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# Mating strategies in flowering plants: the outcrossing–selfing paradigm and beyond

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Comparisons of the causes and consequences of cross- and self-fertilization have dominated research on plant mating since Darwin's seminal work on plant reproduction. Here, I provide examples of these accomplishments, but also illustrate new approaches that emphasize the role of floral design and display in pollen dispersal and fitness gain through male function. Wide variation in outcrossing rate characterizes animal-pollinated plants. In species with large floral displays, part of the selfing component of mixed mating can arise from geitonogamy and be maladaptive because of strong inbreeding depression and pollen discounting. Floral strategies that separate the benefits of floral display from the mating costs associated with geitonogamy can resolve these conflicts by reducing lost mating opportunities through male function. The results from experiments with marker genes and floral manipulations provide evidence for the function of herkogamy and dichogamy in reducing self-pollination and promoting pollen dispersal. Evidence is also presented indicating that increased selfing resulting from changes to floral design, or geitonogamy in large clones, can act as a stimulus for the evolution of dioecy. The scope of future research on mating strategies needs to be broadened to include investigations of functional links among flowers, inflorescences and plant architecture within the framework of life-history evolution.

**Keywords:** floral design; floral display; geitonogamy; gender strategies; pollen dispersal; selfing; outcrossing

## 1. INTRODUCTION

Mating influences the movement of genes in space and their transmission through time. It therefore plays a fundamental role in determining the spatial and temporal patterns of genetic diversity within and between populations and hence their evolutionary dynamics. In flowering plants, the reproductive phase is of particular importance because it represents one of the only opportunities during the life cycle for genes to move among conspecific populations through pollen and seed dispersal (Levin & Kerster 1974). High rates of gene flow can increase effective population size ($N_e$) and reduce population differentiation by natural selection or genetic drift (Slatkin 1985). Variation among plant species in estimates of gene flow (*Nm*) and genetic differentiation often reflect differences in their pollination and mating systems (Govindaraju 1988). For example, average values of *Nm* range from 0.06 in selfing species to 5.4 for outcrossing wind-pollinated species (Hamrick 1987) demonstrating that mating patterns and gene flow are inextricably linked. In selfing species, very low rates of pollen dispersal between populations combined with recurrent cycles of colonization and extinction can result in large reductions in genetic diversity within populations (Ingvarsson 2003). Mating strategies therefore have diverse genetic consequences and play a key role in determining whether populations behave as independent evolutionary units or are components of metapopulations connected by gene flow.

Flowering plants possess exceptionally versatile mating strategies. This variation is a consequence of distinctive features of their biology including: immobility, modular growth, multiple reproductive structures, hermaphroditism, the closed carpel and the life-history diversity that has accompanied extensive ecological radiation (table 1). The functional units of angiosperm mating—flowers and inflorescences—exhibit more variation in size and morphological complexity than the equivalent structures of virtually any other group of organisms. This extensive diversification has largely been driven by requirements for efficient pollination; the sessile habit of plants has necessitated agents for pollen dispersal, resulting in the evolution of numerous floral adaptations associated with the types of vector employed to disperse pollen between plants. However, not all structural variation in flowers and inflorescences is determined by pollen vector divergence. Indeed, there has been a long tradition since Darwin's seminal work (Darwin 1862, 1876, 1877) of interpreting floral traits as adaptations that limit the deleterious consequences of selfing and promote cross-pollination. Today it is widely recognized that many features of flowers and inflorescences influence patterns of pollen dispersal, and in concert with post-pollination mechanisms (e.g. self-incompatibility systems), these reproductive traits comprise the key elements of the mating strategies of flowering plants.

With the advent of allozyme markers, much research on plant mating has focused on the measurement of the

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Table 1. Distinctive features of flowering plants that influence their reproductive habits.

| Feature | influence and reproductive consequences |
|---|---|
| immobility | reliance on pollen vectors for mating has promoted floral diversification associated with biotic and abiotic pollination |
| modularity | clonal growth or large plant size increases the likelihood of geitonogamy with resulting female and male mating costs |
| multiple reproductive structures | opportunities for variation in floral display, reproductive plasticity of gamete and seed packaging, and diverse gender strategies |
| hermaphroditism | selfing results in costs including inbreeding depression, gamete and seed discounting, benefits include reproductive assurance |
| closed carpel | post-pollination physiological mechanisms filter unsatisfactory pollen dispersal and provide opportunities for mate selection |
| life-history diversity | diverse mating patterns associated with variation in longevity, growth form and plant size |

frequency of self-fertilization ($s$), or its complement, the frequency of outcrossing ($t = 1 - s$), of maternal parents within populations. Indeed, a commonly used definition of 'mating system' in plants is the average frequency of cross- versus self-fertilization in a population (but see below). Over the past 30 years, considerable effort has been directed towards estimating these quantities in populations of over 200 plant species, with a particular focus on outcrossing versus selfing species (Barrett & Eckert 1990; Vogler & Kalisz 2001). Why has the comparison of outcrossing and selfing species attracted so much attention? There are important biological reasons why this comparison has been so influential and has stimulated diverse approaches to the study of plant mating systems.

Early on, systematists recognized that many herbaceous groups contained related outcrossing and selfing species with strikingly different floral characters useful for taxonomy. The contrasting patterns of morphological variation within and among populations of species with different 'breeding systems' had profound implications for taxonomic sampling and species circumscription (Davis & Heywood 1965). Also, following Darwin's (1876) lead, population geneticists demonstrated the contrasting effects of outcrossing versus selfing on plant fitness through heterosis and inbreeding depression (Charlesworth & Charlesworth 1987). Models of the joint evolution of selfing and inbreeding depression indicated that predominant outcrossing or selfing were the most likely stable end points of mating-system evolution in plants (Lande & Schemske 1985; Charlesworth *et al.* 1990). Stimulated by these models, attention focused on obtaining empirical estimates of mating patterns and inbreeding depression in natural populations in an effort to corroborate theory (Husband & Schemske 1996). At the same time, considerable evidence was accumulating on the role of outcrossing in determining patterns of genetic diversity and amounts of gene flow among populations (Hamrick & Godt 1996). Also, more recently, measurements of DNA sequence diversity in outcrossers and selfers have provided new insights into genome evolution and mechanisms of selection at the molecular level (Charlesworth & Wright 2001). Collectively, these advances, which I refer to as the outcrossing–selfing paradigm, have made research on plant mating systems one of the most dynamic areas of evolutionary biology.

Despite the impressive progress that has arisen from the outcrossing–selfing paradigm, many unanswered questions about plant mating still remain, particularly concerning the links between floral biology and mating success. Studies of mating commonly ignore the seemingly obvious fact that all seeds have both a maternal and a paternal parent (Charnov 1982) and fitness through male function therefore represents half of the genetic contribution of hermaphrodite plants to future generations (Morgan & Schoen 1997). An accurate view of plant mating therefore demands recognition that individuals not only serve as maternal parents to the outcrossed and selfed seeds they produce, but that genetic transmission through pollen also contributes to mating success (Snow & Lewis 1993). Measurements of mating patterns not only require determination of the classes of mating event (self versus outcross), but also the relative contribution that individuals make to the next generation through female and male gametes (i.e. estimates of fertility). Unfortunately, because of limited variation at allozyme loci, accurate information on the maternal and paternal mating success of plants is available for only a very few populations. Although more variable markers (e.g. amplified fragment length polymorphisms and microsatellites) are now being used for parentage analysis, this field is still in its infancy and has yet to move beyond the descriptive phase (Krauss & Peakall 1998; Gerber *et al.* 2000). This deficiency currently represents a serious obstacle for measuring trait-mediated influences on components of mating and hence selection on reproductive characters.

