

Convergence in the Leaf Shape of Vines: A Test of the Carolina Flora Using Phylogenetic Comparative Methods

CAROL GOODWILLIE^{1,*}, MELYNDA K. MAY¹, JENNIFER W. WEST¹,
AND CHRISTIAN S. MCKEON¹

Abstract - An association between vine habit and cordate leaf shape in higher plants has been reported, but previous comparative analyses have not taken into account phylogenetic history. We surveyed the flora of the Carolinas and used phylogenetic comparative methods to test the hypothesized relationship. We found 25 phylogenetically independent vine taxa in the Carolina flora and, for each, attempted to identify its hypothesized non-vine sister taxon based upon recent phylogenetic studies. Using conservative criteria for vine habit and leaf shape determinations, a sign test revealed a significant association between the two traits. The addition of taxa for which information was slightly more ambiguous increased the strength of the association. Our findings suggest that convergence in leaf shape of vine taxa may result from a selective advantage of cordate leaves in plants with a climbing habit. We discuss possible adaptive explanations for the observed association.

Introduction

Botanists have long recognized that leaf shape is often evolutionarily plastic, as compared to reproductive traits, which tend to be more highly conserved. Yet the evolution of leaf shape has received relatively little study. The remarkable variation in leaf shape within plant taxa and resemblance in leaf shape of unrelated, but ecologically similar, species appear to reflect a variety of selective pressures. Thus, the evolution of leaf shape presents an opportunity to study the process of adaptive diversification.

Comparative studies have uncovered a number of ecological trends in leaf shape. For example, large leaf size is associated with high rainfall and humidity, toothed or lobed leaves are found primarily in temperate regions, and elongated “drip tips” are common in wet rainforests (reviewed in Givnish 1987). Adaptive hypotheses to explain these trends have centered on a few key trade-offs. Much of the variation in leaf shape and size is thought to result from joint selective pressures to minimize water loss, optimize temperature, and maximize light interception in different environments (Givnish and Vermeij 1976, Parkhurst and Loucks 1972). For optimal light interception,

¹Department of Biology, East Carolina University, Howell Science Complex, Greenville, NC 27858. *Corresponding author - goodwilliec@mail.ecu.edu.

leaves may be extended in space and held at particular angles, which in most plants is accomplished by support structures such as branches or woody stems. Because these structures are expensive, leaf size and thickness are expected to be constrained by selective pressure to reduce support costs (Givnish 1986).

Our study concerns adaptation in the leaf shape of vines, and addresses an hypothesis set forth by Givnish and Vermeij (1976). Motivated by the observation of a high incidence of cordate (heart-shaped) leaves in sun-exposed vines, they hypothesized that this leaf shape may have particular adaptive value for climbing species. Givnish and Vermeij argued that cordate leaves offer a mechanical advantage that maximizes light interception without requiring woody support structures used by erect plants for this purpose. As evidence supporting this hypothesis, Givnish and Vermeij (1976) presented data on leaf shape for species sampled from four localities in Costa Rica. A higher proportion of cordate leaves was found among vine species than in species of other plant forms.

Since the time of Givnish and Vermeij's study, evolutionary biologists have recognized the need to consider phylogenetic relationships among taxa when carrying out comparative analyses (Felsenstein 1985). In comparative studies, causal or mechanistic explanations are suggested when two or more traits or factors are correlated across a range of taxa. However, traits shared by closely related taxa may reflect phylogenetic constraint rather than similar selective pressures; therefore, taxa that share traits due to common ancestry cannot be considered independent data points. Hence, failure to take phylogenetics into account may result in an over- or underestimation of trait correlations. Comparative methods have been developed that correct for phylogenetic relatedness (Diniz-Filho et al. 1998, Felsenstein 1985, Martins and Hansen 1997).

