

Pigment Evolution in the Caryophyllales: a Systematic Overview*

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Abstract: While the apparent mutual exclusiveness of anthocyanins and betalains in the Caryophyllales has given rise to considerable taxonomic debate, historical factors affecting the present distribution of these compounds have rarely been discussed. An understanding of pigment evolution in the order is hindered by a number of unresolved systematic issues and a lack of knowledge of the importance of anthocyanins and betalains beyond their roles in pollination and seed dispersal. The hypothesis that betalains arose in an unpigmented ancestor of the Chenopodiinae in response to selection from pollinators cannot be rejected, but scant evidence exists in favor of it. Questions persist regarding whether the most recent ancestor to the Chenopodiinae presented a pigmented floral display and whether the appropriate pollinators were present at this time to select for floral pigmentation. In view of these ambiguities and the possible non-monophyly of the Chenopodiinae we consider some alternative scenarios and suggest potentially rewarding avenues for future research. We discuss roles for anthocyanins and betalains beyond their use as optical attractants, possible costs and benefits associated with producing each pigment type, and the possibility that they may have co-occurred in an ancestor for some period of time.

Key words: Caryophyllales, anthocyanins, betalains, pollination biology, flower color.

One of the best known controversies in angiosperm systematics in the 1960s and 1970s regarded the taxonomic significance of the betalains, a unique class of vacuolar pigments restricted in the angiosperms to 13 families in the order Caryophyllales, and the apparent mutual exclusiveness of these compounds and the anthocyanins (Clement et al., 1994). Anthocyanins are found in two families in the order, the Caryophyllaceae and Molluginaceae, as well as throughout the rest of the angiosperms. Anthocyanins seem to be absent from the rest of the Caryophyllales and have been replaced in almost every aspect of metabolism by betalains. They are produced not only in flower and fruit displays, but often also upon wounding, pathogenic infection and senescence, and in seedlings and young shoots (Piatteli, 1981; Clement et al., 1994; Stafford, 1994). Phytochemical surveys of the Caryophyllales (Bate-Smith, 1962; Bittrich and Amaral, 1991) have

revealed a diversity of flavonoids in betalain-producing plants, suggesting they lack one critical step of anthocyanin biosynthesis, the ability to convert leucoanthocyanidin to anthocyanidin.

The argument among taxonomists and chemists centered on whether or not the presence of betalains delimited a natural taxon (to the exclusion of the anthocyanin families) and at what hierarchical rank this group should be recognized (see Cronquist and Thorne, 1994, for a recent review). In spite of the interest the issue has generated, only rarely have comments appeared in the literature that provide a framework for understanding the evolution of the compounds themselves. Given the rigor introduced by the development of the Hennigian approach to systematics and the advent of methods which have allowed explicit statements about the relationships of anthocyanin and betalain plants (Rodman et al., 1984; Rettig et al., 1992; Manhart and Rettig, 1994; Downie and Palmer, 1994; Rodman, 1994), it is remarkable that no recent attempt has been made to develop or evaluate hypotheses of pigment evolution in the order within a phylogenetic context. We provide here some preliminary statements toward such an evaluation, in the form of a brief review of the relevant biochemical, systematic, and evolutionary issues.

A truly evolutionary approach to the problem should include not only a phylogenetic perspective but also a consideration of the fitness effects of the presence/absence of the different pigment types in past environments. For this reason, we discuss factors (such as floral morphology, presence of pollinators) that may indicate ancestral pollination systems in the order, other possible roles for the compounds beyond pollinator attraction, and the benefits and costs associated with producing each type of compound. An assessment of the importance of alternative functions is hindered not only by the difficulty in reconstructing past ecological interactions and their evolutionary consequences (Frumhoff and Reeve, 1994), but also by the poor state of knowledge of what these functions may be, as well as the gulf between our understand-

* The second author of this review is especially pleased to honor Prof. Schnepf on his birthday. For over 20 years Mabry has had a joint project with Prof. Behnke on the Caryophyllales. Every summer Mabry has lectured on plant chemistry in the Zellenlehre, Universität Heidelberg, and Prof. Schnepf regularly attended these lectures. Although natural product chemistry is not his field, he always posed challenging questions and made stimulating comments.

ing of plant secondary metabolism in general and its fitness effects (Berenbaum, 1995). Nevertheless, the present state of knowledge is sufficient to make a partial critique of past proposals and to map future avenues of research.

