The evolutionary biology of tristyly

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'In their manner of fertilization a more remarkable case than can, perhaps, be found in any other plant or animal. Three plainly different forms occur: each of these is an hermaphrodite, each is distinct in its female organs from the other two forms, and each is furnished with two sets of stamens or males differing from each other in appearance and function. In short, nature has ordained a most complex marriage-arrangement, namely a triple union between three hermaphrodites' (Charles Darwin 1865: 169, describing tristylous *Lythrum* species.)

The evolution, adaptive significance, and breakdown of tristylous breeding systems have intrigued evolutionary biologists since the pioneering work of Darwin (1865, 1877) and Hildebrand (1865, 1899). Tristyly and the more familiar distyly are complex genetic polymorphisms which have traditionally been viewed as floral mechanisms that promote outcrossing and hence reduce levels of inbreeding in plant populations. Populations of tristylous plants are composed of three floral morphs that differ reciprocally in the heights at which stigmas and anthers are positioned within flowers. During this century the floral polymorphisms have been used as model systems for addressing a variety of questions in Mendelian and population genetics, development and population biology (reviewed in Ganders 1979a; Barrett 1992). Given their infrequent occurrence among angiosperm families (see below), why have the polymorphisms proven to be attractive subjects for study? The objective of this review is to provide an answer to this question by illustrating how tristyly can be used to investigate mechanisms of evolution and adaptation in plant populations. The review is divided into four sections, here I summarize the issues dealt with in each, to provide a framework for understanding the questions posed by workers studying tristyly.

Any attempt to investigate the evolutionary significance of a complex genetic polymorphism requires information on its general properties and occurrence. In the first section of this review the taxonomic distribution of tristyly and its morphological, physiological, and genetic characteristics are outlined. In the second section the question of the evolution and function of tristyly is addressed. How did the polymorphism originate and what is its adaptive significance? Next, issues concerned with the maintenance and evolutionary breakdown of the polymorphism are reviewed. A particular focus of this section concerns the relative

importance of genetic drift and frequency-dependent selection in determining the frequencies of floral morphs in natural populations of tristylous species. Evidence is presented which indicates that tristyly can be readily destabilized by interactions between stochastic and deterministic forces leading to the evolution of various derivative breeding systems. Finally, in the last section an attempt is made to glimpse into the future by outlining three areas of enquiry likely to be particularly profitable for solving problems concerned with the evolutionary biology of tristyly.

1. GENERAL FEATURES

In common with distyly, the style–stamen polymorphism in tristylous plants is often accompanied by a sporophytically controlled diallelic self-incompatibility system that enforces phenotypic disassortative mating among the floral morphs (Fig. 1). Trimorphic incompatibility is unique among angiosperm incompatibility systems because the two stamen levels within tristylous flowers produce pollen grains with different incompatibility phenotypes. Since two types of pollen are produced by the same plant, the differences can be viewed as 'non-genetic' and likely result from the contrasting developmental environments experienced during microsporogenesis in each stamen level. The°underlying molecular and biochemical basis of intrafloral incompatibility differentiation is unknown, but is likely to be fundamentally different from the molecular S gene specificities that characterize homomorphic incompatibility systems
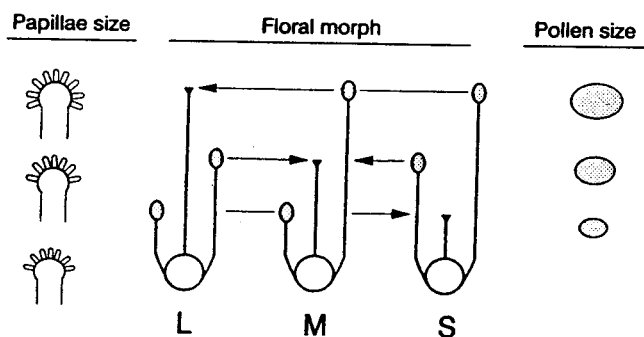

Fig. 1. Schematic diagram of the tristylous genetic polymorphism showing the relative placement of female and male reproductive organs in the long-, mid-, and short-styled morphs (hereafter L, M, and S morphs). Legitimate (compatible) pollinations are indicated by the arrows. In species with strong trimorphic incompatibility the remaining pollinations give little or no seed set. Polymorphisms of stigmatic papillae and pollen size are also illustrated by style length and anther level, respectively.

(Haring *et al.* 1990). Intrafloral incompatibility differentiation in tristylous populations results in complex patterns of compatibility. Of the 24 possible self, intramorph, and intermorph pollen–pistil combinations only six result in full seed set. These involve pollinations between anthers and stigmas of equivalent height and following Darwin (1877) are termed legitimate (Fig. 1). The remaining combinations usually give reduced or no seed set and are termed illegitimate.

Trimorphic incompatibility often varies in expression and in several species controlled pollinations have demonstrated varying degrees of compatibility in the floral morphs following different classes of illegitimate pollination (reviewed by Weller 1992). A common pattern involves the presence of weak incompatibility in the M morph (e.g., *Lythrum salicaria*, Darwin 1877; *Pontederia* spp., Barrett and Anderson 1985). In some tristylous species incompatibility is absent or only very weakly developed as judged by the occurrence of full seed set following illegitimate pollinations (e.g., *Oxalis* spp., Ornduff 1972; *Eichhornia* spp., Barrett 1988*a*; *Decodon verticillatus*, C. G. Eckert and S. C. H. Barrett unpublished data). However, the mating systems of self-compatible tristylous populations may still involve some degree of disassortative mating if differences in pollen tube growth between legitimate and illegitimate pollen occur (e.g., *Eichhornia paniculata*, Cruzan and Barrett 1993). In such cases, the incompatibility system has been described as cryptic, following Bateman (1956).

Accompanying the style–stamen trimorphism in most tristylous species is the occurrence of various ancillary polymorphisms, particularly of the stigmas and pollen of the floral morphs (Fig. 1). Stigmatic papillae often decrease in size associated with shorter style lengths and pollen size increases with stamen height (e.g., *Pontederia sagittata*, Scribailo and Barrett 1991*a*). Pollen production also varies among stamen heights with long-level stamens producing a small number of large-sized pollen grains, mid-level stamens with intermediate numbers of mid-sized pollen grains and short-level stamens producing a large number of small-sized pollen grains (e.g., *Lythrum junceum*, Ornduff 1975). The functional significance of these ancillary polymorphisms is largely uninvestigated. Dulberger (1992) recently reviewed ancillary polymorphisms and proposed that their primary function is in participating directly in the incompatibility mechanism of heterostylous plants. The polymorphisms may also play a role in the pollination process by reducing levels of illegitimate pollination and self-interference (Yeo 1975; Lloyd and Webb 1992*a,b*).

1.1 Taxonomic distribution of tristyly

The most common features of tristylous plants illustrated in Fig. 1 therefore involve three components: (1) a reciprocal style–stamen

trimorphism involving three floral morphs that differ in style length and stamen height; (2) a trimorphic incompatibility system in which pollinations among stigmas and anthers of equivalent height give full seed set; and (3) a suite of ancillary polymorphisms of stigmas and pollen. Using these general criteria most modern workers have recognized that tristyly occurs in eight genera from three unrelated angiosperm families, the Lythraceae, Oxalidaceae, and Pontederiaceae (Table 1). While considerable variation has been reported in the expression of trimorphic incompatibility and in the types of ancillary polymorphisms found in these families, until recently all earlier reports of tristyly from other angiosperm families (e.g., Burck 1895; Bir Bahadur 1978) have been treated as spurious and based on a misunderstanding of the essential features of the polymorphism (see Ganders 1979a,b; Barrett and Richards 1990).

Recent work, however, suggests that there are good grounds for re-evaluating the general criteria used for defining a given species as tristylous. In particular, Lloyd and Webb (1992a) have recently advocated that the term heterostyly, following Hildebrand (1866), should be used in a strictly morphological sense to refer to species that are polymorphic for a reciprocal arrangement of stigma and anther heights at the population level (termed reciprocal herkogamy), irrespective of the type of incompatibility system or occurrence of ancillary polymorphisms. If this approach is adopted species possessing reciprocal herkogamy with three floral morphs differing in the sequence at which stigmas and anthers are presented within flowers can be regarded as tristylous.

With these criteria in mind recent work on *Narcissus* of the Amaryllida-ceae (Lloyd *et al.* 1990; S. C. H. Barrett, D. G. Lloyd, and J. Arroyo, unpublished data) has conclusively demonstrated that *N. triandrus* is tristylous, as originally reported by Henriques (1887) and Fernandes (1935), but later disputed by Bateman (1952a, 1954), Baker (1964) and most modern workers. Bateman was unprepared to accept *N. triandrus* as a legitimate case of tristyly because his studies of a small sample of plants sent to him by Fernandes suggested: (1) style length variation may be continuous in large natural populations; (2) the two anther levels were of uniform height in plants with different style lengths; and (3) the species appeared to possess a multiallelic incompatibility system. In particular, the association between heterostyly and multiallelic incompatibility may have preconditioned Bateman and later workers against accepting that heterostyly could occur in *Narcissus* (Lloyd *et al.* 1990). However, this association may also occur in distylous *Anchusa* spp. (Dulberger 1970a; Schou and Phillip 1984) and possibly *Villarsia* (Ornduff 1988) and recent models of the evolution of heterostyly (discussed below) provide a plausible explanation for the sporadic union of these normally distinct conditions.

Recent quantitative studies of variation in floral traits in natural

populations of *N. triandrus* indicate that most populations are composed of three floral morphs differing in style length (S. C. H. Barrett, D. G. Lloyd, and J. Arroyo, unpublished data). Interestingly, however, while there is a close reciprocal correspondence between anther and stigma heights in the M and S morphs involving three distinct organs levels, in the L morph 'mid-level' anthers are positioned above mid-level organs of the M and S morphs. As a result, anther levels in the L and M morph are of similar height (Fig. 2). This pattern may have contributed to Bateman's assertion that anther levels are of uniform height in the species. While these positional anomalies combined with a putative multiallelic incompatibility system make the tristylous syndrome of *N. triandrus* unique, they in themselves are not sufficient to disqualify *N. triandrus* as being tristylous, if a morphological definition of heterostyly based on reciprocal herkogamy is accepted.

