Molecular phylogenetics has revolutionized our understanding of the Caryophyllales, and yet many relationships have remained uncertain, particularly at deeper levels. We have performed parsimony and maximum likelihood analyses on separate and combined data sets comprising nine plastid genes (∼12,000 bp), two nuclear genes (∼5000 bp), and the plastid inverted repeat (∼24,000 bp), giving a combined analyzed length of 42,006 bp for 36 species of Caryophyllales and four outgroups. We have recovered strong support for deep-level relationships across the order. Two major subclades are well supported, the noncore and core Caryophyllales; Rhabdodendron followed by Simmondsia are sisters to the core Caryophyllales, Limeum and Stegnosperma are successive sisters to the “globular inclusion” clade, Gisekia is a distinct lineage well separated from Rivina within the “raphide” clade, and Rivina and Phytolaccaceae are disparate lineages, with Rivina sister to Nyctaginaceae. The placement of Sarcobatus and relationships within the portulacaceous cohort remain problematic. Within the latter, Halophytum is sister to Basellaceae and Didiereaceae, and the clade comprising Portulaca, Talinum, and Cactaceae is well supported. Classical hypotheses argued that the early Caryophyllales had evolved in open, dry, marginal environments at a time when pollinators were scarce, and, as such, the ancestral caryophyllid flower was wind pollinated with an undifferentiated perianth. We reevaluated these hypotheses in light of our phylogeny and find little support for anemophily as the ancestral condition; however, the early caryophyllid flower is suggested to have possessed an undifferentiated perianth. A subsequent minimum of nine origins of differentiated perianth is inferred. We discuss the evidence for independent origins of differentiated perianth and highlight the research opportunities that this pattern offers to the field of evolutionary developmental genetics.

**Keywords:** character reconstruction, stochastic character mapping, petals, evo-devo, MADS-box.

**Online enhancements:** figures, tables.

**Introduction**

Research interest in Caryophyllales has a long and rich history; core members of this lineage correspond to the old Centrospermae ("central seeded"), a group long recognized by its distinctive placation and embryology (Braun 1864; Eichler 1875–1878). Centrospermae became the focus of research and debate in the 1960s as one of the first groups whose circumscription was modified based on phytochemistry (Cronquist and Thorne 1994). All but two of the 10 families then recognized as belonging to the Centrospermae were discovered to possess betalain pigments instead of anthocyanins (Cronquist 1988). On the basis of this chemo-systematic character, Cactaceae and Didiereaceae were reclassified to the Centrospermae, and several families of dubious affiliation were excluded (Cronquist and Thorne 1994). Subsequent classifications recognized the Caryophyllales as a well-defined group on the basis of numerous morphological, ultrastructural, and chemical characters (Dahlgren 1975; Thorne 1976; Takhtajan 1980; Cronquist 1981, 1988). Just before the emergence of DNA-based molecular systematics, the Caryophyllales sensu stricto comprised 12 families (Takhtajan 1980; Cronquist 1988; Thorne 1992): Phytolaccaceae, Achatocarpaceae, Nyctaginaceae, Aizoaceae, Didiereaceae, Cactaceae, Chenopodiaceae, Amaranthaceae, Portulacaceae, Basellaceae, Molluginaceae, and Caryophyllaceae. In addition, Polygonaceae and Plumbaginaceae have been regarded by numerous systematists as closely related to these 12 families (Cronquist and Thorne 1994). A series of molecular phylogenetic investigations has altered the concept of Caryophyllales provided in earlier classifications. Giannasi et al. (1992) confirmed the close relationship of Polygonaceae and Plumbaginaceae with the Caryophyllales. Albert et al. (1992) and Williams et al. (1994) demonstrated the association of Droseraceae, Nepenthaceae, and Drosophyl-
laceae with Polygonaceae and Plumbaginaceae. The carnivorous clade plus Polygonaceae/Plumbaginaceae was further expanded to include Anisocladaceae, Dioncophyllaceae, Frankeniaceae, and Tamaricaceae (Fay et al. 1997). Numerous analyses have recognized this expanded clade, which is variously termed the noncore Caryophyllales (Cuénoud et al. 2002; APG II 2003), Caryophyllales II (Hilu et al. 2003), and Polygones (Judd et al. 1999), as sister to Caryophyllales sensu stricto (Solis et al. 1999, 2000).

Within Caryophyllales s.s. or the core Caryophyllales, molecular data have resulted in several refinements in phylogeny and classification. Additional families recognized as belonging to the core Caryophyllales include Physenaceae and Asteropeiaeae (Morton et al. 1997), Rhabdodendraceae, and Simmondsiaceae (Fay et al. 1997). Simmondsiaceae are placed as sister to the core Caryophyllales (Cuénoud et al. 2002), with Physenaceae and Asteropeiaeae forming a strongly supported sister group (combined matK/rbcL analysis; Cuénoud et al. 2002). Rhabdodendraceae have been associated with Simmondsiaceae as sister to the core Caryophyllales or as sister to both core and noncore Caryophyllales but with little support for either position (matK analysis; Cuénoud et al. 2002).

Molecular studies have also identified and confirmed a number of polyphylectic groups within the core Caryophyllales. Recognition that Phytolaccaceae are polyphyletic (initially by Rettig et al. [1992]) supports the delimitation of four families: Achatocarpaceae, Barbeuicaceae, Gisekiaceae, and Stegnospermataceae (Cuénoud et al. 2002; APG II 2003). Achatocarpaceae may form a clade together with Caryophyllaceae and Amaranthaceae (combined matK/rbcL analysis; Cuénoud et al. 2002). Stegnospermataceae are placed without support as a successive sister lineage to the remainder of the core Caryophyllales (Cuénoud et al. 2002), following the divergence of Asteropeiaeae, Physenaceae, Achatocarpaceae, Caryophyllaceae, and Amaranthaceae. Barbienia represents a distinct, isolated lineage within the core Caryophyllales but of uncertain position (Cuénoud et al. 2002). Rivina and Petiveria, both formerly of Phytolaccaceae, are polyphyletic (combined matK/rbcL analysis; Cuénoud et al. 2002) with respect to Phytolaccaceae. Rivina has been allied with the family Gisekiaceae, and Petiveria is placed sister to Rivina and Gisekiaceae (combined matK/rbcL analysis; Cuénoud et al. 2002). Similarly in the matK analyses, Hilleria is placed sister to Rivina, and Ledenbergia is sister to those two taxa. Lophiocarpus, originally placed within Phytolaccaceae, is now separated and placed sister to Corbicobia (Cuénoud et al. 2002). Sarcobatis, originally placed in the Chenopodiaceae, was recognized as the family Sarcobatisae (Behnke 1997; APG II 2003) on the basis of distinct sieve-element plastids with respect to Chenopodiaceae (Behnke 1997). This separation was supported by molecular analyses in which Sarcobatisae form a distinct lineage related to the clade containing Aizoaceae, Phytolaccaceae, Nyctaginaceae, Gisekiaceae, and Agdestidaceae (Downie et al. 1997; Cuénoud et al. 2002). The circumscription of Molluginaceae remains problematic; the family is likely to be polyphylectic. Previous authors have suggested that the inclusion of Macarthunia and Polypoda is unlikely on the basis of morphological observations; however, two genera have not been included in previous molecular analyses (Cuénoud et al. 2002). Genera previously included within Molluginaceae (Corbicobia, Limeum, Gisekia) form disparate lineages with respect to the type genus. However, the position of Limeum outside Molluginaceae is unsupported (matK/rbcL; Cuénoud et al. 2002). Mollugo, Adenogramma, Gisebrothmannus, Glinus, Pharnaceum, and Suessenguthiella constitute a monophyletic group that is sister to the portulacaceous cohort (Cuénoud et al. 2002).

