

Mating-system evolution in flowering plants: micro- and macroevolutionary approaches*

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SUMMARY

The hermaphroditism of most plants and their reliance on vectors for pollen dispersal complicates mating patterns in comparison with most animal groups. Since Darwin's early work there has been considerable interest in the causes and consequences of self- and cross-fertilization in plant populations. Most research on this topic has been ahistorical in perspective and conducted almost exclusively within a selectionist framework. It is suggested that a broadened view encompassing both micro- and macroevolutionary analysis is necessary for a comprehensive understanding of how and why mating systems evolve from one state to another. An attempt to illustrate the diverse approaches that can be used in studying mating-system evolution in flowering plants is presented using the heterostylous, aquatic genus *Eichhornia* as a model system. Evidence from molecular phylogenetic reconstruction, large-scale surveys of geographical variation and manipulations of experimental populations are used to address the issue of how often the shift from outcrossing to selfing has occurred and what evolutionary mechanisms are involved. Results indicate that selfing has originated on several occasions in the genus and that interactions between genetic drift and natural selection cause destabilization of heterostyly and the evolution of predominant self-fertilization. It is argued that geographical patterns of intraspecific variation in reproductive traits are likely to provide the critical link between micro- and macroevolutionary approaches to the study of plant mating systems.

Key-words: mating systems, self- and cross-fertilization, tristily, *Eichhornia*, phylogenetic reconstruction, genetic drift, natural selection.

INTRODUCTION

Flowering plants display complex mating patterns in comparison with most animal groups, with individual parents often mating simultaneously with numerous sexual partners during their reproductive lifetime as well as with themselves. This difference arises primarily because of the hermaphroditic nature of most plants, their reliance on biotic or abiotic vectors for pollen dispersal and the fact that gender in plants varies in

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a quantitative manner depending on the sexual system, demography and life-history strategy of populations. Female and male sex organs in plants can be combined or separated at different levels including the flower, the inflorescence, the individual and the population, giving rise to a diverse array of potential ways that gametes can unite to give rise to succeeding generations (Lloyd 1979, 1980a, 1982). How gametes unite depends largely on the pollination biology and compatibility system of individual species and here considerable complexity is also introduced (Waser 1993; Harder & Barrett 1995a; Holsinger 1995). The realized mating pattern in plant populations usually differs from the potential set of male gametes arriving on stigmas following pollen dispersal because post-pollination physiological mechanisms of discrimination act to reject unsuitable partners (Jones 1928; Mulcahy *et al.* 1983; Stephenson & Bertin 1983; Marshall & Folsom 1991; Barrett & Cruzan 1994; Sage *et al.* 1994). Despite the complexity of plant mating patterns, the scientific study of plant mating systems has largely focused on the importance of a single comparison: the relative amounts of self- and cross-fertilization that occur in plant populations (Lloyd 1979, 1980b; Lande & Schemske 1985; Schemske & Lande 1985; Barrett & Eckert 1990; Lloyd & Schoen 1992; Uyenoyama *et al.* 1993). In this review I focus on the diverse approaches that can be used to study selfing and outcrossing in plants and provide some historical background to how such approaches developed.

Why has the study of plant mating systems, beginning with Darwin's contributions (Darwin 1876, 1877), been mostly concerned with understanding the causes and consequences of differences in selfing and outcrossing, rather than on aspects of male fertility and plant paternity? The answer to this question undoubtedly reflects an early lack of appreciation of the significance of the male component of reproductive fitness as well as technical difficulties associated with studying mate diversity in plant populations. However, there are also three compelling biological reasons why the selfing–outcrossing paradigm has received most attention from plant reproductive biologists: (i) the influences of selfing and outcrossing on fitness are well established, with the frequency of selfing having a profound effect on the number and quality of offspring owing to the phenomenon of inbreeding depression (Knight 1799; Darwin 1876; Charlesworth & Charlesworth 1987); (ii) the balance between selfing and outcrossing is the most important determinant of population genetic structure since it affects both the level of genetic diversity within populations and the amount of genetic differentiation among populations of a species (Brown 1979; Hamrick & Godt 1990); (iii) the fitness consequences of selfing and outcrossing have an important influence on floral evolution to the extent that many aspects of floral design are best understood in functional terms as mechanisms to encourage or discourage self-pollination (Darwin 1877; Wyatt 1983; Richards 1986). It is therefore not surprising that the comparison of self- and cross-fertilization has often been considered the central topic of floral biology (Lloyd & Schoen 1992), although this perspective is beginning to change as more attention is given to the importance of floral strategies for maximizing pollen dispersal and male reproductive fitness (Stephenson & Bertin 1983; Harder & Thomson 1989; Harder & Barrett 1995a,b).

Studies on the ecology and evolution of plant mating patterns can be divided into three historical phases. The first, beginning with the early naturalists, was largely descriptive and involved detailed natural history observations on the biology of flowers and inferences on the likely patterns of mating based on studies of pollination mechanisms (reviewed by Baker 1983). This approach continues today, particularly in

tropical regions with less well known floras. While work on mating systems has grown in sophistication over the past few decades, basic studies in floral biology should form the starting point for any study in this area since the pollination process fundamentally determines the frequency and diversity of mating opportunities for any plant.

During the 1970s and 1980s a new approach to the study of plant mating emerged, as population geneticists developed methods for measuring mating patterns in experimental and natural populations (Clegg 1980; Shaw & Brown 1982; Ritland 1983). By using allozyme markers it has become straightforward to estimate the amount of selfing and outcrossing that occurs in populations, enabling workers to go beyond the qualitative inferences obtained from natural history studies of flowers. This advance has been of particular importance for the many self-compatible hermaphroditic species with mixed mating systems where the relative amounts of selfing and outcrossing can be hard to assess from observations of floral biology alone. The application of electrophoretic techniques to the study of plant mating patterns revolutionized the field and has enabled estimation of many important parameters that are featured in models of mating-system evolution (e.g. Clegg & Epperson 1988; Ritland 1990; Holsinger 1992; Kohn & Barrett 1994). Over the past decade, the development of theoretical models and the marriage of marker-gene studies with natural history studies of floral biology has resulted in significant advances in our understanding of the ecology and evolution of plant mating (reviewed in Barrett *et al.* 1992; Lloyd & Schoen, 1992; Schoen & Lloyd 1992; Uyenoyama *et al.* 1993).