Here, we extend the work of Givnish and Vermeij to test the hypothesized relationship between vine habit and cordate leaf shape using phylogenetic information for comparative analysis. In addition, while the previous study focused on the tropical flora of Costa Rica, our data are drawn from the temperate flora of North and South Carolina. Although the use of the Carolina flora places somewhat arbitrary political boundaries on the species included in our study, it represents an independent sample on which to test the hypothesis of Givnish and Vermeij. For each vine taxon found in the Carolina flora, we identified the closest relative (sister taxon) that is not a vine and used each pair as a single data point for examination of the relationship between leaf shape and vine habit. The current study was initiated as a group exercise for a course in plant systematics and evolution at East Carolina University in the fall of 2001.

Materials and Methods

Data collection

All information on the flora of North and South Carolina was obtained from Radford et al. 1968 (hereafter referred to as Radford). We surveyed Radford for all angiosperm taxa with a climbing habit, that is, those described as “vines,” “climbing,” or “twining.” We elected not to include species described as “trailing” or “scrambling” since our hypothesis specifically concerned plant species that use another structure or plant for vertical support. In cases where all or nearly all species of a genus or family were climbing, we counted the entire genus or family as one vine taxon. In some cases, phylogenetic information revealed that local vine taxa were part of a larger vine clade, including several genera or families. In this case, we considered the entire clade as the vine taxon in our analysis.

The sister non-vine taxon was found for each vine taxon or clade represented in the Carolina flora. Determinations of phylogenetic relationship were made using the most recent source of data available, including molecular phylogenetic information in many cases.

We scored all vine taxa and their corresponding non-vine sister taxa for leaf shape. In making leaf shape determinations, we first scored all compound leaves as negative. For simple leaves, we attended primarily to the shape of the leaf base, specifically, the angle between the petiole and leaf base. Those taxa for which this angle was considerably less than 90 degrees (Fig. 1) were scored as positive and all other taxa were scored as negative for cordate leaf shape. For vine taxa and for sister

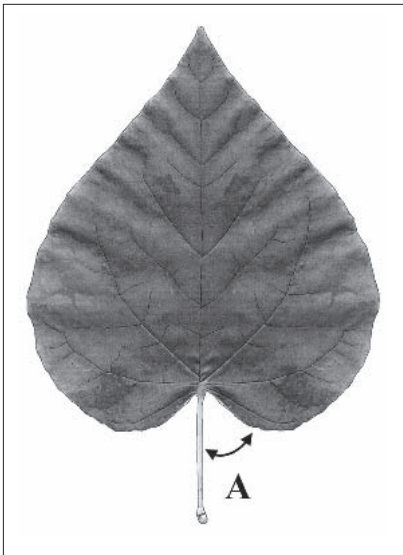


Figure 1. Cordate leaf shape. Note the acute angle that is formed by the petiole and leaf base (A).

non-vine taxa found in the local flora, determinations were based upon descriptions or drawings in Radford. Descriptive terms indicating a petiole to leaf base angle of less than 90 degrees included "cordate," "sagittate" and "hastate." Most of the sister taxa were not found or well-represented in the regional flora. For these, leaf shapes were assessed using descriptions and photographs from other sources, including photographic image sources on the internet such as Vascular Plant Image Gallery (<http://www.cSDL.tamu.edu/FLORA/gallery.htm>, Texas A&M University). Taxa above the species level were scored as positive or negative for cordate leaf shape when the large majority of species (> 80%) were in agreement. Higher taxa with a more even distribution of species with cordate vs. non-cordate leaves were scored as mixed.

Analysis

To test for an association between cordate leaf shape and vine habit, we carried out a sign test on the paired taxa (Zar 1974), a statistical approach that has been used in other phylogenetically corrected comparative analyses (Crawley et al. 1997). This statistic tests for a significant excess of pairs for which leaves of the vine taxon are cordate and those of its non-vine sister are not. Pairs for which leaf shape does not differ between vine and non-vine sisters do not enter into this type of analysis. We chose the sign test, one of the oldest statistical tests to be developed (Arbuthnott 1710), because it is conservative and conceptually simple.

