

The Evolution and Adaptive Significance of Heterostyly

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The origin and adaptive significance of heterostylous breeding systems have fascinated evolutionary biologists since Darwin's early work on the subject. Models of the evolution of heterostyly differ in the emphasis given to different selective forces and in the sequence in which the physiological and morphological components of the polymorphism are thought to arise. Recent field studies of the population biology of heterostylous plants provide support for Darwin's hypothesis that the style-stamen polymorphism promotes disassortative pollination among the floral morphs.

Heterostyly is a genetic polymorphism in which plant populations are composed of two (distyly) or three (tristyly) morphs that differ reciprocally in the heights at which stigmas and anthers are positioned in flowers (Fig. 1). The style-stamen polymorphism is usually accompanied by a sporophytically

controlled, diallelic self-incompatibility system that prevents self and intramorph fertilizations, and by a suite of ancillary morphological features including polymorphisms of stigmatic papilla length, pollen size and the number of pollen grains produced. Heterostyly is reported from approximately 25 angiosperm families and is usually viewed as a floral mechanism that promotes outcrossing.

The problem of the origin and adaptive significance of heterostyly has fascinated evolutionary biologists since Darwin¹ summarized work on the morphological features and breeding relations of heterostylous plants. Studies on heterostyly in this century can be conveniently divided into two phases. The first, lasting until the 1960s, was largely genetical; it was conducted mostly in Europe, and made use of the polymorphism as a model system for studies of mendelian genetics, linkage, supergenes and various frequency-dependent phenomena. This period was dominated by the work of the geneticists

Bateson², Fisher and Mather³, Lewis⁴ and Ernst⁵, and by biosystematic studies by Baker⁶. Most genetic investigations were conducted under garden or glasshouse conditions and involved a small number of herbaceous heterostylous taxa (e.g. *Primula*, *Lythrum*, *Oxalis*, *Linum*)⁷.

Over the past two decades, work on heterostyly has entered a new phase. This has involved a diversification of approaches, with particular emphasis on field studies of the population biology of heterostylous plants⁸ and on the development of theoretical models of the evolution and breakdown of the polymorphism^{9,10}. In addition to population-level studies, work on the structural, developmental and physiological aspects of heteromorphic incompatibility has also provided new insights into the organization of characters making up the syndrome and into the nature of pollen-pistil interactions in heterostylous plants^{11,12}. Recent work has involved a broader range of heterostylous taxa and several new patterns and phenomena have been discovered^{13,14}, with implications for problems concerned with the origin of heterostylous genetic polymorphisms.

Here I review current evidence on two questions concerned with the evolution of heterostyly: how did

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heterostyly originate, and what selective forces maintain the polymorphism?

Origin of heterostyly

The origin of heterostyly is a difficult problem because of the paucity of phylogenetic data on the evolutionary development of the polymorphism in extant plant lineages. Apart from Baker's studies in the Plumbaginaceae⁶, suggesting the build-up of distyly in several steps beginning with incompatibility, few close relatives of heterostylous species display patterns of floral variation that can unambiguously be interpreted as the polymorphism *in statu nascendi*. Despite contrary views¹⁵, there is little genetic or taxonomic evidence that homomorphic and heteromorphic systems of sporophytic self incompatibility are often phylogenetically related¹⁶. Heterostyly is clearly polyphyletic in origin and, with few exceptions, the taxonomic groups in which it occurs do not contain taxa with homomorphic incompatibility¹⁷. The recent claim of multiallelic and diallelic incompatibility within the distylous aquatic genus *Villarsia* is therefore of considerable evolutionary interest¹⁴. If the two systems of incompatibility do indeed occur within this small Australian genus, it would provide an unusual opportunity to investigate their evolutionary relationships.

Recent investigations of pollen-pistil interactions in self-incompatible plants support a separate origin for heteromorphic incompatibility. Studies of pollen tube growth indicate that the nature and sites of inhibition of incompatible pollen tubes are fundamentally different between the two types of sporophytic incompatibility. In heterostylous plants, the sites where cessation of growth occurs usually differ between the morphs and can involve the stigma, style or ovary¹⁸. These patterns contrast with those observed for homomorphic sporophytic systems, where inhibition is usually restricted to the stigmatic surface and involves specific *S* gene recognition factors originating in the anther wall¹⁹. While such factors cannot be entirely ruled out for heterostylous species, there is evidence that heteromorphic characters themselves may play a direct role in mediating incompatibility responses²⁰.

