



The Evolution and Function of Styler Polymorphisms in Flowering Plants

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We recognize four major classes of styler polymorphisms in flowering plants: the heterostylous conditions distyly and tristyly, stigma-height dimorphism, and enantiostyly. These polymorphisms differ in the relative positions of sexual organs and in the number of floral morphs that occur within populations. In heterostyly, stigma and anther heights are reciprocally positioned in the two or three floral morphs; in stigma-height dimorphism the two morphs vary in style length but not anther height; whereas in enantiostyly, flowers differ in whether the style is deflected to the left- or right-side of the flower. We distinguish two forms of enantiostyly depending on whether both style orientations occur on the same plant (monomorphic enantiostyly) or on different plants (dimorphic enantiostyly). Styler polymorphisms have originated independently in numerous animal-pollinated flowering plant families. Both heterostyly and enantiostyly involve distinct floral syndromes suggesting functional convergence in which the position of the pollinator is important for pollen dispersal and male reproductive success. The function of stigma-height dimorphism remains enigmatic although the occurrence of populations with 1:1 style-morph ratios suggest that, like heterostyly and dimorphic enantiostyly, they are maintained by disassortative mating. We interpret these sexual polymorphisms as floral designs that increase the precision of cross-pollination and reduce lost mating opportunities associated with self-interference, especially geitonogamy. A single adaptive explanation based on frequency-dependent male mating proficiency can explain the evolution and maintenance of the four styler polymorphisms in plants.

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INTRODUCTION

A major problem for hermaphrodite plants is to deploy their sexual organs in a manner that simultaneously optimizes maternal and paternal fitness. In most animal-pollinated angiosperms, female and male sexual organs are located in close proximity to one another in the central parts of the flower. Positioning anthers and stigmas at similar locations within a flower increases the precision of cross-pollen transfer since both sexual organs contact similar parts of the pollinator. However, when stigmas and anthers are located at the same position within the flower the two functions of pollen receipt and pollen removal have the potential to interfere with one another leading to lost mating opportunities (Darwin, 1877; van der Pijl, 1978; Lloyd and Yates, 1982). Resolving the conflict between the benefits of proficient pollen dispersal and the costs of reproductive interference probably contributes to the great structural variation in sex-organ position among flowering plants.

The most obvious consequence of reproductive interference is self-fertilization. This can arise in different ways depending on whether self-pollination occurs within (intra-floral) or between flowers (geitonogamy) on a plant (Lloyd and Schoen, 1992). However, in addition to selfing and its effects on fitness through inbreeding depression and pollen discounting (Charlesworth and Charlesworth, 1987; Harder and Wilson, 1998), self-interference can also occur through

more subtle influences that operate pre-zygotically (e.g. stamens or styles obstructing pollen deposition or removal, respectively; stigma and styler clogging with self pollen or pollen tubes; and ovule discounting) each of which can reduce female and/or male fertility (Shore and Barrett, 1984; Bertin and Sullivan, 1988; Waser and Price, 1991; Harder and Barrett, 1995; Sage *et al.*, 1999). Because of the diversity of ways that self-interference can potentially be expressed it is not surprising that mechanisms that reduce its effects should be commonplace. For example, the various forms of herkogamy have been interpreted as floral adaptations that reduce pollen-stigma interference, especially when they occur in self-incompatible species where the benefits of outcrossing are assured through physiological mechanisms (Webb and Lloyd, 1986).

The most common herkogamous floral design is approach herkogamy. Here styles are exerted beyond anthers and stigmas usually contact pollinators first upon their entry into the flower. Less common are species with reverse herkogamy in which styles are shorter in length and stigmas are located below the anthers (Webb and Lloyd, 1986). Populations with approach or reverse herkogamy show the usual patterns of phenotypic variation expected for reproductive structures controlled by the interaction of environmental factors and many genes of small effect. In most species styles are unimodal in length and are typically located at similar positions within the flower when stigmas are receptive. In contrast, in some animal-pollinated species a discontinuous pattern of variation is evident with discrete

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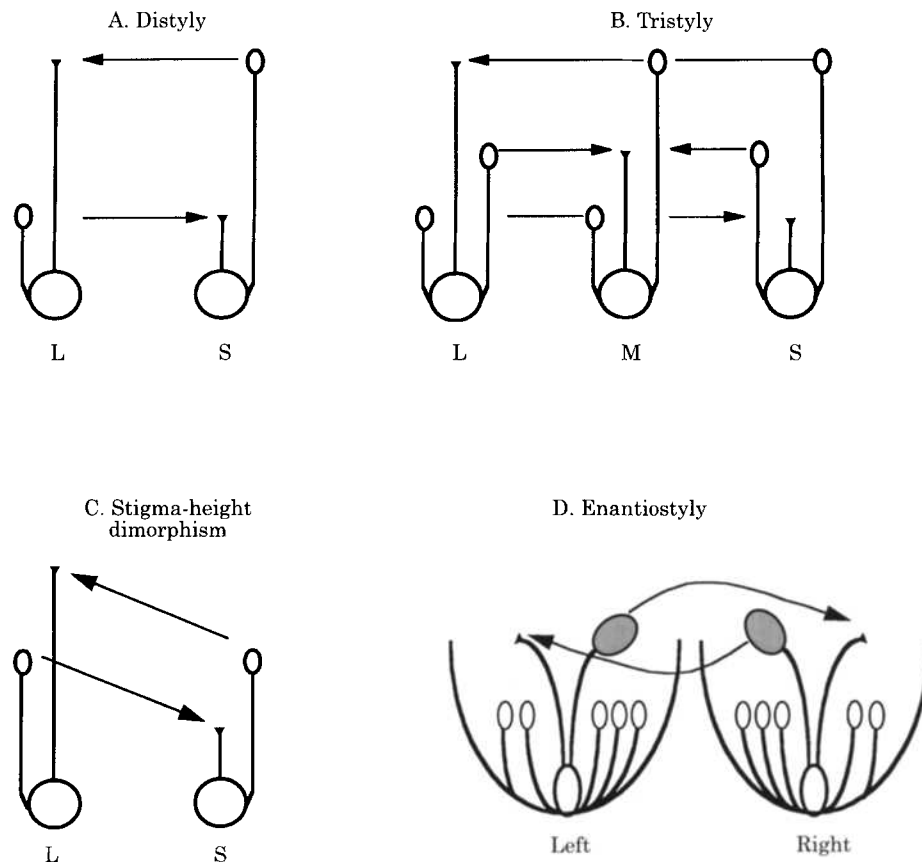


FIG. 1. The four principal styler polymorphisms in flowering plants. A, Distyly; B, tristily; C, stigma-height dimorphism; D, enantiostyly. Arrows indicate intermorph (disassortative) matings promoted by these floral designs and which maintain the polymorphisms with equal frequencies of the floral morphs within populations.

floral morphs occurring within populations differing in style length or orientation. Here we recognize four major classes of styler polymorphism in flowering plants (Fig. 1). The polymorphisms differ in the number of floral morphs within populations and in the particular positions that sexual organs are located within a flower. This paper concerns the evolution and functional significance of these sexual polymorphisms.

