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THE EVOLUTION OF REPRODUCTIVE CHARACTERS IN DIPSACALES

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Improved knowledge of Dipsacales phylogeny provides a solid framework for studies of character evolution. Although the polarity of many characters can now be confidently established, for others it remains unclear. This results largely from uncertainty about the broader relationships of Dipsacales and is especially problematic for characters that differentiate the two basal lineages, Adoxaceae and Caprifoliaceae. Within Caprifoliaceae, changes in stamen number became decoupled from corolla evolution, a reduction from five to four stamens, coinciding with the origin of the Linnina clade. Subsequently, there was a reduction to two stamens within Morinaceae and to three, two, and one within Valerianaceae. In contrast, within Adoxaceae, stamen number covaries with the number of corolla lobes, although the number of stamens was effectively doubled within Adoxina by the division of each stamen. Fleshy fruits may have evolved separately in Adoxaceae and in Caprifolieae. These vary in the number of seeds and in the presence or absence of an endocarp and show repeated patterns in the evolution of color, perhaps underlain by shifts in the timing of developmental events. In Caprifoliaceae, dry fruits include bicarpellate septicidal capsules in Diervilleae and initially tricarpellate, single-seeded achenes in Linnina. Achene fruits exhibit a variety of independently evolved dispersal mechanisms, especially involving modifications of the calyx lobes or bracts subtending the ovary. Within Linnina, a distinctive epicalyx surrounding the ovary appears to have originated through the fusion of supernumerary bracts. It is possible that the epicalyx evolved twice, once in the ancestor of the Morinaceae and again in the *Triplostegia*-Dipsacaceae clade. Within the Dipsacaceae, modifications of the epicalyx parallel those of the ovary and calyx within Valerianaceae, suggesting a transference of function involving homeoheterotopy. Evolutionary adjustments of the epicalyx, calyx, and ovary wall in relation to protection and dispersal may have established an evolutionarily stable configuration.

Keywords: Dipsacales, Adoxaceae, Caprifoliaceae, Morinaceae, Valerianaceae, Dipsacaceae, phylogeny, character evolution, flowers, fruits, transference of function, internal selection, evolutionarily stable configuration.

Introduction

Dipsacales, with more than 1000 species, form a branch within the Asteridae related to the Asterales, Apiales, and several smaller lineages (Bremer et al. 2002). Although phylogenetic relationships among these lineages have resisted resolution, knowledge of relationships within the Dipsacales has advanced to the point that we can confidently begin to trace the evolution of particular morphological characters and to understand how these changes were related to one another and to shifts in function and diversification.

Here, we review and synthesize phylogenetic studies in Dipsacales and use these results to explore the evolution of various reproductive characters. In particular, we highlight the evolution of stamen number in relation to the number of corolla lobes and to flower symmetry. We also focus attention on fruit evolution, emphasizing the color of fleshy fruits and modifications for wind dispersal in taxa with achenes. Of special interest is the origin of a distinctive new structure, the epicalyx, and the ways in which its evolution within Dipsacaceae has paralleled calyx evolution within Valerianaceae.

Phylogeny and New Molecular Evidence

Knowledge of phylogenetic relationships within Dipsacales has improved dramatically over the past 2 decades but especially within the past few years. Analyses have been based on morphological characters and various molecular data sets analyzed both separately and in various combinations (Donoghue 1983*b*; Donoghue et al. 1992, 2001*b*; Judd et al. 1994; Backlund and Donoghue 1996; Backlund and Bremer 1997; Kim et al. 1999; Pyck et al. 1999, 2002; Pyck and Smets 2000; Bell et al. 2001; Pyck 2001; Zhang et al. 2002*a*; Bell and Donoghue 2003).

Overall, these studies show remarkable congruence with respect to the major lineages identified and their relationships to one another. Recently, phylogenetic names have been applied to these major clades (Donoghue et al. 2001*b*; fig. 1*A*). The basal split separates Adoxaceae (including *Viburnum*, *Sambucus*, and Adoxina, which contains *Adoxa* and its relatives) from Caprifoliaceae (including Diervilleae, Caprifolieae, Linnaeeae, Morinaceae, Valerianaceae, and Dipsacaceae). Within Adoxaceae, *Viburnum* is sister to Adoxoideae, which

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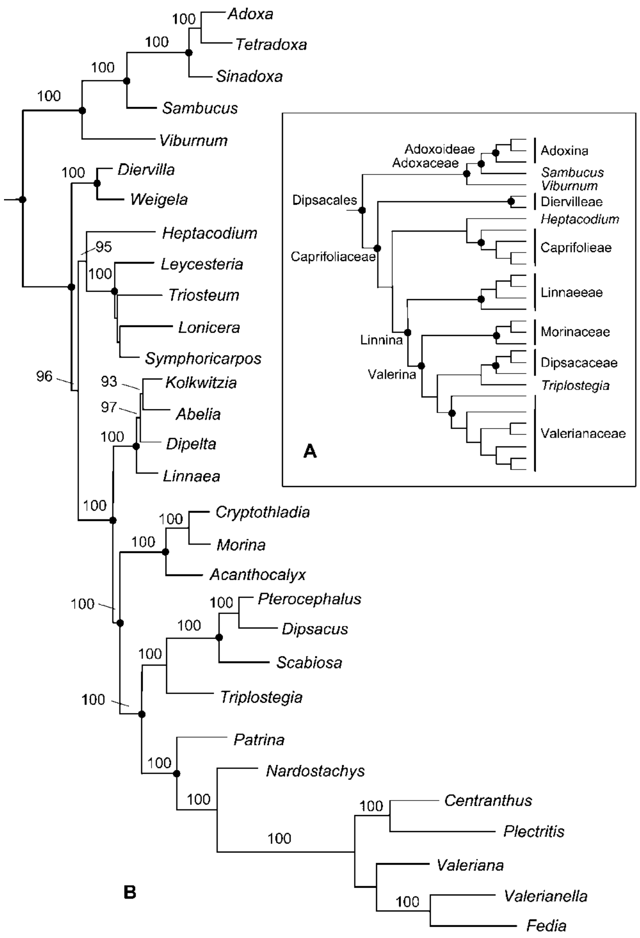

Fig. 1 *A*, Phylogenetic taxonomy of Dipsacales from Donoghue et al. (2001*b*). *B*, Maximum likelihood tree topology ($-\ln L = 46190.77104$) of combined ITS and chloroplast DNA nucleotide sequence data; bootstrap values greater than 90% above the branches.

includes *Sambucus* and the Adoxina clade. Within Caprifoliaceae, Donoghue et al. (2001*b*) provided phylogenetic definitions for the names of two major clades (de Queiroz and Gauthier 1994; Cantino and de Queiroz 2000). The Linnina clade includes Linnaeeae and the Valerina clade, which contains the herbaceous groups Morinaceae, Valerianaceae, and Dipsacaceae. *Triplostegia* now appears to be more closely related to Dipsacaceae than it is to Valerianaceae (in agreement with Thorne 1983; Peng et al. 1995, but contra Cronquist 1981; Backlund and Donoghue 1996; Backlund and Nilsson 1997; Pyck 2001).

Importantly, all phylogenetic analyses have shown that Caprifoliaceae in the traditional sense (consisting of *Viburnum*, *Sambucus*, Diervilleae, Caprifolieae, and Linnaeeae) do not form a clade. In the phylogenetic classification of Donoghue et al. (2001*b*; fig. 1*A*), the most significant change is that the name Caprifoliaceae is applied to the clade that includes Diervilleae, Caprifolieae, and Linnaeeae, as well as the Morinaceae, Valerianaceae, and Dipsacaceae. In the interest of nomenclatural stability, these six names have been retained because in their traditional sense they refer to clades. The classification suggested by Backlund and Pyck (1998; also Backlund and Bremer 1996; Angiosperm Phylogeny Group 1998; Stevens 2001–) is rejected because it requires a variety of name changes only for the sake of adjusting ranks (Diervilleae, Caprifolieae, and Linnaeeae are renamed Diervilleaceae, Caprifoliaceae, and Linnaeaceae, respectively) and because it fails to provide names for several well-supported clades (e.g., the Linnina clade, which includes Linnaeeae, Morinaceae, Valerianaceae, and Dipsacaceae). Similar criticisms apply to other recently proposed classifications (Benko-Iseppon and Morawetz 2000).

Progress has also been made in elucidating phylogenetic relationships within particular Dipsacales clades. Within Adoxaceae, analyses of several data sets have been reported for *Viburnum*, including morphological characters (Donoghue 1983*a*), cpDNA restriction sites (Donoghue and Sytsma 1993), and sequences of the nuclear ribosomal internal transcribed spacer (ITS) region (Donoghue and Baldwin 1993). More recently, analyses have been conducted using the chloroplast locus *mat*K and the nuclear granule-bound starch synthase (*waxy*) genes (Winkworth and Donoghue 2002; R. C. Winkworth and M. J. Donoghue, unpublished data). *Sambucus* has been analyzed using ITS sequences (Eriksson and Donoghue 1998) and Adoxina using morphology and sequences of *rbc*L and ITS (Liu et al. 2000; Donoghue et al. 2001*b*). Within Caprifoliaceae, Kim and Kim (1999) analyzed Diervilleae (*Diervilla* and *Weigela*) using ITS sequences. *Triosteum* has been studied using ITS and *waxy* sequences (Gould and Donoghue 2000) and more recently using cpDNA *trn*L and *mat*K sequences (S. Cullis-Suzuki, C. D. Bell, R. C. Winkworth, and M. J. Donoghue, unpublished data), and *Lonicera* is currently under study using ITS and *mat*K (Li and Donoghue 2002; J. Li and M. J. Donoghue, unpublished data). Bell and Donoghue (2002, 2003) have carried out analyses within Morinaceae and Valerianaceae on the basis of *trn*L, *mat*K, and ITS. For Dipsacaceae, Caputo and Cozzolino (1994) analyzed morphological characters, Mayer and Ehrendorfer (1999, 2000) presented phylogenetic hypothesis for *Scabiosa* and *Pterocephalus* and their relatives, and studies are underway using *trn*L, *mat*K, ITS (C. D. Bell and M. J. Donoghue, unpublished data). In all, ca. 300 species have now been included in one or another phylogenetic analysis.