Table 1 presents the known distribution of betalains and anthocyanins in the Caryophyllales. These groups will be referred to throughout this paper as the Chenopodiinae (betalain-producing plants) and Caryophyllinae (anthocyanin-producing plants) sensu Mabry (1977). A listing of individual genera is provided in Clement et al. (1994). It should be noted that monophyly is not at all certain for many of the taxa listed in Table 1, including the two sub-orders themselves.

Table 1 The distribution of betalains and anthocyanins in the Caryophyllales, showing the proportion of the number of genera in which pigments have been detected to the total number of genera in each group. Taxonomy follows Kubitzki et al. (1993) except for Molluginaceae, which follows Hofmann (1973) excl. Gisekia, Sesuviae.

Chenopodiinae (betalain-producing taxa)	
Achatocarpaceae (0/2)	Halophytaceae (1/1)
Aizoaceae (46/127)	Hectorellaceae (1/2)
Aizooidae (0/6)	Nyctaginaceae (11/31)
Mesembryanthemoideae (2/9)	Abroniae (1/1)
Ruschioideae (41/106)	Boldoae (1/3)
Sesuvioideae (2/4)	Bougainvilleae (1/2)
Tetragonioideae (1/2)	Leucastereae (0/4)
Amaranthaceae (14/69)	Nyctagineae (7/15)
Amaranthoideae (9/55)	Pisoniae (1/3)
Gomphrenoideae (5/14)	Phytolaccaceae (7/18)
Basellaceae (2/4)	Agdestioideae (1/1)
Cactaceae (27/98)	Barbeuioideae (0/1)
Cactoideae (25/91)	Microteoideae (0/2)
Opuntioideae (1/5)	Phytolaccoideae (2/4)
Pereskioideae (1/2)	Rivinoideae (3/9)
Chenopodiaceae (15/98)	<i>incertae sedis</i> – Gisekia
Chenopodioidae (12/44)	Portulacaceae (12/30)
Polycnemoideae (0/3)	Calyptrotheceae (0/1)
Salicornioideae (1/14)	Portulacarieae (2/2)
Salsoloideae (2/38)	Portulacaceae (4/5)
Didiereaceae (4/4)	Talineae (5/21)
	<i>incertae sedis</i> – Talinella
	Stegnospermaceae (1/1)
Caryophyllinae (anthocyanin-producing taxa)	
Caryophyllaceae (15/86)	Molluginaceae (3/11)
Alsinoideae (4/28)	Corbichonieae (0/1)
Caryophylloideae (7/24)	Limeae (1/2)
Paronychioideae (4/34)	Mollugineae (2/8)

Surprisingly little attention has been paid to the evolution of anthocyanins and betalains in the Caryophyllales. Mabry (1973, 1976) held the view that the Chenopodiinae and Caryophyllinae diverged prior to the origin of red-violet floral pigmentation in angiosperms. While this could be interpreted

to mean that an ancestor to the order was “essentially without pigments” (Harborne and Turner, 1984, p. 306), the well-established homology of anthocyanin structural and regulatory genes among monocots and dicots (Koes et al., 1994; Forkmann, 1994) contradicts this. A more reasonable view is that the split of anthocyanin and betalain groups in the Caryophyllales predates not the anthocyanin pathway itself but the use of these pigments in floral displays. In other words, the Caryophyllales must be descended from a line of plants that at one time possessed the genetic framework for anthocyanin production. In the betalain lineages, then, this ability was subsequently lost.

