Genetic control of self-incompatibility and reproductive development in flowering plants

10. Incompatibility in heterostylous plants

SPENCER C.H. BARRETT and MITCHELL B. CRUZAN*
*Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 3B2; * Present address: Department of Botany, University of Tennessee, Knoxville, TN 37996, U.S.A.*

1. Introduction

Self-incompatible flowering plants are often classified into two major categories, depending on whether mating types in populations are morphologically alike or unlike (Fisher and Mather 1943; Lewis 1949; de Nettancourt 1977; Gibbs 1986; Barrett 1988a). In species with homomorphic incompatibility, populations are composed of numerous mating types identical in appearance with incompatibility under the control of one or more S-loci with multiple alleles. In contrast, only two (distyly) or three (tristyly) mating types occur in populations of species with heteromorphic incompatibility; these can be readily distinguished by differences in the position of reproductive parts within flowers (Fig. 1). The two classes of incompatibility differ in their distribution and abundance among angiosperm families. Homomorphic incompatibility is widely distributed with estimates of up to fifty percent of all angiosperms possessing this type of incompatibility (Darlington and Mather 1949; Brewbaker 1957). Heteromorphic incompatibility, on the other hand, is reliably reported from only 25 families and in many of these its occurrence is restricted to a small number of genera (Ganders 1979a; Lloyd and Webb 1992a). The kinds of research conducted on homomorphic and heteromorphic incompatibility systems have taken different courses, reflecting fundamental differences between the two systems in their general properties and use as experimental systems.

As the contents of this volume indicate, much of the work on homomorphic incompatibility has focussed on understanding the physiological, biochemical, cellular and molecular basis of the incompatibility mechanism. In addition, because of the economic importance of many species with this form of incompatibility as crop plants and ornamentals, considerable effort has been directed to overcoming or modifying the expression of incompatibility to facilitate various breeding objectives. Since mating types in homomorphic systems can only be detected by extensive crossing programmes (e.g. Lawrence and O'Donnell 1981), relatively little is known about the population biology and evolution of homomorphic incompatibility, despite theoretical interest in
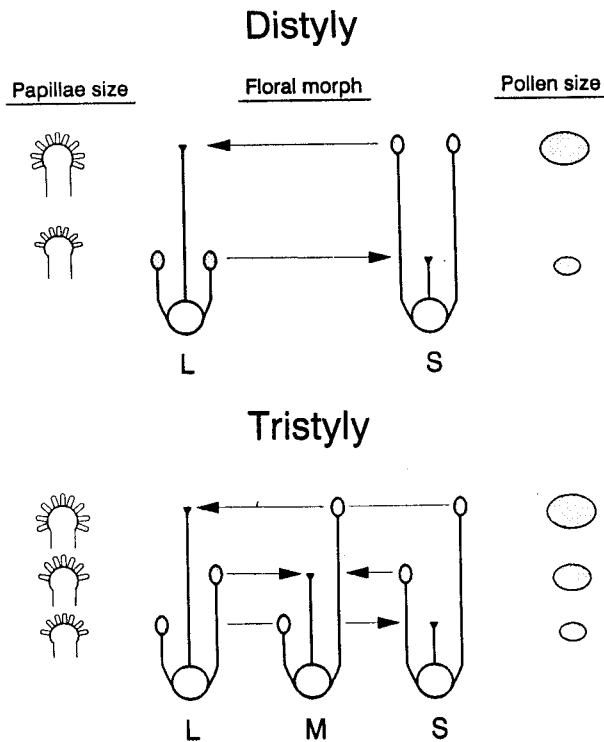
Distyly

| Papillae size | Floral morph | Pollen size |

L   S

Tristyly

L   M   S

Fig. 1. The heterostylous genetic polymorphisms distyly and tristyly. Legitimate (compatible) pollinations are indicated by the arrows; all other pollen-pistil combinations are termed illegitimate and usually yield little or no seed set. In distyly there are two floral morphs whereas in tristyly there are three. L, M, and S refer to the long-, mid-, and short-styled morphs, respectively. Accompanying the reciprocal differences in stigma and anther height in the floral morphs are commonly ancillary polymorphisms of pollen and stigmas. Pollen size and stigmatic papillae length usually increase with stamen height and style length, respectively.

this area (Bateman 1952a; Charlesworth 1988; Uyenoyama 1988a,b,c,d, 1989). Quite a different emphasis is evident in studies of heteromorphic incompatibility, which following Darwin's early lead (Darwin 1877), has largely focussed on evolutionary questions. Because mating types are easily identified under field conditions, much work has been devoted to studies of the genetics and ecology of natural populations in an effort to understand the evolution, adaptive significance and breakdown of the heterostylous syndrome (Vuilleumier 1967; Ganders 1979a; Barrett 1990). In comparison with homomorphic incompatibility, far less is known about the physiological and biochemical basis of incompatibility in heterostylous plants, and as yet, there have been no attempts to investigate the molecular basis of the polymorphism.

Several outstanding issues need to be resolved before a comprehensive understanding of the evolution of heterostyly can be obtained. Incompatibility

is tightly associated with morphological differences between the mating types. This raises the question of whether the polymorphic features of the pistils and pollen of the floral morphs participate directly in the mechanism of incompatibility, or alternatively, whether incompatibility operates independently of morphology, in a manner similar to that found in homomorphic systems. The coupling of morphological and physiological traits also leads to the question of how these characters were assembled during the course of evolution and whether any clear priority is evident in the sequence in which traits became established. A related problem is whether the same selective forces are responsible for the evolution and maintenance of both the morphological and physiological polymorphisms, or whether these traits have entirely separate functions. A wide variety of opinions have been expressed on the evolution and function of heteromorphic incompatibility (reviewed in Barrett 1992a); a major goal of this chapter is to assess the current status in our understanding of some of the issues raised above. To provide the necessary background we begin by outlining the essential features of pollen-pistil interactions in heterostylous plants, using data on the patterns of pollen tube growth and seed set following controlled pollinations. This information is used to illustrate the wide range in expression of heteromorphic incompatibility among angiosperm families, a pattern consistent with the polyphyletic origin of the polymorphism.

2. Pollen-pistil interactions

The heterostylous syndrome is most commonly composed of three elements: 1) a reciprocal arrangement of stigma and anther heights in the floral morphs (hereafter reciprocal herkogamy); 2) a sporophytically-controlled diallelic incompatibility system that prevents self and intramorph fertilization; and 3) a set of ancillary morphological polymorphisms, particularly of the stigmas and pollen of the floral morphs. Through controlled pollinations of distylous and tristylous plants, Darwin (1877) established the essential features of the compatibility relationships of the floral morphs. Compatible pollinations are those that occur between stigmas and anthers of equivalent height (termed legitimate); the remaining pollinations result in reduced or no seed set (termed illegitimate). These relationships are illustrated for distylous and tristylous populations in Fig. 1.

2.1. Compatibility relationships

Controlled self, intramorph and intermorph pollinations have now been conducted on heterostylous taxa from a wide range of taxonomic groups. While the crossing relationships depicted in Fig. 1 are commonly revealed, variation in the strength of the incompatibility system has often been noted. This variation ranges from cases where morph-specific differences in seed set following illegitimate pollination are evident, to taxa in which all morphs set abundant

seed upon illegitimate pollination. In the latter case taxa have usually been described as self-compatible. Figure 2 illustrates the range of compatibility values found in distylous species based on a survey of the literature.
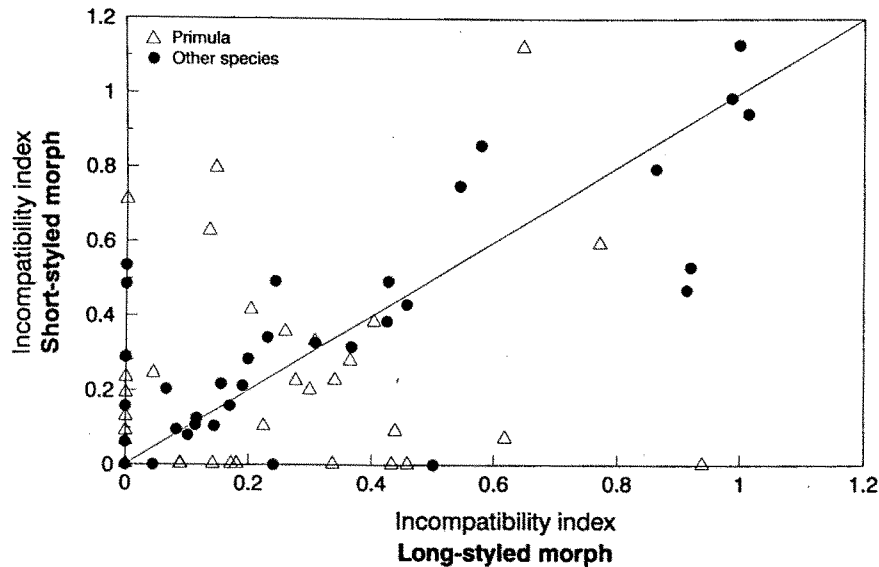