To date, the single most convincing phylogenetic study of the Dipsacales, in terms of both character number (7593 nucleotide sites) and confident resolution, is by Bell et al. (2001). However, this study was entirely based on chloroplast DNA sequences, which leaves open the possibility of incongruence with other data sources. Although it is comforting that cpDNA and morphological analyses have yielded very similar results, the addition of data from nuclear genes would be highly desirable. We have therefore carried out a new analysis combining ITS sequences with the cpDNA sequences from Bell et al. (2001).

ITS sequences were obtained from the accessions used by Bell et al. (2001) with one exception; the sequence for *Lonicera japonica* Thunb. was obtained from Kim and Kim (1999). The ITS region was amplified and sequenced using primers ITS2, ITS3 (in some cases ITS3B), ITS4, and ITS5 (White et al. 1990; Baum et al. 1994) and the standard protocols described in Bell et al. (2001). All newly obtained sequences have been submitted to GenBank (AY236170-AY236196, AJ419710, AJ419711, U88194), and our data matrices and trees are available in TreeBASE (http://www.treebase.org). Maximum likelihood analyses were performed using PAUP* b10 (Swofford 2001) for the Linux operating system. We used a general time reversible model of sequence evolution plus an alpha shape parameter for the gamma distribution to account for rate heterogeneity among sites. Heuristic searches were conducted with tree bisection reconnection branch swapping and the collapse of zero-length branches. Analyses were repeated 100 times with the random addition option. Bootstrap tests (Felsenstein 1985) used 300 replicates with heuristic search settings identical to those of the original search.

Analyses of the ITS sequences alone produced trees that were generally consistent with the cpDNA trees of Bell et al. (2001); the few minor discrepancies were very poorly supported. Our combined analysis of ITS and cpDNA sequences yielded topologies corresponding to those obtained using cpDNA sequences alone and with previous morphological analyses (fig. 1*B*). The trees differ only in the level of support for specific clades. In particular, the addition of ITS sequences increases bootstrap support for the relationship between *Heptacodium* and the Caprifolieae (95%) and for the Valerina clade (100%). ITS data also support the hypothesis that the Diervilleae is the sister group of the remaining Caprifoliaceae (96%). Unfortunately, however, the addition of these data do little to clarify relationships among *Leycestaria*, *Lonicera*, *Symphoricarpos*, and *Triosteum* within Caprifolieae.

The discussion of character evolution below is based on the backbone tree shown in figure 1*B* and, as needed, studies of individual clades noted above. Unless otherwise cited, information on morphological characters has been derived from a variety of general treatments (Eichler 1875; Fritsch 1891; Fukuoka 1972; Hara 1983; Wilkinson 1949; Weberling 1957), from studies of particular taxa (*Adoxa*: Erbar 1994 and Roels and Smets 1994; Linnaeeae: Fukuoka 1968; Morinaceae: Cannon and Cannon 1984), or from studies of individual suites of characters (pollen: Bohnke-Gutlein and Weberling 1981, Blackmore and Cannon 1983, and Donoghue 1985; nectaries: Wagenitz and Liang 1984).

Character Evolution in General

Recent phylogenetic analyses have increased our confidence in inferences of ancestral character states and directions of change in the Dipsacales. For example, we can conclude that the first Dipsacales were woody plants (probably shrubs) and that the herbaceous habit evolved at least five times within the group (in *Triosteum*, Valerina, and Adoxina and probably twice within *Sambucus*; Eriksson and Donoghue 1997); annuals evolved several times within Valerianaceae and Dipsacaceae. We can also be quite certain that the first Dipsacales had opposite, simple leaves without stipules and that compound, whorled, and stipulate leaves each evolved several times. Likewise, the presence of bud scales appears to be ancestral, with naked buds derived on several occasions (e.g., within *Viburnum*).

We can also safely conclude that the Dipsacales originated in the Northern Hemisphere, probably in eastern Asia, and that they first occupied the understory of temperate forests. From there, many lineages spread around the Northern Hemisphere and into the Southern Hemisphere in both the Old and the New World (e.g., within *Viburnum*, *Sambucus*, *Valeriana*, and Dipsacaceae; Tang and Li 1994; Donoghue et al. 2001*a*). In addition, several of the herbaceous lineages moved into alpine habitats, in some cases associated with considerable diversification (e.g., Valerianaceae, in general, but especially in South America; C. D. Bell and M. J. Donoghue, unpublished data, 2002).

With respect to reproductive characters, the first Dipsacales probably produced only perfect, fertile flowers; imperfect flowers (as in some Valerianaceae) and sterile flowers (as in *Viburnum*) evolved several times independently. Ancestrally, Dipsacales flowers had five corolla lobes and five stamens, with reductions occurring on several occasions (see below).

Unfortunately, for a relatively large and important set of reproductive characters the ancestral condition remains unclear. These are, for the most part, characters that differentiate the two basal branches, Adoxaceae and Caprifoliaceae. Ancestrally, Adoxaceae had polysymmetric flowers, with small calyx lobes, rotate corollas, short styles, and lobed stigmas. They also lacked distinct nectaries and had small, prolate pollen grains with a semitectate, reticulate exine. In marked contrast, the earliest Caprifoliaceae had monosymmetric flowers (probably weakly so at first) with larger calyx lobes, tubular corollas, elongate styles, and capitate stigmas. At the base of the corolla they had nectaries composed of unicellular hairs, and their large, rounded pollen grains had a complete, spine-bearing tectum elevated on columellae.

In general, these characters cannot be polarized with confidence owing to continued uncertainty about the broader relationships of the Dipsacales. Several hypotheses have emerged from previous morphological and molecular studies of the Asteridae. Dipsacales have been linked to Apiales, Asterales, or both or to smaller groups, including *Columellia*, *Desfontania*, and various Escalloniaceae and Bruniaceae (Donoghue et al. 1992, 2001*b*; Downie and Palmer 1992; Chase et al. 1993; Olmstead et al. 1993, 2000; Bremer et al. 1994, 2002; Backlund and Donoghue 1996; Backlund and Bremer 1997; Soltis et al. 2000; Bell et al. 2001). However, in all of these studies support for the position of Dipsacales is weak. A better understanding of broader relationships is necessary to make further progress, although, depending on the nature of the variation both among and within the outgroups for these traits, even this may not be sufficient.

For several of the characters that differentiate Adoxaceae from Caprifoliaceae, outgroup uncertainties do not completely preclude further analysis. For example, it is clear that short, rotate corollas are ancestral in Adoxaceae and that longer, tubular corollas evolved several times within *Viburnum*. Associated with this shift was the evolution of a disklike nectary (e.g., *Viburnum farreri*; Erbar 1994). Nectaries consisting of packets of multicellular glandular hairs mark the Adoxina, and in some *Sambucus* species (e.g., *Sambucus chinensis*), whole flowers were converted into nectaries. Likewise, within Caprifoliaceae it is possible to assess the direction of change in several such traits. For example, calyx lobes have been greatly reduced on several occasions (e.g., in association with fleshy fruits in *Lonicera* and its relatives), and pollen grain walls were modified in several ways (e.g., the probable loss of columellae in Diervilleae and of tectal spines within Caprifolieae; Donoghue 1985).

A further problem in assessing character polarity relates to complex character state distributions among the major lineages and lingering phylogenetic uncertainties within Dipsacales. A prime example is carpel number (and the abortion of carpels during ovary development), which varies considerably within both Adoxaceae and Caprifoliaceae. In Adoxaceae, the ancestral state may be three carpels because this is universal in *Viburnum* (though two carpels abort) and also common in *Sambucus*. Three carpels is also a common number within Caprifoliaceae (and ancestral at least within Linnina), but too much variation exists among the basal lineages to assess the direction of change. The Diervilleae, which may be the sister group of the rest of the Caprifoliaceae (figs. 1, 2), are bicarpellate. However, carpel number is highly variable in the Caprifolieae, ranging from two to five. Unfortunately, lack of resolution within Caprifolieae limits our understanding of the evolution of this and several other traits.

In the following section, we avoid such problematic cases and instead focus on sets of characters for which we can be much more certain about the direction of change. Specifically, we will focus on the number of corolla lobes and stamens, on the color of fleshy fruits, and on modifications related to the dispersal of achenes (see below).