Ehrendorfer (1976) elaborated just such a scenario, with the loss of anthocyanin production in an ancestor to the betalain group as the plants evolved in pollinator-poor, arid to semi-arid Cretaceous habitats. Betalains appeared later as a response to the movement of discriminating pollinators into more arid environments and/or the adaptation of Caryophyllalean lineages to more mesic, pollinator-rich environments. Mabry (1977) accepted this interpretation.

These views rely heavily on the monophyly of the Chenopodiinae and on the importance of pollinators in leading to the present-day mutual exclusiveness of these compounds. The first assumption has been called into question in a number of recent phylogenetic studies (Rettig et al., 1992; Downie and Palmer, 1994; Manhart and Rettig, 1994; Rodman, 1994). Cronquist (1977) intimated that the second assumption may be unfounded by stating that “a search for the biological significance of the betalains should concentrate on their repellent (and fungicidal) properties, rather than on their function as flower pigments (p. 187).” Taken together, these issues suggest a number of questions: 1) What evidence is there for or against an ancestor of the Chenopodiinae which did not utilize red-violet pigments in floral display evolving into one which did, prior to the radiation of the group? 2) Were appropriate pollinators indeed absent at a critical point in the evolution of the Caryophyllales, and when did their presence begin to influence the evolution of floral pigments? 3) What other roles could anthocyanins and betalains have played in the early evolution of the order beyond pollinator attraction? 4) Perhaps most importantly, is the Chenopodiinae monophyletic, as has often been assumed?

Ancestral Use of Pigments – Floral Display

Ehrendorfer (1976) held that pollinators were “at least partially” responsible for the origin of betalains. In the strictest sense, this view implies that the most recent common ancestor to the Chenopodiinae presented a pigmented floral display. It is not possible to determine, though, if this is his interpretation or if he means that betalain synthesis originated in an ancestor with little or no floral display and the compounds were then siezed upon as pollinator attractants early in the evolution of a number of different lineages. Betalain-pigmented floral parts appear in the sub-order variously as staminodes, tepals, or bracts (Table 2, Kubitzki et al., 1993), suggesting that the problem of pollinator attraction has been posed in many different lineages and solved in different ways. Additionally, the most dramatic uses of pigments in floral displays are often in rather derived groups – Nyctaginaceae, Cactaceae, Mesembryanthemoideae/Ruschioideae (Aizoaceae). This is true also in the anthocyanin-

Table 2 Floral organs used as optical attractants in the Caryophyllales. These organs are pigmented to some degree unless otherwise noted. The homology of "tepals" among Caryophyllales is still in dispute, especially those of the Portulacaceae and allied families.

Bracts

Amaranthaceae – Gomphrenoideae
Nyctaginaceae – Bougainvilleae

Tepals (free)

Aizoaceae – Aizoioideae, Sesuvioideae, Tetragonioideae
Amaranthaceae – Amaranthoideae; Townsend (1993) describes only *Ptilotus* and *Volkensinia* as pigmented
Basellaceae
Cactaceae
Caryophyllaceae – Paronychioideae (not known to be pigmented)
Chenopodiaceae – Kühn (1993) mentions that some *Anthochlamys* and *Beta* are pigmented
Didiereaceae
Molluginaceae
Phytolaccaceae – Agdestioideae (not known to be pigmented), Phytolacchoideae, Rivinoideae
Portulacaceae

Tepals (fused)

Nyctaginaceae – all tribes except Bougainvilleae; Boldoae and Leucastereae not pigmented

Staminodia

Aizoaceae – Mesembryanthemoideae, Ruschioideae
Caryophyllaceae – Paronychioideae (in part), Alsinoideae (pigments rare), Caryophylloideae
Molluginaceae – occasional in *Corbichonia*, *Glinus*, *Limeum* but only rarely known to be pigmented
Stegnosperrmaceae

Stamens/anthers/appendages

Chenopodiaceae – occasional in *Bassia*, *Salsola*, *Suaeda* (see Blackwell and Powell, 1981; Kühn, 1993)

