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THE DEVELOPMENT OF ENANTIOSTYLY[1]

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Enantiostyly, the deflection of the style either to the left (left-styled) or right (right-styled) side of the floral axis, has evolved in at least ten angiosperm families. Two types of enantiostyly occur: monomorphic enantiostyly, in which individuals exhibit both stylar orientations, and dimorphic enantiostyly, in which the two stylar orientations occur on separate plants. To evaluate architectural or developmental constraints on the evolution of both forms of enantiostyly, we examined inflorescence structure and floral development among unrelated enantiostylous species. We investigated relations between the position of left- and right-styled flowers and inflorescence architecture in four monomorphic enantiostylous species, and we examined the development of enantiostyly in nine monomorphic and dimorphic enantiostylous species from five unrelated lineages. The location of left- and right-styled flowers within inflorescences ranged from highly predictable (in *Solanum rostratum*) to random (in *Heteranthera mexicana*). There were striking differences among taxa in the timing of stylar bending. In *Wachendorfia paniculata*, *Dilatris corymbosa*, and *Philydrum lanuginosum*, the style deflected in the bud, whereas in *Heteranthera* spp., *Monochoria australasica*, *Cyanella lutea*, and *Solanum rostratum*, stylar bending occurred at the beginning of anthesis. Comparisons of organ initiation and development indicated that asymmetries along the left-right axis were expressed very late in development, despite the early initiation of a dorsiventral asymmetry. We suggest that the evolution of dimorphic enantiostyly from monomorphic enantiostyly may be constrained by a lack of left-right positional information in the bud.

Key words: allometry; constraint; enantiostyly; floral development; inflorescence architecture.

Developmental processes can both shape and constrain morphological evolution. Differences in form can occur as a result of changes in the relative timing of developmental processes (heterochrony) and in the dissociation of these processes (Lord, 1981; Guerrant, 1989; Wake, 1991; Diggle, 1992; Richards and Barrett, 1992). However, constraints in the structure, composition, and dynamics of developmental systems can place limits on character evolution (Maynard Smith et al., 1985). For example, a lack of heritable variation in left-right asymmetries can constrain the evolution of form. In *Drosophila*, selection experiments on body asymmetries, including eye facet number (Maynard Smith and Sondhi, 1960), eye size (Coyne, 1987), wing folding behavior (Purnell and Thompson, 1973), and thoracic bristle number (Tuinstra, de Jong, and Scharloo, 1990), have shown that while selection increased the degree of asymmetry, it had no effect on the direction of the asymmetry. These results demonstrate that fluctuating asymmetries may have a heritable component, but that the direction of asymmetry is not heritable, thus limiting the evolution of asymmetric morphologies.

Floral asymmetries often involve radial or dorsiventral asymmetries in perianth structures or sexual organs (for a discussion of the terminology of floral symmetry see Giurfa, Dafni, and Neal [1999]). The patterns of zygomorphy that occur in flowers such as Asterids are likely constrained by early developmental patterns such as petal number, the overall orientation of the bud relative to the stem, and the orientation of dorsiventral differentiation (Ree and Donoghue, 1999). Because of these developmental constraints, only a limited number of forms of asymmetry in the Asterids are possible.

Enantiostylous flowers exhibit another form of asymmetry, a medial-lateral asymmetry (Fig. 1). In enantiostyly, the style of a flower is deflected either to the left (left-styled) or to the right (right-styled) of the floral axis and is therefore a floral polymorphism. Enantiostyly has evolved in at least ten angiosperm families in both the monocotyledons and dicotyledons (Jesson, 2002). Despite the multiple origins of enantiostyly in flowering plants, several similarities in floral morphology occur among unrelated enantiostylous species. For example, enantiostyly is often associated with heteranthery, the specialization of anthers into brightly colored feeding anthers and a cryptically colored pollinating anther (Graham and Barrett, 1995). The pollinating anther is deflected in the opposite direction from the style and likely plays a role in precise pollen transfer. The majority of enantiostylous species exhibit monomorphic enantiostyly (at least 25 genera from ten families; Jesson, 2002), in which left- and right-styled flowers occur on the same individual. Dimorphic enantiostyly, in which individuals are genetically determined to be entirely left- or right-styled (Jesson and Barrett, 2002a), is only reported in five species from three monocotyledon families.

The function of monomorphic enantiostyly has been considered enigmatic (see Barrett, Jesson, and Baker, 2000; Jesson and Barrett, 2002b). Observations of the stigma and the pollinating anther contacting opposite sides of a pollinator's body have led to the suggestion that enantiostyly functions to effect proficient pollination between flowers of opposite style orientation (Dulberger, 1981). However, if a plant has left- and right-styled flowers open simultaneously (as in monomorphic enantiostyly), a pollinator visiting the two opposite forms on the same individual can cause geitonogamous self-pollination. Experimental evidence has shown that selfing rates are significantly lower in plants manipulated to be entirely left- or entirely right-styled than those possessing both floral forms (Barrett, Baker, and Jesson, 2000; Jesson and Barrett, 2002b).

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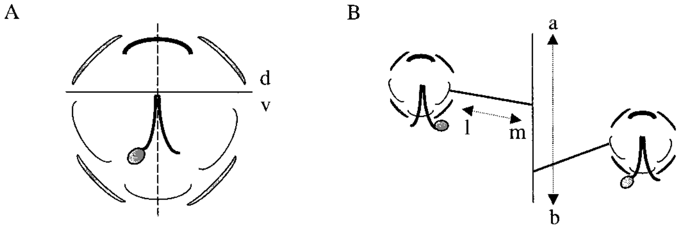


Fig. 1. (A) Medial-lateral asymmetry, in which left and right sides (separated by dashed line) differ, causing asymmetry (enantiostylous flowers). The solid line separates a dorsal region (d) from a ventral region (v) in dorsiventral symmetry (zygomorphy). (B) Asymmetries in flowers are also influenced by apical-basal (a-b) and medial-lateral (m-l) asymmetry of the whole shoot. Dotted lines and arrows delimit apical-basal (a-b) and medial-lateral (m-l) planes of asymmetry that may influence enantiostylous flowers.

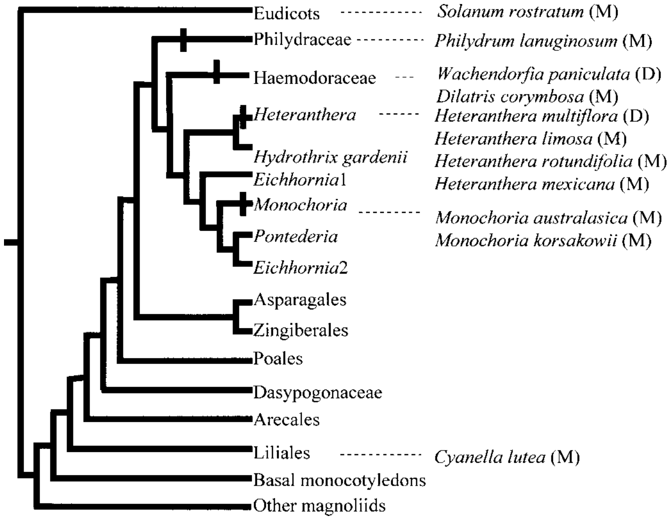


Fig. 2. The phylogenetic relationships of the enantiostylous species examined in this study, summarized from composite comparative information from Soltis, Soltis, and Chase (1999) and Kohn et al. (1996). See Jesson (2002) for more details. Bars indicate likely independent origins of enantiostyly. Origins of enantiostyly also occur within clades in the Liliales (e.g., *Cyanella lutea*) and Eudicots (e.g., *Solanum rostratum*). Due to a likely single origin in each of these clades, they have not been expanded and so bars are not shown. The type of enantiostyly is indicated as M (monomorphic enantiostyly) or D (dimorphic enantiostyly). Taxa followed by a number (e.g., *Eichhornia* 1) indicate non-monophyletic groups.

Thus, it is unclear why a consistent direction of stylar deflection, such as that found in dimorphic enantiostyly, has not evolved more often, given the functional advantages that this polymorphism provides by reducing the costs associated with selfing (Jesson and Barrett, 2002b).