Fig. 2. The relationship between the strength of incompatibility in the long- and short-styled morphs of distylous species based on a literature survey. An index of the strength of incompatibility was obtained for each morph by dividing the mean fruit or seed set resulting from self-pollination (or where not available intramorph pollination) by the value from legitimate pollination. Values of 1 indicate highly self-compatible taxa, values of 0 indicate highly self-incompatible taxa. Data were square root transformed. Points lying on the straight line indicate species in which the strength of the incompatibility is equal in both morphs. Species of *Primula* ($N = 37$, data of Wedderburn and Richards 1990) are plotted separately as open triangles, from the remaining heterostylous species ($N = 54$). There is no significant difference in the number of heterostylous species in which the S morph is more or less incompatible than the L morph (Sign Test – *Primula* spp.: $Z = 0.88$, $p = 0.38$ *ns*; Other taxa: $Z = 1.01$, $p = 0.31$ *ns*) or in the mean difference in the strength of incompatibility between the L and S morphs (Paired *t* test – *Primula* spp.: $t = -0.424$, $p = 0.337$ *ns*; Other taxa: $t = 0.402$, $p = 0.925$ *ns*).

Lewis (1942) proposed that stronger incompatibility in the S morph compared to the L morph in *Primula* could be explained by selection to prevent pollen tubes from traversing the shorter styles of this morph, resulting in self-fertilization. He demonstrated that pollen from long-level stamens was more strongly inhibited than pollen from short-level stamens in illegitimate pollinations of *Primula obconica*. The survey of incompatibility expression in distylous species provides little support for the possibility that this hypothesis is of general significance. There was no greater tendency for the L morph to exhibit weaker incompatibility than the S morph among the sample of distylous species surveyed in Fig. 2 (and see Table 8, D, B. Charlesworth 1979).

Moreover, even among *Primula* spp. this pattern is not evident (see appendix in Wedderburn and Richards 1990).

Patterns of compatibility in tristylous plants are considerably more complex than in distylous species because each morph produces two pollen types differing in incompatibility phenotype and size. Intermorph pollinations that involve stigmas and anthers of different heights are illegitimate, along with all self and intramorph pollinations. Although less numerous, tristylous species appear to exhibit greater variation in the expression of incompatibility compared to distylous taxa. In particular, the M morph can display high levels of compatibility, particularly when pollinated with long-level pollen from either the M or S morphs (e.g. *Lythrum salicaria*, Darwin 1877; Stout 1923, and see Fig. 3).

Barrett and Anderson (1985) proposed a model based on the developmental heterogeneity of mid-level stamens in tristylous flowers to account for the weak incompatibility of the M morph in *Pontederia* spp. They suggested that the contrasting insertion patterns of mid-level stamens in the L and S morphs could give rise to biochemical differences between the two sources of mid-level pollen. If this were true, pistils of the M morph might exhibit a broader physiological range of compatibility, than in the L and S morphs, to facilitate acceptance of the two legitimate pollen types. This range may overlap with that involving pollen from illegitimate anther levels. While there is indeed evidence that mid-level pollen from the L and S morphs differ in incompatibility behaviour (reviewed in Barrett and Anderson 1985), as yet no data demonstrating biochemical differences among the pollen types is available for tristylous plants.

2.2. Incompatibility mechanisms

Pollen germination and pollen tube growth following legitimate and illegitimate pollinations have been studied in several heterostylous taxa, principally *Primula* spp. (Lewis 1942; Shivanna et al. 1981, 1983; Richards and Ibrahim 1982; Stevens and Murray 1982; Wedderburn and Richards 1990) and *Linum* spp. (Darwin 1877; Lewis 1942; Dulberger 1973, 1987; Baker 1975; Ghosh and Shivanna 1980; Murray 1986), but also members of the Boraginaceae (Schou and Philipp 1983; Weller and Ornduff 1989, 1991; Richards and Mitchell 1990), Lythraceae (Schoch-Bodmer 1942, 1945; Esser 1953; Tatebe 1964, Dulberger 1970a), Plumbaginaceae (Baker 1966; Dulberger 1975a), Pontederiaceae (Anderson and Barrett 1986; Scribailo and Barrett 1991a; Cruzan and Barrett 1993), and Rubiaceae (Bawa and Beach 1983; Murray 1990). Dulberger (1992) has recently undertaken a comprehensive review of work on pollen-pistil interactions in relation to floral polymorphisms in heterostylous plants, only a brief review is therefore given here.

Incompatibility responses in heterostylous species are normally determined by the relative position of stigmas and anthers (Fig. 1), rather than whether pollen is self or not. A range of different responses leading to illegitimate pollen failure have been reported including lack of adhesion, hydration, and

germination of pollen, inability of pollen tubes to penetrate the stigmatic zone, and cessation of pollen tube growth in the style and ovary (see Table 5 in Dulberger 1992). For many of the species studied failure at any one stage is often incomplete, with incompatibility resulting from a sequence of barriers, none of which alone is necessarily completely effective. This system of cumulative screening, resulting in a gradual attrititon of illegitimate pollen tubes, has been described as a 'cascade system' by Shivanna et al. (1981, 1983) and differs markedly from the unitary rejection responses often observed in homomorphic sporophytic systems. Not all heterostylous species, however, exhibit variable sites of pollen tube inhibition. In some *Linum* spp., for example, the rejection response is quite localised and occurs just below the stigma in both morphs (Murray 1986; Dulberger 1992).

The variation in incompatibility responses of most heterostylous species is well illustrated by a recent survey of pollen tube growth in *Primula* spp. by Wedderburn and Richards (1990). Of the 52 species they examined, pollen tube inhibition usually occurred at more than one site; when only one site was involved this was the stigma surface. In the majority of species investigated the inhibition sites differed between the morphs. Morph-specific differences in inhibition sites have also been reported in a survey of nine tropical distylous species of Rubiaceae by Bawa and Beach (1983). Inhibition most commonly occurred in the style of the L morph and in the stigmatic tissue of the S morph. This difference is commonly reported in other distylous taxa. In some species, however, including two in the Bawa and Beach survey, the sites of pollen tube inhibition are the same in both morphs. This also occurs in *Luculia gratissima* (Rubiaceae) with inhibition at the base of the stigma (Murray 1990). Interestingly, in this species stigmatic amputation followed by self-pollination of the floral morphs resulted in different incompatibility responses. In the L morph pollen tube growth was inhibited, whereas in the S morph pollen tubes showed normal rates of growth. Murray suggests that the reaction zone in the S morph of *L. gratissima* may be more localized than in the L morph. The floral morphs of heterostylous species have also been shown to differ in their response to bud pollination. While seed set is obtained following geitonogamous pollination of buds 24 h before anthesis in the S morph of distylous *Turnera ulmifolia*, this treatment is largely ineffective in producing selfed seed in the L morph (Shore and Barrett 1985).

In tristylous species the site of cessation of illegitimate pollen tubes can vary from the upper part of the style in all morphs as in *Oxalis* spp. (Weller 1975; Gibbs 1986) to a variety of sites depending on the specific pollen size-style length combination as in *Lythrum salicaria* (Esser 1953), *Lythrum junceum* (Dulberger 1970a) and *Pontederia* spp. (Anderson and Barrett 1986; Scribailo and Barrett 1991a). The occurrence of late-acting pre-zygotic incompatibility in the ovaries of the S morph of *Pontederia* appears to be unique to heterostylous plants with diallelic incompatibility. Ovarian inhibition of incompatible pollen tubes is, however, reported from heterostylous species with putative multiallelic incompatibility systems (see below).