Nectaries

Caryophyllaceae – restricted to *Alsindendron* (Alsinoideae)

producing Caryophyllinae, with showy, pigmented flowers being largely restricted to the Caryophylloideae. The bulk of the Achatocarpaceae, Amaranthaceae, and Chenopodiaceae, as well as some members of all other major lineages, are not at all showy. However, there are a great many plants exhibiting at least a faint pink flush in their flowers. Included among these are much of the Portulacaceae (many of which are quite showy) and Phytolaccaceae, *Stegnosperrma*, the anthocyanic *Hypertelis* (Molluginaceae) and *Spergularia* (Paronychioideae, Caryophyllaceae), and others. Two distinctive pigmented floral displays appear in the Aizoaceae, petaloid staminodes in the Mesembryanthemoideae/Ruschioideae and adaxially pigmented sepals in the other subfamilies (Bittrich and Hartmann, 1988). The primitive form in this family cannot yet be determined. If the two forms delimit sister groups, they may both have evolved from a non-showy ancestor.

Traditionally, the Caryophyllales was thought to have evolved from Ranalean ancestors into something resembling the modern-day Phytolacchoideae (Friedrich, 1956; Buxbaum, 1961) prior to the radiation of the order. Ehrendorfer (1976) noted that this view was difficult to reconcile with the

pigment data. However, the Phytolacchoideae is now widely rejected as the archetype of the order (Rohweder, 1965; Hofmann, 1994), the pseudoapocarpous ovaries being regarded as derived from the more common syncarpous type.

Similarly, the classical view that the general trend in Caryophyllalean flower morphology has been one of reduction has led Leins and Erbar (1994) to conclude that flowers of the Cactaceae and Ruschioideae (Aizoaceae), which are often vividly pigmented, represent the archetype for the order. This is in spite of mounting evidence that these groups are unlikely to be closely related (Gibson and Nobel, 1986; Bittrich and Hartmann, 1988) and seem to actually possess a number of derived characters with respect to the bulk of the Caryophyllales (e.g., one can compare Ruschioideae to the rest of Aizoaceae, Bittrich and Struck, 1989; Cactaceae to the rest of the portulacaceous alliance, Hershkovitz, 1993). The fact that these two groups have diversified in the relatively young American and southern African arid zones (Axelrod and Raven, 1978; Goldblatt, 1978; Mauseth, 1990) also contradicts Leins and Erbar's (1994) view.

The strongest candidate for archetypal floral form in the Chenopodiinae and, indeed, the Caryophyllales as a whole is that of uniseriate pentamery, as found in the Caryophyllaceae, Chenopodiaceae, Amaranthaceae, Achatocarpaceae, Phytolaccaceae, Nyctaginaceae, Basellaceae, much of the Aizoaceae, and the Portulacaceae (Hofmann, 1994). It remains to be resolved then whether in the Chenopodiinae the ancestral flower was of the inconspicuous, herbaceous type (Amaranthaceae, Chenopodiaceae) or was at least somewhat showy, with petaloid white to pink/purple tepals (Phytolacchoideae, Portulacaceae, Aizoaceae). If the ancestral floral form of the Chenopodiinae turns out to have been pigmented, then floral pigmentation may have been lost and regained in several independent lineages.

Ehrendorfer (1976) based much of his argument for the loss of anthocyanin biosynthesis on a shift to anemophily in a "proto-Centrospermous" plant. Even if we accept the inconspicuous flowers of the Chenopodiaceae/Amaranthaceae as an archetype, though, it is hardly necessary to invoke wind pollination. Over a century ago it was noted that the tiny, unpigmented flowers of many chenopodiaceous genera were probably entomogamous (Volkens, 1893). This position has been supported by more recent authors (Blackwell and Powell, 1981; Kühn, 1993). More intensive systematic investigation of the Chenopodiaceae and Amaranthaceae should resolve whether or not anemophily is ancestral in this group.

