Biological Approaches and Evolutionary Trends in Plants

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14 Variation and Evolution of Mating Systems in Seed Plants

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I. Introduction

The mating system involves those attributes of an organism that govern how
gametes are united to form zygotes. The analysis of mating systems is usually con-
cerned with the genetic relatedness of sexual partners and hence the degree of in-
breeding practised by individuals within populations. Mating patterns in plant
populations are influenced by numerous environmental, demographic and genetic
factors. Of these, the type of breeding system (e.g. dioecism, heterostyly) that a
population possesses is of prime importance (Richards, 1986). Quantitative studies
of mating systems, until recently, were largely restricted to cultivated plants.
However, the recent growth of population biology, and the advent of elec-
trophoretic techniques has provided impetus for a growing number of investigations
of mating patterns in natural plant populations (Schemske and Lande, 1985).
Consequently, during the past two decades there has been a rapid increase in stud-
ies on the measurement of mating system parameters, particularly levels of self- and
cross-fertilization.

The mating system is not a static property of an individual, population or
species. Mating patterns are dynamic, and subject to modification by many forces,
operating on both ecological and evolutionary time scales. Interest in the causes of
shifts in mating systems arise because of the important consequences that such
changes have on population genetic structure, selection response, and speciation.
Models of mating system evolution have become increasingly complex as workers
appreciate that selection on mating patterns does not occur in isolation from other
features of the life history and demography of populations (e.g. Lloyd, 1980;

Charlesworth and Charlesworth, 1981; Holsinger, 1986; Campbell and Waser, 1987). A prerequisite for development of realistic models of mating system evolution is accurate information on the mating process and on the ecological and genetic context in which it occurs.

In this paper we examine some of the causes and consequences of mating system variation in flowering plants. We begin by discussing briefly how plant mating systems can be measured and what parameters are of most significance for understanding their evolution. We then present results from a survey of published outcrossing rates in natural populations and discuss their significance for models of mating system change. Finally, we focus on intraspecific studies, discuss their relevance for understanding the processes responsible for evolutionary changes in mating behavior, and conclude by outlining five areas of inquiry that may aid in future attempts to determine the causes of mating system change.

II. Measuring Mating Systems

Inferences on mating parameters, such as the selfing rate, can be made from observations of floral morphology. While this can be a useful guide in some species, such as those that are dioecious or possess cleistogamous flowers, it can be misleading in many self-compatible plants, particularly those with mixed mating systems. In these cases quantitative analysis, requiring large population samples and a statistical model of the mating process, is required to establish the true nature of mating patterns (Clegg, 1980; Ritland, 1983).

There are two types of information required to accurately describe the mating system. These involve measures of fertility and estimates of the kinds of mating events that occur (Brown, 1990). Measures of fertility attempt to estimate the relative contribution that individuals make through male and female gametes to the next generation. Mating events are usually classified as to whether zygotes result from selfing, outcrossing or apomixis. Among the outcrossed fraction it may also be important to determine the degree of biparental inbreeding (Waller and Knight, 1989).

Despite considerable progress in recent years in measuring plant mating systems we are still some way from being able to estimate all of the types of information required for an accurate depiction of the mating process, for even a single natural population. For example, because of the difficulties in measuring male fertility variation and the paternity of outcrossed offspring our knowledge of male mating success is rudimentary at best (Devlin et al., 1989). This deficiency handicaps our ability to test models that invoke sexual selection on the male component of fitness in hermaphrodite plants (Charnov, 1982; Lloyd, 1984). In contrast, because of the relative ease with which mating events can be distinguished for maternal parents, considerable headway has been made in estimating the average frequencies of cross- and self-fertilization for populations of different plant species.

Many aspects of the ecological and genetic relationships of uniting gametes are embodied in the parameters t, the outcrossing rate and s, the selfing rate which are probably the most convenient measures of the mating system in plants (Lloyd,

1980). These parameters are central to testing models for the evolution of several breeding systems, including autogamy (Lloyd, 1979; Lande and Schemske, 1985), heterostyly (Charlesworth and Charlesworth, 1979), dioecy and gynodioecy (Lloyd, 1975; Charlesworth and Charlesworth, 1978a) as well as more generally the evolution of combined versus separate sexes (Lloyd, 1982). This is because in many species the frequency of self-fertilization has a direct bearing on the number and quality of offspring as a result of the phenomenon of inbreeding depression (Charlesworth and Charlesworth, 1987).