Corolla and Stamen Characters

In Dipsacales the number of corolla lobes varies between five (the most common number) and three, and stamens number from 10 to one, with five and four predominating. Parsimony optimization of these characters on the backbone Dipsacales tree implies that the ancestor had five corolla lobes and five stamens (fig. 2*A*). In Adoxaceae, this combination is retained throughout *Viburnum*, in most *Sambucus* species (but see below), and within Caprifoliaceae, the Diervilleae, *Heptacodium*, and Caprifolieae retain this condition (though in some *Symphoricarpos* and *Lonicera* one sees a strictly correlated reduction to four corolla lobes and four stamens). However, within both the Adoxaceae and Caprifoliaceae, there were significant changes in these numbers. Within Caprifoliaceae, the large Linnina clade is marked by a reduction from five to four stamens (fig. 2*B*). This shift entailed the loss of the medially positioned stamen on the dorsal (upper) side of the flower, situated between the two upper corolla lobes. Initially, this loss was not accompanied by a change in the corolla,

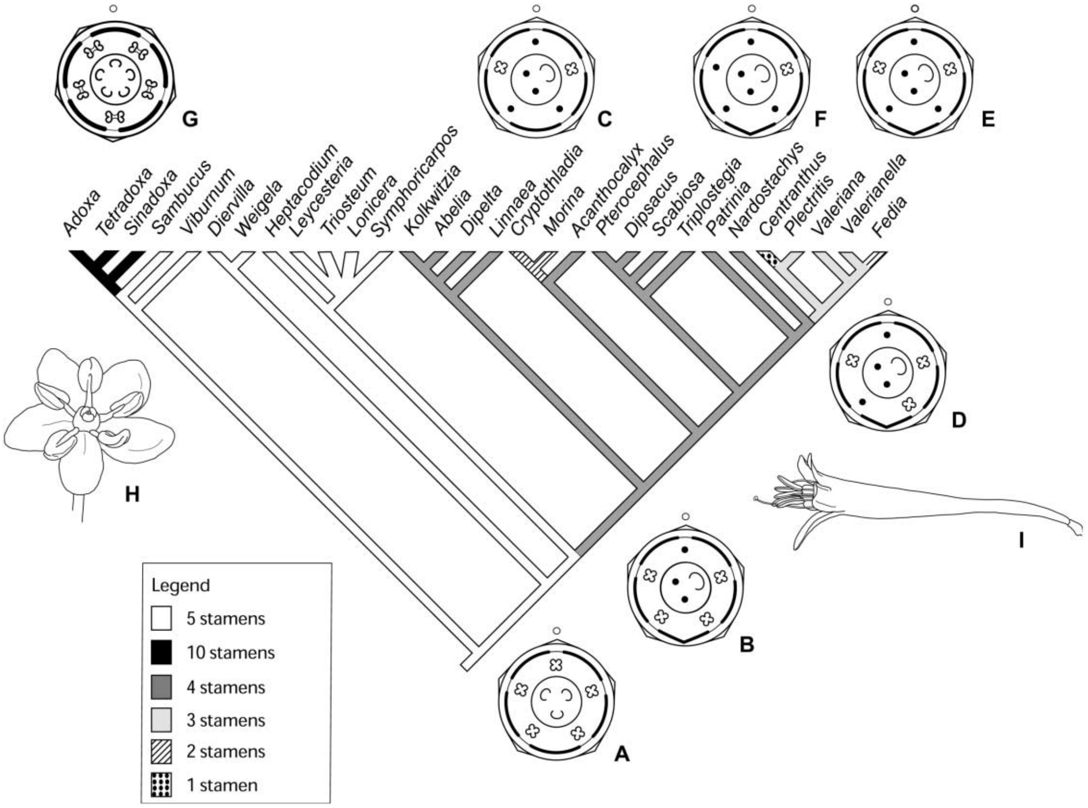

Fig. 2 Phylogeny of Dipsacales showing the evolution of stamen number in relation to corolla characters. Together, the tree and floral diagrams illustrate several major transitions. *A*, Inferred ancestral condition of five stamens and five corolla lobes; *B*, loss of the dorsal stamen with the origin of Linnina; *C*, loss of two stamens within Morinaceae; *D*, loss of one stamen in core Valerianaceae; *E*, loss of one stamen in *Fedia*; *F*, loss of two stamens in *Centranthus*; and *G*, doubling of stamen number through division in Adoxina (five-merous flower shown here; note "lobelioid" orientation). Flower drawings highlight the differences in floral symmetry and form between Adoxaceae (*H*, *Sambucus canadensis*, modified from Ferguson 1966) and Caprifoliaceae (*I*, *Lonicera sempervirens*, modified from Zomlefer 1994).

which retained five lobes, but reductions did occur later within several lineages (e.g., to four corolla lobes in many Dipsacaceae).

Within the Valerina clade, there were several further reductions in stamen number. In Morinaceae, *Acanthocalyx* has retained the ancestral condition of five corolla lobes and four stamens. A reduction to two stamens occurred in the *Morina-Cryptothladia* clade (fig. 2*C*). In this clade, the two uppermost stamens were retained and the lower pair became reduced and sterilized. In the longer corolla tube of *Morina*, the two small staminodia are present in the upper portion of the tube, whereas in the shorter-tubed, apparently cleistogamous flowers of *Cryptothladia*, the staminodia are associated with two nectaries at the very base of the tube. Shortening of the tube in this case may have come about through reduced growth in the stamen-corolla portion of the tube. Dipsacaceae have four stamens, with rare exception (e.g., *Pterocephaldium diandrum*; Caputo and Cozzolino 1994).

Other reductions in stamen number occurred within Valerianaceae. *Patrinia* and *Nardostachys* retained the ancestral condition for Linnina, namely, five corolla lobes, four stamens (though five stamens have occasionally been reported from individual flowers), and monosymmetry at anthesis, although early development in *Patrinia* shows signs of asymmetry (Hofmann and Gottmann 1990; Endress 1999). The large clade containing the core Valerianaceae is marked by a reduction to three stamens (fig. 2*D*). The evolution of this highly unusual configuration entailed the loss of just one of the more ventrally positioned stamens—apparently the one farthest from the fertile carpel—resulting in an asymmetric flower. Within this clade, two other reductions occurred independently. In *Fedia*, the remaining ventral stamen was lost (fig. 2*E*), so that only the pair of dorsal stamens is present, as in the *Morina-Cryptothladia* clade. This loss reestablished floral symmetry, at least with respect to the corolla and stamens. In *Centranthus*, a reduction apparently occurred from three to just one lateral stamen situated in the dorsal portion of the flower adjacent to the fertile carpel (fig. 2*F*). The asymmetry in *Centranthus* is further accentuated by changes in the orientation of the style (curved away from the stamen) and corolla lobes. One of the dorsal lobes that is adjacent to the fertile stamen tends to be somewhat enlarged and oriented upward, whereas the other is oriented downward, such that there often appear to be four ventral lobes. However, as Endress (1999, p. 58) has highlighted, *Centranthus* displays "pendulum symmetry"—the orientation of successive flowers along the monochasium alternates (enantiomorphy) such that the entire entity forms a "monosymmetric superstructure."

Within Adoxaceae, corolla lobe number was reduced several times within *Sambucus* from five to either four or three (Bolli 1994; Eriksson and Donoghue 1997). The basal condition within Adoxina is unclear, but outgroup comparison indicates five lobes; it may have been similar to the condition seen in *Tetradoxa* and *Adoxa*, where both four- and five-merous flowers are produced within an inflorescence. From this condition, there may have been further reduction to three lobes in *Sinadoxa*. In the compressed, headlike *Adoxa* inflorescence, the lateral flowers are oriented in the "lobelioid" fashion (i.e., with the medial petal in the dorsal position; Erbar 1994; Donoghue et al. 1998) and are five-merous (although usually with only three sepals), whereas the terminal upward-facing flower is four-merous (Erbar 1994). In the more elongate infloresences of *Tetradoxa*, the arrangement is more variable (Hara 1981, 1983; Wu 1981; Liang and Zhang 1986; Li and Ning 1987), and at least in some instances, the order is reversed; that is, four-merous flowers are lateral, and the terminal flower is five-merous (M. J. Donoghue, personal observations). *Sinadoxa* (Wu et al. 1981; Li and Ning 1987; Liang 1997) appears to have the most derived architecture, with a spikelike inflorescence bearing tight clusters of tiny flowers. The number of flower parts is somewhat variable within an inflorescence, but three-merous flowers are most common. The gynoecioum is also reduced in *Sinadoxa* to just a single carpel (Wu et al. 1981).

Stamen number also varies within Adoxaceae. Reductions within *Sambucus* to four or three stamens are strictly correlated with corresponding reductions in the number of corolla lobes. In Adoxina, individual stamens appear to have become split between the two thecae of the anther such that the number of stamens was effectively doubled (fig. 2*G*). This interpretation is indicated by the position of the bipartite "stamens" in the sinuses between the corolla lobes, by developmental studies in *Adoxa* showing that each pair originates from a single stamen primordium (Erbar 1994), and by anatomical studies documenting that a single vascular bundle splits into two at whatever level the two halves of the filament separate (Li and Ning 1987). Whereas in *Adoxa* (fig. 3*A*, 3*B*) the filament is divided all the way to the base and in *Sinadoxa* (fig. 3*E*, 3*F*) very close to the base, in *Tetradoxa* the filaments are divided for only about half of their length (fig. 3*C*, 3*D*). If the *Tetradoxa* condition is interpreted as a retained ancestral state (Liang and Zhang 1986; Liang 1997), then the more fully divided stamens of *Adoxa* and *Sinadoxa* were derived independently. Alternatively, the T-shaped *Tetradoxa* stamens may have been derived from the more completely bifid condition (Donoghue et al. 2001*b*). In any case, throughout the Adoxina, stamen number is strictly correlated with the number of corolla lobes and varies accordingly within individual plants. Thus, in flowers with five corolla lobes, there are five split stamens (10 separate anther sacs); in those with four corolla lobes, there are four split stamens; and in *Sinadoxa* with three lobes, there are three split stamens.