The Presence of Appropriate Pollinators

If betalains arose only once, then betalain production may date to the mid-Cretaceous, as pantoporate fossil pollen ascribed to the Chenopodiaceae/Amaranthaceae (Muller, 1981) has been placed as far back as the Maestrichtian, 70 million years ago (mya). On the basis of biogeography, much of the diversification of the order may have occurred prior to, or coincident with, the breakup of Gondwanaland (i.e., as much as 100 mya; Carolin, 1982; Gibson and Nobel, 1986; Bittrich, 1993b; Hershkovitz, 1993). We must then ask whether or not the age of the order is compatible with the age of appropriate pollinators (i.e., those capable of discriminating red-violet or yellow colors). There is evidence for a significant

presence of aculeate hymenopteran and lepidopteran pollinators in the Cretaceous (Crepet et al., 1991). Additionally, many modern members of both groups respond to red-violet pigmented flowers (Willemstein, 1987) and are important pollinators in modern arid environments (Moldenke, 1976; Simpson and Neff, 1987; Struck, 1994). Nevertheless, since the visual and behavioral faculties of their ancestors are unknown, and given the difficulty in associating the appearance of the potential pollinators in time and space with Caryophyllalean ancestors, the influence of insects on the early evolution of the order remains an open issue.

Since members of the Chenopodiinae produce both red-violet betacyanins and yellow betaxanthins, there is also the question of which set of pigments would have been selected for in an ancestor. Betaxanthins are biosynthetically simpler than betacyanins, and when betacyanins have been detected by HPLC in plant tissues they are almost invariably accompanied by betaxanthins (Steglich and Strack, 1990). Additionally, betalamic acid (a precursor to all betalains) is itself an important yellow pigment in some species (Reznik, 1978). However, while betaxanthins often appear as the sole floral pigments (in, for example, members of the Cactaceae, Ruscioideae/Mesembryanthemoideae, Nyctaginaceae), the plants in question are clearly derived from plants that produce both betaxanthins and betacyanins. Again, though, the Portulacaceae may be an exception. The plesiomorphic distribution of betacyanins in floral displays in many groups suggests that they played an important role in the early evolution of the sub-order. Of course, if selection for betalains was originally through a physiological function, and they were only later utilized as visual cues, we have no means of determining the early significance of betacyanins vs. betaxanthins.

Ancestral Use of Pigments – Alternative Roles

We should consider the possible adaptive value of the pigments beyond their use in floral display, as it may be that betalains had their origin prior to the diversification of the Chenopodiinae, but were only later exploited in pollinator attraction. One potential source of selection for pigmentation is fruit color for dispersal via endoornithochory (Willson and Whelan, 1990). However, the distribution of pigmented fruit displays in the order is taxonomically sporadic, in many cases the taxa are clearly derived with reference to some broader group of dry-seeded plants, and, as with floral displays, a diversity of parts are utilized, suggesting multiple independent adaptations.

Functions for betalains which do not require the accumulation of visible amounts of pigmentation, such as indoleacetic acid oxidase inhibitors, green light receptors, and pathogen/herbivore defenses (Cronquist, 1977, 1981; Piatelli, 1980; Stafford, 1994), deserve further attention. Similar roles have been suggested for anthocyanins (McClure, 1975). Unfortunately, empirical evidence is lacking for both betalain- and anthocyanin-containing plants. An argument for alternative roles for betalains comes from their presence in fungi (Steglich and Strack, 1990), where the compounds do not appear to have any adaptive value as pigments *per se*. Again, though, if the betalains are presumed to have functionally replaced the anthocyanins and if the two types of compounds have never co-occurred, we must invoke a relaxation of the selective

maintenance of anthocyanin production, followed by renewed selection once the ability to make anthocyanins had been lost.

Monophyly of the Betalain Group

The Chenopodiinae (Table 1) has little to commend it as a natural group beyond the presence of this unique class of compounds and the apparent lack of anthocyanins. These families were not placed together, to the exclusion of the anthocyanin families, in any system of classification prior to Mabry et al. (1963). And while Cronquist and Thorne (1994) state that Mabry's "position with regard to the taxonomic and evolutionary significance of betalains now appears to be mainstream (p. 11)", the impact of Mabry's interpretation of the chemical data on the modern conception of the Caryophyllales is clear in Cronquist's (1981, 1988) and Bittrich's (1993a) phylogenies for the order which place the anthocyanin families basal to a monophyletic Chenopodiinae.