It has been suggested that selection on the direction of stylar deflection on flowers of an individual may be constrained developmentally or structurally, perhaps by a lack of positional information within the bud (Barrett, Jesson, and Baker, 2000). We were therefore interested in examining how developmental processes may constrain the evolution of enantiostyly, particularly dimorphic enantiostyly. Specifically, we were interested in addressing four questions related to the developmental biology of enantiostyly: (1) For species with monomorphic enantiostyly, what is the arrangement of left- and right-styled flowers on inflorescences? (2) In species where the direction of stylar bending is fixed (i.e., dimorphic enantiostyly), when are the differences between floral forms first visible and how does this compare to species with monomorphic enantiostyly? (3) How early in floral development does the style deflect from the central axis in different enantiostylous species? (4) What are the developmental patterns of the gynoecium (particularly the style) and androecium (the pollinating and feeding anthers) for enantiostylous species occurring in unrelated families?

We investigated the development of enantiostyly by comparing inflorescence architecture and floral organ development among a range of enantiostylous species. We chose to take a broad, comparative approach by examining multiple lineages in which enantiostyly occurs, rather than focusing on a smaller species sample. To examine whether the direction of style deflection in monomorphic enantiostylous plants was predictable, we compared the arrangement of left- and right-styled flowers within inflorescences of three monocotyledon species (*Heteranthera mexicana* and *Monochoria korsakowii* [Pontederiaceae] and *Cyanella lutea* [Tecophilaeaceae]) and one dicotyledon species (*Solanum rostratum* [Solanaceae]). Comparative evidence indicates that enantiostyly has separate evolutionary origins in each of these families (Kohn et al., 1996; Soltis, Soltis, and Chase, 1999; summarized in Fig. 2). To investigate whether asymmetry in sex organs occurs early in development, we compared related dimorphic and monomorphic enantiostylous species of *Heteranthera* in the Pontederiaceae (*Heteranthera multiflora* and *Heteranthera limosa*, respectively) as well as in two unrelated species in which the direction of stylar deflection within the flower was predictable (dimorphic enantiostylous *Wachendorfia paniculata* [Haemodoraceae] and monomorphic enantiostylous *S. rostratum* [Solanaceae]). To examine the role of heterochrony in the timing of floral organ differentiation, particularly in the feeding and pollinating anthers, we compared growth allometries of sex organs in nine enantiostylous taxa representing five distinct lineages (Jesson, 2002).

MATERIALS AND METHODS

Floral structure—We examined floral structure in buds of *Dilatris corymbosa* and *W. paniculata* (Haemodoraceae), *H. limosa*, *H. multiflora*, *H. rotundifolia*, and *M. korsakowii* (Pontederiaceae), *Philydrum lanuginosum* (Philydraceae), *C. lutea* (Tecophilaeaceae), and *S. rostratum* (Solanaceae) (see http://ajbsupp.botany.org/v90/ for details of collection localities). Material from each population was collected as a bulk random sample consisting of at least ten plants. Samples of dimorphic enantiostylous *W. paniculata* and *H. multiflora* (from the bud stage) were collected and separated into labelled vials containing buds from left- or right-styled plants. Because all other species exhibit monomorphic enantiostyly, the identity of buds with respect to style orientation was unknown. All buds were collected and fixed in formalin-glacial acetic acid ethanol (FAA) and stored in 70% alcohol.

For all species, we noted the organization of the organs of the mature flower (bract, sepal, and petal, or tepal, stamen, and gynoecium), as well as the structural features of stamen dimorphism, the position of the pollinating and feeding anthers, the position of stamen insertion, and any patterns of perianth fusion.

Inflorescence patterning—To determine whether patterning of left- and right-styled flowers within an inflorescence was predictable with respect to inflorescence architecture, we recorded the direction of style deflection and flower position in glasshouse populations of three enantiostylous species: *M. korsakowii*, *H. mexicana* (Pontederiaceae), and *S. rostratum* (Solanaceae), and two field populations of *C. lutea* (Tecophilaeaceae). See http://ajbsupp.botany.org/v90/ for details of collection localities and number of plants measured.

Inflorescences of *S. rostratum* are unbranched scorpioid cymes, whereas flowers of *C. lutea*, *M. korsakowii*, and *H. mexicana* are all borne on indeterminate racemes. In all species, we recorded the direction of style deflection and flower position for the first five flowers produced on an inflorescence. For glasshouse populations, we noted the direction of the style for the first five flowers in anthesis, the date of flowering, and the nodal position of the flower on the plant (1 = bottom flower, which is the first to flower). For *C. lutea*, we sampled two natural populations and recorded the direction of deflection of all newly opened flowers on a plant, their nodal position, and the number of flowers previously opened.

We used logistic regression (see Neter, Wasserman, and Kutner [1990]) to examine differences in left- and right-styled flower patterning on an inflorescence. We tested all predictors and interactions and removed terms via stepwise deletion. Tests of significance were obtained by comparing the changes in the full main effects model to a model with the main effects removed or interactions added (equivalent to type III sums of squares). All analyses were conducted using S-plus 4 (Mathsoft, 1997).

For glasshouse populations of *M. korsakowii*, *H. mexicana*, and *S. rostratum*, the model response was the direction of stylar bending (either left or right) on flowers at nodal positions 2–5. The predictors in the model were nodal position (2–5), date of flowering, and direction of the first emerging flower on an inflorescence (nodal position 1). In field populations of *C. lutea*, the flower located at the second nodal position was most frequently open, and hence, its direction of deflection was known. To examine if the direction of deflection of a flower could be predicted by the direction of other flowers on the inflorescence, we considered the flower at the second node as a predictor variable. Other predictor variables also included the nodal position of a flower (i.e., the total number of previous flowers produced). The direction of flowers at nodal positions 1, 3, 4, and 5 (if known) were considered response variables.

Relative growth rates—To investigate the development of flower structure and the relative growth stages of sexual organs, we dissected 15–70 buds per species (see http://ajbsupp.botany.org/v90/) ranging from 1 to 20 mm in length using a Stemi Sv8 (Zeiss, Oberkochen, Germany) dissecting microscope. To examine the initiation of stylar deflection and the relative growth rates of the floral organs, we measured bud length, style length, and filament length of one pollinating stamen and one feeding stamen, the length of one pollinating and feeding anther, and the distance from the stigma to the floral axis. Measurements were made with an ocular micrometer (to the nearest 0.1 mm) or with digital calipers.

To investigate differences in allometric growth rates, we regressed the length of the style and the stamen (filament length + anther length) against the length of the floral bud. All data were log transformed prior to analysis. We used linear least squares regression to calculate the relative growth rates of the different organs. We used analysis of covariance (Sokal and Rohlf, 1995) to compare differences in the slopes and intercepts of the length of pollinating and feeding stamens for all species.

The initiation of left-right asymmetries—To investigate asymmetries during floral development, we used scanning electron microscopy to examine buds of two monomorphic (*H. limosa* and *S. rostratum*) and two dimorphic enantiostylous species (*H. multiflora* and *W. paniculata*). We compared these species because in *H. multiflora*, *W. paniculata*, and *S. rostratum* (see RESULTS), we were able to predict the direction of style deflection of the flowers (either left- or right-styled). To examine differences in dimorphic and monomorphic enantiostyly, bud development in *H. multiflora* was compared to *H. limosa*. Inflorescences and flower buds were fixed in FAA and vacuum infiltrated overnight (138 kPa). Specimens were dehydrated in a graded ethanol series, CO_2 critical point dried using an Autosamdri 814 Critical Point Dryer (Tousimis Research, Rockville, Maryland, USA), gold coated using a Cressington 108 Sputter Coater (Cressington Scientific Instruments, Cranberry Township, Pennsylvannia, USA), and viewed in a Hitachi scanning electron microscope (Model S-2500, Hitachi, Tokyo, Japan) at 10 kV. We viewed 3–12 buds per floral stage across at least three floral stages for each species to compare qualitative trends. The buds viewed had been measured previously in the allometric study.