These observations highlight a basic contrast in corolla and stamen number evolution between the two major lineages of Dipsacales. In Adoxaceae, evolutionary modifications of corolla lobe and stamen number have been coupled, whereas in Caprifoliaceae, these became uncoupled beginning with the evolution of Linnina. In effect, these floral features behave as one character in the Adoxaceae, whereas they may evolve independently within the Caprifoliaceae.

This difference may be related to an underlying change in floral symmetry. Adoxaceae all have polysymmetric corollas (fig. 2*H*; except for the enlarged sterile flowers in *Viburnum*), whereas Caprifoliaceae are weakly to strongly monosymmetric (fig. 2*I*). Specifically, in Diervilleae the corolla is usually noticeably monosymmetric, with the two upper (dorsal) petals differentiated from the three lower (ventral) petals. This condition is retained in *Heptacodium*. In Caprifolieae, the corolla is only weakly monosymmetric in *Leycesteria* and *Symphoricarpos* but with rare exceptions is rather strongly monosym-

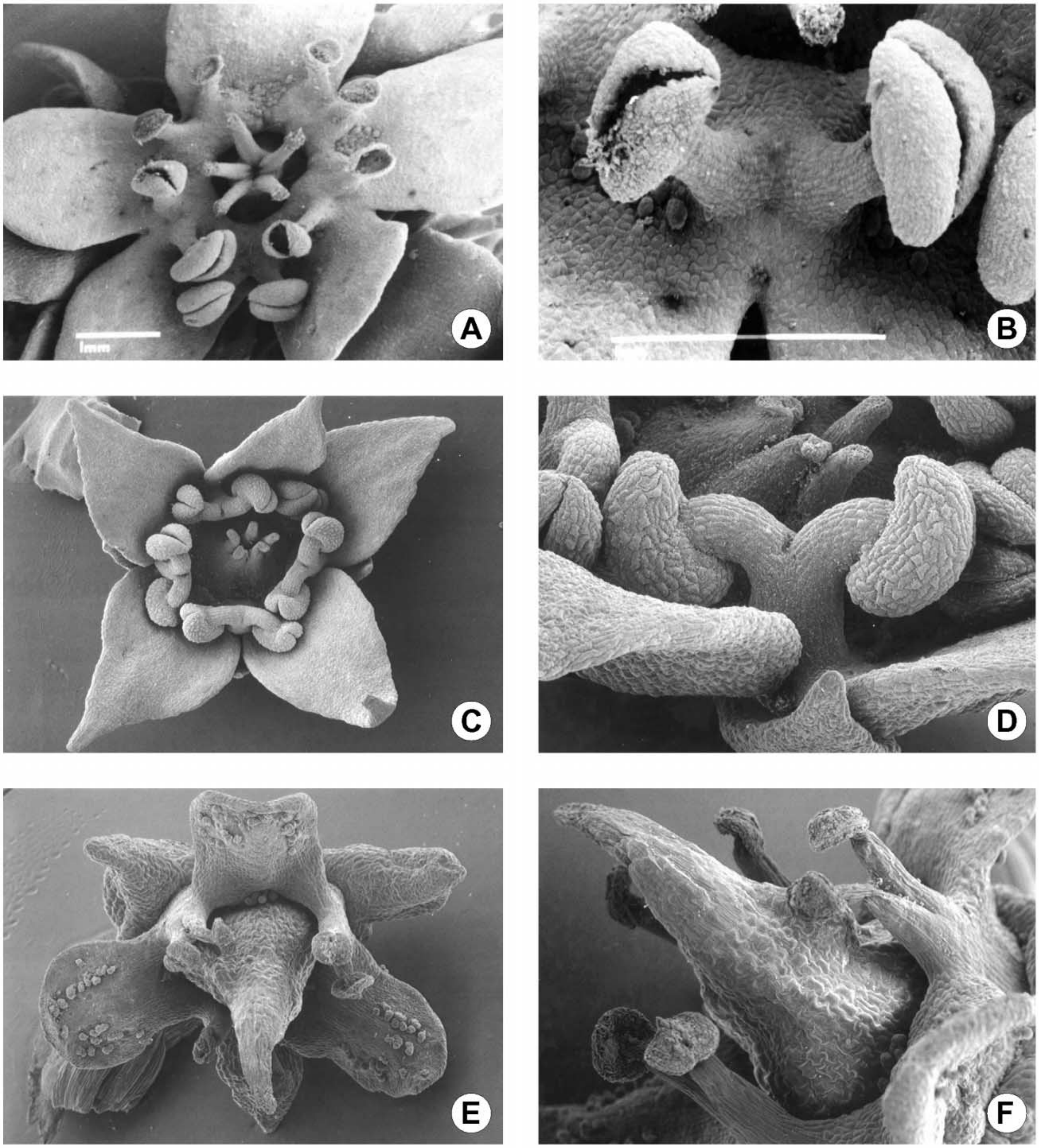

Fig. 3 Scanning electron micrographs illustrating Adoxina flowers and stamen morphology. *A*, *B*, *Adoxa moschatellina* L. (from Erbar 1994) showing five-merous flower with paired stamens; note separate style branches/stigmas and nectaries at the base of each petal. Scale bars = 1 mm. *C*, *D*, *Tetradoxa omeiensis* (H. Hara) C. Y. Wu showing five-merous flower with partially divided, T-shaped stamens. *E*, *F*, *Sinadoxa corydalifolia* C. Y. Wu, Z. L. Wu, and R. F. Huang showing three-merous flower with paired stamens; note single carpel and multicellular glandular trichomes on corolla lobes.

metric in *Triosteum* and *Lonicera*. When the corolla is strongly monosymmetric in the latter two groups, four corolla lobes are oriented upward and the medial ventral lobe is oriented downward. The basic condition in Linnina is monosymmetry, with two up and three down, though in some cases, the corolla is almost polysymmetric (e.g., *Linnaea*, *Patrinia*).

It may be that subdivision of the floral meristem into dorsal and ventral portions, e.g., with respect to the expression of genes such as CYCLOIDEA (Luo et al. 1996; Cronk and Moller 1997; Donoghue et al. 1998), sets the stage for the individuation of stamens in different developmental zones. Pronounced monosymmetry at the base of the Linnina could have facilitated the loss of the medial dorsal stamen. Although such a model may help explain the initial shift to four stamens and also subsequent shifts that occurred symmetrically along the dorsoventral axis, it would not, by itself, explain asymmetric changes (e.g., in the core Valerianaceae and *Centranthus*). Instead, such changes may relate to carpel abortion in Linnina or to differences in the arrangement of flowers at the level of the inflorescence. Eventually, studies of gene expression will be needed to elucidate the mechanisms underlying the evolutionary pattern outlined here.

Fruit Characters

Dipsacales show greater variation in fruit type than many clades of comparable size. Most Adoxaceae have fleshy bird-dispersed "drupes," with an endocarp surrounding the single seed in *Viburnum* or separately surrounding several seeds in Adoxoideae. In Adoxina, the ovary wall is generally drier at maturity, and the fruits have sometimes been described as "dry drupes." These are borne close to the ground, often reaching soil level during development, and in *Adoxa* are reportedly dispersed by birds (Lagerberg 1904) or snails (Muller-Schneider 1967). In *Sinadoxa*, the single carpel matures into a single-seeded achenelike fruit (Wu et al. 1981). At maturity, the calyx lobes appear to become inflated, air-filled sacs, and the main dispersal agent may be water (M. J. Donoghue, personal observation).

Caprifoliaceae exhibit even greater fruit diversity. Fleshy, bird-dispersed fruits are limited to the Caprifolieae. Within this clade, *Leycestaria* has berries (endocarp lacking), with several to numerous seeds. *Lonicera* likewise has berry fruits, though generally with just a few seeds embedded in copious pulp. Drupe fruits, with an endocarp surrounding each seed, are found in *Triosteum* and *Symphoricarpos*. In both taxa, there is programmed carpel abortion and the number of seeds corresponds to the number of fertile carpels. In *Triosteum*, one of the four carpels aborts, and the drupes have three stones, whereas in *Symphoricarpos*, two of the four carpels abort, and there are two stones. The mesocarp in both cases is characteristically rather dry and mealy in texture.

The remaining Caprifoliaceae have dry fruits. In Diervilleae these are elongate bicarpellate capsules, with septicidal dehiscence releasing the sometimes distinctly winged seeds (in *Weigela*). In contrast, *Heptacodium* and all Linnina have achene fruits, bearing just a single seed at maturity.

Before considering fruit evolution in more detail, it is important to note that the ancestral fruit type for Dipsacales is uncertain. In part, this is from uncertainty about outgroups, but, as in the case of carpel number, it also reflects the distribution of fruit types among the basal lineages. Parsimony optimization on the tree in figure 1 (using MacClade 4.0; Maddison and Maddison 2000) implies that the first fruits probably were not achenes. It is possible that the first fruits were fleshy, but the results depend on whether fleshy fruits are broken into drupes versus berries and on exactly how relationships are resolved within Caprifolieae. Another possibility is that the first Dipsacales had capsular fruits with numerous seeds, in which case the larger number of seeds in *Leycestaria* might be viewed as a retained ancestral condition. Again, evaluation of such speculations will require the resolution of relationships within Caprifolieae.