The styler polymorphism heterostyly has been intensively studied since Darwin's pioneering work last century (Darwin, 1877). Populations are composed of two (distyly) or three (tristyly) floral morphs that differ in stigma and anther heights (Fig. 1A,B). Because of the voluminous literature on these polymorphisms (reviewed in Barrett, 1992), we discuss them only briefly, focusing on recent work and unresolved issues concerning their evolutionary origins. The two remaining styler polymorphisms, stigma-height dimorphism and enantiostyly (Fig. 1C,D), are less well known and we therefore concentrate our attention on them. Both polymorphisms have evolved independently in a number of unrelated flowering plant families suggesting convergent selection pressures. The major objective of this paper is to draw attention to these interesting floral designs and review recent empirical and theoretical studies that we have conducted concerning the selective forces that maintain the polymorphisms in diverse taxonomic groups. Our

goal is to show that styler polymorphisms provide valuable model systems for investigating form and function in simply inherited plant reproductive traits.

HETEROSTYLY: WHAT WE KNOW AND DON'T KNOW

Heterostyly is usually composed of a syndrome of traits including differences in style length and anther height, diallelic self-incompatibility, and a suite of ancillary pollen and stigma polymorphisms. Its floral design provides a unique evolutionary solution to the conflicting problems of precision in pollen transfer between plants and the avoidance of pollen-stigma interference (Lloyd and Webb, 1992a). The polymorphism can be viewed as reciprocal herkogamy since populations are composed of floral morphs that differ in the sequence in which stigmas and anthers are presented. For example, in distylous populations (Fig. 1A) the long- and short-styled morphs (hereafter L- and S-morphs) are similar in morphology to the approach and reverse herkogamous conditions found in species monomorphic for style length.

The architecture of the mid-styled morph in tristily (Fig. 1B), with stigmas positioned between long- and short-level stamens, does not appear to occur anywhere else but in tristylous species. The apparent maladaptiveness of this

floral design for non-heterostylous species is probably because when present in a monomorphic state this morphology promotes self-fertilization (Kohn and Barrett, 1992). Where selfing is selectively favoured in monomorphic species it can be achieved more simply without the necessity for the differentiation of two separate anther levels. This example indicates that functional interpretation of the floral architecture of style morphs in heterostylous species requires an appreciation of their mutual interdependence in promoting cross-pollination.

Experimental studies demonstrate that heterostyly functions to promote more effective pollinator-mediated pollen dispersal between plants than would occur in populations uniform in style length (Darwin, 1877; Ganders, 1974; Barrett and Glover, 1985; Kohn and Barrett, 1992; Lloyd and Webb, 1992b; Stone and Thompson, 1994). Due to reciprocal herkogamy, intermorph cross pollinations are favoured over intramorph transfers because of segregated pollen deposition on different pollinator parts (Wolfe and Barrett, 1989; Lloyd and Webb, 1992b). In species with diallelic incompatibility, effective intermorph cross-pollination reduces pollen losses on incompatible stigmas resulting in more efficient male function. Heterostyly is therefore best interpreted as a floral design that increases male mating proficiency by increasing the precision of pollen dispersal between plants.

Recent discoveries of distyly and tristily in angiosperm families in which the polymorphisms were not known to occur [e.g. distyly in Polemoniaceae (Cochrane and Day, 1994) and Lamiaceae (Fig. 2); tristily in Amaryllidaceae (Barrett *et al.*, 1997), Connaraceae (Lemmens, 1989) and Linaceae (Thompson *et al.*, 1996)] serve to emphasize the diverse lineages in which the polymorphisms have evolved. Heterostyly is now reported in at least 28 families and has clearly originated on numerous occasions, especially in groups with an intermediate degree of advancement and tubular flowers (Lloyd and Webb, 1992a). What is particularly curious is the isolated occurrence of the polymorphism in many unrelated lineages. In many families and genera the polymorphism is restricted to a small number of species. For example, within the large families Lamiaceae

and Polemoniaceae heterostyly has apparently evolved in only a single species. This situation contrasts with taxa such as *Primula*, *Oxalis* and Rubiaceae where heterostyly is firmly established with hundreds of species possessing the polymorphism. The origins and contrasting evolutionary success of heterostyly among angiosperm families remains a mystery.

Diverse pollen–pistil interactions in heterostylous species

Much of what we know about heterostylous plants has come from detailed studies of a relatively small number of taxa (e.g. *Primula*, *Lythrum*, *Linum*, *Oxalis*). As more species are studied it is clear that some early generalizations may need to be modified. Several examples concerning pollen–pistil interactions serve to illustrate this point. The occurrence of unusual incompatibility systems in distylous *Villarsia* (Ornduff, 1988) and tristylous *Narcissus* (Sage *et al.*, 1999) indicate that the morphological components of heterostyly are not always associated with intramorph mating barriers typical of species with diallelic incompatibility. Indeed, as more heterostylous species are studied experimentally it is evident that many are self-compatible (reviewed in Barrett and Cruzan, 1994), raising the question of whether reciprocal herkogamy acting alone can promote sufficient disassortative mating to maintain the polymorphism. In some self-compatible taxa, cryptic incompatibility favours intermorph mating through differential pollen tube growth (*Amsinckia*: Weller and Ornduff, 1977; *Eichhornia*: Cruzan and Barrett, 1993; *Decodon*: Eckert and Allen, 1997). Even in species with ‘classical heteromorphic incompatibility’ the sites of pollen tube arrest can vary strikingly both within and among species (reviewed in Dulberger, 1992). In tristylous *Pontederia*, for example, cessation of pollen tube growth in selfs occurs in different parts of the style and ovary, depending on the particular pollen–pistil combination involved (Scribailo and Barrett, 1991).

Future research directions

Two major gaps in our knowledge restrict understanding of the evolution of heterostyly. First, little is known about the phylogenetic history of the vast majority of heterostylous groups and it is therefore difficult to identify the floral characteristics of the immediate ancestors of heterostylous lineages (but see Kohn *et al.*, 1996; Schoen *et al.*, 1997). Few heterostylous families show a clear build-up of the polymorphism and disagreement exists on the order of establishment of morphological and physiological components of the syndrome (Charlesworth and Charlesworth, 1979; Lloyd and Webb, 1992a, b; Richards, 1998). Second, while considerable information on the inheritance of heterostyly is available (reviewed in Lewis and Jones, 1992), almost nothing is known about the molecular and developmental genetics of the polymorphism. As is evident from this volume, this situation is in striking contrast to the burgeoning literature on the molecular genetics of homomorphic incompatibility systems. What accounts for this difference, given that heterostyly has been used as a model