The contrasting patterns of pollen tube growth and inhibition in the floral morphs of heterostylous species are usually associated with morph-specific differences in pollen size. This has led to the suggestion that size differences may be involved in the incompatibility mechanism. In legitimate pollinations of distylous species, pollen tubes of the larger pollen from long-level anthers often grow considerably faster than those of the smaller pollen from short-level anthers. In illegitimate pollinations, long-level pollen is usually inhibited earlier than short-level pollen. The physiological bases for these associations are not known. Furthermore, as recently pointed out by Dulberger (1992), in some heterostylous species (e.g. *Linum* spp. Lewis 1942; Dulberger 1973) differences in pollen tube growth occur *without* size dimorphisms of pollen, while in others (e.g. *Melochia tomentosa* Martin 1967; *Faramea* spp. Bawa and Beach 1983) pollen size dimorphism is associated with *similar* inhibition sites in the floral morphs. Dulberger (1992) has suggested that if the association between pollen size heteromorphism and differing inhibition sites has a functional basis, then neither the storage product nor the grain size proper are likely to be involved, although it must be strongly linked with morph-specific size or dry weight. She suggests that a protein or growth factor deriving from an early premeiotic stage of microsporogenesis, during differential growth of the sporogenous cells or the pollen mother cells, may be involved.

Other attempts to relate the incompatibility mechanisms of heterostylous species to interactions between heteromorphic characters have focussed primarily on pollen-stigma interactions. Early investigations by Lewis (1943) led him to propose that pollen germination in *Linum grandiflorum* was controlled by differences in the osmotic potential between the pollen and stigmas of the floral morphs. A more extensive survey of *Linum* spp. by Murray (1986) involving intra- and interspecific pollinations has, however, cast doubt on Lewis's original hypothesis. Although the stigmatic cells of the floral morphs of *Linum* species showed differences in osmotic pressure, the differences could not be used to predict the compatibility of crosses. Microscopic examination of stigma surfaces in *Linum* spp. (Dulberger 1987; Ghosh and Shivanna 1980) and several other heterostylous species (e.g. *Primula* spp. Heslop-Harrison et al. 1981; Schou 1984; *Pontederia* Scribailo and Barrett 1991b) have revealed differences in the amount of stigmatic exudate between the morphs. Since pollen types differ in their ability to take up atmospheric moisture (Shivanna et al. 1983), the availability of moisture on the stigmatic surface and ability of pollen to absorb it may contribute to illegitimate pollen failure in some heterostylous species. Other cases where interaction between pollen grains and the stigma surface appears to favour the adhesion and/or hydration of legitimate pollen are discussed in Dulberger (1975a, 1992), Mattsson (1983) and Richards and Mitchell (1990).

3. Variation in the expression of incompatibility

3.1. Genetic mechanisms

Genetic modifications to the incompatibility systems of heterostylous plants take several distinct forms. The most well known involve the origin of homostyles, through recombination in the supergene governing the heterostylous syndrome in distylous species (Ernst 1955; Dowrick 1956; B. and D. Charlesworth 1979; Shore and Barrett 1985; Lewis and Jones 1992). While homostyles are usually fully self-compatible, it is important to recognize that their pistils and pollen retain the expected incompatibility reactions for illegitimate pollinations when crossed to heterostylous morphs (Dowrick 1956; Baker 1975; Wedderburn and Richards 1992). Retention of incompatibility is particularly remarkable in homostylous forms of the *Turnera ulmifolia* complex since populations are reproductively as well as geographically isolated from heterostylous populations (Barrett and Shore 1987). Under these conditions it might be expected that mutations abolishing incompatibility would rapidly accumulate since incompatibility serves no function in self-compatible homostylous populations. The persistence of residual incompatibility may perhaps be explained by selection to maintain floral traits, if incompatibility is a pleiotropic effect of style and stamen growth.

A second way in which incompatibility can be altered is when modifier genes non-allelic to the heterostyly genes change morphological features of the syndrome. In *Primula sinensis* two genes, Primrose Queen and fertile double, are known which affect the relative heights of stigmas and anthers, respectively. Significantly, these changes are also accompanied by modifications to incompatibility. For example, shortening of the style in normally long-styled forms of Primrose Queen results in an incompatibility response intermediate between the L and S morph (De Winton and Haldane 1933; Beale 1939). These findings are consistent with the view that morphological factors play an important role in mediating the incompatibility mechanism in heterostylous plants (Mather and De Winton 1941; Dulberger 1975b, 1992).

Modifications to trimorphic incompatibility that accompany the evolution of distyly from tristyly in *Oxalis* spp. (reviewed by Weller 1992) provide additional evidence for the close association between morphological and physiological traits in heterostylous plants. In populations from which the M morph is absent, the incompatibility reactions of mid-level pollen produced by the L and S morphs become altered in opposite directions. Pollen from mid-level anthers of the L morph is compatible with stigmas of the S morph, whereas the corresponding pollen of the S morph is compatible with L stigmas. These changes are associated with an increase in 'mid-level' anther height in the S morph and a decrease in the L morph. As recently pointed out by Lloyd and Webb (1992a), these patterns provide evidence against the occurrence of a shared recognition system involving pollen and style specifities in heterostylous plants, as found in species with homomorphic incompatibility.

The most common form of incompatibility modification in heterostylous plants involves the occurrence of sporadic self-compatible variants. In these variants floral morphology is usually unchanged despite relaxation or complete loss of the incompatibility system. Controlled pollination programmes of heterostylous species often reveal such individuals, but rarely has the genetic basis of self-compatibility been investigated. In tristylous *Pontederia cordata*, Barrett and Anderson (1985) found a large amount of variation in the expression of trimorphic incompatibility among a sample of 36 clones from a natural population (Fig. 3). The use of clonal replicates provided evidence that some of this variation had a genetic basis. Controlled self-pollinations and the assessment of self-compatibility in parents and offspring suggested that overall differences between floral morphs in the expression of incompatibility was more likely to be due to the pleiotropic effects of major genes controlling tristyly than linked modifiers. In contrast, variation among clones within a morph appeared to be largely polygenic in origin.

Shore and Barrett (1986) reported genetic data from various self-compatible variants of the L and S morphs of *Turnera ulmifolia*. Through the use of reciprocal crosses they demonstrated that individual variants exhibited either null stylar activitity or aberrant pollen behaviour. In no instance was the genetic control of self-compatibility simple or the result of segregation at the distyly locus. Of particular interest was the finding that the strength of self-incompatibility was modified considerably in plants resulting from wide interpopulation crosses. This suggests that genetic background plays an important role in regulating the expression of the self-incompatibility reaction.

In the *Turnera* study only limited progress was made in selecting for increased self-compatibility. However, long-term artificial selection for self-compatibility in ornamental forms of the L morph of *Primula sinensis* during the past century has apparently led to a striking decrease in the strength of incompatibility (Table 7 in Mather and De Winton 1941). It has often been assumed that the high levels of self-compatibility found in some heterostylous species (Fig. 2) result from relaxation in self-incompatibility brought about by similar genetic mechanisms, but involving selection for reproductive assurance. However, it may not be safe to assume that a rigid strongly expressed incompatibility system necessarily represents an ancestral feature in most heterostylous taxa, as discussed more fully below.

3.2. Environmental influences

Virtually all experimental work on incompatibility expression in heterostylous species has been conducted under artificial conditions, most commonly in glasshouses or growth rooms. The extent to which the variation that is revealed has a direct bearing on the mating systems of natural populations has yet to be determined. It is possible that if the temperature and humidity conditions under experimental situations do not reflect those commonly experienced by plants in the field, the observed seed set values following illegitimate pollination may
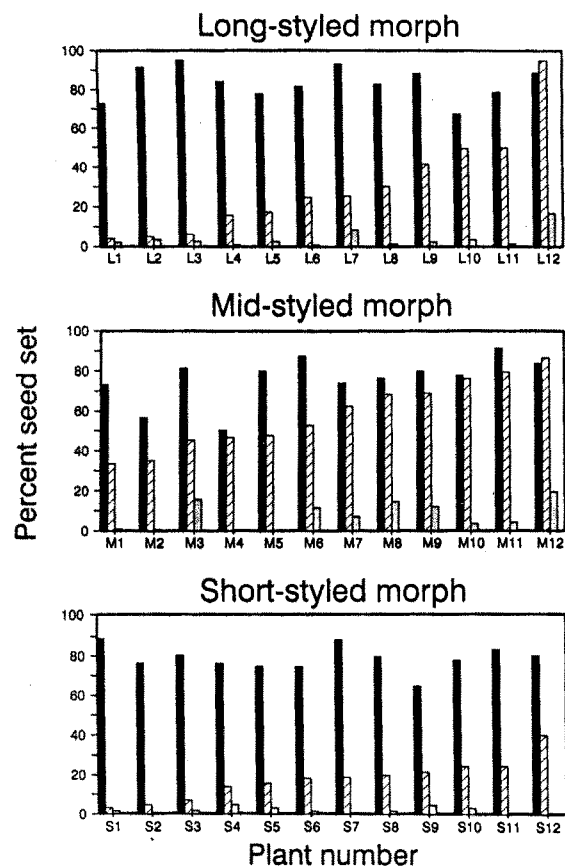
Fig. 3. Variation in the strength of trimorphic incompatibility in *Pontederia cordata* based on seed set data following controlled pollinations. A total of 36 clones (12 per morph) were sampled from a natural population and each clone was pollinated with three sources of pollen: legitimate pollen (shaded bars) and self pollen from the alternate anther levels within a flower. The most compatible anther levels (L morph – mid-level, M morph – long-level, S morph – mid-level) are indicated by hatched bars and the least compatible anther levels (L morph – short-level, M morph – short-level, S morph – long-level) by stippled bars. In addition to the within flower variation displayed by alternate anther levels, two basic patterns are evident in the strength of incompatibility : 1. between morph differences, with the M morph having the weakest incompatibility system; 2. wide variation in the strength of incompatibility among clones within a morph e.g. compare L1 versus L12. Note there is no association between the level of compatibility displayed by different anther levels within a plant. After Barrett and Anderson (1985).