For example, the traditional explanation for the spatial and temporal segregation of sex-organs within and between flowers is that these floral strategies function as 'anti-selfing mechanisms', reducing the debilitating effects of selfing and inbreeding depression. However, these conditions commonly occur in species that are already partly protected from the costs of selfing by physiological self-incompatibility. This apparent redundancy of function can be resolved by considering alternative interpretations based on a plant's paternal role as a mating partner (Lloyd & Webb 1986; Webb & Lloyd 1986). Although evidence supporting this view is emerging, our limited knowledge of how floral traits directly influence pollen dispersal and male siring success has thwarted progress in this area (but see Elle & Meagher 2000; Morgan & Conner 2001). Ignoring fitness returns through male reproductive function complicates attempts to measure selection on floral traits and has clouded the current debate on the evolution of pollination systems (Johnson & Steiner 2000).

Here, I review recent work on plant mating strategies in an effort to illustrate recent progress in this field and to address unresolved questions. I begin by looking at general patterns obtained from surveys of maternal outcrossing rate and consider explanations for the widespread occurrence of mixed mating. Next, I review how mating success through female and male function can be influenced by the structural and phenological variation in the sex function of flowers and inflorescences. A particular emphasis here is to go beyond the descriptive population-level estimates of outcrossing reviewed in the preceding section, by highlighting studies employing experimental manipulations and marker-gene analysis. This approach enables a fine-scale view of the pollination process in which the floral traits governing mating are more readily identified. In this section I also illustrate how comparative and phylogenetic analysis can inform understanding of the evolution and adaptive significance of floral traits by revealing the nature of trait correlations and how they might have arisen. Finally, in an effort to place mating strategies in a broader ecological and life-history context, I consider how growth habit and clonal architecture may affect mating patterns, potentially leading to evolutionary transitions between sexual systems.

## 2. INTERSPECIFIC AND INTRASPECIFIC VARIATION IN OUTCROSSING RATES

The simple dichotomy in mating systems between selfing and outcrossing that is often used as a heuristic tool is clearly an over-simplification. As originally pointed out by Herbert Baker (1959, p. 178):

> Between the extremes represented by habitual outbreeders and inbreeders lie the probable majority of flowering plants which show varying degrees of outcrossing.

Indeed, empirical evidence on the distribution of population-level maternal outcrossing rates (reviewed in Barrett & Eckert 1990; Vogler & Kalisz 2001) confirms Baker's assertion as populations of many species experience significant amounts of selfing and outcrossing (mixed mating). In animal-pollinated species the distribution of outcrossing rates is continuous (figure 1*a*) with no hint of the bimodality predicted by most genetic models of mating-system evolution. Moreover, this pattern has also been demonstrated within species (e.g. *Eichhornia paniculata*, figure 1*b*) where selfing evolves from outcrossing. Although recent models of mating-system evolution incorporating ecological factors (e.g. pollination biology (Johnston 1998) and environment-dependent inbreeding depression (Cheptou & Mathias 2001)) can explain the maintenance of partial selfing, empirical evidence showing that stable mixed mating represents an adaptive strategy in natural populations is surprisingly limited. The well-known case of cleistogamy and chasmogamy in *Impatiens* is clearly one example (Lu 2000), but there are few convincing studies in which it has been shown that selfing is adaptive in species with mixed mating. In fact, even examples of floral mechanisms that appear to provide reproductive assurance may not be as clear cut as previously assumed, especially where seed discounting and inbreeding depression occur (Herlihy & Eckert 2002).

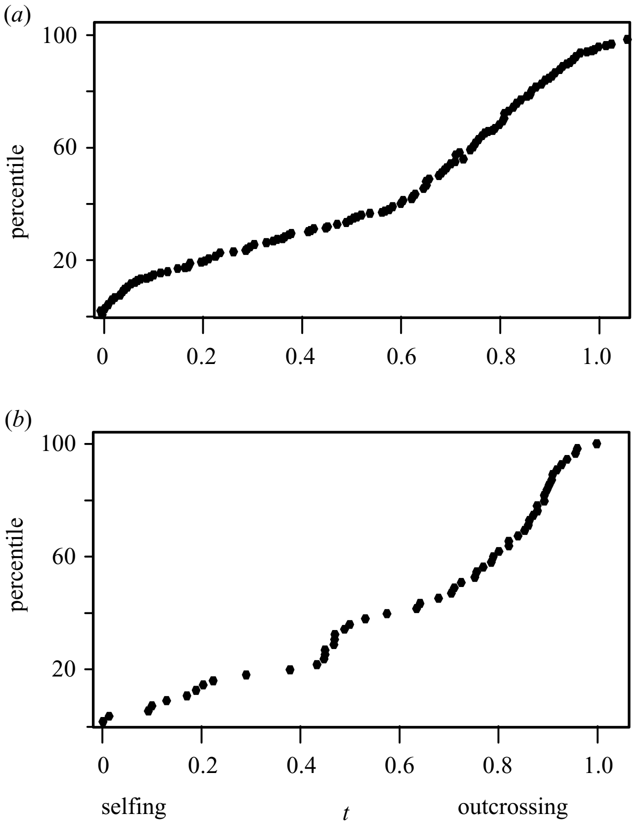

Figure 1. Outcrossing rates exhibit wide interspecific and intraspecific variation. (*a*) One hundred and sixty-nine animal-pollinated species; survey data from Barrett & Eckert (1990) and Barrett *et al.* 1996*a*, after Vogler & Kalisz (2001); (*b*) Fifty-five populations of *Eichhornia paniculata*; after Barrett & Husband (1990) and Husband & Barrett (1991). Values plotted are mean estimates of the population maternal outcrossing rate obtained using allozyme markers.

In many species the selfing component of mixed mating may represent a non-adaptive cost associated with the large floral displays required to attract animal pollinators. Significant amounts of selfed seed may arise from inter-flower selfing (geitonogamy) and provide little benefit to fitness because of strong inbreeding depression and pollen discounting. As Lloyd (1992) has shown theoretically, in most cases geitonogamy is unlikely to be favoured directly by selection, yet this form of selfing is likely to be common in self-compatible plants with many flowers in bloom simultaneously (de Jong *et al.* 1993; Harder & Barrett 1995). The hypothesis that selfing arises as a non-adaptive cost of being showy can be evaluated by using genetic markers to measure changes in the inbreeding coefficient from parents to offspring (Ritland 1990). Recent studies using this approach in three unrelated animal-pollinated species with contrasting mating strategies provide evidence for the non-adaptive basis to selfing (figure 2). Populations of *Decodon verticillatus* (Lythraceae), *Narcissus longispathus* (Amaryllidaceae), and *Sagittaria latifolia* (Alismataceae) each experience significant self-fertilization, but strong selection prevents selfed offspring from reaching reproductive maturity as a result of inbreeding depression. The three species exhibit different forms of clonal growth but

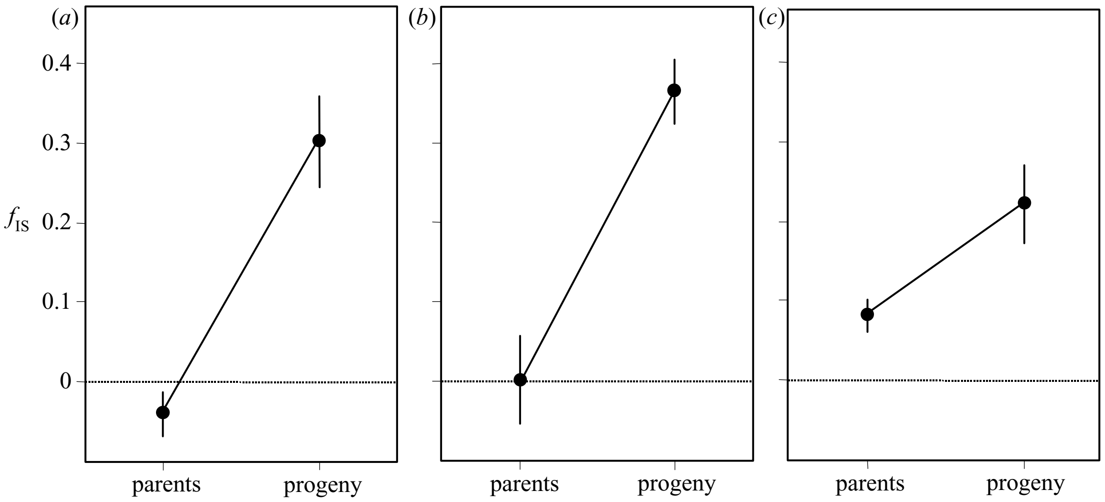

Figure 2. Selection against selfed progeny in populations of three animal-pollinated plant species with mixed mating systems. In each species there are significant changes in the mean inbreeding coefficient between parents and progeny estimated using allozyme markers. (*a*) Tristylous *Decodon verticillatus*: 10 populations, data in Eckert & Barrett (1994); (*b*) sexually monomorphic *Narcissus longispathus*: six populations, data in Barrett *et al.* (2003); and (*c*) monoecious *Sagittaria latifolia*: six populations, data in Dorken *et al.* (2002). Standard errors of the parental generation were estimated by bootstrapping data from Ritland's multilocus outcrossing rate program and those for progeny were the sum of the standard errors from estimates of selfing rates and parental $F$ (see Ritland 1990).