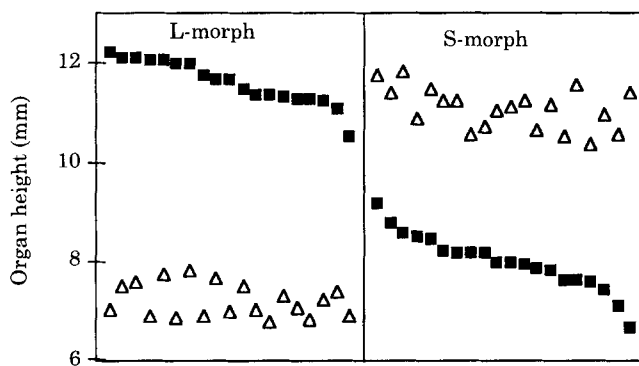


Fig. 2. Reciprocal herkogamy in distylous *Salvia brandegeei* (Lamiaceae). Range of variation in stigma (■) and anther height (△) in a sample of 40 flowers from plants originating from Cherry Canyon, Santa Rosa Island, California. Note the reciprocal correspondance of stigma and anther heights in the long- and short-styled morphs (Barrett, Wilken and Cole, 2000).

system by many influential geneticists since Bateson and Gregory first elucidated the simple inheritance of distyly in 1905?

The limited progress in analysing the genetic architecture of heterostyly using molecular approaches is largely because the problem is difficult. Firstly, the loci governing the polymorphism have not been genetically mapped in any species. Moreover, little is known about the precise number of loci involved (see Kurian and Richards, 1997), or their organization and developmental regulation. Recent genetic mapping techniques with RAPD or AFLP markers offer the best approach to locating the genes governing heterostyly. Of particular interest will be to determine whether a supergene model involving several tightly linked genes is sufficient to explain the polymorphism, or whether regulatory genes with morph-limited expression also play an important role in determining the unique features of the floral morphs. Secondly, the biochemical basis of self and cross rejection of pollen from like morphs is unclear although it seems unlikely that recognition specificities of the type found in homomorphic systems are involved. Thirdly, the extent to which the morphological differences between style morphs participate in the incompatibility reactions is still uncertain (see Dulberger, 1992). Unlike homomorphic incompatibility systems, identification of morph-specific incompatibility proteins in heterostylous plants (see Wong *et al.*, 1994a, b; Athanasiou and Shore, 1997) is more difficult because of the potentially confounding problems of morphology. Protein differences between morphs may have nothing to do with incompatibility *per se* but instead may simply be associated with differential organ growth. Lastly, the likelihood that the mechanisms of incompatibility in the floral morphs differ from one another (reviewed in Dulberger, 1992; Barrett and Cruzan, 1994) adds another level of complexity to the problem that does not occur with homomorphic systems.

These uncertainties, combined with the paucity of economically important heterostylous crops (cf. *Brassica*, *Nicotiana*), have undoubtedly discouraged many workers from investigating the problem. Nevertheless, given the long historical interest in heterostyly and the unusual opportunity the polymorphism provides for linking genes, development, morphology, and fitness it is unlikely that our current state of ignorance of the molecular genetics of heterostyly will continue.

STIGMA-HEIGHT DIMORPHISM: SEXUAL DISHARMONY OR STABLE FLORAL STRATEGY?

The defining feature of heterostyly is the reciprocal positioning of anthers and stigmas in the floral morphs. As Darwin (1877) first appreciated, this floral design makes functional sense when interpreted as an adaptation that promotes cross-pollination by animal pollinators. A second less well-known floral design, stigma-height dimorphism, also involves discrete style-length morphs and shares some similarities with distyly. However, it differs in important ways that merit its recognition as a distinct styler polymorphism. Species with this polymorphism are composed

of populations with two floral morphs that differ principally in the heights at which stigmas are located within the flower. In the L-morph, stigmas are usually located above the stamens, whereas in the S-morph the stigmas are located below the anthers (Fig. 1C). In common with distyly, it seems most likely that the ancestral condition was approach herkogamy (see Lloyd and Webb, 1992a), with populations monomorphic for this phenotype invaded by short-styled variants resulting in a polymorphic state.

From a morphological standpoint the most important distinguishing feature of stigma-height dimorphism is that the stamen levels in the two floral morphs are of similar height. Hence unlike distyly there is no clear reciprocal correspondence of anther and stigma heights in the floral morphs. From a functional perspective this arrangement of sexual organs presents a puzzle. If pollen is deposited on a single region of a pollinator's body, as might be predicted from what is known about the mechanics of pollination in heterostylous species, why are two distinct stigma heights maintained in populations of these species?

Because of the apparent sexual disharmony in sex-organ positioning most workers have assumed that stigma-height dimorphism is an unstable condition representing a transitional stage in the evolution of distyly. Theoretical models of the evolution of distyly generally show that: (1) the polymorphism is difficult to maintain (Charlesworth and Charlesworth, 1979); and (2) selection of mutations giving anther-height dimorphism rapidly spread to fixation in populations resulting in complete reciprocal herkogamy (Lloyd and Webb, 1992b). Indeed, the rarity of stigma-height dimorphism in flowering plants has been interpreted as evidence that the condition is rapidly passed through during the evolution of distyly. However, comparative data on the distribution of the polymorphism among angiosperm families casts doubt on whether stigma-height dimorphism is always an ephemeral state inextricably linked to the evolution of distyly.

Stigma-height dimorphism is reported from several heterostylous families (e.g. Boraginaceae—*Anchusa* spp.: Dulberger, 1970; Philipp and Schou, 1981 and *Lithodora* spp.: S. C. H. Barrett, J. D. Thompson and D. Manicacci, unpubl. res.; Linaceae—*Linum grandiflorum*: Darwin, 1877; Dulberger, 1992; but see Lloyd and Webb, 1992a, p. 166), and in some instances (e.g. *Primula boveana*, Primulaceae: Al Wadi and Richards, 1993; Richards, 1998) may indeed be a stage in the build-up of distyly. However, in other cases the polymorphism is reported from families in which heterostyly is not known to occur (e.g. Epacridaceae—*Epacris impressa*: O'Brien and Calder, 1989; Ericaceae—*Kalmiopsis leachiana* (Fig. 3); Liliaceae—*Chlorogalum angustifolium*: Jernstedt, 1982; Haemodoraceae—*Anigozanthos humilis*: S. D. Hopper, pers. comm.) but virtually nothing is known about the reproductive biology of stigma-height dimorphism in these non-heterostylous families. This latter group represents a heterogeneous assortment of different animal-pollinated species, none of which possess traits typically associated with the heterostylous syndrome. How the polymorphism is maintained is unclear but its occurrence in these families at least demonstrates that the

origin of stigma-height dimorphism does not necessarily lead inexorably to the evolution of distyly.

Evolutionarily stable stigma-height dimorphism

We have recently begun to investigate the ecology and evolution of stigma-height dimorphism in *Narcissus* (Amaryllidaceae), a genus of insect-pollinated geophytes native to the Mediterranean basin. We chose *Narcissus* because unlike many of the taxa listed above, the polymorphism is particularly well established, occurring in at least a dozen species distributed among three sections of the genus (Apodanthae, Jonquillae, Tazettae). Clearly in this group at least the polymorphism is not an evolutionarily transient state and its maintenance among diverse lineages within *Narcissus* requires a functional explanation.