Several additional taxa in three other angiosperm families have also recently been claimed as tristylous. Lemmens (1989) working primarily on herbarium material reported tristyly in *Agelaea, Jollydora* and *Manotes* of the Connaraceae and suggested that evolutionary trends in breeding systems in the family were similar to those documented in the Lythraceae and Oxalidaceae (see below). Reports of tristyly in tropical *Hugonia* and *Roucheria* of the Linaceae have also been made (see Ganders 1979*a*; Lloyd *et al.* 1990). Particularly intriguing is a recent report by Zavada and Lowrey (1992) of tristyly in the south African shrub *Dais continifolia* (Thymelaeaceae) since these workers examined live material from a natural population. Field studies suggested the presence of three floral morphs differing in style length, stigmatic papillae size, and pollen exine sculpturing. Clearly, detailed population-level studies of these taxa are highly desirable, particularly since distyly is known (Connaraceae, Baker 1962; Linaceae, Lloyd *et al.* 1990) or has been reported (Thymelaeaceae, Darwin 1877) from other members of these three families. It is possible that with further exploration of tropical floras the number of tristylous families will increase from the three that are widely accepted in the literature on heterostylous plants.

1.2 Genetics of tristyly

The inheritance of tristyly has been investigated by controlled crosses among the floral morphs and observations of progeny ratios in *Lythrum* spp. (Barlow 1923; Fisher and Mather 1943; Dulberger 1967), *Decodon verticillatus* (C. G. Eckert and S. C. H. Barrett, unpublished data), *Oxalis* spp. (Von Ubisch 1926; Fyfe 1950, 1956; Sved 1965; Weller 1976*a*; Leach 1983; Bennett *et al.* 1986), *Eichhornia* spp. (S. C. H. Barrett, unpublished data) and *Narcissus triandrus* (Fernandes 1964). Despite the polyphyletic origin of tristyly a similar two-locus model appears to govern

Table 1

Tristylous taxa and their general features including distribution, life form, incompatibility expression (− = absent; + = weak; ++ = strong), presence of ancillary features and types of breeding-system modifications reported in the literature. Putative tristylous taxa have recently been reported from the Connaraceae, Linaceae, and Thymelaeaceae (see text)

Taxon	Number of tristylous spp. of total	Distribution	Life form	Incompatibility
Lythraceae				
Decodon verticillatus	1/1	North America	Clonal aquatic	−
Lythrum	5/35	Eurasia	Perennial herbs	+ +
Nesaea	3/50	Africa	Perennial herbs	?
Oxalidaceae				
Averrhoa	2/2	Tropical	Tree	?
Biophytum	1?/50	Tropical	Tree	+ +
Oxalis	Many/800	Cosmopolitan	Perennial herbs	− to + +
Pontederiaceae				
Eichhornia	3/8	Neotropics	Annual or clonal aquatics	− to +
Pontederia	4/5	Neotropics and North America	Clonal aquatics	+ to + +
Amaryllidaceae				
Narcissus triandrus	1/30	Spain and Portugal	Perennial bulbs	+ +

| Ancillary features	Breeding-system modifications	Sources
Weak pollen size trimorphism	Tri-, di-, monomorphic populations, semi-homostylous variants	Eckert and Barrett (1992) and unpublished data
Stigma polymorphisms, moderate pollen size trimorphism	Tri-, di-, and monomorphic populations, semi-homostyly and derived distyly	Darwin (1877), Koehne (1903), Dulberger (1970b), Ornduff (1979)
?	Derived distyly?	Koehne (1903), Ornduff (1978)
?	Tri- and dimorphic populations	Ganders (1979a), Weller (1992)
?	Tri-, and dimorphic populations, semi-homostylous variants	Mayura Devi (1964), Mayura Devi and Hashim (1966)
Moderate pollen size trimorphism	Tri-, di-, and monomorphic populations, semi-homostyly and derived distyly	Darwin (1877), Mulcahy (1964), Ornduff (1972), Weller (1992)
Weak-moderate pollen size trimorphism	Tri-, di-, and monomorphic populations, semi-homostyly	Barrett (1977b, 1978, 1985, 1988a)
Stigma polymorphisms, strong pollen size trimorphism	Tri-, di-, and monomorphic populations, no variants observed	Ornduff (1966), Barrett (1977a), Barrett and Anderson (1985), Scribailo and Barrett (1991a,b)
No ancillary polymorphisms	Tri- and dimorphic populations	Fernandes (1935, 1964), Lloyd et al. (1990), Barrett (unpublished data)
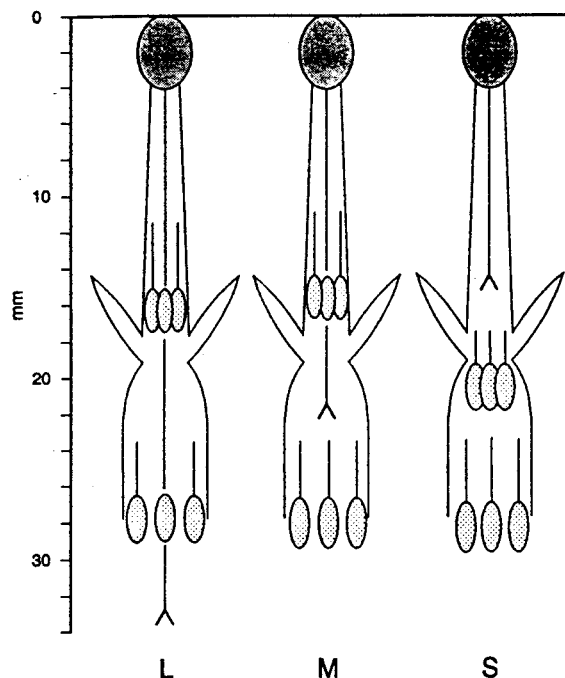
Fig. 2. The style-stamen trimorphism in *Narcissus triandrus*. The size and relative placement of reproductive parts illustrated are mean values for each organ level based on a sample of 50 flowers per morph sampled from a single population in southern Spain. Flowers of *N. triandrus* are pendulous, hence the aspect shown.

the genetic control of the polymorphism, at least in the Lythraceae, Oxalidaceae, and Pontederiaceae (reviewed in Charlesworth 1979; Lewis and Jones 1992). Under this general model two diallelic loci (S, M), with the S locus epistatic to the M locus, occur. Genotypes for the floral morphs and their equilibrium frequencies (discussed below) are given in Table 2. Three additional genotypes homozygous at the S locus are possible in self-compatible tristylous species as a result of self-fertilization (*SSmm, SSMm, SSMM*). Polyploidy with double reduction in *L. salicaria* (Fisher 1941, 1944; Fisher and Martin 1947), various degrees of linkage in *Oxalis* spp. (Fyfe 1950; Weller 1976a) and *Eichhornia* spp. (S. C. H. Barrett, unpublished data), and dominance reversal with the S morph recessive to the L morph but epistatic to the M morph in *Oxalis* spp. (Von Ubisch 1926; Fyfe 1956; Mulcahy 1964; Lewis and Jones 1992) contribute to variations of the basic two-locus model.

Recently, Bennett *et al.* (1986) have proposed a three-locus model to account for progeny data obtained from controlled crosses of *Oxalis rosea*. Some plants of the S morph are dominant to non-short-styled

Table 2

Genotypes and their equilibrium frequencies in a diploid tristylous population with disassortative mating under the two-locus model of inheritance (after Heuch and Lie 1985).

Floral morph	Long	Mid		Short		
Genotype	ssmm	ssMm	ssMM	Ssmm	SsMm	SsMM
Genotype frequency	0.333	0.309	0.024	0.179	0.131	0.024
Phenotype frequency	0.333	0.333		0.333		

plants while others are recessive. The authors suggest that the S morph is under the control of two loci (A and S). In plants segregating for A, a on an SS background, the S morph appears recessive while in plants segregating for S, s on an aa background it appears dominant. Among non-shorts, the M morph is dominant to the L morph. Alleles at the three loci (S, M, A) show independent assortment. Under this model there are $3^3 = 27$ style length genotypes, seven for the L morph, 14 for the M morph and six for the S morph (see Table 4 in Bennett *et al.* 1986). The suggestion that a third locus may be involved in the genetic control of tristyly is of interest in connection with models concerned with the evolution of tristyly (see below).

Little is known about the underlying genetical architecture of tristyly. While generalizations concerning supergene control of the heterostylous syndrome are frequently made, based on Ernst's (1955) studies of *Primula*, the existence of supergenes in tristylous species remains an unresolved issue. Some workers visualize the S and M 'loci' as supergenes each containing several tightly linked loci responsible for the morphological and physiological components of the tristylous syndrome (Ornduff 1972; Lewis 1975; Lewis and Jones 1992). Under this model rare variants with one set of anthers at a similar height as the stigma (termed semi-homostyles) are interpreted as products of recombination. It is also possible that the genetic architecture of tristyly is fundamentally different from distylous species and that supergenes are not involved. Charlesworth (1979) suggested that the expression of some tristylous characters may result from the pleiotropic effects of the S and M loci and concluded that there was no compelling evidence for supergenes in tristylous species. In a model of the evolution of tristyly she proposed that stigma position may automatically determine both its incompatibility reaction and anther height within a flower. Anther height could, in turn, determine the incompatibility reaction of pollen. Under this model semi-homostyles result from the action of modifier genes non-allelic to the S and M loci.

Unfortunately, little data on the genetic basis of semi-homostyly is available to clearly distinguish between the two models. Most semi-homostyles, however, do not appear to possess simple combinations of tristylous characters suggesting that they have not arisen through recombination. Molecular techniques may eventually resolve the issue of whether supergenes actually occur in tristylous plants.

2. EVOLUTION AND ADAPTIVE SIGNIFICANCE

Solving the puzzle of the evolution and adaptive significance of tristyly probably represents one of the most challenging problems in studies of plant breeding-system evolution. The complexity of tristyly, its rarity, and the absence of immediate relatives exhibiting intermediate stages in the build-up of the polymorphism complicates attempts to determine with any confidence the evolutionary processes responsible for its origin, in the handful of unrelated plant families in which it occurs. Notwithstanding these problems some headway has recently been made on this topic through the development of theoretical models and by experimental work on tristylous species. Before this work is reviewed, however, some common misconceptions concerning the evolution of tristyly need to be briefly considered.