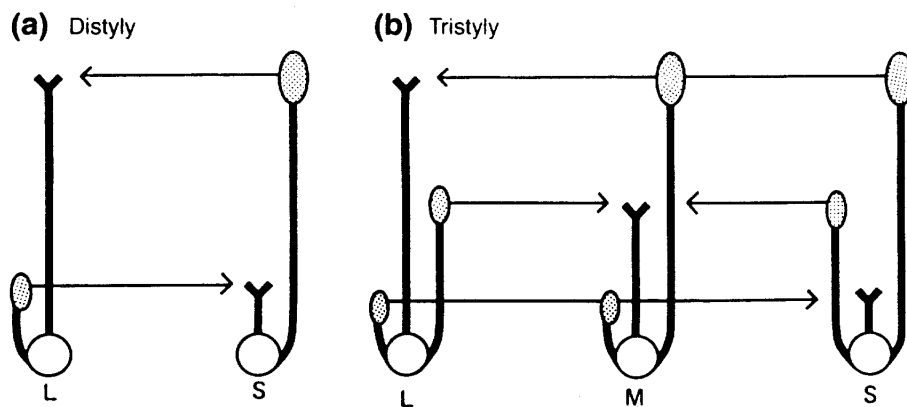


Fig. 1. The heterostylous genetic polymorphisms distyly and tristyly. Legitimate (compatible) pollinations are indicated by the arrows; all other pollen-pistil combinations result in little or no seed set. L, M and S refer to the long-, mid- and short-styled morphs, respectively. (a) Distyly is controlled by a single locus with two alleles. The L morph is usually of genotype *ss* and the S morph *Ss*. (b) In tristyly, the most common mode of inheritance involves two diallelic loci (*S* and *M*), with *S* epistatic to *M*.

Molecular techniques are eventually likely to resolve problems of the phylogenetic relationships between different self-incompatibility systems. Until then, however, the existing data do not provide strong support for a strictly conserved *S* gene for all sporophytic systems¹⁶ or a monophyletic origin for self incompatibility in the angiosperms²¹.

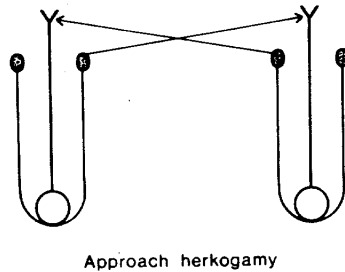
The heterostylous syndrome consists of three associated sets of traits: reciprocal herkogamy (herkogamy is the spatial separation of anthers and stigma within a flower), self and intramorph incompatibility, and an array of ancillary floral polymorphisms. Several hypotheses have been formulated concerning the sequence in which these characters have evolved. Mather and de Winton²² proposed that the morphological and physiological components of heterostyly arose simultaneously; more recently, Dulberger¹¹ suggested that differential growth of styles and stamens could be involved in synthesis of incompatibility proteins. However, the occurrence of diallelic incompatibility in the herb *Armeria*⁹, which is monomorphic for style and stamen length, casts doubt on the simultaneous origin hypothesis. Despite the absence of sound phylogenetic data for the family, comparative information from *Armeria* and related taxa in the Plumbaginaceae has frequently been used as evidence that establishment of diallelic incompatibility precedes the evolution of reciprocal herkogamy in heterostylous plants^{8,9}. This view has received widespread support from modern workers, who have generally argued that prevention of self fertilization – the selective force

most often invoked to account for the evolution of heterostyly – is more likely to be achieved by self incompatibility than through reciprocal herkogamy acting alone.

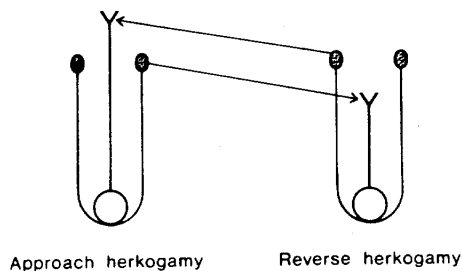
Charlesworth and Charlesworth⁹ provided the first quantitative model of the evolution of heterostyly. The model involves establishment of diallelic self incompatibility, through selection for inbreeding avoidance, followed by evolution of reciprocal herkogamy and associated floral polymorphisms, to increase the efficiency of pollen transfer between the incompatibility groups. The initial stages in the model involve a functionally gynodioecious stage (gynodioecy is the occurrence of hermaphrodite and male plants within a population) in which a pollen-sterile mutant spreads if the product of the selfing rate (*s*) and inbreeding depression (δ) exceeds 0.5. The ancestral condition in the model is a self-compatible phenotype with anthers and stigmas at the same level. This poses problems for the model since, by analogy with homostylous descendants of heterostylous plants, it seems likely that this phenotype would be largely autogamous. If this were true, populations would be unlikely to maintain a sufficient genetic load of deleterious recessives to satisfy the constraint that $s\delta > 0.5$.

