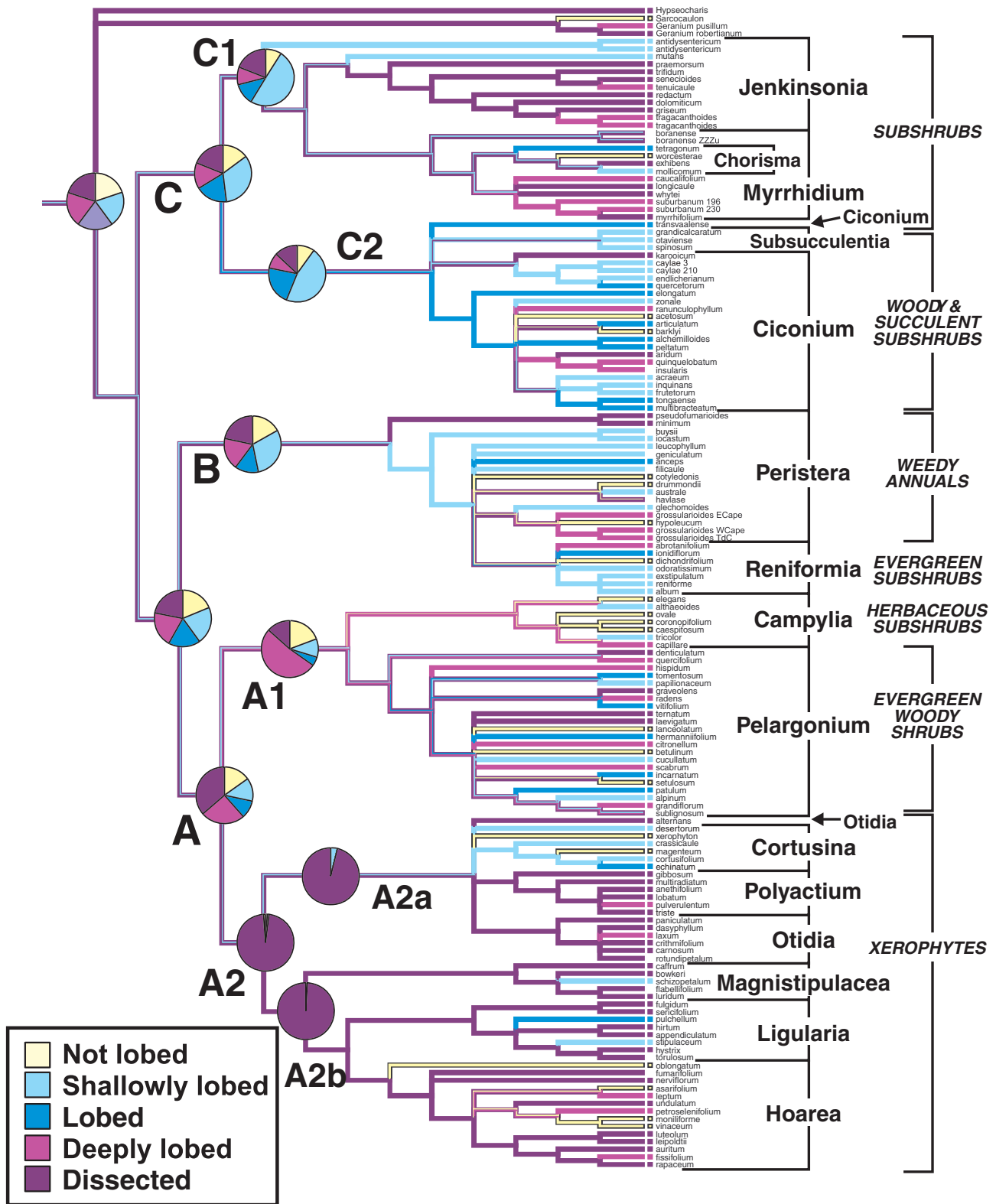
Trait lability as indicated by the number of transformations across the tree was high for the dataset biased toward lobing



**Figure 4.** Two methods of reconstruction of venation type shown on 50% majority rule consensus tree (see text for details). Branch color indicates parsimony optimization of ancestral character states assuming character-state evolution is unordered. Pie charts at major nodes show posterior probabilities from the stochastic analysis of ancestral state reconstructions.