Results

We identified 25 phylogenetically independent vine taxa in 25 families. Sister taxa were identified from recent phylogenetic analyses for most of these taxa (Table 1). Information on exact sister relationships was unavailable for a few taxa, but in most of these cases, leaf shape of the sister taxon could nevertheless be inferred. For instance, the sister to *Adlumia* in the Fumariaceae could not be determined because a recent phylogenetic treatment of the family has not been published. However, since the family is predominantly non-climbing, and leaf shape within the family is nearly always pinnately compound, it could be reasonably assumed that the non-vine sister genus or genera has non-cordate leaves. Because species level phylogenies were not available, similar assumptions were made about the sisters to *Solanum dulcamara* and the climbing section of *Polygonum*. For some taxa, the sister non-vine clade was found to include a number of genera or families (Table 1).

In a number of cases a taxon or clade was mixed for leaf shape; that is, it included a substantial proportion of both cordate and non-cordate leaves (e.g., the genus, *Smilax*). In a small number of taxa, leaf shape for an individual species was ambiguous in that leaves were variable or

Table 1. Vine taxa identified in Radford, and sister non-vine taxa, as hypothesized in the systematic reference listed. Parentheses in vine column enclose the clade (comprising one or several taxa) to which the local vine taxa or taxon belongs. For those taxa with no systematic references listed, information on exact sister relationships was not available (see text for explanation). For leaf shape, + denotes cordate leaves, - denotes non-cordate leaves, +/- denotes mixed or ambiguous leaf shape determination (see text).

Family	Vine taxon or clade	Vine leaf shape	Sister non-vine taxon or clade	Non-vine leaf shape	Systematic reference
Anacardiaceae	<i>Toxicodendron radicans</i> (<i>Toxicodendron</i>)	-	<i>Searsia</i> , <i>Schinus</i>	-	Miller et al. 2001
Apocynaceae	<i>Trachelospermum difforme</i> (<i>Periploca</i> , <i>Secamone</i> , <i>Trachelospermum</i> and genera)	-	<i>Pachypodium</i> , <i>Funtumia</i> and other genera	-	Sennblad et al. 1998
Araliaceae	<i>Hedera helix</i> (<i>Hedera</i>)	+/-	<i>Brassaiopsis</i> , <i>Trevesia</i>	-	Wen et al. 2001
Aristolochiaceae	<i>Aristolochia macrophylla</i>	+	<i>Asarum</i> , <i>Saruma</i>	+	Qiu et al. 2000
Asclepiadaceae	<i>Matalea</i> spp., <i>Cynanchum</i> spp. (<i>Matalea</i> , <i>Gonolobus</i> , <i>Araujia</i> , <i>Dregea</i> and other genera)	+/-	<i>Kopsia</i> , <i>Rauwolfia</i> , <i>Tabernaemontana</i> and other genera	-	Civeyrel et al. 1998
Asteraceae	<i>Mikania scandens</i>	+	<i>Ageratina</i>	-	Schmidt and Schilling 2000
Bignoniaceae	<i>Campsis radicans</i> (<i>Campsis</i> , <i>Pandorea</i>)	-	<i>Amphitecna</i> , <i>Tabebuia</i> , <i>Oroxylum</i> , <i>Crescentia</i> , and other genera unknown	-	Spangler and Olmstead 1999
Cannabaceae	<i>Anisostichus capreolata</i>	-	<i>Cannabis</i>	-	Song et al. 2001
Caprifoliaceae	<i>Lonitcera</i> spp.	+	<i>Lonitcera</i> spp.	-	Simmons et al. 2001
Celastraceae	<i>Celastrus</i> (<i>Celastrus</i>)	-	Included in unresolved clade comprising <i>Euonymus</i> , <i>Schaefferia</i> , <i>Acanthothamnu</i>	-	
Convolvulaceae	<i>Convolvulus arvensis</i> , <i>Jacquemontia tammifolia</i> , <i>Calystegia</i> spp., <i>Ipomoea</i> spp., <i>Bonamia</i> spp. (Convolvulaceae)	+	Solanaceae	-	Olmstead et al. 1993

Table 1, continued.

