

## THE COMPARATIVE BIOLOGY OF MIRROR-IMAGE FLOWERS

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Enantiostyly, the deflection of the style to the left or right side of the floral axis, is a sexual polymorphism reported from at least 10 angiosperm families in both monocotyledons and dicotyledons. To investigate variation in the enantiostylous condition, we examined sex-organ reciprocity and the arrangements of left- and right-styled flowers in 15 enantiostylous species from eight genera in six families. Enantiostyly can be expressed at three distinct levels of structural organization. In “monomorphic enantiostyly,” individual plants produce both floral forms, either mixed within an inflorescence or segregated between left- and right-styled inflorescences. In “dimorphic enantiostyly,” plants are exclusively left- or right-styled and this condition is a genetic polymorphism. Enantiostyly is most commonly associated with the reciprocal deflection of a pollinating anther (“reciprocal enantiostyly”), although there is a continuum in stigma-anther position, ranging from no reciprocal anther to complete reciprocity. Comparative analyses of a composite phylogeny of the monocotyledons indicate that in this group enantiostyly has evolved at least 11 times, often simultaneously with the gain of heteranthery. Both concentrated changes and maximum likelihood tests indicated that heteranthery and the loss of nectaries are significantly associated with the presence of enantiostyly, indicating a syndrome of traits where pollen is the main reward and anthers are divided into two functions: pollination and nutrition. The association of enantiostyly with heteranthery, the loss of nectaries, and the reciprocal placement of a pollinating anther suggest that the polymorphism functions to increase the precision of cross-pollen transfer and to reduce interference of stigmas and anthers within or between flowers on the same plant.

*Keywords:* asymmetry, cross-pollination, enantiostyly, floral syndromes, monocotyledons, phylogeny reconstruction, plant sexual polymorphism.

### Introduction

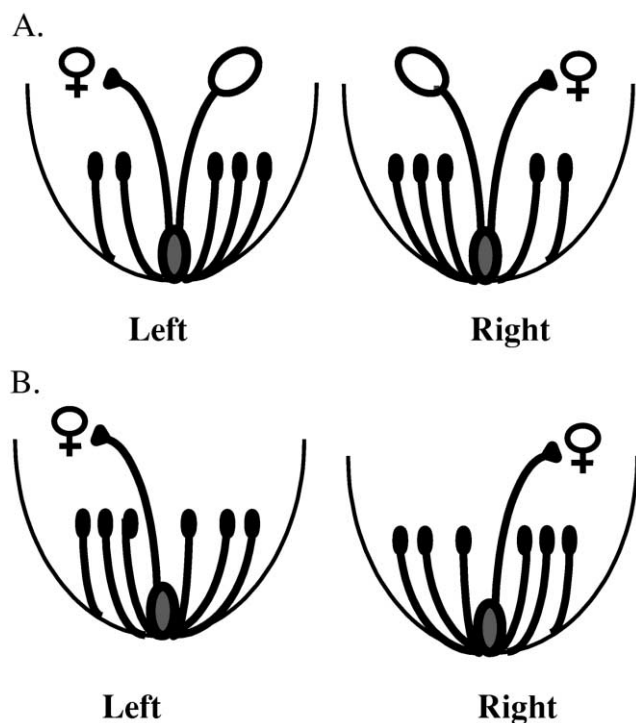
The enormous diversity of floral form exhibited by animal-pollinated plants largely reflects adaptations that function to maximize mating success in different pollination environments. For example, floral traits influence the position of pollinators, controlling the amount and efficiency of pollen removal, transfer, and deposition (Robertson and Lloyd 1991; Harder and Barrett 1993). These traits include variation in perianth structure (e.g., zygomorphy; Donoghue et al. 1998; Endress 1999), the relative positions of sexual organs (e.g., heterostyly and herkogamy; Webb and Lloyd 1986; Barrett 1992), and the location of the reward (e.g., specialized floral spurs; Hodges and Arnold 1995). Although variation in floral characters is immense, unrelated groups often share common traits, suggesting that they have evolved by similar selective pressures. Many unrelated plants visited by similar pollinators exhibit enough convergence in floral form that syndromes of traits have been recognized (Grant and Grant 1965; Faegri and Van der Pijl 1979; Bruneau 1997).

Although there has been much debate on the utility of floral syndromes (reviewed in Johnson and Steiner 2000), the mul-

iple origins of associated characters can provide information about both evolutionary sequences and convergent adaptive function. For example, in the stylar polymorphism heterostyly, the reciprocal positioning of stigma and anther heights and ancillary polymorphisms found in numerous unrelated families are commonly associated with actinomorphic tubular corollas, the presence of nectar, longitudinal anther dehiscence, and centrally presented stigmas (Lloyd and Webb 1992a). These characters are part of a syndrome in which long-tongued pollinators probe for nectar and the sexual organs contact the pollinator's body in succession as the proboscis enters and leaves the flower. This association led Lloyd and Webb (1992a) to argue that heterostyly has evolved from approach herkogamous flowers that exhibit the same characters. Other functional associations include the suite of floral traits associated with wind pollination (Linder 1998), buzz pollination (Buchman 1983), and cleistogamy (Lord 1981).

Four stylar polymorphisms are commonly reported in flowering plants: distyly, tristyly, stigma-height dimorphism, and enantiostyly (reviewed in Barrett et al. 2000b; but see Li et al. 2001). The first three are style-length polymorphisms, and in both distyly and tristyly, the nature, the evolution, and the function of the polymorphisms have been extensively documented (reviewed in Barrett 1992). In contrast, enantiostyly, an asymmetry polymorphism in which the style is deflected either to the left or right side of the flower, is not well understood. The occurrence of mirror-image flowers in diverse, unrelated families such as the Solanaceae, Caesalpinaceae, and

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**Fig. 1** Enantiostyly can be expressed in two ways depending on the arrangement of anthers in the flower. *A*, Reciprocal enantiostyly in which stylar deflection is accompanied by the reciprocal deflection of the style and a pollinating anther. *B*, Nonreciprocal enantiostyly. Here, the style is deflected from the central axis of the flower either to the left or to the right, and there is no anther dimorphism.

Haemodoraceae implies that enantiostyly has evolved on multiple occasions, probably as a result of convergent selective pressures associated with cross-pollination (Graham and Barrett 1995).

Although mirror-image flowers have been recognized for more than a century (Todd 1882), there has been no synthesis of information on the nature of enantiostyly or its systematic distribution, evolution, and functional significance. Here, we provide a comprehensive review of the biology of enantiostyly. Our review consists of three major sections. In the first, we examine variation in the forms of enantiostyly that occur among angiosperm families both in the arrangement of sex organs within a flower and in the distribution of left- and right-styled flowers within and between inflorescences and plants. This analysis leads to a classification of the various forms of enantiostyly that exist in nature. In the second section, we examine the distribution of enantiostyly among angiosperm families. Using a composite phylogeny of the monocotyledons based on molecular data, we investigate the number of evolutionary origins of enantiostyly within this large monophyletic clade. We focus in particular on the monocotyledons for two reasons. First, the availability of recent phylogenetic information facilitates comparative analysis. Second, enantiostyly is especially well developed in this clade and is likely to have evolved on multiple occasions (Graham and Barrett 1995; Kohn et al. 1996). In the final section, we review the mor-

phological characters associated with enantiostyly and investigate whether enantiostyly is associated with a suite of traits that might constitute a floral syndrome.

## The Nature of Enantiostyly

### A Classification of Enantiostyly

Mirror-image flowers appear to have been first reported by Todd (1882), who observed that flowers with left- and right-deflected styles occurred on the same inflorescence in *Solanum rostratum* and *Cassia chamaecrista* (*Chamaecrista fasciculata*), while Wilson (1887) reported in *Wachendorfia paniculata* that individual plants were fixed for either left- or right-styled flowers. The term “enantiostyly” seems to have been first used by Knuth (1906), who defined it as “the occurrence of right-styled and left-styled flowers” (p. 107). Knuth (1906) recognized three types of enantiostyly: (1) without a division of labor among anthers, (2) with a division of labor among anthers but without “preferential crossing of opposite floral forms,” and (3) with a division of labor among anthers and “regular crossing between flowers of opposite forms” (p. 107). Crossing between flowers of opposite stylar orientation is likely to be influenced by the degree of reciprocity in the positioning of the stigma and pollinating anther. If a stigma and anther are reciprocally positioned on opposite sides of the flower, it is likely that pollen transfer would be most effective between flowers of opposite form. This reciprocity of sexual organs is functionally similar to the reciprocal arrangement of anthers and stigmas in heterostylous species.