RESULTS

Mature flower structure—The floral structure of the enantiostylous species investigated differed considerably (Fig. 3). The perianths of *D. corymbosa*, *M. australasica*, and *Heteranthera* spp. are all actinomorphic, whereas *W. paniculata* and *S. rostratum* are weakly zygomorphic and *P. lanuginosum* and *C. lutea* are more strongly zygomorphic. The perianth structure of these monocotyledon species ranges from free tepals in *D. corymbosa* and *C. lutea*, to tepals connate at the base in *Heteranthera* spp., *M. australasica*, and *W. paniculata*, to tepals fused to bracteoles in *P. lanuginosum*. In the dicotyledon *S. rostratum*, the calyx and corolla are fused.

The number of feeding (brightly colored) and pollinating (cryptically colored) anthers also differs among species. In species of *Heteranthera* examined, all possess a single, purple-blue pollinating anther and two yellow feeding anthers. The pollinating anther is located at the ventral position in the flower adjacent to the tepal. *Monochoria australasica* does not have a pollinating anther, but rather has six yellow feeding anthers that are arranged conically in the center of the flower. Interestingly, each neighboring stamen in *M. australasica* is longer than the previous one so that the stamens are arranged in a spiral pattern in either a clockwise or counterclockwise direction. *Cyanella lutea* also possesses six stamens: one large yellow pollinating anther that is adjacent to the tepal nearest the stem and five smaller yellow feeding anthers.

Dilatris corymbosa has two dark-colored pollinating anthers and a single, orange feeding anther that is always adjacent to the tepals furthest from the stem. *Wachendorfia paniculata* does not have differentiated feeding and pollinating stamens. All three stamens have anthers of equal size and color; however, one stamen has a longer filament and is always in a position reciprocal to the style. This stamen is positioned nearest to the stem of the inflorescence. There is also no division of labor in the anthers of *P. lanuginosum*, because flowers only have one stamen. *Solanum rostratum*, like *Heteranthera* spp. and *C. lutea*, has dimorphic stamens. In *S. rostratum*, the single pollinating anther is dark yellow or brown and is fused to the corolla in a ventral position in the flower. The four feeding anthers are bright yellow and are located in a cone in the center of the flower.

Inflorescence patterning—Among the four monomorphic enantiostylous species investigated there were striking differences in the extent to which stylar orientation could be predicted by flower position within inflorescences. This variation ranged from almost complete predictability to an apparently random arrangement of left- and right-styled flowers within an inflorescence. The patterning of left- and right-styled flowers on an inflorescence in *S. rostratum* is predictable (Fig. 4; Table 1) as the direction of the style is always deflected towards the stem. Shoot growth patterns of *S. rostratum* are monochasial and determinate. Inflorescences are quickly overtopped by one lateral shoot from the node below. The position of this lateral developing shoot alternates between distal axils on the left and right sides of the inflorescence. Flowers that originate from a left axil lateral shoot are right-styled, while flowers originating from a right axil lateral shoot are left-styled. Thus, the direction of deflection alternates along the inflorescence cyme and along the vegetative stem of the plant. Observations of stylar deflection for the first five developing flowers on an inflorescence demonstrated this pattern of alternation between left-

Fig. 3. Mature flower structure and floral diagrams of (A) *Dilatris corymbosa* (Haemodoraceae); (B) *Monochoria australasica* (Pontederiaceae); (C) *Heteranthera limosa* (Pontederiaceae) (mature flower structure of the three species of *Heteranthera* is similar); (D) *Wachendorfia paniculata* (Haemodoraceae); (E) *Solanum rostratum* (Solanaceae); (F) *Philydrum lanuginosum* (Philydraceae); (G) *Cyanella lutea* (Tecophilaeaceae). Floral structures on floral diagrams from outer whorls to inner whorls are represented as follows: tepals (two whorls of three, except in E, where five petals and sepals are fused); anthers (black circles = pollinating anther; white circles = feeding anthers); large open circle = gynoecium; small circle with dots (in A, D, F, and G) = insertion point of flower on inflorescence; hatched lines = bracts (in F). *Wachendorfia paniculata* does not have a morphologically distinct pollinating anther. However, one anther is always in a reciprocal position to the stigma. The insertion point of the flower on the inflorescence is not shown in B, C, and E as it is centrally located on the flower.

and right-styled flowers. However, the first flower produced was either left- or right-styled. This led to a highly significant interaction between the direction of the focal flower and the nodal position of the flower (Table 1). Because of the monochasial pattern of inflorescence architecture, the ratio of flowers on an individual was 1 : 1. Of the 30 plants examined, the proportion of right-styled flowers on a plant was 0.53 (1 SE = 0.05; N = 150 flowers).

In *M. korsakowii*, there were no significant predictors of stylar deflection. While there was a qualitative pattern of the direction of stylar deflection alternating at different nodes (Fig. 4), the interaction between the direction of the focal flower and the nodal position of the flower was not significant and removed by stepwise elimination. The mean proportion of right-styled flowers in the 50 plants examined was 0.45 (1 SE = 0.02; N = 250 flowers).

In *C. lutea*, the direction of stylar deflection qualitatively appeared to alternate along successive floral nodes (Fig. 4).

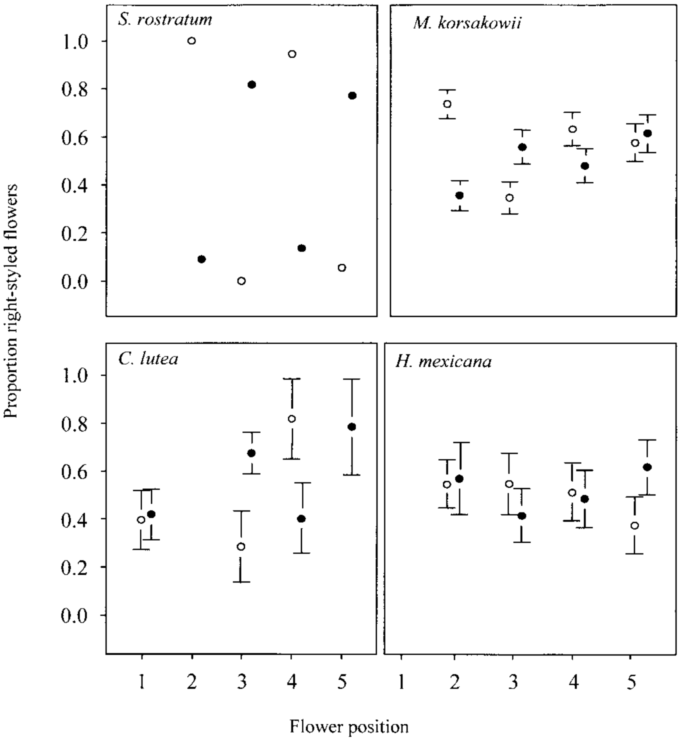


Fig. 4. The proportion of right-styled flowers by position within inflorescences of *Solanum rostratum* (Solanaceae), *Monochoria korsakowii* (Pontederiaceae), *Cyanella lutea* (Tecophilaeaceae), and *Heteranthera mexicana* (Pontederiaceae). The data are based on the first five flowers produced on an inflorescence. Open symbols denote the proportion of right-styled flowers on a plant in which the focal flower was left-styled. Closed symbols denote the proportion of right-styled flowers if the focal flower was right-styled. The focal flower was at the first node for *Solanum rostratum*, *Monochoria korsakowii*, and *Heteranthera mexicana* and at the second node for *Cyanella lutea*. Error bars are not shown for *Solanum rostratum* because there was virtually no variance within the data. Symbols indicate means ± 1 SE.

However, the interaction between the direction of the focal flower and the nodal position of the flower accounted for 0.02 deviance units out of a total of 142.31, thus explaining only 0.001% of the total variance (Table 1). Hence, in this species there is only a limited amount of patterning of left- and right-styled flowers within an inflorescence. In *C. lutea*, there was no effect of population or plant on the proportion of left- and right-styled flowers within an inflorescence. The mean proportion of right-styled flowers on a plant was 0.57 at Brandweg (1 SE = 0.05, N = 320 flowers) and 0.41 at MacGregor (1 SE = 0.05, N = 195 flowers).