Morphological, chemical and ultrastructural work (reviewed in Hershkovitz, 1991a, b, c and Bittrich, 1993a) have identified a number of distinctive, well-supported lineages within the order – Chenopodiaceae/Amaranthaceae, Phytolaccaceae/Nyctaginaceae, the "portulacaceous alliance" (Portulacaceae/Basellaceae/Cactaceae/Didiereaceae), derived Caryophyllaceae – but neither these approaches nor recent molecular studies have resolved relationships among the major groups. They have also failed to confidently place a number of smaller taxa – Achatocarpaceae, Halophytaceae, Stegnospermaceae, *Barbeuia*, *Lophiocarpus*, and the possibly unnatural Molluginaceae.

Molecular studies in the Caryophyllales have identified a new synapomorphy for the order (loss of the chloroplast *rp12* intron, Downie et al., 1991; Downie and Palmer, 1994) and suggested new outgroups (the Nepenthaceae and Droseraceae, Chase et al., 1993; Williams et al., 1994), but due to small sample sizes these studies have had only a limited impact on infra-ordinal systematics. Downie and Palmer (1994) identified a number of potentially informative structural rearrangements in the chloroplast genome and the *rbcl* evidence (Rettig et al., 1992; Manhart and Rettig, 1994) strongly supports the non-monophyly of the Chenopodiinae and Caryophyllinae (Fig. 1). While many nodes of the most-parsimonious *rbcl* tree have low bootstrap and decay values (Manhart and Rettig, 1994), a reanalysis of this dataset (Clement and Mabry, unpublished) shows that the shortest tree allowing a monophyletic Chenopodiinae is 14 steps longer than the most-parsimonious tree.

Much of the lack of resolution in the *rbcl* tree seems to be due to the extremely long branch separating the order from the rest of the angiosperms. This is, in fact, the longest internal branch found among dicots in the *rbcl* analyses which have sampled a significant number of Caryophyllalean taxa (Albert et al., 1992; Williams et al., 1994; each study including 100 species of dicots). It is surpassed only by three monocot branches and the branch leading to the angiosperms in the Chase et al. (1993; 475 species) seed plant survey. Eliminating the outgroups used by Rettig et al. (1992) and Manhart and Rettig (1994) from the analysis leads to a dramatic increase in bootstrap and decay index values within the order (Clement and Mabry, unpublished). In some cases, it may be best for workers pursuing higher level molecular systematics in the