In common with other groups with stigma-height dimorphism there has been confusion in the literature concerning the nature of stylar variation in *Narcissus* and its relationship to heterostyly (reviewed in Barrett *et al.*, 1996; Baker *et al.*, 2000a). Our investigations indicate that despite the widespread distribution of stigma-height dimorphism in *Narcissus*, heterostyly is only reliably reported in two species, distylous *N. albimarginatus* of section Apodanthae (Arroyo and Barrett, 2000) and tristylous *N. triandrus* of section Ganymedes (Barrett *et al.*, 1997). The rarity of heterostyly in *Narcissus* implies that there are constraints on the evolution of reciprocal herkogamy in this group. The nature of these constraints is discussed more fully in Barrett *et al.* (1996); here we consider the nature of stigma-height dimorphism and its associated self-sterility system, and how the polymorphism is maintained in natural populations.

Reproductive consequence of self-sterility

Stylar polymorphisms in *Narcissus* are associated with a late-acting self-sterility system (Bateman, 1954; Dulberger, 1964; Barrett *et al.*, 1997; Sage *et al.*, 1999). In all species studied, pollen-tube growth rates of self and outcross pollen are similar, with self-sterility expressed in the ovary.

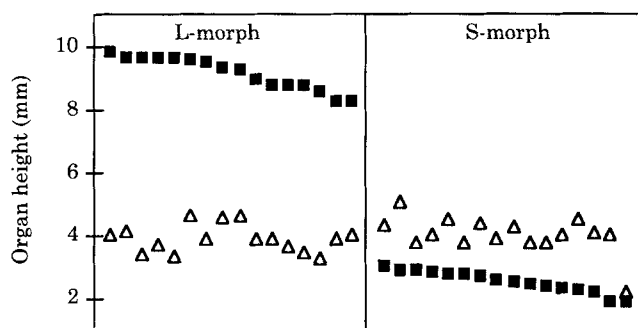


FIG. 3. Stigma-height dimorphism in *Kalmiopsis leachiana* var. *leachiana* (Ericaceae). Range of variation in stigma (■) and anther height (△) in a sample of 32 flowers from a population at Kalmiopsis Wilderness, Siskiyou National Forest, Curry County, S.W. Oregon, USA. While stigma heights in the long- and short-styled morphs differ significantly, anther heights do not.

Self-pollinations result in little seed set, cross-pollinations produce variable quantities of seed, and prior self-pollination causes significant reductions in outcrossed seed set. Detailed investigations of pollen–pistil interactions in tristylous *N. triandrus* have revealed that self-sterility is the result of a novel type of self-incompatibility that does not readily fit into classical models (Sage *et al.*, 1999).

Low seed set following self-pollination in *N. triandrus* is caused by a reduction in ovule availability resulting from embryo-sac degeneration. Self-pollen tube recognition in the pistil appears to elicit responses that prevent normal ovule development, thus reducing levels of pollen-tube penetration and double fertilization. While the physiological and molecular mechanisms responsible are not known they are probably associated with contrasting ‘long-distance’ signalling phenomena associated with cross- vs. self-pollen tube growth (see O’Neill, 1997). Species with stylar dimorphism also exhibit ovarian self-sterility and it seems likely that the mechanism responsible is the same as in *N. triandrus*. However, further work is needed to confirm this expectation and to determine the role, if any, that inbreeding depression might also play in self-sterility.

Since self-pollination can reduce the availability of ovules for outcrossing (ovule discounting—Barrett *et al.*, 1996), then floral designs that reduce levels of self-interference should be selectively advantageous in the genus. In this regard it is of interest to note that most *Narcissus* species with stylar polymorphisms produce multi-flowered inflorescences whereas the majority of those with stylar monomorphism are solitary flowered. This suggests that the costs associated with geitonogamous self-pollination in multi-flowered species may be reduced to some extent by the evolution of herkogamous polymorphisms.

The origin of stylar polymorphisms in *Narcissus* may have been initially stimulated by the spread of short-styled variants in ancestral long-styled populations because their morphology reduced levels of self-interference, especially self-pollination and ovule discounting. This seems plausible because the S-morph possesses much greater stigma–anther separation than the L-morph in all *Narcissus* species examined (see Table 13.1 in Barrett *et al.*, 1996), and all species with stigma-height dimorphism possess narrow floral tubes in which the sexual organs of the L-morph are in close proximity. Field studies of *Narcissus* spp. confirm that the L-morph is indeed more susceptible to self-pollination than the S-morph (Arroyo and Dafni, 1995; Baker *et al.*, unpubl. res.). However, our comparisons of seed set have failed to detect morph-specific differences in female fertility (e.g. Baker *et al.*, 2000b), so the ecological and evolutionary significance of self-interference and particularly ovule discounting in *Narcissus* remains to be established under field conditions.

Another reproductive consequence of the type of self-sterility found in *Narcissus* concerns its influence on mating patterns and the frequency of style morphs in populations. Studies of the genetics of stigma-height dimorphism in *N. tazetta* (Dulberger, 1964, and unpubl. res.) indicate that the inheritance of style length conforms to the single-locus two allele control common to most distylous species, with the allele for short styles dominant. With this inheritance

pattern and equivalent amounts of disassortative mating in the style morphs 1:1 (isoplethic) style–morph ratios are expected in populations, as in distylous species. However, unlike most distylous species there are no barriers to intramorph crossing in *Narcissus* and hence the mating system of populations can involve variable amounts of assortative and disassortative mating. As a result style–morph ratios are predicted to be a great deal more variable than in distylous species, and indeed this is what is generally found in *Narcissus* species with stigma-height dimorphism (Arroyo and Dafni, 1995; Barrett *et al.*, 1996).

Variation in style–morph ratios

A particularly striking case of wide variation in style–morph ratios involves *N. assoanus* (section *Jonquillae*), a diminutive species widespread in S. Spain and S.W. France that is pollinated by butterflies, hawkmoths and solitary bees. A survey of 46 populations in the Languedoc-Roussillon region of S.W. France revealed two contrasting patterns (Baker *et al.*, 2000a; Fig. 4). Populations occurring in the fragmented landscapes surrounding Montpellier were often small in size and exhibited strongly L-biased morph

ratios, a common feature of *Narcissus* species with stigma-height dimorphism (see Barrett *et al.*, 1996). In contrast, populations further inland, particularly in the N.W. portion of the region sampled, were much larger in size and all exhibited isoplethic morph ratios. No populations monomorphic for style morph were encountered, although these are reported in other *Narcissus* species with stigma-height dimorphism and are maintained because of the intramorph compatibility system in the genus (e.g. see Fig. 13.8 in Barrett *et al.*, 1996).

