

1

Gentianaceae in context

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INTRODUCTION

What does it take to recognize a family such as Gentianaceae? This is both an evolutionary biological question and one of perception and emphasis. Tracing back to the descriptor of Gentianaceae, Antoine Laurent de Jussieu (1789), gentians were distinguished as the Natural Order “Gentianeae”, one of 15 such orders in Jussieu’s class VIII, “*Dicotyledones monopetalae, corolla hypogyna*”. According to Lindley (1853), Jussieu usually derived the names for his Natural Orders from genera deemed well representative in their general structure. We therefore have Gentianaceae from Linnaeus’s *Gentiana*, defined by being dicotyledonous, sympetalous, and hypogynous. However, in the twenty-first century it is easy to see that many angiosperms, both those phylogenetically close and those phylogenetically distant from each other, could fit this bauplan. In one such example, Jussieu included *Mitreola* and *Spigelia* in Gentianaceae; in another, he included *Potalia*. These opinions were both pre-evolutionary (Darwin, 1859) and pre-phylogenetic (Hennig, 1966; Kluge & Farris, 1969), and were therefore based on different emphases of perceived morphological similarities and differences. In the first case, the hemi-apocarpous nature of the *Mitreola* and *Mitrasacme* gynoecium (Endress *et al.*, 1983; Conn *et al.*, 1996) matched the nascently apocarpous but postgenitally fused ovaries of many Gentianaceae (Padmanabhan *et al.*, 1978). *Spigelia* does not display this trait, but it does have generalized sympetalous and hypogynous flowers. *Potalia*, on the other hand, has a 4-lobed, decussate calyx, an 8–10-lobed sympetalous corolla with 8–10 adnate stamens, and a secondarily syncarpous gynoecium (Leeuwenberg & Leenhouts, 1980; L. Struwe & V. A. Albert, unpubl.). Moreover, all of these divergent taxa save *Gentiana* have been commonly assigned to Loganiaceae since Bentham (1857). To

confuse matters still further, *Fagraea*, a non-polymerous but secondarily syncarpous relative of *Potalia* (Struwe & Albert, 1997; Struwe *et al.*, 1998), was included in Jussieu's original circumscription of yet another family, Apocynaceae.

To quote *Sesame Street*, "One of these things is not like the other, one of these things just doesn't belong". In Jussieu's classification of Gentianaceae, differences and similarities must have been given unlike emphases (weights), a condition that could describe most if not all decisions made by the classical system-builders. Modern biological data can come to the aid of artificial classifications, but reliably so only if these data are viewed in an explicit phylogenetic context. For example, the distributions of certain phytochemicals among the angiosperms can suggest natural groupings. In the case of Gentianaceae, the combination of secoiridoid and xanthone production is a defining feature for most taxa (Jensen, 1992; Jensen & Schripsema, 2002 (Chapter 6, this volume)); only *Exacum* and close relatives apparently lack xanthenes (Saccifolieae have not yet been investigated). *Mitreola* lacks xanthenes and *Spigelia* species lack secoiridoids and xanthenes of the gentian types, whereas *Potalia* and *Fagraea* can be readily accommodated within Gentianaceae since completely comparable phytochemicals are produced (Jensen, 1992; Jensen & Schripsema, 2002). However, it is equally and ironically true, with reference to Jussieu, that the pollen of *Potalia* is more like that of some Apocynaceae rather than Gentianaceae, whereas *Fagraea* pollen is readily accommodated among that of the gentians (Nilsson, 2002 (Chapter 4, this volume)). Therefore, reliance on only single or few comparable traits cannot provide confidence in a natural classification. For example, the presence/absence of certain phytochemicals may result from duplication and divergence within single gene families (cf. Helariutta *et al.*, 1996; Eckermann *et al.*, 1998), and this could occur convergently among plants of different lineages (e.g., glucosinolates in Brassicales and Euphorbiaceae; Rodman *et al.*, 1998). Likewise, gross morphological similarities and differences can be phylogenetically deceptive (e.g., connation of petals into a tube in, for example, Asteridae, Cucurbitaceae, and Liliaceae, which are phylogenetically dispersed; APG, 1998; Soltis *et al.*, 2000).