On theoretical grounds it seems reasonable to assume that distyly represents an intermediate stage in the evolution of tristyly from a monomorphic ancestor. The presence of distylous taxa in the Lythraceae and Oxalidaceae, however, cannot be used as evidence for this pathway as has been done by some workers (e.g., Richards 1986). Studies of distylous *Lythrum* and *Oxalis* spp. indicate that this condition is derived from tristyly, through loss of one of the floral morphs (reviewed in Weller 1992 and see below). The absence of ancestral distylous taxa in tristylous families suggests that either this stage is rapidly passed through during the evolution of tristyly or, alternatively, that some other pathway is involved. Moreover, the absence of occasional tristylous taxa from the approximately 24 exclusively distylous families also suggests that if distyly is an intermediate stage in the evolution of tristyly, there are obviously severe constraints to the origin of tristyly in distylous families. These problems should remind us that while the two heterostylous polymorphisms are often lumped together, it is possible that the evolutionary processes responsible for their origins may be different. If this turns out to be true, it will undoubtedly be because of the more complex genetic and developmental basis of floral trimorphism.

It has often been assumed that because all tristylous species possess two stamen levels within a flower, two series of whorls of stamens are a precondition for the evolution of tristyly to occur (Yeo 1975). This is

not necessarily the case, however, since in tristylous taxa of the Pontederiaceae each stamen level has members from the first and second series (Richards and Barrett 1984, 1987). Two distinct stamen series may therefore not be necessary for the evolution of tristyly, although developmental studies of tristylous taxa suggest that a within-flower stamen dimorphism is probably a prerequisite (Richards and Barrett 1992).

2.1 Theoretical considerations

Distyly and tristyly have most commonly been interpreted as outbreeding mechanisms that reduce the harmful effects of close inbreeding in populations. This view is well illustrated by the following quote by R. A. Fisher in his analytical treatment of the differing efficacies of various plant breeding systems in reducing inbreeding:

The three chief methods of avoiding self-fertilization in hermaphrodite plants are distylism, tristylism, and a system of self-sterility allelomorphs. All of these are effective in preventing self-fertilization, but unequally effective in lowering, the frequency of mating between near relatives (Fisher 1949: 109.)

While this perspective is important for understanding the genetic consequences of mating in heterostylous populations, any adaptive explanation to account for the evolution of tristyly must deal with both the morphological and physiological components of the heterostylous syndrome. This is necessary because: (1) self-fertilization is largely prevented in heterostylous plants by physiological self-incompatibility, leaving unanswered the question of the function of the reciprocal style-stamen polymorphism; and (2) in comparison with multiallelic self-incompatibility, heteromorphic incompatibility prevents mating with 1/2 (distyly) to 1/3 (tristyly) of the remaining plants in the population. Hence its efficacy as an outbreeding mechanism is problematic. Darwin (1877, pp. 263–5) saw these difficulties and this led him to formulate an hypothesis for the evolution and adaptive significance of heterostyly based on its role as a pollination mechanism, rather than as an outbreeding device *per se*. According to Darwin, the style–stamen polymorphism in heterostylous plants functions to increase the proficiency of cross-pollination between anthers and stigmas of equivalent height. This would be more likely to occur because the two organ levels come into contact with the same part of a pollinators body. Incompatibility, rather than being viewed as an outbreeding mechanism, was seen as an 'incidental and purposeless' (Darwin 1877, p. 265) result of differential selection for pollen tube growth in the style type to which pollen was most frequently transferred.

Theoretical models of the evolution of heterostyly formulated in the modern era differ in the emphasis placed on anti-selfing (e.g., D. Charlesworth and B. Charlesworth 1979) versus pollen transfer (e.g., Lloyd and Webb 1992b) as selective forces, and in the sequence in which the morphological and physiological components of the polymorphism are thought to rise. Most modern workers have favored the essential features of the Charlesworths' model, with diallelic incompatibility evolving first as a selfing avoidance mechanism, with inbreeding depression the primary selective force. The reciprocal style–stamen polymorphism then arises secondarily to promote efficient pollen transfer between the small number of incompatibility types, hence reducing pollen wastage. A. J. Bateman (1952b) in his treatment of angiosperm incompatibility systems appears to have been first to articulate the idea that heterostyly reduces the loss of pollen on incompatible stigmas (although see Lloyd and Webb 1992b, pp. 204–5). In considering a homomorphic incompatibility system with only two mating types he stated:

Since, however, half the pollinations would be incompatible there would be a large wastage of pollen which could be overcome by a morphological mechanism which reduced the chances of incompatible pollinations. Heterostyly as in *Primula* may be such a mechanism, though I am not aware that it has been proven experimentally. In which case selection might favour the evolution of heterostyly from such a homostyled incompatibility (Bateman 1952b: 298.)

However, Lloyd and Webb (1992a,b), following Darwin's original idea, suggest instead that the style–stamen polymorphism evolved prior to incompatibility to promote efficient pollen transfer and increase fitness through male reproductive function. According to this view, incompatibility then develops secondarily, either as a pleiotropic effect of selection for increased pollen competitive ability, or as an anti-selfing device arising when most interplant pollen transfer is already intermorph through effective functioning of the style–stamen polymorphism. Hence in this scenario the 'cost of diallelic incompatibility,' with respect to the inability of plants to fertilize other individuals of the same morph, is reduced.

Most discussions of the evolution and selection of heterostyly deal implicitly, or explicitly as in the models above, with the evolution of distyly. Only Charlesworth (1979) has formulated specific models to account for the evolution of tristyly. While the selective forces in her models are similar to those investigated in the Charlesworths' distyly model, the underlying genetic and developmental assumptions are fundamentally different and take account of the more complex nature of tristylous flowers. Charlesworth (1979) explores two basic models starting with an ancestral form which is self-compatible with a long style and mid- and short-level anthers. The models assume: (1) three mutational

steps are required for tristyly to establish; (2) the pleiotropic effects of the S and M loci largely govern the expression of tristylous characters, rather than many separate loci organized as supergenes (see above); (3) tristyly is governed by three loci, one of which is fixed in contemporary populations of tristylous species. The two models differ in the sequence in which the morphological and physiological characters become associated and in the relative importance of selfing and inbreeding depression and pollen transfer. Both models involve an intermediate distylous condition with L and S morphs, implying that the M morph is the last phenotype to establish.

2.2 Empirical approaches

What evidence exists among tristylous families for the evolutionary pathways and sequence of character assembly postulated in models of the evolution of heterostyly? Can experimental approaches be used to investigate the role of tristyly as an anti-selfing device or as a mechanism to increase the proficiency of cross-pollination among the floral morphs?

2.2.1 Evolutionary pathways

At present comparative data from the three well-established tristylous families are largely uninformative concerning the evolutionary pathways by which tristyly has evolved. Sound phylogenetic data are unavailable for the dicotyledonous Lythraceae and Oxalidaceae and in neither family are the immediate ancestors of tristylous taxa known. Most workers have concluded that tristyly, associated with strong trimorphic incompatibility, represents the ancestral breeding system in lineages where they occur (e.g., Ornduff 1972; Weller 1992). In these families, tristylous genera also contain dimorphic and monomorphic species, however, genetic and morphological evidence indicates that these conditions represent stages in the breakdown rather than the build-up of the polymorphism (Darwin 1877; Ornduff 1972; Weller 1992; and see below).

Evolutionary patterns in the monocotyledonous Pontederiaceae are more interesting because of the small size of the family (6–8 genera, approximately 35 species), the limited number of heterostylous taxa (Table 1), and the availability of phylogenetic data based on morphological (Eckenwalder and Barrett 1986) and molecular characters (S. W. Graham, J. R. Kohn and S. C. H. Barrett, unpublished data). Nevertheless, difficulties of interpretation still remain. The most perplexing issue concerns the sequence in which floral trimorphism and incompatibility evolved in the family. Phylogenetic data support the hypothesis that *Eichhornia* is ancestral to *Pontederia*. While tristylous *Eichhornia* spp., with the exception of *E. azurea* (Barrett 1978), are highly self-fertile with weak expression of ancillary pollen and stigma characters (Barrett

1988*a*), in *Pontederia* spp. the polymorphism is associated with strong trimorphic incompatibility and well developed ancillary polymorphisms (Ornduff 1966; Price and Barrett 1982: Scribailo and Barrett 1991*b*). Does the occurrence of self-compatible tristyly in *Eichhornia* represent the ancestral condition anticipated in Lloyd and Webb's model of the evolution of heterostyly or, alternatively, has self-compatibility in tristylous *Eichhornia* spp. arisen as a derived condition through relaxation in the strength of the incompatibility system? Unfortunately, because of ambiguities in the interpretation of character state evolution there is no clear answer to these questions. Incompatibility systems are frequently subject to genetic modification (Nettancourt 1977; Barrett 1988*b*), making interpretation of the sequence in which physiological and morphological characters have evolved in heterostylous groups difficult to resolve. It seems unlikely, however, because of the complex nature of trimorphic incompatibility, that such a system could evolve in a monomorphic ancestor, prior to the evolution of floral trimorphism. Whether incompatibility develops simultaneously with the establishment of the style–stamen polymorphism or follows through selective discrimination among pollen types in self-compatible tristylous species remains unclear. Resolution of these problems depends, in part, on determining the functional interdependence of characters in the tristylous syndrome and their genetic bases. This information is critical for sound phylogenetic analysis of polymorphisms composed of complex character syndromes.

Patterns of floral variation in *Narcissus* spp. may provide valuable data for understanding the evolutionary build-up of tristyly in the Amaryllidaceae. *Narcissus* is comprised of approximately 30 spp. of bulbous perennials native to the Mediterranean region. The majority are insect-pollinated and monomorphic for style length with two stamen levels and stigmas generally exserted beyond the stamens (approach herkogamy *sensu* Webb and Lloyd 1986). Field studies in Spain and Portugal have also revealed *Narcissus* populations with stylar dimorphism and trimorphism (S. C. H. Barrett, D. G. Lloyd, and J. Arroyo, unpublished data). Species with dimorphic populations are distributed among three sections of the genus (Tazettae: e.g., *N. papyraceus, N. tazetta,* Dulberger 1964; Jonquillae: e.g., *N. assoanus, N. gaditanus, N. jonquilla;* Apodanthe: *N. calcicola, N. rupicola*). Populations are composed of two phenotypes that differ principally in whether stigmas are positioned above or below the two stamen levels. Since only small differences in anther height are evident between the L and S morphs, and there is also a lack of reciprocal correspondence between stigma and anther heights, this condition is best regarded as stylar dimorphism rather than true heterostyly. In *N. triandrus* (Section *Ganymedes*) populations dimorphic and trimorphic for style length occur with a reciprocal correspondence between stigma and anther heights (and see Fernandes

1965). Phylogenetic data would be helpful in establishing how stylar monomorphism, dimorphism, and trimorphism are evolutionarily related to one another and whether they represent stages in the build-up of tristyly. The isolated occurrence of tristyly in section *Ganymedes* makes it more probable that this condition represents a derived trait within the genus. If this is true the ancestral character states may be more easily identified than in the remaining tristylous families.