each produces attractive floral displays. In *D. verticillatus* and *S. latifolia* the contribution of geitonogamy to selfing is known. Using allozyme markers, Eckert (2000) estimated that in *D. verticillatus* 82% of the selfed seed produced results from geitonogamy. Moreover, in *S. latifolia* all selfing is geitonogamous because the species is monoecious and therefore produces unisexual flowers (Dorken *et al.* 2002). These results raise the question of how widespread this phenomenon is. It would be worthwhile to evaluate the survey data on outcrossing rates (figure 1*a*) to determine the susceptibility of other species to geitonogamy, and to assess genetic evidence for selection against selfed offspring. A significant amount of the observed variation in outcrossing may arise as an incidental outcome of geitonogamy, through inefficient pollen dispersal in self-compatible species.

A growing number of studies document intraspecific variation in outcrossing rate, but rarely are the proximate mechanisms causing this variation determined. For example, the tristylous annual *E. paniculata* (Pontederiaceae) displays an exceptionally wide range of population-level outcrossing rates ranging from near zero to complete outcrossing (figure 1*b*). Although part of this variation clearly results from the spread of modifier genes that alter stamen position causing autonomous intrafloral selfing, demographic factors also play a role through their influence on pollinator visitation (Barrett & Husband 1990). Because of this it is difficult to tease apart the direct influences of floral traits versus demographic factors on mating patterns because these factors are often confounded. In *E. paniculata*, tristyly is commonly associated with large dense populations, whereas high selfing is more frequently found under the opposite demographic conditions. Moreover, mating patterns are further complicated because the selfing rate of stamen variants can vary depending on the style-morph composition of local populations (Kohn & Barrett 1994). This type of context-dependent mating is likely to be common whenever populations vary widely in their size and density, or contain phenotypes with contrasting sex roles, as occurs in species with mating polymorphisms maintained by frequency-dependent selection.

The observed variation in outcrossing rates among angiosperm species may also be influenced by the sampling bias of researchers interested in mating-system evolution. Most species examined so far are self-compatible, presumably because investigators assume invariance in the outcrossing rates of self-incompatible and dioecious species. A recent study of the annual colonizer *Crepis sancta* (Asteraceae) illustrates that this assumption is not always warranted and that environmental conditions can play a role in modifying mating patterns even in self-incompatible species (see Cheptou *et al.* 2002). *Crepis sancta* was formerly considered to be strongly self-sterile; however, controlled crosses indicated variation in the expression of incompatibility. Significantly, 'pseudo self-fertility' was correlated with the successional stage in which populations occur. Plants from late successional stages are strongly self-incompatible and predominantly outcrossing, whereas those from early successional stages exhibit significant selfing. Unlike *E. paniculata*, this pattern was not associated with low-density conditions promoting autogamy. Instead, mating patterns in *C. sancta* appear to be largely governed by the influence of successional stage on plant size and the number of flowers produced per plant. In early succession, plants are larger (presumably because of less competition) with showier floral displays, and as a result they self more through geitonogamy. As predicted by theoretical models (Harder & Barrett 1996), floral display size in *C. sancta* also has a significant influence on the correlation of outcrossed paternity. The highest number of paternal parents occurred in the oldest populations with lower mate number evident in younger successional stages. Cheptou and colleagues suggest that this pattern arises because between-plant pollinator movement varies with display size, resulting in more pollen dispersal among plants with fewer flowers. This example illustrates the complex interactions that can occur between ecological conditions, plant size and mating patterns, even in species with self-incompatibility.

## 3. ADAPTIVE FLORAL DESIGN AND DISPLAY

The primary function of flowers and inflorescences is to promote successful mating. In biotically pollinated species, this occurs during brief interactions with pollinators when pollen is imported and exported from flowers. Effective cross-pollen dispersal largely depends on the types of pollinator visiting flowers and the manipulation of their foraging behaviour by plant reproductive traits. Although the characteristics of individual flowers, including their structure, colour, scent and rewards (floral design), and the number of flowers open at one time and their arrangement in inflorescences (floral display) govern these interactions, the consequences of variation in floral design and display for pollination and mating are still largely unexplored. Unfortunately, our ignorance of this topic has limited understanding of how selection operates on floral traits and of the mechanisms driving the reproductive diversification of angiosperms. The lack of functional analyses of floral design and display may be because diverse trait combinations often appear to promote equivalent mating patterns. Similar levels of maternal outcrossing are indeed achieved in species with strikingly different floral strategies, but explaining the adaptive basis of floral design and display requires determining how particular traits influence both female and male components of mating.

Much floral diversity resides in lineage-specific variation in the size, shape and colour of petals (or tepals), the main attractive structures of plants. This variation is largely associated with pollen vector divergence and functions to attract and position pollinators for effective contact with the sexual organs. Despite this floral complexity, sex organs are deployed in a restricted number of ways, reflecting functional constraints associated with how pollinators contact these structures and influence pollen dispersal and mating success. Unfortunately, there are no general theories that predict the optimal positioning of sex organs for particular floral designs or pollination syndromes, despite recurrent patterns among angiosperm families and models that emphasize the role of pollen transfer in the evolution of mating strategies (Lloyd & Webb 1992*a*; Barrett *et al.* 1996*b*; Holsinger 1996; Jesson *et al.* 2003).

### (a) ***Herkogamy***

The spatial separation of pollen presentation and pollen receipt (herkogamy) is nearly ubiquitous in animal-pollinated plants. The distance separating stigmas and anthers influences maternal outcrossing rates in diverse self-compatible species (see Belaoussoff & Shore 1995; Karron *et al.* 1997; Brunet & Eckert 1998). Several different forms of herkogamy are recognized (Webb & Lloyd 1986), but by far the most common is approach herkogamy in which stigmas are located above anthers and contact pollinators first upon their entry into flowers. The opposite arrangement in which anthers are positioned above stigmas (reverse herkogamy) is less widespread, although it occurs commonly in lepidopteran-pollinated species with narrow floral tubes, an association that still requires a satisfactory functional explanation. Why do the different forms of herkogamy differ in abundance and how does the particular sequence of stigmas and anthers influence mating patterns?