**Figure 5.** Reconstruction of margin type shown on 50% majority rule consensus tree. Branch color indicates parsimony optimization of ancestral character states assuming character-state evolution is unordered. Pie charts at major nodes show posterior probabilities from the stochastic analysis of ancestral state reconstructions.



**Figure 6.** Reconstruction of degree of dissection shown on 50% majority rule consensus tree. Branch color indicates parsimony optimization of ancestral character states based on an assumption of unordered character states. Pie charts at major nodes show posterior probabilities from the stochastic analysis of ancestral state reconstructions.

**Table 3.** Character association statistics for specific state associations,  $d_{ij}$ . State codings are listed in Figure 3. Bold face indicates associations between specific states that occurred more frequently (positive) or less frequently (negative) than expected if the characters are evolving independently.

Venation		0	1			
Lobing	0	-0.004	0.004			
	1	<b>-0.063</b>	<b>0.063</b>			
	2	-0.028	0.028			
	3	0.002	-0.002			
	4	<b>0.094</b>	<b>-0.094</b>			
Funct. leaf size	0	<b>0.074</b>	<b>-0.074</b>			
	1	0.015	-0.015			
	2	<b>-0.055</b>	<b>0.055</b>			
3	-0.033	0.033				
Functional leaf size		0	1	2	3	
Lobing	0	<b>-0.024</b>	0.008	0.014	0.002	
	1	<b>-0.041</b>	-0.006	0.021	0.025	
	2	-0.023	0.013	0.007	0.003	
	3	0.001	-0.001	0.009	-0.009	
	4	<b>0.086</b>	-0.014	<b>-0.052</b>	-0.021	
Margin		0	1	2	3	4
Venation	0	<b>0.067</b>	-0.023	0.018	<b>-0.064</b>	0.002
	1	<b>-0.067</b>	0.023	-0.018	<b>0.064</b>	-0.002
Funct. leaf size	0	<b>0.067</b>	-0.038	0.022	<b>-0.049</b>	-0.002
	1	-0.027	0.008	-0.002	0.018	0.003
	2	-0.025	0.030	-0.014	0.011	-0.002
	3	-0.015	0.000	-0.006	0.021	0.000
Lobing	0	-0.014	0.021	-0.006	-0.001	0.000
	1	<b>-0.047</b>	0.005	-0.012	<b>0.055</b>	-0.001
	2	-0.015	0.019	-0.001	-0.003	-0.000
	3	0.006	-0.002	-0.008	-0.001	0.004
4	<b>0.070</b>	<b>-0.044</b>	0.0278	<b>-0.051</b>	-0.003	

(Table 2), with transitions from three-fourth lobed to dissected-to-midrib and the reverse favored (Fig. 3G).

#### Functional leaf size

Categories assigned to functional leaf size were relatively broad; a species with leaves in multiple size categories was assigned to the largest diameter category. Functional leaf size was by far the most labile character, with relatively uniform numbers of transitions among states. None of the clades at major nodes had ancestral state reconstructions with high posterior probabilities for any one state (reconstructions not shown). As expected, the number of transformations among states was high (Fig. 3H).

#### Correlated evolution among characters

For tests of association between any two characters, SIMMAP calculates a  $D$  statistic that measures the difference between expected associations if characters are evolving independently and the ob-

served associations.  $D$  values reflect overall character association across all possible state combinations. For tests of character associations below,  $D$  values ranged between 0.346 and 0.42. For all tests,  $P = 0.000$ , indicating that in 505 null simulations for each association, SIMMAP did not generate any mappings (assuming independence) with  $D$  values greater than or equal to a  $D$  value obtained from simulated mappings forced to conform to observed character states. Therefore, null hypotheses of independent evolution between any two characters were rejected for all overall tests of character association. The lack of independence in character evolution among characters was driven by specific associations among just a few character states.

#### Venation and lamina dissection

Pinnate venation was positively associated with lamina dissected to midrib whereas palmate venation was not likely to be found in these leaves (Table 3). Conversely, leaves that were slightly lobed are positively associated with palmate venation.