The portulacaceous cohort of Basellaceae, Cactaceae, Didieraceae, and Portulacaceae was initially proposed by Thorne (1976) and is supported by non-DNA characters such as presence of a floral involucre, succulent tissue, mucilage, and Crassulacean acid metabolism (Cuénoud et al. 2002; Nyffeler 2007). The monophyly of the cohort was implied by early molecular analyses (Rettig et al. 1992; Downie et al. 1997); however, relationships within the group are unclear and complicated by the gross paraphyly of Portulacaceae (suggested by Carolin [1987] and Hershkovitz [1993]). The addition of molecular data has resulted in some clarification. In an analysis of ITS sequences, Hershkovitz and Zimmer (1997) suggested that Cactaceae were embedded within Portulacaceae and sister to Portulaca, Anacampseros, and relatives and portions of Talinum (the ACPT clade, from Anacampserotaeae, Cactaceae, Portulaca, and Talinum; Nyffeler 2007). These findings were confirmed and extended by Applequist and Wallace (2001) in an analysis of ndhF sequences; Talinum with Tlinnella, Portulaca with Anacampseros, and the Cactaceae form three distinct lineages within a well-supported clade. Although the monophyly of the ACPT clade seems clear, as summarized by Nyffeler (2007), the pattern of branching within the clade has varied among analyses. Outside of the ACPT clade, Hershkovitz and Zimmer (1997) found that Basellaceae and Didieraceae form a distinct monophyletic group and are sister to portulacaceous genera Portulararia and Ceraria. In addition, Applequist and Wallace (2001) described this same clade as consisting of three distinct lineages: Basellaceae; Didieraceae with Calyptrocteca, Ceraria, and Portulararia; and a strongly supported assemblage of genera including Claytonia, Montia, Calandrinia, Montioptia, Cistanthe, Calytridium, and Phemeranthus.

Despite these advances in the understanding of subordinal relationships in Caryophyllales, a number of uncertainties remain. The positions of Limeum, Stegnosperma, and Barbeuia are all ambiguous. The relative position of Rhabdodendron and Simmondsia as possible sisters to either the core Caryophyllales or Caryophyllales s.l. is unclear. Relationships within the portulacaceous cohort are largely unresolved, particularly within the clade containing Basellaceae, Didieraceae, and allied genera (Applequist and Wallace 2001). The clade including Phytolaccaceae, Sarcobataceae, Nyctaginaceae, Gisekiaceae, and Agdestidaceae also lacks internal resolution. Additionally, some relationships have been suggested only on the basis of single-gene analyses (Cuénoud et al. 2002), with many nodes scattered throughout the Caryophyllales lacking strong support. In an effort to resolve the remaining problematic deep-level relationships within Caryophyllales, we constructed a much larger data set than employed previously. Our data set comprised eight plastid genes from single-copy (SC) regions, two nuclear genes, and the entire plastid inverted repeat (IR; a combined analyzed length of 42,006 bp) for 40 taxa representing 31 families of the Caryophyllales and three families as outgroups.
its evolutionary origins. Ehrendorfer (1976) outlined a plausible scenario to explain the coincident evolution of several unique characteristics found in the Caryophyllales, including floral variation and betalain pigmentation. He proposed that ancestral taxa in Caryophyllales occupied “open, warm, dry and windy habitats with mineral soils” (Ehrendorfer 1976, p. 104). Reasons for assuming this ancestral habitat derive from the observation that many of the families in Caryophyllales currently inhabit xeric, marginal environments. Ehrendorfer (1976) argued that if the ancestral habitats were xeric, there would be strong selection for anemophily because pollinating insects would have been scarce in areas of little pioneer plant growth. He states that pollinators may have been scarce because the time of the origin of the core Caryophyllales (104–111 Myr BP; Wikstrom et al. 2001) predates the major diversification of insect pollinator lineages. He then argues that much of the floral variation and novel pigmentation in the core Caryophyllales could be interpreted as the consequence of this anemophilous ancestry, with reversals to zoophily in extant lineages. However, as this study will demonstrate, the phylogenetic concept of the Caryophyllales has changed considerably since Ehrendorfer (1976). We evaluate Ehrendorfer’s hypotheses in light of a much-altered phylogeny by examining patterns of pollination biology and perianth differentiation. We discuss the evolution of perianth differentiation in the context of the literature on perianth development within Caryophyllales and use our phylogeny to identify broad trends in perianth evolution across the clade. Finally, we discuss the research opportunities that these patterns of morphological variation offer to the field of evolutionary developmental genetics.

Material and Methods

Taxon Sampling

In this analysis 31 families of Caryophyllales sensu APG II (2003; Cuénoud et al. 2002) were represented. Some families are monotypic (e.g., Drosophyllaceae, Halophytaceae, Stegnospermateae); others comprise only one genus (e.g., Asteraceae, Nepenthaceae, Ancistrocladaceae, Frankeniaceae) or two or three genera (Achatocarpaceae, Dioncophyllaceae, Drosaceae, Limeaceae, Talinaceae). For larger potentially polyphyletic or paraphyletic families (e.g., Portulacaceae), multiple genera were sampled to represent more of the phylogenetic diversity. The final data set included 36 taxa of Caryophyllales, with an additional four taxa (Tetracera and Hibbertia representing Dilleniaceae, Berberidopsid, and Vitis) sampled as outgroups. Species, voucher information, and GenBank accession numbers are given in tables A1–A9 in the online edition of the International Journal of Plant Sciences. In some instances sequence data were combined from multiple species to represent a family; this was judged not to significantly affect a family-level analysis, but the instances are listed here: Aizoaceae (Delosperma napiforme, Delosperma echnatum, Delosperma cooperi), Amaranthaceae (Celosia argentea, Celosia cristata), Cactaceae (Opuntia microdasys, Opuntia dillenii), Didiereaceae (Alluaudia ascendens, Alluaudia procura), Dilleniaceae (Hibbertia volubilis, Hibbertia cuneiformis), Gisekiaceae (Gisekeia africana, Gisekeia pharmacoideas), Molluginaceae (Limeum africam, Limeum aethopicum), Plumbaginaceae (Limonium gibertia, Limonium arborescens, Plumbago zeylanica, Plumbago auriculata), Polygonaceae (Polygonum sagittatum, Polygonum virgincum), and Portulacaceae (Claytonia virginica, Claytonia perfoliata).

DNA Isolation and Amplification

We isolated DNA following standard CTAB protocols (Doyle and Doyle 1987) and using Qiagen DNA extraction kits (Qiagen, Valencia, CA). To augment depleted DNA stocks, we carried out multiple displacement amplification (MDA) using the Genomiphi kit (Amersham, Piscataway, NJ) according to the manufacturer’s instructions (Brockington et al. 2008). MDA-treated DNA was diluted 1 : 10 before further PCR amplification of targeted genes.

We targeted 11 specific genes for sequencing (nine plastid genes from the large and small SC regions and two nuclear genes); all targeted genes and primers used for PCR and sequencing are provided in figure A12 in the online edition of the International Journal of Plant Sciences. All PCR reactions contained Taq DNA polymerase (New England Biolabs, Ipswich, MA) and 10X Thermopol reaction buffer supplied by the manufacturer. The reaction volume was 25 μL, and the final concentration of the components was Tag buffer (pH 8.8), MgCl₂ (1.5 mM), 200 μM dNTP, forward and reverse primers (1 μM), 1X Taq polymerase, and 1 μL of DNA. PCR cycling was carried out in an Eppendorf Mastercycler (Eppendorf, Westbury, NY) at 95°C for 3 min, followed by 30–35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, with a final extension time of 7 min at 72°C. PCR products were purified using ExoSAP, and sequences were generated on an ABI 3730 XL DNA sequencer (Applied Biosystems, Fullerton, CA) following the manufacturer’s protocol. Sequences were submitted to GenBank (numbers given in tables A1–A9).