Family	Vine taxon or clade	Vine leaf shape	Sister non-vine taxon or clade	Non-vine leaf shape	Systematic reference
Cucurbitaceae	<i>Cucurbita pepo</i> , <i>Cucumis melo</i> , <i>Lagenaria vulgaris</i> , <i>Citrillus</i> <i>vulgaris</i> , <i>Cayaponia boykinii</i> , <i>Melothria pendula</i> , <i>Sicyos angulatus</i> , <i>Echinocystis lobata</i> (Cucurbitaceae)	+	Begoniaceae OR Coriaceae, Corynocarpaceae, Datisaceae and Begoniaceae	+	Swensen et al. 1994 Wagstaff and Dawson 1999
Dioscoreaceae	<i>Dioscorea</i> spp. (<i>Dioscorea</i> and <i>Trichopus</i>)	+	<i>Tacca</i>	-	Caddick et al. 2002
Fumariaceae	<i>Adlumia fungosa</i> (<i>Adlumia</i>)	-	genera within Fumariaceae	-	Backlund et al. 2000
Gelsemiaceae	<i>Gelsemium</i> spp.	-	<i>Mostuea</i>	-	Dahlgren and Bremer 1985
Lardizabalaceae	<i>Akebia quinata</i> (Lardizabalaceae and Sargentodoxaceae)	-	Ranunculaceae, Berberidaceae, Papaveraceae and others	-	Loconte and Estes 1989
Menispermaceae	<i>Menispermum canadensis</i> , <i>Cocculus carolinus</i> (Menispermaceae)	+	Berberidaceae and Ranunculaceae	+/-	Loconte and Estes 1989
Passifloraceae	<i>Passiflora</i> spp. (Passifloraceae)	-	Flacourtiaceae	-	Rodman 1991
Polygonaceae	<i>Polygonum</i> spp. (<i>Polygonum</i> section <i>Tiniaria</i>)	+	Other <i>Polygonum</i> sections	-	
Ranunculaceae	<i>Clematis</i> spp. (<i>Clematis</i>)	+/-	<i>Anemone</i> , <i>Pulsatilla</i>	+/-	Ro et al. 1997
Rhamnaceae	<i>Berberemia scanden</i>	-	<i>Berberemia</i> spp.	-	Richardson et al. 2000
Schisandraceae	<i>Schisandra glabra</i> (<i>Schisandraceae</i>)	-	Illiciaceae	-	Rodman 1984
Smilacaceae	<i>Smilax</i> spp. (Smilacaceae, Philesiaceae)	+/-	Liliaceae, Calochortaceae and others	-	Chase et al. 1995
Solanaceae	<i>Solanum dulcamara</i>	+/-	<i>Solanum</i> spp.	-	
Vitaceae	<i>Vitis</i> spp., <i>Ampelopsis</i> spp. (Vitaceae)	+	Leeaceae	-	Fishbein et al. 2001

only marginally cordate (e.g., *Solanum dulcamara*). To account for these uncertainties, the analysis was carried out first with all ambiguous or mixed cases omitted (a conservative analysis) and second, with ambiguous or mixed taxa counted as positive for cordate leaf shape. For the Cucurbitaceae, two recent studies conflicted in their determination of the sister non-vine taxon; this was considered an ambiguous data point as well.

The local flora included a number of climbing species in the family Fabaceae. We elected to omit the family from our analysis for a number of reasons. First, in most cases they qualified only marginally as vine taxa. For example, several species of *Vicia* were described as “trailing or climbing.” Second, the recent phylogenetic treatments of this large family do not include a number of the relevant genera, hence, information on sister taxa was unavailable in many cases. Third, all of the climbing or trailing species had compound leaves, as do most species within the family; it is unlikely that a vine/non-vine sister pair would enter into the sign test analysis. However, because a small minority of taxa in the Fabaceae do have simple leaves, a slight possibility exists that we may have omitted a vine taxon for which the non-vine sister had cordate leaves.