Studies of plant mating systems have recently entered a new phase as two distinct but complementary approaches have been applied to the field. First, through developments in phylogenetic analysis and molecular systematics, workers have begun to reconstruct the evolutionary history of plant reproductive traits associated with the pollination biology and mating systems of species (Hart 1985; Eckenwalder & Barrett 1986; Donoghue 1989; Cox 1990; Sytsma *et al.* 1991; Rieseberg *et al.* 1992; Armbruster 1993; Weller *et al.* 1995). As recently pointed out by Kohn *et al.* (1996), studies on the evolution of plant reproductive systems are well suited to historical reconstruction. First, closely related taxa often show considerable intra- and interspecific variation in traits influencing mating patterns. Secondly, theoretical models with specific hypotheses concerning the order of character state change are available for testing. Lastly, a considerable body of population-level ecological and genetic data exists for some plant groups providing an important opportunity to link micro- and macroevolutionary approaches to the study of plant evolution. Phylogenetic studies of mating-system evolution in plants are in their infancy, but it seems likely that they will emerge as a major growth area over the next few years.

With increased interest in the evolutionary ecology of plant reproduction (reviewed in Wyatt 1992) has come a second development involving experimental work and, in particular, the use of manipulative experiments involving natural and experimental plant populations. Two main lines of enquiry have been followed in this research. First, workers have sought to investigate the fitness consequences of particular patterns of mating by comparing the performance of offspring obtained by controlled crosses among selfed and outcrossed individuals and those of different degrees of relatedness. While this approach has a long and distinguished tradition, going back over two centuries to the work of Knight and Darwin, these modern comparisons have been made under a wide range of environmental conditions that attempt to reflect more closely the ecological context in which selection occurs (e.g. Schemske 1983; Kohn 1988;

Dudash 1990; Schmitt & Ehrhardt 1990; Schmitt & Gamble 1990; Fenster 1991a,b; Waser 1993; Uyenoyama *et al.* 1993).

A second innovation involves attempts to investigate the direct influence of reproductive traits on pollen dispersal and patterns of mating. This has been achieved by manipulating both floral characters and the demographic features of populations such as their size, structure and phenotypic attributes. By using pollen markers (size, colour or pollen surrogates) to measure pollen transfer within and between plants (e.g. Waser & Price 1984; Thomson 1986; Feinsinger & Busby 1987; L. D. Harder & S. C. H. Barrett, unpublished) or genetic markers to estimate mating parameters (e.g. Kohn & Barrett 1992a,b; Schoen & Lloyd 1992; Barrett *et al.* 1994; Leclerc-Potvin & Ritland 1994) some assessment can be made as to the causal agents responsible for particular patterns of pollen dispersal and mating. In these studies the underlying approach is a mechanistic and largely reductionist approach in which an attempt is made to decompose the mating process into its elementary causative agents by the use of manipulative experiments. While these types of experiments may seem some way from the descriptive natural history traditions of reproductive biology it is important to realize that such traditional approaches lie at the heart of these modern experimental approaches since the specific details of floral biology and pollination play a central role in governing mating patterns.

In this paper I review recent research that we have conducted on the heterostylous, aquatic genus *Eichhornia* (Pontederiaceae) that illustrates some of the diverse approaches that can be used to investigate mating-system evolution in plants. I begin by using comparative approaches at the species level to explore the evolution of self-fertilization and, in particular, ask whether phylogenetic analysis can assist in determining if selfing has had multiple origins within the genus. I then show how population-level enquiry involving large-scale surveys of geographical variation can provide a powerful tool for addressing questions concerning the relative importance of different evolutionary mechanisms responsible for mating-system variation and evolution within *Eichhornia* species. Having established patterns at the species and population level, I then review recent studies that have used experimental approaches to test hypotheses concerning the role of selection operating within populations. This hierarchical approach using comparative and experimental analysis at the interspecific, interpopulation and intrapopulation level provides opportunities for integrating both micro- and macroevolution sources of data in addressing issues concerned with the evolution of self-fertilization.

PHYLOGENETIC RECONSTRUCTION OF THE EVOLUTION OF SELF-FERTILIZATION

The evolution of predominant self-fertilization from outcrossing is one of the most pervasive themes in floral biology, since the acquisition of the selfing syndrome can have profound ecological, genetic and evolutionary consequences (Stebbins 1970; Jain 1976). Two important questions arise when studying a particular taxonomic group—how often has predominant selfing arisen and what selective mechanisms are involved? Among heterostylous families the evolution of selfing has occurred repeatedly via the origin of homostyly. Homostylous species are monomorphic for floral traits with individuals usually self-fertile and possessing anthers and stigmas at the same height within a flower. As a result of these features homostyles set most of their seed through self-pollination.

It has generally been assumed, following Darwin (1877), that homostylous species are evolutionarily derived from heterostylous ancestors but the factors responsible for the change in mating pattern from outcrossing to selfing have been much debated (Ganders 1979; Richards 1986; Charlesworth & Charlesworth 1987; Barrett 1989).