### Venation and functional leaf size

Laminae in the smallest functional leaf size category (0–5 mm) were more likely to have pinnate venation; large laminae (> 20 mm) were found on leaves with palmate venation (Table 3).

### Functional leaf size and lamina dissection

Significant associations were observed between a few specific states: the smallest functional leaves were significantly positively associated with laminae that were dissected to midrib, and negatively associated with leaves that were entire or only slightly lobed (Table 3). The third largest functional leaf size class, 20–40 mm, was negatively associated with leaves that were dissected to the midrib.

### Margin type

Entire margins were significantly associated with pinnate venation, the smallest functional leaf sizes, and with leaves dissected to midrib. Crenate margins tended to be found on palmately veined, shallowly lobed leaves.

## Discussion

Across *Pelargonium*, different leaf-shape traits varied dramatically in their degree of evolutionary lability. To the extent that selection is acting, it is not “seeing” the entire leaf as a module, but instead is filtering individual shape-determining components at different rates. Alternatively, if all changes are drift based, the evidence indicates limited integration of leaf-shape traits. What are the reasons for such profound differences in rates of evolution among shape-determining elements of a single organ? Unfortunately, phylogenetic methods that detect patterns of correlated change cannot provide much insight into the underlying causes of such patterns (i.e., genetic architecture or selection). Nor can we assess the relative roles of lack of variation versus internal selection on developmental cohesion (Schwenk and Wagner 2004) in the evolution of leaf shape in *Pelargonium* until we understand the genetic architecture of shape-determining elements in this genus. We can, however, consider the potential selective advantage of leaf-shape traits in functional terms, based on theoretical expectations; most of these ideas have not been field-tested. Below we emphasize trait evolution, discussing clade-specific patterns only where changes in states appear closely associated with major clades.

### LEAF TRAITS WITH RELATIVELY LOW LEVELS OF EVOLUTIONARY LABILITY

For *Pelargonium*, the least labile traits describe the overall form of the leaf, that is the apex, base, and outline shape of the lamina (disregarding the extent of lobing). Ancestral states were reconstructed as rounded for leaf apices and ovate for leaf outlines for

nodes of all major clades and subclades considered in this study. Limited variation among terminal taxa, as well as the low number of reconstructed transitions, suggests that these characters are more constrained than others. Givnish (1979) proposed that an ovate leaf outline, with its widest point below the midpoint of the lamina, could be a relatively efficient shape with respect to the photosynthetic surface area supported by the midrib if leaves are arranged in spiral phyllotactic patterns, as they are in most species of *Pelargonium*. For leaf apices, the functional consequences for particular shapes have received less attention, with the exception of those apices modified into “drip trips” that function to conduct water from the leaf in regions with high annual rainfall (Ivey and DeSilva 2001); *Pelargonium*, however, largely has rounded leaf apices.

The shape of the leaf base was slightly less conserved. Cordate bases, that is those with an inverted heart shape, were reconstructed as the ancestral state for all clades except Clade A2a. Cordate leaf bases may lead to increased self-shading (Givnish 1984), but under high levels of radiation and temperature that often coincide with lower rainfall, the inefficiency of self-shading may outweigh the reduced risk of overheating. Wyatt and Antonovics (1981) found that leaf bases shifted from obovate in the east to cordate in the western regions of the range of *Asclepias tuberosa*; they attributed this shift to declining rainfall. In *Pelargonium*, the opposite situation seems to apply: Clade A2a, a xerophytic clade of stem succulents and geophytes, is the only clade in which cordate leaf bases do not prevail.

An unexpected finding of this study was the reconstruction of different venation types in two major clades. Whether the characteristic venation patterns, palmate in Clade C and pinnate in Clade A, diverged in response to a drying period that occurred near the deep divergence of the lineage 30 Mya (Bakker et al. 2005) is uncertain. High posterior probabilities at the major clade level occurred despite evolutionary transitions in venation in nested subclades. For example, in Clade C1, the smaller clades comprising section *Myrrhidium* and some members of section *Jenkinsonia* show independent shifts to pinnate venation (Fig. 4). Interestingly, both of these C1 subclades include species that tend to be distributed in the arid winter rainfall region whereas those in the palmately veined Clade C2 are more widely distributed, predominantly in the summer rainfall region.