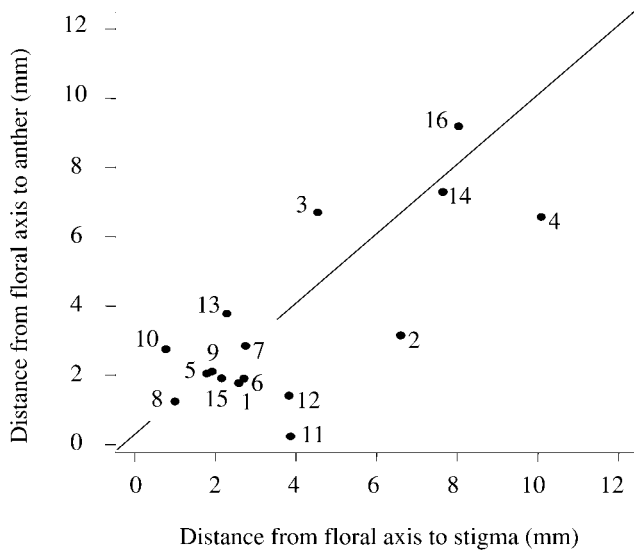
Our surveys of enantiostylous species (Jesson et al. 2003b) indicate that many species possess a single stamen deflected to the opposite side of the flower to the style. We refer to this condition as “reciprocal enantiostyly” (fig. 1). This stamen is usually larger and more cryptically colored (often the same color as the perianth) than other stamens in the flower and is usually associated with pollination by pollen-collecting bees. However, as Knuth (1906) recognized, not all enantiostylous species possess a reciprocal arrangement of the stigma and pollinating anther, and in some groups, there is no division of

**Table 1**

**Ratio of Left- and Right-Styled Inflorescences in Nine Populations of *Monochoria australasica* from Northern Territory and Northern Queensland, Australia**

Population	Number sampled	Ratio left : right
Tortilla Flats	92	0.55
Pell	19	0.57
South Adelaide 1	32	0.56
South Adelaide 2	122	0.41
South Adelaide 3	48	0.48
Cooktown	33	0.48
Hann Roadhouse	19	0.47
Musgrave	79	0.48
Hann River Crossing	62	0.53

Note. An attempt was made to sample a single inflorescence from each flowering plant in the population during May and June 2001. *G*-tests for goodness of fit to a 1 : 1 ratio:  $G_{\text{pooled}} = 0.07$ ,  $P = 0.79$ ;  $G_{\text{het}} = 6.97$ ,  $P = 0.54$ .



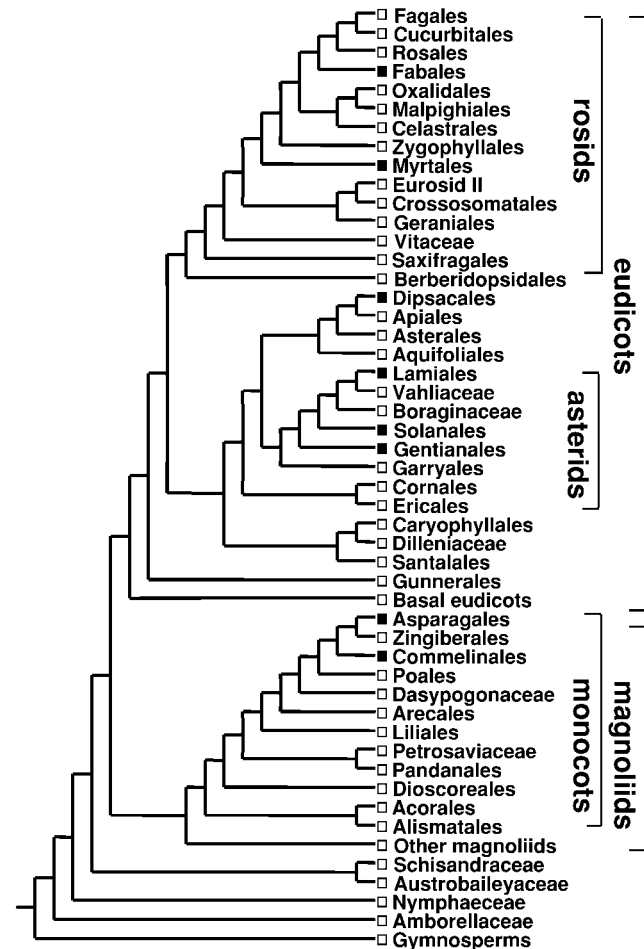
**Fig. 2** Reciprocity in the deflection of style and stamen for 15 enantiostylous species and one nonenantiostylous species. Measurements were made from the floral axis to the appropriate sex organ. Numbers refer to individual species (localities and number of flowers measured are in parentheses below): 1, *Commelina* sp. (University of Toronto glasshouse population [UTGH], Toronto,  $n = 20$ ); 2, *Cyanella lutea* (MacGregor, Cape Province, South Africa,  $n = 50$ ); 3, *Dilatriis corymbosa* (Silvermine Nature Reserve [SMNR], Cape Town, South Africa,  $n = 90$ ); 4, *Dilatriis viscosa* (Jonkershoek Nature Reserve, Stellenbosch, South Africa,  $n = 45$ ); 5, *Dilatriis pilansii* (SMNR,  $n = 40$ ); 6, *Exacum* sp. (UTGH,  $n = 56$ ); 7, *Heteranthera mexicana* (UTGH,  $n = 100$ ); 8, *Heteranthera multiflora* (Poplar Bluff, Mo.,  $n = 55$ ); 9, *Heteranthera rotundifolia* (Poplar Bluff,  $n = 30$ ); 10, *Heteranthera limosa* (Poplar Bluff,  $n = 30$ ); 11 *Monochoria australasica* (Tortilla Flats, Northern Territory, Australia,  $n = 42$ ); 12, *Saintpaulia ionantha* (UTGH,  $n = 32$ ); 13, *Wachendorfia brachyandra* (Oranjekloof Nature Reserve, Cape Town,  $n = 40$ ); 14, *Wachendorfia paniculata* (SMNR,  $n = 88$ ); 15, *Wachendorfia parviflora* (Red Hill, Simonstown, South Africa,  $n = 42$ ); 16, *Wachendorfia thyrsiflora* (Oranjekloof Nature Reserve,  $n = 68$ ). *Dilatriis pilansii* is not truly enantiostylous, but styles are deflected in a random direction away from the floral axis. In species of *Dilatriis* and *Commelina*, two anthers function as “pollinating anthers”—one adjacent to the style and one opposite the style. Measurements were made of the latter anther. Two or three flowers were measured per plant.

labor in anther function. We refer to this condition as “non-reciprocal enantiostyly.”

As earlier workers recognized, there are two fundamentally different patterns of organization of left- and right-styled flowers on individual plants (Jesson et al. 2003b). We refer to these two distinct conditions as “monomorphic enantiostyly” and “dimorphic enantiostyly.” Monomorphic enantiostyly is the presence of left- and right-styled flowers on the same plant, also termed “somatic enantiostyly” (Fenster 1995; Graham and Barrett 1995; Wang et al. 1995). Thus, all plants in a population have the same floral phenotype, and the population is monomorphic for this stylar condition. Dimorphic enantiostyly is the presence of left- and right-styled individuals within a population. These individuals are fixed for stylar ori-

entation, and this condition is a true genetic polymorphism (Jesson and Barrett 2002b).

Monomorphic enantiostyly is expressed in two distinct ways. Virtually all monomorphic enantiostylous species have left- and right-styled flowers on the same inflorescence. Representative examples include *Monochoria korsakowii* (Pontederiaceae), *S. rostratum* (Solananaceae), *Cyanella lutea* (Tepophilaceae), *C. fasciculata* (Caesalpiniaceae), *Philydrum lanuginosum* (Philydraceae), and *Dilatriis corymbosa* (Haemodoraceae). However, in *Monochoria australasica* (Pontederiaceae), a perennial aquatic from tropical Australia, inflorescences are entirely left-styled or entirely right-styled, and individual plants can produce both left- and right-styled inflorescences. *Monochoria australasica* therefore exhibits monomorphic enantiostyly, but the variation in left- and right-styled flowers occurs between inflorescences. Many individuals of *M. australasica* consist of a single ramet in which only one inflorescence is open on a given day. These individuals therefore function on a given day as left- or right-styled plants. However, larger plants exhibit clonal growth, and several ramets of a clone can flower simultaneously. In these individuals, both left-



**Fig. 3** An ordinal phylogeny of the angiosperms (from Soltis et al. 1999). Black boxes indicate confirmed cases of the occurrence of enantiostyly in an order.



and right-styled inflorescences can occur together within a plant. *Monochoria australasica* represents the only known example of inflorescence-level monomorphic enantio styly in flowering plants.

