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The genetics of mirror-image flowers

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Conspicuous asymmetries in forms that are polymorphic within a species can be genetically or environmentally determined. Here, we present a genetic analysis of the inheritance of dimorphic enantiostyly, a sexual polymorphism in which all flowers on a plant have styles that are consistently deflected either to the left or the right side of the floral axis. Using *Heteranthera multiflora* (Pontederiaceae), a short-lived herb, we conducted crosses within and between left- and right-styled plants and scored progeny ratios of the style morphs in F_1, F_2 and F_3 generations. Crosses conducted in the parental generation between morphs or right-styled plants resulted in right-styled progeny, whereas crosses between left-styled plants resulted in left-styled progeny. When putative heterozygous F_1 plants were selfed, the resulting F_2 segregation ratios were not significantly different from a 3 : 1 ratio for right- and left-styled plants. Crosses between left- and right-styled plants in the F_2 generation yielded F_3 progeny with either a 1 : 1 ratio of left- and right-styled plants or right-styled progeny. Our results are consistent with a model in which a single Mendelian locus with two alleles, with the right-styled allele (*R*) dominant to the left-styled allele (*r*), governs stylar deflection. The simple inheritance of dimorphic enantiostyly has implications for the evolution and maintenance of this unusual sexual polymorphism.

Keywords: asymmetry; enantiostyly; *Heteranthera*; major gene; stylar polymorphisms

1. INTRODUCTION

Conspicuous left–right asymmetries in morphological structures are a widely recognized feature of many organisms and have evolved multiple times (Palmer 1996). Two broad types of morphological asymmetries are generally distinguished. In species with directional asymmetry, the asymmetry in most individuals is fixed towards the same side. Conversely, antisymmetrical species usually have equal frequencies of 'right-handed' (dextral) and 'left-handed' (sinistral) individuals. The consistent bias to one side found in directional asymmetry indicates determination by an internal, genetic trigger, whereas in antisymmetry the trigger is thought to be most often environmentally determined (Palmer 1996). There are a few known cases in which a population or species is polymorphic for asymmetry and yet the direction of the asymmetry is genetically inherited. For example, in snails that are polymorphic for coiling direction, one or two (tightly linked) maternally inherited genes control the direction of coiling, with the dominant allele either dextral or sinistral, depending on the species (Murray & Clarke 1976; Asami *et al.* 1998). In a small freshwater goby, *Rhinogobius flumineus*, the direction of distortion of mouth opening is controlled by a one-locus two-allele system, in which dextrality is dominant over sinistrality, and the homozygous dominant genotype is lethal (Seki *et al.* 2000).

Polymorphisms in conspicuous left–right asymmetries are not well understood in plants (Endress 2001). In species with spiral phyllotaxis, successive vegetative or floral organs are offset in a way that creates a left or right chirality, with equal frequencies of both forms usually found (Hudson 2000). Studies of leaf positioning in tobacco and *Arabidopsis thaliana* have found no evidence for a genetic basis for spiral phyllotaxis, suggesting that the control of direction results from random accidents of development (Allard 1945; Hudson 2000). In some angiosperm species, petals of flowers or floral buds are contorted to the left or to the right of the main axis. Left- and right-contorted structures can be found on the same individual, or fixed to one direction in entire species, genera or families (Endress 1999, 2001). However, examples where this asymmetry is fixed for an individual, but is polymorphic within a species are unknown.