Ganders⁸ argued that the ancestral condition is more likely to involve a self-compatible monomorphic population with anthers and stigmas separated. This floral morphology usually promotes outcrossing, and populations possessing it would be more likely to carry a significant genetic load. He

1. Ancestor



2. Stigma-height Polymorphism



3. Reciprocal Herkogamy

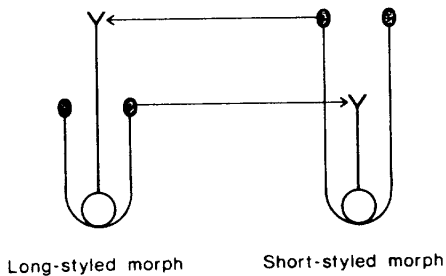


Fig. 2. The principal stages in the evolution of reciprocal herkogamy according to the model of Lloyd and Webb²¹. Arrows indicate the directions of most proficient pollen transfer. After Ref. 24.

suggested that a change in pollinators, perhaps associated with a bottleneck or colonizing event, could cause a large increase in the selfing rate, resulting in high inbreeding depression. Under these conditions, and where $s\delta > 0.5$, the series of events postulated by Charlesworth and Charlesworth⁹ could lead to the evolution of diallelic self incompatibility. Following this scenario, once incompatibility becomes established, style- and stamen-length mutants linked to the incompatibility locus are selected, because they favour compatible pollen transfer between the two mating types and reduce the rate of illegitimate pollination. While this general model has been unchallenged for the past decade, little empirical evidence that bears directly upon the

postulated sequence of events has been obtained, although several demonstrations of inbreeding depression in natural populations of self-compatible outcrossing plants have been made²³. Unfortunately, since the functionally gynodioecious stage postulated in the model is not associated with observable morphological changes, it is unlikely to be detected in natural populations.

Recently, Lloyd and Webb^{24,25} have challenged the widely held view that diallelic self incompatibility preceded the evolution of reciprocal herkogamy. Instead, they revive Darwin's¹ original suggestion that reciprocal herkogamy evolved first through selection to increase the efficiency of pollen transfer between morphs (Fig. 2). According to their models, once a stamen–style polymorphism establishes, self incompatibility develops by gradual adjustment of pollen tube growth to the different styler environments of the morphs.

Lloyd and Webb's ideas are based on several assumptions: (1) the ancestors of heterostylous plants exhibited approach herkogamy (stigmas above and separated from anthers); (2) the initial step in the evolution of reciprocal herkogamy was the evolution of a stigma-height polymorphism; and (3) heteromorphic self incompatibility is fundamentally distinct and has a separate origin from homomorphic self incompatibility. The first of these seems reasonable since approach herkogamy is common in outcrossing self-compatible plants, and only minor genetic and developmental changes would be required to establish a style-length variant. As discussed above, studies of the general properties of homomorphic and heteromorphic incompatibility systems also suggest independent origins – a view also shared by D. Charlesworth¹⁶. The major points of disagreement between the two models therefore centre on whether reciprocal herkogamy precedes the evolution of self incompatibility or vice versa, and whether the initial step could have involved a stigma-height polymorphism.

Charlesworth and Charlesworth⁹ found that a polymorphism for stigma height was difficult to maintain and concluded that the evol-

ution of heterostyly in a self-compatible species was unlikely to be initiated by a change in stigma position. In their models they mainly considered how changes in stigma height would affect pollen transfer and female fertility. Lloyd and Webb²⁵ focus instead on how such a change would influence pollen donation and male fertility. In their models the initial spread of a reverse herkogamous mutant is favoured because its male fitness is higher than that of the average approach herkogamous plant. Why this should be so is not obvious, but it may occur because the mutant's female organs interfere less with male donation than in approach herkogamous plants. As the mutant spreads, owing to its frequency-dependent mating advantage, levels of disassortative pollination between the floral morphs increase, with selection for more efficient pollen donation resulting in subsequent changes in anther height. While it is well known that even low levels of disassortative mating result in polymorphic equilibria in self-compatible heterostylous populations²⁶, it is less clear a simple change in stigma height is likely to give rise to this pattern of mating.