Quantitative estimates of outcrossing rate were first obtained from predominantly self-pollinating crops using morphological markers and a model which assumes that each zygote results from either self-fertilization, with fixed probability s, or fertilization by a pollen grain chosen at random from the population with probability $t = 1 - s$ (Jones, 1916; Fyfe and Bailey, 1951). Since then the mixed-mating model has been used to estimate outcrossing rates for a wide range of agricultural and natural populations of both inbreeding and outbreeding species. Brown et al. (1985) have reviewed the basic assumptions of the model and some of the difficulties that have emerged during its application.

Several refinements have been made to techniques for estimating outcrossing rates in recent years. Isozyme loci have largely replaced the use of morphological marker genes (Brown and Allard, 1970). This is because morphological markers are often difficult to find, exhibit dominant expression reducing statistical power, and there is also the possibility that the genes themselves (e.g. flower color loci) may directly influence the mating process. Allozyme variants have, in contrast, several distinct advantages. They are codominantly expressed, highly polymorphic, and unlikely to be under strong selection. Because of these advantages workers surveying allozyme variation in plant populations can now routinely estimate outcrossing rates, so long as samples are collected separately from individual maternal parents and not pooled.

The large number of marker loci available using allozyme polymorphisms has also led to the development of more sophisticated estimation procedures using multiple, rather than single loci (Green et al., 1980). A single estimate of outcrossing based on the joint behavior of several marker loci, increases the probability of detecting an outcrossing event, in comparison with single locus estimates (Shaw et al., 1981). In addition, multilocus estimates are less affected by selection and non-random outcrossing than single locus estimates, and under most circumstances, multilocus measures, using three to four loci, provide a reliable average estimate of the outcrossing rate at the population level (Ritland and Jain, 1981). More difficult problems are encountered, however, in estimating the outcrossing rates of individual plants within populations. As a result, less information on plant to plant variation in outcrossing rate is available for natural populations (Ritland and Ganders, 1985; Morgan and Barrett, 1990). Further discussion of the strengths and weaknesses of various estimation procedures used for measuring mating patterns in natural populations are given in Clegg (1980), Ritland (1983), and Brown (1990). A

recent paper by Ritland (1990) provides details of computer algorithims used for estimating mating system parameters.

III. Models of Mating System Evolution

Theoretical interest in the evolution of self-fertilization began with Fisher's (1941) demonstration that a variant practising some degree of selfing will spread in an outbreeding population, as long as its ability to disseminate pollen to other individuals is not affected by its propensity to self-fertilize. The reason is simply that while the average individual in an outcrossing population transmits one haploid genome through each seed and one through each successful pollen grain, a self-fertilized individual passes on two haploid genomes through each seed while still transmitting one in each successful outcrossing pollen grain. An allele for complete self-fertilization is, therefore, one and a half times more likely to be transmitted to the next generation than an alternate allele for random mating. Given the observation that many flowering plants are predominantly outcrossing, theoretical work has sought to explain why selfing has not spread in more species through the 'automatic selection' of genes influencing the rate of self-fertilization (Jain, 1976) .

The spread of a selfing variant in an outcrossing population may be prevented if the proportion of pollen wasted or 'discounted' by self-pollination is equal to or higher than the variant's selfing rate (Lloyd, 1979; Holsinger et al., 1984; Holsinger, 1988a). While this effect is of theoretical interest, there are, to date, no published estimates of pollen discounting in natural populations so it is difficult to evaluate its role in constraining the evolution of self-fertilization.