Enantiostyly is an asymmetry polymorphism involving the reproductive structures of angiosperm flowers. The female sex organ (style) is deflected to the left (left styled) or to the right (right styled) of the floral axis and a reciprocally positioned pollinating anther is commonly located on the opposite side of the flower to the deflected style. This arrangement results in 'mirror-image flowers' and these have been known for over a century although their evolution and functional significance is still poorly understood (Todd 1882; Knuth 1906; Ornduff & Dulberger 1978; Dulberger 1981; Fenster 1995; Jesson & Barrett 2002*a*). Left- and right-styled flowers can occur on the same individual or on separate individuals, and these two conditions are referred to as 'monomorphic' and 'dimorphic' enantiostyly, respectively, with individual species exhibiting a single condition only (Barrett *et al.* 2000). Dimorphic enantiostyly is considerably less common than monomorphic enantiostyly, and is reported from only seven species in three monocotyledonous families (Haemodoraceae, Jesson & Barrett 2002*b*; Pontederiaceae, this study; Tecophilaeaceae, Dulberger & Ornduff 1980). By contrast, monomorphic enantiostyly occurs in at least 10 families, including both monocotyledons and dicotyledons (reviewed in Jesson 2002). The contrasting frequency and systematic distribution of the two types of enantiostyly imply differing constraints on their evolutionary origins.

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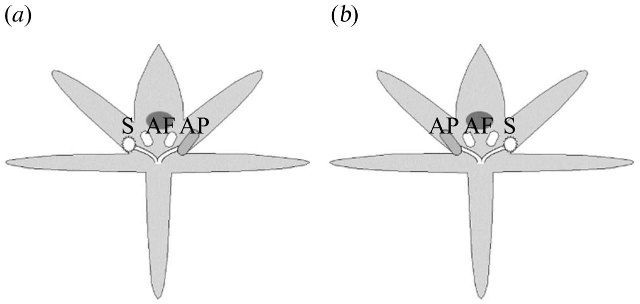

Figure 1. Flowers of (*a*) the left- and (*b*) the right-styled morphs of *Heteranthera multiflora* illustrating the position of the sexual organs. The styles (S) are deflected to the left or right side of the flower and a pollinating anther (AP) is positioned reciprocally in the opposite position. Two feeding anthers (AF) are located below a centrally positioned nectar guide.

Here, we investigate the inheritance of dimorphic enantiostyly in *Heteranthera multiflora* (Pontederiaceae), a short-lived herb that occurs in ephemeral aquatic habitats from South America to the central USA (Horn 1985). We chose *H. multiflora* because, in contrast to all other taxa with dimorphic enantiostyly, this species is relatively short lived and is easily cultured and crossed. Moreover, populations of this species are often fixed (monomorphic) for a single-style deflection. Because style deflection is a stable feature of these populations, it suggested to us that it may have a genetic basis, rather than being solely the result of environmental influences. Stylar monomorphism also enabled us to begin our genetic analyses with homozygous material of the two style morphs, thus ensuring the production of heterozygotes in crosses between parental genotypes. Our study addressed two principal questions: (i) is the direction of stylar deflection under genetic control in *H. multiflora*; and (ii) if so, what is the pattern of inheritance of the left- and right-styled morphs in this species?

2. METHODS

(a) *Floral biology*

Plants of *H. multiflora* are self-compatible and regeneration each year is predominantly from seed. The small, pale purple flowers open after noon, and field and glasshouse observations indicated that anthers often dehisce prior to this time (L. K. Jesson, personal observation). Inflorescences submerged in water are completely cleistogamous and never open (Horn 1985; L. K. Jesson, personal observation). These observations indicate that at least in some populations of *H. multiflora,* self-pollination occurs. However, like many enantiostylous species, *H. multiflora* has one cryptically coloured blue anther, 'pollinating anther' and two brightly coloured yellow anthers, 'feeding anthers'. The pollinating anther is positioned in a reciprocal position to the style, on the opposite side of the floral axis (figure 1). To characterize the left- and right-styled morphs, we measured using digital calipers the distance between the floral axis and the stigma and pollinating anther on one flower from each of 30 plants of each morph from the material examined below.

(b) *Sampling*

We collected seed families of parental material for the crossing programme from five populations (A–E) in the vicinity of Poplar Bluff, Missouri, USA in August 1997. All populations were located at least 10 km from each other. Populations were growing in rice fields or irrigation ditches, with the exception of population E, which was from a natural slough. All populations were fixed for one style direction or the other, with the exception of population A, which had a morph ratio of 90% right-styled. Two families from each population were sown and used as parental material for the crossing programme. Segregation of both style morphs occurred in two seed families from population A, all other seed families bred true for the direction of style deflection.