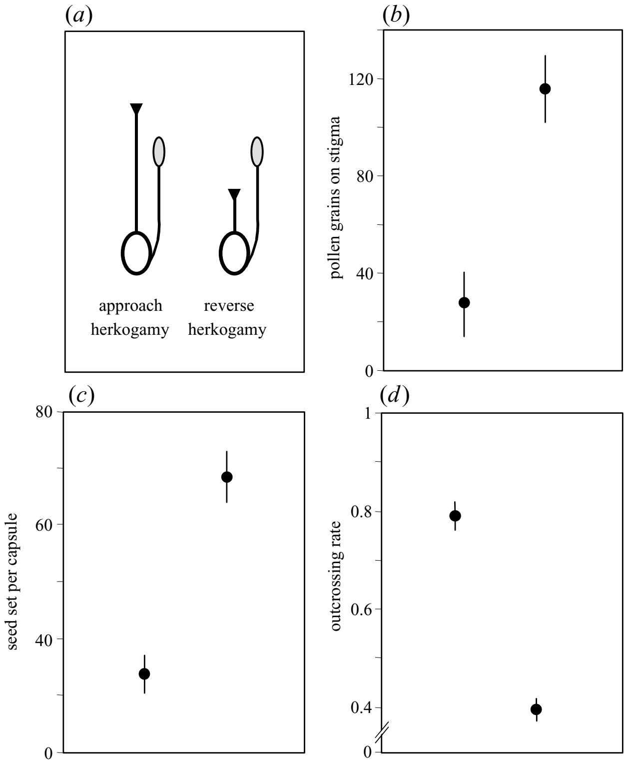

Figure 3. The reproductive consequences of approach herkogamy versus reverse herkogamy. (*a*) Arrangement of sex organs in the two herkogamous phenotypes; (*b*) number of pollen grains deposited on stigmas; (*c*) seed set per fruit; and (*d*) maternal outcrossing rates. The two phenotypes were created by floral manipulations of tristylous *Eichhornia paniculata*. Before anther dehiscence, short- and long-level anthers were removed from the long- and short-styled morphs, respectively, resulting in two floral phenotypes differing only in style length. Reproductive parameters were measured in three to six replicate garden arrays of 36 plants containing a single phenotype visited primarily by bumblebees. Maternal outcrossing rates were estimated by using polymorphism at the *PGI-2* locus. Values presented are means and confidence intervals. (After A. M. Baker and S. C. H. Barrett (unpublished data).)

This question was recently addressed experimentally by manipulating the flowers of tristylous *E. paniculata* to create approach and reverse herkogamous phenotypes and measuring the reproductive consequences of these alternative floral designs following bee pollination (figure 3). The results from this study indicate that the two forms of herkogamy have strikingly different effects on the pollen loads, seed set and outcrossing rates of maternal parents. Approach herkogamous flowers captured less total pollen but exhibited significantly higher outcrossing than reverse herkogamous flowers, because the location of stigmas below anthers in reverse herkogamous flowers made them more susceptible to pollinator-mediated intrafloral self-pollination. These results support Webb & Lloyd's (1986) suggestion that for most floral architectures, approach herkogamy functions more effectively than reverse herkogamy in preventing self-pollination explaining, at least for self-compatible groups, why the two main forms of herkogamy differ in frequency.

Many species that possess herkogamy are also self-incompatible. This has led to the proposal that herkogamy more generally serves to reduce interference between maternal and paternal functions, rather than acting solely as a mechanism to prevent selfing and inbreeding depression (Webb & Lloyd 1986). Sexual interference can take several forms, but of particular significance are floral strategies that promote more effective pollen dispersal among plants and reduce male gamete wastage through pollen discounting (Barrett 2002). A recent experimental study by Fetscher (2001) on the adaptive significance of movement herkogamy in *Mimulus aurantiacus* (Scrophulariaceae) provides experimental evidence for the pollen dispersal hypothesis. She found that rapid developmental changes to stigma lobes following pollination increased herkogamy and promoted greater pollen dispersal compared with plants in which stigma closure was prevented. Significantly, she also found no evidence that this form of herkogamy functions to limit self-pollination as earlier workers had assumed. Distinguishing between these alternatives is important because of their contrasting effects on plant fitness. Floral mechanisms that prevent selfing primarily increase maternal fitness through the production of high-quality offspring, whereas those that reduce conflict between female and male sex function largely promote paternal fitness through outcross siring success. Selection to reduce interference can therefore be a distinct selective force and is not always an ancillary consequence of the avoidance of selfing.

Most outcrossing species exhibit one form of herkogamy only. However, species in at least 40 animal-pollinated families possess herkogamy polymorphisms in which populations contain two or three complimentary floral phenotypes (morphs) with reciprocal arrangements of sexual organs (reciprocal herkogamy). The widespread occurrence of herkogamy polymorphisms suggests that populations of animal-pollinated plants may often experience conditions under which the effectiveness of cross-pollen dispersal is compromised. Under these circumstances, populations may be readily invaded by simply inherited variants with altered sex-organ positions if their establishment improves the pollen economy of populations. Because most species with herkogamy polymorphisms are self-incompatible it is unlikely that these complex floral designs become established as anti-selfing mechanisms. Rather, it seems more likely that these adaptations have evolved to increase mating proficiency through male function in comparison with what could be achieved in their sexually monomorphic ancestors (see Lloyd & Webb 1992*b*; Barrett *et al.* 2000).

The most well-known examples of reciprocal herkogamy are the heterostylous polymorphisms distyly and tristyly (Darwin 1877; Barrett 1992*a*). Functional studies of heterostylous species indicate that sex-organ reciprocity serves to promote cross-pollen transfer between floral morphs, with floral polymorphism maintained by negative frequency-dependent selection resulting from disassortative mating (Charlesworth & Charlesworth 1979; Lloyd & Webb 1992*a*). Recent experimental work on two other herkogamy polymorphisms, enantiostyly (Jesson & Barrett 2002*a*) and stigma-height dimorphism (Thompson *et al.* 2003), indicates that they also function in a similar manner. Hence herkogamy polymorphisms represent alternative evolutionary solutions to the problem faced by many animal-pollinated plants: to achieve effective cross-pollen transfer without incurring the gamete losses associated with interference between female and male sexual organs.

Enantiostyly provides an example of a convergent mating strategy promoting pollen dispersal in bee-pollinated species. Phylogenetic analysis of the monocotyledons indicates that in this group alone enantiostyly has evolved at least 11 times (Jesson & Barrett 2003). In these curious floral designs, commonly referred to as mirror-image flowers, the style is deflected either to the left or right with a pollinating anther positioned on the opposite side of the flower. There are two fundamentally distinct forms of enantiostyly, depending on whether left- and right-styled flowers occur on the same plant (monomorphic enantiostyly), or occur on different plants (dimorphic enantiostyly) (Barrett *et al.* 2000; Barrett 2002). Dimorphically enantiostylous populations often contain equal frequencies of the two style morphs (Jesson & Barrett 2002*b*), and the inheritance of this polymorphism, at least in *Heteranthera multiflora* (Pontederiaceae), involves a single-locus with the right-styled allele dominant to the left (Jesson & Barrett 2002*c*). Comparative evidence supports an evolutionary sequence beginning with a straight-styled ancestor and progressing to monomorphic enantiostyly and occasionally to dimorphic enantiostyly (Jesson & Barrett 2003). Theoretical models indicate that the primary selective force driving these evolutionary transitions is likely to be the more proficient cross-pollen transfer that arises from the presence of reciprocal flower forms in a population (Jesson *et al.* 2003).