The presence of monomorphic enantio styly at the inflorescence level should serve as a caution for researchers conducting population-level surveys of enantio styly. In nine populations of *M. australasica* we sampled in the Northern Territories and Queensland, we found that the ratios of left- and right-styled inflorescences in a population were consistently 1 : 1 (table 1). Isoplethic morph ratios have been interpreted as resulting from negative frequency-dependent selection between morphs in species with dimorphic enantio styly (Jesson and Barrett 2002a). However, as demonstrated here, a 1 : 1 ratio in a population does not necessarily indicate that the species possesses dimorphic enantio styly. Rather, in the case of *M. australasica*, this pattern likely results from environmental or developmental factors. This occurs in many animals where 1 : 1 ratios involving directional asymmetries in morphological structures can be environmentally determined (Palmer 1996).

In species with dimorphic enantio styly, all inflorescences on a plant contain flowers that have the same stylar orientation. In *Heteranthera multiflora*, dimorphic enantio styly is controlled by a single diallelic Mendelian locus with right deflection dominant to left (Jesson and Barrett 2002b). In *Barbaretta aurea* and *Wachendorfia thyrsiflora*, populations that are monomorphic for style deflection occur. In these species, stylar monomorphism is the consequence of founder effects and extensive clonality (Ornduff 1974; Jesson and Barrett 2002a). Flowers on a single clone are always uniform in style orientation despite the occurrence of many inflorescences per genet. This provides further evidence for the genetic control of stylar bending in these clonal species.

In some species, the direction of deflection is uniform for all individuals. However, this is not a form of enantio styly because both forms of stylar deflection do not occur within a species. For example, we sampled five populations of *Tenicroa exuviata* (Liliaceae) within Cape Province, South Africa, during September 1998. All plants were entirely right-styled ( $n = 20$  to 100 for each population). This form of deflection may simply represent a type of herkogamy in which the stigma is deflected away from the anther or, less likely, is a breakdown of enantio styly because of the loss of one morph from an ancestral species with dimorphic enantio styly. In theory, dimorphic enantio styly species could evolve from this form of herkogamy through the invasion of a variant with the alternate style orientation. In distyly, it has been hypothesized by Lloyd and Webb (1992b) that the polymorphism evolved because of the invasion of a short-styled variant into an approach herkogamous population (where the style is exerted beyond the anthers). It is possible that enantio styly evolved via an analogous pathway (Jesson et al. 2003a).

### Patterns of Sex-Organ Reciprocity in Enantio styly Species

To investigate variation in the reciprocal positioning of sex organs, we compared sex-organ reciprocity among 15 enantio styly species from eight genera in six families (fig. 2). Our results indicate that while many species have complete reciprocal positioning of the stigma and one anther (usually the pollinating anther), in others this anther is not deflected to the same extent as the style. Indeed, there is a continuum from no deflection of an anther to reciprocal positioning of both stigma and anther. The four dimorphic enantio styly species included in this sample (*Wachendorfia brachyandra*, *W. paniculata*, *W. thyrsiflora*, and *H. multiflora*) (fig. 2, species 13, 14, 16, and 8, respectively) all exhibit some degree of reciprocal herkogamy. In contrast, in *Saintpaulia ionantha*, the style is deflected away from a central cone of anthers that are of equal size, and there is no reciprocity between stigma and anther position (fig. 2, species 12). It is important to note that in enantio styly species heteranthery is not a requirement for sex-organ reciprocity. For example, despite the reciprocal positioning of the stigma and one anther in *Wachendorfia*, these species do not have a division of labor among the anthers—all anthers are of equal size. In contrast, *Heteranthera* flowers possess conspicuous heteranthery despite weaker reciprocity between the stigma and pollinating anther. Although the reciprocal arrangement of stigma and anther position is an important feature of most enantio styly species, especially those with dimorphic enantio styly, it is not a sufficiently consistent condition to define the polymorphism.

Enantio styly is therefore a stylar polymorphism that is expressed at a number of different levels of structural organization from the flower to the whole plant. Monomorphic enantio styly can occur with variation between flowers on an inflorescence or between inflorescences on an individual. Dimorphic enantio styly involves variation in stylar deflection between individuals in a population. Flowers of enantio styly species either can have the style alone deflected or can have both the style and one anther deflected in opposite positions. The presence of an anther in a reciprocal position to the style in most enantio styly species strongly suggests that this floral polymorphism functions to increase the precision of pollen transfer between flowers of opposite type. Indeed, experimental evidence indicates that pollen transfer between left- and right-styled flowers (and vice versa) is greater than transfers between flowers of the same form (Bowers 1975; Jesson and Barrett 2002c). For species with nonreciprocal enantio styly, this explanation is not sufficient, and therefore alternative hypotheses are warranted. In nonreciprocal enantio styly species, the style orientation may serve to reduce sexual interference between pollen deposition and receipt and may also

**Fig. 4** A phylogeny of the monocotyledons with Commelinaceae, Haemodoraceae, Pontederiaceae, and Tecophilaeaceae expanded. The most parsimonious reconstructions of the ancestral states of enantio styly are mapped onto the phylogeny. Enantio styly has evolved a minimum of 11 times, with at least one reversion to the straight-styled condition. Branch shading and boxes by terminal taxa indicate stylar condition, black indicates straight-styled, hatch marks indicate monomorphic enantio styly, and white indicates dimorphic enantio styly. Gray bars indicate an equivocal reconstruction. Taxa with unknown stylar condition do not have a box at the tip of the phylogeny.

reduce damage to female function during buzz pollination (Dulberger 1981).

### The Taxonomic Distribution of Enantiostyly in the Angiosperms

Enantiostyly is widely distributed among flowering plant families in both the dicotyledons and the monocotyledons. It is documented in at least 10 families of angiosperms, representing eight orders (fig. 3). All enantiostylous taxa are animal pollinated, mostly by insects, particularly pollen-collecting bees. In the dicotyledons, enantiostyly is found in diverse groups including the Solanaceae (*Solanum* section *Androcera*; Whalen 1979), Caesalpiniaceae (*Chamaecrista* and *Cassia*; Todd 1882; Dulberger 1981; Fenster 1995), Gesneriaceae (*Saintpaulia*; Harrison et al. 1999), and Gentianaceae (*Exacum*; Lloyd and Webb 1992a). In the monocotyledons, enantiostyly is well known from species in the Pontederiaceae (*Heteranthera*, *Monochoria*; Iyengar 1923; Graham and Barrett 1995; Wang et al. 1995), Philydraceae (Simpson 1990; Graham and Barrett 1995), and Haemodoraceae (Ornduff and Dulberger 1978; Simpson 1990; Jesson and Barrett 2002a).

When enantiostyly is reported, it has most commonly been described as the monomorphic form of the polymorphism, e.g., Solanaceae (Bowers 1975), Caesalpiniaceae (Fenster 1995), and Gesneriaceae (Harrison et al. 1999). Most monomorphic enantiostylous species also have reciprocal enantiostyly. Lloyd and Webb (1992a) proposed that reciprocal enantiostyly evolved from nonreciprocal enantiostyly. They based this hypothesis on the assumption that more species are known with a stylar deflection alone than with a stamen deflection alone. To our knowledge, nonreciprocal enantiostyly is only reported in *Exacum* spp. (Gentianaceae; Lloyd and Webb 1992a), *Saintpaulia* spp. (Gesneriaceae; Harrison et al. 1999), *Monochoria australasica* (Pontederiaceae; fig. 2), *Lachnanthes* spp. (Haemodoraceae; Simpson 1990), and possibly some genera of Commelinaceae (*Coleotrype*, *Porandra*, and *Amischotolype*; Evans et al. 2000). The occurrence of a stamen deflection without stylar deflection is exceptionally rare and is only reported from species of *Murdannia* (Commelinaceae; Evans et al. 2000). Hence, the majority of reports of enantiostyly in angiosperm families involve monomorphic enantiostyly with a reciprocal deflection of the style and a stamen.

Dimorphic enantiostyly is much rarer and is only reported from three closely related families (Haemodoraceae, Pontederiaceae, and Tecophilaeaceae). All dimorphic enantiostylous

species possess reciprocal enantiostyly, and in each case, the family also includes species with monomorphic enantiostyly. Within the Haemodoraceae, only *Wachendorfia* and *Barbaretta* are known to possess dimorphic enantiostyly (Ornduff 1974; Ornduff and Dulberger 1978; Jesson and Barrett 2002a), while many genera exhibit monomorphic enantiostyly (e.g., *Schiekia*, *Lachnanthes*, *Haemodorum*, *Dilatris*; Simpson 1990). In the Pontederiaceae, all species of *Monochoria* and most species of *Heteranthera* display monomorphic enantiostyly (Graham and Barrett 1995), with the exception of *Heteranthera multiflora*, which exhibits dimorphic enantiostyly (Jesson and Barrett 2002b), and *Heteranthera dubia*, which is straight-styled (i.e., nonenantiostylous).