Critical testing of the Lloyd and Webb model therefore requires information on the influence of stigma-height variation on disassortative mating and patterns of male fertility. Few non-heterostylous species are known with well-developed stigma-height polymorphisms⁹, and it may be that experimental manipulation of the floral morphology of heterostylous and homostylous groups will provide the best opportunities for testing these ideas.

Lloyd and Webb's models^{24,25} differ from those of the Charlesworths⁹ in being largely non-genetic and emphasizing aspects of floral biology. Nonetheless, they appear to provide explanations for a number of genetic phenomena that have up to now puzzled workers in the field. Apart from two notable exceptions (*Limonium*⁶ and *Hypericum*²⁷), all work on the inheritance of heterostyly has demonstrated dominance of the S morph over the L morph²⁸. If the ancestral condition was approach herkogamy (L morph), as Lloyd and Webb suggest, it would

be considerably easier for a reverse herkogamous mutant (S morph) to invade the population (for the well-known population genetic reasons first elaborated by Fisher²⁹) if this phenotype were governed by a dominant rather than a recessive allele. It is worth noting, however, that this explanation is only relevant for fully outcrossing populations, since with partial selfing the opportunities for the spread of recessive alleles are increased considerably. A second feature of the genetic data, in accord with the models, is the failure to detect recombination between loci controlling stylar incompatibility and style length in the *Primula* supergene¹⁰. If incompatibility was based largely on pollen responses to different stylar environments, rather than on synthesis of specific stylar incompatibility specificities, an additional gene governing stylar incompatibility reactions would not be expected.

Studies of pollen tube growth and seed set patterns in illegitimate pollinations of tristylous *Pontederia*^{18,30,31} have given results that are consistent with the hypothesis that incompatibility in heterostylous plants operates through failure of pollen tubes to grow in particular stylar environments, rather than from recognition of shared pollen and style specificities. The cessation in growth of illegitimate pollen tubes in *Pontederia* depends on the particular combination of pollen size and style length that is employed (Fig. 3). Illegitimate pollinations, with pollen smaller than the legitimate size class, result in pollen tubes that terminate growth at different distances in the style without reaching the ovary. Where pollen larger than the legitimate size class is used, two responses are observed. In the case of the M morph, ovules are commonly fertilized. In the S morph, however, pollen tubes enter the ovary but grow past the micropyle, perhaps because they fail to respond to an ovular signal that apparently causes legitimate pollen tubes to grow in a directional manner towards the ovule, resulting in fertilization. The absence in *Pontederia* of a unitary rejection response, as occurs in homomorphic systems, and the contrasting behaviours of different combinations

of pollen size and style length, suggest that incompatibility reactions may have evolved separately in the style morphs and that they do not share matching recognition factors in the pollen and style.

Adaptive significance of heterostyly

What is the evidence for Darwin's original suggestion that reciprocal herkogamy actively promotes legitimate pollination (cross-pollination between anthers and stigmas of equivalent height in the floral morphs) in heterostylous populations? Two kinds of experimental data have been collected to assess the hypothesis; both employ the conspicuous size heteromorphism of pollen produced by different stamen levels in heterostylous plants. Studies of pollen deposition on pollinating insects (mostly bees) have provided evidence for the segregation of pollen types in both distylous³² and tristylous species³³. The limited data provide some support for the idea that during feeding, pollen is deposited on different regions of a pollinator's body. Many factors result in disturbance of pollen after its initial placement on pollinators, however, and it is of more critical importance to determine whether deposition of pollen on stigmas supports Darwin's hypothesis.

Over the past 15 years, approximately 20 field studies on a wide range of heterostylous taxa have compared the amounts of legitimate pollen on naturally pollinated

stigmas of the morphs with expectations based on random pollination⁸. This procedure involves determination of the proportion of pollen types in the population using estimates of the frequency and flower production of morphs. The majority of these 'pollen flow studies' have been interpreted as providing either no evidence or only weak evidence in support of the Darwinian hypothesis. Observed pollen loads were frequently consistent with the expectations of random pollination, or in some cases involved an excess of illegitimate pollen on stigmas³⁴.