Experimental evidence supporting the cross-pollen transfer hypothesis comes from comparisons of mating patterns and fertility in arrays of *Solanum rostratum,* a species pollinated by bumble-bees that exhibits monomorphic enantiostyly (Jesson & Barrett 2002*a*; L. K. Jesson and S. C. H. Barrett, unpublished data). In the experiments illustrated in figure 4, plants of *S. rostratum* were manipulated to produce three treatments: arrays with left-styled plants, arrays with right-styled plants, and arrays containing a 1 : 1 ratio of left- and right-styled plants. In this way it was possible to examine the reproductive consequences of stylar monomorphism versus polymorphism. Outcrossing rates were similar among the three treatments because herkogamy reduced the likelihood of self-pollination in an equivalent manner for all plants (figure 4*a*). However, the female fertility of plants in dimorphic arrays was significantly higher than in arrays containing plants of a single stylar orientation because dimorphic enantiostyly increased the amount of pollen transferred between plants (figure 4*b*). An analysis of mating events in dimorphic arrays indicated that most seed resulted from crosses between plants of opposite style orientation (Jesson & Barrett 2002*a*), because of the segregated pollen pools deposited by the pollinating anther on opposite sides of bumble-bees' bodies. These results illustrate that an exclusive focus on the maternal

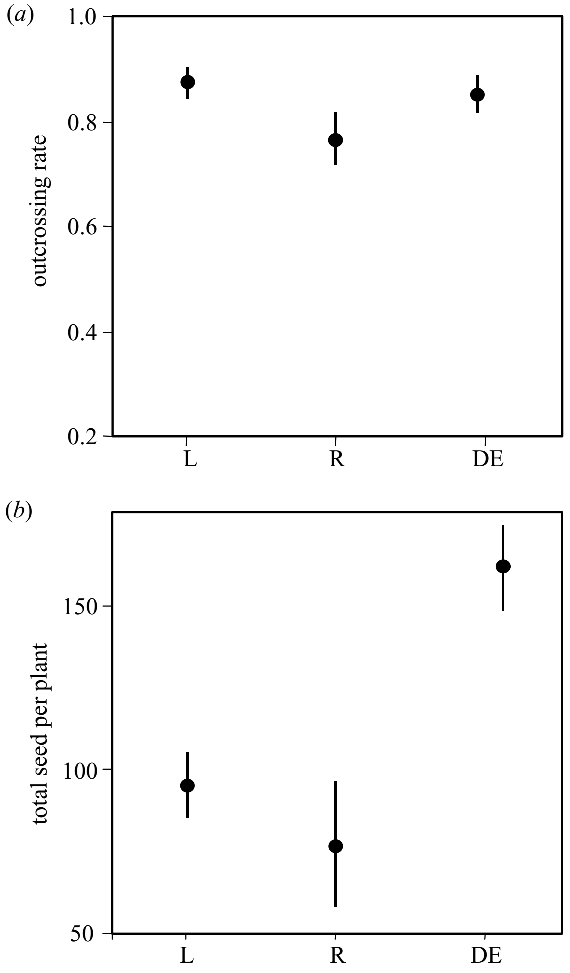

Figure 4. Dimorphic enantiostyly increases pollen transfer between plants in comparison with stylar monomorphism. (*a*) Maternal outcrossing rates; and (*b*) seed set per plant, in experimental arrays of *Solanum rostratum* visited by bumblebees. Three experimental treatments were compared: arrays containing plants with all left-styled flowers (L) or all right-styled flowers (R) and arrays with a 1 : 1 ratio of L and R plants (DE). The treatments were created by removing flowers of the appropriate style orientation. Floral displays were maintained at an equivalent size (six flowers per plant) in all treatments and there were 16 plants in each array with four replicates. Maternal outcrossing rates were estimated using polymorphism at the *PGI-1* locus. (After Jesson & Barrett (2002*a*); L. K. Jesson and S. C. H. Barrett, unpublished data.)

outcrossing rate would miss the important functional benefit that this polymorphism provides through the promotion of more effective cross-pollen dispersal. Although floral polymorphism clearly enhances the fitness of plants as male parents, in pollen-limited environments it may also benefit female function as well.

Heterostyly and dimorphic enantiostyly both promote disassortative mating; however, the floral biologies of species with these herkogamy polymorphisms are strikingly different. Heterostyly is most often associated with actinomorphic flowers, with floral tubes and nectar as the main reward, whereas flowers of enantiostylous species are usually non-tubular, nectarless and exhibit a functional division of labour between pollinating and feeding anthers (heteranthery). Not surprisingly, these differences are associated with contrasting pollination biologies. Heterostylous flowers are serviced by long-tongued pollinators with pollen spatially segregated along the length of the pollinator's proboscis and body; whereas enantiostylous flowers are commonly buzz-pollinated with pollen segregated sideways (i.e. left and right) on the pollinator's body. These two herkogamy polymorphisms therefore represent fundamentally different mechanical solutions to the problem of effective cross-pollen dispersal in flowers with alternative floral rewards. The groups in which these herkogamy polymorphisms have evolved differ strikingly in floral characteristics and pollination systems. Nevertheless, the same functional solution of efficient cross-pollen transfer through the geometry of flower-pollinator contacts has been achieved. Such adaptive convergence often characterizes the evolution of mating strategies in plants.

### (**b**) ***Dichogamy***

Dichogamy is the temporal separation of pollen presentation and stigma receptivity within and between flowers on a plant. It is an exceptionally widespread floral strategy occurring in many outcrossing species, regardless of pollination system. Despite its abundance, dichogamy has been neglected by floral biologists, particularly in wind- and water-pollinated species, where it plays a crucial role in governing pollen dispersal and mating patterns. Few experimental studies have investigated the reproductive consequences of the temporal segregation of sex function (but see Griffin *et al.* 2000) and dichogamy has been almost universally interpreted as an anti-selfing mechanism. However, in common with herkogamy, many dichogamous species are also self-incompatible. Indeed, a survey by Bertin (1993) revealed that dichogamy occurred as commonly in self-incompatible as self-compatible species. The frequent occurrence of dichogamy in self-incompatible species has motivated alternative adaptive explanations based on reducing sexual interference between female and male function (Lloyd & Webb 1986; Bertin 1993). However, interpretations of the adaptive significance of dichogamy require recognition that this floral strategy occurs in several distinct forms with contrasting influences on pollen dispersal and mating.

The two main types of dichogamy are protandry, in which pollen is dispersed before stigmas are receptive, and protogyny, in which the reverse sequence occurs. Because only protogyny guarantees a period of stigma receptivity free from self-pollen it seems probable that this form of dichogamy functions primarily as an anti-selfing mechanism, whereas protandry is likely to be less effective in this regard and may function instead to reduce sexual interference (Lloyd & Webb 1986). If this interpretation is correct, the two forms of dichogamy might be expected to have contrasting associations with the compatibility systems of plants. Protogyny should be more frequently associated with self-compatibility where it would serve to reduce inbreeding, whereas protandry should be more commonly found in self-incompatible species as they are protected from the deleterious effects of selfing (Bertin 1993). A recent comparative study (M. B. Routley, R. I. Bertin and B. C. Husband, unpublished data) investigated this hypothesis by mapping these traits onto a family-level molecular phylogeny of the angiosperms and examining the correlated evolution of the two forms of dichogamy with self-incompatibility. Routley and colleagues found strong support for the association between protogyny and self-compatibility, whereas protandry was more commonly associated with self-incompatibility. Their analysis supports the view that dichogamy functions to promote both maternal and paternal fitness with benefits to different gender roles depending largely on the sequence of sex function.

What evidence is there that by reducing sexual interference, protandry functions to promote increased paternal fitness? Recent experimental work has addressed this question by determining the mating consequences of the sexual segregation that characterizes synchronized protandry (Harder *et al.* 2000; Routley & Husband 2003). In this common form of dichogamy the vertical inflorescence is the reproductive unit and protandrous flowers mature sequentially from the bottom to the top of the inflorescence. Pollinators, usually bees, forage upwards on inflorescences so that lower female-phase flowers receive outcross pollen and upper male-phase flowers disperse pollen to other plants. By segregating sex function among flowers, synchronized protandry may allow plants to increase floral display size, thus attracting more pollinators without paying the mating costs commonly associated with geitonogamy.

In experimental arrays of bee-pollinated *E. paniculata* and *Chamerion angustifolium* (Onagraceae), Harder *et al.* (2000) and Routley & Husband (2003), respectively, compared the outcrossed siring success of two contrasting inflorescence treatments by using floral manipulations and genetic markers. In adichogamous inflorescences, flowers presented pollen and stigmas simultaneously whereas in inflorescences with synchronized protandry, as described above, sex function is segregated. In both studies, protandrous inflorescences sired significantly more offspring in arrays than adichogamous inflorescences despite equivalent gametic outputs. Both studies therefore support the sexual interference hypothesis for the adaptive significance of synchronized protandry.