Dulberger and Ornduff (1980) reported that *Cyanella lutea* (Tecophilaeaceae) exhibits both left- and right-styled flowers within an inflorescence, and hence the species exhibits monomorphic enantiostyly. In contrast, they reported that *Cyanella alba* is dimorphic for style orientation. However, in *C. alba*, dimorphic enantiostyly does not seem to be a completely fixed condition. In two glasshouse populations of *C. alba*, up to 20% of the plants that were examined had inflorescences with both left- and right-styled flowers (Dulberger and Ornduff 1980). Moreover, in a recent survey of a large population of *C. alba* at Wuppertal, South Africa, L. D. Harder (personal communication) observed that most plants produced both left- and right-styled flowers. Clearly, further studies of *C. alba* are needed to determine the type or types of enantiostyly present in this species. The restricted occurrence of dimorphic enantiostyly and its occurrence in families with monomorphic enantiostyly has led to the hypothesis that dimorphic enantiostyly has probably evolved from monomorphic enantiostyly (Barrett et al. 2000b).

### The Evolution of Enantiostyly in the Monocotyledons

The wide and scattered distribution of enantiostyly in the angiosperms (fig. 3) implies that the polymorphism has had multiple evolutionary origins. Moreover, co-occurrence of more than one type of enantiostyly within a group also suggests that transitions from one enantiostylous condition to another have probably occurred. We were therefore interested in examining the number of evolutionary origins of enantiostyly and the pathways by which the floral polymorphism has evolved. Composite phylogenies, made by combining published phylogenetic trees into a single tree, can be useful tools for examining evolutionary questions because they increase the

Table 2

Most Parsimonious Number of Transitions between Forms of Enantiostyly and the Straight-Styled Condition

	Straight-styled	Nonreciprocal enantiostyly	Reciprocal enantiostyly	Dimorphic enantiostyly
Straight-styled	...	1–2	7–9	0–1
Nonreciprocal enantiostyly	0–0	...	0–1	0–0
Reciprocal enantiostyly	1–2	2–3	...	0–2
Dimorphic enantiostyly	0–1	0–0	0–1	...

Note. Values separated by a dash are minimum and maximum values, respectively. “Straight-styled” is a morphology in which the style is not deflected from the center of the flower; “nonreciprocal enantiostyly” and “reciprocal enantiostyly” are both forms of monomorphic enantiostyly (see fig. 1 for details). In “dimorphic enantiostyly,” all individuals are fixed for one stylar direction, and the style and an anther are reciprocally deflected.

number of taxa in the tree and enhance the power of phylogenetic analyses (Donoghue and Ackerly 1996). We constructed a composite tree of the monocotyledons to specifically investigate three questions: (1) What is the ancestral stylar condition in the monocotyledons? (2) How many times has enantiostyly evolved? (3) Is there evidence for evolutionary transitions between nonreciprocal enantiostyly, reciprocal enantiostyly, and dimorphic enantiostyly?

In assembling the composite tree, we based the backbone of the phylogeny on a phylogeny from Soltis et al. (1999). We then expanded clades in which enantiostylous taxa were reported. When more than one tree was available, e.g., Haemodoraceae (Simpson 1990; Hopper et al. 1999), we selected the tree based on molecular data because floral traits were often characters used in assembling the morphological trees. To obtain the greatest information about phylogenetic relationships, we used a published most parsimonious tree rather than a consensus tree (Weiblen et al. 2000). In the case of the Tecophilaeaceae, we pruned the relevant information from a larger tree of the Cyanastroideae (Brummitt et al. 1998). Recent molecular data provides strong evidence for the positioning of the Haemodoraceae and the Pontederiaceae as sister taxa (Graham et al. 2001). We therefore grafted a topology of the Commelinales from S. W. Graham (unpublished data) onto the composite phylogeny. Further information about the phylogenetic trees that we used can be found in Jesson (2002).

We scored all taxa for the following conditions: straight-styled, nonreciprocal monomorphic enantiostyly, reciprocal monomorphic enantiostyly, and dimorphic enantiostyly (all known dimorphic enantiostylous species have reciprocal enantiostyly). Species that have two laterally positioned pollinating anthers (*Dilatris*, *Aneilema*, and *Commelina*) were scored as reciprocal enantiostyly because one anther is in a reciprocal position to the stigma. We could not include dimorphic *Heteranthera multiflora* in the phylogeny because it was not represented in available trees of the Pontederiaceae. After coding, two terminal taxa were still polymorphic for enantiostyly (*Murdannia* and *Cyanella*). Because reconstruction of ancestral states is difficult for polymorphic taxa (Maddison and Maddison 1992), we split polymorphic taxa into separate clades. *Murdannia* was split into two clades (nonenantiostyly and enantiostyly) and *Cyanella* was split into three (nonenantiostyly, reciprocal monomorphic enantiostyly, and dimorphic enantiostyly). This subsequent polytomy was resolved separately for each analysis, and we compared the results for consistency.

We used MacClade (Maddison and Maddison 1992) to reconstruct ancestral states and to infer state changes. Composite trees enhance the power of phylogenetic analyses but may also compound bias or uncertainty (Sanderson et al. 1998; Weiblen et al. 2000). For this reason, we conducted sensitivity analyses that compared (1) changes in 100 randomly generated trees, (2) resolutions obtained by changing the backbone tree of the phylogeny to one most parsimonious tree from the Chase et al. (1993) supertree, and (3) another most parsimonious tree within the Pontederiaceae (from Kohn et al. 1996). This gave information on the sensitivity of our analyses to major topological changes or minor topological changes within either the backbone tree or one of the grafted trees.

Analysis of the composite tree of the monocotyledons in-

**Table 3**  
Results of Maddison's (1990) Concentrated Changes Test for Correlations between Floral Characters Associated with Enantiostyly

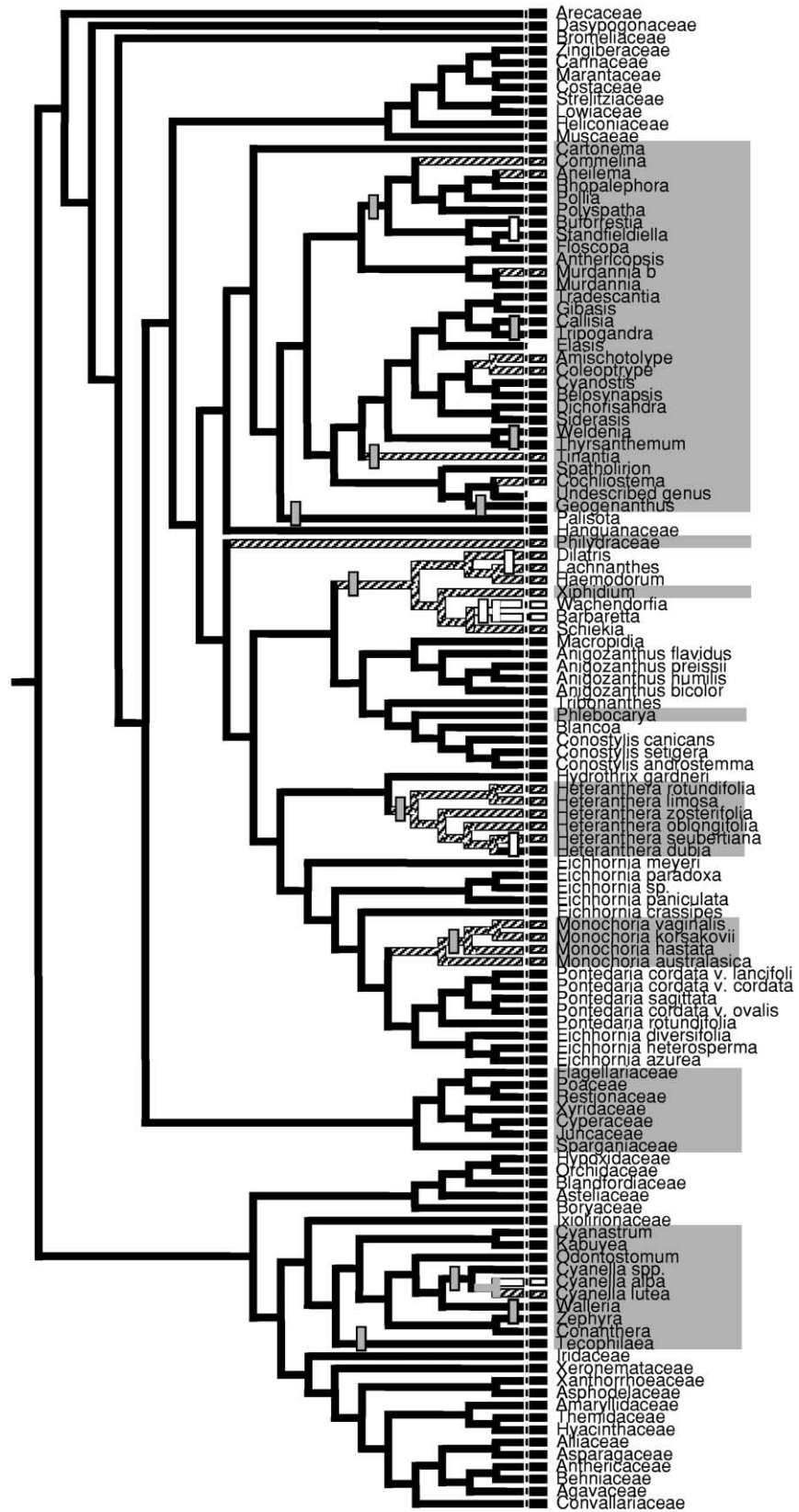
	Graham et al.		Kohn et al.		Chase et al.	
	G-L	P	G-L	P	G-L	P
Enantiostyly	11-1		11-1		11-1	
Heteranthery	6-0	<u>0.012</u>	6-0	<u>0.012</u>	6-0	<u>0.006</u>
Loss of nectaries	10-1	<u>0.000</u>	10-1	<u>0.000</u>	10-1	<u>0.000</u>
Poricidal anthers	2-0	1	2-0	1	2-0	0.4
Zygomorphy:						
N	7-0	0.1	6-0	0.4	8-0	<u>0.003</u>
E	8-0	<u>0.018</u>	7-0	0.08	9-0	<u>0.012</u>