Unfortunately, most pollen flow studies have employed inappropriate experimental designs to assess the adaptive significance of heterostyly. To evaluate properly whether reciprocal herkogamy promotes legitimate pollination, the intraflower illegitimate component of the pollen load should be removed by emasculation^{8,25}. Emasculation is necessary because pollen transfer between anthers and stigmas within a flower is unaffected by the polymorphism, and this component of the pollen load obscures the critical parameter to be measured, i.e. the illegitimate outcrossed fraction of the pollen load. In the only two studies where this procedure has been undertaken on all morphs – in the distylous herb *Jepsonia heterandra*³⁵ and tristylous aquatic *Pontederia cordata*³⁶ – experimental support for the Darwinian hypothesis was obtained. Pollen loads were composed of an excess of

A. Pollen Tube Growth

| | | PISTIL | | |
|--------|---|--------|------------|------------|
| | | L | M | S |
| POLLEN | i | 13.7 | 7.2 | 1.7 |
| | m | 7.9 | 7.6 | 1.7 |
| | s | 1.1 | 1.0 | 1.3 |

B. Seed Set

| | | PISTIL | | |
|--------|---|-------------|-------------|-------------|
| | | L | M | S |
| POLLEN | i | 81.2 | 72.6 | 7.4 |
| | m | 11.1 | 91.8 | 27.5 |
| | s | 0.7 | 16.4 | 78.4 |

Fig. 3. Pollen–pistil interactions in self-incompatible, tristylous *Pontederia sagittata*. (A) Mean distance (mm) at which pollen tubes terminate growth in the style, following controlled pollinations. (B) Mean percentage seed set. Values in bold type are the legitimate combinations. For details see Refs 30 and 31.

legitimate pollen over that expected from random pollination.

Recently, Lloyd and Webb²⁵ have developed a novel way of analysing pollen deposition patterns in heterostylous populations. By comparing legitimate and illegitimate pollen transfer from the perspective of pollen donation and pollen receipt, data can be converted into probabilities of a single pollen grain of each type being deposited on stigmas of each morph. Using this method, they reanalysed pollen load data for *Jepsonia* and *Pontederia*. The results obtained for the two species were in remarkable accord, and provide strong support for the Darwinian hypothesis. In both taxa the average proficiencies of legitimate transfer and receipt were approximately twice those of the corresponding illegitimate combinations. More experimental studies, employing emasculation and the new analytical techniques, are required to determine if the patterns observed in *Jepsonia* and *Pontederia* are of general significance.

Another way of evaluating Darwin's hypothesis involves measuring mating patterns in *self-compatible* heterostylous populations. Progeny tests and the use of genetic markers can be employed to estimate outcrossing rates and, more importantly, levels of disassortative (intermorph) mating. This approach has recently been undertaken in two tristylous populations of *Eichhornia paniculata*³⁷⁻³⁹, a close relative of the infamous weed water hyacinth *E. crassipes*. High rates of outcrossing were detected in each population. Furthermore, estimates of disassortative mating indicate that most outcrossing is between the style morphs, with low levels of assortative (intra-morph) mating in each population.

Care is required in interpreting this kind of data, however, since other reproductive factors unrelated to reciprocal herkogamy (e.g. differences in the growth rate of legitimate over illegitimate pollen tubes, or the selective abortion of illegitimate offspring) could also play a role in promoting disassortative mating. Interestingly, however, the abortion of developing embryos in *E. paniculata* is very low (< 5%) and is therefore unlikely to contribute significantly to post-zygotic control of mating patterns⁴⁰. In addition,

preliminary marker gene studies of pollen mixtures have failed to detect differences between the siring ability of illegitimate or legitimate outcross pollen³⁷. If post-pollination interactions do not contribute significantly to maintaining high levels of disassortative mating in *E. paniculata*, the most reasonable alternative explanation is that reciprocal herkogamy is effective at promoting intermorph pollinations in the species. However, since the marker gene approach cannot be used to distinguish legitimate from illegitimate intermorph matings in tristylous populations³⁸, the data provide only partial support for the Darwinian hypothesis. Further support can only be obtained from pollen load studies in species with conspicuous pollen-size heteromorphism.

In conclusion, for the only two species for which there are adequate data to test Darwin's hypothesis properly, a twofold difference was found between the proficiency of legitimate compared to illegitimate pollen transfer. These results from *Jepsonia* and *Pontederia* are encouraging, and represent a strong advantage compared to the results of most other field studies of genetic polymorphisms⁴¹. Far from there being little direct evidence in favour of the Darwinian hypothesis, as previous workers have generally concluded, few selection hypotheses involving polymorphic traits can claim this degree of support²⁵. While there is obviously much more to learn about heterostyly, particularly with respect to its origin and spread, recent theoretical developments and the availability of a broader range of experimental systems provides workers in the field with exciting new challenges for investigating the evolution and adaptive significance of these complex floral polymorphisms.

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