The most likely source of interference between female and male function in species with many flowers in bloom simultaneously is geitonogamous pollen discounting (Harder & Barrett 1995). Self-pollen transported between flowers on a plant is not available for export to other plants in the population and this can reduce paternal fitness. In the study by Harder *et al.* (2000) there was evidence that the reduced siring success of adichogamous inflorecences was indeed associated with this mating cost. By reducing levels of geitonogamy and enhancing outcrossed siring success, inflorescence-level protandry may allow animal-pollinated plants to maintain larger floral displays than could be achieved in their adichogamous ancestors. These functional linkages between floral design and display seem likely to be commonplace in animal-pollinated plants, although our current understanding of these associations is surprisingly limited.

### (c) *Gender strategies*

Herkogamy and dichogamy also play a major role in promoting the wide diversity of gender strategies found among angiosperm families. This is because the spatial and temporal segregation of sex function influences the functional gender of plants. For example, both these floral conditions occur in monoecious species because female and male function are spatially separated in pistillate and staminate flowers (interfloral herkogamy) and these often bloom at different times. Although this segregation of sex function has most often been interpreted as a means of avoiding selfing and inbreeding depression, this is unlikely to be a general explanation because comparative surveys of the incidence of monoecy and self-incompatibility indicate that monoecy occurs as often in self-incompatible as self-compatible plants (Bertin 1993). Moreover, studies of mating patterns in self-compatible, monoecious species indicate that the segregation of sex function among flowers on inflorescences certainly does not guarantee that selfing is prevented (Dorken *et al.* (2002) and see below). Because monecious plants can readily alter female and male investment in response to environmental conditions, reproductive plasticity may in fact represent this sexual system's main virtue.

How might the floral design and display of hermaphrodite plants influence the evolution of gender strategies? A recent study of the evolution of dioecy in *Wurmbea* (Colchicaceae) illustrates how phylogenetic analyses of floral traits can provide insights into this question (Case *et al.* 2003). *Wurmbea* is composed of *ca.* 41 species of perennial geophytes near-equally distributed between Africa and Australia that are largely pollinated by flies and small bees. All African species are hermaphroditic, whereas gender dimorphism occurs in 5 out of the 22 Australian species. Population-level studies of intraspecific variation in the sexual systems of *W. dioicia* (Barrett 1992*b*) and *W. biglandulosa* (Vaughton & Ramsey 2002) indicate that gender dimorphism has evolved through the gynodioecy pathway with females invading hermaphroditic populations because of outbreeding advantage (Charlesworth 1999). However, in hermaphroditic populations the inbreeding depression necessary for the spread of male sterility genes depends on increased selfing rates, raising the question of what factors might favour this shift in mating pattern.

Case *et al.* (2003) investigated this question by reconstructing the evolutionary history of reproductive traits in *Wurmbea* by using a combined morphological and molecular phylogeny. The historical reconstructions indicated that in Australia, dioecy probably originated from hermaphroditism on a minimum of four separate occasions (figure 5*a*). Two features of floral design and display were examined because of their potential influence on changes to the selfing rate. First, in *Wurmbea*, styles are either straight or recurved (figure 5*b*) and this determines the degree of herkogamy and propensity for pollinator-facilitated intra-floral self-pollination. Second, floral display size shows striking variation among Australian *Wurmbea* species, with significantly lower flower numbers per plant in hermaphroditic than in dioecious species. If selection for elevated display size had occurred in hermaphroditic populations, this could have resulted in increased geitonogamy and the inbreeding depression essential for the evolution of gender dimorphism. However, larger display sizes in dioecious species could also reflect a release from the 'geitonogamy constraint' that may commonly operate in self-compatible species pollinated by generalist insects such as flies and small bees. Distinguishing whether these character associations are causes or

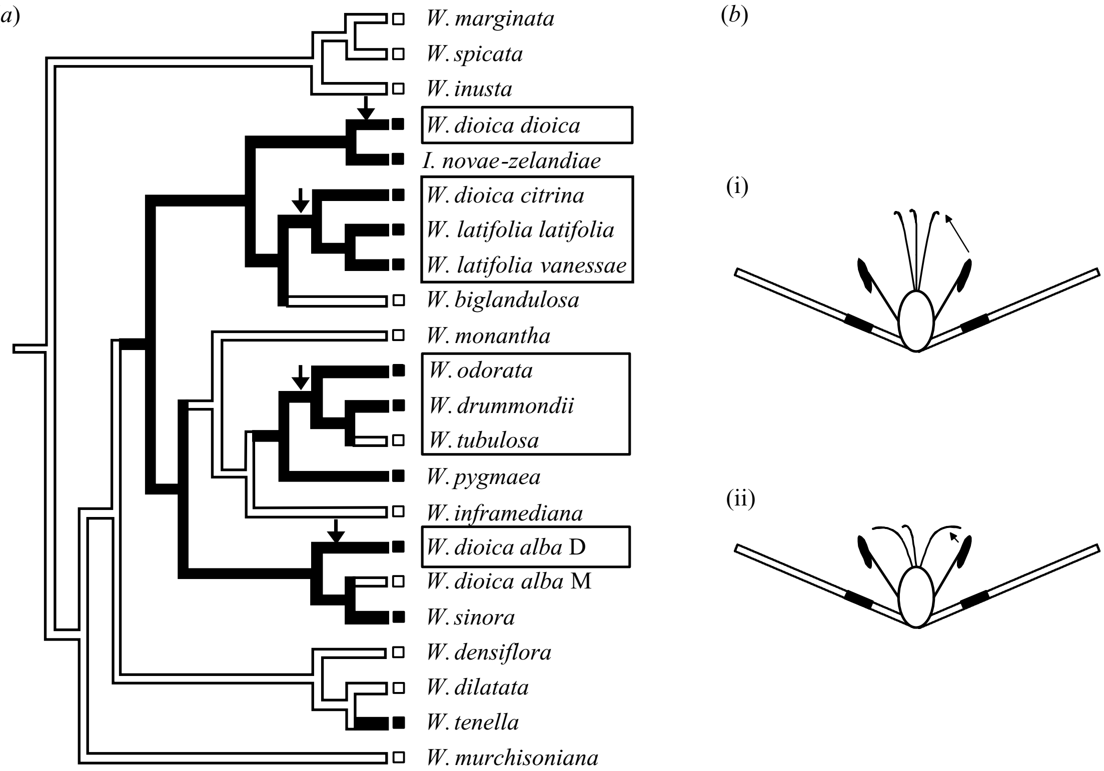

Figure 5. Changes in style morphology promoting increased self-pollination precede the origins of dioecy in *Wurmbea*. (*a*) The phylogenetic tree illustrates a historical reconstruction of character transitions in style morphology (black branches, recurved styles; white branches, straight styles) and sexual system (transitions from gender monomorphism to dimorphism indicated by black arrows, dioecious species in boxes) using ACCTRAN optimization. The tree is pruned from the single most parsimonious tree from combined morphological (45 characters) and molecular (two segments *psb*BTNH and *trn*LF of the large single-copy region of the chloroplast genome) data. (*b*)(i) Straight and (ii) recurved styles. Arrows illustrate the differences in herkogamy between the two stylar conditions. (After Case *et al.* (2003).)

consequences of gender dimorphism is therefore necessary. This requires that their order of establishment in lineages be determined.

The results of character optimizations of style morphology and floral display size in *Wurmbea* revealed a striking difference in the inferred role that each has played in the evolution of dioecy (Case *et al.* 2003). Increased flower number was never found to precede the origin of gender dimorphism in *Wurmbea,* casting doubt on the geitonogamy hypothesis. By contrast, reduced stigma–anther separation resulting from the evolution of recurved styles preceded all four transitions to dimorphism (figure 5). This implicates pollinator-mediated intra-floral self-pollination as the probable mechanism leading to inbreeding depression. Field studies of the pollination biology of hermaphroditic and dioecious populations of *W. dioica* have provided additional support for this hypothesis (A. C. Case and S. C. H. Barrett, unpublished data). There were also four instances of style recurvature in hermaphroditic *Wurmbea* species but in each case the plants have small solitary flowers that appear to be predominantly selfing. In one of these species, the rare, diminutive, New Zealand endemic *Iphigenia* (*Wurmbea*) *novae-zelandiae*, selfing probably aided in establishment after long-distance dispersal from Australia.