The possible association between tristyly and a putative multiallelic incompatibility system in *N. triandrus* has an important bearing on models for the evolution of heterostyly. A recent controlled crossing programme has confirmed Bateman's (1952a, 1954) earlier findings that the patterns of compatibility among the floral morphs are inconsistent with the operation of diallelic incompatibility (S. C. H. Barrett, unpublished data). Data on seed set and pollen tube growth implicate the operation of late-acting multiallelic gametophytic incompatibility and/or early acting inbreeding depression. While self-pollinations result in low levels of seed set, intra- and intermorph pollinations are equally fertile (Fig. 3B). Since *all* cross pollen is effective at producing seeds, the reciprocal style–stamen trimorphism in *N. triandrus* cannot have evolved to reduce levels of 'illegitimate' pollen transfer among the floral morphs. Rather, given the widespread occurrence of incompatibility in *Narcissus* (Bateman 1954; S. C. H. Barrett unpublished data), it seems more likely that heterostyly evolved after the establishment of multiallelic incompatibility as a mechanism to increase the proficiency of cross-pollination among plants. The putative association of multiallelic incompatibility with heterostyly in *N. triandrus*, as well as in *Anchusa* spp., is a major anomaly for models of the evolution of heterostyly that postulate that the style–stamen polymorphism evolved *after* the establishment of diallelic incompatibility to reduce levels of illegitimate pollination and pollen wastage. The association can be accommodated, however, under Lloyd and Webb's model (1992a,b), since the selective forces they invoke to explain the evolution of the style–stamen polymorphism are unrelated to whether the ancestral condition is self-incompatible or self-compatible.

2.2.2 Selective mechanisms

Experimental studies of the floral biology of tristylous populations have been employed to investigate hypotheses concerned with the adaptive significance of tristyly. Of particular value are self-compatible tristylous species, since in the absence of strong trimorphic incompatibility it is possible to assess the direct effects of the style–stamen polymorphism on reproductive parameters. Furthermore, by manipulating the morph structure of populations the reproductive performance of morphs under trimorphic versus monomorphic conditions can be compared. In so doing, it is possible to identify the reproductive consequences of the style–stamen
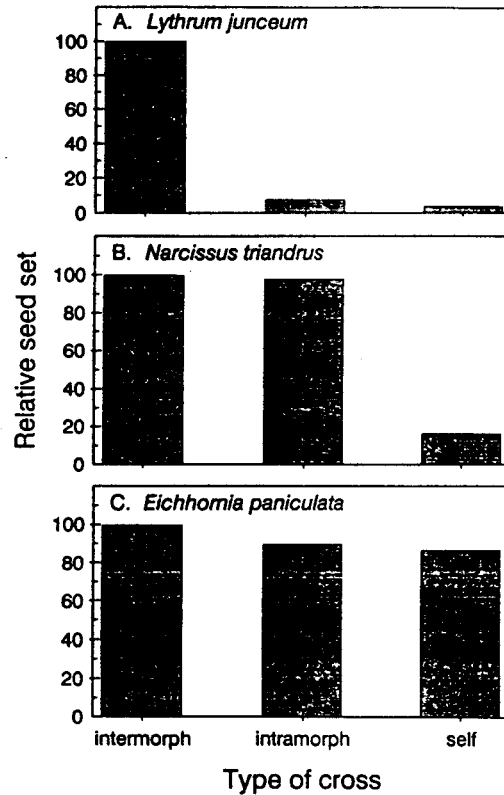
Fig. 3. The relative seed fertility of intermorph, intramorph and self-pollinations in three tristylous species with contrasting systems of compatibility. (A) *Lythrum junceum* (data from Dulberger 1970*b*). (B) *Narcissus triandrus* (S. C. H. Barrett, unpublished data). (C) *Eichhornia paniculata* (data from Kohn and Barrett 1992*a*). *Lythrum junceum* possess a conventional trimorphic incompatibility system, *N. triandrus* a putative multiallelic incompatibility system, and *E. paniculata* is highly self-compatible.

polymorphism, as well as gaining insights into the effects of each morph's floral architecture in isolation.

This approach has been used by Kohn and Barrett (1992*a*) employing genetic markers and the experimental manipulation of garden populations of *Eichhornia paniculata*. In this species self-, intramorph, and intermorph pollinations give similar levels of seed set (Fig. 3C). While marker gene studies of pollen competition in *E. paniculata* indicate a siring advantage to legitimate pollen, the advantage by itself cannot account for the observed levels of intermorph mating discussed below (Glover and

Barrett, 1986; Cruzan and Barrett 1993). Outcrossing rates, levels of intermorph mating and seed set were compared in replicate trimorphic versus monomorphic arrays to investigate the two primary hypotheses (anti-selfing and improved cross-pollination) concerned with the function of the style–stamen polymorphism. In trimorphic arrays there were no significant differences in the outcrossing rate or seed set of the floral morphs. Outcrossing rates averaged 81 per cent with a large proportion (95 per cent) of outcrossed seed the result of intermorph mating. If outcrossing events were random, expected rates of intermorph mating should have averaged 69 per cent in each array. Hence, the significantly higher levels of intermorph mating provide some support for the Darwinian hypothesis that heterostyly functions to promote cross-pollination among the floral morphs. Marker gene studies in natural populations of *E. paniculata* have also revealed high levels of intermorph mating (Barrett *et al.* 1987; Morgan and Barrett 1990) indicating that results from the garden arrays are of relevance to field conditions.

Dramatic differences in the reproductive performance of the floral morphs were evident when they were present in monomorphic versus trimorphic arrays. In general, floral monomorphism decreased both outcrossing rates and seed set but the magnitude of the differences varied greatly among the morphs. In the L morph, outcrossing rates were only slightly lower in monomorphic than trimorphic populations, but seed set was reduced by over 50 per cent. In contrast, seed set in the M morph was similar between the two array types, whereas outcrossing rates dropped to 30 per cent. In the S morph both outcrossing rates and seed set were substantially lower in monomorphic arrays. These results indicate how variation among morphs in the relative placement of female and male reproductive organs cause profound differences in the mating patterns and fertility of the morphs when in isolation. Such differences disappear, however, when the morphs occur together in polymorphic populations.

The differences among floral morphs in their reproductive performance complicates assessment of the anti-selfing or improved cross-pollination hypotheses for the adaptive significance of tristyly. Support for either hypothesis depends on which floral morph is assumed to most closely resemble the ancestral phenotype in a tristylous species. If this is assumed to be the L morph, as in Charlesworth's (1979) models for the evolution of tristyly, and by Lloyd and Webb (1992a,b) for heterostylous groups in general, then the large increase in seed set, compared to the relatively small increase in outcrossing rate, supports the idea that the selective basis for the evolution of the style–stamen polymorphism was increased pollen transfer, rather than higher levels of outcrossing. Evidence for improved pollen transfer in trimorphic versus monomorphic arrays was

evident from data on pollen loads. Stigmas of the L morph captured many more pollen grains in trimorphic arrays, despite lower visitation rates by pollinators (Kohn and Barrett 1992a).

The assumption that the ancestral phenotype is more similar to the L morph than the remaining two morphs is supported by two lines of evidence. First, among non-heterostylous plants reverse herkogamy, where stigmas are positioned below anthers (as in the S morph), is far less common than approach herkogamy, where stigmas are positioned above anthers (Webb and Lloyd 1986). Second, the phenotype of the M morph is virtually unknown outside of tristylous species and is therefore unlikely to represent the ancestral phenotype. Nevertheless, despite these arguments the strength of the conclusion that increased seed set in the L morph supports the pollen transfer model rests on how closely this morph resembles the ancestral phenotype. Since adjustments in the floral architecture of morphs seem likely during the evolutionary refinement of the polymorphism, the differences between contemporary morphs and their immediate monomorphic ancestors may be reproductively significant. These problems highlight some of the problems in testing selection hypotheses in contemporary populations where only educated guesses concerning the characteristics of ancestral phenotypes can be made.

An alternative approach to studying the adaptive significance of heterostyly is to ask whether the style–stamen polymorphism promotes legitimate pollination in natural populations of heterostylous species, as predicted by the Darwinian hypothesis. Two types of data, pollen deposition on pollinators and pollen loads on stigmas, have been collected to assess the hypothesis; both employ the conspicuous size heteromorphism of pollen produced by different stamen levels in heterostylous populations. Unfortunately, in most tristylous taxa considerable overlap in size occurs between pollen types originating from different anther levels complicating attempts to determine unambiguously their source (Mulcahy and Caporello 1970; Ornduff 1975). In *Pontederia* spp., however, there is no significant overlap in the size of the three pollen types (Glover and Barrett 1983; Price and Barrett 1984) allowing experimental tests of the Darwinian hypothesis.

Inspection of deposition patterns of *P. cordata* pollen on pollinating insects (mostly bees) have provided evidence for the segregation of the three pollen types on different regions of the bodies of pollinators (Wolfe and Barrett 1989). Since grooming and other activities can disturb the position of pollen deposited on pollinators, segregated distributions cannot by themselves prove that pollen deposition on stigmas is also segregated. To do this stigmatic pollen loads of naturally pollinated flowers need to be examined. The rationale behind this approach is that a more proficient transfer of legitimate pollen to stigmas would be indicated by a higher proportion of legitimate pollen in stigmatic loads,

than expected from random pollination. Random expectations can be obtained by estimating the relative frequencies of the three pollen types in the population based on the frequencies and flower production of the morphs.

Most studies of heterostylous populations have not been able to fully evaluate whether the style–stamen polymorphism promotes legitimate pollination, since only pollen loads of unmanipulated flowers have been examined. To evaluate the function of the polymorphism the effects of intrafloral pollen transfer need to be removed by emasculating the flowers from which stigmas are to be collected (Ganders 1979a). 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 prevents reliable estimates from being made of the amounts of illegitimate outcrossed pollen that are transferred by pollinators.

To date, field emasculations have been undertaken only in a single population of one tristylous species; however, the data from this study has provided encouraging results. Barrett and Glover (1985) found that pollen loads of emasculated flowers of *Pontederia cordata* were composed of a significant excess of legitimate pollen over that expected from random pollination. Data from this study have recently been subjected to a more sophisticated analysis by Lloyd and Webb (1992b) that considers not only the receipt, but also the donation of legitimate and illegitimate pollen by the floral morphs (Table 3). Data from pollen loads were converted into the probabilities of a single pollen grain of each type being deposited on a given morph. When this was done it was found that legitimate pollen transfer exceeded illegitimate transfer for all pollen–stigma combinations. The average proficiency of legitimate pollen transfer was nearly double that of illegitimate transfer (Table 3). The approximate doubling of the average proficiency of legitimate transfer compared with illegitimate transfer would appear to constitute a powerful selective force maintaining the style–stamen polymorphism in *P. cordata* and provides the most convincing evidence in support of the Darwinian hypothesis of the functional significance of tristyly.