In *H. mexicana*, we found no significant predictors for the direction of stylar deflection (Fig. 4; logistic regression results not shown). As with the other species, the proportion of left- and right-styled flowers on a plant was near equal. The proportion of right-styled flowers on a plant in *H. mexicana* was 0.52 (1 SE = 0.02; N = 250 flowers). Five plants of *H. mexicana* were observed to have a single flower with a straight style.

Allometric growth patterns—The pattern and timing of floral development varies considerably between species. In *W. paniculata* and *D. corymbosa*, bending was first observed at a bud length of 5 and 7 mm, respectively (approximately 25% and 40% of the mature length, respectively; Fig. 5). In *P. lanuginosum*, this occurred at a length of approximately 9 mm (45% of mature flower length; Fig. 5). In contrast, no evidence of stylar bending was found prior to anthesis in *C. lutea*, *H. limosa*, *H. rotundifolia*, *H. multiflora*, *M. australasica*, and *S. rostratum*.

Differences in the length of feeding and pollinating stamens were due to differences in intercepts, not relative growth rates (Table 2). Differences in intercepts of the regression were likely due to differences in initiation of stamen filaments or anthers. In all species measured, stamen length was not significantly influenced by the interaction of stamen type × bud length, indicating that there were no differences in relative growth rates of the two stamen types (Table 2). In contrast, there were significant differences in stamen length between the two stamen types in *D. corymbosa*, *H. limosa*, *H. rotundifolia*, *C. lutea*, and *S. rostratum* (Table 2). Thus, the intercept of the regression of stamen length on bud length varied with the function of the anther. In all species tested, the growth of the pollinating stamen was initiated earlier than the feeding stamen.

The initiation of left-right asymmetries—There was an obvious developmental asymmetry in the stigmas of dimorphic enantiostylous *H. multiflora* from the stage of stigma initiation onwards (Figs. 6–10, especially Fig. 7). The initiation of stigmatic papillae occurred in *H. multiflora* at a stage when the pollinating anther and the developing gynoecium were approximately of equal heights (Fig. 7; pollinating anther not shown for clarity). At this stage, the stigmatic surface grows unevenly, resulting in an apparent tilt away from the pollinating anther (Fig. 7; arrow). This asymmetric growth was not found to be a left-right asymmetry as the pollinating anther was located at the base of the flower adjacent to the lowest tepal. Rather, this asymmetry was along the dorsiventral axis. At this stage, differences in the size of the locules were also

TABLE 1. Logistic regression of the direction of style deflection in three enantiostylous species. Style direction of the focal flower is the direction of the first flower produced in *Monochoria korsakowii* and *Solanum rostratum* and the second flower in *Cyanella lutea*. **$0.001 < P < 0.01$; ***$P < 0.001$. NA indicates a predictor not used in model; a dash indicates a predictor removed by stepwise elimination.

Predictor	*S. rostratum*		*M. korsakowii*		*C. lutea*	
	df	Deviance	df	Deviance	df	Deviance
Total	159	221.40	500	690.48	102	142.31
Population	NA	NA	NA	NA	1	22.88
Plant within population	—	—	100	14.83	54	65.69
Position of node	3	0.08	—	—	3	0.40
Direction of focal flower	1	0.32	1	2.58	1	2.40
Direction of focal flower × position of node	3	129.85***	—	—	3	0.02**

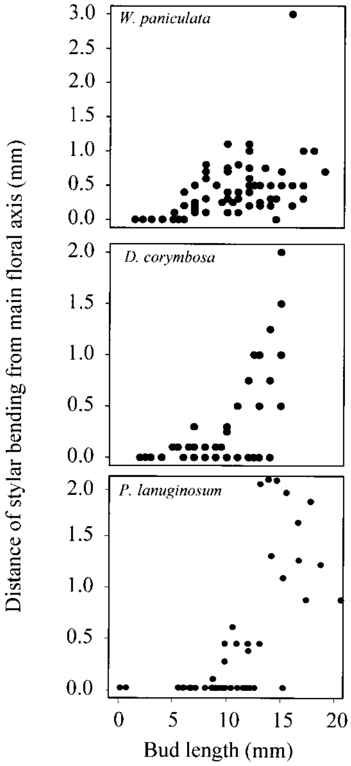


Fig. 5. The deflection of the style from the floral axis at different bud lengths in *Wachendorfia paniculata*, *Dilatris corymbosa*, and *Philydrum lanuginosum*. The remaining six species that were examined did not exhibit any stylar deflection in the bud.

evident. The locule opposite the pollinating anther was much larger (Figs. 7–8; asterisks) than the locules adjacent to the pollinating anther (Fig. 8; dot). This difference became more pronounced during floral development (Fig. 9). Late in development, tilting of the stigmatic surface was evident either to the left or to the right relative to the stem of the inflorescence (Fig. 10 and inset). This directionality was only seen in the largest buds and likely occurred less than 24 h prior to anthesis. In *H. multiflora*, the direction of the stigmatic surface was always opposite to the direction of stylar deflection (i.e., styles deflected to the left will have a stigmatic surface tilted to the right). This directionality in growth was the first indicator of the left- or right-deflection of enantiostyly.

Similar developmental characteristics (asymmetric stigmatic growth and differences in locule size) were also observed in monomorphic enantiostylous *H. limosa* (Figs. 11–14). However, the initiation of directionality in the stigmatic surface appeared to be at an earlier developmental stage than in *H. multiflora* (Fig. 12; arrowhead). In *H. limosa*, stigmatic papillae were also initiated earlier than in *H. multiflora* (Fig. 13; anthers removed for clarity). Asymmetries in locule size were not as evident as in *H. multiflora* (Fig. 14).

In *W. paniculata*, the stamen that becomes positioned reciprocally to the style later in development was deflected away from the other stamen primordia after initiation (Fig. 15, A*). However, there was no indication of which direction any particular anther would deflect. No asymmetries in the gynoecium were visible at initiation or during the early stages of development (Fig. 16). It was not until very late stages of development that the style deflected away from the pollinating anther either to the left or the right of the floral axis (Fig. 17). This was the first sign of a morphological difference between the buds of left- and right-styled morphs. Another unique structural feature in *W. paniculata* that did not occur in other species was evident at later stages of development; the reciprocal anther and the style deflected in opposite directions, changing the direction of the stamen and style (Figs. 17–18).

In *S. rostratum*, the anthers, sepals, and petals were initiated well before the gynoecium. At this stage, the developing pollinating anther was similar in size and shape to the other developing anthers; however, it was distinguishable by its position away from the other anthers (Fig. 19). This positioning of the pollinating anther was evident in all buds examined at this stage, suggesting that it was not an artifact of specimen preparation for viewing under scanning electron microscopy (SEM). At a later stage of development, the pollinating stamen was much larger in size than the other stamens (Fig. 20). During early stages of development, there were no indications of bending in either the gynoecium or the stamens. No clear morphological differences were found between left- and right-styled flowers in the bud, suggesting that the differences that occur in the mature flower structure arise very late in floral development or at the beginning of anthesis.

DISCUSSION

Enantiostyly has originated on multiple occasions in diverse evolutionary lineages, yet unrelated enantiostylous species share several similarities in their floral development. In this discussion, we compare the development of enantiostyly in these species and examine possible developmental constraints that influence the evolution of monomorphic and dimorphic enantiostyly. We also compare the patterns of development of the enantiostylous syndrome with heterostyly because this other well-known style-length polymorphism has been the subject of more intensive developmental investigations.

Comparisons of the development of enantiostyly—Enantiostylous species from diverse evolutionary origins exhibit obvious differences in the structure of their mature flowers. For example, in some species there is a morphological division of anthers into feeding and pollinating functions (e.g., *S. rostratum*), while in others there are no differences in anther function or structure (*M. australasica*) or there is only one stamen (*P. lanuginosum*). Analysis of the growth allometries of these species suggest that the heterochronic processes of initiation and termination of stamen growth (rather than differences in growth rate) account for the structural variation of stamens, although this was not apparent in scanning electron micrographs of *W. paniculata* and *S. rostratum* (results not shown). It is possible that nonlinear growth early in development accounts for this difference, or that perhaps small, undetected changes become augmented later in the developmental process.