have little ecological relevance. Lewis (1942) demonstrated temperature effects on legitimate and illegitimate pollen tube growth in distylous *Primula* spp. Studies on the effects of atmospheric humidity on the hydration and germination of pollen in illegitimate pollinations of *Primula* spp. also suggest that environmental effects may play a role in incompatibility expression in this genus

(Shivanna et al. 1983; Richards 1986). Clearly it would be of value to perform controlled hand pollinations of heterostylous plants in the field to establish whether the results obtained under artificial conditions are representative.

An additional consideration in assessing the significance of weak incompatibility for the mating system of populations concerns the size and composition of pollen loads in open-pollinated plants. Data in Fig. 2 mostly involve hand pollinations in which large quantities of illegitimate pollen usually from a single male donor are applied to stigmas. Under field conditions, however, stigmas most commonly capture smaller pollen loads composed of both legitimate and illegitimate pollen from many donors (e.g. Ganders 1979a; Barrett and Glover 1985; Piper and Charlesworth 1986). Since differences in the growth rate of pollen tubes between legitimate and illegitimate pollen commonly occur (see below), most ovules may still be fertilized by legitimate pollen despite weak incompatibility. Alternatively, in mixed pollinations it is possible that interactions between pollen tubes of different origins could occur. Faster growing legitimate pollen tubes could potentially improve conditions for illegitimate pollen tube growth, resulting in increased levels of illegitimate fertilization than would be predicted on the basis of single donor hand pollination. Murray (1990) found evidence for interactions between compatible and incompatible pollen tubes in mixed pollinations of *Luculia gratissima*. When compatible pollen was followed by incompatible pollen, the latter showed inflated pollen tube tips and a pattern of uncontrolled growth not observed when growing alone. This observation suggests that there may be a recognition of legitimate versus illegitimate which either stimulates or inhibits pollen tube growth (B.G. Murray, pers. comm.). Clearly, more studies of pollen-pistil interactions in heterostylous species using mixed pollinations are required. Mentor effects and the occurrence of 'cryptic self-fertility' in non-heterostylous species are discussed by Bertin and Sullivan (1988) but as yet such phenomena are largely unexplored in heterostylous species.

4. Evolution of heteromorphic incompatibility

4.1. Independent origins

Since heteromorphic incompatibility is under sporophytic control it might be expected to share features with homomorphic sporophytic incompatibility, including a common evolutionary origin. Despite persistent statements to the contrary (Crowe 1964; Beach and Kress 1980; Muenchow 1982; Wyatt 1983; Zavada 1984), however, there is considerable evidence indicating that the two systems of incompatibility are fundamentally distinct with independent origins. The arguments against an evolutionary relationship between heteromorphic and homomorphic incompatibility have been discussed in detail by several authors (e.g. D. and B. Charlesworth 1979; Ganders 1979a; Charlesworth 1982; Gibbs 1986; Barrett 1988a; Lloyd and Webb 1992a); only a brief summary is given here.

One of the major arguments in favour of a common origin for homomorphic and heteromorphic incompatibility was the idea that self-incompatibility arose only once in the angiosperms (Whitehouse 1950), with a strictly conserved *S*-locus present in all self-incompatible families. Not only is there little phylogenetic support for this proposition for homomorphic and heteromorphic sporophytic systems occur in different families (see Charlesworth 1985), but recent molecular evidence casts doubt on whether the *S*-locus even in gametophytic and sporophytic sytems of homomorphic incompatibility is evolutionarily homologous (Haring et al. 1990). The scattered taxonomic distribution of heteromorphic incompatibility strongly suggests that it is polyphyletic, with over 20 independent origins among angiosperm families (see Fig. 2 in Barrett 1992b). The diversity of origins has important implications for attempts to seek general mechanisms to account for incompatibility phenomena in heterostylous plants. It may be unwise to assume that the same physiological mechanisms govern incompatibility phenomena in all heterostylous species.

Studies of pollen-pistil interactions in heterostylous plants indicate that the properties of heteromorphic incompatibility are qualitatively distinct from those of homomorphic sporophytic incompatibility. As discussed above, in the former, multiple inhibition sites occur including the stigma, style and ovary, and these commonly differ between the floral morphs; whereas in the latter, inhibition on the stigma surface involving tapetal bound recognition factors, is the basis of the incompatibility mechanism. (Dickinson and Lewis 1973; Heslop-Harrison 1975). Other differences based on genetic characteristics of the two systems include the number of alleles maintained at the incompatibility 'loci', the nature of allelic interactions (e.g. dominance, independence, or competition), and the types of mutations modifying incompatibility (Charlesworth 1982; but see Shore and Barrett 1986). These distinctive features, combined with the close association between morphological polymorphisms and incompatibility in heterostylous plants and complete absence of such associations in homomorphic systems amount to a powerful case against a common origin for the two systems of incompatibility.

While the evidence against a common origin for heteromorphic and homomorphic incompatibility comes from diverse sources, workers have still sought to establish parallels between the molecular and physiological mechanisms governing incompatibility reactions in the two systems. For example, the difficulty of explaining how a sporophytically-controlled pollen reaction could occur in the style or ovary, rather than on the stigma surface, has been viewed as a particular problem in understanding the mechanisms of incompatibility in heterostylous plants (Richards 1986, p. 279). This concern presumably stems from the underlying assumption that pollen wall proteins, similar to those in homomorphic sporophytic incompatibility, play a similar role in heterostylous species. It is possible, however, that either mRNA of paternal origin could be translated for a considerable time after pollen germination (Stevens and Murray 1982), that pollen tube growth procedes after the reaction on the stigma has occurred, or that no inhibition actually occurs on

the stigma (Scribailo and Barrett 1991a). The broader issue of whether a protein-based recognition system with matching pollen and style specificities occurs in heterostylous species, as in homomorphic incompatibility, is therefore still an open question.

Based on a review of studies of stigma and style extracts in *Primula* spp. by Golynskaya et al. (1976), Shivanna et al. (1981), and Richards and Ibrahim (1982), Richards (1986, p. 280) proposed that up to six gene products may be involved in the incompatibility reaction, with different recognition proteins responsible for the multiple inhibition sites found in the floral morphs. However, the evidence to support this remarkable claim is weak at best. Stevens and Murray (1982) were unable to decide whether recognition factors or physiological differences between pollen tubes and pistils were responsible for the incompatibility reaction in *P. obconica*. They did conclude, however, that pollen wall proteins derived from the breakdown of the tapetum played no apparent role in the self-incompatibility reaction. Recently, Lloyd and Webb (1992a) have also questioned whether a mutual recognition system, based on molecular specifities expressed by *S*-gene products in the pollen and style, occurs in heterostylous species. They propose instead that incompatibility operates through the failure of each class of pollen tube (legitimate versus illegitimate) to grow in a particular stylar environment in a manner similar to that observed in certain inter-specific crosses (Hogenboom 1975; Williams and Rouse 1990). According to this view the incompatibility reactions found in a given morph develop separately *after* the evolution of reciprocal herkogamy and as a result need not share a common physiological or molecular basis. This perspective raises the critical issue of evolutionary priority and the functional interdependence of morphological and physiological traits in heterostylous species.