3. MAINTENANCE AND BREAKDOWN

Frequency-dependent selection operating during the mating cycle acts to maintain mating types in populations of self-incompatible plants. In distylous populations the simple inheritance (one diallelic locus with dominance) and strong disassortative mating results in a 1:1 ratio of floral morphs in a single generation. In tristylous populations, however, the more complex genetic system complicates the problem of predicting equilibrium morph frequencies. J. B. S. Haldane appears to have been the first to point this out:

Table 3

Stigmatic pollen loads and the proficiencies of legitimate and illegitimate pollen transfer in emasculated flowers of Pontederia cordata. *(A) Average number of pollen grains of the three types deposited on stigmas of the floral morphs by pollinators (data from Barrett and Glover 1985). (B) The transfer probabilities of a pollen grain of a given type being deposited on stigmas of the floral morphs (after Lloyd and Webb 1992b). Data on pollen number per flower from Price and Barrett (1982).*

(A) Pollen loads on stigmas

| Pollen type (number/flower) | Style | | |
	L	M	S
l (4573)	**62.5**	26.0	10.3
m (6463)	46.5	**74.9**	31.0
s (23707)	123.6	164.2	**138.7**

$$T_{ij} = \frac{\text{Amount of pollen } i \text{ on stigmas of } j}{\text{Total amount of pollen } i \text{ produced}}$$

(B) Transfer probabilities

| Pollen type | Style | | |
	L	M	S
l	**6.55×10^{-3}**	2.32×10^{-3}	1.33×10^{-3}
m	3.22×10^{-3}	**4.42×10^{-3}**	2.65×10^{-3}
s	2.82×10^{-3}	3.19×10^{-3}	**3.90×10^{-3}**

$$\text{Average proficiency } P = \frac{\left(\prod_{i=j} T_{ij}\right)^{(1/3)}}{\left(\prod_{i \neq j} T_{ij}\right)^{(1/6)}} = 1.94$$

Lythrum populations seem to vary rather more round their mean than do those of dimorphically heterostylic plants. This is to be expected. For the tendency to return towards equilibrium is far greater in the case of dimorphic than of trimorphic heterostylism (Haldane 1936: 396.)

Two contrasting approaches have been used to address the problem of equilibrium morph frequencies. Most effort has gone into determining through analytical means or computer simulation the equilibrium morph ratios for various hypothetical or known genetic systems controlling tristyly (reviewed below). More recently, however, sex allocation theory has been used to explain morph frequencies in heterostylous plants (Casper and Charnov 1982). The models assume that autosomal genes separate from the loci controlling heterostyly influence morph frequencies, giving rise to equilibria in which all individuals have equal fitness. Casper (1992) has recently reviewed the limited empirical evidence for the presence of such genes. To date, only Taylor (1984) has developed a sex allocation model specifically for a tristylous species. He suggests that before more progress in this area can be made, empirical data on allocation costs and the shape of male and female fitness functions in tristylous populations are required. These deficiencies in our knowledge also apply to the application of sex allocation theory to non-heterostylous plant populations.

3.1 Theoretical analysis of equilibrium morph frequencies

Early surveys of floral morph frequencies in tristylous populations of *Lythrum salicaria* (Darwin 1877; Haldane 1936), as well as controversies concerning the inheritance of tristyly (East 1927, 1932; Fisher 1935), undoubtedly led Fisher (1941, 1944) to undertake the first theoretical analysis of equilibrium conditions in tristylous populations. He demonstrated that equal morph frequencies (isoplethy) could be maintained from generation to generation by frequency-dependent mating, a process akin to that producing equal sex ratios in dioecious species. His analysis, however, did not exclude the possibility of different equilibria, and for other hypothetical mechanisms of the inheritance of incompatibility, various stable equilibria involving anisoplethy are possible (Finney 1952, 1983; Spieth and Novitski 1969; Spieth 1971; Moran 1962). Later, Heuch (1979a) developed a general theorem for tristylous populations. His analyses indicated that provided that there are no fitness differences among the floral morphs, an isoplethic equilibrium is the only possible condition in large populations with disassortative mating (Table 2, Fig. 4A).

In these theoretical analyses two contrasting mating models have been employed. Under the 'pollen elimination model' (limited competition
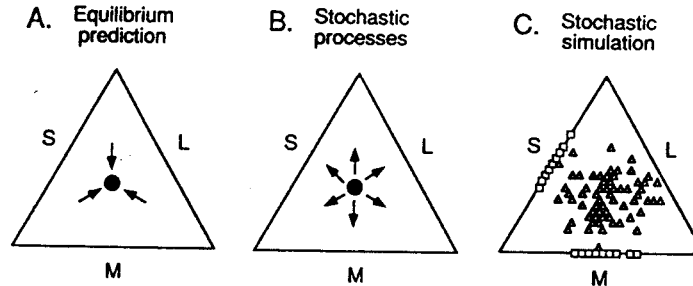
Fig. 4. Patterns of morph frequency variation in tristylous populations. The three large triangles represent the hypothetical space that can be occupied by populations of tristylous species. Within the triangles a point represents the floral morph frequencies of a single population. The distance from a point to an axis is proportional to the frequency of a morph in the population. Populations with equal morph frequencies are equidistant from all axes, those lacking one morph (dimorphic) lie on the axes, those lacking two morphs (monomorphic) occur at the vertices. Trimorphic and dimorphic populations are represented as small triangles and squares, respectively. (A) The equilibrium prediction of equal morph frequencies in a tristylous population. (B) The disruptive effect of stochastic processes on equilibrium morph frequencies. (C) The results of a stochastic computer simulation involving 100 populations of size 30 with strong disassortative mating ($d = 0.95$) after 100 years. Note that the scatter of points is not random, dimorphic populations are primarily LM and to a lesser extent LS and there is an absence of trimorphic populations near the three apices of the triangle. (After Eckert and Barrett 1992.)

model of Spieth (1971)) it is assumed that gametes are drawn at random from the potential pool of compatible mates. In contrast, under the 'zygote elimination model' (mass action model of Karlin and Feldman (1968)) legitimate mating occurs with a frequency directly proportional to the product of the frequencies of the mating types involved. For incompatibility systems with two mating types (distyly) the different models give identical results, for tristylous systems, however, the two models often result in different equilibria, depending on the genetic system involved (Spieth 1971).

Heuch's (1979a) general theorem and various derivations discussed below deal only with the calculation of equilibrium phenotype (morph) frequencies in populations of tristylous plants. More recently, Heuch and Lie (1985) have shown how equilibrium genotype frequencies may also be determined for both diploid and tetraploid populations, with and without linkage between the S and M loci. Equilibrium genotype frequencies for the six style length genotypes for a diploid tristylous population undergoing complete disassortative mating are given in Table 2. As can be seen, while the three floral morphs are equally represented,

the equilibrium genotype frequencies vary widely. Extensive progeny testing is required to determine genotype frequencies in natural populations and to date only limited data are available (Barrett *et al.* 1987).

Using modifications of Heuch's (1979*a*) general theorem a variety of theoretical models have been developed to explore how various ecological and genetic factors influence floral morph frequencies in tristylous populations (Heuch 1979*b*, 1980; Barrett *et al.* 1983, 1989; Eckert and Barrett 1992; Husband and Barrett 1992*a*). The models have dealt primarily with variation in mating system, fertility, life history, clonal propagation and effects of finite population size. In general, they indicate that the significance of stochastic forces depends on the balance between effective population size and the frequency-dependent selection of style morph alleles that results from disassortative mating. Increased levels of self-fertilization or random mating, as well as perenniality and clonal propagation, restrict the rate of recruitment of offspring resulting from disassortative mating and allow stochastic forces to have a greater influence on morph frequencies. This body of theoretical work provides the necessary background in which to interpret empirical surveys of floral morph frequencies in natural populations. The models can be used to distinguish between the relative importance of stochastic and deterministic forces in explaining patterns of morph frequency variation.

3.2 Population surveys of floral morph frequencies

Large-scale surveys of floral morph frequencies have been conducted in populations of seven tristylous species (Fig. 5 and *Eichhornia crassipes*). Each displays a distinctive pattern of morph frequency variation reflecting its own unique ecology and evolutionary history. Despite this variation some similarities between species exist; where this occurs common causal mechanisms are likely to play a role. Although data on the frequencies of floral morphs in other tristylous taxa have been reported (e.g., *Oxalis* spp., Faberge 1959; Ornduff 1964, 1972, 1974; Weller 1980; *Lythrum junceum*, Dulberger 1970*b*; *Eichhornia azurea*, Barrett 1978; *Pontederia rotundifolia*, Barrett 1977*a*; *P. sagittata*, Glover and Barrett 1983) in most cases the samples are too limited to infer much about the evolutionary forces influencing the maintenance of the polymorphism.