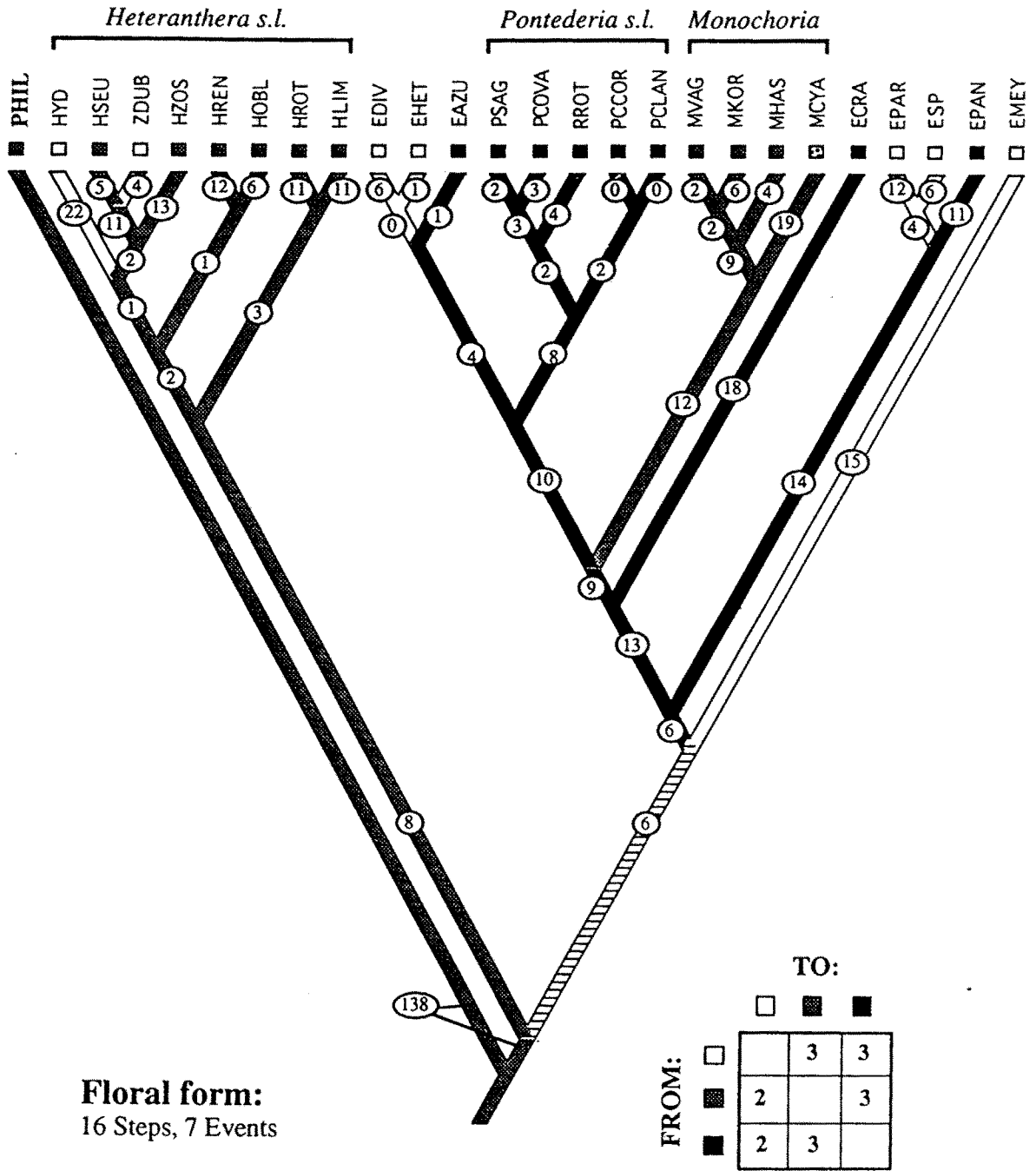
To examine the origins of selfing we have used phylogenetic analysis to investigate the evolution and breakdown of heterostyly in the small tristylous monocotyledonous family Pontederiaceae. This taxon is particularly suitable for phylogenetic reconstruction of the evolution of reproductive traits for at least three reasons: (i) it contains considerable floral variation associated with outcrossing and selfing floral syndromes both within and between species, particularly in *Eichhornia* (Barrett 1988); (ii) it is relatively small and apparently monophyletic (Graham & Barrett 1995); (iii) considerable microevolutionary information is available for several species of *Eichhornia* that display mating-system variation. Our studies have focused on the evolutionary relationships among the three major floral syndromes in the family—tristyly, enantiostyly and homostyly. In this discussion, however, I only consider the evolutionary breakdown of tristily to homostyly. Details of other possible evolutionary relationships among the floral syndromes are discussed more fully in Graham & Barrett (1995).

Population-level studies of tristylous *Eichhornia paniculata* reviewed below suggest that the tristylous polymorphism has broken down repeatedly to give rise to selfing, homostylous species. However, a phylogenetic analysis of the family involving 34 taxa and 42 morphological characters undertaken by Eckenwalder & Barrett (1986) indicated that the shift from tristily to homostyly occurred just once giving rise to a group of selfing homostylous species. These authors warned against accepting the interpretation of a single origin for homostyly in *Eichhornia* because they suspected that the homostylous species were linked as a monophyletic group because of convergent evolution of characters associated with the selfing syndrome. Phylogenetic reconstruction using morphological characters is likely to be compromised in groups where repeated transitions to selfing have occurred since this evolutionary shift is typically accompanied by multiple parallel changes to a broad range of reproductive and life-history traits. This violates the critical assumption of character independence that is implicit in phylogenetic reconstruction.

To avoid the distorting effects of the selfing syndrome on phylogenetic reconstruction we have recently used molecular data to help elucidate the patterns of mating-system evolution in the Pontederiaceae (Graham & Barrett 1995; Kohn *et al.* 1996). This source of data is likely to be relatively free of the multiple convergences caused by correlated selection on reproductive traits that can occur using morphological characters. Figure 1 illustrates a phylogenetic reconstruction of the family using PAUP version 3.1.1 (Swofford 1993) based on combined sequence data from the two chloroplast genes *rbcL* and *ndhF*. Twenty-five taxa (23 species and three varieties of *Pontederia cordata*) are included in the analysis representing approximately two-thirds of the species in the family. The tree illustrated is the shortest found with a length of 459 steps (CI=0.549, excluding uninformative characters; RI=0.776). Reconstruction of character evolution on the tree was performed using MacClade version 3 (Maddison & Maddison 1992).