Of the 25 original pairs, we found 11 in which neither the vine nor its non-vine sister had cordate leaves, and two pairs in which both sister taxa had primarily cordate leaves. Using conservative criteria, we identified six sister pairs in which only the vine taxon had cordate leaves and we found no pairs in which the reverse was true. With these data, a sign test of the hypothesized cordate-vine association yields a P value of 0.0313. When the ambiguous taxa were included as cordate, the number of pairs showing the hypothesized association between climbing habit and leaf shape increased to 10 with no pairs exhibiting the reverse pattern, yielding a P value of 0.00195.

Discussion

Our sampling of the flora of the Carolinas yields evidence for an association between cordate leaf shape and a climbing habit. While this relationship has been noted anecdotally (Richards 1964) and demonstrated as a simple correlation in a tropical flora (Givnish and Vermeij 1976), our study is the first to confirm the result using methods that take into account phylogenetic relationships.

By reducing our sample of species to only phylogenetically independent vine taxa and their non-vine sisters, our study is likely to be more conservative than a simple comparative analysis. For instance, a straightforward correlation would have considered as independent data points each of approximately 15 twining members of the Convolvulaceae in the

Carolina flora, the majority of which have cordate leaves, increasing the perceived strength of the relationship between leaf shape and vine habit. A strong case has been made for the potential bias in ignoring similarity due to common descent in comparative studies (Felsenstein 1985). More recently, however, it has been argued that phylogenetic comparative methods may be too conservative in that they consider only the origins of traits and ignore their maintenance in evolutionary lineages (Martins 2000, Westoby et al. 1995). For instance, it might be argued that cordate leaves in each of the vine species of the Convolvulaceae, though not independently derived, are nevertheless independently maintained due to similar selective pressures related to a vine habit. To reduce each of these taxa to a single data point may exclude relevant data. Given the potentially conservative nature of the analytical method, the finding of a significant relationship is striking.

Beyond the statistical evidence for the hypothesized association, examination of individual taxa yields some fascinating examples of convergence in leaf shape in climbing species. For instance, the only climbing member (*Mikania scandens*) of the very large assemblage of composites (Asteraceae) in the local flora has prominent cordate leaves, in striking contrast to the lanceolate, dentate or finely dissected leaves that are more typical of the family. Of the approximately 430 non-climbing species of Asteraceae found in the local flora, only 15 have leaf bases described as "cordate," and in fact some of these cases are only "slightly cordate." Similarly, the majority of species of *Polygonum* (Polygonaceae) in the local flora are erect with lanceolate leaves, but twining or climbing species in the section *Tiniaria* are characterized by strongly hastate or cordate leaves. A third notable example concerns a monocot taxon. A common and distinguishing feature of the monocots is parallel leaf venation, and leaf shape in erect monocot species is generally linear and blade-like (e.g., grasses, sedges, irises) or narrowly lanceolate (e.g., some lilies). In contrast, broad leaves with hastate or cordate bases are characteristic of *Dioscorea*, a monocot genus composed predominantly of vine species, including the yam (*Dioscorea batatas*). Interestingly, the nomenclature of some of the vine taxa we encountered appears to reflect this convergence in leaf shape. For instance, the name *Mikania cynanchifolium* (a vine taxon that is closely related to the local species, *M. scandens*) refers to its similarity in leaf shape to *Cynanchum*, a vine genus in the Asclepiadaceae. Similarly, *Dioscoreophyllum*, a genus in the predominantly twining dicot family Menispermaceae, makes reference to its resemblance in leaf shape to the monocot vine genus, *Dioscorea*.