The data on floral morph frequencies illustrated in Fig. 5 clearly indicate that tristylous species vary in the extent to which the three floral morphs are maintained in populations. Life history characteristics largely govern this variation. In highly clonal species in which sexual reproduction is limited, founder events play a major role in determining the morph structure of populations. In such populations progress towards any deterministic equilibrium can be very slow and those that acquire skewed morph frequencies through founder effects may bear the imprint of
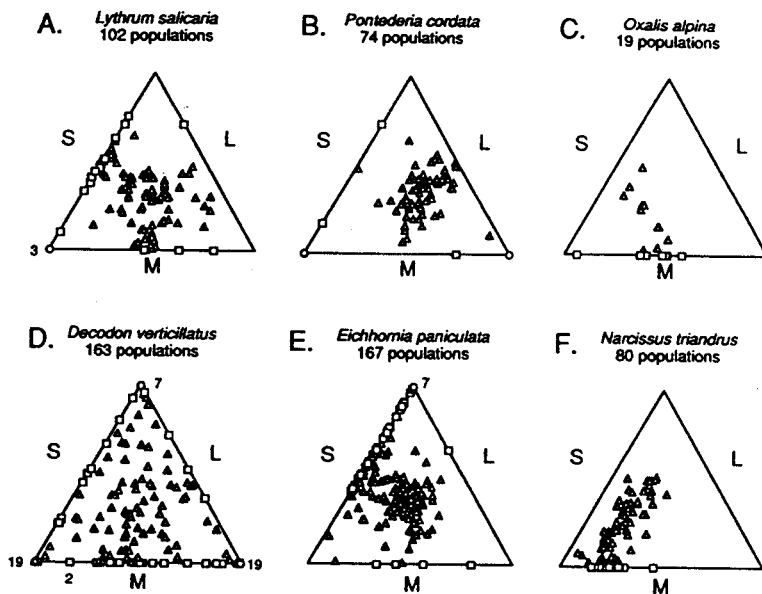
Fig. 5. Large-scale surveys indicating the patterns of morph frequency variation in populations of six tristylous species: (A) *Lythrum salicaria*; (B) *Pontederia cordata*; (C) *Oxalis alpina*; (D) *Decodon verticillatus*; (E) *Eichhornia paniculata*; (F) *Narcissus triandrus*. Data for each species are from Eckert and Barrett (1992), Barrett *et al.* (1983), Weller (1979), Eckert and Barrett (1992), Barrett *et al.* (1989) and unpublished data, and Barrett, unpublished data, respectively. The number of populations sampled for each species is indicated.

historical accident for long periods (Eckert and Barrett 1992). In an extensive geographical survey of floral morph representation in 196 New World populations of the clonal aquatic weed *E. crassipes*, Barrett and Forno (1982) found that 77.0 per cent were monomorphic, 18.4 per cent were dimorphic and only 4.6 per cent contained the three floral morphs. The high dispersal of free-floating vegetative fragments combined with the species rampant powers of clonal propagation have undoubtedly given rise to these patterns. Similar processes in the clonal aquatic *Decodon verticillatus* likely account for the high proportion of monomorphic (32 per cent) and dimorphic (20 per cent) populations and the wide dispersion of population morph frequencies (Fig. 5D). Skewed morph ratios in populations of clonal *Oxalis* spp. have also been interpreted as resulting from founder events and the restricted occurrence of sexual reproduction (Ornduff 1972, 1974).

3.2.1 Stochastic forces

It has sometimes been suggested that the random loss of floral morphs from tristylous populations should produce dimorphic populations composed of the L and M, L and S, or M and S morphs with equal probabilities (e.g., Charlesworth 1979). Similarly, the possibility of a consistent bias towards any of the floral morphs through chance events was discounted by Barrett et al. (1983) in explaining skewed morph frequency data in Pontederia cordata (Fig. 5B). However, the intuitive idea that stochastic forces acting alone should give rise to random patterns of morph frequency variation has not stood up to theoretical scrutiny. Stochastic models have shown that founder events and genetic drift can give rise to distinctive patterns of morph frequency variation in tristylous species because of the particular mode of inheritance of tristyly (Heuch 1980; Heuch and Lie 1985; Morgan and Barrett 1988; Barrett et al. 1989; Eckert and Barrett 1992; Husband and Barrett 1992a). This variation primarily involves the more frequent loss of the S morph and, to a lesser extent, the M morph from tristylous populations (Fig. 4B, C). The S morph is more vulnerable to stochastic loss since it alone produces gametes with the dominant S allele required to produce the S morph. Both the S and M morphs can segregate sM gametes required for the M morph, and all three morphs produce sm gametes needed for the L morph. As a result of stochastic forces dimorphic populations should most often be composed of the L and M morphs and less often the L and S morphs. Populations dimorphic for the M and S morphs are predicted by stochastic models to be very rare in nature (Fig. 4C). Thus while frequency-dependent selection provides the dominant force maintaining floral trimorphism, stochastic forces have the potential to destabilize the polymorphism in distinctive ways with potential consequences for the future course of floral evolution.

What evidence exists for the role of stochastic forces in influencing morph frequency variation, and is genetic drift strong enough in natural populations to influence the frequencies of alleles under selection? If drift is an important evolutionary force we might predict that the likelihood of morph loss should decrease with increasing population size. Moreover, morph loss should most frequently involve the S morph and to a lesser extent the M morph. We might also anticipate that where founder events owing to migration or local colonization have occurred these should most often lead to dimorphic populations composed of the L and M morphs. In L. salicaria and E. paniculata data on floral morph frequencies and population size are consistent with the hypothesis that stochastic forces play an important role in determining the morph structure of populations (Fig. 6). In each species there was a negative relationship between morph loss and population size. In L. salicaria

A. *Lythrum salicaria*
102 populations

Percent of populations in each size class

☑ Trimorphic   ■ Nontrimorphic

B. *Eichhornia paniculata*
167 populations
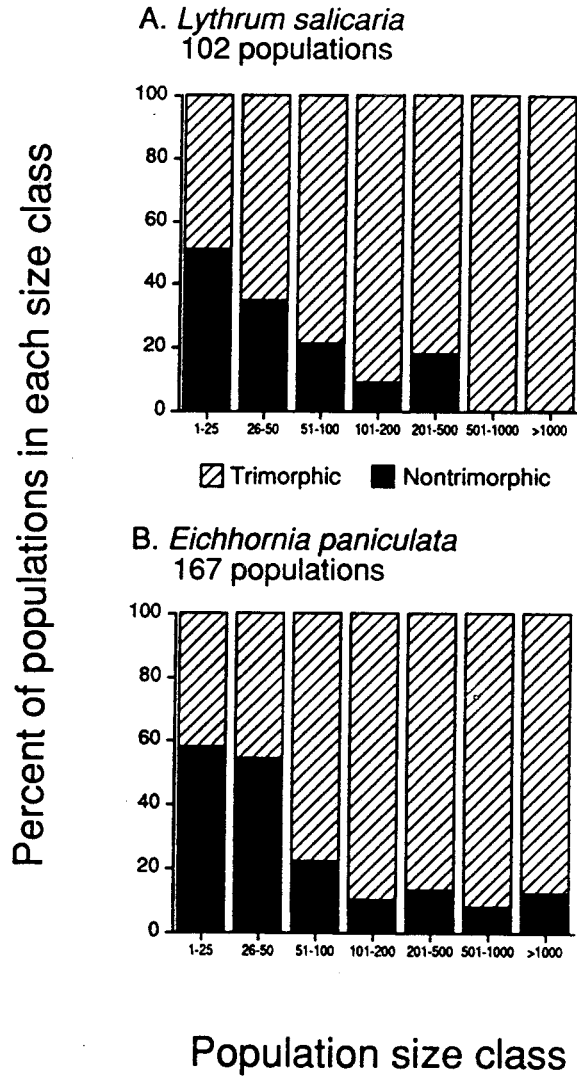

Population size class

Fig. 6. Population size and morph structure in two tristylous species: (A) *Lythrum salicaria*, and (B) *Eichhornia paniculata*. The percentage of trimorphic and non-trimorphic populations occurring within seven population size classes are shown. The size classes were chosen to maximize the sample sizes within each class. In both species the proportion of populations lacking a morph differ significantly among size classes. (After Eckert and Barrett (1992) and Husband and Barrett (1992a).)

75 per cent of the dimorphic populations sampled in an Ontario survey lacked the S morph, 20 per cent the M morph and only 5 per cent the L morph (Fig. 5A). The scattered geographical distribution of dimorphic populations indicated that morph loss has occurred repeatedly in the region (Eckert and Barrett 1992). Similar patterns are evident in *E. paniculata*. Virtually all dimorphic populations of the species in NE Brazil are composed of the L and M morphs and these are widely distributed (Barrett *et al.* 1989; Husband and Barrett 1993; and Fig. 5E). A survey of temporal changes in morph frequency over two consecutive years in 76 populations of *E. paniculata* in NE Brazil revealed that 12 trimorphic populations lost morphs during this period. The L, M, and S morphs were lost 2, 6, and 11 times, respectively, a pattern consistent with the random loss of morphs from populations (Husband and Barrett 1992*a*). While dimorphic LS populations were more common (44 per cent) than LM populations (37 per cent) in the survey of *D. verticillatus* (Fig. 5D), the much lower frequency of MS populations (19 per cent) is as predicted by the stochastic model.

The estimates of population size reported in Fig. 6 represent the census number (N) of individuals within populations; however, measures of the effective population size (N_e) are more relevant for investigating evolutionary mechanisms (Wright 1931; Kimura and Crow 1963). The few estimates available for plant populations suggest that values of N_e may often be considerably smaller than N, due to non-random mating and high variation in fertility (Crawford 1984; Heywood 1986). Data from a recent study of N_e in 10 populations of *E. paniculata* support this view (Husband and Barrett 1992*b*). Estimates of N_e were inferred from year to year changes in allele frequency at isozyme loci (see Waples 1989) and from five demographic variables. Genetic estimates averaged 15.8 (range 3.4–70.6), a fraction (mean $N_e/N = 0.106$) of the census number. This value is well below the critical size of about 40, below which drift overwhelms the frequency-dependent selection maintaining the tristylous polymorphism (Heuch 1980; Husband and Barrett 1992*a*, *b*). Applying the average N_e/N value to the 167 populations of *E. paniculata* surveyed for morph frequency and population size (Figs 5E, 6B) indicates that 72 per cent had effective sizes below this number. This suggests that stochastic forces are likely to play a major role in destabilizing tristyly in *E. paniculata*.

Founder events during migration in *Eichhornia* and *Lythrum* also support the stochastic model of morph loss for tristylous systems. Long-distance dispersal of *E. paniculata* to Jamaica apparently did not involve the S morph, since only the L and M morphs are represented on the island (Glover and Barrett 1987; Husband and Barrett 1991). Similarly, in *E. crassipes* introduced populations in Central and North America as well as the Old World are composed of the L and M morphs. The S

morph is restricted to the species' native range in lowland South America and was presumably not represented among the founding genotypes introduced to the adventive range by humans. In *Lythrum* all tristylous species in the genus are native to Eurasia. Comparative morphological evidence indicates that the uniformly distylous North American members of section *Euhyssopifolia* most closely resemble the L and M morphs of their Old World tristylous ancestors (Ornduff 1979). This suggests that the S morph may have been lost during the migration of *Lythrum* to North America. In each of these examples the most parsimonious explanation to account for the derived dimorphism involving the L and M morphs is stochastic loss of the S morph during migration.