4.2. Evolutionary models

Darwin (1877, p. 265) viewed the evolution of incompatibility in heterostylous plants as an 'incidental and purposeless' result of disruptive selection for pollen tube growth in the style type to which pollen was most often transferred. He came to this conclusion after reasoning that any system that effectively prevents an individual from mating with a large segment of the population can hardly function as an outbreeding mechanism *per se*. Most modern workers, however, have interpreted heteromorphic incompatibility as an outbreeding device 'reinforced' by the morphological features of the polymorphism. For example, Vuilleumier (1967), while not favouring any particular order of priority, suggested that 'whichever system arose first was later *reinforced* by the other to produce more efficiency in outbreeding'. A problem with this perspective is that the term reinforcement conveys little meaning in functional terms. More recently, several workers (e.g. Yeo 1975; Ganders 1979a) have proposed that to understand the evolution of heterostyly it is necessary that the component parts of the syndrome are distinguished and attempts made to determine their

functional significance. Theoretical models can then be used to explore the types of selective forces that could lead to the evolution and maintenance of the polymorphisms.

The two principal groups of quantitative models of the evolution of heterostyly differ in the sequence in which the morphological and physiological components of the heterostylous syndrome are thought to arise (Fig. 4). In models of the evolution of distyly, developed by D. and B. Charlesworth (1979), diallelic incompatibility evolves first as a selfing avoidance mechanism, with inbreeding depression the selective agent. Reciprocal herkogamy then follows to promote efficient pollen transfer between the two mating types, hence reducing pollen wastage on incompatible stigmas. While the Charlesworths found that under some circumstances reciprocal herkogamy could establish without a preexisting incompatibility system they doubted 'whether any heterostyled species has evolved without an incompatibility system' (D. and B. Charlesworth 1979, p. 473). Most students of heterostyly have favoured the idea

1. Selfing Avoidance Model
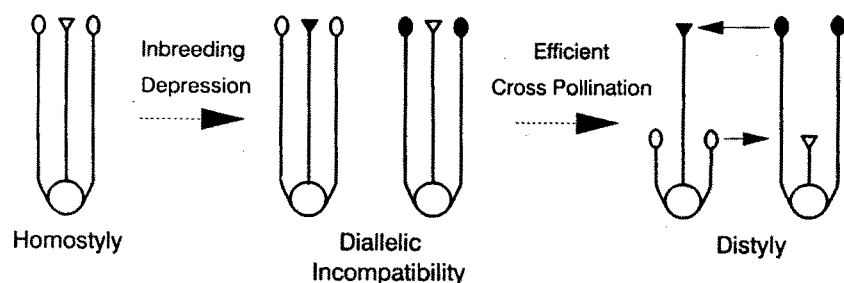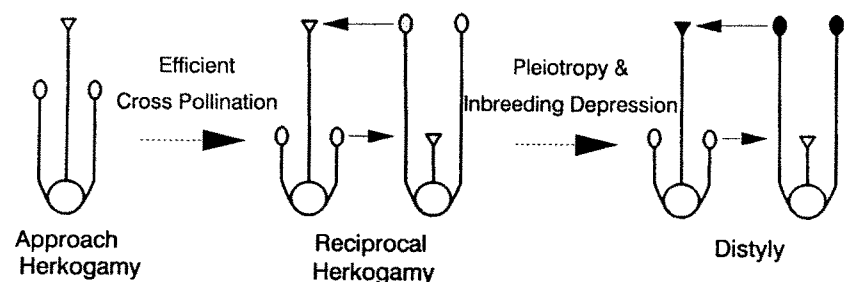

2. Pollen Transfer Model

Fig. 4. Major phenotypic stages in theoretical models of the evolution of distyly. The selfing avoidance and pollen transfer models follow D. and B. Charlesworth (1979) and Lloyd and Webb (1992b), respectively. Only the major stages for each model are shown for simplicity. Phenotypes with uniform pollen and stigmas are self-compatible whereas those with shaded pollen or stigmas exhibit diallelic incompatibility. The models differ in the ancestral phenotypes proposed, the sequence in which reciprocal herkogamy and diallelic incompatibility become established, and in the emphasis placed on various selective forces. After Barrett (1992b).

that incompatibility establishes before the style-stamen polymorphism (e.g. Bateman 1952a; Baker 1964; Yeo 1975; Ganders 1979a; Lewis 1982), although aside from Baker's (1966) studies of the Plumbaginaceae, there is little comparative data to support this sequence.

Lloyd and Webb (1992a,b) have recently resurrected the original proposal of Darwin (1877 pp. 260-8) on the evolution of morphological and physiological traits in heterostylous species: In their models of the evolution of distyly, they suggest that reciprocal herkogamy most often evolves prior to incompatibility to promote pollen transfer among plants, thus increasing fitness through male reproductive function. Diallelic incompatibility then develops secondarily, 'due to a combination of intramorph failures arising incidentally from specialization for legitimate pollination and active selection restricting self-fertilization' (Lloyd and Webb 1992a, p. 171). An important result of Lloyd and Webb's quantitative models is the finding that the presence of reciprocal herkogamy makes it considerably easier to maintain self-incompatibility because the mating restrictions imposed by a diallelic system are reduced considerably.

The most contentious aspect of the Lloyd and Webb model concerns whether a simple change in stigma height resulting in a stigma-height polymorphism could by itself give rise to significant levels of disassortative pollen transfer between the morphs. This idea was considered unlikely by the Charlesworths and there is now a need to investigate by experimental means whether the proposal has any validity. A recent study by J.L. Stone and J.D. Thomson (unpub. ms.) using artificial flowers and captive bumble bees demonstrated significant levels of disassortative pollen transfer between flowers with different stigma heights. The mechanism responsible for such an effect was not determined but presumably results from details of pollinator positioning within flowers, as suggested by Lloyd and Webb (1992b).

While in the Charlesworth's models diallelic incompatibility is largely a prerequisite for the evolution of reciprocal herkogamy, in the models of Lloyd and Webb the selection pressures invoked are independent of whether ancestral populations are self-compatible or self-incompatible. Another difference concerns the nature of the incompatibility systems that are likely to evolve. Diallelic incompatibility results from mutations with large effect at separate pollen and style loci in the Charlesworth's model. This implies that incompatibility is likely to be quite strong at the outset and is under major gene control. In contrast, incompatibility in the Lloyd and Webb model results, in part, from polygenic modification of the ability of pollen types to grow in styles of different length. Hence the strength of the incompatibility reaction is likely to increase gradually, depending on the transfer efficiency of pollen and deleterious effects of self-fertilization.

Less progress has been made on the theoretical aspects of the evolution of tristyly with only a single quantitative study (Charlesworth 1979). Because of the complexity of trimorphic incompatibility, particularly the occurrence of intrafloral incompatibility differentiation, it is more difficult to imagine how such a system could evolve prior to the establishment of morphological

differences among the mating types. Several scenarios were studied by Charlesworth, the most promising of which involved the assumption that incompatibility was a pleiotropic effect of genes governing style and stamen length. The possibility that in tristylous species a gradient in the expression of incompatibility could establish from differential organ growth is an attractive idea. It more readily explains intrafloral incompatibility differentiation and modifications to incompatibility associated with the evolution of distyly (discussed above), than a model based on fixed recognition specificities.

Observations of aberrant floral phenotypes in several tristylous species provide some support for the idea that the morphological and physiological systems in tristylous plants are connected physiologically, rather than through genetic linkage. Sved (1965) reported that a short-styled plant of *Oxalis pescaprae* growing under heat stress produced mid-styled flowers with altered compatibility relationships, as judged by crosses conducted with other plants. Similarly, in *Pontederia sagittata* some genotypes of the long-styled morph when grown under glasshouse conditions were found to produce a high frequency of modified flowers with shortened mid-length styles. Self-pollination of these normally self-incompatible flowers regularly produced seed (R.W. Scribailo and S.C.H. Barrett, unpubl. data). Interestingly, in the occasional distylous family with two stamen levels within a flower there is some evidence that pollen produced by the alternate anther levels differs in size (e.g. *Erythroxylum* – Ganders 1979b). This pattern might be a simple developmental outcome of differential stamen growth but its effect could be to produce pollen with slightly different physiological properties. Such variation might provide the necessary starting point for disruptive selection on pollen phenotype that could lead to the evolution of incompatibility differentiation. Studies of pollen size variation and pollen tube growth in non-heterostylous species with two stamen levels would be of interest in this regard (R. Dulberger, pers. comm.). It is conceivable that a gradient model might also apply to some distylous plants. For example, the reports of altered incompatibility expression associated with shortened style length in Primrose Queen (discussed above) would appear to be consistent with such a model.