Following its divergence from Clade C, The Small Chromosome Clade (A + B) continued to diversify from the mid-Miocene. Although tropical climates prevailed in southern Africa during this period, ancestral lineages of the modern Cape Flora, including the Small Chromosome Clade (A + B) of *Pelargonium*, may have been harbored in mountainous regions (Linder 2003; Linder and Hardy 2004). Clade B includes section *Peristera*, a paraphyletic grade of widespread, herbaceous annuals and section *Reniformia*, a small clade of perennial subshrubs that has

secondarily evolved woody stems, in addition to one stem succulent native to the island of St. Helena (*P. cotyledonis*) (Bakker et al. 1998). Venation reconstructions in Clade B were less certain than either Clades A or C, with posterior probabilities of 0.63 for a reconstruction of palmate venation. This lower certainty for venation arises from the presence of a small sister clade containing the pinnate species, *P. minimum* and *P. pseudofumaroides*; most of the remaining members of Clade B have palmate venation.

Clade A, the highly divergent “winter rainfall clade,” had high posterior probabilities for ancestral pinnate venation. Although the climate at the time of divergence of Clades A and B may have been tropical, much of the subsequent diversification of this clade, comprising 80% of *Pelargonium* species, occurred during periods of increasing aridification. This drying trend is attributed to the northward shift of the cold Benguela Current (Bakker et al. 2005), thought to have appeared about 18–20 Mya and become firmly established 12–15 Mya (Cowling and Richardson 1995; Linder and Hardy 2004). The reconstruction of pinnate venation as ancestral for Clade A suggests that pinnate venation was present prior to the evolution of variation in growth form that subsequently occurred during the radiation of the “xerophytic” Clade A2.

The general association of venation type with regions of divergent rainfall patterns suggests that venation type may be associated with different functional roles. Recent studies have shown that major veins have greater flow rates and less axial conductance than minor veins (Zwieniecki et al. 2002), such that they function to distribute water over longer distances within the leaf; their arrangement determines how equitably this occurs (Roth-Nebelsick et al. 2001; Sack and Holbrook 2006). Nevertheless, because most of the hydraulic resistance within the leaf occurs in the minor veins and mesophyll (Sack et al. 2004), total hydraulic resistance of the leaf is independent of major vein density (i.e., first and second order veins) (Sack and Frole 2006), and thus presumably also independent of major vein pattern. However, there are likely to be advantages, hydraulic and otherwise, of one venation pattern over another, at least under certain conditions. Recent studies by Sack et al. (Sack et al. 2008) have suggested that leaves with palmate venation are better able to tolerate disruption of the leaf water supply due to mechanical vein breakage (e.g., herbivory), because the higher numbers of first-order veins, the defining feature of palmate venation, provide redundancy. Significant functional effects of differences in vein patterns also may turn out to be related to changes in other leaf traits that are correlated with venation pattern, for example minor vein density and investment in support structure (Niinemets et al. 2007).

Our analyses suggest that leaf shape, as expressed in the extent of lamina dissection, is evolutionarily linked to venation type

in *Pelargonium*. We observed significant positive associations between pinnate venation and dissection of the leaf lamina to the midrib and between pinnate venation and the smallest functional leaf size category, and conversely, a positive association between palmate venation and slightly lobed leaves. Furthermore, pinnate venation and highly dissected leaves in *Pelargonium* are associated with occupation of increasingly arid environments.

#### LEAF TRAITS WITH INTERMEDIATE LEVELS OF EVOLUTIONARY LABILITY: LEAF MARGIN AND LEAF SIZE

Most clades of *Pelargonium* are characterized by toothed margins, either rounded (i.e., crenate) or pointed (dentate/serrate). Posterior probabilities for reconstructions of specific margin types are highest at the subclade level. Both subclades in Clade C are reconstructed with ancestrally toothed margins, but in Clade C1 these teeth are crenate and in Clade C2 they tend to be dentate or serrate. Likewise, the subclades of Clade A are characterized by different margins: dentate/serrate in the evergreen shrubs and subshrubs of Clade A1, entire in Clade A2b but mixed in Clade A2a.