3.2.2 Deterministic forces

Inspection of the morph frequency triangles illustrated in Fig. 5 reveals several large-scale patterns that are difficult to account for by stochastic processes acting alone. In these cases selective mechanisms are implicated. Computer models incorporating fitness differences among the floral morphs resulting from variation in fertility, mating patterns, and life history can provide valuable information for investigating the selective mechanisms responsible for observed patterns of morph frequency variation. By combining these theoretical approaches with field studies it is sometimes possible to distinguish the relative importance of stochastic and deterministic forces in controlling morph frequency variation. Several examples help to illustrate this approach.

In trimorphic populations of *E. paniculata* in NE Brazil, there is a marked deficiency of the S morph in comparison with the isoplethic expectation (Fig. 5E). Yet stochastic models in small tristylous populations clearly demonstrate that a significant bias in morph frequencies should not generally occur (Fig. 4C; and see Eckert and Barrett 1992). Heuch (1980) suggested that local loss of the S morph *within* tristylous populations could occur through random processes if neighborhood sizes were small due to restricted pollen and seed dispersal. With a high degree of population substructure local neighborhoods might often become dimorphic (L, M), whereas if selection were operating against the S morph, the frequencies of different subpopulations may be closer to the overall population average.

Two lines of evidence are inconsistent with a model of random loss of the S morph within substructured populations. Analysis of the spatial autocorrelation of floral morphs in tristylous populations of *E. paniculata* failed to reveal any significant evidence for spatial structure (Husband and Barrett 1992c). This is not altogether unexpected since most populations are small (< 100) and inhabit ephemeral pools and ditches in which the tiny seeds of *E. paniculata* are easily dispersed in water. Second, if stochastic forces were solely responsible for the reduced

average frequency of the S morph then we would expect that the M morph to be lower in average frequency than the L morph. This is not the case; the average frequencies of the two morphs in trimorphic populations are nearly equal (L = 0.374, M = 0.370, S = 0.256; N = 58 populations, Barrett *et al.* 1989).

Field observations and experimental studies of *E. paniculata* suggest that selective factors contribute to the deficiency of the S morph in tristylous populations. Where long-tongued pollinators that normally service populations are absent, the S morph suffers reduced female fertility as a result of its concealed female reproductive parts. Computer calculations indicate that with the observed reductions in fertility, the S morph will be driven down in frequency and ultimately lost from populations (Barrett *et al.* 1989). Loss of the S morph occurs more rapidly if the strength of disassortative mating is reduced (Fig. 7). Since *E. paniculata* is highly self- and intramorph compatible (Fig. 3C), alterations in pollinator service resulting from local demographic or environmental conditions make populations more vulnerable to changes in mating pattern than in tristylous species with strong incompatibility (Barrett and Husband 1990). Similar arguments also apply to self-compatible *D. verticillatus* and weak disassortative mating undoubtedly contributes to morph loss in this species also (Fig. 5D).

Controlled pollinations of the floral morphs of *E. paniculata* have consistently demonstrated reduced fertility of the S morph following self- and intramorph pollinations (Kohn and Barrett 1992a). While the genetic
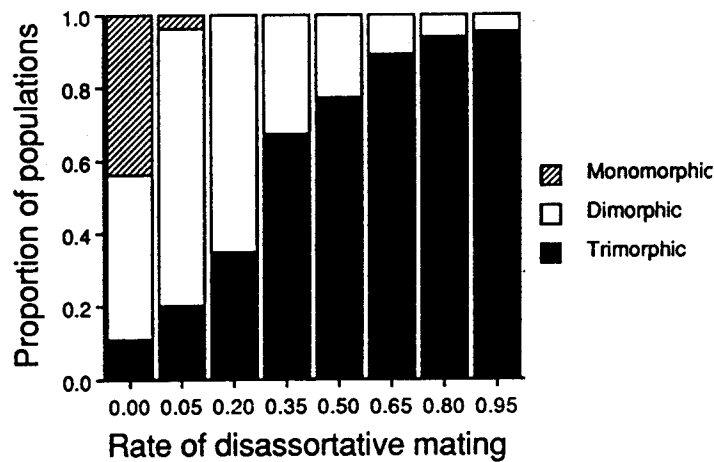

Fig. 7. The effect of different rates of disassortative mating on stochastic morph loss in simulated tristylous populations. The bars are divided into the proportion of 100 populations of size 50 that were trimorphic, dimorphic, or monomorphic after 100 years. See Eckert and Barrett (1992).

basis of this effect is not well understood (e.g., inbreeding depression and/or weak incompatibility), it is possible that in small populations experiencing inbreeding the S morph may suffer disproportionately, affecting its frequency relative to that of the L and M morphs. Additional studies of the mating patterns and fertility of floral morphs are required to establish the particular selective mechanisms responsible for the low frequency of the S morph. Regardless of the mechanism(s) involved, the fact that the S morph often occurs at a lower frequency than the remaining morphs makes it even more susceptible to stochastic loss from small trimorphic populations.

Comparison of morph frequency data for tristylous populations of *E. paniculata* (Fig. 5E) and the related *Pontederia cordata* (Fig. 5B) illustrates a striking difference between the two species. In *P. cordata* the S morph is often in excess of isoplethic expectations while, as discussed above, in *E. paniculata* the reverse pattern occurs. The predominance of the S morph in *P. cordata* is associated with a deficiency of the L morph and occurs irrespective of locality, habitat type or population size (L = 0.255, M = 0.340, S = 0.405; N = 69 populations). Barrett *et al.* (1983) proposed that the observed anisoplethic morph structure in *P. cordata* resulted from mating asymmetries among the morphs due to differences in pollen production. Earlier, Price and Barrett (1982) had unexpectedly revealed that mid-level anthers of the S morph produce on average nearly twice as much pollen as the equivalent anther level in the L morph. Using computer calculations, Barrett *et al.* (1983) demonstrated that the observed differences in pollen production among the morphs could account for the excess of the S morph and deficiency of the L morph owing to preferential fertilization of M ovules by pollen of the S morph. Experimental manipulations of pollen transport and progeny tests in natural populations provided limited support for the male fertility hypothesis but were not decisive.

Subsequently, Morgan and Barrett (1988) demonstrated that in non-equilibrium populations morph frequencies are strongly influenced by the style morph genotypes of founding individuals. They found that when populations were initiated by genotypes capable of producing trimorphism, non-equilibrium populations had an excess of the S morph and a deficiency of the L morph, as observed. Since seedling establishment is highly episodic in *P. cordata*, populations may frequently be composed of a restricted number of style length genotypes that are descendants of the initial founding morphs. Progress towards equilibrium is therefore likely to be slow because of ecological restrictions (water depth) on further establishment. Thus it seems quite possible that in small non-equilibrium populations historical factors account for much of the observed anisoplethy, whereas in large populations male fertility differences among the morphs are more likely to be involved.

Unfortunately, experimental tests of hypotheses based on male fertility are difficult to undertake in natural populations of *P. cordata* using genetic markers. A recent study of male fertility differences in experimental populations of *E. paniculata* demonstrated that the S morph sired 2–3 times more ovules of the M morph than the L morph (Kohn and Barrett 1992*b*). However, in this study individuals of each floral morph were homozygous for three different alleles at the marker locus. Hence the paternal morph responsible for siring each seed could be determined unambiguously. Molecular markers, such as RAPDs or various mini-satellite DNA sequences (Schaal *et al.* 1991), may eventually enable critical tests of the male fertility hypothesis in natural populations of *P. cordata*.

Among tristylous species that have been surveyed extensively, the patterns of morph frequency variation in *Narcissus triandrus* are most distinctive. A survey of 80 populations in Spain and Portugal revealed that the L morph predominates in most populations with an average frequency in trimorphic populations of 0.567, compared to 0.219 for the M morph, and 0.214 for the S morph. Fifteen per cent of the populations sampled were dimorphic, all were missing the M morph and in the vast majority the L morph predominated ($L = 0.712$, $S = 0.288$; $N = 12$ populations). When data on morph ratios are plotted it is evident that populations of *N. triandrus* occupy a restricted and distinct region of morph frequency space in comparison with most other tristylous species (Fig. 5F). Data for *N. triandrus* bear some resemblance to *Oxalis alpina* (Fig. 5C), where all dimorphic populations are missing the M morph and the L morph often occurs at the highest frequency.

The strongly anisoplethic morph ratios in *N. triandrus* probably result from the unique association of tristyly with a putative multiallelic incompatibility system in the species. Since floral morphology and incompatibility are largely uncoupled in populations, equilibrium morph frequencies are likely to be quite different from those in tristylous species with conventional self- and intramorph incompatibility (Fig. 3A). All cross-pollinations in *N. triandrus* are equally compatible (Fig. 3B), hence morph ratios will be governed by the genetic system controlling tristyly and the relative fitness of the floral morphs as male and female parents. Estimates of fruit and seed set in tristylous populations indicate that the female fertility of the floral morphs are similar (S. C. H. Barrett, unpublished data); therefore, it seems probable that their effectiveness as pollen parents may be more important. The anomalous features of floral morphology in *N. triandrus* (Fig. 2) may provide clues for determining which aspects of the mating process favor the L morph. Elsewhere this phenotype predominates in populations of *Anchusa officinalis*, a distylous species with a putative multiallelic incompatibility (Philipp and Schou 1981) and in the few non-heterostylous species with

stigma-height polymorphisms (e.g., *Chlorogalum angustifolium*, Jernstedt 1982; *Epacris impressa*, O'Brien and Calder 1989).

The evolutionary processes responsible for dimorphic populations in *N. triandrus* and *O. alpina* (Fig. 5) are likely to be quite different. Extensive research by Stephen Weller (reviewed in Weller 1992) involving comparative data on the morphology, cytology, reproductive biology, and genetics of trimorphic and dimorphic populations of *O. alpina* has provided strong evidence that dimorphism is a derived condition in this species, and probably in other taxa of section *Ionoxalis* that are exclusively distylous. Further, theoretical models have confirmed that genetic modifications to incompatibility in the L and S morphs revealed by Weller's experimental studies can account for the selective elimination of the M morph from tristylous populations (Charlesworth 1979: Heuch 1979*a*).

The unique features of the tristylous reproductive system of *N. triandrus* and its isolated occurrence in the genus suggest that the dimorphic state of some populations may represent an ancestral condition. The disassociation of floral polymorphism and incompatibility in *Narcissus* removes most of the genetic and developmental constraints envisioned by Charlesworth (1979) in accounting for the evolution of tristyly in taxa with trimorphic incompatibility. Furthermore, the anomalous stamen positions of the floral morphs, in comparison with other tristylous species, provides a simple explanation that might readily explain the origins of trimorphism from dimorphism in *N. triandrus*.