The amplification, sequencing, and annotation of plastomes method (Dhingra and Folta 2005) was used to obtain the sequence of the plastid genome IR for 35 genera of Caryophyllales (the IR for Physena was not sequenced) and two members of Dilleniaceae. The published complete plastid sequence of Spinacia (Schmitz-Linneweber et al. 2001) and Plumbago (Moore et al. 2007) provided the IR sequence for these two taxa. The IR sequences were subsequently annotated using DOGMA (Wyman et al. 2004) and were submitted to GenBank (numbers given in tables A1–A9).

Alignment and Phylogenetic Analysis

Sequences were automatically aligned using Clustal X (Thompson et al. 1997) and then manually adjusted. Coding regions were aligned by predicted amino acid sequence. Regions at the beginnings and ends of genes for which sequences were incomplete, together with regions that were difficult to align, were excluded from the analysis. The total aligned lengths and the aligned analyzed lengths are given in table 1. Using the new sequences generated here, together with those previously published (cited in tables A1–A9), we constructed six different data partitions: (1) individual plastid genes from the SC regions, (2) combined plastid genes from the SC regions, (3) two nuclear ribosomal RNA genes (18S rDNA and 26S rDNA), (4) plastid IR, (5) combined plastid SC and nu-
Table 1

### Information on Parsimony and Likelihood Analyses for Each Data Partition

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<th>Data partition</th>
<th>Total aligned length (bp)</th>
<th>Analyzed aligned length (bp)</th>
<th>No. MP trees</th>
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<th>Consistency index of MP tree</th>
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<td>SC plastid + nuclear + IR</td>
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All data partitions were subject to the following phylogenetic analyses. We used maximum parsimony (MP) and maximum likelihood (ML) to infer phylogeny. MP analyses were implemented in PAUP*, version 4.0 (Swofford 2000). Shortest trees were obtained using a heuristic search and 1000 replicates of random taxon addition with tree-bisection-reconnection (TBR) branch swapping, saving all shortest trees per replicate. Bootstrap support (BS) for relationships (Felsenstein 1985) was estimated from 1000 bootstrap replicates using 10 random taxon additions per replicate, with TBR branch swapping and saving all trees.

For ML analyses we employed the program GARLI (Genetic Algorithm for Rapid Likelihood Inference, version 0.942; Zwickl 2000). GARLI conducts ML heuristic phylogenetic searches under the GTR model of nucleotide substitution, in addition to models that incorporate among-site rate variation, assuming a gamma distribution (\(\Gamma\)), a proportion of invariable sites (I), or both. Analyses were run with default options, except that the “significandtopochange” parameter was reduced to 0.01 to make searches more stringent. ML bootstrap analyses were conducted with the default parameters and 100 replicates. We performed a strict consensus of five replicate GARLI analyses, and topological differences resulting in collapsed nodes were annotated on the representative ML tree.

Bayesian analyses were performed on the combined partition to generate trees for stochastic character mapping. Models of nucleotide substitution were determined using MrModeltest (Nylander 2004). The Akaike Information Criterion was used to select GTR + I + G as an appropriate model based on the relative informational distance between the ranked models. Analyses were implemented in MrBayes, version 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Two independent analyses each ran for 5 million generations, using four Markov chains, with all other parameters at default values; trees were sampled every thousandth generation, with a burn-in of 200,000 generations. Stationarity of the Markov Monte Carlo chain was determined by the average standard deviation of split frequencies between runs (after 5 million generations, the average standard deviation was 0.004%) and by examination of the posterior in Tracer, version 1.3 (Rambaut and Drummond 2003). A majority rule consensus of post-burn-in trees was generated in PAUP*, version 4.0 (Swofford 2000), using the resulting posterior distribution of the trees.

**Character Reconstructions**

Parsimony-based reconstructions were achieved using standard unweighted parsimony character optimization and performed within Mesquite (Maddison and Maddison 2008). Reconstructions focused on the core Caryophyllales and were carried out using the MP topology derived from the total evidence data set. Reconstructions were further modeled by means of stochastic mapping techniques as described by Huelsenbeck et al. (2003) and implemented in SIMMAP (Bollback 2006). This approach estimates the rates at which a discrete character undergoes state changes as it evolves through time. Bayesian estimation has several advantages over traditional parsimony-based reconstruction. First, it allows one to average over equally likely topologies, which is valuable because the positions of some taxa are poorly supported (e.g., *Limeum*) or poorly resolved (e.g., taxa within the portulacaceous cohort and “raphide” clade). Second, it allows more than one character change per branch and is therefore a useful methodology for character reconstruction in the Caryophyllales, a clade in which long branches are common.

Posterior mapping requires the specification of prior values. The prior on the bias parameter was fixed at 1/\(k\), where \(k\) is the number of states (this being the recommended approach in SIMMAP for characters of more than two states; Renner et al. 2007). We applied an empirical Bayesian approach in choosing appropriate priors for the substitution rate parameters following the method of Couvreur (2008) and Couvreur et al. (2008). The gamma distribution of the substitution rate is governed by two hyperparameters defining the mean, \(E(\Gamma)\), and the standard deviation, \(SD(\Gamma)\). The values of these hyperparameters for the prior gamma distribution were selected independently for each character using the “number of realizations sampled from priors” function in SIMMAP with 10,000 draws. A series of trials was performed (10,000 realizations in...
each) that systematically sampled for values of \( E(T) \) between 1 and 30, in combination with SD(T) values of either 1 or 5. The posterior distribution of these combinations was visualized in Tracer, version 1.3, and further plotted as graphs of frequency against rate (see fig. A11 in the online edition of the *International Journal of Plant Sciences*). The posterior distribution curves derived from these trials allowed the selection of values of \( E(T) \) that gave highest sampling and allowed optimization of the \( E(T) \) value (Couvreur 2008; Couvreur et al. 2008). A trial was also performed without specifying priors and allowing rates to be determined by branch lengths (as performed by Renner et al. [2007]); however, the posterior distribution curves were generally highly skewed (fig. A11), and thus this form of prior selection was not employed in subsequent analyses. Following exploration of different combinations of \( E(T) \) and SD(T), the prior \( E(T) \) values chosen for the characters were as follows: perianth \( E(T) = 17 \), and pollination \( E(T) = 7 \) (marked with an asterisk in fig. A11). For all of these values of \( E(T) \), an SD(T) value of 5 was applied in subsequent analyses, allowing a large standard deviation to accommodate uncertainty in mean rate of substitution.

Following specification of priors, the rate and number of state transformations were estimated by 100 realizations on the 4800 post-burn-in trees (with branch lengths) from the Bayesian analyses. As recommended, branch lengths were re-scaled so that the total tree length was 1 but the branch length proportions were maintained. The ancestral state at different nodes was assessed using a hierarchical Bayesian ancestral state reconstruction method implemented in the “posterior ancestral states” function of SIMMAP (Bollback 2006). The nodes for which ancestral states were estimated are labeled in figure 3. The estimations of the posterior probability of ancestral character states at each node are listed in tables A12 and A13 in the online edition of the *International Journal of Plant Sciences* and presented graphically on the nodes in figure 3.

With parsimony reconstruction analyses, when more than one character state was present in the family, the representative taxon was coded as having more than one character state. In stochastic mapping analyses using SIMMAP, terminals cannot be coded as having more than one state, so in instances where more than one character state was present in the family, the representative taxon was coded as unknown (?). Information on pollination was derived primarily from entries in Kubitzki et al. (1993) and Kubitzki and Bayer (2003, 2007); pollination was coded as entomophilous or anemophilous. In the case where observations on pollinators have not been made, the character state determination was unknown (?). All coding information is listed in tables A10 and A11 in the online edition of the *International Journal of Plant Sciences*.