The explanation for the maintenance of outcrossing which has attracted most attention, involves the common observation that offspring resulting from close inbreeding are usually inferior to those from crosses between unrelated individuals (Darwin, 1876; Charlesworth and Charlesworth, 1987). If selfed progeny survive and reproduce only half as well as outcrossed progeny, selfing may no longer be advantageous. Although this relationship appears simple, the origin and maintenance of inbreeding depression in natural populations, and therefore its importance in determining levels of outcrossing has been the subject of much debate (Lande and Schemske, 1985; Campbell, 1986; Charlesworth and Charlesworth, 1987; Holsinger, 1988b).

Lande and Schemske (1985) proposed that predominant selfing and predominant outcrossing should be alternative states of the mating system in most plant populations. Their genetic models predict a bimodal distribution of outcrossing rates, owing to selection for the maintenance of outcrossing in historically large populations, with substantial inbreeding depression, and selection for selfing when increased inbreeding, through bottlenecks or pollinator failure, reduces inbreeding depression below one-half:

$$\text{i.e.} \quad \delta = (1 - \frac{\text{fitness of selfed offspring}}{\text{fitness of outcrossed offspring}}) < \frac{1}{2}$$

Schemske and Lande (1985) tested their models by surveying the distribution of outcrossing rates for 55 species of flowering plants. Although the distribution of t was somewhat bimodal, providing support for their models, a significant proportion of species (31%) exhibited mixed mating systems (0.21 < t < 0.81).

Several explanations have been advanced to account for the discrepancy between Lande and Schemske's theoretical predictions and the empirical evidence (Waller, 1986; Schemske and Lande, 1987). One possibility is that Lande and Schemske's models do not accurately depict the joint evolution of inbreeding depression and self-fertilization. More recent models indicate that the precise endpoint of mating system evolution may depend on 1) the architecture and strength of selection on genes controlling inbreeding depression and self-fertilization (Charlesworth and Charlesworth, 1987, 1990; Holsinger, 1988b; Charlesworth et al., 1990); 2) the level of biparental inbreeding in populations (Uyenoyama, 1986); and 3) spatial variation in the strength of inbreeding depression (Holsinger, 1986). Unfortunately, at the present time the accumulation of theoretical ideas on inbreeding depression and mating system evolution has far outstripped empirical data from in natural plant populations.

What other factors besides inbreeding depression might be important in shaping the evolution of plant mating systems? Lloyd (1979) considered the role of reproductive assurance in favoring the evolution of self-fertilization. He argued that when pollen vectors are unreliable some selfing may be selected, despite levels of inbreeding depression exceeding one-half. Furthermore, he pointed out that it will always be advantageous for individuals to self unfertilized ovules after opportunities for outcrossing are exhausted. 'Delayed self-fertilization' should, therefore, evolve regardless of the level of inbreeding depression. Reproductive assurance is likely to be important under conditions of low density, and in short-lived species such as annuals that occur in environments with unreliable pollinator service (Baker, 1955; Stebbins, 1957; Jain, 1976). The main difficulty with empirical work on this problem is that most of the conditions favoring the evolution of selfing, such as small population size, low plant density, poor pollinator service and reduced inbreeding depression, often occur together. Teasing out their direct effects on the selfing rate is, therefore, a major challenge and probably requires experimental approaches.

IV. A Comparative Approach

A. Mating system surveys

Comparisons of the taxonomic distribution of a trait is a valuable tool in evolutionary biology. Finding similar traits in diverse taxa may indicate convergent responses to similar selection pressures (Pagel and Harvey, 1988). As discussed above, this approach was used by Schemske and Lande (1985) who surveyed outcrossing rates to assess their models for the evolution of self-fertilization. In the last five years the number of published estimates of t has more than doubled. Here

we compile this larger data set to assess the importance of several genetic, ecological and life-history factors thought to be important in mating system evolution.

We collected outcrossing rates determined by analyzing the segregation of morphological (17% of 155 studies) or allozyme markers in five or more progeny arrays from either natural populations (94% of 155 studies) or from plants transplanted from nature into a common garden. Estimates derived from comparing the performance of selfed, outcrossed and open-pollinated progeny (Charlesworth, 1988), or calculated from F-statistics (Ritland, 1983), were excluded. We included multilocus estimates of t whenever available. In cases where more than one locus was used, and multilocus estimates were not given, we averaged t across loci. When estimates were available for more than one population of a given species, t was averaged across populations . To construct the distribution of t across taxa, estimates were classified into five equal classes following Schemske and Lande (1985). The data involved in this survey are available from the authors on request.