Genetic mating models of the maintenance of stigma-height dimorphism help interpret the variation in style–morph ratios in *Narcissus* (Baker *et al.*, 2000b). When levels of disassortative mating are higher than assortative mating the polymorphism is always maintained, although, as discussed above, equivalent levels of disassortative mating in the two morphs are required for isoplethic ratios. However, if one morph exhibits a higher level of assortative mating than the other it is driven up in frequency giving rise to biased style–morph ratios. These findings imply that patterns of cross-pollen transfer differ between populations of *N. assoanus* in the two areas sampled, perhaps because of their different pollination environments. We have yet to

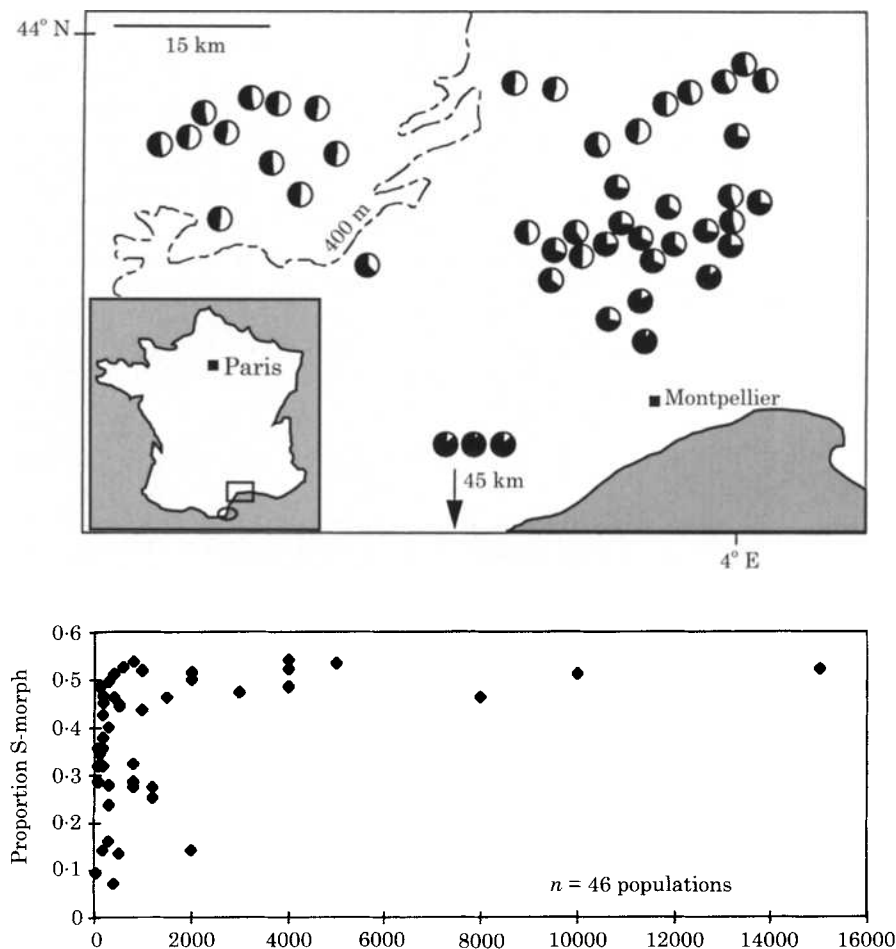


FIG. 4. Stigma-height dimorphism in *Narcissus assoanus* (Amaryllidaceae). A, Population samples of the long- and short-styled morph at 46 sites in S.W. France. The frequencies of the L-morph (black) and S-morph (white) for each population are indicated. B, The relation between the proportion of short-styled plants in populations and their size. After Baker *et al.* (2000a).

determine which aspects of the pollination process are involved, or why population size should be associated with different levels of assortative and disassortative mating in the style morphs. However, the occurrence of 1:1 style-morph ratios indicates that under the appropriate conditions symmetrical disassortative mating, as occurs in distyly, can be achieved in populations with a stigma-height dimorphism. This observation is significant because it is a requirement for some models of the evolution of distyly (see Lloyd and Webb, 1992b).

Stigma-height dimorphism in *Narcissus* is probably maintained because, in common with heterostyly, it increases the proficiency of cross-pollination by reducing self-pollination and self-interference. How precisely this is achieved without clear sex-organ reciprocity is unclear. In *N. assoanus*, it is possible that while pollen pick-up by its long-tongued pollinators occurs on a restricted location of the proboscis, pollen is subsequently redistributed through proboscis coiling facilitating effective deposition on both stigma heights (L. D. Harder, pers. comm.). Alternatively, pollen may be deposited over a broad surface area of the pollinator during nectar feeding allowing maintenance of a wider range of variation in stigma height than usually occurs in most species. Detailed studies of the mechanics of the pollination process in species with stigma-height polymorphism would be valuable in addressing these questions.

ENANTIOSTYLY: MIRROR IMAGE FLOWERS AND THEIR FUNCTION

The final stylar polymorphism we recognize in flowering plants is enantiostyly, a form of floral asymmetry in which the style is deflected away from the main axis of the flower either to the left (left-styled) or right (right-styled) side (Fig. 1D). While mirror image flowers have been known for over a century, only recently has it been appreciated that they come in two quite distinct forms which we term monomorphic and dimorphic enantiostyly (Fig. 5). Monomorphic enantiostyly, unlike heterostyly and stigma-height dimorphism, is not a genetic polymorphism since populations are composed of a single phenotype with both left- and right-styled flowers occurring on the same plant. This

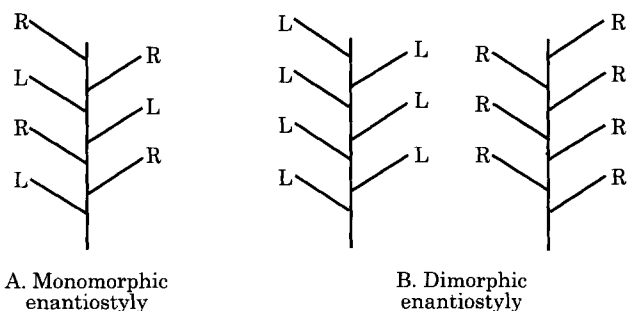


FIG. 5. The two forms of enantiostyly in flowering plants. A, Monomorphic enantiostyly; B, dimorphic enantiostyly. L and R refer to flowers with styles that are located on the left and right side of flowers, respectively. The two forms of enantiostyly have strikingly different frequencies among flowering plant taxa (see text for details).

condition is therefore best viewed as a somatic floral polymorphism. In contrast, dimorphic enantiostyly appears to be a true genetic polymorphism since populations are composed of two distinct types of individuals; those that produce all left-styled flowers and those that produce only right-styled flowers. As yet the genetic basis of dimorphic enantiostyly has not been determined but its clear cut phenotypic expression suggests that stylar orientation, like heterostyly and stigma-height dimorphism, may be under the control of a single Mendelian locus.