Here, we highlight two aspects of fruit evolution for which we can be relatively certain about the direction of change. In the fleshy fruits, we focus on repeated changes in color, and for dry fruits, we concentrate on dispersal-related modifications of the achenes.

Fruit-Color Evolution

The fleshy fruits of Adoxaceae are usually dark purple or red at maturity; in some, they are more distinctly blue and covered by a waxy bloom (e.g., in *Viburnum* sect. *Lentago*) or, rarely, in some cultivated forms, even yellow. In Adoxina, the fruits tend to remain greenish at maturity. It is also worth noting that several colors, such as light metallic blue and white, as seen, e.g., in *Cornus*, do not occur in Adoxaceae.

Within both *Viburnum* and *Sambucus*, phylogenetic analyses indicate that dark purple is ancestral and red derived. In *Viburnum*, red fruits characterize section *Opulus* and a clade consisting almost entirely of Asian species from the large, paraphyletic section *Odontotinus* (Donoghue 1983*b*). Whether the red condition evolved once or twice is still unclear, but in either case, there have been several independent reversals to purple. Specifically, both the purple-fruited Asian species, *Viburnum melanocarpum*, and the New World species, *Viburnum acerifolium*, appear to be nested within the otherwise Asian red-fruited clade (Baldwin et al. 1995; R. C. Winkworth and M. J. Donoghue, unpublished data). The phylogenetic position of *Viburnum cylindricum* and its relatives of sect. *Megalotinus* is more uncertain. Mature fruit color is unclear for some species, but there may be additional reversals in this group. Within *Sambucus*, red fruits evolved in the derived paniculate clade (sect. *Botryosambucus*) and may also have independently evolved in the herbaceous sect. *Scyphidanthe* (Eriksson and Donoghue 1997).

Observations on fruit development in *Viburnum* suggest a possible mechanism for shifts in color. In the ancestral developmental sequence, retained for example in sects. *Solenotinus*, *Pseudotinus*, and *Viburnum*, the wall of the fruit matures from green, to yellow, to red, and finally to purple. The red phase is protracted, and fruits turn asynchronously to purple at the very end of the sequence. As a result, toward the end of the ripening period, a range of fruit colors is present in the infructescence. This sequence indicates the possibility that red fruits were attained through paedomorphosis. Studies of the absolute time from flowering to fruit maturity (Donoghue 1980) indicate that time to maturity is not markedly different in purple- and red-fruited species. This suggests neotony rather than progenesis, which is consistent with observations on fruit abscission (red fruits tend to remain attached longer) and on endocarp shape (red fruits have flattened stones resembling early stages in the inferred ancestral sequence of endocarp development). In any case, the evolution of red fruits may have involved changes in the timing of developmental events (heterochrony). Fruit-color development in *Sambucus* has not been observed in detail, but a similar mechanism may help explain the evolution of red fruits in this group as well.

The fleshy fruits of the Caprifolieae exhibit an even wider range of colors at maturity. Unfortunately, continued uncertainty about relationships within this clade, as well as limited sampling within *Lonicera*, restrict our ability to infer evolutionary sequences. However, the independent transition from red to bright white fruits within both *Symphoricarpos* and *Triosteum* is a particularly striking pattern. In this case, white is not part of the development sequence leading to red, which argues against the heterochronic explanation invoked for Adoxaceae.

Modifications for Achene Dispersal

In the Caprifoliaceae, achenes with a single seed are present in *Heptacodium* and in the large Linnina clade (though in *Dipelta*, and rarely in *Linnaea*, there are two seeds at maturity). The linkage of *Heptacodium* with Caprifolieae in recent analyses is surprising from the standpoint of fruit evolution because this implies either the independent evolution of achenes or a transition from achenes to fleshy fruits in the line leading to Caprifolieae (but see Zhang et al. 2002*b* on the possible hybrid origin of *Heptacodium*). In Linnina, where it has been studied in detail, evolution of the achene was evidently correlated with the abortion of two of the three carpels and the development of just a single ovule within the remaining fertile carpel (Wilkinson 1949). Carpel abortion occurs at a relatively late stage in ovary development in the Linnaeeae, Morinaceae, and Valerianaceae, as evidenced by the presence of two empty locules at fruit maturity in many species. In fact, in some species, these empty compartments have been co-opted in various ways in connection with dispersal (e.g., inflated for water dispersal in some *Valerianella*). In Dipsacaceae, remnants of the ancestral tricarpellate condition have been lost, and the ovary is described as "pseudomonomerous."

Among the achene-producing Caprifoliaceae, there are various adaptations for wind dispersal. In many cases, this has entailed modifications of the calyx. One of the most striking of these modifications is enlargement of the calyx lobes into wings as the fruits mature. Many botanists will have encountered the enlarged, often red-colored calyx wings of cultivated *Abelia*. A strikingly similar morphology is found in *Heptacodium*, and enlarged calyx lobes are also prominent in *Cryptothladia* infructescences.

Valerianaceae exhibit an especially wide array of calyx modifications, including loss (Eriksen 1989; fig. 5). Especially well known is the production of a feathery pappus-like structure in such species as *Valeriana officianalis* and *Centranthus ruber*. Others produce a leafy calyx (e.g., in *Nardostachys*), form "hooks" or a "crown-shaped" structure, or become inflated (e.g., in *Valerianella*). In other groups, the ovary wall itself is modified, especially for passive external transport by animals. *Kolkwitzia* (Linnaeeae) provides an excellent example, with the ovary wall covered by sharp, spinelike projections.

In Linnina, we encounter several important modifications of a set (possibly two to three pairs ancestrally) of so-called supernumerary bracts in the inflorescence (Troll and Weberling 1966; Fukuoka 1969; Weberling 1989). Their presence in the ancestor of the Linnina clade presumably resulted from the loss of flowers in the axils of bracts in previously more complex inflorescences. In any case, their existence made possible a close association with the ovary and a variety of modifications related to dispersal. For example, in *Linnaea*, two whorls of sterile bracts closely surround the ovary; these are covered with glandular trichomes that secrete a sticky substance. The most striking modification is found in the clade including *Dipelta* (three extant species native to China) and its fossil relative *Diplodipelta* (two species from the Eocene and Miocene of western North America; Manchester and Donoghue 1995). In these plants, members of two pairs of supernumerary bracts surround the ovary in flower and become greatly enlarged during fruit development. At maturity, these form membranous, papery wings that surround the fruit. In *Dipelta* the fruits are borne singly within the dispersal unit, whereas in *Diplodipelta* they were paired.

Evolution of the Epicalyx

It seems likely, on the basis of phylogenetic and developmental studies, that the epicalyx—a tubular structure that encloses the ovary in Morinaceae, *Triplostegia*, and Dipsacaceae—originated through the fusion of supernumerary bracts (Hilger and Hoppe 1984; Roels and Smets 1996; see below). However, the fact that phylogenetic analyses do not directly link Morinaceae with the *Triplostegia*-Dipsacaceae clade suggests a complicated history, involving either two separate origins of the epicalyx or a single origin and subsequent loss (fig. 4). A complex evolutionary scenario is consistent with subtle structural differences. Morinaceae are characterized by the presence of a single epicalyx, with usually 12 prominent ribs. In contrast, in Dipsacaceae the epicalyx is marked by the presence of eight ribs (reduced to four in *Knautia*). In *Triplostegia*, there are two epicalices. The inner one has eight ribs and is presumed to be homologous with the single epicalyx in Dipsacaceae. The outer epicalyx is four lobed and bears glandular hairs that presumably function in dispersal by animals.

Two scenarios for the evolution of the epicalyx are consistent with our present knowledge of phylogeny, morphology, and development (fig. 4). For the sake of argument, these each assume that ancestral Linnina had three pairs of supernumerary bracts subtending each flower (also see Roels and Smets 1996). In the first scenario (fig. 4*A*), the epicalyx originates separately in the Morinaceae and in the *Triplostegia*-Dipsacaceae clade. For Morinaceae, with 12 ribs, this assumes the fusion of all three sets of supernumerary bracts (two ribs per bract, four ribs per pair). For Dipsacaceae and the inner epicalyx of *Triplostegia* with eight ribs, we picture the fusion of the upper two pairs of bracts (Hilger and Hoppe 1984), leaving the third pair to form the outer epicalyx in *Triplostegia* (these bracts, or the outer epicalyx, were then lost in Dipsacaceae). Detailed developmental studies in *Dipsacus* and *Knautia* document that the epicalyx does originate as four primordia, which later become continuous. Valerianaceae entirely lack an epicalyx (Roels and Smets 1996). *Patrinia*, with three sets of bracts subtending the ovary, may have retained the ancestral condition, with bracts then further reduced in number within core Valerianaceae.