Increased selfing rates in *Wurmbea* therefore appear to initiate two contrasting evolutionary trajectories of mating-system evolution in hermaphrodite populations. With limited pollination some species follow the pathway to autogamous selfing, culminating in small flowers and low allocation of sexual resources to floral display. Alternatively, populations of other species are susceptible to the spread of unisexuals through outbreeding advantage leading to the establishment of gender dimorphism. Free from the 'geitonogamy constraint', and subject to sexual selection, reproductive investment in these species may increase significantly, particularly to floral display in male plants. Harder & Barrett (1996, pp. 178–179) suggested that which of these alternatives occurs in hermaphrodite species may be linked to the pollination biology of populations, particularly the extent to which pollination service was *insufficient* (resulting in increased selfing) or *inferior* (resulting in gender dimorphism), respectively. No doubt other factors, including the availability of sterility mutations, genetic load and resource supply, also play a role, but altered pollination conditions leading to unsatisfactory pollen dispersal may be the critical proximate ecological factor initiating either change.

## 4. MATING AND PLANT LIFE HISTORIES

Plants vary considerably in growth form and longevity and as a result possess diverse and often complex life histories. Modular construction and striking variation among individuals in size introduces both problems and opportunities for investigating the evolution of mating strategies. The reiteration of plant parts including multiple reproductive structures represents a fundamental distinction from the unitary development of most animals and complicates measurement of reproductive success. Estimates of whole-plant fitness are difficult to obtain, except for a small subset of plants with short life cycles and simple growth forms.

The functional links among growth form, life histories and mating strategies are not well understood beyond the association between longevity and maternal outcrossing rates (Baker 1959; Lloyd 1980). Comparative analysis of the distribution of outcrossing rates among plants with different growth forms and longevities indicates that the selfing rates of annual species tend to be higher than those of perennial herbs, and herbaceous plants self more frequently than woody plants (Barrett *et al.* 1996*a*). Models based on the cumulative effects of repeated episodes of inbreeding depression, mitotic mutation and the contribution of strongly recessive mutations help to explain why perennials outcross more than annuals (Morgan *et al.* 1997; Morgan 2001). Empirical studies of inbreeding depression (Husband & Schemske 1996) generally indicate that long-lived perennials, particularly forest trees, harbour substantial genetic loads explaining, in part, the maintenance of high outcrossing rates. Because the demographies of annuals and perennials differ in many respects, it seems likely that other factors influencing inbreeding depression (e.g. periodic bottlenecks (Lande & Schemske 1985) and density regulation (Cheptou & Diekmann 2002)) may also contribute to the observed differences in outcrossing rate among plant life forms.

The modular construction of plants allows a wide diversity of growth architectures, with implications for the spatial and temporal distribution of flowers and inflorescences. Clonal plants change their size and shape as they grow and this can have important consequences for pollen dispersal and outcross mating opportunities (Handel 1985). The multiplication and simultaneous blooming of reproductive shoots within a clone can result in considerable geitonogamous pollen transfer, leading to both female and male mating costs because of inbreeding depression and pollen discounting, respectively. As discussed earlier, floral strategies such as heterostyly and dichogamy that decouple the benefits of large floral displays for pollinator attraction from the mating costs associated with geitonogamy may serve this role in animal-pollinated clonal plants. Comparative studies would be valuable to determine if recurrent associations between particular mating and clonal strategies are evident among unrelated angiosperm groups.

A wide range of clonal strategies occur in angiosperms and these vary in the extent to which flowering shoots of a single genet remain intact and clumped in their distribution or are intermingled and physiologically autonomous (e.g. phalanx versus guerilla strategies, respectively (Lovett Doust 1981)). The mating consequences of these contrasting clonal strategies are likely to be quite different (Charpentier 2002). For example, many highly clonal aquatics (e.g. monoecious *Hydrocharis morsus-ranae* (Hydrocharitaceae) and tristylous *Eichhornia crassipes* (Pontederiaceae)) exhibit fragmentary growth due to the weak stolon connections that join daughter rosettes of a clone. This mechanism of growth enables the dispersal of floating vegetative propagules in water currents but it also provides opportunities for the spatial mixing of genotypes and reduces opportunities for the inevitable geitonogamy that would occur in large intact clones. Nevertheless, many self-compatible species do form extensive clonal colonies and a high degree of temporal asynchrony between female and male sex function is required to prevent geitonogamy. This is particularly the case in abiotically pollinated species as pollen dispersal can be random and nondirectional. In many clonal species it may not be possible to achieve precise control of sex expression when flowering shoots are spatially segregated and physiologically autonomous.

Studies of mating patterns in the water-pollinated, monoecious seagrass *Zostera marina* (Zosteraceae) revealed elevated levels of geitonogamy in large clonal patches of low diversity, implying restricted pollen dispersal (Reusch 2001). Dioecy is common in clonal aquatics, including seagrasses, and it seems probable that unisexuality has been selected in some groups because of mating costs associated with large clone size. This evolutionary scenario may explain variation in gender strategies in the clonal aquatic *S. latifolia* (Alismataceae). Recent investigations of this species (Sarkissian *et al.* 2001; Dorken *et al.* 2002; Dorken & Barrett 2003) illustrate how knowledge of the interactions among life history, clonality and mating can inform our understanding of the maintenance of combined versus separate sexes.

*Sagittaria latifolia* is unusual among flowering plants in possessing both monecious and dioecious sexual systems. Populations of the two sexual systems are fully interfertile and genetic evidence indicates that unisexuality is simply inherited (M. E. Dorken and S. C. H. Barrett, unpublished data). Monecious and dioecious populations often occur in close geographical proximity but evidence from allozymes indicates that despite their inter-fertility there is limited gene flow between populations of the two sexual systems (Dorken *et al.* 2002). The maintenance of two gender strategies in *S. latifolia* can be explained by ecology; populations of the two sexual systems occupy distinct wetland habitats and have different life histories.

Monoecious populations of *S. latifolia* primarily colonize ephemeral habitats exposed to frequent disturbance, whereas dioecious populations inhabit large, stable marshlands dominated by tall emergent aquatics. Population surveys indicate that monoecious populations are susceptible to frequent local extirpation, whereas dioecious populations are longer lived and as a result clone sizes can be considerable, particularly in habitats with limited sexual recruitment. Comparative glasshouse and field transplant studies of life-history traits in *S. latifolia* indicate that plants from monoecious populations are smaller in size, flower earlier, and produce more flowers, clonal ramets and corms than plants from dioecious populations, which are larger in size and produce heavier corms (Dorken & Barrett 2003). These differences are those predicted by life-history theory for plants occupying habitats differing in the relative importance of disturbance and competition, respectively (Crowley & McLetchie 2002). The maintenance of these associations between life history and sexual system are best understood by considering the mating systems of populations; moreover these associations provide clues about the potential selective mechanisms responsible for the evolution of gender dimorphism in this species.

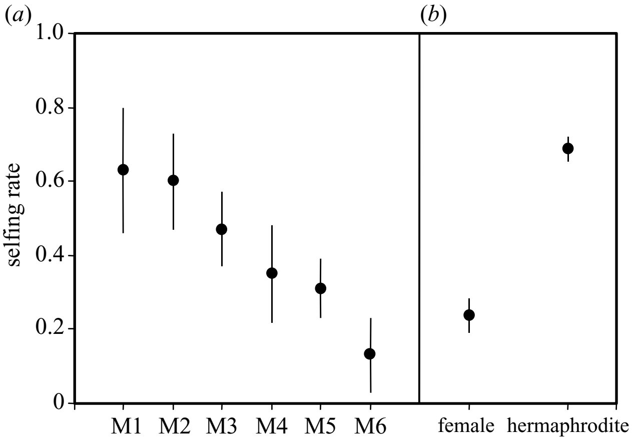

Figure 6. Clonal growth promotes geitonogamous selfing in monoecious populations of *Sagittaria latifolia*. (*a*) Mean geitonogamous selfing rates among six monoecious populations from Ontario, Canada, after Dorken *et al.* (2002). (*b*) Mean geitonogamous selfing rates of females and hermaphrodites in a population of *S. latifolia* at Barron River, Ontario. Clone sizes in this population were typical of dioecious populations in the region. Maternal outcrossing rates and their standard errors (1000 bootstraps) were estimated from 742 progeny sampled from 54 maternal families (female $n = 28$; hermaphrodite $n = 26$) using polymorphism at *PGI* and *IDH*. The smaller standard errors in (*b*) compared with (*a*) reflect the larger sample sizes used for estimating gender-specific selfing rates.