Enantiostyly has evolved independently in at least a dozen flowering plant families and is often associated with other floral traits including dimorphic anthers, vibrational pollen collection by bees, lack of nectaries, and outwardly-facing flowers (Graham and Barrett, 1995). The presence of this pollination syndrome in unrelated groups suggests functional convergence in which the position of the pollinator is important for pollen dispersal and male reproductive success. However, as discussed below, the occurrence of two forms of enantiostyly complicates simple adaptive explanations for the origin and maintenance of these particular stylar polymorphisms.

Most workers have interpreted enantiostyly as a floral design that promotes outcrossing through pollinator-mediated intermorph pollinations (Todd, 1882; Wilson, 1887; Iyengar, 1923; Ornduff, 1974; Ornduff and Dulberger, 1978; Kohn *et al.*, 1996). Pollen removed by a pollinator from a flower of one type is more likely to be deposited on the opposite type in a manner analogous to the functioning of heterostyly. Equal ratios of left- and right-styled individuals in populations of *Wachendorfia paniculata* (Ornduff, 1974; Jesson and Barrett, unpubl. res.), a dimorphically enantiostylous species from the Cape Province of South Africa, is consistent with the view that intermorph cross-pollination resulting in disassortative mating plays an important role in the maintenance of the polymorphism.

However, for species with monomorphic enantiostyly this interpretation is more problematic, since by having both flower types on the same plant a pollinator could potentially visit successive flowers causing geitonogamous self-pollination. Geitonogamy is generally viewed as a 'non-adaptive' cost of large floral displays since it can result in inbreeding depression and pollen discounting (Lloyd, 1992; Harder and Barrett, 1995). Because of the purported costs of geitonogamy in species with monomorphic enantiostyly the function of the polymorphism has remained enigmatic (Bowers, 1975; Dulberger and Ornduff, 1980; Fenster, 1995; Graham and Barrett, 1995). One approach to clarifying this problem is to determine the evolutionary relationships between the two forms of enantiostyly and to consider what the stylar condition of their immediate ancestors is likely to have been.

Evolutionary origins of enantiostyly

The systematic distribution and abundance of the two forms of enantiostyly are strikingly different. Monomorphic enantiostyly is widely distributed occurring in both dicotyledonous (e.g. Leguminosae, Solanaceae and

Gesneriaceae) and monocotyledonous families (e.g. Comelinaceae, Haemodoraceae, Philydraceae, Pontederiaceae, Tecophilaeaceae). In contrast, dimorphic enantiostyly is only reliably reported from *Wachendorfia* and *Barbaretta* (Ornduff, 1974; Ornduff and Dulberger, 1978) of the Haemodoraceae; *Heteranthera multiflora* (Jesson and Barrett, unpubl. res.) and possibly *Monochoria cyanea* (S. W. Barrett, pers. comm.) for the Pontederiaceae, and *Cyanella alba* (Dulberger and Ornduff, 1980) of the Tecophilaeaceae. Thus dimorphic enantiostyly is only known in a handful of species in three monocotyledonous families, each of which also contains species with monomorphic enantiostyly.

These distribution patterns in conjunction with phylogenetic evidence (e.g. Simpson, 1990, for Haemodoraceae) support the hypothesis that dimorphic enantiostyly is derived from monomorphic enantiostyly rather than the more unlikely sequence involving the reverse polarity. In addition, phylogenies of Pontederiaceae (Graham and Barrett, 1995) and Solanaceae (Olmstead and Palmer, 1997) suggest that species with monomorphic enantiostyly have probably evolved from straight-styled ancestors. If these evolutionary sequences are accepted then it is important to determine how these three floral designs influence geitonogamy in comparison with one another. We have recently begun to investigate this problem using both theoretical and experimental approaches.

Theoretical models

To understand the evolution of monomorphic enantiostyly from a straight-styled ancestor we compared predicted levels of geitonogamy for the two conditions using phenotypic selection models (Jesson and Barrett, unpubl. res.). The models of geitonogamous pollen transfer were modified from those existing in the literature (e.g. de Jong *et al.*, 1992; Barrett *et al.*, 1994) to take into account expected differences in pollen transfer between flowers on a plant for the two floral designs. We assumed that because of segregated pollen deposition on different sides of a pollinator's body in enantiostyly (see Bowers, 1975) there would be negligible pollen transfer between flowers of the same stylar orientation. This is illustrated in Fig. 6, which compared geitonogamous pollen transfer between flowers in non-enantiostylous *vs.* monomorphically enantiostylous plants. Despite the earlier assumption that monomorphic enantiostyly promotes geitonogamy, our model indicates that, in comparison with a non-enantiostylous population, levels of geitonogamous pollen transfer are in fact reduced (Fig. 7). This is because for a given inflorescence size the number of potential pollen transfers between flowers for monomorphic enantiostyly can be reduced to a half, depending on the ratio of left and right-styled flowers on a plant. In contrast, for a non-enantiostylous population all flowers on a plant can potentially donate and receive pollen from one another

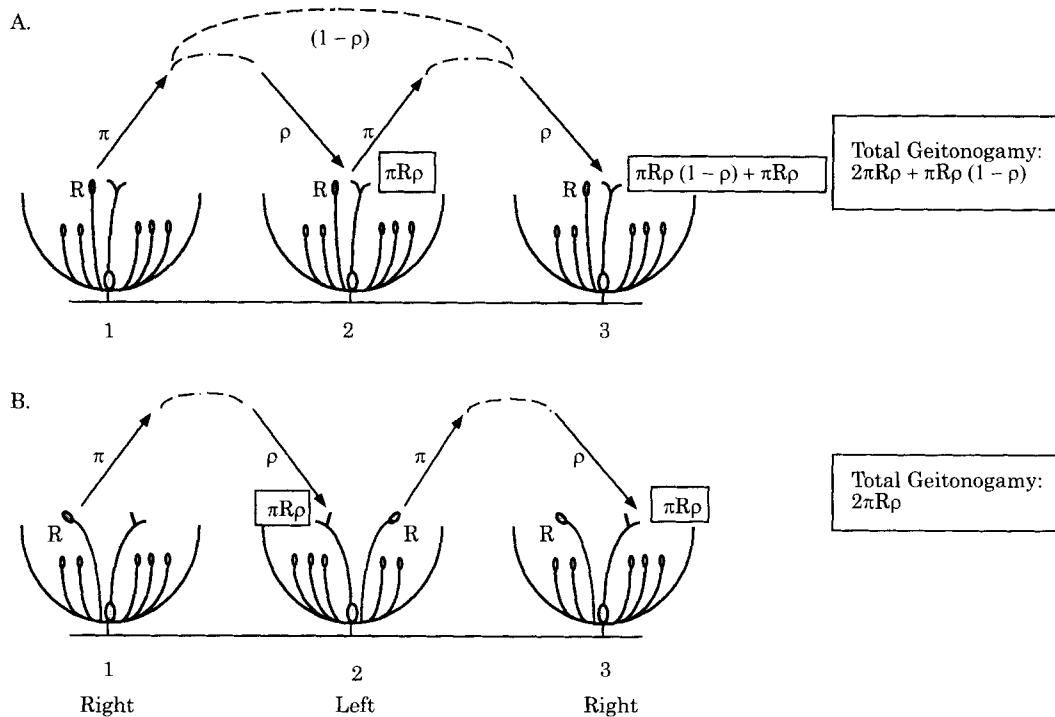


FIG. 6. An example of geitonogamous pollen transfer on a three-flowered plant. Levels of geitonogamy for each flower are indicated in boxes. Total geitonogamy is the sum of geitonogamy experienced by each flower. A, A non-enantiostylous plant; B, a monomorphically enantiostylous plant. R , Number of pollen grains removed from each flower; π , proportion of pollen which is deposited on the pollinator's body; and ρ , proportion of pollen on a pollinator's body that is deposited onto the stigma. We assume for this example there is no intrafloral pollen transfer. In monomorphically enantiostylous plants, the sequence of visitation to left- and right-styled flowers will vary. While this will change the final equation, geitonogamy in a monomorphically enantiostylous plant will always be less than a non-enantiostylous plant.