Our approach to coding perianth requires further clarification because there are many types of differentiated perianth within Caryophyllales, and their homology is not always clear. Occurrences of differentiated perianth were given different character states, where there are clear, documented differences in development of the differentiated perianth. Data on perianth and development were collated from the available literature (see fig. A11). As reviewed by Ronse De Craene (2008), criteria used to determine these differences in the literature include meristic variation, sequence of organ initiation, difference in appearance at maturity, and presence of morphological intermediates. We were, however, interested in estimating the minimum number of origins of the differentiated perianth under parsimony and therefore applied a stringent approach to character coding, minimizing the number of character states to four: undifferentiated (0), differentiated with stamen-derived petals (1), differentiated with an involucrare-derived outer whorl (2), and differentiated perianth of uncertain affinity (3). We employed a conservative approach to character coding, assigning states 1 and 2 only to taxa in which development morphological data were most conclusive. Where we were uncertain, we assigned taxa to character state 3. We emphasize that this coding does not reflect our belief that these instances of differentiated perianth are necessarily homologous, but in coding them as identical, we ensure that estimation of the number of origins of differentiated perianth is conservative.

**Results**

*Individual Plastid SC Data Sets*

MP and ML trees from individual data sets are largely congruent with each other (figs. A1–A7 in the online edition of the *International Journal of Plant Sciences*; tree statistics shown in table 1). Consistent with the approximate nature of the GARLI approach to ML phylogeny estimation, replicate GARLI analyses on the individual gene data sets do, on occasion, recover slightly different topologies. Taking into account nodes either that are unsupported or that collapse in the strict consensus, however, there are few instances of conflicting relationships between trees derived from different individual gene data sets. These examples of conflict include the following: in the matK MP tree, *Delosperma* and *Gisekia* were recovered as sister groups (BS 51%); in the MP and ML ndhF tree, *Spinacia* and *Stellararia* were resolved as sister groups to the exclusion of *Celosia* (MP BS 100%); in the MP and ML rbcL tree, *Gisekia* was sister to *Rivina* (MP BS 100%), *Delosperma* was sister to *Phytolacca* (MP BS 89%), and *Stellararia* was sister to the Amanthaceae (MP BS 53%); and in the ML rpoC2 tree alone, *Phytolacca* and *Sarcobatus* were recovered as sister groups (ML BS 79%). Importantly, these anomalous relationships are not recovered or not supported in any other data sets, in either single-gene or combined partitions. None of the trees derived from these individual plastid gene data sets gives good resolution across the tree, and deeper-level relationships in particular are poorly supported.

*Inverted Repeat Data Set*

As with the individual plastid gene data sets, the IR partition generates MP and ML trees that are congruent (fig. A8 in the online edition of the *International Journal of Plant Sciences*). Parsimony analyses recovered a single tree; replicate GARLI analyses recovered trees that differed only in the topology of the “succulent” clade. The IR tree differs from the previous analyses in the placement of *Sarcobatus*, which is resolved as sister to *Nyctaginaceae* and Phytolaccaceae with strong support (ML BS 100%). Furthermore, analyses of individual plastid genes resolve *Talinum* as sister to *Portulaca* and Cactaceae whereas the IR data set recovers *Portulaca* and *Talinum* as sister to each other.
The combined plastid gene data set generated a single most parsimonious tree. Replicate GARLI analyses recovered the same ML topology (fig. A9 in the online edition of the *International Journal of Plant Sciences*). Levels of BS are higher in general in the ML tree than in the MP tree. Again, the MP and ML trees are largely congruent, although in the MP tree *Sarcobatus* and *Rivina* are sister to each other whereas in the ML tree *Sarcobatus* is placed without support with Physotilacaceae and Nycaginaceae. In the MP tree *Limeum* is placed without support as sister to *Mollugo* and the “succulent” clade, and *Stegnosperma* is placed as sister to the “globular inclusion” clade; in the ML tree, *Limeum* and *Stegnosperma* are placed as successive sisters to the “globular inclusion” clade. As with the individual plastid gene trees, *Talinum* is resolved as sister to *Portulaca* and Cactaceae, but relationships among Didieraceae, Basellaceae, Halophytum, and Claytonia are either poorly supported or unresolved.

**Combined Plastid SC and Nuclear Genes**

The addition of the nuclear gene data set to the combined plastid genes has little effect on topology (fig. A10 in the online edition of the *International Journal of Plant Sciences*). In contrast to analyses of the combined plastid gene data set, both the MP and the ML analyses with the nuclear data recover *Stegnosperma* and *Limeum* as successive sisters to the globular inclusion clade. Again the MP and ML trees differ in their placement of *Sarcobatus* in the same way as in the combined plastid tree topologies, i.e., as sister to *Rivina* in the MP tree but sister to *Rivina* and Nycaginaceae in the ML tree; both placements have low BS (~60%). As in the IR ML tree topology, the MP recovers *Portulaca* and *Talinum* as sister to Cactaceae but without support; in the ML tree, however, *Talinum* is sister to *Portulaca* plus Cactaceae.

**Total Evidence Data Set**

The total evidence data partition generated a single MP (fig. 2) tree that agrees in topology with the ML tree (fig. 1), except for the placement of *Sarcobattes*. The MP and ML trees derived from the total evidence data set show more congruence with each other than the congruence found between MP and ML trees derived for any other data partition. As in previous combined analyses, in the MP tree, *Sarcobatus* and *Rivina* are placed sister to each other (BS 63%) while in the ML tree, *Sarcobatus* is placed without support as sister to Nycaginaceae plus *Rivina*. The position of *Sarcobatus* therefore remains uncertain in these analyses. The ML topology was chosen as the basis of subsequent character reconstruction analyses because it is less prone to the problem of long-branch attraction (Felsenstein 1978) and because the bootstrap values are higher than in the MP tree. The full topology of the tree is therefore described in detail here.

The noncore Caryophyllales form a strongly supported (BS 100%) monophyletic group with two subclades. One clade comprises Plumbaginaceae with Polygonaceae resolved as sister to Frankeniaceae plus Tamaricaceae (all with BS 100%). The second clade, containing the carnivorous taxa and relatives, comprises Drosophyllaceae with Ancistrocladaceae and Dioncophyllaceae (BS 100%) and Nepenthaceae with Droseraceae (BS 59%).

The core Caryophyllales form a strongly supported group (BS 100%), with Rhabdodendraceae as sister to the rest (BS 100%). Following the divergence of Rhabdodendraceae, the backbone of the tree is strongly supported and characterized by a grade of successively branching taxa, in the following order: Simmondsiaceae; Asteropeiaceae with Physenaceae; a clade comprising Caryophyllaceae, Achatocarpaceae, and Amaranthaceae (BS 100%); and Stegnospermataceae (BS 100%). Subsequently, *Limeum* is placed as sister to the remaining members of Caryophyllales, which form two clades. In the first of these two clades, the topology is as follows: the earliest-diverging group is Aizoaceae, followed by *Gisekia*, *Phytolaccaceae*, *Sarcobatus*, *Rivina*, and *Nycaginaceae*. In the second clade, Molluginaceae are sister to a group comprising Cactaceae, Portulacaceae, Didiereaceae, Basellaceae, *Halophytum*, and *Claytonia*. Within this group, *Portulaca* and *Talinum* are strongly supported as sister to Cactaceae; however, relationships among Didiereaceae, Basellaceae, *Halophytum*, and *Claytonia* are poorly supported.

For each of the character reconstructions, multiple state transitions are inferred within the core Caryophyllales. The patterns of character evolution derived from parsimony reconstruction and the inferred ancestral states derived from stochastic mapping analyses are illustrated in figure 3.

**Discussion**

Several broad molecular phylogenetic analyses have examined intraordinal relationships across the entire Caryophyllales sensu lato. Rettig et al. (1992) conducted an *rbcL* analysis of 12 families, Downie and Palmer (1994) inferred phylogeny from chloroplast genome structural changes and IR restriction site variation in 11 families of Caryophyllales, and Downie et al. (1997) compared sequences of ORF2280 (ycf2) across 11 families. However, the most comprehensive study is that of Cuénoud et al. (2002), who generated a partial *matK* sequence phylogeny (30 families, 121 genera). In Cuénoud et al. (2002) a subset of the *matK* data was combined with previously published genes to generate a combined *matK/rbcL* phylogeny (19 families, 53 genera) and a four-gene analysis that also incorporated *atpB* and 18S rDNA sequences (19 families, 25 genera). Although the taxonomic sampling of the *matK* phylogeny was extensive and dramatically improved our understanding of the Caryophyllales phylogeny, the study suffered from restricted taxon sampling in the combined analyses, with just over half of the families in the core Caryophyllales represented in the *matK/rbcL* and *matK/rbcL/atpB/18S* data sets. Parsimony was the only optimality criterion used in these analyses, and there were several soft incongruences among the *matK*, *matK/rbcL*, and *matK/rbcL/atpB/18S* trees. Our analyses resolve many of these remaining uncertainties.