(c) *Crosses*

We performed crosses in a pollinator-free glasshouse at the University of Toronto. Flowers were individually tagged and fruits were collected at maturity after two to three weeks. We stored seed for at least six weeks prior to sowing to obtain high germination rates. Because under glasshouse conditions at Toronto, *H. multiflora* selfs prior to anthesis, we emasculated the maternal parent the day before flower opening and checked the stigma for pollen grains. To further ensure complete cross-fertilization, we used polymorphism for the enzyme 6-phosphogluconate dehydrogenase (PGD) to identify morph-specific markers in the parental material. We conducted crosses between individuals that were homozygous for different *PGD* alleles. The genotypes of F_1 progeny were checked to ensure that progeny from crosses were heterozygous for *PGD*. Because most parental families from a population were fixed for stylar deflection, all crosses between style morphs involved different populations.

We performed hand-crosses within and between morphs of the parental material using each morph as both a maternal and paternal parent. Flowers on the parental material that were not crossed were self-fertilized. We then selfed progeny from the F_1 generation to examine segregation in the F_2 generation. To produce an F_3 generation, we then crossed left- and right-styled F_2 plants. We tested F_2 segregation against a 3 : 1 model of Mendelian expectation for a single diallelic locus with dominance using *G*-tests (Sokal & Rohlf 1995). Segregation patterns from F_3 progeny were likewise tested against a 1 : 1 model.

To verify the stability of style deflection we scored all flowers on at least one inflorescence of all progeny. Style deflection on a second inflorescence was scored for 106 plants, and 55 plants were scored for three or more inflorescences. We found no evidence that plants produced inflorescences of opposite style deflection although some plants (4.1% (from 242 plants)) had occasional flowers deflected in the opposite direction to the majority of flowers on a plant. The factor(s) responsible for this variation are not known but the instability may result from accidents of development and/or the variable penetrance and expressivity of alleles governing stylar deflection.

3. RESULTS

(a) *Stylar dimorphism*

Measurements of the distance from the floral axis to both the stigma and the pollinating anther demonstrate that individuals of *H. multiflora* are readily characterized into one of two distinct style morphs (figure 2). Left-styled phenotypes have a style deflected to the left of the floral axis and a pollinating anther deflected to the right of the floral axis. The situation is reversed in the right-styled

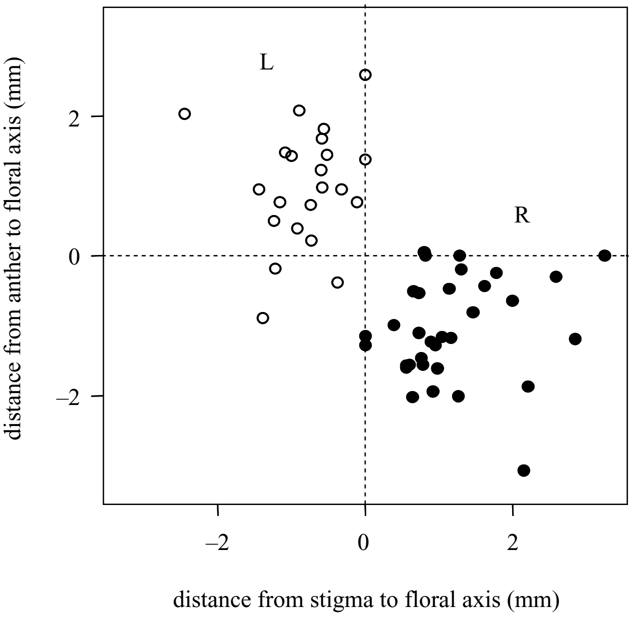

Figure 2. Floral measurements (mm) of the distance from the stigma and pollinating anther to the floral axis in left-styled (open circles) and right-styled (closed circles) morphs of *Heteranthera multiflora*.

morph. There were no instances involving flowers with no deflection of both sexual organs from the floral axis.