The phylogenetic reconstruction of reproductive characters illustrated in Fig. 1 indicates that tristily evolved near the base of the family and was subsequently lost on at least three occasions, with at least two losses giving rise to homostylous species. This result therefore conflicts with that obtained from the phylogenetic analysis of morphological data discussed above. However, it is more in accord with microevolutionary

evidence reviewed below which suggests that the shift from tristylly to homostyly is readily achieved. Two phylogenetically distinct clades of *Eichhornia* each consisting of a tristylous species (*E. azurea* or *E. paniculata*) and two selfing species are evident in the phylogeny. In addition, tristylous *E. crassipes* and the selfing *E. meyeri* are in neither of these two groups. Although the apparent polyphyly of *Eichhornia* complicates phylogenetic interpretation and also has interesting systematic implications for the status of the genus, it is clear from the molecular data that homostylous species of *Eichhornia* are unlikely to be of monophyletic origin. Recently, D. J. Schoen *et al.* (unpublished) have also provided evidence for the multiple breakdown of heterostyly to homostyly in



Step Matrix for "Relaxed-Dollo"
Optimization Scheme for Floral Form

Amsinckia (Boraginaceae) based on molecular phylogenetic data. The apparent ease with which the transition from outcrossing to selfing is achieved in heterostylous groups is undoubtedly associated with the relatively simple genetic basis of homostyly involving either recombination or major gene changes at modifier loci (Lewis & Jones 1992; Fenster & Barrett 1994).

POPULATION SURVEYS OF GEOGRAPHICAL VARIATION

While microevolutionary analysis of selective mechanisms obviously requires detailed demographic and genetic studies conducted *within* local populations, investigators should not ignore the fact that an unduly myopic view concentrating solely on the local population rather than a metapopulation, regional or geographical perspective may result in missed opportunities for evolutionary analyses (Husband & Barrett 1996). For example, patterns of reproductive-trait variation of significance to the mating system can often be revealed at the species level by examining large numbers of populations over a broad geographical area or across important ecological gradients. Mating systems may be evolutionarily stable over much of the range of a species with 'hot spots' of variation restricted to particular ecological or geographical zones. These situations may, however, be of critical importance for understanding the processes of evolutionary differentiation and speciation via mating-system divergence. They can therefore provide useful insights bridging microevolutionary processes with macroevolutionary patterns. It has certainly been the experience of our laboratory that an initial broad-scale survey of geographical variation in reproductive traits combined with the collection of simple ecological and demographic information almost always repays the effort by revealing novel variation of evolutionary significance. In several cases, e.g. gender variation in cosexual and dioecious *Wurmbea dioica* (Barrett 1992) and floral variation in heterostylous *Decodon verticillatus* (Eckert & Barrett 1992, 1994), *Narcissus* spp. (Barrett *et al.* 1995) and *Turnera* spp. (Barrett & Shore 1987), studies of just a few populations would not have detected the important patterns of variation that later formed the basis for in-depth ecological-genetic studies of selection in local populations.

Fig. 1. Phylogenetic reconstruction of reproductive characters in the Pontederiaceae using the most parsimonious tree found from a combined analysis of the chloroplast genes *ndhF* and *rbcL*. Modified from Graham & Barrett (1995). The number of steps on each branch indicates the reconstructed number of base substitutions and indels for these two genes on the tree (using ACCTRAN optimization in PAUP version 3.1.1.; Swofford 1993). Shifts in floral syndrome (tristyly, enantiostyly and homostyly) on the tree were reconstructed using MacClade version 3 (Maddison & Maddison 1992). The floral syndromes were optimized onto the tree using a relaxed-Dollo (*sensu* Swofford & Olsen 1990) optimization scheme. The step-matrix employed (shown) gives a slightly smaller weight to the loss of tristily or enantiostyly (i.e. a shift to homostyly or floral monomorphism) than to the gain or interconversion between these two flower types. See Graham & Barrett (1995) for the rationale for using an unequally weighted optimization scheme. Taxa were coded as tristylous ■, enantiostylous ▣, or florally monomorphic/homostylous □. *Monochoria cyanea* was coded as enantiostylous or monomorphic (i.e. uncertain: ◻). ◻ = Equivocal reconstruction. Two to three shifts from predominantly outcrossing, tristylous species to predominantly selfing, homostylous taxa are required. A trichotomy involving *E. azurea*, *E. heterosperma* and *E. diversifolia* was arbitrarily resolved; alternative resolutions of this trichotomy would yield an extra loss of tristily on the tree (not shown). Abbreviations of taxa used: PHIL, *Philydrum lanuginosum* (Philydraceae); EAZU, *Eichhornia azurea*; ECRA, *E. crassipes*; EDIV, *E. diversifolia*; EHET, *E. heterosperma*; EMEY, *E. meyeri*; EPAN, *E. paniculata*; EPAR, *E. paradoxa*; ESP, *Eichhornia* sp.; HLIM, *H. limosa*; HOBL, *H. oblongifolia*; HROT, *H. rotundifolia*; HSEU, *H. seubertiana*; HZOS, *H. zosterifolia*; HYD, *Hydrothrix gardneri*; PCCOR, *Pontederia cordata* var. *cordata*; PCLAN, *P. cordata* var. *lancifolia*; PCOVA, *P. cordata* var. *ovalis*; PSAG, *P. sagittata*; MCYA, *Monochoria cyanea*; MHAS, *M. hastata*; MKOR, *M. korsakovii*; MVAG, *M. vaginalis*; RROT, *Pontederia (Reussia) rotundifolia*; ZDUB, *Heteranthera (Zosterella) dubia*.