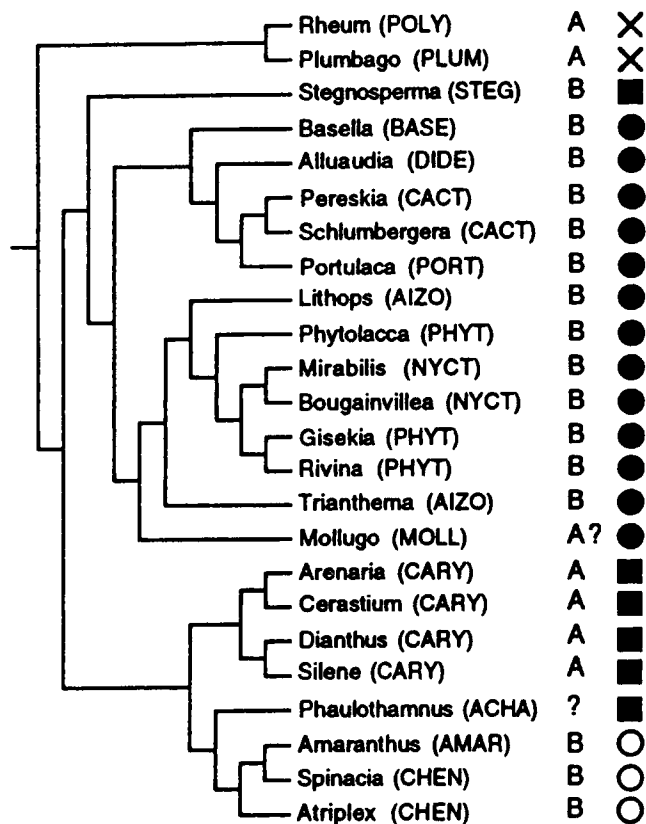


Fig. 1 The most parsimonious *rbcl* tree (Manhart and Rettig, 1994). The symbols represent the presence of anthocyanins (A) or betalains (B), pigments unknown (?), pigments reported without experimental data (A?), absence of the unique sieve-element plastid type (X), presence of the unique plastid type with polygonal protein inclusion (solid square), globular protein inclusion (solid circle) or no protein inclusion (empty circle). Pigment and plastid type distribution taken from Clement et al. (1994) and Behnke (1994).

order to forego outgroups and concentrate on nearest-neighbor instead of sister-group relationships. For example, a rooted phylogeny is not necessary to establish non-monophyly of the Chenopodiinae and Caryophyllinae. There is no place to "re-attach" the *Plumbago/Rheum* branch in Fig. 1 that allows monophyly of either sub-order.

It is intriguing that *Mollugo* comes out in the *rbcl* analysis near the Aizoaceae, where it formerly was placed (Pax and Hoffmann, 1934; Hofmann, 1973; Bittrich and Hartmann, 1988). This arrangement allows a parsimonious explanation of sieve element plastid evolution, since *Mollugo* is embedded in a group of taxa also containing globular sieve element plastid inclusions (Fig. 1; Behnke, 1994). It will be interesting to see whether *rbcl* sequences for the other tribes of the family (Limeae, with angular protein inclusions and anthocyanins, Behnke et al., 1983, and Corbichoniae) come out in this group or with the angular inclusion-containing Caryophyllaceae, since the monophyly of the Molluginaceae is not at all certain (Endress and Bittrich, 1993).

Mutual Exclusiveness of the Anthocyanins and Betalains

The possible paraphyly of the Chenopodiinae and the vagaries involved in constructing a scheme for the evolution of anthocyanins and betalains in the Caryophyllales lead us to look for alternatives regarding the mutual exclusiveness/functional equivalence of the two pigment types. It is possible that the two classes of compounds co-occurred for some time in an ancestor of the order, or a portion of the order, each perhaps maintained under moderate or weak selection, with stochastic losses of one pigment type or the other in various lineages. Such a view is consonant with, but does not restrict us to, the possibility of adaptive functions for the compounds beyond their use as optical attractants. It may be that certain lineages within the ancestral group (producing *both* pigment types) more readily made the genetic, developmental, and physiological adjustments necessary to capitalize on the new class of compounds, while some others did not cross this threshold. In each case, then, the ability to synthesize the less adaptive (and presumably physiologically costly) pigment type was eventually lost. In this light, it is intriguing that a carbon-based ring structure, such as found in anthocyanins, would be replaced by a nitrogen-containing compound, and that these pathways would be expressed under analogous conditions. The production of a single betacyanin molecule results in the metabolic loss of two reduced nitrogens that would have been freed by converting the tyrosine molecules, on which betacyanins are based, into the C_9 moieties of two anthocyanin molecules via phenylalanine. On the other hand, four malonyl CoA molecules are saved by *not* producing the two molecules of anthocyanin. As previously stated, though, the physiological roles of the two compound classes have not been thoroughly investigated and, in general, true metabolic costs are not easily assessed.