B. Interspecific variation in outcrossing rate

Outcrossing rates for 129 species of seed plants are presented in Figure 1A. The species are distributed among 67 genera from 33 families. The majority of our sample involved dicotyledonous angiosperms; the remainder were monocotyledons (12%) and gymnosperms (13%). Schemske and Lande's survey of outcrossing rates showed a highly non uniform distribution of t , with a deficiency of species in the intermediate categories. Our larger sample including more than twice as many species shows the same pattern ($X^2 = 52.90$, $d.f. = 4$, $P < .001$). When gymnosperms are removed from the analysis the distribution remains non uniform ($X^2 = 21.74$, $d.f. = 4$, $P < .001$). These results are consistent with the prediction that predominant selfing and predominant outcrossing are alternative stable endpoints of mating system evolution.

C. Outcrossing rate and longevity

Further support for the importance of inbreeding depression for mating system evolution is revealed by subdividing the distribution of t by life form (Fig. 1B). Since inbreeding depression is the expression of genetic load, we would expect life forms accumulating high genetic load to practice predominant outcrossing. If we assume that the per cell, per year mutation rate is constant across species, one would expect longer-lived organisms to accumulate higher genetic load. There is some evidence that genetic load is indeed higher in longer-lived species (Wiens, 1984; Ledig, 1986). Our data shows a significant association between outcrossing and longevity. The distribution of t for woody perennials ($N = 51$ species) is more strongly skewed towards outcrossing than that for herbaceous perennials and an-

nuals ($N = 76$ species; Kolgomorov-Smirnov 2-sample test [Sokal and Rohlf, 1981, p. 443] $D = 0.498$, $P < .001$). The same effect is not evident in the comparison of herbaceous perennials ($N = 24$) and annuals ($N = 54$; $D = 0.292$, $P > .10$). This may be due to the smaller sample sizes involved, or may indicate factors in addition to longevity influence the accumulation of genetic load. For instance, the higher levels of gene flow in many trees should create larger effective population sizes which would, in turn, protect rare deleterious mutations from loss due to genetic drift (Loveless and Hamrick, 1984). Ledig (1986) also suggested that trees may accumulate more genetic load through somatic mutation by virtue of their large size (see also Klekowski, 1988). An additional explanation that may account for the correlation between longevity and outcrossing is also associated with differences in size among life forms. Because long-lived plants are often large, they would be more likely to self-fertilize (see below). As a result there may be stronger selection for mechanisms restricting self-fertilization (Maynard-Smith, 1978).

D. Outcrossing rate and pollination mode

Aide (1986) pointed out that when Schemske and Lande's original distribution of outcrossing rates was subdivided according to pollination mode, wind-pollinated species showed a clear bimodal distribution whereas animal-pollinated species did not. The distribution presented in Figure 1C shows the same effect, although there is also some trend towards bimodality among animal-pollinated species as well ($X^2 = 20.68$, $d.f. = 4$, $P < .05$). Aide (1986) explained the difference between the two pollination modes by suggesting that, while selection on outcrossing rates was strongly directional, as Lande and Schemske predicted, the intermediate t values recorded in animal-pollinated species were more influenced by environmental factors. Animal vectors are likely to show considerable spatial and temporal variation in abundance and behavior. Wind, on the other hand, would be expected to disseminate pollen more predictably.

Some support for this interpretation is provided by the much lower interpopulation variation in t values observed for wind-pollinated species compared to animal-pollinated species. Among the sample of wind-pollinated species ($N = 35$) only *Larix laricina* displayed much interpopulation variation in t ($t = .54 -.91$, Knowles et al., 1985). In contrast, among animal-pollinated species ($N = 94$), 14 exhibited t values spanning an equivalent range, and of those, six spanned twice that range.