Note. To test for sensitivity of the analysis, two topologies of the Pontederiaceae (Kohn et al. 1996; Graham et al. 2001) and the Chase et al. (1993) backbone topology were compared. All other topologies were kept consistent. Gains and losses (G-L) in the enantiostyly row are the number of gains and losses of enantiostyly on the whole tree. All other gains and losses are the number of gains and losses of enantiostyly on branches distinguished by character 1 of the correlated character. Probability values (*P*) have been adjusted for multiple comparisons. Numbers underlined indicate statistical significance. N indicates that equivocal branches were not included in the analysis; E includes equivocal branches.

indicates that the most parsimonious ancestral condition to enantiostyly is straight-styled and that enantiostyly arose at least 11 independent times, with at least one reversal to a straight-styled condition (fig. 4). The number of origins did not change substantially when compared with other resolutions of the supertree; e.g., using the Chase et al. (1993) topology of the monocotyledons, the number of gains ranged from 10 to 11 and the number of losses ranged from 1 to 2. However, comparisons with 100 randomly generated trees indicate that this result is sensitive to major changes in tree topology (using 100 randomly generated trees, the number of gains ranged from 15 to 26 and the number of losses from 0 to 10).

The reconstructions also indicate that numerous transitions have occurred between the various enantiostylous conditions (fig. 4; table 2). The most parsimonious reconstruction indicates that transitions from nonreciprocal monomorphic enantiostyly to reciprocal monomorphic enantiostyly occurred 0 to 1 time and occurred in reverse 1 to 2 times (depending on the resolution of the *Cyanella* clade). Transitions between reciprocal monomorphic enantiostyly and dimorphic enantiostyly may have occurred 1 to 2 times, and transitions from dimorphic enantiostyly to reciprocal monomorphic enantiostyly occurred a minimum of once. This reconstruction also indicates that transitions from straight-styled to reciprocal monomorphic enantiostyly has occurred as often as transitions from straight-styled to nonreciprocal monomorphic enantiostyly (table 2) at once each. Transitions from reciprocal monomorphic enantiostyly to nonreciprocal monomorphic enantiostyly occurred unambiguously in *Lachnanthes*.

There were no unequivocal evolutionary transitions from nonreciprocal monomorphic enantiostyly to reciprocal monomorphic enantiostyly. Thus, we found no evidence to support Lloyd and Webb's (1992a) hypothesis that reciprocal enantiostyly with reciprocal placement of stigmas and anthers has





evolved from enantiostyly with deflection of the style alone. It is possible that this sequence has occurred, but the intermediate steps are not represented in this phylogeny. This could happen if the rate of character change exceeds the rate of speciation (Weiblen et al. 2000) or if taxa that possessed an intermediate stage were not included in the phylogeny. Most species with reciprocal enantiostyly also have heteranthery. Therefore, it may be possible to examine questions about evolutionary sequences in enantiostylous taxa by examining the evolution of other correlated traits.

### Characters Associated with Enantiostyly

As mentioned earlier, many enantiostylous species have other floral characters in common. Indeed, Dulberger (1981) suggested that enantiostyly may be part of a “floral syndrome” associated with the following traits: (1) nectarless flowers with upper “feeding anthers” and lower “pollinating anthers,” (2) poricidal anther dehiscence, (3) orientation of anthers leading to deposition of pollen from the “feeding” anthers on the ventral parts of the insect and of the “pollinating” anthers on its back or side, (4) curved styles and stigmas touching the zone on which pollen has been deposited from the “pollinating” anthers, and (5) minute stigmas. Graham and Barrett (1995) examined the known enantiostylous species in the monocotyledons for the presence of these characters as well as for zygomorphy (bilateral symmetry of the perianth) and outward floral orientation. They found repeated associations between dimorphic anthers, zygomorphy, and an outward floral orientation. Graham and Barrett (1995) suggested that the frequent association between these traits and enantiostyly might be due to the presence of a pollination syndrome in which consistent positioning of insect pollinators is important for effective cross-pollen transfer.

#### *Is There a Syndrome of Traits Associated with Enantiostyly?*

We were therefore interested in examining Dulberger's (1981) hypothesis that enantiostyly was part of a syndrome of traits important for cross-pollen transfer. We used the composite phylogeny of the monocotyledons (fig. 4) to test for the correlation of a suite of traits, using both Maddison's (1990) concentrated changes test (CCT) and Pagel's (1994) maximum likelihood (ML) test. Because both tests compare characters with two-state options, we scored taxa in the phylogeny for the following binary characters: floral symmetry (radially symmetric vs. bilaterally symmetric or irregular flowers), anther function (functions of stamens equal vs. division of labor into feeding anthers and pollinating anthers), anther dehiscence

(longitudinal vs. pore, porelike, or apical or basal slits), and nectaries (present vs. absent). Scoring these characters was based on published accounts, including taxonomic descriptions, floras, codings for morphological phylogenies, and articles in reproductive biology (Buchman 1983; Givnish et al. 1999; Evans et al. 2000). When published accounts differed, we chose the most detailed account (Scott [1991] lists *Cyanella* as zygomorphic despite published descriptions of Tecophilaeaceae as actinomorphic) or coded the character as polymorphic.

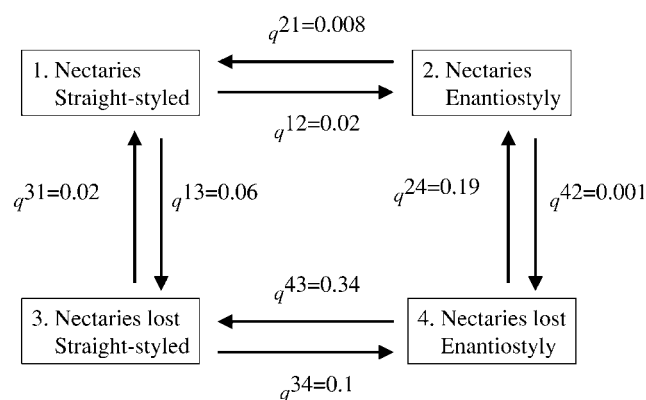
We tested correlations between taxa using the CCT on the basis of the most parsimonious resolution obtained with equally weighted losses and gains (Maddison and Maddison 1992). We performed the CCT with 1000 simulations of actual changes. When the most parsimonious reconstruction of a branch was equivocal, we examined all possible resolutions. To adjust for multiple comparisons within a phylogeny, we multiplied the resulting probability value by the number of comparisons (Hibbett and Donoghue 2001). We examined the sensitivity of the analysis to mild deviations in tree topology by changing the topology of the Pontederiaceae to a previously published resolution obtained from chloroplast DNA restriction-site variation alone (Kohn et al. 1996) and to the Chase et al. (1993) topology.