The techniques of molecular phylogenetics have provided an opening to trace gentian phylogeny using traits of the genotype. Menyanthaceae, accepted as a subfamily of Gentianaceae by Gilg (1895), are now firmly nested inside Asterales from the standpoint of DNA data (Olmstead *et al.*, 1992; Gustafsson *et al.*, 1996; Gustafsson & Albert, 1999). Phenotypic characters are still of great importance to Gentianaceae circumscription

and classification, but it is the sum of all available evidence, cast in a phylogenetic framework, that most efficiently and objectively informs us about natural groupings of taxa. In the new, monophyletic classification of Gentianaceae presented in this book, *Mitreola*, *Spigelia*, and Menyanthaceae are excluded, while *Potalia* and *Fagraea* are included. These informed decisions are addressed below with respect to the gentian bauplan (architectural organization), its context, and its within-family variations, as well as the numerous structural gestalts (forms) shared among Gentianaceae, with other members of the gentian parent lineage (the order Gentianales and subclass Asteridae), and even with other angiosperms. We conclude with new, phylogenetically grounded perspectives on gentian diversity.

ASTERIDAE AND GENTIANALES: THE PARENT LINEAGE

The Asteridae comprises the numerous tube-flowered eudicot groups as well as some lineages of choripetalous plants that have sometimes been placed elsewhere (e.g., Cornales and Apiales; e.g., Cronquist, 1981). The subclass is strongly supported as monophyletic based on molecular systematic studies (APG, 1998; Soltis *et al.*, 2000). Two major subclades can be recognized in Asteridae, one including the Asterales (including Menyanthaceae), Dipsacales, and Apiales, and the second comprising the Lamiales, Oleales, Solanales, and Gentianales (Soltis *et al.*, 2000). Higher-order phylogenetic analyses identify the Gentianales as a large monophyletic group composed of three major and two minor subclades; these correspond to Gentianaceae (in the sense recognized in this book), Rubiaceae, Apocynaceae, Gelsemiaceae, and Loganiaceae (Struwe *et al.*, 1994, 1998; Backlund *et al.*, 2000). There are several important points to be made about this collection of taxa. First, pre-phylogenetic systems have commonly excluded the Rubiaceae based on their near ubiquitously epigynous flowers (e.g., Cronquist, 1981). This *ad hoc* emphasis placed on ovary position can be traced all the way back to Jussieu's system (1789), in which sympetalous angiosperms with hypogynous versus epigynous flowers were separated. Recent studies have shown that epigyny has evolved a number of times among otherwise hypogynous angiosperm groups (Gustafsson & Albert, 1999), and should therefore not necessarily be taken as a cardinal character at higher classificatory levels. Second, recent research has shown that the Asclepiadaceae, with their complex flowers, are merely an apomorphic derivation within Apocynaceae representing the epitome of floral synorganization (Endress *et al.*, 1996; Sennblad & Bremer, 1996; Civeyrel *et al.*, 1998;

Potgieter & Albert, 2002). Trends of character evolution leading up to the asclepiad condition can be clearly traced among various clades of Apocynaceae (MacFarlane, 1933; Endress *et al.*, 1996; Potgieter & Albert, 2002). Third, the heterogeneous Loganiaceae has been phylogenetically disassembled since Leeuwenberg and Leenhouts' 1980 treatment. *Buddleja* and Buddlejaceae have moved from Gentianales to Lamiales, near Verbenaceae (Oxelman *et al.*, 1999). Likewise, phylogenetic, morphological, and phytochemical evidence has moved *Desfontainia* to Dipsacales (Jensen, 1992; Struwe *et al.*, 1994; Backlund & Bremer, 1997), *Retzia* to Stilbaceae (Lamiales), and *Sanango* to Gesneriaceae (Bremer *et al.*, 1994; Jensen, 1994; Smith *et al.*, 1997; Oxelman *et al.*, 1999), to name just a few. Other former Loganiaceae (Struwe, 2002a, 2003) remain within Gentianales; *Potalia*, *Fagraea*, and *Anthocleista* (recall Jussieu!) form a monophyletic group nested far within the Gentianaceae (Struwe *et al.*, 1994, 1998; Struwe & Albert, 1997), and *Gelsemium* and *Mostuea* together form the sister group to Apocynaceae, now recognized as Gelsemiaceae (Struwe *et al.*, 1994; Backlund *et al.*, 2000; Struwe, 2003).