One possible alternative scenario entails a single origin of the epicalyx at the base of the Valerina clade and its subsequent

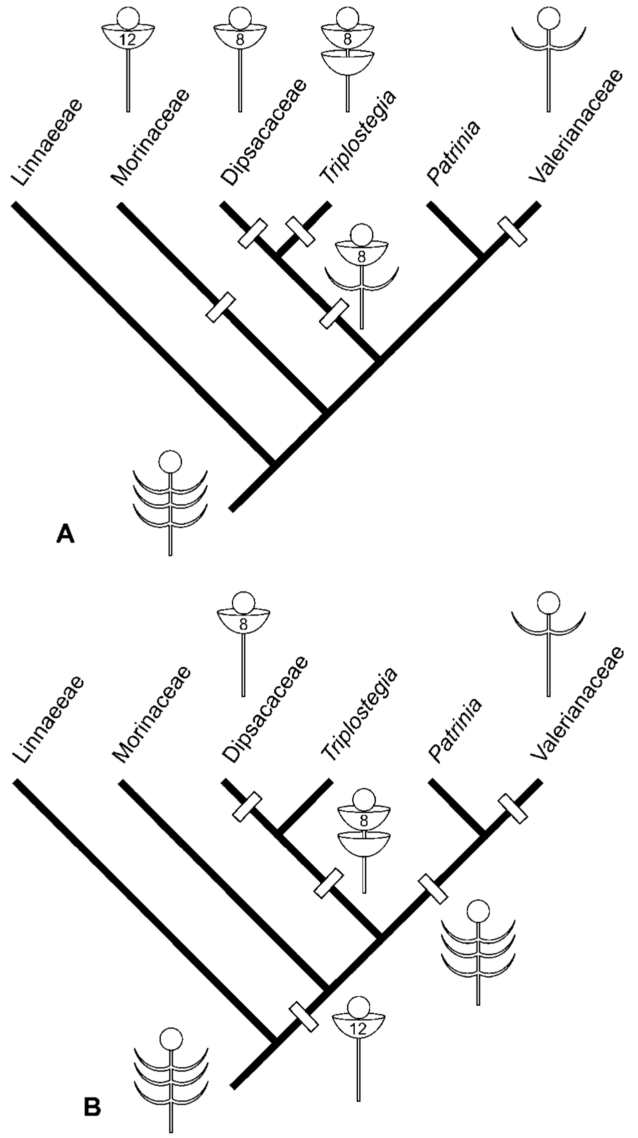

Fig. 4 Two scenarios for the evolution of the epicalyx in the Valerina clade. Both assume a hypothetical ancestor having three pairs of supernumerary bracts subtending the flower, and both take five steps when all events are equally weighted (fusion of bracts, loss of bracts, and so on). *A*, Independent evolution of the epicalyx in Morinaceae (with 12 ribs) and in *Triplostegia*-Dipsacaceae (with eight ribs); note outer epicalyx in *Triplostegia* and retention of the ancestral condition in *Patrinia*. *B*, A single origin of the epicalyx followed by dissolution (in *Triplostegia*-Dipsacaceae) and reversion to the ancestral condition (in *Patrinia* and the remaining Valerianaceae).

loss in Valerianaceae (fig. 4*B*). We view this sequence as somewhat less plausible, as it involves the deconstruction of the epicalyx in both the Dipsacaceae-*Triplostegia* lineage and in the Valerianaceae. Although the complete loss of the epicalyx may not present difficulties, the presence of bracts in Valerianaceae, especially the ancestral bract arrangement in *Patrinia*, implies the disassociation of formerly fused parts.

Whatever its origin, the epicalyx provided new functional opportunities and has been extensively modified for both protection and dispersal. Such modifications are especially obvious in the Dipsacaceae. Here, the true ovary wall has been greatly reduced and the epicalyx has largely taken over the protective function. At the same time, often elaborate outgrowths at the apex of the epicalyx appear to be adaptations for dispersal, and in this sense, the epicalyx has assumed the function of the calyx in other Valerina. In this case, however, the true calyx has often been maintained and apparently modified to function in concert with the epicalyx. Such modifications provide examples of "internal selection," in which the relevant environment for selection of a particular structure is provided by other structures within the suite of functionally interacting characters (Whyte 1965; Wagner and Schwenk 2000). The resulting functional integration may have established what Wagner and Schwenk (2000; also Schwenk and Wagner 2001) termed an "evolutionarily stable configuration." In such a system, the worth of an individual character change is evaluated largely in terms of its contribution to the entire functional unit, a relationship that may tend to maintain phenotypic stability even in the face of significant environmental change.

Epicalyx modifications may also provide examples of "transference of function" (Corner 1958; Baum and Donoghue 2002). Ovule protection and some germination-related functions seem to have been largely transferred to the basal portion of the epicalyx, which becomes progressively lignified, with corresponding reduction of the ovary wall (Verlaque 1984; Mayer 1995), while dispersal functions have been, at least in part, assumed by various expansions of the upper portion (Mayer and Ehrendorfer 1999). Similarities between various epicalyx modifications within Dipsacaceae and modifications of the calyx and ovary within the closely related Valerianaceae are quite striking in some cases (fig. 5). In *Scabiosa* and its relatives, a specialized diaphragm (produced by a meristem inside the apex of the epicalyx) forms a tight collar surrounding the specialized calyx stalk, effectively closing off the epicalyx (e.g., see Mayer and Ehrendorfer 1999). In some species, the diaphragm region of the epicalyx is further elaborated in the form of an epidiaphragm and corona, which together may form an upright or flattened, membranous, funnel-like outgrowth. These epicalyx structures function in concert with the often highly modified calyx lobes (which may be, for example, elevated on a stalk, increased in number, or covered with bristles or plumose hairs) and ovary (which sometimes forms a sclerified "ovarian cone" that may secure it within the epicalyx) (Caputo and Cozollino 1994). In some species distinct pits (or foveoles) are also formed near the apex of the basal portion of the epicalyx, in the zone of the diaphragm. Again, the results are sometimes strikingly similar to ovary modifications within Valerianaceae.

It is possible that these structures were selected de novo and that their development is underlain by different genes and developmental programs. Alternatively, the evolution of the epicalyx may have entailed the transfer of function through "homeoheterotopy" (Baum and Donoghue 2002). That is, genes expressed in the ovary wall and calyx before the origin of the epicalyx (as in the Valerianaceae and/or the Linnaeeae) may have been recruited to the newly originated and directly adjacent epicalyx. Such a common genetic basis may have enabled the evolution of a parallel suite of modifications in the two structures. It may also favor the scenario in figure 4*A*,

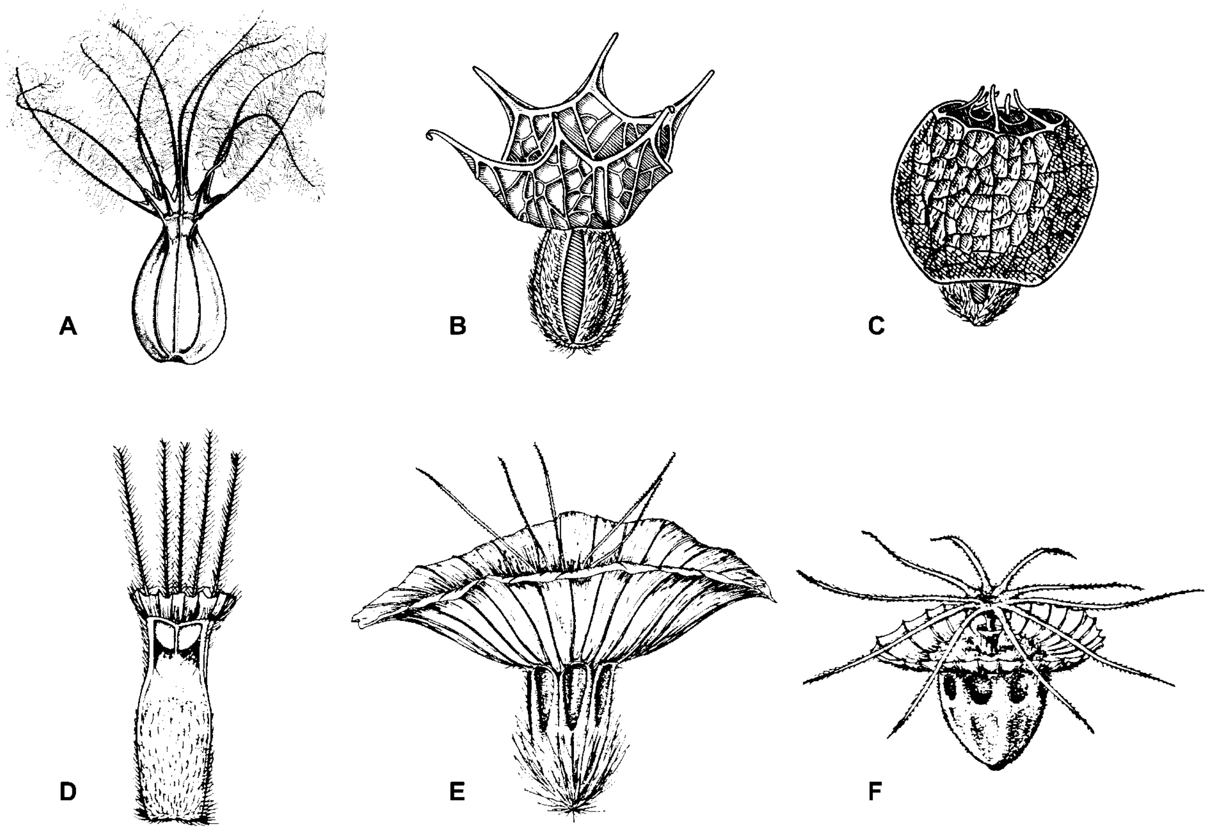

Fig. 5 Examples of "fruit" morphology, illustrating similar modifications of the ovary/calyx in Valerianaceae and of the epicalyx/calyx in Dipsacaceae. *A–C*, Valerianaceae, showing calyx and ovary wall modifications. *A*, *Valeriana tuberosa* with pappus-like calyx; *B*, *Valerianella coronata* with crown-shaped calyx; *C*, *Valerianella vesicaria* with inflated calyx. *D–F*, Dipsacaceae, showing calyx (protruding) and epicalyx (lower portion, with pitting, completely surrounding the ovary; upper portion with diaphragm and related structures). *D*, *Pycnocomon intermedia*; *E*, *Scabiosa rotata*; *F*, *Tremastelma palaestinum*. *A–C*, From Weberling (1989). *D–F*, From Mayer and Ehrendorfer (1999).

with the epicalyx originating by transference of the fusion of structures outward from the calyx to the subtending bracts. Carefully executed comparative developmental genetic analyses may provide a means to distinguish between these alternative explanations.