(b) *Progeny ratios*

Progeny ratios in F_1, F_2 and F_3 families were consistent with a model in which style deflection is governed by a single Mendelian locus with the right-styled allele (*R*) dominant to the left-styled allele (*r*). All selfs (five families, $n = 142$ progeny) or intramorph crosses (two families, $n = 95$ progeny) of left- or right-styled plants in the parental generation bred true whereas intermorph crosses (eight families, $n = 242$ progeny) produced only right-styled plants. All F_2 segregations conformed to the expected 3 : 1 ratio of right- to left-styled plants (table 1). Two patterns of segregation were obtained among intermorph crosses of F_2 plants depending on whether right-styled plants were putatively homozygous (*RR*: one family, $n = 15$ progeny, all right styled) or heterozygous (*Rr*: two families, $n = 43$ progeny, $G_{\text{het}} = 0.533$, $p = 0.46$, deviation from 1 : 1, $G_{\text{pooled}} = 0.611$, $p = 0.43$).

4. DISCUSSION

Unlike most other polymorphic left-right asymmetries, our results indicate that the direction in which styles are deflected in *H. multiflora* is not randomly determined but is instead controlled by a simple Mendelian system of inheritance. Segregation ratios of style morphs in progeny from controlled crosses indicate that dimorphic enantiostyly in *H. multiflora* is governed by a single locus with two alleles with the right-styled allele (*R*) dominant to the left-styled allele (*r*). Our study represents the first demonstration, to our knowledge, of the genetics of dimorphic enantiostyly and is the only example that we are aware of involving a 'gene for handedness' in plants.

Table 1. Segregation of left- and right-styled plants of *Heteranthera multiflora* in the F_2 generation following self-fertilization of the F_1 obtained by crossing left- and right-styled plants. (Replicated goodness-of-fit tests were not significantly different from the expected ratio of 3 : 1 for F_2 segregations. Heterogeneity $G_{\text{het}} = 1.273$, $p = 0.93$; deviation from 3 : 1, $G_{\text{pooled}} = 0.139$, $p = 0.71$.)

cross number	parental cross	F_2 proportion left	right	number of progeny	G
1	C1-3L x E1-8R	0.26	0.74	34	0.038
2	C2-1L x B2-10R	0.27	0.73	62	0.189
3	C2-9L x B2-1R	0.22	0.78	27	0.114
4	E1-7R x C2-1L	0.22	0.78	81	0.342
5	C2-5L x E1-8R	0.17	0.83	18	0.728
6	E1-8R x C1-1L	0.25	0.75	20	0
	total	0.24	0.76	242	1.41

Major genes also govern the inheritance of other types of stylar polymorphism (e.g. heterostyly; see Charlesworth & Charlesworth 1979; Lewis & Jones 1992). With single-locus control of stylar polymorphism and equivalent levels of disassortative mating between the style morphs a 1 : 1 ratio would be predicted in natural populations. This expectation has been confirmed for many heterostylous species (reviewed in Barrett 1992). The only extensive survey of style-morph ratios in enantiostylous species also revealed a pattern consistent with this expectation (Jesson & Barrett 2002*b*). All 24 populations of the predominantly outcrossing *Wachendorfia paniculata* (Haemodoraceae), contained left- and right-styled plants in frequencies that were not significantly different from equality. This suggests that enantiostyly is also simply inherited in *W. paniculata* and functions to promote cross-pollination between the two floral forms. Experimental studies using pollen dyes and marker genes have provided evidence that enantiostyly promotes cross-pollination between flowers of opposite style deflection in a manner functionally analogous to heterostyly (Bowers 1975; Barrett *et al.* 2000; Jesson & Barrett 2002*a*).

Several features of the life history and floral biology of *H. multiflora* in Missouri, USA, suggest that populations are not predominantly outcrossing, but instead experience considerable selfing. In these populations enantiostyly may not function effectively as a mechanism promoting cross-pollination. Populations occur in weedy habitats and are commonly monomorphic for style orientation. Colonization events involving a single style morph followed by selfing best explain the pattern of style-morph structure we observed. Indeed, a similar pattern of stylar monomorphism also occurs in *Wachendorfia parviflora*, a species with dimorphic enantiostyly, small flowers and high selfing rates (Jesson & Barrett 2002*b*).