Despite the fact that many heterogeneous taxa were dispensed with as a result of phylogenetic consideration of Gentianales, such considerable diversity remains so as to make definition of an ordinal bauplan difficult. All recognized families except Rubiaceae have wood with intraxylary phloem (Struwe *et al.*, 1994). Solanaceae, which may be relatively closely related to Gentianales, also have this trait. Most Gentianales produce secoiridoid or complex indole alkaloid compounds, but several taxa of the strictly circumscribed Loganiaceae, as well as all investigated Gentianaceae, do not produce indole alkaloids (Jensen, 1992; Struwe *et al.*, 1994; Jensen & Schripsema, 2002). Nearly all Gentianales have opposite leaves with interpetiolar stipules or stipular lines, and colleters (small glandular structures) appear almost universally on the insides of leaf bases and/or calyces (Struwe *et al.*, 1994). Sympetaly is also nearly universal (save, e.g., *Theligonum* and *Dialypetalanthus*; Rutishauser *et al.*, 1998; Piesschaert *et al.*, 1997; Savolainen *et al.*, 2000), but then again, so is it in Gentianales' parent subclass, the Asteridae. The bottom line is that global morphological similarities among Gentianales families (i.e., synapomorphies) are not much to go on compared with such easily recognizable differences as inferior versus superior ovaries with placentation that varies both among and within the families of the order. Still, overlapping patterns of similarity and molecular phylogenetic evidence do identify Gentianales, and morphological evolution within the order's type family, Gentianaceae, should be evaluated from this context.

CHARACTERIZATION OF THE GENTIANACEAE LINEAGE

One of the historic problems with classifying Loganiaceae was that most taxa assigned to the family had rather generalized or plesiomorphic traits (see Leeuwenberg & Leenhouts, 1980). Thus, Loganiaceae became something of a grab-bag for taxa of Gentianales (and other orders) that didn't fit clearly anywhere else. As a whole, the same argument could be made for Gentianaceae, despite the fact that, based on molecular data, Gentianaceae are strongly supported both as monophyletic and as the second branching clade in Gentianales after Rubiaceae (Backlund *et al.*, 2000). Gentians simply do not bear readily stereotyped (e.g., highly syn-organized or otherwise jointly apomorphic) flowers, nor do they have a unified ovary or fruit structure or unique trait such as latex that would have pleased the early system-builders. The recent phylogenetic research that laid the foundation for the new classification of gentians presented in this book has in fact increased their already considerable morphological heterogeneity, legitimizing a perception of the family as becoming a sort of "natural grab-bag" in its own right. Three cases bear special mention.

Saccifolium

The addition to Gentianaceae of *Saccifolium* (Struwe *et al.*, 1998; Thiv *et al.*, 1999) alone adds saccate leaves (of still unknown functional relevance), a glandular disk inside the calyx possibly homologous to colleters, and a rare instance of imbricate corolla aestivation (most gentians are contort).

Saprophytes

In the same basal-most clade as *Saccifolium* lies *Voyriella*, a reduced, achlorophyllous mycotroph, as well as two to three genera that are heterostylous but that otherwise have generalized gentian flowers. Indeed, saprophytes (or parasites) have evolved at least four other times in Gentianaceae, in some *Sebaea* and *Cotylanthera* species (of Exaceae, the second-most basal clade of gentians; Struwe *et al.*, 2002 (Chapter 2, this volume)), in *Bartonia* and in *Obolaria* (separately placed within the derived Gentianeae; Struwe *et al.*, 2002), and in *Voyria*, which remains difficult to classify within the family (Albert & Struwe, 1997; Struwe & Albert, 2000).