**Phylogenetic Analyses**

The earliest-diverging lineages in the core Caryophyllales are clarified and well supported. Notably, Rhabdodendraceae followed by Simmondsiaceae are supported as sisters to the rest of the core Caryophyllales (both with BS 100%; fig. 1). The position of *Rhabdodendron* had previously been ambiguous, recovered either as sister to both core and noncore Car-
Fig. 1  Maximum likelihood (ML) tree resulting from GARLI analysis of total evidence data set (two nuclear genes, nine plastid genes from the single-copy region, and the inverted repeat) for 36 members of the Caryophyllales and four outgroups. Numbers above branches are bootstrap values (−lnL score 210,420.96).
Fig. 2 Phylogram of single most parsimonious tree based on the total evidence data set (two nuclear genes, nine plastid genes from the single-copy region, and the inverted repeat) for 36 members of the Caryophyllales and four outgroups. Numbers above branches are bootstrap values (length 27,604, consistency index 0.605, retention index 0.538).
Fig. 3 Parsimony reconstruction (illustrated on a maximum parsimony tree) and stochastic character mapping (illustrated on a Bayesian consensus tree). a, Reconstruction of perianth evolution. b, Pollination syndromes.
yophyllales (in combined matK and rbcL analyses; Cuénoud et al. 2002) or as weakly supported as sister to Simmondsia, at the base of the core Caryophyllales (in matK analysis; Cuénoud et al. 2002). Following the divergence of Rhabdodendra-ceae and Simmondsiaceae, our analyses strongly support a clade of Physenaceae and Asteropaeaceae (BS 100%) as sister to the remaining core Caryophyllales. Relatively little is known about these early-diverging lineages of core Caryophyllales from the perspective of morphology, and lack of data for these critical early lineages prevents a clear understanding of ancestral states within the core Caryophyllales.

In the matK analysis of Cuénoud et al. (2002), Caryophyllaceae and Achatocarpaceae plus Amaranthaceae s.l. branch successively as sister to the rest of the core Caryophyllales and do not form a clade with Achatocarpaceae and Amaranthaceae s.l., as suggested in the matK/rbcL analyses. In our analyses, the clade comprising Caryophyllaceae, Achatocarpaceae, and Amaranthaceae s.l. receives strong support (BS 100%), agreeing with the combined analyses of Cuénoud et al. (2002). Morphological synapomorphies for this clade remain elusive, probably in part because Achatocarpaceae are poorly studied.

Molecular studies have consistently recovered a distinct clade within the core Caryophyllales (Giannasi et al. 1992; Rettig et al. 1992; Downie et al. 1997; Cuénoud et al. 2002), termed the globular inclusion clade (Aizoaceae, Phytolaccaceae, Nyctaginaceae, Gisekiaaceae, Molluginaceae, Portulacaceae, Didiereaceae, Basellaceae, Cactaceae; Cuénoud et al. 2002), on account of distinctive plastid characteristics (as found by Behnke [1994]). Consistent with previous analyses, Spegnosperma and Limeum are recovered as successive sisters to this globular inclusion clade but with greater support (BS 98%) than in earlier studies. Within the globular inclusion clade, two subclades are recovered that correspond to the raphide clade (Judd et al. 1999) and the succulent clade but with no support in the matK analysis; Cue´noud et al. (2000), whose single-gene analyses variously place Gisekia as sister to Aizoaceae (matK) or Rivina (rbcL) and matK/rbcL analyses and moderate support (BS 70%) in the four-gene analysis.

Within the raphide clade, Gisekia is strongly supported as sister to Phytolaccaceae, Rivina, Sarcobatus, and Nyctaginaceae (BS 100%). This contradicts the findings of Cuénoud et al. (2002), whose single-gene analyses variously place Gisekia as sister to Aizoaceae (matK) or Rivina (rbcL) and matK/ rbcL). Out of the eight plastid genes that we analyzed, only rbcL supports a sister relationship between Gisekia and Rivina. In addition, we provide further evidence and support for the separation of Rivina (Rivinoideae) from Phytolaccaceae and its placement as sister to Nyctaginaceae (BS 77%). The placement of Sarcobatus in our analyses is problematic, and its position varies in relation to Nyctaginaceae, Rivina, and Phytolaccaceae. The raphide clade is undersampled in this study, and while we suggest alternative placements for Gisekia and Sarcobatus, we recognize that increased taxon sampling (e.g., Agdestis, which has been associated with Sarcobatus by Cuénoud et al. [2002]) could affect these findings.

Taxa within the portulacaceous cohort have traditionally been treated at the rank of family; however, the degree of paralogy in Portulacaceae suggests that phylogenetic resolution should be conceptually envisioned as an infrafamilial problem (Hershkovitz and Zimmer 1997). For example, in analyses of ITS, the genetic divergence of Cactaceae from Portulacaceae is equal to or less than that between many pairs of genera in Portulacaceae (Hershkovitz and Zimmer 1997). Two methodo-
logical constraints limited our ability to address the question of phylogenetic relationships among Cactaceae and its portulacaceous relatives. First, the large amount of sequencing for each taxon limited the total number of taxa that could be sampled; this undersampling is particularly acute in the succulent clade, given the degree of paraphyly inherent in Portulacaceae. Second, the broad scope of the taxon sampling, i.e., the whole of Caryophyllales s.l., meant that only slower-evolving coding genes rather than more rapidly evolving regions, such as intergenic spacers, could be sampled to permit alignment across the order. Consistent with the low levels of genetic divergence in this clade, very little informative variation was obtained for members of the portulacaceous cohort from these coding regions (despite sequencing more than 40,000 bp). There are fewer than 20 substitutions on the branches leading to the clade containing Halophytum, Allau-dia, and Basella and fewer than 50 substitutions on the branch leading to Portulaca and Talinum.

Limitations aside, within the portulacaceous cohort, the monophyly of the ACPT clade comprising Cactaceae (Pe- reskia plus Opuntia), Portulaca, and Talinum is strongly sup-
ported (BS 100%) in analyses of all partitions, with the exception of the IR partition. Historically, however, different analyses have recovered different patterns within the ACPT clade, depending on taxon sampling and phylogenetic markers used (reviewed in Nyffeler 2007). In our analyses, different partitions and analytical methods also gave different branching patterns within the ACPT clade. Parsimony analyses of the IR partition recovered a branching pattern (with support less than 50%) similar to the morphological cladistic analysis of Carolin (1987). Parsimony and GARLI analyses of the plastid gene partition found Talinum sister to Portulaca plus Cactaceae, as proposed by the morphological cladistic analyses of Hershkovitz (1993) and the Bayesian molecular analysis of Nyffeler (2007). Parsimony and GARLI analyses of the total evidence and GARLI analyses of IR data sets recovered a well-supported branching pattern not found in previous analyses: Cactaceae sister to Portulaca plus Talinum.