The general proposition that incompatibility in heterostylous species is a direct physiological outcome of differences between the floral morphs in development and morphology was first proposed by Mather and De Winton (1941). Unlike the distyly models discussed above, they argued that the morphological and physiological components of heterostyly arose simultaneously, hence an obligatory association between them would be expected in evolution. Dulberger (1975b, 1992) has extended these ideas by suggesting that in most heterostylous species polymorphisms in style length, pollen and stigmatic papillae all contribute to the physiological control of incompatibility. She suggests that morph-specific stylar recognition substances inhibitory to the male gametophyte are synthesized during cell wall extension in the morphs. If incompatibility is, in part, a physiological manifestation of the differential growth of styles there may not be 'incompatibility loci' equivalent to

those in homomorphic systems. In this regard it is of interest that the extensive studies of Ernst (1955) on recombination in the distyly supergene of *Primula* failed to separate either style length from its incompatibility reaction or pollen size and incompatibility. This suggests that separate loci specifically governing incompatibility may not occur within the distyly supergene. While style length and incompatibility reaction can be separated in *Mitchella repens* (Ganders 1975a), this may result from other genetic loci independent of the supergene and does not refute the hypothesis that supergene control of these traits may involve the same gene (D. and B. Charlesworth 1979).

Recent work on heterostyly has involved a wider range of taxonomic diversity, particularly involving non-European taxa (reviewed in Barrett and Richards 1990). Several patterns that have been reported are relevant to models of the evolution of heterostyly. While simple refutation of any of the above models is not possible, and indeed is unwise given the probable independent origin of heterostyly in different families, the information can be used to address particular features of the models. By reviewing experimental work on two particular types of compatibility system not traditionally dealt with in discussions of heterostyly, we hope to broaden future discussions concerned with the evolution of incompatibility in heterostylous plants.

5. Heterostyly and self-compatibility

The phylogenetic status of self-compatibility in heterostylous groups is of relevance to models of the evolution of heterostyly. While in Lloyd and Webb's models, self-compatible heterostyly could represent an ancestral condition, in the Charlesworth's models self-compatibility is more likely to result from the loss of a pre-existing diallelic incompatibility system. Experimental studies have revealed many taxa in which heterostyly is associated with high levels of self-compatibility. These include *Amsinckia* (Ray and Chisaki 1957; Ganders 1975b), *Cryptantha* (Casper 1985), *Decodon* (Eckert and Barrett 1994), *Eichhornia* (Barrett 1988b), *Melochia* (Martin 1967), *Nivenia* (Goldblatt and Bernhardt 1990), *Oplonia* (Ornduff 1979), *Oxalis* (Ornduff 1972) and *Quinchamalium* (Riveros et al. 1987). Theoretical work indicates that as long as some degree of disassortative mating occurs in populations of these taxa, heterostyly should be maintained as a stable breeding system (Heuch 1979). In several of the genera listed above, closely related species with stronger heteromorphic incompatibility systems occur (e.g. *Eichhornia*, *Oplonia*, *Oxalis*). The self-compatible status of individual species has, therefore, usually been interpreted as the result of a weakening and eventual loss of diallelic incompatibility. However, in other genera (e.g. *Amsinckia*, *Cryptantha*, *Decodon*, *Nivenia*) all heterostylous species examined are self-compatible (note *Decodon* is monotypic), raising the possibility that strong diallelic incompatibility may not occur in these genera and that heterostyly could have conceivably evolved without incompatibility. However, in *Amsinckia* and

Cryptantha (Boraginaceae) and *Decodon* (Lythraceae), genera elsewhere in the families possess diallelic incompatibility so that even in these cases self-compatibility may be derived. Unfortunately, the absence of sound phylogenies for these and most other heterostylous taxa hampers interpretation of the sequence of character assembly so as yet critical data supporting an ancestral status for self-compatibility is unavailable for heterostylous groups.

Patterns of compatibility and incompatibility are particularly intriguing in the Pontederiaceae because there are few heterostylous taxa (*Pontederia* and *Eichhornia*) and phylogenetic information is available for the family (Eckenwalder and Barrett 1986; J.R. Kohn, S.W. Graham and S.C.H. Barrett, unpub. data). Morphological and molecular data support the hypothesis that *Pontederia* is derived from *Eichhornia*. In *Pontederia*, tristyly is associated with strong incompatibility and well developed pollen and stigma polymorphisms, whereas in *Eichhornia* incompatibility varies greatly in expression. *Eichhornia crassipes* and *E. paniculata* are highly self-compatible with weakly developed ancillary polymorphisms; in contrast the tristylous syndrome of *E. azurea* resembles that found in *Pontederia*. Does this variation represent an evolutionary build-up of trimorphic incompatibility in the *Eichhornia-Pontederia* clade, with self-compatible tristyly the ancestral condition? Or is self-compatibility in *Eichhornia* a derived condition through evolutionary loss of incompatibility? Since trimorphic incompatibility varies greatly in expression (Fig. 3) and is likely to be easily modified in evolutionary time clear answers to these questions are difficult to obtain.

In the preceeding discussion, *Amsinckia* and *Eichhornia* have both been described as highly self-compatible on the basis of similar levels of seed set upon self- and cross-pollination. However, from a functional perspective this description is misleading since in both taxa, species occur with 'cryptic incompatibility systems' (*sensu* Bateman 1956) whereby legitimate pollen fertilizes significantly more ovules than illegitimate pollen when the two occur together in pollen mixtures (*Amsinckia grandiflora* – Weller and Ornduff 1977; 1989; *A. douglasiana* – Casper et al. 1988; *Eichhornia paniculata* – Cruzan and Barrett 1993). The advantage of legitimate pollen is apparently the result of its faster pollen tube growth, rather than post-zygotic mechanisms involving selective abortion (although see Weller and Ornduff 1991). The presence of cryptic incompatibility in heterostylous groups raises a number of issues concerned with its relationship to more stringent forms of heteromorphic incompatibility and functional role in natural populations. In addition, work on the mechanisms responsible for cryptic incompatibility may provide insights into the nature and evolutionary basis of diallelic incompatibility.

Studies of the competitive relationships among pollen types using equal mixtures of genetically-marked pollen indicates that legitimate pollen consistently outcompetes illegitimate pollen regardless of whether or not it is self or outcross in origin. Moreover, in tristylous *E. paniculata*, the ranking of competitive ability of the two illegitimate pollen types for any given morph is the same as their ability to set seed in illegitimate pollinations in related *Pontederia*

spp. with strong trimorphic incompatibility. For example in illegitimate pollinations of *Pontederia*, mid-level pollen is the most compatible pollen in the L and S morphs whereas in the M morph long-level pollen is most compatible (Fig. 3). Similarly, in the L and S morphs of *E. paniculata* mid-level pollen outcompetes short- or long-level pollen, respectively in illegitimate pollen mixtures whereas in the M morph long-level pollen outcompetes short-level pollen (Fig. 5). These parallels in pollen tube growth and seed set data indicate that cryptic incompatibility in *E. paniculata* simply represents a weakened version of trimorphic incompatibility. However, as discussed above, whether this condition represents an incipient or derived state in relation to the strong incompatibility systems found in most heterostylous species is not clear.
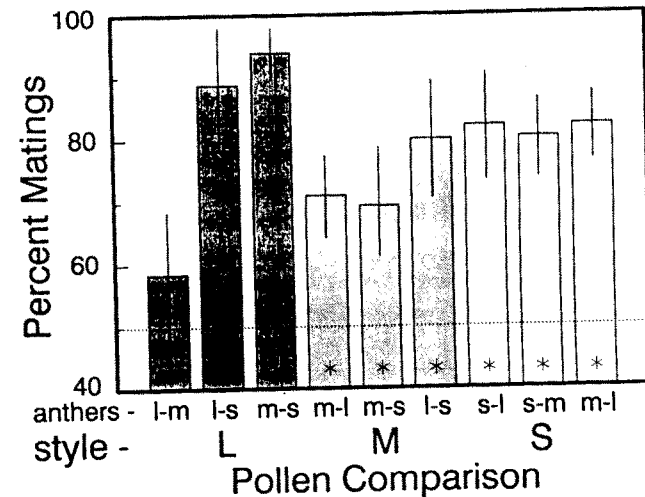

Fig. 5. Siring success following controlled pollinations of the three floral morphs of tristylous *Eichhornia paniculata* with genetically-marked (*AAT-3*) pollen mixtures. Bars indicate the frequency of matings obtained by the first pollen type listed from pollinations using pairs of pollen types in equal mixtures for each recipient morph. Vertical bars represent two standard errors of the mean and asterisks indicate means that differ (*P* <0.05 from *G* tests) from the random expectation of 50% (dotted line). After Cruzan and Barrett (1993).