Potaliinae

Another divergent and controversial gentian group comprises *Potalia*, *Anthocleista*, and *Fagraea*, which were for a long time assigned to the Loganiaceae as tribe Potalieae. Jussieu's early perceptions on *Potalia* (see above) were not heeded by later system-builders, including von Martius (1827), who erected Potaliaceae for all three genera. *Fagraea* was, ironically, the easiest to fit within the gentian bauplan, as was recognized by Bureau (1856) and Fosberg and Sachet (1974, 1980). Cladistic analyses of non-molecular data placed Potaliinae, now recognized at subtribal level, within Gentianaceae (Bremer & Struwe, 1992; Struwe *et al.*, 1994; Struwe & Albert, 1997), but owing to their shared possession of mainly generalized (plesiomorphic) phenotypic features only the combined presence of seco-iridoids and xanthenes has rendered the Potaliinae decisively gentian. This relationship was strongly corroborated by molecular evidence (Downie & Palmer, 1992; Olmstead *et al.*, 1993; Struwe *et al.*, 1998; Thiv *et al.*, 1999). Appearances can be deceptive: Potaliinae, which often become large trees, are apparently closely related to small herbs (e.g., *Faroua* and *Neurotheca*) as well as to shrubs and small trees (*Lisianthus* spp.). Furthermore, the floral and palynological features of *Potalia* and *Anthocleista* are divergent (see above), and all three genera bear fleshy berries in contrast to the dry or leathery (in *Symbolanthus*) capsules of most other gentians. Fruit fleshiness might be linked with congenital syncarpy in the group (L. Struwe & V. A. Albert, unpubl.), another anomalous feature. By displaying a mosaic mainly of autapomorphic (uniquely derived) and plesiomorphic (generalized Gentianales) features, the Potaliinae had been a classic example of misclassification due to crypticity in shared derived (i.e., phylogenetically informative) traits that might otherwise have been diagnostic.

Summary

In conclusion, a Gentianaceae bauplan is difficult to identify. Other than in cryptic phytochemical features, Gentianaceae are not particularly divergent from basal Apocynaceae, Gelsemiaceae, or Loganiaceae *sensu stricto*. Opposite leaves with colleters and stipular lines to ocreas, 4–5-part calyces with colleters, 4–5-part corollas with contort aestivation, and postgenitally fused gynoechia characterize most taxa, but the exceptions are notable. The major clades within Gentianaceae, classified in this book as tribes and subtribes, are in some cases diagnosable by morphological features that could be taken to represent *unterbaupläne*. Indeed, these architectural variants in

many cases hold much better within their respective lineages than do gentians among the various clades of Gentianales. For example, seeds with star-shaped testa cells, ovules positioned on entire inner surface of ovaries, helically twisting anthers, bilamellate stigmas, capitate stigmas, and stipitate gynoecea with sessile stigmas do well to identify most major gentian clades. Gentianaceae genera as classified in this book, however, are often more easily recognized as structural gestalts set off hierarchically by supporting molecular phylogenetic data. In other cases, it is species within genera that vary in this fashion. Since basic morphological, palynological, and phytochemical differences among Gentianaceae tribes, subtribes, and genera are covered in other chapters of this book, we will focus here on the patterns of occurrence of several common structural gestalts (themes) and their evolutionary correlates. Some of these will be seen to be perceptually related to emphases struck in previous, non-natural classifications of Gentianaceae and Gentianales. Our descriptions are not meant to be exhaustive treatises, but rather highlights of relevant themes.

GENTIANACEAE GESTALTS: EVOLUTION OF SHAPES AND OF CLASSIFICATIONS

Pollination syndromes

Features associated with pollination syndromes have long been recognized as structural gestalts that may or may not reflect phylogenetic relationships (see, e.g., Endress, 1994, for an in-depth treatment). For example, flowers with narrow corolla tubes and perpendicularly flattened corolla limbs (also known as salverform corollas) occur in several major gentian lineages, including *Voyria* (e.g., *Voyria caerulea*), Exaceae (e.g., *Tachiadenus carinatus*), Chironieae (*Centaurium erythraea*), and Gentianeae (e.g., *Gentiana verna*). Indeed, this flower form is not uncommon among other Gentianales (e.g., *Vinca* in Apocynaceae and *Ixora* in Rubiaceae), Asteridae (e.g., *Primula* in Primulaceae and *Syringa* in Oleaceae), and other angiosperms (e.g., *Dianthus* in Caryophyllaceae). Salverform flowers, which provide a landing platform for visiting insect pollinators such as butterflies, have therefore evolved convergently at several nested hierarchical levels, including during gentian diversification (i.e., they do not appear to be a phylogenetically primitive gentian trait).