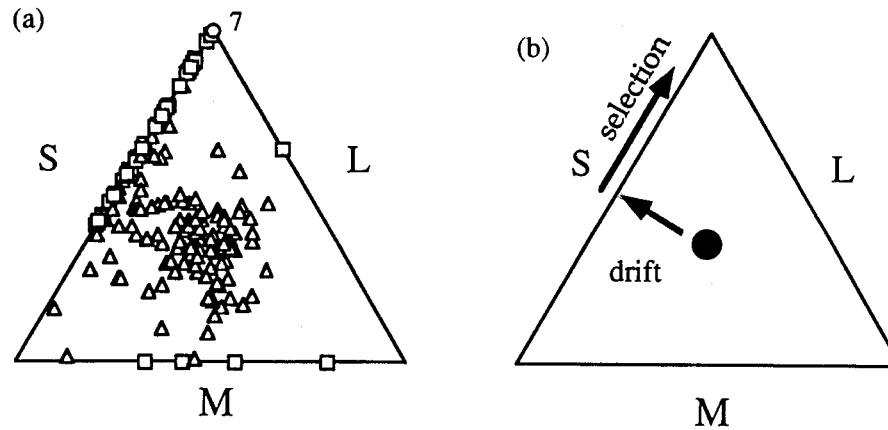


Fig. 2. Evolutionary significance of variation in style morph frequencies in tristylous *Eichhornia paniculata* (Pontederiaceae). (a) Style morph frequencies in 167 populations sampled from NE Brazil. Each side of the triangle represents one of the three style morphs (long-, mid- and short-styled morph = L, M, S, respectively), and each point represents the morph frequencies of a single population. The distance of a point from a side or the triangle is proportional to the frequency of that morph in that population. Points in the centre of the triangle have even morph frequencies (1:1:1). Trimorphic, dimorphic and monomorphic populations are represented by triangles, squares and circles, respectively. Seven populations in the sample were monomorphic for the M morph. Data are from Barrett *et al.* (1989) and Husband & Barrett (1993). (b) Model of the evolutionary breakdown of tristily to homostyly in *E. paniculata*. Arrows indicate the evolutionary pathway from outcrossing (floral trimorphism) to selfing (floral monomorphism). Genetic drift is proposed as the primary mechanism responsible for the transition from trimorphism to dimorphism and selection is implicated as the main driving force for the evolution of floral monomorphism from dimorphism.

Nowhere has the population-survey approach been more informative than in our work on mating-system variation within each of the three tristylous *Eichhornia* species (*E. azurea*, *E. crassipes*, *E. paniculata*, reviewed in Barrett 1988). Population studies have revealed the presence of selfing homostylous variants in geographically or ecologically marginal areas in each species indicating that the polymorphism is susceptible to evolutionary modification under contemporary ecological conditions. This intraspecific variation represents the critical linkage to the macroevolutionary patterns evident at the species level within *Eichhornia* and also provides opportunities for investigations of the selective mechanisms responsible for the evolution of self-fertilization in the genus. We have exploited this variation most effectively in *E. paniculata*, an annual emergent aquatic of seasonally flooded pools and ditches native to NE Brazil, Jamaica and Cuba. Below I briefly summarize the major findings of the population-level work we have conducted on this species.

Figure 2a illustrates the results of a survey of style-morph frequencies in 167 populations of *E. paniculata* from NE Brazil. Four patterns are clearly evident from the data: (i) the majority of populations are trimorphic but a significant number (29.3%) were missing one or two of the floral morphs; (ii) in many trimorphic populations the short-styled morph was under-represented in comparison with the equilibrium expectation (1:1:1); (iii) the vast majority of dimorphic populations are missing the short-styled morph, and the mid-styled morph predominates over the long-styled morph; (iv) monomorphic populations are composed exclusively of the mid-styled morph. These patterns, which could not have been detected by surveying a small number of populations, have enabled the formulation of a model of the breakdown process which has guided much of our research on this species over the past decade (Fig. 2b). The model involves two stages, first the loss of the short-styled morph from tristylous populations, and, secondly, the spread to fixation of the mid-styled morph in

non-trimorphic populations. The interaction of genetic drift and natural selection are implicated as the major evolutionary agents responsible for these changes resulting in the destabilization of tristylly and the evolution of floral monomorphism.

Loss of the short-styled morph from tristylous populations of *E. paniculata* appears to largely involve genetic drift. Theoretical work on the effects of finite population size on the maintenance of tristylly indicate that this morph is more likely to be lost from populations owing to stochastic influences (Heuch 1980; Barrett *et al.* 1989; Husband & Barrett 1992a). Such effects seem likely in this species since population bottlenecks commonly occur owing to the ephemeral nature of its aquatic habitats. Furthermore, studies on effective population size in *E. paniculata* have shown that many populations are below the critical size required for the maintenance of the polymorphism (Husband & Barrett 1992b). In some populations stochastic loss of the short-styled morph may also be aided by fertility selection against the short-styled morph owing to the irregular visitation of suitable long-tongued bees that are required to pollinate this morph (Barrett *et al.* 1989). However, at present evidence for the specific selective mechanisms that can account for the frequent deficiency of the short-styled morph *within* many trimorphic populations is unclear (reviewed in further detail in Barrett 1993).

Our surveys of *E. paniculata* have revealed that the average size of dimorphic and monomorphic populations are smaller than trimorphic populations and that many occur in ecologically or geographically marginal sites (Barrett *et al.* 1989; Husband & Barrett 1993). Fitness comparisons of the long- and mid-styled morphs have shown a substantial fitness advantage to the mid-styled morph under these conditions (Barrett *et al.* 1989). Close inspection of the patterns of floral variation in these populations have revealed the functional basis of this fitness advantage. Many of the mid-styled plants possess selfing phenotypes owing to alterations to the positions of stamens within flowers (Barrett 1988; Seburn *et al.* 1990; Barrett & Harder 1992). The stamen alterations are under the control of one or a few recessive modifier genes, with different recessives modifying stamen position in contrasting parts of the species' geographical range (Fenster & Barrett 1994). These changes to stamen position result in the autonomous self-pollination of flowers giving plants reproductive assurance under conditions of unreliable pollinator service.