Estimates of mating patterns in monoecious populations of *S. latifolia* indicate that the incidence of selfing in some populations is high (figure 6*a*). As discussed earlier, selfing must arise from geitonogamy and, as flowering shoots of *S. latifolia* are strongly protogynous, selfing probably results largely from inter-inflorescence pollen transfer mediated by the generalist pollinators (bees, flies and butterflies) of this species. As clone size increases, more inter-inflorescence geitonogamy will occur as flowering shoots do not exhibit sex-phase synchrony. The vulnerability of large hermaphrodite clones to geitonogamy is illustrated in figure 6*b*. Here, selfing rates were measured in a dioecious population containing female, male and hermaphroditic individuals. Except for their large size the hermaphrodite clones in this population have the same sex expression as plants from monoecious populations. These hermaphrodites are referred to as 'inconstant males', occur commonly at low frequency in dioecious populations of *S. latifolia*, and are widely reported from other species with gender dimorphism. They are of particular value in *S. latifolia* because they decouple the functional association between sexual system and clone size that normally occurs in this species. This enables an assessment of selfing in clones of large size.

The high selfing rate of the 'monoecious phenotype' of *S. latifolia* at the Barron River population illustrates how maladaptive hermaphroditism can be when it is associated with the large clone sizes typical of dioecious populations. This result implies that there may be an upper limit to clone size in monoecious populations, above which this gender strategy becomes destabilized leading to the evolution of separate sexes. Clone sizes in monoecious populations rarely attain the dimensions evident in many dioecious populations because the habitats they occupy are usually short-lived. Nevertheless, in some monoecious populations (figure 6*a*), over half the seed produced results from geitonogamous selfing and, because of strong inbreeding depression, the necessary conditions favouring the spread of unisexual variants are met (Dorken *et al.* 2002). Habitat stability promoting extensive clonal expansion and limited sexual recruitment may have acted as a stimulus for the evolution of dioecy in other monoecious species.

## 5. BEYOND THE OUTCROSSING–SELFING PARADIGM

Over the past century the most influential research connecting floral biology and plant mating has been associated with the outcrossing–selfing paradigm. This work was largely motivated by Darwin's pioneering studies on floral adaptations, inbreeding depression and outcrossing mechanisms (Darwin 1862, 1876, 1877, respectively). Many of Darwin's original insights have remained remarkably durable and form a large part of the conceptual basis for current research on mating strategies. More recently, however, our understanding of this topic has broadened considerably because of the development of a strong theoretical foundation, combined with the integration of ideas and approaches from diverse fields, particularly evolutionary ecology and comparative biology. What new directions might be profitably pursued in future research on plant mating strategies?

The recent application of hypervariable genetic markers for parentage analysis (e.g. Reusch 2000), new advances in mating-system estimation (e.g. Ritland 2002), and the adoption of field experiments in which mating patterns are manipulated (e.g. Muis & Eckert 2003), now provide exciting opportunities to move beyond the descriptive population-level estimates of outcrossing that have been a central feature of the outcrossing-selfing paradigm. Future studies of plant parentage using microsatellite markers in combination with path analysis (Burd & Callaghan 2002) and selection gradient analysis (Morgan & Conner 2001) may allow an examination of the direct and indirect contributions of floral traits to fitness. These procedures should enable the measurement of selection through female and male function. Experiments in which plants with contrasting phenotypes are compared under different ecological conditions could provide valuable insights into the function of reproductive traits and the resilience of mating strategies to environmental change.

Most work linking floral biology to mating strategies has focused on floral design, particularly the strategic deployment of sex organs within flowers. Much less is known about the evolutionary ecology of floral displays despite their fundamental role in pollinator attraction and mating success (Schoen & Dubuc 1990; Fishbein & Venable 1996; Harder & Barrett 1996). Moreover, functional interactions among flowers, inflorescences and plant architecture have rarely been considered (but see Diggle 1997; Bond & Maze 1999). Floral displays depend on the rate at which flowers open, their longevity and spatial arrangement within inflorescences. Few studies have simultaneously investigated variation in these components of display or whether plants adjust the size of their displays in response to local pollination environments (L. D. Harder and S. D. Johnson, unpublished manuscript). Another neglected feature of floral displays concerns the adaptive basis of variation in flower size versus number that exists among angiosperm groups (Worley *et al.* 2000). This variation is assumed to reflect trade-offs but empirical evidence demonstrating negative genetic correlations between flower size and number is surprisingly limited (Worley & Barrett 2000, 2001). Future studies are required to determine how selection governs the optimal combination of flower size and number and the extent to which the hierarchical allocation of resources (Venable 1996; Worley *et al.* 2003) influences investment decisions within and among inflorescences. Work on the joint evolution of floral design and display will help to move the study of mating strategies beyond the outcrossing–selfing paradigm and into the broader framework of life-history evolution.

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## *Discussion*

A. J. Richards (*School of Biology, University of Newcastle, Newcastle upon Tyne, UK*). Would you explain the high proportion of dioecy in certain biomes such as tropical rain forest by avoidance of selfing?

S. C. H. Barrett. The avoidance of selfing probably plays the most important role in the evolution of dioecy, regardless of biome. However, other factors such as optimal resource allocation are also certainly involved.

M. W. Bayliss (*Biotechnology Consultant, Wokingham, UK*). Has Professor Barrett looked at variation in extent of inbreeding depression in different experimental populations, e.g. in the *Sagittaria* example?

S. C. H. Barrett. We have not investigated inbreeding depression in experimental populations of *Sagittaria* but we have measured this quantity in the more important situation—natural populations (Dorken *et al.* 2002). As indicated in my talk, inbreeding depression in *Sagittaria* can be very strong under field conditions.

D. Charlesworth (*Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh, UK*). It is worth pointing out that the view that mixed mating is 'non-adaptive' does not mean that there is no selection involved. Such cases fit very well into the concept/theories that mixed mating can arise when there is a trade-off (at the whole plant level) between male and female reproductive functions.

R. J. Abbott (*Division of Environmental and Evolutionary Biology, School of Biology, University of St Andrews, St Andrews, UK*). How important is sex in the clonal species Professor Barrett discussed? What happens to the sexual system at the distribution margins of these species?

S. C. H. Barrett. The two species that I discussed that form extensive clones were the aquatics *Decodon verticillatus* (Lythraceae) and *Sagittaria latifolia* (Alismataceae). Both can reproduce by seed but the frequency and geographical distribution of sex varies. In tristylous *Decodon*, populations at the northern periphery of the range are largely clonal and composed of a single style morph. Experimental studies by Christopher Eckert (Queens University, Canada) have shown that a sterility gene affecting pollen function is the cause of the sterility. This case represents one of the best examples of the evolutionary loss of sex in a clonal species. Two distinct patterns are evident in *S. latifolia*, depending on whether populations are monoecious or dioecious. In Ontario, monoecious populations dominate at range limits and these populations reproduce by seed and clonal propagules. By contrast, in eastern Canada, populations are dioecious and highly clonal with limited sexual recruitment.

### *Additional reference*

Dorken, M. E., Friedman, J. & Barrett, S. C. H. 2002 The evolution and maintenance of monoecy and dioecy in *Sagittaria latifolia* (Alismataceae). *Evolution* **56**, 31–41.