Total evidence, plastid gene, and plastid plus nuclear and IR data sets all place Halophytum as sister to Basellaceae and Didiereaceae. This affiliation is consistent with Savolainen et al.’s (2000) analysis of rbcL sequences (albeit with low taxon sampling in the Caryophyllales) and was suggested by Bittrich (1993) on the basis of pollen morphology and by Hunziker et al. (2000) because of shared similarities in basic chromosome number (x=12). The position of Claytonia, however, is unstable in our analyses and is generally not in agreement with studies with better taxon sampling (Hershkovitz and Zimmer 1997; Applequist and Wallace 2001; Nyffeler 2007). Claytonia together with associated portulacaceous genera were placed in a clade with Basellaceae and Didiereaceae with reasonable support (BS 80%; Applequist and Wallace 2001). However, in our study only MP analyses of the matK and IR data sets were able to recover this relationship. Indi-
vidual plastid genes placed Claytonia in a variety of positions while the combined data sets invariably placed Claytonia as...
Our phylogeny differs considerably from the concept of the Caryophyllales that stimulated the speculations of Ehrendorfer (1976). The Caryophyllales sensu Ehrendorfer essentially correspond to the core Caryophyllales presented in this study; however, the composition and phylogeny of this clade have changed considerably. None of the four currently recognized early-diverging lineages was recognized as belonging to the Caryophyllales in the 1970s. Ehrendorfer was strongly influenced by the idea that the Chenopodiaceae (Amaranthaceae s.l.), with their reduced anemophilous flowers, were representative of the ancestral Caryophyllid type. Consequently, he argued that anemophily was the ancestral condition because the early Caryophyllales had evolved in open, dry, marginal environments at a time when pollinators were scarce. These hypotheses are difficult to prove or disprove (Clement and Mabry 1996); however, our phylogeny confirms that the Amaranthaceae constitute a relatively derived lineage. If pollinators were scarce at the time of origin of the Caryophyllales, this might also apply as a general limitation to other lineages of eudicots diverging at that time, but in any case, the relative timing and location of diversification in eudicot lineages and their respective pollinator lineages are unclear at present. Friedman and Barrett (2008) demonstrate a strong correlation between the occurrence of open habitat and anemophily and provide support for the prevalence of anemophily in open habitats; however, this correlation may not necessarily be due to pollinator scarcity but rather to the selective advantage of wind pollination in an open environment. Moreover, parsimony reconstruction of the ancestral habitat would be ambiguous, given that the extant members of early-diverging lineages of the core Caryophyllales occupy tropical understory (Asteraceae and Rhabdodendraceae) or have a global holartic distribution (Caryophyllaceae).

Using the current phylogeny, parsimony-based character reconstruction and stochastic character mapping do not provide support for the hypothesis that the Caryophyllales were ancestrally wind pollinated. Rhabdodendron, which is sister to all other core Caryophyllales, is described as visited by pollen-collecting bees (Prance 2003) while extensive field observations suggest that Asteraceae are also entomophilous (Birkhawsh et al. 2004). It is notable, however, that together with the wind-pollinated Simmondsia, two other early-diverging lineages do at least exhibit morphological characteristics that are reminiscent of wind-pollinated flowers. Despite reports of bee visitation, Rhabdodendron exhibits very long anthers and sepaloid petals, lacks a nectary, possesses a gynoecium with only one or two ovules and a single seed in fruit, and has a relatively long stigma (P. K. Endress, personal communication); perianth parts also fall off as the flower opens (Nelson and Prance 1984). Physena exhibits very long anthers and no petaloid organs, lacks a nectary, and has a large stigmatic surface. Coding Physena as wind pollinated, however, does not alter the conclusion of the character mapping analyses, and thus there is little support for an anemophilous ancestry in the core Caryophyllales. Indeed, as noted by Clement and Mabry (1996), even if one accepts the highly reduced inconspicuous flowers of Amaranthaceae s.l. as archetypal, it is not necessary to invoke wind pollination because it has already been noted that many of the diminutive flowers in Amaranthaceae are probably entomophilous (Blackwell and Powell 1981; Kuhn 1993).

Reconstruction of Pollination Mechanism

Despite recovering entomophily as ancestral, our character reconstruction analyses suggest that an undifferentiated perianth arose early within the core Caryophyllales (in agreement with Ehrendorfer 1976; Ronse De Craene 2008). This perianth type has been strongly correlated with anemophily (Friedman and Barrett 2008). Parsimony reconstruction infers that the evolution of this undifferentiated condition evolved after the divergence of Rhabdodendron, while the stochastic mapping analyses recover the basalmost node in core Caryophyllales as either undifferentiated or differentiated, with equal probability. Subsequent nodes along the backbone of the tree until the divergence of Molluginaceae are recovered as undifferentiated (with greater than 0.99 posterior probability).

A discussion of perianth evolution within the core Caryophyllales is complicated by the great diversity of floral structure within the order and the uncertainty in defining the correspondence of these structures both within Caryophyllales and with respect to floral organs in other eudicots. Observations by Ronse De Craene (2007, 2008) suggest that although petal organs in eudicots may appear homologous with respect to position and superficial appearance, the variable expression of features reminiscent of either stamens or bracts means that petals in different lineages of core eudicots are of uncertain homology and may have been differently derived. This viewpoint argues against the widespread notion that the petals within eudicots are invariably derived from stamens and makes it difficult to homologize between perianth parts even within the core eudicots (Ronse De Craene 2007, 2008). In the Caryophyllales, these difficulties are compounded by different floral structures and the limitations of established terminology. The specific terms “petal,” “sepal,” “corolla,” and “calyx” are not usefully applied to Caryophyllid taxa because they imply not only the characteristics and function of an organ but also the position of the organ (Endress 1994; Jaramillo and Kramer 2007). In core eudicots, for example, the term “petal” implies both the showiness of the perianth part and the position of the organ in the second whorl of the flower (Jaramillo and Kramer 2007). However, in comparing the differentiated perianth found in many Caryophyllales with the bipartite perianth of most core eudicots, the positional feature alone is not necessarily a sufficient criterion of homology, and terms such as “petal” that imply positional correspondence are misleading. Similarly, the term “bipartite perianth” should also be avoided because this implies the presence of distinct perianth whorls. While distinct perianth whorls may be found in some families in the Caryophyllales, e.g., Limeaceae (Hofmann.
1973) and Caryophyllaceae (Rohweder 1967), differentiation in a spiral phyllotaxis occurs in Cactaceae. For the purposes of this discussion, therefore, we use the term “differentiated” to describe a perianth that comprises at least two distinct types of organ that perform the functions commonly ascribed to the calyx and corolla. We refer to members of a differentiated perianth as either petaloid or sepaloid (i.e., resembling the petals or sepals of other core eudicots and putatively performing similar functions without necessarily being homologous by positional criterion alone). Finally, because the terms “petaloid” and “sepaloid” refer only to a superficial resemblance and putatively similar function, within Caryophyllales we apply these terms to structures that are clearly nonhomologous in other respects. The terms “sepaloid tepal” and “petaloid tepal” are applied to the quincuncial perianth parts that are present in core Caryophyllales, while “petaloid staminodes” refer to perianth parts that are clearly androecium derived.

Multiple Origins of Perianth Differentiation

Our analyses suggest that there have been a minimum of nine independent origins of a differentiated perianth within the Caryophyllales. This is more than the minimum suggested by Ronse De Craene (2008), who, in a broad survey of eudicots, cites five origins of petals in the core Caryophyllales, occurring in Stegnospermataceae, Aizoaceae, Portulacaceae clade, Caryophyllaceae, and Molluginaceae. Considering the reconstructions provided by Ronse De Craene (2008), the difference in our respective estimations of perianth differentiation can be attributed to several factors. Most significantly, coding and definition of the perianth differ in our studies; e.g., Nyctaginaceae and the portulacaceous cohort are coded as petals absent (Ronse De Craene 2008; fig. 3), but by our definition, both Mirabilis jalapa (Nyctaginaceae) and the portulacaceous cohort have a differentiated perianth and are listed as polymorphic and differentiated, respectively. Similarly, Glimus is a member of the Molluginaceae that possesses putatively staminal petals (Hofmann 1994, pp. 137, 141) but is coded as petals absent by Ronse De Craene (2008). In our analysis, Molluginaceae are coded as polymorphic, exhibiting both taxa with a uniseriate, undifferentiated perianth and taxa with differentiated perianth. A different tree topology may also be a factor contributing to the different results, e.g., Rhabdodendron is sister to the core Caryophyllales (this study), and the placement of Corbichonia and Glimus as successive sisters to the “globuloid” clade by Ronse De Craene (2008) is erroneous based on current understanding; Corbichonia is most likely sister to the raphide clade (Cuénoud et al. 2002), and Glimus (Molluginaceae) is sister to the portulacaceous clade (BS 70% [according to Cuénoud et al. 2002] and BS 100% [this study]). Finally, because of the broader scope of the study by Ronse De Craene (2008; i.e., all eudicots), lineages of core Caryophyllales with differentiated perianth are also undersampled, with both Asteropeia and Limenum excluded from his study. Consequently, differences in emphasis, sampling, coding, and tree topology may all have contributed to the differences between Ronse De Craene’s (2008) results and those we report here.