Under field conditions heterozygous genotypes (*Rr*) of *H. multiflora* will only be produced in dimorphic populations where crossing between the style morphs occurs. The rate of outcrossing in *H. multiflora* is not known, but two segregating families were detected in a single population we sampled indicating some degree of outcrossing. Further investigations of *H. multiflora*, particularly in the neotropics, would be valuable to examine the pollination and mating biology of populations and the relative frequency of stylar monomorphism versus dimorphism. The maintenance of pollinating and feeding anthers in *H. multiflora*, traits commonly associated with the enantiostylous floral syndrome (Jesson 2002), implies that significant pollinator-mediated cross-pollination may occur in some populations of this species.

Among the hundreds of plants of *H. multiflora* we surveyed in our field and glasshouse studies, we observed no obvious recombinants in which the style and pollinating anther were deflected in the same direction. Although one gene may be responsible for the reciprocal stigma and anther deflection, it is also possible that two genes are involved with the gene controlling anther position tightly linked to the gene determining style position. This would be analogous to the genetic control of distyly, where genes governing a suite of traits including style length and anther position are inherited together as a single linkage group in a Mendelian fashion. Recombination at this heterostyly 'supergene' can give rise to homostylous phenotypes with similar style and anther positions (Lewis & Jones 1992).

The simple Mendelian inheritance of dimorphic enantiostyly in *H. multiflora* has implications for the evolution of this polymorphism. Comparative evidence reviewed elsewhere (Barrett *et al.* 2000; Jesson 2002), strongly suggests that in the three monocotyledonous families with dimorphic enantiostyly the polymorphism is derived from monomorphic enantiostyly. *Heteranthera multiflora* is the only species in the Pontederiaceae with dimorphic enantiostyly whereas monomorphic enantiostyly occurs in at least a dozen species of *Heteranthera* and *Monochoria* (Graham & Barrett 1995; S. C. H. Barrett, unpublished data).

The transition from monomorphic to dimorphic enantiostyly could potentially occur through either of two contrasting evolutionary pathways. In the first, disruptive selection on the direction of stylar deflection could result in plants fixed for opposite style orientations. This assumes that heritable genetic variation in the ratios of left- and right-styled flowers occurs in ancestral populations with monomorphic enantiostyly. Alternatively, the origin and spread of a mutation of large effect that fixed the direction of stylar deflection on a plant could give rise to one of the two style morphs, but a second mutation would be required to produce the opposite style morph. Given that we have demonstrated major gene control of dimorphic enantiostyly, the latter scenario seems more plausible to us. However, two separate origins of plants fixed for styles in opposite directions seems likely to be rather infrequent and this may help explain, in part, the extreme rarity of dimorphic enantiostyly in the angiosperms.

The rarity of dimorphic enantiostyly may also arise from constraints associated with a lack of positional information required to establish a left–right axis in plants. The molecular and developmental mechanisms of left–right determination in organisms are largely unknown (Palmer 1996). Moreover, the evolution of asymmetrical forms can be limited in some groups by a lack of heritable variation in the direction of asymmetry (Maynard Smith & Sondhi 1960; Coyne 1987; Tuinstra *et al.* 1990). For a style to be deflected in a consistent direction away from the mid-plane of a flower, the left–right axis of all flowers must be consistently orientated with respect to apical–basal and dorsi–ventral axes. Some signal that differentiates left from right of the mid-plane is also required. In vertebrates, the role of monocilia in early embryonic development has been implicated as the trigger establishing a left–right axis (Capdevila *et al.* 2000). However, mechanisms breaking symmetry in plants are more obscure and many asymmetries are thought to result from random accidents during development and have no genetic control (Hudson 2000). Molecular and developmental characterization of the enantiostyly locus could provide an opportunity to determine how plants 'tell left from right' and to integrate studies of genes, development, morphology and fitness.

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