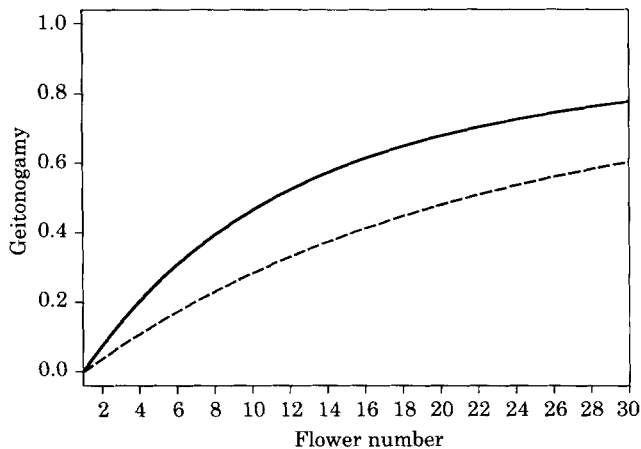


FIG. 7. The relation of flower number with the proportion of pollen grains produced by a plant which are deposited geitonogamously for a non-enantiostylous plant (—) and a monomorphically enantiostylous plant (---). $\pi R = 1000$, $\rho = 0.15$. We assume the proportion of left- and right-styled flowers on a monomorphically enantiostylous plant is 0.5, and there is no intrafloral selfing. (Jesson and Barrett, unpubl. res.)

and hence levels of geitonogamy are likely to be greater. Obviously, if this depiction of pollen transfer is correct then dimorphic enantiostyly should further reduce levels of geitonogamy in comparison with monomorphic enantiostyly. It was presumably this contrast that earlier workers had in mind when considering the apparent maladaptiveness of monomorphic enantiostyly in 'promoting' geitonogamy. However, as discussed above, this problem is resolved by recognizing that monomorphic enantiostyly has most probably evolved from a non-enantiostylous ancestor with straight styles.

Experimental studies

We have begun to employ floral manipulations to evaluate these ideas concerning geitonogamous pollen dispersal and its effects on mating patterns in enantiostylous plants. For example, by using genetic markers and experimental arrays of the bee-pollinated, monomorphically enantiostylous *Monochoria korsakovii* (Pontederiaceae) we demonstrated that geitonogamous selfing rates were two- to three-times higher in inflorescences with mixtures of left- and right-styled flowers, in comparison with similar-sized manipulated inflorescences containing flowers with only one style orientation (Barrett *et al.*, 2000). This result is consistent with the hypothesis that dimorphic enantiostyly reduces levels of geitonogamous self-pollination to a greater extent than monomorphic enantiostyly.

We have also conducted other manipulative experiments under field conditions in South Africa using several enantiostylous species of Haemodoraceae (Jesson and Barrett, unpubl. res.). Here the approach has been to compare levels of geitonogamous pollination between different floral designs by staining pollen with dyes injected into anthers just before anthesis (see Peakall, 1989 for method). All but one focal flower within an inflorescence is stained and the number of stained pollen grains transferred

to stigmas of the focal flower at the end of each day recorded. We contrasted geitonogamous pollination in non-enantiostylous *Dilatis pillansii* with the monomorphically enantiostylous *D. corymbosa*. After controlling for inflorescence size, our results revealed higher levels of geitonogamous pollination in *D. pillansii* than *D. corymbosa*, although the difference we obtained fell short of statistical significance (adjusted mean pollen deposition: *D. pillansii* = 12.62, s.e. = 0.20; *D. corymbosa* = 9.81, s.e. = 0.75; Poisson regression $P = 0.19$).

One of the problems with this type of comparison is that the species we compared differ in several floral traits, in addition to style condition, that may influence pollen dispersal patterns (e.g. perianth morphology, inflorescence architecture). To avoid the confounding problem of species differences we have recently attempted to simulate a straight-styled condition in *D. corymbosa* by altering style orientation using cotton thread tethers and comparing levels of geitonogamous pollination between this condition and non-manipulated inflorescences. While these types of floral manipulations are challenging to perform, particularly under field conditions, we believe that in the absence of genetic variants for style condition they are necessary for field testing of hypotheses concerning the functional basis of enantiostyly.

Why is dimorphic enantiostyly so rare?

The final issue that we address here is why dimorphic enantiostyly is so rare in flowering plants. Only a few evolutionary transitions from monomorphic to dimorphic enantiostyly are evident in the monocotyledons. The rarity of dimorphic enantiostyly is, at first, puzzling because of the functional benefits in reducing geitonogamy that the polymorphism would appear to bring over monomorphic enantiostyly. Moreover, since both left and right-styled flowers exist in monomorphic enantiostyly no new morphogenetic capability is required to produce the dimorphic condition only genetic fixation of the alternate states. In some ways this problem shares similarities with the evolution of dioecy from monoecy. The ancestral state involves plants producing two types of flowers (female and male) and in the derived condition the two flower types are segregated on different plants. Renner and Ricklefs (1995) have argued that one of the reasons that dioecy apparently evolves via this route so commonly is because the developmental machinery necessary to produce the dimorphic condition is already present in the ancestral state. Clearly in the case of dimorphic enantiostyly there must be more involved than this or else evolutionary transitions would occur more often.

In species with small daily inflorescence displays, the strength of selection to reduce geitonogamy will be weak or non-existent and hence dimorphic enantiostyly may serve little functional benefit. This is most obvious in the case of solitary-flowered plants. With monomorphic enantiostylous flowers could be left- or right-styled through random accidents of development but as long as style orientation occurred at around a 1:1 ratio at the population level there would be no added benefits to genetic fixation. This

situation is evident in *Heteranthera limosa* (Pontederiaceae) which produces a single flower per shoot each day and whether it is left- or right-styled is apparently random. In contrast, the advantages of dimorphic enantiostyly in reducing geitonogamous pollination will be most apparent in species with large daily floral displays. It is perhaps no coincidence that dimorphically enantiostylous *Wachendorfia thrysiflora*, *W. paniculata* and *Heteranthera multiflora* have large floral displays. Comparative analysis of floral display size in enantiostylous groups and their non-enantiostylous relatives would be valuable for exploring these ideas further.