Interestingly, betalains may be more effective than anthocyanins as pigments on a per molecule basis, since they have considerably higher molar absorptivities (ϵ) in the visible light range. For example, the molar absorptivity for betanidin ($\log \epsilon_{\text{MeOH-HCl}} = 4.63$, H. Wyler and A. Dreiding, unpublished data) vs. that of delphinidin ($\log \epsilon_{\text{EtOH-HCl}} = 4.49$, Harborne, 1967) indicates that the same degree of absorbance can be obtained from betanidin at only three-fourths the concentration of delphinidin. This is only a rough comparison, due to the different solvents used, but it suggests that much smaller amounts of betacyanin are needed to be equally effective as pigments.

Stafford (1994) raises some questions concerning the evolution of the apparently analogous expression of anthocyanins and betalains. One possibility is that this pattern is due to conservation of the regulatory pathway as a group of plants made the shift from one set of pigments to the other. It is possible that once plants began to experience selection for betalain pigmentation in flowers or fruits the most efficient way of incorporating the requisite structural genes into the course of development was to exploit the regulatory signaling mechanisms formerly associated with anthocyanin expression. This then would have led to betalain biosynthesis being turned on under circumstances in which anthocyanin pathway had once been turned on, regardless of whether the betalains were always performing an analogous function.

In summary, the possible paraphyly of the Chenopodiinae and the difficulty in determining the role of floral pigmentation in the history of the Caryophyllales do not necessarily complicate our view of the evolution of anthocyanins and betalains if we allow that these pigments may have co-occurred in an ancestor for some time and that these compounds may perform other significant functions beyond that of optical attraction. There is much room for work on these issues. Higher level Caryophyllalean systematics remains in an unsatisfactory state despite the considerable interest this group has generated over the years. Immediate pay-offs will likely come from gene sequencing studies in the form of well-supported molecular phylogenies and placement of the remaining tribes of the Molluginaceae and several anomalous genera (*Barbeuia*, *Lophiocarpus*, *Halophytum*). For example, data from the chloroplast *orf2280* gene places *Sarcobatus* (allied with the Chenopodiaceae, but possessing globular sieve element plastid inclusions, Behnke, 1994) near the Phytolaccaceae (Downie et al., 1995). A phylogeny from a nuclear-encoded gene would be valuable since the relationship of the Caryophyllaceae with the Chenopodiaceae and Amaranthaceae suggested by chloroplast DNA data is hard to reconcile with Chang and Mabry's (1974) RNA-DNA hybridization data, which shows Caryophyllaceae 16S ribosomal RNA as highly divergent from that of spinach. The accumulation of gene sequence data may also lead to a confident placing of the "root" for the order.

In addition to the basic physiological work that could be done to further elucidate the roles of anthocyanins and betalains, molecular techniques have much to offer. Cloning of the genes involved in betalain synthesis should provide evidence on the evolution of this pathway. The origin of the 3,4-DOPA dioxygenase is of particular interest, since aromatic ring-cleavage enzymes have been characterized from only one other group of plants (the legume genus *Stizolobium*, Saito and Komamine, 1978), and this aspect of plant metabolism is poorly understood (Ellis, 1971, 1973). The analogous expression of anthocyanins and betalains could be studied by documenting whether flavonoids accumulate along with betalains at wounding/infection sites, as well as in flowers and fruits, which would suggest a common regulatory mechanism. Transient assays using clones of anthocyanin regulatory genes could then be used to determine if these genes directly lead to betalain formation. A number of anthocyanin structural genes have been isolated from *Dianthus* (Caryophyllaceae, Forkmann, 1994), but not the gene responsible for the conversion of leucoanthocyanidin to anthocyanidin (ANS). A sequence for this gene would be especially valuable, since if it is radically different from the ANS genes found throughout angiosperms this would suggest a separate evolutionary origin within the Caryophyllales (i.e., loss of the original ANS and its eventual functional replacement). A phylogenetic analysis would show if this gene is directly related to other ANS genes or is even a member of the dioxygenase family to which these genes belong (Britsch et al., 1993). Regulation of anthocyanin gene expression has not been investigated in *Dianthus* yet. If this system turns out to depart significantly from the common angiosperm models (Goodrich et al., 1993; Quattrocchio et al., 1994), this might also suggest the loss and regain of part of the anthocyanin pathway.

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