Hawkmoth pollination, which involves longer and narrower, lightly colored corolla tubes, may occur in *Tachiadenus* (Exaceae) and the recently described *Aripuana* (Helieae). This syndrome is certainly present in other

Gentianales such as *Stephanotis* (Apocynaceae) and *Posoqueria* (Rubiaceae), and therefore, like salverform flowers, follows a similarly repetitive (homoplastic) phylogenetic distribution among Gentianaceae and Gentianales.

Features suggestive of bird pollination, not unexpectedly, also occur homoplastically within Gentianaceae and Gentianales. Although they are not common, tubular or salverform flowers with red-orange-yellow coloration are known among several apparently independent subclades of Helieae represented by *Calolisianthus*, *Celiantha*, *Lagenanthus*, *Neblinantha*, *Tachia*, and *Rogersonanthus* (*R. coccineus*, described by Struwe & Albert, 1998), and “Roraimaea” (ined., L. Struwe, S. Nilsson, & V. A. Albert, unpubl.). Additionally, some species of *Symbolanthus* (also Helieae), which typically have greenish-yellow or pink-red flowers (e.g., *S. elisabethae*, *S. pulcherrimus*, and *S.* “tetrapterus”), are known to be visited by hummingbirds (Struwe, 2000b). Bird pollination is also known among other families of otherwise insect-pollinated Gentianales, e.g., Loganiaceae (e.g., *Spigelia marilandica* and *Labordia waiolani*) and Rubiaceae (e.g., *Retiniphyllum*).

The aforementioned *Symbolanthus* case, however, becomes complicated since the gullet-shaped flowers of some yellow to green or white-flowered species (e.g., *Symbolanthus vasculosus*) are probably also visited by bats (cf. the case in *Chelonanthus alatus*; Machado *et al.*, 1998). Moreover, not all *Calolisianthus*, *Rogersonanthus*, or *Tachia* species have red-yellow-colored flowers, and, returning to the issue of salverform corollas, other species of *Gentiana* (e.g., *Gentiana acaulis*) have bell-shaped flowers typical of bumblebee pollination. Therefore, form and phylogeny in Gentianaceae show a poor correlation with respect to pollination syndromes. Pollination syndromes do, however, correlate at one level with extensive intra-lineage diversification among genera, for example, within Helieae.

Fruit form

Most Gentianaceae have dry, capsular fruits, and this could be one main reason why the fleshy-fruited Potaliinae had been excluded from the family for so long. *Potalia*, *Anthocleista*, and *Fagraea* almost always bear fleshy, indehiscent berries (rarely dehiscent owing to the enormous size of the fruit in *Fagraea auriculata*; Leenhouts, 1962), as do some genera of Loganiaceae *sensu stricto* (*Gardneria* and *Strychnos*). The fleshiness of Potaliinae berries appears to correlate with congenital syncarpy, a trait only rarely seen among other gentians (i.e., dry-fruited *Aripuana*; Struwe *et al.*, 1997), which characteristically have postgenitally fused ovaries. The state of ovary fusion in fleshy-fruited species of *Crawfordia* and *Tripterosperrum* (of

Gentianeae) has not been thoroughly investigated, but leathery-fruited *Symbolanthus* (Helieae) definitely has postgenital fusion (L. Struwe & K. Gould, unpubl.). In Potaliinae, the ovaries (but not always the mature fruits) of *Anthocleista* and *Potalia* have the same bilobed, inrolled placentas as do many other taxa of the family (e.g., Helieae), whereas *Potalia* placentas appear to be more axile, a finding that requires further study. Fruits of at least West African species of *Anthocleista* are known to be eaten by hammerheaded bats (Bradbury, 1984), and it is plausible that the fleshy, whitish, upright, and open flowers may be pollinated by the same animals. The animal dispersers of *Potalia* seeds are unknown, but their smaller flowers with small to tiny openings borne on electric-yellow cymes seem more likely bird pollinated. *Fagraea* species have many potential pollination syndromes from hawkmoth to bat pollination, but, at least in the widespread Pacific taxa *Fagraea berteriana* and *F. gracilipes*, seed dispersal by birds seems most likely, although some bat-pollinated species may also be bat dispersed (Ridley, 1930).