Another possible constraint on the evolution of dimorphic enantiostyly is that there is little heritable variation for the amount of styler orientation within a plant. Our observations of the production of left- and righted-styled flowers in glasshouse populations of several enantiostylous species (e.g. *Monochoria korsakovii*, *Heteranthera mexicana*, *Solanum rostratum*) have not revealed individuals with biased styler ratios. Most plants produce close to equal proportions of left and right-styled flowers. In *M. korsakovii*, these are randomly produced with regard to developmental position within an inflorescence (Fig. 8). Lack of heritable variation for style orientation would strongly constrain selection for entirely left- or right-styled morphs. Elsewhere, attempts to select on the direction of asymmetry in *Drosophila* have proven difficult (Maynard *et al.*, 1960; Coyne, 1987; Tuinstra *et al.*, 1990). It is possible that genes providing the appropriate positional information to distinguish both ventral from dorsal and left from right sides of the flower may be particularly difficult to assemble, especially in species with radially symmetric flowers (see Coen and Meyerowitz, 1991).

DISCUSSION

The four styler polymorphisms we recognize all involve discrete variation in style positioning within the flowers of animal-pollinated angiosperms. In all heterostylous, and most enantiostylous species, this variation covaries with some form of stamen-position polymorphism. Among heterostylous plants the degree of polymorphism in style length is usually more pronounced than that of stamens (Lloyd and Webb, 1992a). In species with stigma-height dimorphism stamen differentiation is generally absent (although see Baker *et al.*, 2000a). To our knowledge no species is known in which stamens are polymorphic in position but styles are of uniform length or orientation. These findings raise the question of why the contrasting patterns of variation in female vs. male sexual organs should occur?

In species with styler polymorphisms anther position may be under stronger stabilizing selection than stigma height. Variants arising with altered stamen position may be more strongly selected against than those with novel stigma heights. This could be because variation in stigma height has less influence on female fertility than any corresponding variation in anther height has on male fertility. This interpretation is consistent with the view that in outcrossing species selection on male function typically

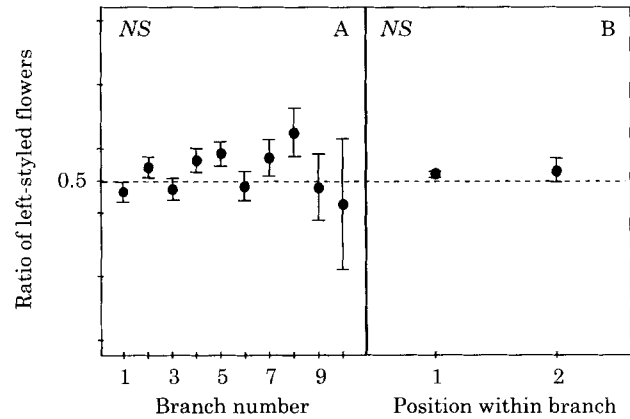


FIG. 8. The relation between branch number (A), and position on a branch (B) for left- and right-styled flowers of *Monochoria korsakovii* (Pontederiaceae), a monomorphically enantiostylous species. Plants were grown under glasshouse conditions ($n = 70$) and the style orientation of all flowers were recorded daily. Logistic ANOVA indicated no significant position effects with respect to flower type.

plays a more significant role in floral design than female function (Bell, 1985; but see Wilson *et al.*, 1994). From a developmental perspective changes in style length through cell division and elongation may be relatively easy to achieve; on the other hand alterations in anther height, particularly in tubular flowers with epipetalous stamens, may be more difficult.

In all four styler polymorphisms populations with equal frequencies of the style morphs are reported. The occurrence of equal morph frequencies is indicative of a balanced polymorphism maintained by frequency-dependent selection. The primary mechanism that appears to maintain the polymorphisms is disassortative mating, the higher frequency of intermorph than intramorph mating. Although experimental data are only available for heterostylous species, it seems reasonable to suggest that more proficient pollinator-mediated cross-pollination between morphs is largely responsible for this pattern of non-random mating. The evolution of styler polymorphisms may resolve the conflict between the problems of precision in pollen transfer between plants and the avoidance of self-interference. Increased fitness returns through male fertility over what could be achieved by floral monomorphism may provide the conditions required for the invasion of populations by styler variants. If this interpretation is correct then a single adaptive explanation based on frequency-dependent male mating proficiency can explain the evolution and maintenance of all four styler polymorphisms in plants.

We know little about the factors responsible for the evolution of one type of styler polymorphism over another in a given lineage. Transitions between heterostylous conditions and between stigma-height dimorphism and distyly are predicted on theoretical grounds (Charlesworth, 1979; Charlesworth and Charlesworth, 1979; Lloyd and Webb, 1992a, b; Richards, 1998), and there is empirical evidence to indicate that such changes in styler condition can indeed occur. In contrast, the evolution of enantiostyly involves

independent origins, presumably because this syndrome is composed of a range of floral traits that are not usually found associated with the style-length polymorphisms (e.g. nectarless, buzz-pollinated flowers).

In the Pontederiaceae, one of the few families in which both heterostyly and enantiostyly co-occur, phylogenetic evidence indicates separate origins for the two polymorphisms in association with quite different pollination syndromes (Graham and Barrett, 1995; Kohn *et al.*, 1996). However, even among the enantiostylous species in this family the polymorphism is not homologous with separate origins likely in the *Heteranthera* and *Monochoria* clades. Why this small family is particularly prone to the origination of styler polymorphisms is unclear, but the possibility of two separate transitions from monomorphic to dimorphic enantiostyly in *Heteranthera* and *Monochoria*, in addition to the well studied build-up and breakdown of tristily (see Barrett, 1988; Kohn *et al.*, 1996), make this group particularly valuable for studies of the evolution of styler polymorphisms.

This review has not dealt with styler polymorphisms involving stigmatic structure (*Armeria*: Baker, 1966), styler colouration (*Eichhornia*: Barrett, 1977) or styler hooking (*Jasminum*: Thompson and Dommée, unpubl. res.) all of which are reported from heterostylous groups. But do other polymorphisms exist that involve the relative positioning of sex-organs in animal-pollinated flowers? Recent studies of *Hemimeris* (Scrophulariaceae) in the Cape Province of South Africa indicate a novel styler polymorphism involving the reciprocal placement of styles and stamens in a vertical plane. The two floral morphs that occur in populations differ in whether styles or stamens occur at the top or bottom of the zygomorphic flowers of this insect-pollinated species (Steiner and Pauw, pers. comm.). How the polymorphism functions is not known but the reciprocal positioning of sex organs certainly suggests parallels with several of the styler polymorphisms discussed here. Increased appreciation of how floral designs can reduce self interference and promote more proficient pollen dispersal among plants will probably reveal other floral mechanisms that have evolved to serve this role.

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