Taken together, our study of the Carolina flora and Givnish and Vermeij's (1976) survey of Costa Rican habitats provide strong evidence to support earlier anecdotal observations (Richards 1964) of

convergence in leaf shape of vine species. The mechanistic explanation for the association is less clear, but Givnish and Vermeij (1976) proposed a plausible hypothesis. The essential feature of the vine habit is its reduced allocation to support structures. While erect or woody plants may maximize light interception by holding leaves on extended branches or stems, vines must arrive at an alternative solution. Givnish and Vermeij suggest that the long petioles that are characteristic of vine leaves serve to minimize shading by the support plant or structure, and large leaves maximize light interception. However, large leaves create a heavy load for petioles to bear, and the stress is greater as the load arm increases in length. Relative to a horizontal petiole, a petiole held at an upward angle can more easily support the weight of a large leaf. When held on an erect petiole, a cordate leaf base, i.e., one that extends backward from the point of petiole insertion, offers advantages because it counterbalances the weight of the distal end of the leaf.

Although we find a significant association between vine habit and cordate leaves in the Carolina flora, our survey reveals that there are many exceptional taxon pairs that do not exhibit the trend. In particular, many of the vine taxa do not have cordate leaves. A couple of explanations for this result seem likely. First, although plasticity in leaf shape is observed in many families, some show conservation in this trait, and phylogenetic constraint may prevent vine taxa in some families from adopting this strategy. Examples in our survey may include *Adlumia* in the Fumariaceae, a family that is characterized as a whole by pinnately divided leaves, and many vine taxa within the Fabaceae, a family in which the vast majority of taxa have compound leaves. A second explanation comes from Givnish and Vermeij (1976). In their study of the tropical rain forest, they argued that vine taxa with cordate leaves occur primarily in the upper, high-light layers of the forest, but that at lower levels conditions favor different leaf morphologies. Variation in leaf shape in the vines of the Carolinas may reflect variation in the light environment, a factor that we did not consider in our study.

A minority of the exceptions to the significant association we observed are those in which both sister taxa have cordate leaves. One example is the vine genus, *Aristolochia*, and its near relatives in the non-vine genera *Asarum* and *Saruma*. Another is the family Menispermaceae, which is composed primarily of climbing species, and its predominantly non-vine sister clade, which includes the Berberidaceae and Ranunculaceae. In both cases, the non-vine sister taxon has a high or at least substantial proportion of species with cordate leaves. Interestingly, most of these cordate-leaved species are acaulescent; they have greatly reduced stems, and the leaves are held aloft from the ground on long vertical petioles. In this arrangement, the cantilever provided by a cordate base might also be beneficial in bal-

ancing the leaf load. A familiar example of an acaulescent plant with cordate leaves is the violet (Violaceae).

Our simple comparison of vine vs. non-vine taxa in the Carolinas provides a starting point for understanding the selective factors that act on leaf shape in vines. A more detailed examination of the reasons behind the relationship might take into account petiole length and ecological factors (e.g., light availability). In our analysis, we were also constrained by the availability of (and perhaps by the accuracy of) phylogenetic information. More complete phylogenetic treatments of taxa that contain both vine and non-vine members might yield new insights. For example, does vine habit evolve more readily in plant taxa with some leaf shapes than in taxa with others? The non-vine genus, *Ageratina*, the sister to the vine genus, *Mikana*, has broadly ovate leaves, a shape that is relatively uncommon in the Asteraceae family. This suggests the intriguing possibility that a nearly cordate leaf shape in their common ancestor in some sense paved the way for the evolution of a vine habit in *Mikania*.

Acknowledgments

We thank the other members of the Plant Systematics and Evolution course who participated in the initial survey of the Carolina flora: B. Bailes, S. Bateman, L. Phillips, E. Scanlan, and M. Scibetta.