Not only is it *not* strange to find fleshy-fruitedness to be derived within a Gentianales clade, it is also not at all unexpected to find this feature associated with congenital syncarpy. Within Apocynaceae, *Carissa* and *Akocanthera* had previously been thought to occupy a rather basal position in this otherwise strongly apocarpous family (Leeuwenberg, 1994; Endress *et al.*, 1996). Instead, molecular data have proven them to be highly derived phylogenetically, occupying a position proximal to higher Apocynaceae, periplocoids, and asclepiadoids (Potgieter & Albert, 2002). Dry-fruited, apocarpous taxa such as *Aspidosperma* occupy the basal-most branches of the family, which suggests these features to be the ancestral conditions for the clade (Potgieter & Albert, 2002). After the overturning of such conventional wisdom in Apocynaceae, the derived status of Potaliinae in Gentianaceae seems hardly more controversial. Instead, it just reinforces an evolutionary hypothesis that congenital syncarpy may be a prerequisite to fleshiness in otherwise capsular clades. The fleshy, apocarpous fruits common in Apocynaceae (e.g., among the Alyxieae; Endress *et al.*, 2000) appear to have evolved on a different ontogenetic trajectory, directly from apocarpous, dry-fruited lineages. Regardless, one can easily see that fleshy-fruitedness is a homoplastic trait in both Gentianaceae and Apocynaceae, correlated more with fruit dispersal syndrome than with phylogeny. The homoplastic distribution of fruit fleshiness in Gentianales had been demonstrated already for Rubiaceae (e.g., Bremer & Eriksson, 1992), for which fruit features had previously served as cardinal characters for classification (Robbrecht, 1988). Similar cases in which fruit fleshiness is derived within

an asterid clade come from taxa endemic to the Hawaiian Islands; the baccate-fruited genera *Clermontia* and *Cyanea* (Campanulaceae) are derived from dry-fruited lobelioid ancestors (Givnish *et al.*, 1995), and the mints with fleshy nutlets (*Phyllostegia* and *Stenogyne*, Lamiaceae) descend from a dry-fruited lineage characterized by several independent derivations of fruit fleshiness (Lindqvist *et al.*, 2000).

Plant habit

A further possible reason why Potaliinae were excluded from earlier Gentianaceae classifications could concern their woody habit. *Potalia* species range from sparsely stemmed shrubs to 12 m tall trees, *Anthocleista* species are also usually single-stemmed trees but are often much taller and considerably more branched, and *Fagraea* species range from lianas to shrubs to tall, branched trees up to 30 m in height. However, other Gentianaceae, for example members of neotropical Helieae, may also form small trees (*Macrocarpaea* spp. can be up to 10 m tall and 10 cm in diameter at the base; J. R. Grant & L. Struwe, unpubl.). Indeed, it was after examining some of these taxa that Fosberg and Sachet (1980) noted the strong similarity between *Fagraea* and genera such as *Macrocarpaea*. Although the primitive state for Gentianaceae may be woody (e.g., the Saccifolieae and Exaceae are largely suffrutescent perennials), short-lived, herbaceous plants with little secondary growth are derived in Chironieae, Faroinae, and Gentianeae, but so are trees in Potaliinae and Helieae. The latter pattern appears *not* to be the case for Apocynaceae, in which many of the basal-most taxa (e.g., *Aspidosperma*) are tall trees, whereas a number of derived taxa (such as *Asclepias*) are herbaceous. The same appears true of Loganiaceae *sensu stricto*, in which the basal lineages are woody and arborescent (e.g., *Antonia*), while some derived taxa such as *Mitreola* and *Mitrasacme* are herbaceous, and others are woody (e.g., *Geniostoma*). Woodiness and arborescence in Gentianales are obviously sequentially correlated, but the phylogenetic status of arborescence appears to be independent of the acquisition of woodiness in Gentianaceae versus Apocynaceae and Loganiaceae.

To conclude, taking a bauplan, adding various gestalts, and having multiple originations of features (i.e., parallelisms and reversals) is a simple way to parameterize diversity. A classification can be a model that employs these parameters while still retaining biological information for practical storage and retrieval. A phylogenetic system, such as that presented in this book, is precisely the sort that permits stable, well-grounded assessments of diversity (Stevens, 1997; Struwe & Albert, 1997) both within Gentianaceae