Not all animal-pollinated species with mixed mating systems exhibit conspicuous variation in outcrossing rates. For example, in *Eucalyptus* outcrossing rates measured in 10 species were similar both within and among ($\overline{X} = .76$, $SD = .08$) species (Moran and Bell, 1983). These data provide a challenge to the models of Lande and Schemske, particularly since strong inbreeding depression occurs in
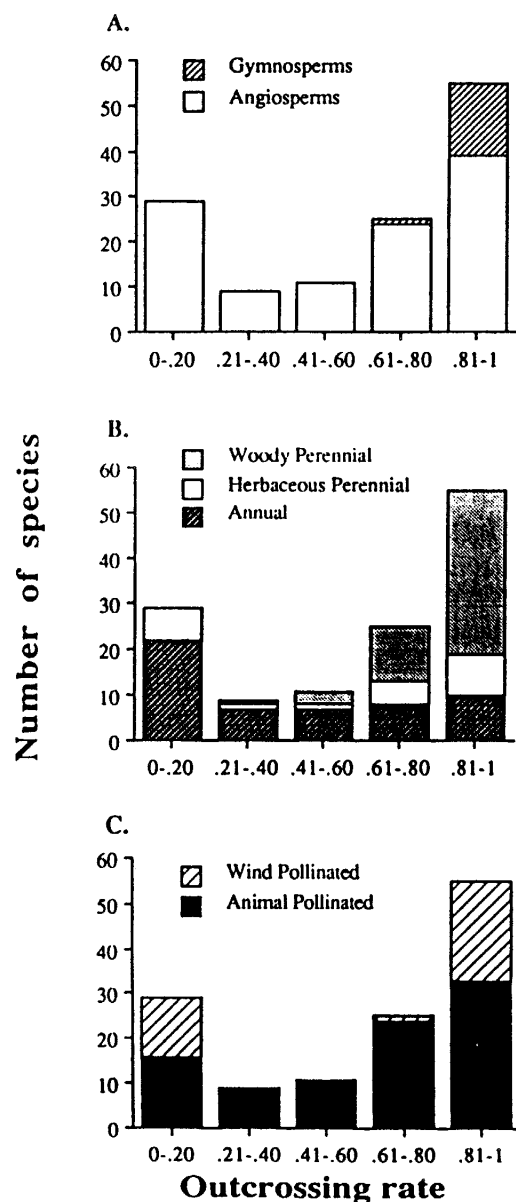
FIG. 1 The distribution of outcrossing rates in populations of 129 species of seed plants subdivided into A. angiosperms and gymnosperms; B. life forms; and C. animal- and wind-pollinated species.

several species of the genus (Hopper and Moran, 1981). This anomaly may be explained if there is little genetic variation within *Eucalyptus* for floral mechanisms that would increase outcrossing rates, a phylogenetic constraint that may have arisen during the evolution of this group's specialized floral morphology.

Unreliability of pollen vectors may also favor selfing rates higher than predicted by considering inbreeding depression alone, because selfing provides reproductive assurance. This should be most important in annuals where the evolutionary risks of pollinator failure are highest. This prediction is borne out by the distribution of t in Figure 1B. It is also apparent, however, that many animal-pollinated perennials practice moderate amounts of self-fertilization. This may be maladaptive and simply result from the vagaries of pollinator behavior. It may, on the other hand, result from selection for delayed selfing associated with reproductive assurance. Unfortunately, there has been little empirical work on the adaptive significance of delayed self-fertilization (but see Cruden and Lyon, 1989), and it remains an open question whether such mechanisms even occur in wind-pollinated plants.

Few of the species in our sample are known to have floral mechanisms which obviously promote delayed selfing. Genotypes of *Lupinus nanus* showed levels of autofertility that were higher than their floral morphology or outcrossing rates would suggest (Horovitz and Harding, 1972). This kind of autofertility may result from selection for delayed self-fertilization. Mechanisms involving pollen-pistil interactions also play a role in regulating the degree of selfing and outcrossing (Weller and Ornduff, 1977; Glover and Barrett, 1986; Seavey and Bawa, 1986; Bowman, 1987). Whether or not these mechanisms are sensitive to the supply of outcrossed pollen remains largely unexplored. Studies testing for increases in t when the supply of outcross pollen is experimentally augmented would be valuable for assessing the prevalence and functional significance of delayed selfing.