We also tested for the presence of trait correlations using Discrete, which implements the ML method of Pagel (1994). Because Discrete requires that all terminal taxa be scored for the characters of interest, we coded all species with missing values according to the trait inferred using equally weighted parsimony optimization. The test was performed using a backbone topology from Graham et al. (2001), with the branch lengths of all species set to 1. We first estimated the ML values of the transition parameters for each trait using a model of independent evolution of enantiostyly. We then compared this with a model of dependence in which the evolution of enantiostyly correlated with the evolution of the trait of interest. Probability values were tested by 100 iterations using Monte Carlo simulation.

#### *Enantiostyly, Heteranthery, and the Loss of Nectaries*

Our analyses revealed several significant associations between floral traits and enantiostyly. Using both the CCT and ML methods, there was a highly significant association between heteranthery and enantiostyly (table 3; fig. 5; ML ratio = 11.92,  $P < 0.001$ ). For the CCT, this association was resilient to minor and major changes in tree topology. There were three unambiguous instances of the evolution of heteranthery before enantiostyly (*Cyanella* and the *Commelina-Aneilema* clade) and one instance of the evolution of enantiostyly before heteranthery (*Monochoria australasica*). All

**Fig. 5** A parsimonious reconstruction of the loss of nectaries and the gain of heteranthery and enantiostyly. The polymorphism has evolved at least 11 times, often coincident with heteranthery. Branches and boxes by terminal taxa indicate styler condition, black indicates straight-styled, hatching indicates monomorphic enantiostyly, white indicates dimorphic enantiostyly, and gray indicates equivocal reconstruction. Gray horizontal bars show gains of heteranthery and white bars show losses of heteranthery. Gray boxes show taxa in which nectaries have been lost. Unambiguous reconstructions suggest heteranthery has evolved simultaneously with enantiostyly three times, before enantiostyly three times, and after enantiostyly once. Concentrated changes tests indicate a significant association with heteranthery ( $P = 0.006$ ) and loss of nectaries ( $P < 0.001$ ).



**Fig. 6** Transition rate parameters ( $q$ ) for gain and loss of enantiostyly and nectaries, given the presence or absence of the other character. Transition probabilities were estimated using Discrete (Pagel 1994). The transition probabilities for the gain of enantiostyly were allowed to vary according to the presence or absence of nectaries (i.e., the gain of enantiostyly can depend on the presence of nectaries).

other instances of the gain of heteranthy and enantiostyly occur on the same branch.

A loss of nectaries was also associated with the origins of enantiostyly for both CCT and ML methods (table 3; ML ratio = 5.10,  $P = 0.025$ ). The result for the CCT was insensitive to changes in topology. Mapping the loss of nectaries onto the phylogeny of the monocotyledons (fig. 5) indicated that heteranthy had been gained nine to 10 times in clades that have lost nectaries, but only one to two times in clades with nectaries. Of the 11 gains of enantiostyly in the monocotyledons, nine to 10 of these occurred after nectaries had been lost.

Using Discrete, we tested for a model of conditional evolution of enantiostyly where a change in enantiostyly is dependent on the loss of nectaries. The model parameters that Discrete estimates are the instantaneous transformation probabilities for each character, given a particular state in the other character. We therefore compared two models; in the first, transition probabilities for the gain of enantiostyly are the same regardless of whether nectaries are present or absent (i.e., the gain of enantiostyly is independent of nectaries). The second model allowed the transition probabilities for the gain of enantiostyly to vary according to the presence or absence of nectaries (i.e., the gain of enantiostyly can depend on the presence of nectaries). An examination of the transition matrix produced by the dependent model of the ML analysis (fig. 6) indicates that there is only a slight tendency for enantiostyly to evolve more rapidly after nectaries have been lost (i.e.,  $q_{34} > q_{12}$ ). Using the likelihood ratio statistic as  $-2 \log(H_0/H_1)$ , where  $H_0$  and  $H_1$  are the independent and dependent models, respectively, we found no significant difference between the two models (ML ratio = 0.034,  $P = 0.85$ ). Thus, while there is evidence for correlated evolution between loss of nectaries and a gain in enantiostyly, there is no evidence for a model of conditional evolution.

Both the CCT and ML methods showed no association between enantiostyly and poricidal anthers (ML ratio = 2.26,  $P = 0.32$ ) and only a significant association with zygomorphy when the Chase et al. (1993) tree was used as the backbone

phylogeny (table 3; ML ratio using Soltis et al. [1999], backbone = 4.34,  $P = 0.12$ ).

The CCT examines the changes in a dependent character (in this case, enantiostyly) after the evolution of the trait that is considered independent. For our analyses, we included traits that arose simultaneously because we were primarily interested in the association of traits and only secondarily interested in a cause-and-effect scenario. The association between heteranthy and enantiostyly was lost if simultaneous occurrences of enantiostyly and heteranthy were not counted. Of the 11 origins of enantiostyly, four of these were coincident with heteranthy (fig. 5). There is one unequivocal occurrence of heteranthy evolving after enantiostyly (*Monochoria*) and two unequivocal occurrences of heteranthy before enantiostyly (*Aneilema* and *Commelina*). We used Discrete to test for a model of conditional gain of enantiostyly after the gain of heteranthy. We found no significant difference between a model in which the gain of enantiostyly was independent of the character state of heteranthy and a model that allowed differential probabilities for the gain of enantiostyly dependent on heteranthy (ML ratio = 0.05,  $df = 1$ ,  $P = 0.82$ ). Thus, it is difficult to make any strong conclusion about the sequence in which enantiostyly, reciprocity of stigma-anther position, and heteranthy appeared.

The occurrence of enantiostyly and heteranthy on the same branches could be due to a number of factors. Intermediate taxa may not have been included in the phylogeny, either because of sampling or because they are extinct. It is also possible that enantiostyly and heteranthy evolved simultaneously (as the result of a single developmental shift) or that the two traits arose in rapid succession. The consequences to reproductive fitness of moving a stigma or anther are likely to be sufficiently strong that any variant with a greater level of reciprocity would likely have a much higher reproductive success. Analogous arguments have been made for the rapid evolution of reciprocal herkogamy from groups with stigma-height dimorphism (Lloyd and Webb 1992a).

It is important to recognize that the construction of a composite phylogeny from many phylogenetic trees is likely to compound any uncertainties or errors (Donoghue and Ackerman 1996; Sanderson et al. 1998). These can occur through uncertainties in topology and the coding of morphological traits for the phylogeny. We were also restricted by the availability of published phylogenies. For example, our phylogeny of the Tecophilaeaceae was pruned from within a larger phylogeny of the Cyanastroideae. Although we attempted to examine the sensitivities of our analyses to major assumptions and errors, it is not possible to examine all of the ways in which errors could be compounded (Sanderson et al. 1998). For the most part, however, our analyses are not sensitive to changes in topology. It is obvious that the focal tree we used for this analysis is not the "true" evolutionary tree (Donoghue et al. 1998), thus we restrict our discussion of results to those that are insensitive to major and minor changes in tree topology.

Although the interpretation of our data deserves some caution, our results indicate that there is an association of several floral traits with enantiostyly. Enantiostyly is commonly associated with a syndrome in which pollen is the main reward and pollination occurs primarily through the function of a

cryptically colored pollinating anther. Nectaries are consistently lost before the evolution of a division of labor in the anthers and before the origin of enantiostyly, although there is no evidence that the gain of enantiostyly is conditional on the loss of nectaries. Although there are perhaps many evolutionary reasons for this sequence, it is very likely that the loss of nectaries is developmentally much less complex than changes to the size and shape of anthers and filaments necessary for the evolution of anther dimorphism or changes in cell shape or size required for the deflection of a stigma or anther.

These results indicate that poricidal anthers and zygomorphy are not regularly associated with enantiostyly. Zygomorphy is often found in plant syndromes where consistent positioning of a pollinator is important (Faegri and van der Pijl 1979). It is possible that the location of the pollen reward by species that have lost nectaries also serves to orient the pollinator during flower visits consistently. The evolution of zygomorphy has occurred numerous times in the monocotyledons (results not shown). The large number of zygomorphic taxa in the monocotyledons also makes the conditions for a significant association more stringent because there is a much greater likelihood of enantiostyly occurring on a zygomorphic branch simply by chance (Maddison and Maddison 1992).