The origin and spread of selfing variants in *Eichhornia* provides vital clues to the evolution of homostyly and for understanding the distribution of mating systems in the genus. In *E. paniculata* a range of selfing phenotypes differing in floral morphology occur in natural populations. These include those in outcrossing populations which are identical to heterostylous morphs except for a single altered stamen, to those in highly selfing populations which are small-flowered with three stamens adjacent to the stigma. In the latter case this morphology closely resembles that found in homostylous species of the genus and indicates that these species have probably originated through the spread of selfing variants in ancestral tristylous taxa. If homostyly originated in this manner it further supports the results of the molecular phylogenetic analysis indicating multiple shifts from tristylly to homostyly.

Microevolutionary studies of *E. paniculata* suggest that the number of origins of selfing may be considerably greater than can be revealed by phylogenetic analysis where species are the units considered. First, the genetic studies indicating different recessive modifier loci responsible for the selfing phenotype in different parts of the species' range imply multiple origins of selfing within *E. paniculata*. Secondly, an allozyme survey of 24 loci in 44 populations by Husband & Barrett (1993) is also consistent with a

hypothesis of multiple origin for homostyly within the species. Analyses of genetic distance and the distribution of rare alleles indicated that populations with selfing variants occurred in three genetically distinct parts of the range with selfing populations more similar to neighbouring trimorphic populations than to one another (Fig. 3). Given the relatively simple and different genetic basis to selfing in *E. paniculata* in different parts of its range it would seem more parsimonious to interpret the scattered distribution of selfing variants as evidence for multiple origins of the selfing phenotype rather than migration from a single ancestral population. This information, in conjunction with theoretical studies (Charlesworth 1979; Barrett *et al.* 1989; Husband & Barrett 1992a,b) that demonstrate the instability of the tristylous polymorphism in the face of the kinds of ecological and demographic pressures that these largely colonizing aquatic plants experience, makes it particularly likely that shifts from outcrossing to selfing have occurred repeatedly within individual species. Genealogical studies using phylogenetic approaches at the population level are needed to enable more accurate estimates of the number of evolutionary events that occur below the species level. At present such studies are just beginning (reviewed in Avise 1994) and as yet have not been applied to questions concerned with the evolution of mating systems in plants.

MANIPULATIVE EXPERIMENTS ON PLANT MATING PATTERNS

Experimental studies of microevolution in plant populations have a long and well-established tradition beginning with work in genecology and experimental taxonomy (Heslop-Harrison 1964; Langlet 1971), through to more recent work in population biology and evolutionary ecology (Solbrig *et al.* 1979; Solbrig 1980; Silvertown & Lovett Doust 1993). The immobility of plants, the ease with which they can be cloned and manipulated, and their propensity for local adaptation have undoubtedly been the main reasons why they have been so successfully exploited as model systems for microevolutionary study. Curiously, despite the long interest in plant reproduction there have been relatively few manipulative experiments of plant populations in which investigators have attempted to understand the proximate factors that influence mating patterns. Recently, however, several workers have employed experimental populations and genetic markers to address a variety of questions concerned with mating-system evolution in plants (e.g. Schoen & Clegg 1985; Epperson & Clegg 1987; Abbott & Irwin 1988; Stanton *et al.* 1989, 1991; Kohn & Barrett 1992a,b; Rausher *et al.* 1993). We have also used this approach to further our understanding of the selective mechanisms responsible for the maintenance of outcrossing in some populations of *E. paniculata* and the spread of selfing variants in others.

In natural populations of *E. paniculata* selfing variants are usually absent from populations containing all three floral morphs but they predominate in non-trimorphic populations (Husband & Barrett 1993). What selective mechanisms might account for the markedly non-random distribution of selfing phenotypes? Explaining the high frequency of selfing in dimorphic and monomorphic populations is relatively straightforward given the ecological and demographic characteristics of many non-trimorphic populations and the observed fitness advantages experienced by selfing phenotypes under these conditions (Barrett *et al.* 1989; Barrett & Husband 1990). However, a more difficult problem concerns the question of why selfing variants fail to spread in most trimorphic populations. We have recently addressed this issue using manipulations of floral morph structure in experimental garden populations of *E. paniculata* (Kohn &