The incompatibility system in *E. paniculata* and other self-compatible heterostylous species in which a clear prepotency of legitimate pollen over illegitimate pollen occurs can be viewed as facultative. This is because incompatibility operates in a quantitative manner, depending on the local pollen environment. Work on *E. paniculata* indicates that the siring success of legitimate pollen is strongly influenced by the size of the stigmatic pollen load (M.B. Cruzan and S.C.H. Barrett, unpubl. data). With large pollen loads intense pollen competition leads to the near exclusion of illegitimate matings, whereas with smaller pollen loads a mixture of legitimate and illegitimate offspring are produced. This ability to respond to the level of pollen deposition

results in a flexible mating system that takes advantage of high pollinator activity, by producing largely outcrossed progeny, but does not sacrifice fecundity when pollinators are less frequent. Context-dependent behaviour of this type may be more likely to occur in the mating systems of animal-pollinated species subject to wide fluctuations in population size and levels of pollinator service. In this regard it is of interest to note that *Amsinckia* and *E. paniculata* are both annual taxa in which populations sizes vary dramatically.

The functional similarities between pollen-pistil interactions in self-compatible and self-incompatible heterostylous species would suggest that selection could readily modify the level of discrimination among pollen types resulting in changes to the strength of the incompatibility system. To investigate to what extent phenotypic variation in stylar discrimination occurs in natural populations we screened a large number of individuals of the L morph of *E. paniculata* by applying a standard pollen mixture from multiple pollen donors involving equal proportions of the three pollen types. Progeny tests indicated continuous variation ranging from individuals where all offspring were sired by
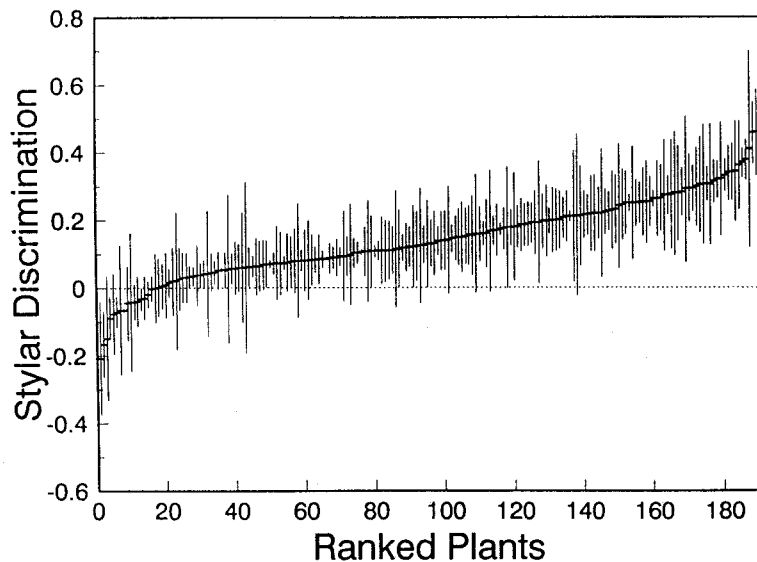

Fig. 6. Variation in stylar discrimination among 192 plants of the long-styled morph of tristylous *Eichhornia paniculata* derived from a single natural population (B-46) from N.E. Brazil. Each point is the mean, and the vertical bar the range, of values obtained from four pollinations per plant of an equal mixture of the three pollen types (large-, medium-, and small-sized). Values above zero indicate that legitimate (large-sized pollen) had the greatest fertilization success, while those below zero indicate that mid-sized pollen outcompeted the other two pollen types. To reduce the effect of among donor variation, pollen mixtures were from random groups of five donors per anther level with each mixture used to pollinate groups of 24 recipients. Each plant therefore received a mixture of pollen obtained from 15 male donors. Siring success was measured by the use of allozyme markers (see Cruzan and Barrett 1993 for methods). Unpublished data of M.B. Cruzan and S.C.H. Barrett.

legitimate pollen to a few individuals in which illegitimate pollen outcompeted legitimate pollen (Fig. 6). If at least some of this variation is under genetic control it would provide the necessary substrate for a selection response involving either an increase or decrease in the strength of the incompatibility system.

The most likely selective force responsible for increasing the strength of incompatibility is the avoidance of selfing owing to inbreeding depresssion. Hence, the overall strength of the incompatibility system in heterostylous plants may be determined on the one hand by the relative importance of selfing avoidance and the genetic load of populations and, on the other, by fertility assurance and the likelihood of reproductive failure. The significance of these two opposing forces is likely to be largely determined by ecological factors associated with the demography and life history of individual taxa.

6. Heterostyly and intramorph compatibility

Darwin and most modern workers have defined heterostyly in terms of the joint occurrence of a style-stamen polymorphism linked to an incompatibility system that prevents self, and particularly, intramorph matings. In several heterostylous taxa, however, the floral morphs posses a self-recognition system that appears to share more similarities to groups with homomorphic incompatibility since most outcrossed matings whether intermorph or intramorph are fertile. While infrequent, these curious associations are of relevance to models of the evolution of heterostyly. Under a model in which diallelic incompatibility evolves first, and reciprocal herkogamy follows to reduce illegitimate pollen transfer between the small number of mating groups, such a combination of traits would not be expected to occur. If, on the other hand, reciprocal herkogamy evolves primarily to increase the proficiency of cross-pollination among plants, these associations are no longer so anomalous (Lloyd and Webb 1992a). Because of the potential evolutionary significance of floral heteromorphism in species with intramorph compatibility we review below the limited data available for the three genera in which these associations have been reported.

Controlled pollinations among the floral morphs of *Anchusa hybrida* (Dulberger 1970b), *A. officinalis* (Philipp and Schou 1981; Schou and Philipp 1984), *Narcissus tazetta* (Dulberger 1964), *N. triandrus* (Bateman 1952; S.C.H. Barrett, W.W. Cole and M.B. Cruzan, unpub. ms.) and the S morph of *Villarsia parnassiifolia* (Ornduff 1988a) have revealed similar patterns. Self-pollinations result in little or no seed set whereas intramorph and intermorph pollinations produce variable amounts of seed, but with similar overall mean values for the two classes of pollination. The lack of association between genes controlling the mating system and those governing style length differences suggest that the self-recognition systems in these taxa may be quantitative in nature. How many loci control self-recognition, and if an incompatibility system is present, whether it

is sporophytic, gametophytic or a combination of both, are not known. In *A. officinalis*, Schou and Philipp (1984) found no cross-incompatibility among siblings indicating that one or a few incompatibility loci were unlikely to be involved. It is quite possible that early-acting inbreeding depression may play a role in determining the variable patterns of seed set observed following self- and cross-pollinations, particularly since the mechanisms of self-rejection at least in *Anchusa* and *Narcissus* occur in the ovary (see Barrett 1988a; Charlesworth 1992). It seems unlikely, however, that inbreeding depression *alone* can account for the absence (*A. officinalis*) or very low levels of seed set (*Narcissus* spp.) found upon self-pollination. Not only would the genetic load required to produce such high levels of zygote death be intolerable (see appendix in Waser and Price 1991), but also one might expect more variation in seed set among selfed individuals under a strictly inbreeding depression hypothesis (Seavey and Bawa 1986) than is actually observed. Clearly, further genetic and anatomical studies are required to establish the nature of the self-recognition systems present in these species.

Studies of pollen tube growth in *Anchusa officinalis* (Schou and Philipp 1983), *Narcissus tazetta* (Dulberger 1964) and *N. triandrus* (Bateman 1954; S.C.H. Barrett, W.W. Cole and M.B. Cruzan, unpub. ms.) indicate that the site of the incompatibility reaction is the ovary. Rates of pollen tube growth in the style are similar between self and outcross pollen and self pollen tubes regularly enter the micropyle; however the precise nature of the cellular events leading to subsequent degeneration and collapse of ovules is not clear. Of particular importance is to establish whether normal fertilization occurs or whether the rejection reaction is pre-zygotic as in *Pontederia* (Scribailo and Barrett 1991a). Resolving this issue has bearing on controversies concerning the existence of post-zygotic incompatibility and its relationship to inbreeding depression (Seavey and Bawa 1986), as well as to the broader question of the definition and concept of incompatibility in flowering plants (Lundquist 1964; de Nettancourt 1977; Uyenoyama 1988a,b,c,d, 1989).