### Concluding Remarks

Enantiostyly is one of several stylar polymorphisms in the angiosperms (Barrett et al. 2000b; Li et al. 2001). These stylar polymorphisms are distributed among many unrelated families, which implies multiple independent origins. Barrett et al. (2000b) proposed that all stylar polymorphisms have evolved to serve the same basic function: to resolve the conflict between effective cross-pollination while avoiding stigma-anther interference (also Webb and Lloyd 1986). In this section, we compare and contrast enantiostyly with the other stylar polymorphisms and examine how the differences in floral form and associated traits exhibited in enantiostylous species are likely to influence the function of this polymorphism.

Like reciprocal enantiostyly, distylous and tristylous species are characterized by a reciprocal placement of their sexual organs. However, in common with nonreciprocal enantiostyly, species with stigma-height dimorphism have two stigma positions, but anthers are uniform in position (Baker et al. 2000). The lack of reciprocity has led to the hypothesis that stigma-height dimorphism represents an intermediate step in the evolution of heterostyly (Lloyd and Webb 1992b). Although it has also been suggested that nonreciprocal enantiostyly is an intermediate stage in the evolution of reciprocal enantiostyly, our analysis provided no compelling evidence to support this hypothesis. However, stigma-height dimorphism is also documented in families without heterostyly and is therefore clearly not always a transient evolutionary condition (Barrett et al. 2000b). Similarly, nonreciprocal enantiostyly also occurs in families without reciprocal enantiostyly (e.g., *Exacum*, Gentianaceae; Webb and Lloyd 1986). Thus, despite a lack of reciprocity in sexual organs, both stigma-height dimorphism and nonreciprocal enantiostyly are clearly stable sexual polymorphisms, but how do they function to promote mating success? It seems likely that in both cases they reduce costs as-

sociated with sexual interference between female and male reproductive function.

The most fundamental difference between enantiostyly and the other stylar conditions is that monomorphic enantiostyly is not a genetic polymorphism because both left- and right-styled flowers are produced on the same plant. This variation complicates adaptive explanations on the basis of the cross-pollination hypothesis. Reciprocal enantiostyly has been most often interpreted as a mechanism that promotes cross-pollination in a manner functionally analogous to heterostyly because flowers of one type should be fertilized more often by flowers of the opposite form (Todd 1882; Wilson 1887; Iyengar 1923; Ornduff 1974; Ornduff and Dulberger 1978). However, in reciprocal monomorphic enantiostyly, a pollinator can potentially visit alternate flower forms on the same plant, causing geitonogamous self-pollination (Helme and Linder 1992; Fenster 1995; Graham and Barrett 1995). This could influence both female fitness through inbreeding depression and male fitness through pollen discounting (Charlesworth and Charlesworth 1987; Harder and Wilson 1998).

Experimental evidence indicates that monomorphic enantiostyly increases geitonogamous mating when compared with a dimorphic enantiostylous condition (Barrett et al. 2000a; Jesson and Barrett 2002c; and see Fenster 1995). However, as pointed out elsewhere (Dulberger and Ornduff 1980; Barrett et al. 2000b), if compared with plants that are not enantiostylous, monomorphic enantiostyly may in fact reduce levels of geitonogamy. The appropriate comparison depends on which stylar condition is the most likely ancestral state. The reconstructions of the phylogenetic relationships within the monocotyledons suggest that in these taxa, monomorphic enantiostyly has evolved from a straight-styled ancestor and that there are very few, if any, transitions from dimorphic to monomorphic enantiostyly. The phylogenetic evidence also suggests that dimorphic enantiostyly has been derived from monomorphic enantiostyly in at least one family (Haemodoraceae). Thus, the function of monomorphic enantiostyly may not be as enigmatic as it first appears and like dimorphic enantiostyly may largely function to promote cross-pollination (Jesson and Barrett 2002a, 2002c).

Webb and Lloyd (1986) proposed that the reciprocal nature of both heterostyly and enantiostyly may represent a strategy to reconcile the conflict between avoiding self-interference of anthers and stigmas and promoting effective cross-pollination. Traits that reduce interference between female and male function are likely to be especially important when one or many of the anthers provide the main floral reward to pollinators. Heteranthery divides the anthers spatially within a flower so that the pollen involved in male function (from the pollinating anther) is not involved in attraction or reward. The association of enantiostyly with heteranthery separates female, male, and attractive functions so that the process of pollen collection does not interfere with pollen deposition or receipt. An example of this interference may occur in buzz-pollinated species where the vibrations of large-bodied bees may damage the gynoecium (Dulberger 1981). This hypothesis would also explain the function of nonreciprocal enantiostyly, especially in species where pollen is the main reward (e.g., *Saintpaulia* and *Exacum*).

Although our analyses of the monocotyledons found no evidence for a consistent association with buzz pollination (as

assessed by the presence of poricidal anthers), the association with loss of nectaries and gain of heteranthery suggests that enantiostyly functions to reduce stigma-anther interference by moving the style away from the path of the pollinator. The most parsimonious reconstructions of the evolution of enantiostyly in the monocotyledons indicated that at least four transitions were associated with the gain of heteranthery on the same branch (fig. 5). Although we cannot determine cause and effect in this situation, this may be because the selective association between these two traits is relatively strong (Frumhoff and Reeve 1994). It is also possible that selection to move the stigma away from the anthers results in strong selection for an anther to be positioned in a reciprocal position to increase the precision of pollination, or that the division of anthers into those with nutritional versus pollination function results in selection for stigma-anther separation. However, the former scenario does not explain the evolution of heteranthery, only the reciprocity between stigma and anther position.

The association of enantiostyly with traits such as heteranthery and a lack of nectar indicates that strong selective pressure for a consistent placement of the pollinator is important. Most pollinators of enantiostylous species are large-bodied, pollen-collecting bees (but see Jesson and Barrett 2002a). Selection for precise positioning of the pollinator is also important in heterostylous species; however, in contrast to enantiostyly, species with these polymorphisms are usually pollinated by long-tongued, nectar-probing animals. Thus, it seems that the fundamental differences between enantiostyly and the other style-length polymorphisms is due to differences in the main floral rewards, i.e., nectar versus pollen collection.

Heterostyly is commonly found in species with actinomorphic, nectariferous flowers with prominent floral tubes. Although nontubular heterostylous species do exist (e.g., *Fagopyrum*, *Turnera*), the fact that the reward is usually located deep within the flower means that pollen can be segregated spatially along the length of a pollinator's proboscis and body. In contrast, enantiostylous species usually have pollen as the main reward and are not tubular in structure. Indeed, they are generally open in design and outward facing, and pollinators tend to collect the reward with short mandibles rather than with a long proboscis. With this pattern of foraging, pollen cannot be deposited lengthways along the pollinator's body; instead, pollen is segregated sideways (i.e., left and right) on the pollinator's body. Therefore, we propose that heterostyly and enantiostyly represent fundamentally different mechanical solutions to the problem of effective cross-pollination in flowers with alternate (nectar vs. pollen) floral rewards.

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### Literature Cited

- Baker AM, JD Thompson, SCH Barrett 2000 Evolution and maintenance of stigma-height dimorphism in *Narcissus*. I. Floral variation and style-morph ratios. *Heredity* 84:502–513.
- Barrett SCH, ed 1992 Evolution and function of heterostyly. Springer, Berlin.
- Barrett SCH, AM Baker, LK Jesson 2000a Mating strategies in monocotyledons. Pages 258–269 in KL Wilson, D Morrison, eds. Systematics and evolution of monocots. CSIRO, Melbourne.
- Barrett SCH, LK Jesson, AM Baker 2000b The evolution and function of stylar polymorphisms in flowering plants. *Ann Bot* 85(suppl): 253–265.
- Bowers KA 1975 The pollination ecology of *Solanum rostratum* (Solanaceae). *Am J Bot* 62:633–638.
- Brummitt RK, H Banks, MAT Johnson, KA Docherty, K Jones, MW Chase, PJ Rudall 1998 Taxonomy of Cyanastroideae (Tecophilaeaceae): a multidisciplinary approach. *Kew Bull* 53:769–803.
- Bruneau A 1997 Evolution and homology of bird pollination syndromes in *Erythrina* (Caesalpinaceae). *Am J Bot* 64:54–72.
- Buchman SL 1983 Buzz pollination in angiosperms. Pages 73–113 in CE Jones, RJ Little, eds. Handbook of experimental pollination biology. Van Nostrand, New York.
- Charlesworth D, B Charlesworth 1987 Inbreeding depression and its evolutionary consequences. *Annu Rev Ecol Syst* 18:237–268.
- Chase MW, DE Soltis, RG Olmstead, D Morgan, DH Les, BD Mishler, MR Duvall, et al 1993 Phylogenetics of seed plants: an analysis of nucleotide-sequences from the plastid gene *rbcL*. *Ann Mo Bot Gard* 80:528–580.
- Donoghue MJ, DD Ackerly 1996 Phylogenetic uncertainties and sensitivity analyses in comparative biology. *Philos Trans R Soc Lond B Biol Sci* 351:1241–1249.
- Donoghue MJ, RH Ree, DA Baum 1998 Phylogeny and the evolution of flower symmetry in the Asteridae. *Trends Plant Sci* 3:311–317.
- Dulberger R 1981 The floral biology of *Cassia didymobotrya* and *C. auriculata* (Caesalpinaceae). *Am J Bot* 68:1350–1360.
- Dulberger R, R Ornduff 1980 Floral morphology and reproductive biology of four species of *Cyanella* (Tecophilaeaceae). *New Phytol* 86:45–56.
- Endress P 1999 Symmetry in flowers: diversity and evolution. *Int J Plant Sci* 160(suppl):S3–S23.
- Evans TM, RB Faden, MG Simpson, KJ Sysma 2000 Phylogenetic relationships in the Commelinaceae. I. A cladistic analysis of morphological data. *Syst Bot* 25:668–691.
- Faegri K, L van der Pijl 1979 The principles of pollination ecology. Pergamon, New York.
- Fenster CB 1995 Mirror image flowers and their effect on outcrossing rate in *Chamaecrista fasciculata* (Caesalpinaceae). *Am J Bot* 82: 46–50.
- Frumhoff PC, HK Reeve 1994 Using phylogenies to test hypotheses of adaptation: a critique of some current proposals. *Evolution* 48: 172–180.
- Givnish TJ, TM Evans, JC Pires, KJ Sysma 1999 Polyphyly and convergent morphological evolution in Commelinales and Commelinidae: evidence from *rbcL* sequence data. *Mol Phylogenet Evol* 12: 360–385.
- Graham SW, SCH Barrett 1995 Phylogenetic systematics of the Pontederiales: implications for breeding-system evolution. Pages 415–