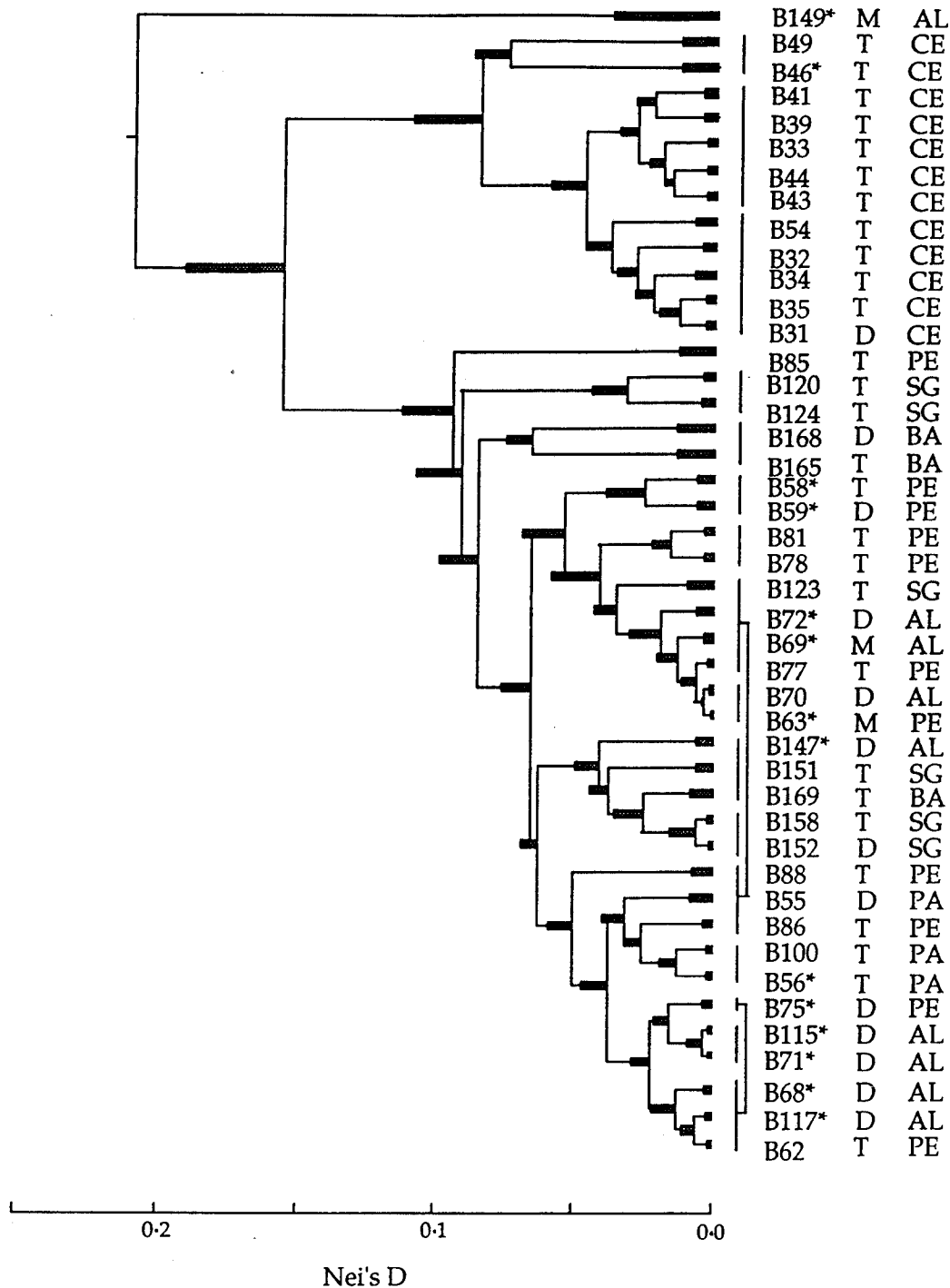


Fig. 3. Evidence for multiple origins of selfing in *Eichhornia paniculata* based on a survey of allozyme variation at 24 isozyme loci in 44 populations from NE Brazil. The genetic relationships among populations are illustrated by a UPGMA dendrogram of Nei's genetic distance (Nei 1978). Shaded bars on the dendrogram branches represent one standard error (Ritland 1989), vertical bars adjacent to the population codes identify statistically distinct clusters of populations, based on the criteria that the branch length for that cluster is greater than twice the standard error. The style morph structure (T, trimorphic; D, dimorphic; M, monomorphic) and state (AL, Alagoas; BA, Bahia; CE, Ceará; PA, Paraíba; PE, Pernambuco; SE, Sergipe) are listed for each population. Asterisks indicate the populations containing selfing variants of the mid-styled morph. The populations are dispersed throughout the dendrogram with selfing variants occurring in several statistically distinct clusters of populations. After Husband & Barrett (1993).

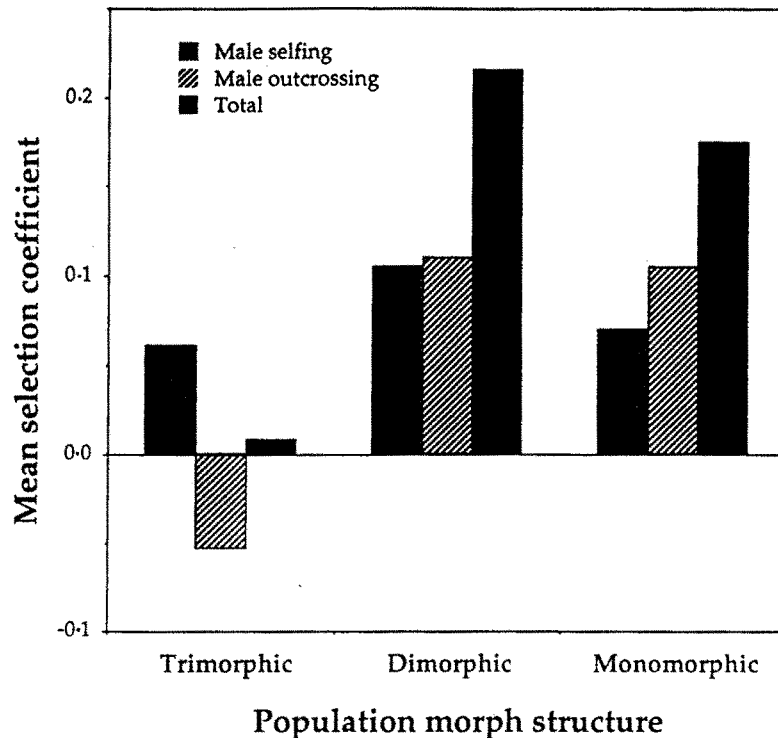


Fig. 4. Genetic transmission through pollen and ovules of the selfing variant (M') of *Eichhornia paniculata* depends on the morph structure of experimental populations. Bars indicate the mean selection coefficient [$I = 1 - (W_m)/(W_{m'})$] favouring the M' over the M morph under different experimental population morph structures. The total selection coefficient was decomposed into male selfing [$(S_{m'} - S_m)/W_m$] and male outcrossing [$(P_{m'} - P_m)/W_m$] sources to illustrate the strength and direction of selection on different components of reproductive success. In trimorphic populations genetic transmission of the M' and M morphs are not significantly different whereas in dimorphic and monomorphic populations male gamete transmission by the M' morph was approximately 1.5 times greater than the M morph. After Kohn & Barrett (1994).