Literature Cited

- Arbuthnott, J. 1710. An argument for divine providence taken from the constant regularity in the births of both sexes. *Philosophical Transactions* 27:724–735.
- Backlund, M., B. Oxelman, and B. Bremer. 2000. Phylogenetic relationships within the Gentianales based on *ndhF* and *rbcL* sequences, with particular reference to the Loganiaceae. *American Journal Botany* 87:1029–1043.
- Caddick, L.R., P. Wilkin, P.J. Rudall, T.A.J. Hedderson, and M.W. Chase. 2002. Yams reclassified: A recircumscription of Dioscoreaceae and Dioscoreales. *Taxon* 51:103–114.
- Chase, M.W., D.W. Stevenson, P. Wilkin, and P.J. Rudall. 1995. Monocot systematics: A combined analysis. Pp. 685–730. *In* P.J. Rudall, P.J. Cribb, D.F. Cutler, and C.J. Humphries (Eds.). *Monocotyledons: Systematics and Evolution*. Royal Botanic Gardens, UK. 750 pp.
- Civeyrel, L., A. Le Thomas, K. Ferguson, and M.W. Chase. 1998. Critical reexamination of palynological characters used to delimit Asclepiadaceae in comparison to the molecular phylogeny obtained from plastid *matK* sequences. *Molecular Phylogenetics and Evolution* 9:517–527.
- Crawley, M.J., P.H. Harvey, and A. Purvis. 1997. Comparative ecology of the native and alien floras of the British Isles. Pp. 36–53. *In* J. Silvertown, M. Franco, and J.L. Harper (Eds.). *Plant Life Histories: Ecology, Phylogeny and Evolution*. Cambridge University Press, Cambridge, UK. 313 pp.

- Dahlgren, R., and K. Bremer. 1985. Major clades of the angiosperms. *Cladistics* 1:349–368.
- Diniz-Filho, J.A.F., C.E.R. De Sant'ana, and L.M. Bini. 1998. An eigenvector method for estimating phylogenetic inertia. *Evolution* 52:1247–1262.
- Felsenstein, J. 1985. Phylogenies and the comparative method. *American Naturalist* 125:1–15.
- Fishbein, M.C., C. Hibsich-Jetter, D.E. Soltis, and L. Hufford. 2001. Phylogeny of Saxifragales (Angiosperms: Eudicots): Analysis of a rapid, ancient radiation. *Systematic Biology* 50:817–847.
- Givnish, T.J. 1986. Economics of support. Pp 413–420. *In* T.J. Givnish (Ed.), *On the Economy of Plant Form and Function*. Cambridge University Press, Cambridge UK. 717 pp.
- Givnish, T.J. 1987. Comparative studies of leaf form: Assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytologist* 106 (Suppl.):131–160.
- Givnish, T.J., and G.J. Vermeij. 1976. Sizes and shapes of liane leaves. *American Naturalist* 100:743–778.
- Loconte H., and J.R. Estes. 1989. Phylogenetic systematics of Berberidaceae and Ranunculales (Magnoliidae). *Systematic Botany* 14:565–579.
- Martins, E.P., and T.F. Hansen. 1997. Phylogenies and the comparative method: A general approach to incorporating phylogenetic information into the analysis of interspecific data. *American Naturalist* 149:646–667.
- Martins, E.P., and T.F. Hansen. 2000. Adaptation and the comparative method. *Trends in Ecology and Evolution* 15:296–299.
- Miller, A.J., D.A. Young, and J. Wen. 2001. Phylogeny and biogeography of *Rhus* (Anacardiaceae) based on ITS sequence data. *International Journal of Plant Sciences* 162:1401–1407.
- Olmstead, R.G., B. Bremer, K.M. Scott, and J.D. Palmer. 1993. A parsimony analysis of the Asteridae *sensu lato* based on the rbcL sequence. *Annals of the Missouri Botanical Garden* 80:700–722.
- Parkhurst, D.F., and O.L. Loucks. 1972. Optimal leaf size in relation to environment. *Journal of Ecology* 60:505–537.
- Qiu, Y.-L., J. Lee, F. Bernasconi-Quadroni, D.E. Soltis, P.S. Soltis, M. Zanis, E. A. Zimmer, Z. Chen, V. Savolainen, and M.W. Chase. 2000. Phylogeny of basal angiosperms: Analysis of five genes from three genomes. *International Journal of Plant Sciences* 161(Suppl.):S3–S27.
- Radford, A.E., H.E. Ahles, and C.R. Bell. 1968. *Manual of the Vascular Flora of the Carolinas*. University of North Carolina Press, Chapel Hill, NC. 1183 pp.
- Richards, P.W. 1964. *The Tropical Rainforest*. Cambridge University Press, London, UK. 450 pp.
- Richardson, J.E., M.F. Fay, Q.C.B. Cronk, D. Bowman, and M.W. Chase. 2000. A phylogenetic analysis of Rhamnaceae using rbcL and trnL-F plastid DNA sequences. *American Journal of Botany* 87:1309–1324.
- Ro, K.E., C.S. Keener, and B.A. McPherson. 1997. Molecular phylogenetic study of the Ranunculaceae: Utility of the nuclear 26S ribosomal DNA in inferring intrafamilial relationships. *Molecular Phylogenetics and Evolution* 8:117–127.
- Rodman, J.E. 1984. A taxonomic analysis and revised classification of Centrospermae. *Systematic Botany* 9:297–323.