- 441 in PJ Rudall, PJ Cribb, DF Cutler, CJ Humphries, eds. *Monocotyledons: systematics and evolution*. Royal Botanic Gardens, Kew.
- Graham SW, D Cherniawsky, VL Biron, HS Rai 2001 Commelinoid monocot phylogeny revisited, using a large chloroplast data set. Abstract. Botany 2001 "Plants and People" meeting, Albuquerque, August 12–16.
- Grant V, KA Grant 1965 Flower pollination in the phlox family. Columbia University Press, New York.
- Harder LD, SCH Barrett 1993 Pollen removal from tristylous *Pontederia cordata* (Pontederiaceae): effects of anther position and pollinator specialization. *Ecology* 74:1059–1072.
- Harder LD, WG Wilson 1998 A clarification of pollen discounting and its joint effects with inbreeding depression on mating system evolution. *Am Nat* 152:684–695.
- Harrison C, M Möller, Q Cronk 1999 Evolution and development of floral diversity in *Streptocarpus* and *Saintpaulia*. *Ann Bot* 84:49–60.
- Helme NA, HP Linder 1992 Morphology, evolution and taxonomy of *Wachendorfia* (Haemodoraceae). *Bothalia* 22:59–75.
- Hibbett DS, MJ Donoghue 2001 Analysis of character correlations among wood decay mechanisms, mating systems, and substrate ranges in homobasidiomycetes. *Syst Biol* 50:215–242.
- Hodges SA, ML Arnold 1995 Spurring plant diversification: are floral nectar spurs a key innovation? *Proc R Soc Lond Ser B* 262:343–348.
- Hopper SD, MF Fay, M Rossetto, MW Chase 1999 A molecular phylogenetic analysis of the bloodroot and kangaroo paw family, Haemodoraceae: taxonomic, biogeographic and conservation implications. *Biol J Linn Soc* 131:285–299.
- Iyengar MO 1923 On the biology of the flowers of *Monochoria*. *J Indian Bot Soc* 3:170–177.
- Jesson LK 2002 The evolution and functional significance of enantiostyly in flowering plants. PhD thesis. University of Toronto.
- Jesson LK, SCH Barrett 2002a Enantiostyly in *Wachendorfia* (Haemodoraceae): the influence of reproductive systems on the maintenance of the polymorphism. *Am J Bot* 89:253–263.
- 2002b The genetics of mirror-image flowers. *Proc R Soc Lond Ser B* 269:1835–1839.
- 2002c Solving the puzzle of mirror-image flowers. *Nature* 417:707.
- Jesson LK, SCH Barrett, T Day 2003a A theoretical investigation of the evolution and maintenance of mirror-image flowers. *Am Nat* 161:916–930.
- Jesson LK, J Kang, SL Wagner, SCH Barrett, ND Dengler 2003b The development of enantiostyly. *Am J Bot* 90:181–194.
- Johnson SD, KE Steiner 2000 Generalization versus specialization in plant pollination systems. *Trends Ecol Evol* 15:140–143.
- Knuth P 1906 *Handbook of flower pollination*. Clarendon, Oxford.
- Kohn JR, SW Graham, B Morton, JJ Doyle, SCH Barrett 1996 Reconstruction of the evolution of reproductive characters in Pontederiaceae using phylogenetic evidence from chloroplast DNA restriction-site variation. *Evolution* 50:1454–1469.
- Li Q-J, Z-F Xu, WJ Kress, Y-M Xia, L Zang, X-B Deng, J-Y Gao 2001 Flexible style that encourages outcrossing. *Nature* 410:432.
- Linder HP 1998 Morphology and the evolution of wind pollination. Pages 123–135 in SJ Owens, PJ Rudall, eds. *Reproductive biology in systematics, conservation and economic biology*. Royal Botanic Gardens, Kew.
- Lloyd DG, CJ Webb 1992a The evolution of heterostyly. Pages 151–178 in SCH Barrett, ed. *Evolution and function of heterostyly*. Springer, Berlin.
- 1992b The selection of heterostyly. Pages 179–208 in SCH Barrett, ed. *Evolution and function of heterostyly*. Springer, Berlin.
- Lord EM 1981 Cleistogamy: a tool for the study of floral morphogenesis, function and evolution. *Bot Rev* 47:421–449.
- Maddison WP 1990 A method for testing the correlated evolution of two binary characters: are gains and losses concentrated on certain branches of a phylogenetic tree? *Evolution* 44:539–557.
- Maddison WP, DR Maddison 1992 *MacClade: analysis of phylogeny and character evolution*. Sinauer, Sunderland, Mass.
- Ornduff R 1974 Heterostyly in South African flowering plants: a conspectus. *J S Afr Bot* 40:169–187.
- Ornduff R, R Dulberger 1978 Floral enantiomorphy and the reproductive system of *Wachendorfia paniculata* (Haemodoraceae). *New Phytol* 80:427–434.
- Pagel M 1994 Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc R Soc Lond Ser B* 255:37–45.
- Palmer AR 1996 From symmetry to asymmetry: phylogenetic patterns of asymmetry variation in animals and their evolutionary significance. *Proc Natl Acad Sci USA* 93:14279–14286.
- Robertson AW, DG Lloyd 1991 Herkogamy, dichogamy and self-pollination in six species of *Myosotis* (Boraginaceae). *Evol Trends Plants* 5:53–63.
- Sanderson MJ, A Purvis, C Henze 1998 Phylogenetic supertrees: assembling the trees of life. *Trends Ecol Evol* 13:105–109.
- Scott G 1991 A revision of *Cyanella* (Tecophilaeaceae) excluding *C. amboensis*. *S Afr J Bot* 57:34–54.
- Simpson MG 1990 Phylogeny and classification of the Haemodoraceae. *Ann Mo Bot Gard* 77:722–784.
- Soltis PS, DE Soltis, MW Chase 1999 Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. *Nature* 402:402–404.
- Todd JE 1882 On the flowers of *Solanum rostratum* and *Cassia chamaecrista*. *Am Nat* 16:281–287.
- Wang G, R Muira, T Kusanagi 1995 The enantiostyly and the pollination biology of *Monochoria korsakowii* (Pontederiaceae). *Acta Phytotaxon Geobot* 46:55–65.
- Webb CJ, DG Lloyd 1986 The avoidance of interference between the presentation of pollen and stigmas in angiosperms. II. Herkogamy. *N Z J Bot* 34:164–178.
- Weiblen GD, RK Oyama, MJ Donoghue 2000 Phylogenetic analysis of dioecy in monocotyledons. *Am Nat* 155:46–58.
- Whalen MD 1979 Taxonomy of *Solanum* section *Androcercas*. *Gentes Herb* 11:359–426.
- Wilson J 1887 On the dimorphism of the flowers of *Wachendorfia paniculata*. *Trans Proc Bot Soc Edinb* 17:73–77.