There are also striking differences in the role of inflorescence architecture on the arrangement of left- and right-styled flowers in enantiostylous species. In *S. rostratum*, the direction of a flower is almost completely predictable once the direction of the style of the first flower is determined, whereas in *H. mexicana*, the position of left- and right-styled flowers appear to be randomly determined. In *C. lutea* and *M. korsakowii*, inflorescence architecture and environmental factors can account for some of the variation in the position of left- and

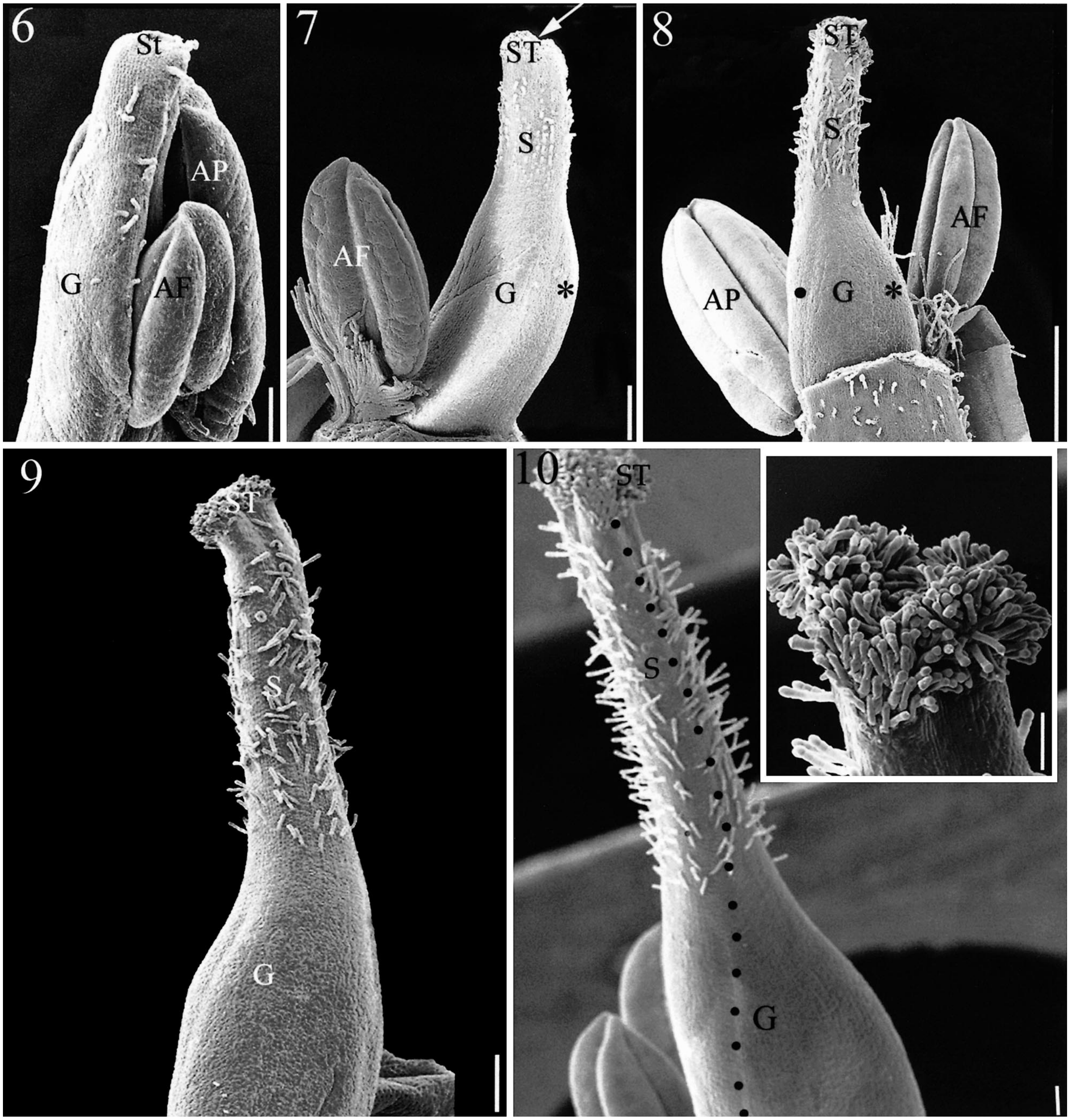
TABLE 2. Regression parameters of style length and length of the pollinating (PS) and the feeding (FS) stamens. Floral organ lengths were regressed against bud length in nine species. N is the number of buds measured, a is the estimate of the regression intercept, b is the estimate of the slope, and SE is standard error. Differences in the slopes and intercepts of the two anther types were tested using analysis of covariance. *0.05 $> P >$ 0.01; ***$P <$ 0.01. For *Wachendorfia paniculata*, stamen 1 is in a reciprocal position to the stigma and stamen 2 is in a nonreciprocal position.

Taxon/Organ	N	a	SE	b	SE	r^2	F intercept	F slope
Haemodoraceae								
Dilatris corymbosa								
Style	57	−2.73	0.16	1.77	0.07	0.92		
PS	58	−1.18	0.17	1.31	0.05	0.83		
FS	58	−2.09	0.22	1.31	0.07	0.92	4.90*	2.18
Wachendorfia paniculata								
Style	86	−1.86	0.11	2.22	0.08	0.90		
Stamen 1	86	−1.16	0.10	1.12	0.05	0.90		
Stamen 2	86	−1.22	0.99	−1.22	0.05	0.88	15.91***	1.50
Pontederiaceae								
Heteranthera limosa								
Style	19	−1.98	0.10	1.46	0.07	0.96		
PS	16	−1.29	0.29	1.06	0.14	0.74		
FS	16	−1.68	0.26	1.15	0.13	0.80	10.25**	1.43
H. rotundifolia								
Style	56	0.93	0.05	1.14	0.07	0.83		
PS	56	0.23	0.11	0.63	0.05	0.75		
FS	56	−0.37	0.11	0.45	0.05	0.61	19.63***	0.20
H. multiflora								
Style	87	−1.55	0.08	1.23	0.06	0.83		
PS	87	0.14	0.06	0.41	0.04	0.58		
FS	87	−0.34	0.07	0.45	0.05	0.50	95.78***	2.72
Monochoria australasica								
Style	59	−0.91	0.11	1.35	0.07	0.87		
Stamen	59	0.42	0.08	0.54	0.05	0.71		
Philydraceae								
Philydrum lanuginosum								
Style	69	−0.49	0.05	1.18	0.05	0.90		
Stamen	62	−0.64	0.09	0.71	0.04	0.82		
Tecophilaeaceae								
Cyanella lutea								
Style	55	−5.59	0.40	3.21	0.19	0.84		
PS	55	−1.42	0.09	1.54	0.05	0.95		
FS	54	−2.01	0.11	1.68	0.06	0.94	149.11***	4.02
Solanaceae								
Solanum rostratum								
Style	22	2.28	0.54	1.94	0.11	0.94		
PS	15	0.08	0.14	0.89	0.07	0.92		
FS	15	−0.16	0.13	0.86	0.06	0.92	34.78***	0.77

right-styled flowers, as the direction of stylar deflection is not completely predictable. This suggests that the cues to stylar bending may be determined differently among species of independent evolutionary origins.