Conclusions

The Dipsacales provide examples of a number of evolutionary phenomena that may be of general interest for understanding morphological evolution. One of these is the apparent change in the relationship between the stamens and the corolla. In Adoxaceae, the number of stamens (or stamen pairs) strictly covaries with the number of corolla lobes, whereas in Caprifoliaceae, these have been decoupled. The establishment of monosymmetry may have predisposed the system to this decoupling and the individuation of repeated units within a whorl. Also of special interest is the emergence of a new morphological structure, the epicalyx. This, we suggest, provides an example of exaptation (Gould and Vrba 1982) in which the supernumerary bracts present in the Linnina clade were co-opted to build the new structure. The transference of functions to the epicalyx and the subsequent evolution of a functionally integrated unit consisting of the ovary, calyx, and epicalyx, may provide an example of internal selection leading to an evolutionarily stable configuration. Detailed developmental studies are needed to elucidate the mechanisms underlying these major evolutionary changes.

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Literature Cited

Angiosperm Phylogeny Group 1998 An ordinal classification for the families of flowering plants. Ann Mo Bot Gard 85:531–553.

Backlund A, K Bremer 1996 To be or not to be: principles of classification and monotypic plant families. Taxon 47:391–400.

——— 1997 Phylogeny of the Asteridae s. str. based on *rbc*L sequences, with particular reference to the Dipsacales. Plant Syst Evol 207:225–254.

Backlund A, MJ Donoghue 1996 Morphology and phylogeny of the order Dipsacales. *In* A. Backlund, ed. Phylogeny of the Dipsacales. PhD diss. Uppsala University.

Backlund A, S Nilsson 1997 Pollen morphology and the systematic position of *Triplostegia* (Dipsacales). Taxon 46:21–31.

Backlund A, N Pyck 1998 Diervillaceae and Linnaeaceae, two new families of caprifoloids. Taxon 47:657–661.

Baldwin BG, MJ Sanderson, JM Porter, MF Wojciechowski, CS Campbell, MJ Donoghue 1995 The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Ann Mo Bot Gard 82:247–277.

Baum DA, MJ Donoghue 2002 Transference of function, heterotopy, and the evolution of plant development. Pages 52–69 *in* Q Cronk, R Bateman, J Hawkins, eds. Developmental genetics and plant evolution. Taylor & Francis, London.

Baum DA, KJ Sytsma, PC Hoch 1994 A phylogenetic analysis of *Epilobium* (Onagraceae) based on nuclear ribosomal DNA sequences. Syst Bot 19:363–388.

Bell CD, MJ Donoghue 2002 Phylogeny and biogeography of Valerianaceae (Dipsacales), with special reference to the South American species. Botany 2002, abstract 451. http://www.botany2002.org/section12/abstracts/185.shtml.

——— 2003 Phylogeny and biogeography of Morinaceae (Dipsacales) based on nuclear and chloroplast DNA sequences. Org Div Evol 3:227–237.

Bell CD, EJ Edwards, ST Kim, MJ Donoghue 2001 Dipsacales phylogeny based on chloroplast DNA sequences. Harv Pap Bot 6:481–499.

Benko-Iseppon AM, W Morawetz 2000 Viburnales: cytological features and a new circumscription. Taxon 49:5–16.

Blackmore S, MJ Cannon 1983 Palynology and systematics of Morinaceae. Rev Palaeobot Palynol 40:207–266.

Bohnke-Gutlein E, F Weberling 1981 Palynologische untersuchungen an Caprifoliaceae. I. Sambuceae, Viburneae und Diervilleae. Trop Subtrop Pflanz 34:131–189.

Bolli R 1994 Revision of the genus *Sambucus*. Diss Bot 223:1–227.

Bremer B, K Bremer, N Heidari, P Erixon, RG Olmstead, AA Anderberg, M Kallersjo, E Barkhordarian 2002 Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. Mol Phylogenet Evol 24:274–301.

Bremer BR, G Olmstead, L Struwe, JA Sweere 1994 *rbc*L sequences support exclusion of *Retzia*, *Desfontainia*, and *Nicodemia* (Buddlejaceae) from Gentianales. Plant Syst Evol 190:213–230.

Cannon MJ, JFM Cannon 1984 A revision of the Morinaceae (Magnoliophyta-Dipsacales). Bull Br Mus Nat Hist (Bot) 12:1–35.

Cantino PK, K de Queiroz 2000 PhyloCode: a phylogenetic code of biological nomenclature. http://www.ohiou.edu/phylocode/.

Caputo G, S Cozzolino 1994 A cladistic analysis of Dipsacaceae (Dipsacales). Plant Syst Evol 189:41–61.

Chase MW, DE Soltis, RG Olmstead, D Morgan, DH Les, BD Mishler, MR Duvall, RA Price, et al 1993 Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbc*L. Ann Mo Bot Gard 80:528–580.

Corner EJH 1958 Transference of function. Bot J Linn Soc 56:33–40.

Cronk Q, M Moller 1997 Genetics of floral symmetry revealed. Trends Ecol Evol 12:85–86.

Cronquist A 1981 An integrated system of classification of flowering plants. Columbia University Press, New York.

de Queiroz K, J Gauthier 1994 Toward a phylogenetic system of biological nomenclature. Trends Ecol Evol 9:27–31.

Donoghue MJ 1980 Flowering times in *Viburnum*. Arnoldia 40:2–22.

——— 1983*a* The phylogenetic relationships of *Viburnum*. Pages 143–166 *in* NI Platnick, VA Funk, eds. Advances in cladistics. Vol 2. Columbia University Press, New York.

——— 1983*b* A preliminary analysis of phylogenetic relationships in *Viburnum* (Caprifoliaceae s.l.). Syst Bot 8:45–58.

——— 1985 Pollen diversity and exine evolution in *Viburnum* and the Caprifoliaceae sensu lato. J Arnold Arbor Harv Univ 66:421–469.

Donoghue MJ, BG Baldwin 1993 Phylogenetic analysis of *Viburnum* based on ribosomal DNA sequences from the internal transcribed spacer regions. Am J Bot 80(suppl):146.

Donoghue MJ, CD Bell, J Li 2001*a* Phylogenetic patterns in Northern Hemisphere plant geography. Int J Plant Sci 162(suppl):S41–S52.

Donoghue MJ, T Eriksson, PA Reeves, RG Olmstead 2001*b* Phylogeny and phylogenetic taxonomy of Dipsacales, with special reference to *Sinadoxa* and *Tetradoxa* (Adoxaceae). Harv Pap Bot 6: 459–479.

Donoghue MJ, RG Olmstead, JF Smith, JD Palmer 1992 Phylogenetic relationships of Dipsacales based on *rbc*L sequences. Ann Mo Bot Gard 79:333–345.

Donoghue MJ, RH Ree, DA Baum 1998 Phylogeny and the evolution of flower symmetry in the Asteridae. Trends Plant Sci 3:311–317.

Donoghue MJ, K Sytsma 1993 Phylogenetic analysis of *Viburnum* based on chloroplast DNA restriction-site data. Am J Bot 80(suppl): 146.

Downie SR, JD Palmer 1992 Restriction site mapping of the chloroplast DNA inverted repeat: a molecular phylogeny of the Asteridae. Ann Mo Bot Gard 79:266–283.

Eichler AW 1875 Blüthendiagramme, I, II. Engelmann, Leipzig.

Endress PK 1999 Symmetry in flowers: diversity and evolution. Int J Plant Sci 160(suppl):S3–S23.

Erbar C 1994 Contributions to the affinities of *Adoxa* from the viewpoint of floral development. Bot Jahrb Syst 116:259–282.

Eriksen B 1989 Notes on generic and infrageneric delimitation in the Valerianaceae. Nord J Bot 9:179–187.

Eriksson T, MJ Donoghue 1997 Phylogenetic analyses of *Sambucus* and *Adoxa* (Adoxoideae, Adoxaceae) based on nuclear ribosomal ITS sequences and preliminary morphological data. Syst Bot 22: 555–573.

Felsenstein J 1985 Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

Ferguson IK 1966 The genera of Caprifoliaceae in the southeastern United States. J Arnold Arbor Harv Univ 47:106–116.

Fritsch K 1891 Caprifoliaceae. Die Nat Pflanz 4:159–169.