Barrett 1994). The results of these experiments have provided novel insights into the complexities of plant mating in showing that fitness differences between alternate floral variants can be highly context-dependent and that studies on the evolution of selfing need to consider the fitness of selfing variants both as female and male parents.

Our experiment addressed the issue of whether the non-random distribution of selfing variants could be explained, in part, by the effects of population morph structure on the relative gamete transmission of mid- and modified mid-styled plants (hereafter M and M' morphs). Genetic transmission through ovules and self and outcross pollen of the two phenotypes were compared in trimorphic, dimorphic (short-styled morph absent), and monomorphic (long- and short-styled morphs absent) populations hence simulating the common population morph structures observed in nature (see Fig. 2a). Since each of the four phenotypes used in the experiments were uniquely marked by alleles at allozyme loci we were able to estimate male reproductive success, as well as more traditional measures such as the frequency of selfing and fecundity of each morph.

Neither population morph structure nor floral phenotype had any significant influence on female reproductive success (fruit and seed set). However, as illustrated in Fig. 4, both factors had strong effects on relative transmission via male gametes (pollen). The frequency of self-fertilization in the M' morph was consistently higher than in the M morph under all morph structures, and the frequency of self-fertilization by both morphs increased as the morph diversity of experimental populations decreased. In trimorphic populations, total genetic transmission of the M and M' morphs were not

significantly different. The small, non-significant increase in selfing by the M' relative to the M morph was balanced by decreased outcross siring success, particularly on the short-styled morph. In populations lacking the short-styled morph, male gamete transmission by the M' morph was 1.5 times greater than the M morph because of both increased selfing and increased success through outcross pollen donation. Hence, patterns of gamete transmission strongly favoured the M' morph only in the absence of the short-styled morph, a result consistent with the distribution of the selfing phenotype in NE Brazil.

Our results emphasize that it is the interaction between floral morphology and aspects of population structure, rather than morphology alone which is likely to determine the frequency of self-fertilization in self-compatible species. Although floral morphology may control the *total* amount of self pollen deposited, the *fraction* of the total stigmatic pollen load that it represents is determined by how much cross pollen arrives from other plants (Holsinger 1991, 1992). Where outcross pollen deposition rates are high, a given amount of self pollen on the stigma will cause less selfing than when outcross pollen deposition rates are low. Hence, the fitness of a variant phenotype that increases self-pollen deposition is likely to depend upon the outcross pollen transmission dynamics of the population in which it originates. This, in turn, will be governed by the pollination biology and patterns of pollen dispersal in the population. These ecological aspects of plant mating have been neglected in recent studies of the evolution of selfing because of the strongly genetical emphasis taken by many population biologists interested in mating-system evolution. While the evolutionary fate of genes modifying the mating system in the direction of increased selfing will be strongly influenced by fitness differences between selfing and outcrossing owing to inbreeding depression, we should not ignore details of pollen transfer since they are also likely to be of importance for understanding the evolutionary dynamics of plant mating systems (Lloyd 1992; Harder & Barrett 1995a; Holsinger 1995).

CONCLUSIONS

This brief review of some recent research conducted in my laboratory on mating-system evolution in plants has attempted to illustrate a variety of different approaches that can be employed to address the issue of how and why self-fertilization evolves from outcrossing. Traditional approaches to this question have tended to focus at the intraspecific level and have most often involved detailed ecological or genetic studies on a small number of populations. In our investigations on the evolution of selfing in *Eichhornia* we have found it profitable to widen our sampling to include the study of broad-scale patterns at the interpopulation and interspecific level. This approach has introduced a historical component to our work and has forced us to consider the importance of evolutionary mechanisms other than natural selection in causing the patterns of variation evident in the genus. The role of stochastic evolutionary processes in destabilizing the tristylous genetic polymorphism in *Eichhornia* would have been difficult to determine by studies on a small number of populations since the diagnostic signature of morph loss requires considerable sampling effort to detect. Recent studies on other unrelated heterostylous taxa have also demonstrated the importance of genetic drift and founder events in causing such large-scale patterns of regional variation (e.g. *Decodon verticillatus*, Eckert & Barrett 1995; *Lythrum salicaria*, Eckert & Barrett 1992; Eckert *et al.* 1996). To what extent founder events and genetic drift have played a role

in determining the patterns of mating-system variation in other groups is not known (but see Jain 1976; Templeton 1981; Barrett 1989). However, it seems improbable that such effects are not more general given the importance of colonization events and episodes of small population size in many plant species (reviewed in Barrett & Kohn 1991).

The historical component to the analysis of mating-system evolution becomes even more important when one considers the systematic distribution of mating systems in a particular taxonomic group. Our attempts at phylogenetic reconstruction of reproductive characters in the Pontederiaceae reflects part of a growing realization among reproductive biologists that the largely ahistorical perspective of earlier work represents a one-sided view of the problem of mating-system evolution. Perhaps one of the major challenges for future studies will be to integrate the large amount of microevolutionary data on mating systems that exists for many of the best studied plant taxa (e.g. *Amsinckia*, *Arenaria*, *Avena*, *Clarkia*, *Impatiens*, *Ipomoea*, *Limnanthes*, *Lupinus*, *Mimulus*, *Plantago*, *Senecio*, *Silene*, *Thymus*) with macroevolutionary information that becomes available from phylogenetic research of these genera. To take full advantage of these new sources of data it will become more important to understand the intraspecific patterns of variation that are exhibited by individual species. The discovery of selfing homostyles *within* each of the tristylous *Eichhornia* species, for example, provided us with important insights for understanding the broader phylogenetic patterns that occur within the genus. Studies of geographical variation have largely been neglected by mating-system workers interested in the mechanisms of selection. In the coming years more attention should be paid to geographical variation not only because of the possibility of revealing areas of 'evolutionary activity' for in depth demographic and genetic studies of selection, but also because such variation may provide the critical link between micro- and macroevolutionary analyses of mating-system evolution.

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