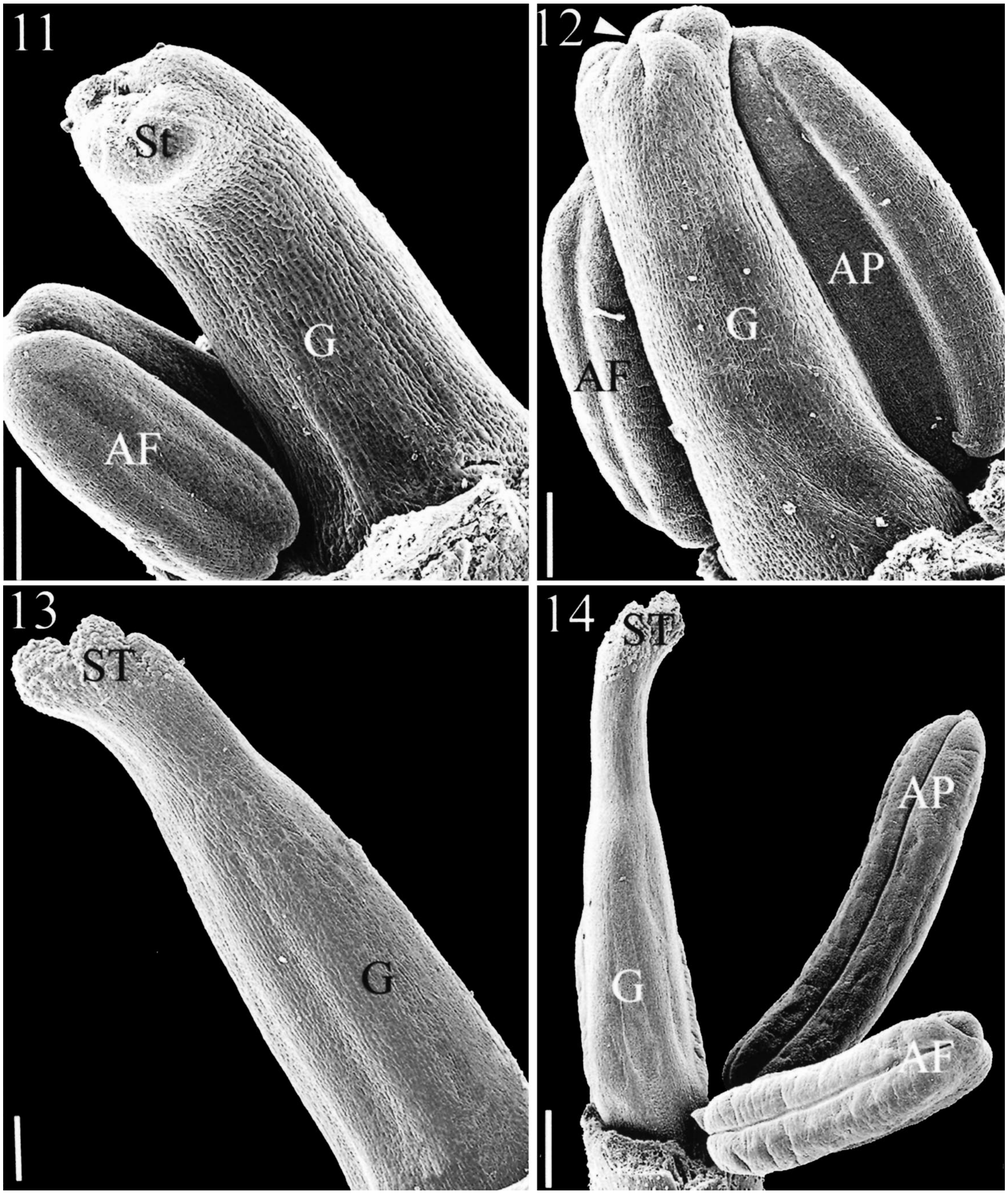
Broad similarities also exist among enantiostylous species in the structure and development of their flowers. In all species examined enantiostyly is initiated relatively late in floral development. In *W. paniculata*, *D. corymbosa*, and *P. lanuginosum*, bending of the style was observed in the floral bud when buds were approximately 25–45% of full size. In all other species studied, the style was not deflected in the bud, suggesting that stylar bending occurs just prior to or simultaneous with the opening of the bud at anthesis. The expression of enantiostyly late in floral development has been noted in other enantiostylous taxa. In *Monochoria hastata* (syn. *M. hastaefolia*; Pontederiaceae) and *Cassia didymobotrya* (Leguminosae), the bending of the style takes place 6–12 h prior to anthesis (Iyengar, 1923; Dulberger, 1981, respectively). The bending of the pollinating stamen also occurs during this time in *M. hastata* and *M. vaginalis* (Iyengar, 1923). It is possible that developmental constraints, such as limited space within a bud, lead to the consistent late appearance of enantiostyly during development. Thus, enantiostyly may only be expressed when the tight packaging that occurs in early bud development is released during floral expansion.

A left- or right-deflection in position of the pollinating stamen was not seen in the bud in any species examined, and thus, must occur very late in development. Despite the reciprocal positioning of the style and a pollinating anther at anthesis, the initiation of androcial and gynoecial structures oc-



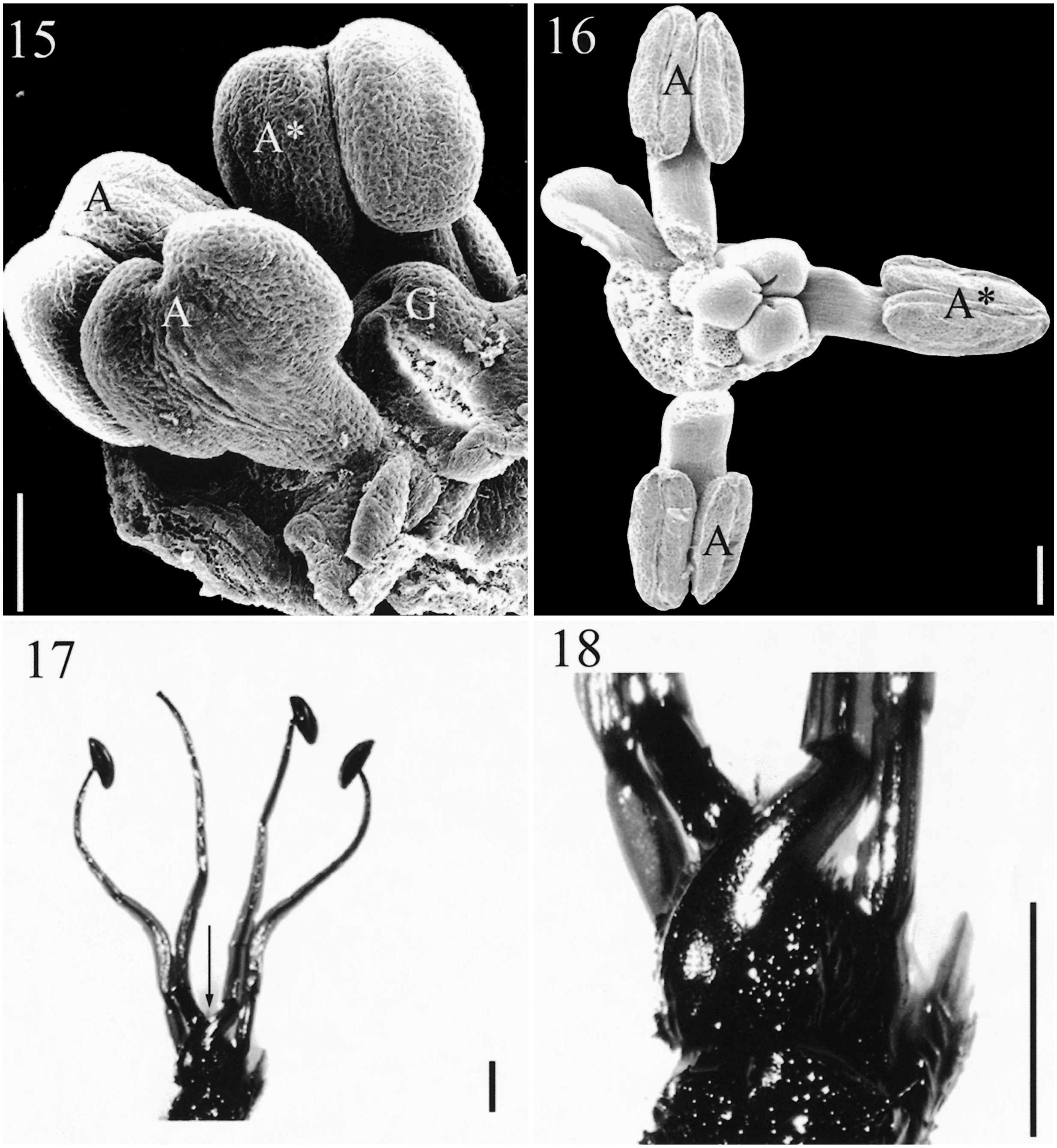
Figs. 6–10. Scanning electron micrographs of *Heteranthera multiflora* flower buds. For all micrographs, tepals were removed to reveal floral organs. **6.** Initiation of the stigmatic surface. The gynoecium is approximately the same size as the pollinating anther. Asymmetric growth of the stigmatic surface has not yet begun at this stage. The ovary (with three locules) is asymmetrical, with the side facing the pollinating anther flattened. One feeding anther has been removed for clarity. Bud size = 1 mm. **7.** A side view of the gynoecium and one feeding anther (the pollinating anther and other feeding anthers have been removed). The stigmatic surface is asymmetrical with the surface tilting away from the pollinating anther (pollinating anther not shown). The side of the ovary opposite the pollinating anther is larger (*). Bud size = 4 mm. **8.** The stigma has bent towards the dorsal surface of the flower. Two locules are shown in view; the larger locule opposite the pollinating anther (*) and one adjacent flattened locule (●) are shown. Bud size = 4 mm. **9–10.** Gynoecium just prior to anthesis. The stigma and style rotate around the longitudinal axis of the gynoecium (dotted line) so that the stigma is directed away from the direction of stylar bending. Inset is an enlarged view of the stigma. All three stamens have been removed for clarity. Bud size = 9 mm. Bars = 200 μm. The background was removed from Figs. 6–9 with Photoshop 5.0 (Adobe Systems, San Jose, California, USA).