The contrasting distributions of outcrossing rates between animal- and wind-pollinated species in our sample is intriguing. However, it is important to consider the possibility that this pattern is simply a sampling artifact. Outcrossing rates for wind-pollinated species come mostly from either habitually selfing, weedy grasses, or conifers which are predominantly outcrossing and possess high genetic loads (Ledig, 1986). Perhaps as a wider array of wind-pollinated families, particularly those with herbaceous taxa, are studied mixed mating systems will be revealed. Clearly, grasses producing both cleistogamous (selfing) and chasmogamous (presumably outcrossing?) flowers are potential candidates (Campbell *et al.*, 1983; Schoen, 1984). However, the most telling evidence for the evolutionary stability of mixed mating systems in wind-pollinated species would come from hermaphroditic species where outcrossing and selfing entail similar energetic costs (Schoen and Lloyd, 1984).

E. Problems with a comparative approach

The potential bias in our sample of outcrossing rates from wind-pollinated species is common to applications of the comparative method. Indeed this problem is likely

to be a confounding influence throughout our analysis. Ideally each point in the frequency distributions in Figure 1 should represent an independent evolution of a certain level of outcrossing. This is unlikely. While the comparative approach seeks robust generalities, we are faced with the problem of distinguishing similarity due to convergent evolution from that due to common ancestry (Pagel and Harvey, 1988). For example, if the ancestral conifer from which the Pinaceae differentiated was highly outcrossing, the mating systems of modern taxa may be bound by phylogenetic inertia due to a lack of genetic variance for t throughout the lineage (see also above). One might argue, however, that it is unlikely that no genetic variance exists for mating system modification in the family, since selfing is thought to have evolved repeatedly within many other taxonomic groups (Stebbins, 1974; Jain, 1976). Following this view it seems reasonable to assume that outcrossing is actively maintained by selection in modern conifers, presumably because of their high genetic loads.

Nevertheless, estimating the distribution of t and searching for correlations with life history and pollination mode should ideally involve a stratified random sample of outcrossing rates from as many families as possible. In contrast, among the relatively small number of families represented in our sample, some (e.g. Myrtaceae) are clearly overrepresented. To partly remedy this bias we also constructed distributions of t among genera and families choosing, at random, one species per genus and one genus per family as representatives. The distributions for all three taxonomic levels were not significantly different (3x5 contingency table: $G = 5.15$, $d.f.$ $= 8$, $P = .74$). Still, attempting to minimize the confounding effect of phylogeny is usually a poor substitute for analyses that make use of information provided by cladistic approaches (Donoghue, 1989). Unfortunately, for most examples of mating system change phylogenetic data are unavailable or are fraught with difficulties of interpretation owing to the occurrence of homoplasy (Eckenwalder and Barrett, 1986; Wyatt, 1988).

V. Intraspecific Variation in Outcrossing Rates

Intraspecific variation in outcrossing rate may be more valuable for identifying the factors responsible for mating system change. Experimental approaches are often feasible, and work at the population level avoids some of the uncontrolled variables that hamper comparisons between species. Our survey of outcrossing rates added several species to those already discussed by Schemske and Lande (1985), in which variation in t spanned much of the range from predominant selfing to near complete outcrossing. In addition, a larger number of other species displayed moderate but still significant variation among populations in outcrossing levels. Most data on variation in outcrossing rates comes from comparisons among populations. We concentrate on this variation here with the knowledge that more meaningful data will come from studies of individual differences in mating patterns within populations.

Factors responsible for variation in outcrossing rates can be roughly classified as either genetic or ecological (Table 1). In practice, however, particular levels of outcrossing usually result from interaction between local environmental conditions, and the demographic, life history and genetic characteristics of populations. Historical factors and phylogenetic constraints may also play an indirect role by influencing the genetic structure of populations and the breeding system possessed by a particular species. Because of the complexity of these interactions it can be difficult to isolate the causes of mating system variation within species, particularly if experimental approaches cannot be employed (see Barrett and Husband, 1990).