Fukuoka N 1968 Phylogeny of the tribe Linnaeeae. Acta Phytotaxon Geobot 23:82–94.

——— 1969 Inflorescence of Linnaeeae (Caprifoliaceae). Acta Phytotaxon Geobot 23:153–162.

——— 1972 Taxonomic study of Caprifoliaceae. Mem Fac Sci Kyoto Univ Ser Biol 6:15–58.

Gould KR, MJ Donoghue 2000 Phylogeny and biogeography of *Triosteum* (Caprifoliaceae). Harv Pap Bot 5:157–166.

Gould SJ, E Vrba 1982 Exaptation: a missing term in the science of form. Paleobiology 8:4–15.

Hara H 1981 A new species of *Adoxa* from Mt. Omei of China. J Jpn Bot 56:271–274.

——— 1983 A revision of the Caprifoliaceae of Japan with reference to allied plants in other districts and the Adoxaceae. Academic Scientific, Tokyo.

Hilger VHH, M Hoppe 1984 Die Entwicklung des Aussenkelchs von *Scabiosa*, Sektionen *Sclerostemma* und *Trochocephalus* (Dipsacaceae). Beitr Biol Pflanz 59:55–73.

Hofmann U, J Gottmann 1990 *Morina* L. and *Triplostegia* Wall ex DC. im Vergleich mit Valerianaceae und Dipsacaceae. Bot Jahrb Syst 111:499–553.

Judd WS, RW Sanders, MJ Donoghue 1994 Angiosperm family pairs: preliminary phylogenetic analyses. Harv Pap Bot 5:1–51.

Kim T, B Sun, CW Park, Y Suh 1999 Phylogenetic implications of *mat*K sequences in Caprifoliaceae. Am J Bot 86(suppl):3202.

Kim YD, SH Kim 1999 Phylogeny of *Weigela* and *Diervilla* (Caprifoliaceae) based on nuclear rDNA ITS sequences: biogeographic and taxonomic implications. J Plant Res 112:331–341.

Lagerberg T 1904 Organografiska studier ofver *Adoxa moschatellina* L. Arkiv Bot 3:1–28.

Li J, MJ Donoghue 2002 Phylogenetics of *Lonicera* (Caprifoliaceae) based on sequences of internal transcribed spacer region of nuclear ribosomal DNA. Botany 2002, abstract 653. http://www.botany2002.org/section12/abstracts/121.shtml.

Li SH, ZH Ning 1987 Studies on the family Adoxaceae. Bull Bot Res 7:93–109.

Liang H 1997 A comparative study of floral vasculature in Adoxaceae. Acta Bot Yunnanica 19:260–264.

Liang H, X Zhang 1986 Floral anatomy of *Tetradoxa omeiensis*. Acta Bot Yunnanica 8:436–440.

Liu JQ, ZD Chen, AM Lu 2000 The phylogenetic relationships of an endemic genus *Sinadoxa* in the Qinghai-Xizang plateau: evidence from ITS sequence analysis. 42:656–658.

Luo D, R Carpenter, C Vincent, L Copsey, E Coen 1996 Origin of floral asymmetry. Nature 383:794–799.

Maddison DR, WP Maddison 2000 MacClade: analysis of phylogeny and character evolution, version 4.0. Sinauer, Sunderland, Mass.

Manchester SR, MJ Donoghue 1995 Winged fruits of Linnaeeae (Caprifoliaceae) in the tertiary of western North America: *Diplodipelta* gen. nov. Int J Plant Sci 156:709–722.

Mayer V 1995 The epicalyx in fruits of *Scabiosa* and *Tremastelma* (Dipsacaceae): anatomy and ecological significance. Bot Jahrb Syst 117:333–357.

Mayer V, F Ehrendorfer 1999 Fruit differentiation, palynology, and systematics in the *Scabiosa* group of genera and *Pseudoscabiosa* (Dipsacales). Plant Syst Evol 216:135–166.

——— 2000 Fruit differentiation, palynology, and systematics in *Pterocephalus* Adanson and *Pterocephalodes* gen. nov. (Dipsacaceae). Bot J Linn Soc 132:47–78.

Muller-Schneider P 1967 Zur verbreitungsbiologie des Moschuskrautes (*Adoxa moschatellina*). Vegetatio 15:27–32.

Olmstead RG, B Bremer, KM Scott, JD Palmer 1993 A parsimony analysis of the Asteridae *sensu lato* based on *rbc*L sequences. Ann Mo Bot Gard 80:700–722.

Olmstead RG, KJ Kim, RK Jansen, SJ Wagstaff 2000 The phylogeny of the Asteridae *sensu lato* based on chloroplast *ndh*F gene sequences. Mol Phylogenet Evol 16:96–112.

Peng C-I, H Tobe, M Takahashi 1995 Reproductive morphology and relationships of *Triplostegia* (Dipsacales). Bot Jahrb Syst 116:505–516.

Pyck N 2001 Phylogenetic relationships within Dipsacales: a combined molecular and morphological approach. PhD thesis. Katholieke University, Louvain, Belgium.

Pyck N, P Roels, E Smets 1999 Tribal relationships in Caprifoliaceae: evidence from a cladistic analysis using *ndh*F sequences. Syst Geogr Plants 69:145–159.

Pyck N, E Smets 2000 A search for the phylogenetic position of *Heptacodium* (Dipsacales). Plant Syst Evol 225:185–199.

Pyck N, A Van Lysebetten, J Stessens, E Smets 2002 The phylogeny of Patrinieae sensu Graebner (Valerianaceae) revisited: additional evidence from *ndh*F sequence data. Plant Syst Evol 233:29–46.

Roels P, E Smets 1994 A comparative floral ontogenetical study between *Adoxa moschatellina* and *Sambucus ebulus*. Belg J Bot 127: 157–170.

——— 1996 A floral ontogenetic study in the Dipsacales. Int J Plant Sci 157:203–218.

Schwenk K, GP Wagner 2001 Function and the evolution of phenotypic stability: connecting pattern and process. Am Zool 41:552–563.

Soltis DE, PS Soltis, MW Chase, ME Mort, DC Albach, M Zanis, V Savolainen, et al 2000 Angiosperm phylogeny inferred from 18S rDNA, *rbc*L, and *atp*B sequences. Bot J Linn Soc 133:381–461.

Stevens PF 2001– Angiosperm phylogeny web site, version 4, May 2003 (and more or less continuously updated since). http://www.mobot.org/MOBOT/research/APweb/.

Swofford DL 2001 PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland, Mass.

Tang Y, L Li 1994 The phytogeography of Caprifoliaceae s. str. with its implications for understanding eastern Asiatic flora. Acta Phytotax Sinica 32:197–218.

Thorne RF 1983 Proposed new realignments in the angiosperms. Nord J Bot 3:85–117.

Troll W, F Weberling 1966 Die infloreszenzen der Caprifoliaceen und ihre systematische Bedeutung. Abh Math Naturwiss Klasse Akad Wiss Lit Mainz 1966:455–605.

Verlaque R 1984 A biosystematic and phylogenetic study of the Dipsacaceae. Pages 307–320 *in* W Grant, ed. Plant biosystematics. Academic Press, Toronto.

Wagenitz G, B Laing 1984 Die Nektarien der Dipsacales und ihre systematische Bedeutung. Bot Jahrb Syst 104:91–113.

Wagner GP, K Schwenk 2000 Evolutionarily stable configurations: functional integration and the evolution of phenotypic stability. Pages 155–217 *in* MK Hecht, RJ MacIntyre, MT Clegg, eds. Evolutionary biology. Vol 31. Kluwer Academic, New York.

Weberling F 1957 Morphologische untersuchungen zur systematik der Caprifoliaceen. Abh Math Naturwiss Klasse Akad Wiss Lit Mainz 1957:1–50.

——— 1989 Morphology of flowers and inflorescences. Cambridge University Press, Cambridge.

White TJ, T Bruns, S Lee, J Taylor 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322 *in* M Innis, D Gelfand, J Sninsky, T White, eds. PRC protocols: a guide to methods and applications. Academic Press, San Diego, Calif.

Whyte LL 1965 Internal factors in evolution. Braziller, New York.

Wilkinson AM 1949 Floral anatomy and morphology of *Triosteum* and of the Caprifoliaceae in general. Am J Bot 36:481–489.

Winkworth RC, MJ Donoghue 2002 *Viburnum* phylogeny based on granule-bound starch synthase genes. Botany 2002, abstract 626. http://www.botany2002.org/section12/abstracts/184.shtml.

Wu CY 1981 Another new genus of Adoxaceae, with special references on the infrafamiliar evolution and the systematic position of the family. Acta Bot Yunnanica 3:383–388.

Wu CY, ZL Wu, RF Huang 1981 *Sinadoxa* C. Y. Wu, Z. L. Wu et R. F. Huang, genus novum familiae Adoxacearum. Acta Phytotax Sinica 19:203–210.

Zhang WH, ZD Chen, JH Li, HB Chen, YC Tang 2002*a* Phylogeny of the Dipsacales s.l. based on chloroplast *trn*L-F and *ndh*F sequences. Mol Phylogenet Evol 26:176–189.

Zhang ZY, ZK Zhou, ZJ Gu 2002*b* Karyomorphology of *Heptacodium* (Caprifoliaceae s. str.) and its phylogenetic implications. Taxon 51:499–505.

Zomlefer WB 1994 Flowering plant families. University of North Carolina Press, Chapel Hill.