Figure Abbreviations: A = anther; AF = feeding anther; AP = pollinating anther; G = gynoecium; S = style; St = stigmatic surface; ST = stigma.



Figs. 11–14. Scanning electron micrographs of *Heteranthera limosa* flower buds. **11.** Initiation of stigmatic surface. The pollinating anther (not in view) is located behind the gynoecium and is the same height as the feeding anther. Bud size = 1 mm. **12.** The gynoecium and pollinating anther are still equal in height; however, the stigmatic surface is beginning to grow asymmetrically away from the pollinating anther (arrowhead). Bud size = 2 mm. **13.** A developing gynoecium with elongating style and developing stigmatic papillae is shown. Unlike *Heteranthera multiflora*, there are no obvious differences in locule size in *Heteranthera limosa*. The pollinating anther and feeding anthers have been removed. The pollinating anther was located on the left side of the gynoecium. Bud size = 3 mm. **14.** Lateral view of floral organs 0–24 h prior to anthesis. The stamens have been bent back to reveal the gynoecium. Note that the stigmatic surface tilts in the direction away from the pollinating anther. Bud size = 7 mm. Bars = 200 μm. The background was removed with Photoshop 5.0 (Adobe Systems).

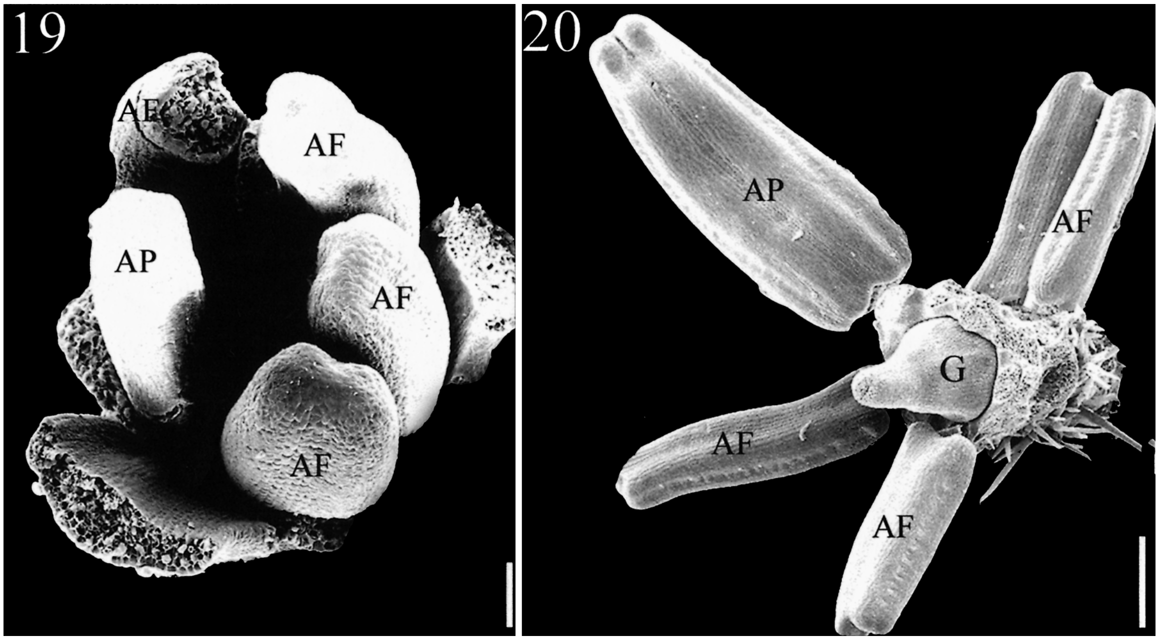
cur at different developmental stages. In all species examined, the stamens were initiated and differentiated into anther and filament well before gynoecial differentiation into the ovary, style, and stigma. Thus, the reciprocal positioning of the stigma and anther that occurs in many unrelated taxa is likely to be due to convergent selection, rather than through allometric processes in early organ development. This convergent selection on stigma and anther height has been documented in other species. For example, Hufford (1988a, b) examined diversification of organ lengths in the genus *Eucnide*. He found that while stigma and anther heights were always similar in mature organs, the growth rates and timing of organ initiation were



Figs. 15–18. Scanning electron micrographs of buds and light micrographs of mature sex organs of *Wachendorfia paniculata*. **15.** A young bud with the tepals removed to reveal the anthers and the developing gynoecium. One anther (A*) is positioned away from the other two anthers and is in the reciprocal position to the stigma in the mature flower. No asymmetries in the gynoecium are evident at this stage. Bud size = 4 mm. **16.** Overview of an older bud. Stigma and style (not in view) and anthers are more developed. Bud size = 7 mm. **17.** Mature sex organs of *Wachendorfia paniculata*. A stamen and the gynoecium have crossed (arrow) resulting in reciprocal positioning of the anther. The style has a reverse ''s'' shape that is characteristic of mature *Wachendorfia paniculata* flowers. **18.** Base of gynoecium and stamen after crossing. Bud size = 15 mm. Bars = 100 μm (Fig. 15); 500 μm (Fig. 16); 2 mm (Figs. 17–18).

very diverse within this clade. This indicates that similarities in the mature structures of flowers can result from quite distinct developmental processes.

Axes of asymmetry are present soon after organ initiation in *H. limosa*, *H. multiflora*, *S. rostratum*, and *W. paniculata*. However, the axis of asymmetry lies in the dorsiventral plane, rather than in the medial-lateral plane. In *H. multiflora*, *H. limosa*, and *S. rostratum*, the pollinating anther is consistently located on the ventral side of the flower, while in *W. paniculata*, it is located on the dorsal side. The early initiation of dorsiventral asymmetries is common in many zygomorphic species, and in many species, zygomorphy is expressed at floral initiation (Endress, 1999). In other species, dorsiventral asymmetries can be expressed later in development due to differences in heterochrony or the late development of structures such as glands or spurs (for review see Tucker [1999]).



Figs. 19–20. Scanning election micrographs of *Solanum rostratum* flower buds. **19.** Young bud in which the pollinating anther is positioned apart from the feeding anthers. The gynoecium has not yet emerged. Bud size = 5 mm. **20.** A later stage of floral development with a developing gynoecium. The pollinating anther is larger that the feeding anthers (only three feeding anthers are shown). Sepals and petals have been removed. Bud size = 10 mm. Bars = 200 μm. The background was removed with Photoshop 5.0 (Adobe Systems).