The presence of leaf teeth in a flora has long been associated with differences in habitat, particularly mean annual temperature (Bailey and Sinnott 1916). This association occurs to the extent that the size and shape of marginal teeth are used as physiognomic indicators of paleoclimates (Wolfe 1978; Wilf 1997). In extant, woody mesic floras, greater average number of teeth and tooth area per site are correlated with colder mean annual temperatures (Royer et al. 2005). Whether this is the explanation for toothed margins in *Pelargonium* remains to be determined. However, the association of entire margins in one of the more xerophytic lineages is consistent with previous observations. The 8–12 Mya estimates established by Bakker et al. (2005) for radiation of the xerophytic Clade A2 are consistent with the origin of this clade near the time of establishment of the winter rainfall patterns in southern Africa (Linder and Hardy 2004). This time frame also corresponds to the period of origin of a few other well-known southern African clades (*Ehrharta*, 9.8–8.7 Mya; *Phyllica* 7–8 Mya, Ruschioideae, 3.8–8.7 Mya) (Richardson et al. 2001; Linder 2003; Verboom et al. 2003; Klak et al. 2004; Linder and Hardy 2004). Diversification within Clade A2 during this period resulted in Clade A2a, three currently recognized sections of stem succulents and geophytes characterized by a mix of leaf margin types, and Clade A2b, reconstructed with ancestral entire margins for its geophytes (sections *Hoarea* and *Magnistipulacea*) and subshrubs (section *Ligularia*).

Intermediate levels of lability were also seen in total leaf (lamina) size. Leaf size categories were broad, which may explain why posterior probabilities for total lamina size class were not especially high. Both parsimony and the Bayesian analysis

reconstructed most major clades with ancestral states of microphyll. When the leaf size was assumed to evolve in an unordered sequence, Clade A2a shows a majority of nodes reconstructed as equivocal, reflecting the greater range of leaf sizes and diversity of growth forms. However, if this trait is treated as ordered, then this node is reconstructed as microphyllous in the majority of trees.

#### **LEAF TRAITS WITH HIGH LEVELS OF EVOLUTIONARY LABILITY: DEGREE OF DISSECTION AND FUNCTIONAL LEAF SIZE**

At the outset we observed that different species within sections differed dramatically in lobing. Thus it followed that the two traits most closely related to the total extent of lobing, the degree of dissection between major lobes and functional leaf size, were highly labile and homoplasious across the genus. We did not expect a priori to find associations between these two traits because small, unlobed leaves can have small functional leaf sizes. However, in *Pelargonium*, very small functional leaf sizes were correlated with the greatest degree of dissection, that is dissected to the midrib; conversely, leaves with relatively large functional leaf sizes, 20–40 mm, were correlated with slightly lobed leaves.

Character-state reconstructions were generally equivocal for both the degree of lamina dissection and functional leaf size for most clades. However, despite the uncertain ancestral reconstruction of dissection for Clade C1, two more internal clades, section *Jenkinsonia* and a clade consisting of most members of section *Myrrhidium*, show shifts to highly dissected laminas. Interestingly, these are the same subclades characterized by pinnate venation within the otherwise palmately veined Clade C (compare Figs. 4 and 6). Functional leaf size was highly variable within both.

In stark contrast to other clades, the ancestral states of the xerophytic Clade A2 and its subclades were reconstructed with high posterior probabilities for dissected to midrib (dataset biased toward lobing) or a combination of highly dissected and dissected to midrib (dataset biased away from lobing). The evolution of more dissected leaves in the xerophytic clade is consistent with community-level studies showing associations of smaller functional leaf sizes with dry, sunny, or nutrient poor habitats, and increasing elevation (see references cited in Givnish 1984; Cunningham et al. 1999; Fonseca et al. 2000; McDonald et al. 2003; Thuiller et al. 2004) and compound leaves in areas with high summer temperatures and low moisture availability (Stowe and Brown 1981). Small leaves are thought to be associated with arid environments because smaller leaf dimensions reduce boundary layer thickness and thus help leaves avoid excessive leaf temperatures when transpiration is restricted (Parkhurst and Loucks 1972). In general, smaller leaves track ambient temperatures more closely

than large leaves, but recent work by Leigh et al. (Leigh et al. 2006; Leigh 2008) has shown that the thermal profiles of specific regions of the leaf are not determined solely by distance to an edge, as predicted by boundary layer effects, but are also strongly influenced by distance from the midvein and petiole, suggesting that the water content of specific regions of the leaf dramatically affects local temperature. Clearly, leaf shape and venation pattern will influence the distance of a leaf region to a major vein as much as they influence distances to an edge.