Despite these differences, the five independent origins of petals described by Ronse De Craene (2008) are included in the nine independent derivations of differentiated perianth inferred in our study. These nine origins occur in Asteropeiaeae, Caryophyllaceae (although several genera do not have differentiated perianth), Stegnospermataceae, some species of Limenum, Corbichonia (not sequenced in this study), the subfamilies Mesembryanthemoideae and Ruschioidae within Aizoaceae, Mirabilis in Nyctaginaceae, Glimus in Molluginaceae, and the portulacaceous cohort (Portulacaceae, Didiereaceae, Basellaceae), including Cactaceae. The number of origins of differentiated perianth could well increase, depending on the final placement of the enigmatic Macarthuria and increased resolution of phylogenetic relationships within Caryophyllaceae. Developmental evidence (where available) is consistent with these independent origins of differentiated perianth indicated by character reconstruction analyses. We discuss the developmental evidence for perianth differentiation by different mechanisms in these nine lineages: through differentiation of putatively homologous organs and through the recruitment of floral structures derived either from the androecium or from the preceding bracts.

Recruitment of Preceding Bracts

The secondary recruitment of preceding bracts to from perianth parts has occurred twice within the globular inclusion clade, once in Nyctaginaceae and again in the portulacaceous cohort (fig. 3). Developmental studies suggest that the mechanism underlying the recruitment of preceding bracts is different in these distinct lineages. Within Nyctaginaceae, an involucre may have evolved more than once, occurring also in Abronia, Allionia, Boerhavia, Bougainvillaea, Mirabilis, Nyctaginia, and Tripterocalyx (Douglas and Manos 2007). In Boerhavia, Sharma (1963) describes an involucre surrounding five lateral flowers and one central flower. In Mirabilis, however, there appears to be a tendency toward reduction in floral number. In Mirabilis nyctagonus, the first three leaves of the involucre subtend axillary flowers (Hofmann 1994). In Mirabilis jalapa, each flower has a differentiated perianth with a calyx of five fused parts that has been secondarily derived from an involucre of bracts (fig. 4L). This variation within Mirabilis suggests that the apparent bipartite perianth in M. jalapa may have been derived through reduction in floral number. Subsequent loss of five lateral flowers is inferred, leaving a single central flower with the involucre appearing as a pseudocalyx (Vanvinckenroye et al. 1993). In Nyctaginaceae, floral loss within an involucre of bracts would appear to result in an apparently differentiated perianth, although the association of the involucre (calyx) with the rest of the flower is weak (Rohweder and Huber 1974).

Portulacaceae s.l., Didiereaceae, and Basellaceae share a distinct floral morphology that emerges following the divergence of Molluginaceae (fig. 3). These lineages possess flowers with an involucre that, in contrast to Mirabilis (Nyctaginaceae), comprises two leafy phyllomes that are inserted below the petaloid members of the perianth. Importantly, their developmental origin is probably different from the involucre found in Nyctaginaceae because Hofmann (1994) comments that axillary products are never formed in the axes of these phyllomes. They are termed “involucral phyllomes” by Hofmann (1994), reflecting the belief that these organs are additional phyllomes inserted be-
Fig. 4  Diverse forms of perianth in the core Caryophyllales. A, Bougainvillea sp. (Nyctaginaceae); B, Claytonia sp. (photo by Ron Wolf; Portulacaceae s.l.); C, Aptenia cordifolia (Aizoaceae); D, Opuntia humifusa (Cactaceae); E, Stegnosperma sp. (photo by Debra Valov; Stegnospermataceae); F, Sesuvium portulacastrum (Aizoaceae); G, Hypertelis salsoloides (Molluginaceae); H, Chenopodium sp. (photo by Brian Johnston; Amaranthaceae); I, Portulaca oleracea (photo by Kurt Neubig; Portulacaceae); J, Stellaria media (photo by Kurt Neubig; Caryophyllaceae); K, Phytolacca americana (photo by Kurt Neubig; Phytolaccaceae); L, Mirabilis jalapa (photo by Walter Judd; Nyctaginaceae).
tween the bracteoles and the sepals; however, there have been other interpretations as to the nature of these phyllomes. Sharma (1954; reviewed in Milby 1980), who examined vascular anatomy in *Portulaca* and *Talimun*, concluded that the flowers are essentially dimerous, with the pentamerosed petaloid perianth inferred as a derived condition. These alternatives will merit further developmental study as phyllogenetic understanding within this group is clarified, but it is valuable to consider these different interpretations in light of the current phylogeny and perianth reconstruction analysis (fig. 3). The ancestral floral condition of the portulacaceous cohort is uniseriate pentamery; therefore, Sharma’s (1954) interpretation suggests reduction to a dimerous state, followed by a reversal to a pentamerosed condition. Irrespective of the developmental origin of these two phyllomes, in many species they cover the developing floral meristem very early in development and thus perform the function of a calyx in a differentiated perianth (Hofmann 1994). Subfunctionalization of perianth roles may have facilitated the high degree of petaloidy in the inner quincuncial perianth members of these families (fig. 4B, 4I, 4L): “the involucral phyllomes cover the inner bud very early and take over the function of the calyx. Therefore, the sepals [uniseriate pendants] behave like petals” (Hofmann 1994, p. 138).

Within the portulacaceous cohort, a very different floral structure is found in Cactaceae. In contrast to the perianth of Portulacaceae s.l., Didiereaceae, and Basellaceae, Cactaceae exhibit a great increase in perianth parts. Increases in floral meristem and generally modified floral form make it challenging to determine correspondence between perianth in Cactaceae and its closest relatives—in this study, *Portulaca* and *Talimun*. The perianth parts of Cactaceae are suggested to be bracteal rather than staminodial in their homology (Buxbaum 1950–1955, pp. 122, 123; Ronse De Craene 2007, 2008) and are arranged in a spiral phyllotaxy. The perianth may have arisen by inclusion and differentiation of supernumerary bracts (Ronse De Craene 2008) or simply by formation of additional bracts. Differentiation of the perianth occurs with outer sepaloid parts and highly petaloid inner parts (fig. 4D). This high degree of differentiation, together with a spiral phyllotaxy, is unusual within the Caryophyllales; however, Ronse De Craene (2008) highlights that this combination of floral characters (large, spirally arising petals with a multistaminate androecium) occurs in several derived lineages in the core eudicots. Endress (2002) suggested that increases in numbers of stamens and/or carpels may result in increase in size of the flower, greater plasticity, and irregular petal development. This developmental interpretation is consistent with our reconstruction analyses, which do not argue for an independent origin of differentiated perianth in Cactaceae; rather, an increase in meristem size and merosity of reproductive organs may be in part responsible for the unusual perianth in Cactaceae.