Despite the early initiation of dorsal-ventral asymmetry, we found no consistent differences in left-right axes early in development. In buds of *H. multiflora* that were greater than 90% of mature length, the stigma rotated so that the tilt of the stigma was away from the direction of stylar bending. This was the earliest indication that we noticed for the direction of stylar bending. Thus, despite the genetic determination of dimorphic enantiostyly in *H. multiflora* (Jesson and Barrett, 2002a), we found no differences between the style morphs until very late in development. Tucker (1999) also noted that stylar bending in several enantiostylous species in the Leguminosae (e.g., *C. didymobotrya* and *C. fasciculata*) occurred late in development. This is not necessarily the case in other enantiostylous species. Iyengar (1923) found that both *M. hastata* and *M. vaginalis* exhibited one or two unequal spurs on the filaments of all stamens, with the largest spur always occurring on the side of deflection of the pollinating stamen. These spurs were unequal before bending of the stamen and were hence an early indicator of the direction of stylar bending. We did not observe any spurs in *M. australasica*, although this species does not possess a pollinating anther. Thus, it would be interesting to contrast floral development among species of *Monochoria* as this may reveal other differences in the timing of initiation of asymmetries.

Constraints on the evolution of monomorphic and dimorphic enantiostyly—Dimorphic enantiostyly appears to have evolved from monomorphic enantiostyly only two or three times (Barrett, Jesson, and Baker, 2000). Within the Pontederiaceae, we found no significant differences in development between dimorphic enantiostylous *H. multiflora* and monomorphic enantiostylous *H. limosa*. While the type of enantiostyly differs at the plant level in these two species, it remains very similar at the developmental level and in the mature flower structure. In *H. multiflora*, it appears that fixing the direction of stylar deflection does not require separate developmental processes. In this way, enantiostyly may have similarities to the evolution of dioecy from monoecy. In monoecious species, plants produce both male and female flowers, whereas plants with dioecious species produce flowers of only one sex. Renner and Ricklefs (1995) have argued that one of the reasons that dioecy commonly evolves from monoecy is because the developmental machinery is already present in the ancestral state. However, the infrequent occurrence of dimorphic enantiostyly (compared to dioecy) suggests the genetic determination of left from right (or vice versa) required for a transition from monomorphic to dimorphic enantiostyly is much more difficult than the origin of dioecy from monoecy. Alternatively, selection for dimorphic enantiostyly may not be as strong as for dioecy (but see Jesson and Barrett, 2002b, c).

Clues to the constraints in the positional information determining left from right may be found by examining the patterning of left- and right-styled flowers on an inflorescence. In *S. rostratum*, the direction of style bending is almost completely predictable (Fig. 4). Consecutive flowers on a plant alternate between being left- or right-styled, with the style always turned towards the axis of the raceme (termed pendulum asymmetry; see Charlton, 1998). This pattern was first noticed in *S. rostratum* by Todd (1882) and Knuth (1906) and also occurs in enantiostylous species of *Cassia* and *Chamaecrista* (Tucker, 1999). While it is unknown how the direction of deflection of the first flower is initiated, the consistent patterning of flowers suggests that the position of the flower in relation to the axis of the inflorescence plays a critical role in determining stylar direction. The consistent deflection of a style towards the axis of the cyme implies that an axis of symmetry that is already present in the shoot plays a role in determining left from right. Thus, in *S. rostratum*, it is possible that monomorphic enantiostyly did not evolve through the development of a new axis of symmetry, but rather developed from positional cues that occurred from an already established axis.

In other species, the direction of deflection is not as predictable. While flowers of *C. lutea* on a given node are more likely to deflect to the opposite direction than the previous older flower, this pattern is not consistent. Therefore, factors other than pendulum asymmetry clearly play a role. We found no consistent predictors of stylar direction within inflorescences in *H. mexicana*. While stylar direction seems to be completely random in this species, it is likely that other factors not examined in this study (such as hormonal or environmental cues) may determine style direction. Further work is needed to determine whether the patterning of left and right styles within an inflorescence is the result of random accidents of development.

In species such as *S. rostratum* in which stylar deflection is predictable, left- and right-styled flowers are determined by the position of the flower relative to the axis of the inflorescence. Fixing the direction of deflection (as in dimorphic enantiostyly) would necessitate changing the required positional cue to one that is consistently left or right regardless of flower position. While aborting 50% of the flowers on a plant would produce entirely left- and entirely right-styled individuals in this species, the fitness consequences of this are likely to be severe.

Coen and workers (Carpenter and Coen, 1990; Luo et al., 1996) have shown that positional information in zygomorphic flowers is conveyed by two genes: *CYCLOIDEA* and *DICHOTOMA*. These genes establish a distinction between dorsal, lateral, and ventral organs, as well as dorsiventral asymmetry within individual organs. However, enantiostyly also requires the establishment of a medial-lateral axis (or the utilization of an existing apical-basal axis; see Fig. 1). Monomorphic enantiostyly may result from differential growth rates along apical-basal or medial-lateral axes. Dimorphic enantiostyly, however, requires differential growth rates consistently on one side. Therefore, additional genes or signaling pathways must be required to establish left from right as well as medial from lateral and dorsal from ventral. If this is true, then the evolution of dimorphic enantiostyly may be constrained by the absence of gene products that perceive and transduce appropriate positional information or by a lack of cues to provide such positional information.

Comparisons with the development of other stylar polymorphisms—Three other stylar polymorphisms are well documented in flowering plants: distyly, tristyly, and stigma-height dimorphism (Barrett, Jesson, and Baker, 2000). While there have been no studies of the development of stigma-height dimorphism, the organogenesis of distylous and tristylous flowers have been investigated in some detail (Richards and Barrett, 1984, 1987, 1992; Richards and Koptur, 1993; Faivre, 2000). As in enantiostyly, heterostylous species from different evolutionary origins show many ontogenetic differences. In tristylous species, the dimorphism of stamen height found within a morph is evident at initiation. The different organ heights can occur in a radial arrangement in two different stamen series (as in the Lythraceae and the Oxalidaceae) or in a dorsiventral arrangement (as in the Pontederiaceae) so that stamens in the same series can have different heights (Richards and Barrett, 1992). In the enantiostylous species studied, the different organ heights also occur in a dorsiventral arrangement. Richards and Barrett (1992) found no evidence that differences in stamen height within a flower were due to changes in growth rate, a pattern also found in this study. We found no evidence in enantiostylous species of differences in the relative growth of the feeding and the pollinating anther in all species examined. Instead, our allometric studies showed evidence for differences in stamen development from initiation, although this was not seen in SEM studies.

An examination of the development of stylar polymorphisms in an evolutionary context is important as it can be used to test evolutionary hypotheses. Faivre (2000) compared the ontogeny of species with putative ancestral floral morphologies to heterostylous flowers. She found that transitions from an approach herkogamous morphology (in which the stigma is positioned above the anthers) to a heterostylous one was possible, as predicted in theoretical models by Lloyd and Webb (1992). However, the change in stylar growth patterns between a species with approach herkogamy and a closely related heterostylous species led Faivre (2000) to suggest that a further developmental step was probably required for the evolution of heterostyly to occur. In monomorphic enantiostylous *H. limosa* and dimorphic enantiostylous *H. multiflora*, we found few differences in organ initiation and development. This gives some support to the hypothesis that dimorphic enantiostyly evolved from monomorphic enantiostyly, rather than from a separate evolutionary transition in which differences in development would more likely occur. It would be useful to compare development in other closely related pairs of monomorphic and dimorphic enantiostylous species to see if this pattern of similarity remains consistent.

The development of enantiostyly in species of diverse evolutionary origins is characterized by similar developmental processes. The position of sex organs in mature flowers is determined by bending of the style and pollinating stamen and these events occur late in development. Furthermore, variations in stamen size are not due to differences in growth rates. This suggests that the diverse ways in which enantiostyly can be expressed in flowering plants may be constrained by either developmental or physical structures. Despite this, the patterning of left- and right-styled flowers within inflorescences varies markedly between families. It is possible that such differences may play a role in limiting the evolution of dimorphic enantiostyly to only three monocotyledon families.

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