In *Narcissus*, when stigmas are pollinated with self pollen prior to the application of outcross pollen, seed set is reduced significantly (Dulberger 1964; see Fig. 7). This suggests that ovules have either been fertilized by self-pollen tubes or have been rendered non-functional in some other way. A similar pattern was observed by Crowe (1971) in *Borago officinalis* and was used as evidence by her to support the existence of a post-zygotic incompatibility mechanism in the species (and see Waser and Price 1991). In contrast, in the few heterostylous species with diallelic incompatibility systems that have been studied, the prior application of illegitimate pollen to cross-pollinated stigmas has little effect on subsequent seed set (Shore and Barrett 1984; Barrett and Glover 1985, although see Nicholls 1987), perhaps because the morphological polymorphisms serve, in part, to reduce levels of pollen-stigma interference (Yeo 1975; Lloyd and Webb 1992a). Regardless of whether incompatibility is pre- or post-zygotic in *Anchusa* and *Narcissus* it is not difficult to see why ovarian incompatibility involving the irreversible sterilization of ovules by
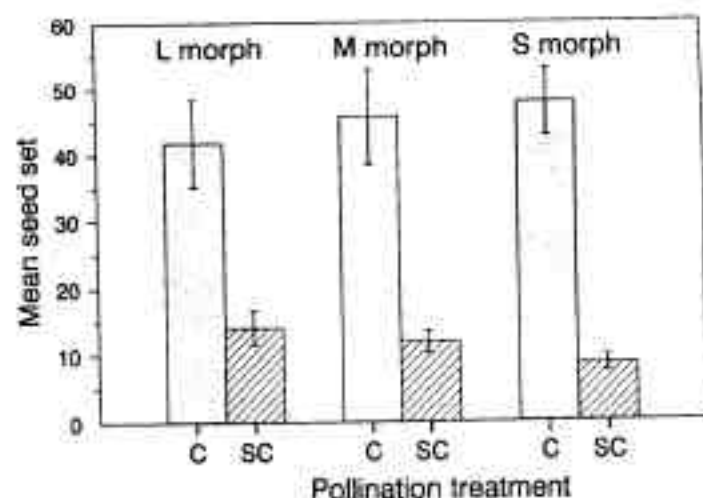
Fig. 7. The inhibitory effects of self pollen on the seed set of cross-pollinated flowers in the floral morphs of tristylous *Narcissus triandrus*. On each of 12 two-flowered plants per morph two pollination treatments were conducted. One flower was self-pollinated followed 24 hours later by cross pollen (SC); the other was cross-pollinated (C). Flowers in *N. triandrus* last for 5-10 days. The prior application of self pollen reduced seed set by 74% in comparison with cross-pollinated controls indicating that ovules had either been self-fertilized or rendered non-functional in some other way. S.C.H. Barrett, W.W. Cole and M.B. Cruzan, unpub. ms.

'incompatible' pollen would be maladaptive in species with only two or three mating types in a population (see Dulberger 1964).

While the three taxa with intramorph compatibility are quite unrelated it is of interest that they share several features in common that distinguish them from most other heterostylous groups. In all three, style length polymorphisms are well developed but differences in anther height are either absent (*N. tazetta*), weakly developed (*Anchusa* and *Villarsia*) or involve positional anomalies (*N. triandrus*, S.C.H. Barrett, D.G. Lloyd and J. Arroyo, unpub. ms.). In each case the variation results in less reciprocal correspondence between anthers and stigmas than is normally found in other heterostylous species. Associated with these patterns are either an absence or weak development of ancillary polymorphisms of pollen and stigmas. Where this occurs in other heterostylous taxa it is most often associated with the breakdown of heterostyly and occurrence of self-compatibility (Ornduff 1972; Barrett 1988b).

The incompatibility systems in *Villarsia* are particularly unusual for a heterostylous group. Studies by Ornduff (1982, 1986, 1988a,b) have revealed three patterns among western Australian members of the genus: 1) distyly with conventional diallelic incompatibility and ancillary pollen and stigma polymorphisms (e.g. *V. capitata*); 2) distyly in which the the L morph apparently possesses a conventional self- and intramorph incompatibility system and

the S morph exhibits self-incompatibility but high levels of intramorph compatibility (*V. parnassiifolia*); and 3) floral monomorphism associated with strong self-incompatibility and a high degree of cross-compatibility among individuals within a population (*V. albiflora*). The evolutionary relationships between these systems are not known and more work is required to determine the true nature of the incompatibility reactions. Cladistic studies would also be valuable to establish the likely phylogenetic relationships of taxa.

7. Future studies

This review has stressed that incompatibility in heterostylous plants is manifested by a diversity of pollen-pistil interactions, despite similar morphological traits associated with the evolution of reciprocal herkogamy. Unitary rejection responses typical of many species with homomorphic sporophytic incompatibility are absent from most heterostylous groups. Instead, great variation in the nature of incompatibility reactions are evident within and among families and between the floral morphs. Since heteromorphic incompatibility is polyphyletic in origin it is not unexpected that incompatibility responses in unrelated families might vary, because of developmental constraints imposed by phylogenetic history (Scribailo and Barrett 1991b). However, the consistent finding of morph-specific differences in the region of the pistil where male gametophytes are inhibited strongly implicates the role of floral morphology in the incompatibility mechanism. Moreover, the diversity of inhibition sites reported lends support to Lloyd and Webb's (1992a) suggestion that incompatibility may evolve separately in the floral morphs. The variation in expression of incompatibility also suggests that genetic systems controlling partial or leaky incompatibility may be more common than is often supposed. Whether the maintenance of such variation in natural populations reflects an adaptive response to selection for mixed mating or is a simple outcome of developmental and physiological variation arising from the functioning of heteromorphic characters is not clear. Whatever the cause it may be unwise to assume that such variation represents an unstable evolutionary state and that refinement to either well-developed incompatibility or the evolution of full self-compatibility will necessarily occur.

Little progress has been made in characterizing the cellular and biochemical basis of incompatibility in heterostylous plants and there have also been no attempts as yet to investigate the molecular genetics of heterostyly. In the future molecular techniques could be used to determine the number, location and organization of genes controlling floral polymorphism and incompatibility behaviour. Of particular interest will be to establish whether 'incompatibility loci', analogous to those found in homomorphic systems (reviewed in Haring et al. 1990), occur in heterostylous species. If incompatibility in heterostylous plants evolves independently in each morph through selection on polygenic

variation in pollen tube performance, a common molecular mechanism may not exist. An implication of this model is that incompatibility loci are likely to be more difficult to locate and hence molecular analyses will be a good deal more complex than so far conducted on homomorphic systems. Molecular studies are also likely to eventually resolve debates concerning the evolutionary relationships between heteromorphic incompatibility and other types of incompatibility.

Heterostylous populations frequently contain floral variants with altered style and stamen positions and incompatibility characteristics. The occurrence of this variation provides valuable opportunities to explore further the functional relationships between morphology and incompatibility. A particularly interesting form of floral variation that could be further exploited involves intraplant differences among flowers as a result of development accidents (Richards and Barrett 1992). Observations of variation in mass-flowering heterostylous species frequently reveal occasional flowers with anomalous style and stamen lengths. If incompatiblity responses are governed by differential organ growth, as Dulberger (1975b, 1992) has proposed, we might anticipate that these floral phenodeviants would display modified incompatibility expression, as discussed above. Controlled pollinations of these flowers and observations of pollen tube growth in comparison with control flowers could be potentially revealing. Artificial variation of this type can also be produced by manipulative experiments with plant hormones. Work on the effects of hormones on differential organ growth in concert with assays of endogenous hormone levels could provide useful information on the physiological mechanisms governing incompatibility responses in heterostylous plants.

Finally, considerable scope exists for further genetic and ecological studies of incompatibility in natural populations of heterostylous plants. Since the expression of incompatibility can vary greatly within and among the floral morphs it is of importance to establish the actual levels of self-and cross-fertilization that occur under field conditions through the use of marker genes (Barrett et al. 1992). Incompatibility in some self-compatible heterostylous species may be facultative depending on pollinator service, the delivery of legitimate and illegitimate pollen, and differential pollen tube growth. Where differences in pollen tube growth between the various pollen types occur, as in *Amsinckia* spp. and *Eichhornia paniculata*, it would be of interest to determine through selection experiments if the degree of discrimination can be altered and whether correlated responses are evident in morphological traits. Selection for a high degree of discrimination might eventually change a facultative system of incompatibility into one in which illegitimate fertilizations are prevented altogether. Since considerable variation in the expression of incompatibility is apparent within natural populations such experiments, if tractable, could provide important insights into how incompatibility evolves in heterostylous plants.

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