The phenotypes of the L and M morphs in tristylous populations of *N. triandrus* differ in style length, not stamen position (Fig. 2). The genetic and developmental changes required to produce a mid-styled phenotype from the L morph are therefore likely to be minor, involving a simple shortening of the style. Under conditions where improved cross-pollination is advantageous, perhaps owing to the presence of specific pollinators, variant phenotypes of the L morph with mid-length styles may be favored. The variants could benefit by increased pollen dispersal from their upper level anthers, owing to reduced stylar interference, and improved levels of pollen transfer to their own mid-level stigmas from lower level anthers of the S morph. According to this model, dimorphic populations would be vulnerable to invasion by variant L phenotypes with shortened styles wherever appropriate genetic variation in style length occurs and pollinators capable of mediating segregated pollen transfer are present to service populations.

Several field observations have a bearing on these ideas. Dimorphic populations of *N. triandrus* are restricted to the cooler central and northern portions of the range of the species in Spain and Portugal. They appear to be serviced by different pollinators (e.g., *Bombus* spp.) from those commonly observed in tristylous populations (e.g., *Anthophora*

spp.) from warmer Mediterranean regions (S. C. H. Barrett, unpublished observations). In addition, tristylous populations in the region of dimorphism contain very low frequencies of the M morph and mid-styled individuals in populations typically exhibit wide variation in style length. This suggests that style length is poorly canalized and that these phenotypes may represent 'naive variants' of recent origin. Further ecological and genetic studies are required to determine the evolutionary relationships between floral dimorphism and trimorphism in *N. triandrus*. Of particular importance will be evidence that can distinguish between the hypothesis that dimorphic populations of *N. triandrus* represent an ancestral condition or whether like *O. alpina*, floral dimorphism has arisen through selective elimination of the M morph from tristylous populations.

3.3 Evolutionary breakdown

Compared to our limited knowledge of the evolution of tristyly much more is known about the breakdown of the polymorphism and evolution of various derivative breeding systems. This topic has recently been reviewed in detail by Weller (1992) and only a brief summary is given here. While changes in breeding system are reported from distylous groups (reviewed in B. Charlesworth and D. Charlesworth 1979; Ganders 1979a; Barrett 1989), the prevalence of genetic modifications to tristyly suggest that because of its greater complexity this particular polymorphism is inherently less stable. In most tristylous species investigated in any detail discrete phenotypic variants with modified floral characters have been found (e.g., Stout 1925; Esser 1953; Mayura Devi and Hashim 1966; Ornduff 1972; Barrett 1988a). Moreover, since trimorphic incompatibility often varies in expression, the potential for self-fertilization and deviations from strict disassortative mating is present in most populations. Under suitable ecological and demographic conditions this morphological and physiological variation provides the necessary genetic substrate for evolution to alternative reproductive modes.

The breakdown of tristyly, as the term implies, involves the dissolution of the polymorphism through the permanent loss of floral morphs from populations. While segregation in genotypes heterozygous at the style length loci can reintroduce morphs not initially present in a population, there is no evidence (except possibly in *N. triandrus*, see above) that mutation at the style length loci is capable of giving rise *de novo* of missing morphs. As discussed earlier, morph loss from tristylous populations occurs through a variety of different ecological processes. In many cases this is not associated with evolutionary change because of limited sexual reproduction (e.g., *E. crassipes* and *D. verticillatus*) or where non-trimorphism is transient because of reintroduction of missing style length

alleles through gene flow (Halkka and Halkka 1974, but see Heuch 1980). Evolutionary modifications to tristyly are more likely to occur in geographically isolated populations undergoing regular sexual reproduction (e.g., *E. crassipes* in Costa Rica, Barrett 1979; *E. paniculata* in Jamaica, Barrett 1985). The establishment of monomorphic populations in distylous species is often short-lived because diallelic incompatibility effectively curtails further reproduction. In tristylous species, however, the loss of a single morph may not impair reproductive success and floral dimorphism may set the stage for subsequent evolutionary modifications to occur. Even where two morphs are permanently lost from populations the common occurrence of weak incompatibility, particularly in the M morph, may enable populations to persist long enough to allow selection on floral traits that increase fertility.

Tristylous breeding systems have been modified in a variety of different ways (e.g., see Fig. 10 in Ganders 1979a). The variation can largely be grouped into two contrasting shifts in mating system: (1) the evolution of distyly by loss of one of the floral morphs and subsequent genetic modifications to floral traits; and (2) the evolution of predominant self-fertilization through the spread and fixation of semi-homostylous variants. The particular pathways and genetic mechanisms by which these different breeding systems have established varies among tristylous taxa.

In the Lythraceae and Oxalidaceae distyly appears to arise most often through the loss of the M morph, although the S morph may also be involved. The shift to distyly may or may not be accompanied by the loss of a stamen set and can occur in both self-compatible or self-incompatible taxa. In the latter case loss of incompatibility differentiation between the two stamen levels of the L and S morphs is evident (e.g., *Oxalis alpina*, Weller 1976b). Exclusively distylous genera with two stamen levels and floral morphs corresponding to the L and S morphs are interpreted as being derived from tristylous ancestors through loss of the M morph (e.g., Lythraceae: *Pemphis acidula*, Lewis and Rao 1971; Oxalidaceae: *Sarcotheca celebica*, Lack and Kevan 1987). Distylous breeding systems are absent from the Pontederiaceae; Weller (1992) suggests that this may reflect differences in the nature of incompatibility systems in the families but what these differences may be is unclear.

The shift to floral monomorphism and varying degrees of self-fertilization has occurred on numerous occasions in each of the three well-established tristylous families via the evolution of semi-homostyly. Semi-homostyles occur as sporadic variants in otherwise tristylous species (e.g., *Biophytum sensitivum*, Mayura Devi and Hashim 1966) or the condition may be fixed at the population or species level (e.g., *E. paniculata* and *E. diversifolia*, respectively, Barrett 1988a). The variants are most commonly self-compatible and because of the close proximity of anthers and stigmas they are largely self-pollinating. Semihomostyly

is usually associated with reductions in the size and showiness of flowers (Ornduff 1972) as well as changes in the relative allocation of resources to male and female function (Morgan and Barrett 1989).

The evolutionary breakdown of tristyly to semi-homostyly has been investigated most thoroughly in the Pontederiaceae where it represents the major pathway of breeding-system evolution. Studies of the ecological genetics of *E. paniculata* populations have provided insights into the selective mechanisms responsible for the breakdown of tristyly and on the genetic consequences of increased inbreeding (Barrett *et al.* 1989; Barrett and Husband 1990). Genetic bottlenecks resulting from long-distance dispersal and colonization of ephemeral aquatic habitats with uncertain moisture regimes play a major part in disrupting the maintenance of tristyly in this species.

4. FUTURE PROSPECTS

A diversity of evolutionary patterns and processes are evident among the small number of plant species possessing tristylous genetic polymorphisms. Real tristylous populations often deviate from the 1:1:1 morph ratio predicted by equilibrium models and the elegant symmetry in mating relationships depicted in Fig. 1 may, more often than not, be violated owing to genetic modifications to floral traits. The distinctive features of the tristylous syndrome of *Narcissus triandrus* and, in particular, the association of tristyly with a putative multiallelic incompatibility system should warn against the oversimplified view of the polymorphism frequently portrayed in textbooks. Despite this variation, however, several recurrent patterns are evident among unrelated taxa. The remarkable similarities in the inheritance of tristyly, combined with the striking convergence in morphological features of the syndrome, imply that similar evolutionary processes are responsible for the polyphyletic origin of the polymorphism. A major challenge for future studies will be to clarify what the nature of these processes are likely to have been. Research on the following three topics would seem to be particularly warranted.

Information on the molecular and developmental genetics of tristyly is required to determine the number, location, organization, and regulation of genes controlling floral organogenesis and incompatibility. Supergene models are most often used to explain the close association between characters of the heterostylous syndrome. Other models should also be considered for tristylous plants. It is possible that regulatory genes, operating in a morph- or tissue-specific manner in ways similar to sex-limited gene expression, could govern the expression of tristylic traits. Such genes might operate by switching development along alternate pathways or by controlling hormone gradients influencing differential

organ growth and perhaps incompatibility expression. Experimental studies with hormones would be particularly interesting to see whether manipulations of organ levels within a flower are capable of altering incompatibility responses. The availability of a range of genetic variants with modified floral traits might also provide opportunities for molecular studies in this area. Rapid advances in molecular genetics will undoubtedly resolve many of the outstanding issues concerned with the genetic architecture of tristyly. Information from such work is required for developing further models of the evolution of tristyly.

Studies of the population biology of tristylous plants have provided some information on the selection of tristyly and its adaptive significance. However, the research has been limited to a few studies, primarily on members of the Pontederiaceae, and further studies on additional tristylous species are desirable. Tristylous plants also offer outstanding opportunities for manipulative field experiments aimed at investigating the reproductive and genetic consequences of variable floral morphology. The polymorphic nature of tristylous flowers, involving discrete variation in reproductive organ position and pollen size, enables quantitative studies of the fine scale details of the pollination process in ways not feasible in monomorphic species (Barrett and Wolfe 1986; Harder and Barrett 1993). Studies of this type, involving experimental manipulations of tristylous flowers and of the morph structure of populations, offer more powerful approaches to the study of selection than many of the correlative approaches used to date to investigate floral evolution.

One of the most persistent themes to emerge from this review concerns our ignorance of the phylogenetic relationships of most tristylous taxa, and their immediate ancestors. Most work on heterostyly has focused at the population level with little serious consideration given to the historical context of evolutionary change. This situation is not unique to heterostylous breeding systems, but reflects the fact that most workers in the modern era interested in the evolution of plant breeding systems have adopted microevolutionary approaches (but see Donoghue 1989). In order that hypotheses on the evolution and breakdown of tristyly can be tested more rigorously, phylogenetic analyses using cladistic approaches need to be undertaken. Studies of this type should benefit greatly from advances in molecular systematics because of the array of new molecular characters that can be employed in phylogenetic reconstruction (Soltis et al. 1991). These approaches are particularly critical for the Lythraceae and Oxalidaceae since these families are large and the phylogenetic relationships of taxa poorly understood. Phylogenetic studies are particularly important for heterostylous groups since theoretical models of the evolution and breakdown of the polymorphisms frequently differ in the direction and sequence of character transformation that are invoked. By using cladistic approaches it should be possible to provide more detailed

information on the pathways of evolution in tristylous groups. Such information may help guide future experimental work on the selective mechanisms responsible for the evolution and breakdown of these complex floral polymorphisms.

ACKNOWLEDGMENTS

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