**Petaloid Modification of the Androecium**

Reconstruction analyses suggest that perianth differentiation through sterilization and petaloid modification of the outer members of a centrifugally initiating androecium has arisen a minimum of three times in Caryophyllales (fig. 3): clear examples occur in *Aizoaceae* (fig. 4C), Molluginaceae, and *Corbichonia* (not sampled in this study but shown to be a distinct lineage within the raphide clade; Cuénoud et al. 2002). In *Glinus*, *Corbichonia*, and *Aizoaceae*, the petaloid structures can be readily interpreted as differentiated staminodial structures (Ronse De Craene 2008). For example, within *Aizoaceae* subfamilies, Ruschioideae, and Mesembryanthemoideae, androecial development proceeds centrifugally, and the basipetal members become progressively more sterile and petaloid, with intermediates conceptually linking the outermost petals to the inner fertile stamens (fig. 4C). A similar situation has been described in *Glinus* in the Molluginaceae (Hofmann 1994) and in *Corbichonia* (Ronse De Craene 2007).

Petaloid members of the differentiated perianth in Caryophyllaceae, *Limeum*, Stegnosperma, and *Macarthuria* have also been attributed to the androecium (Hofmann 1973; Ronse De Craene 2007, 2008). The reconstruction analyses suggest that these differentiated perianths have occurred independently and thus merit further developmental study. The assessment of homology between the petaloid members of the differentiated perianth and the androecium is complicated by a high degree of variability in androecium organization, processes of reduction, and differences in phyllotaxy. However, several lines of evidence suggest an androecial origin of the petals in Caryophyllaceae (Rohweder 1967; reviewed in Ronse De Craene et al. 1998; Ronse De Craene 2007, 2008). Ronse De Craene et al. (1998) review the presence and absence of petals in 52 genera of Caryophyllaceae: nine genera lack petals, 11 genera have both species with petals and species without, while the remaining 32 genera in the survey possess petals. It remains unclear whether the absence of petals is ancestral in Caryophyllaceae or whether instances of petal loss have occurred. The most comprehensive molecular phylogeny of Caryophyllaceae to date (Fior et al. 2006) sampled only two genera with apetalous members (*Paronychia* and *Sagina*), but none of the entirely apetalous genera were sampled.

**Differentiation of Homologous Perianth Parts**

Despite the high degree of variation in floral structure found in different lineages of Caryophyllales, there are key common elements. Almost all lineages within the order possess five perianth members that are organized in a uniseriate quincuncial arrangement (with the exception of Cactaceae, which is multiseriate). Occasionally, one of the members in this series has been lost, to give a tetramerous perianth, e.g., in *Tetragonia* and *Aptenia* in *Aizoaceae*, Rivinoideae, and Didiereaceae, but these cases of tetramery are clearly derived from pentamerosed ancestors. These uniseriate quincuncial perianth members are probably homologous, given their constancy, position in the flower, and common phyllotaxy. These putatively homologous organs have, however, undergone considerable differentiation in certain lineages, which often correlates with the emergence of a differentiated perianth. For example, in *Aizoaceae*, members of the early-diverging subfamilies Sesuvioideae and Aizooidae possess a quincuncial uniseriate perianth whose members are petaloid on the adaxial surface and sepaloid on the abaxial side. In the derived subfamilies Mesembryanthemoideae and Ruschioideae, the androecium is polyandrous, and the outer members of centrifugally initiating stamens are sterile, resulting in a differentiated perianth with androecial-derived petaloid organs. Concomitantly, the outer quincuncial uniseriate perianth loses...
all petaloid characteristics and resembles only a calyx. In instances where differentiation of the perianth has been achieved through recruitment of involucral bracts and/or bracteoles (Portulacaceae and Mirabilis), the involucral organs act as a calyx, and the now-inner uniseriate quincuncial perianth members are considerably more showy and petaloid (cf. the showy petaloid perianth of the portulacaceous cohort [fig. 4B, 4D, 4I] with the diminutive simple perianth of some genera in Molluginaceae). Seemingly homologous perianth parts within Caryophyllales can be petaloid, e.g., Nyctaginaceae (fig. 4A, 4L) and Portulacaceae (fig. 4J); sepaloid, e.g., *Limeum*, *Stegeosperma* (fig. 4E), Molluginaceae, Ruschioideae, and Mesembryanthemoidae (fig. 4C), Caryophyllaceae (fig. 4J), and *Simmondsia*; or chimeric, e.g., Sesuvioideae/Aizoioideae (fig. 4F) and *Hypertelis* (fig. 4G).

**Caryophyllales as a System for Floral Evo-Devo**

Nine origins of a differentiated perianth, the concomitant evolution of petaloidy from either androecial or bracteal organs, and varying degrees of petaloid differentiation in homologous structures across the order make the Caryophyllales a valuable system for exploring the evolutionary developmental genetics of petaloidy in core eudicots. In the majority of core eudicots whose petal developmental genetics have been examined (e.g., in *Arabidopsis thaliana*, *Antirrhinum majus*, *Solanum lycopersicoides*, *Nicotiana tabacum*, *Petunia hybrida*), differentiation of the petals is strongly influenced by MADS-box transcription factors: APETALA3 (AP3) and PISTILLATA (PI; in *A. thaliana*) and their orthologues (Irish and Kramer 1998; Kramer and Irish 1999). In these core eudicot species, AP3 and PI orthologues are expressed throughout the development of the petal, and their ubiquitous expression in the petal has been shown to be necessary for normal petal development in *A. thaliana* and *A. majus* (Bowman et al. 1989; Sommer et al. 1991; Zachgo et al. 1995). It seems apparent that these genes play a conserved role in petal identity in the core eudicots examined so far, yet core eudicot petals have also traditionally been considered to be homologous, stamen-derived organs; this homology has been invoked to explain such developmental genetic similarities (Irish and Kramer 1998). More recently, however, the assertion that petals in core eudicots are largely homologous and predominantly stamen derived has been questioned (Ronse De Craene 2007). Although in Caryophyllales the homology of the perianth parts to petals in other core eudicots is uncertain, it is clear that many lineages (Sesuvioideae, Nyctaginaceae, Portulacaceae, Cactaceae) possess petaloid organs that are bracteal rather than staminal in origin. Furthermore, the occurrences of stamen-derived petals within Caryophyllales (Caryophyllaceae, Aizoaceae, *Glinus*, and *Corbichonia*) are phylogenetically derived, independent events. These independent occurrences are valuable for further study because there are very few examples of petals within core eudicots that are unquestionably stamen derived (Ronse De Craene 2007). The pattern of petaloid evolution in the Caryophyllales therefore presents a unique opportunity to address long-standing questions regarding differences and/or similarities in the developmental genetics of bracteopetals and andropetals (Ronse De Craene 2008; rearticulated by Ronse De Craene (2008), this question remains highly pertinent in studies of floral diversification. The improved phylogenetic understanding reported here provides opportunities for comparing bracteopetalous and andropetalous lineages that have arisen more recently than both basal angiosperms (traditionally considered to bear bracteopetals) and the early-diverging eudicot lineages (with their presumed andropetals). The Caryophyllales are a well-defined clade within core eudicots, but, in a sense, the patterns of perianth evolution discussed here recapitulate (on a smaller phylogenetic scale) the evolutionary trends traditionally thought to have taken place across the angiosperms as a whole (Bessey 1915; Takhtajan 1991). Therefore, despite uncertainty surrounding the precise correspondence of the caryophyllid perianth with the perianth of other eudicots, evo-devo investigations in the Caryophyllales may have far-reaching implications for our understanding of petal evolution and perianth differentiation. Is there latent developmental genetic homology underlying these derived and oft-seemingly dissimilar occurrences of perianth differentiation in Caryophyllales? What is the involvement of AP3 and PI orthologues in these bracteopetals in Caryophyllales? How do expression and function of AP3 and PI orthologues in caryophyllid bracteopetals compare with their expression and function in the derived instances of andropetals? How do expression and function of AP3 and PI orthologues compare in the different occurrences of andropetals? Evolutionary developmental approaches to these questions are currently under way (Brockington et al. 2007) and may shed light on the evolutionary origins and homology of these diverse perianth forms in relation to the perianth in other core eudicots.

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**Literature Cited**