Whether an additional role exists for leaf dissection per se in photosynthesis and water-use efficiency beyond general effects on functional leaf size is not yet established (Niinemets and Sack 2006). To investigate this question directly in *Pelargonium*, Nicotra et al. (2008) used paired species from several sections representing phylogenetically independent contrasts for leaf dissection. Increasing dissection was correlated with greater rates of photosynthesis and water loss. Shape-dependent differences in photosynthesis were most pronounced under cool, moist growing conditions, that is conditions under which most winter-growing *Pelargonium* species are actively photosynthesizing in the field. Higher rates of photosynthesis in more dissected leaves have been found in a limited number of intraspecific studies comparing ecotypes (Gurevitch 1988, 1992), morphotypes (Lynn and Waldren 2002), and cultivars (Stiller et al. 2004).

#### **CONCLUDING REMARKS**

Previous studies considering leaf-shape evolution have tended to treat leaf shape as one trait, for example lobed or not lobed, wide versus narrow. In such studies, shifts in shape from the ancestral condition are generally interpreted as evolutionary responses of functional leaf size to changes in the abiotic environment (e.g., Ezcurra et al. 1997; Hufford and McMahon 2004; Mummehoff et al. 2005). Our results show *Pelargonium* exhibits different levels of evolutionary lability and independence in different elements of leaf shape, the two most variable being the degree of dissection (lobing) and functional leaf size. Only in the xerophytic clade does the degree of lobing carry strong phylogenetic signal, but even among the xerophytes the extent of lobing varies.

Did leaf dissection evolve in response to external selection by abiotic factors? The general occurrence of more dissected leaves in more xerophytic clades coupled with observations of higher photosynthetic rates in more dissected species within clades might suggest so. This hypothesis, however, does not address why leaf dissection varies markedly within clades that share the same growth form. Perhaps within-clade lability has arisen via strong selection for functional leaf size in response to specific climatic or microclimatic conditions that drove local niche differentiation. Also to be explained, however, is the observation that species with leaves of different shapes can be found growing within a meter



of each other in the field. We cannot rule out the possibility that at least some of the variation in leaf dissection within clades is nonadaptive, especially relative to the adaptive advantages provided by different growth form, for example stem succulence, geophytism, or woodiness. Differences in growth form are much less labile than those in leaf dissection (Jones and Price 1996; Bakker et al. 1999b).

Significant associations between pinnate venation, highly dissected laminas, the very smallest functional leaf sizes, and entire margins suggest that these traits have coevolved in *Pelargonium*, giving rise to a generalized xerophytic leaf-form characteristic of the most speciose clade. When particular suites of traits co-occur only among terminal taxa of speciose clades, “key innovation” is frequently invoked as a possible hypothesis. Our historical analysis shows that in *Pelargonium*, shape-determining elements in the xerophytic lineages were acquired gradually, distributed across a series of nested nodes in the tree, indicating “culmination in a combination of traits that serve to increase diversification” (Donoghue 2005). Although leaves function as single, complex modules, current diversity in *Pelargonium* reflects long-term evolutionary integration of independent and differentially labile shape-determining elements.

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## Supporting Information

The following supporting information is available for this article:

**Table S1.** Posterior probabilities of character-state reconstructions for ancestral nodes based on Bayesian analysis of “most lobed” dataset.

**Figure S1.** Illustrations of character states. A. Determination of overall outline (dashed) superimposed on leaf of *P. echinatum*. B. Categories of lobing. Note that degree of dissection is independent of venation type. C. Venation patterns considered pinnate and palmate.

**Figure S2.** Reconstruction of total leaf size categories shown on 50% majority rule consensus tree. Branch color indicates parsimony optimization of ancestral character states. Pie diagrams at major nodes show posterior probabilities from the stochastic analysis of ancestral state reconstructions (excluding *P. nanum*).

Supporting Information may be found in the online version of this article.

(This link will take you to the article abstract).

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