- Rodman, J.E. 1991. A taxonomic analysis of glucosinolate-producing plants. Part 2: Cladistics. *Systematic Botany* 16:619–629.
- Schmidt, G.J., and E.E. Schilling. 2000. Phylogeny and biogeography of *Eupatorium* (Asteraceae: Eupatorieae) based on nuclear ITS sequence data. *American Journal of Botany* 87:716–726.
- Sennblad, B., M.E. Endress, and B. Bremer. 1998. Morphological and molecular data in phylogenetic fraternity: The tribe Wrightieae (Apocynaceae) revisited. *American Journal of Botany* 85:1143–1158.
- Simmons, M.P., V. Savolainen, C.C. Clevinger, R.H. Archer, and J.I. Davis. 2001. Phylogeny of the Celastraceae inferred from the phytochrome B, rbcL, atpB, 26S nuclear ribosomal DNA and morphology. *Molecular Phylogenetics and Evolution* 19:353–366.
- Song, B.H., X.Q. Wang, F.Z. Li, and D.Y. Hong. 2001. Further evidence for paraphyly in the Celtidaceae from the chloroplast gene matK. *Plant Systematics and Evolution* 228:107–115.
- Spangler, R.E. and R.G. Olmstead. 1999. Phylogenetic analysis of Bignoniaceae based on the cpDNA gene sequences rbcL and ndhF. *Annals of the Missouri Botanical Garden* 86:33–46.
- Swensen, S.M., B.C. Mullin, and M.W. Chase. 1994. Phylogenetic affinities of Datisceae based on an analysis of nuclear sequences from the plastid rbcL gene. *Systematic Botany* 19:157–168.
- Wagstaff, S.J., and M.I. Dawson. 2000. Classification, origin and patterns of diversification of *Corynocarpus* (Corynocarpaceae) inferred from DNA sequence. *Systematic Botany* 25:134–149.
- Wen, J., G.M. Plunkett, A.D. Mitchell, and S.J. Wagstaff. 2001. The evolution of Araliaceae: A phylogenetic analysis based on ITS sequence of nuclear ribosomal DNA. *Systematic Botany* 26:144–167.
- Westoby, M., M.R. Leishman, and J.M. Lord. 1995. On misinterpreting the “phylogenetic correction.” *Journal of Ecology* 83:531–534.
- Zar, J.H. 1974. *Biostatistical Analysis*, 2nd Edition. Prentice Hall, Englewood Cliffs, NJ. 718 pp.