TABLE 1

Genetic, demographic and environmental factors that contribute to intraspecific variation of outcrossing rate in natural plant populations

Factor	Species	Mean t	Range[1] of t	Relation[2] with t	Ref[3]
I. GENETIC FACTORS					
A. Quantitative floral traits					
Flower Size	Lycopersicon pimpinellifolium	.13	.00 -.40[†]	+	1
Herkogamy	Turnera ulmifolia	.34	.04 -.79[†]	+[NS]	2
Dichogamy	Clarkia tembloriensis	.55	.08 -.83	+	3
	Gilia achilleifolia	.57	.15 -.95	+	4
B. Polymorphic floral traits					
Flower color					
pigmentation	Ipomoea purpurea	.65	.60 -.86[†]	+	5
blue reflectance	Lupinus nanus	.43	.27 -.34	+	6
Heterostyly					
frequency of	Amsinckia spectabilis	.29	.03 -.53	–	7
homostyles	Eichhornia paniculata	.55	.00 -.96	–	8
	Primula vulgaris	.85	.08 - 1.1[†]	–	9
Gynodioecy					
frequency of	Bidens menziesii	.62	.58 -.65	+	10
females	Plantago coronopus	.86	.62 -.98	+	11
Capitulum polymorphism					
frequency of radiate morph	Senecio vulgaris	.04	.03 -.04[†]	+	12

TABLE 1   (Cont'd)

Factor	Species	Mean t	Range of t	Relation with t	Ref
II. DEMOGRAPHIC FACTORS					
Plant size	Malva moschata	.65	.22 -1.0†	–	13
Plant density					
animal	Cavanillesia plantanifolia	.46	.35 -.57†	+	14
pollination	Echium plantagineum	.88	.73 -.97	+	15
	Helianthus annuus	.75	.65 -.86	–	16
wind	Pinus ponderosa	.92	.85 -.96	+	17
pollination	Plantago coronopus	.70	.62 -.92	+	11
Population size	Eichhornia paniculata	.55	.00 -.96	+	8
III. ENVIRONMENTAL FACTORS					
Moisture	Abies lasiocarpa	.89	.65 -.99†	+	18
	Bromus mollis	.09	.04 -.16†	+NS	19
	Hordeum spontaneum	.02	.00 -.02	+	20
	Picea englemanni	.86	.85 -.93†	+	18
Altitude	Abies balsamea	.89	.78 -.99	–	21

1: Ranges marked with '†' are from within-population studies; all others are among-population ranges.
2: The relationship of each factor with t is indicated as '+' = positive; and '–' = negative (NS ' denotes a non-significant but consistent trend).
3: References: 1 = Rick et al., 1978; 2 = Barrett and Shore, 1987; 3 = Vasek and Harding, 1976; 4 = Schoen 1982; 5 = Brown and Clegg, 1984; 6 = Horovitz and Harding, 1972; 7 = Ganders et al., 1985; 8 = Barrett and Husband, 1990; 9 = Piper et al., 1982; 10 = Sun and Ganders, 1988; 11 = Wolff, 1988; 12= Marshall and Abbott, 1982; 13 = Crawford, 1984; 14 = Murawski et al., unpubl. MS.; 15 = Burdon and Brown, 1986; Burdon et al., 1988; 16 = Ellstrand et al., 1978; 17 = Farris and Mitton, 1984; 18 = Shea, 1987; 19 = Brown et al., 1974; 20 = Brown et al., 1978; 21=Neale and Adams, 1985.

A. Genetic control of mating system variation

There is considerable evidence from cultivated plants of both major gene control and quantitative inheritance of floral traits that directly influence levels of outcrossing. In particular, considerable work has been conducted on the genetics of self-incompatibility and self-compatibility and on sex expression (Frankel and Galun, 1977; Nettancourt, 1977). This variation has enabled crop breeders to modify mating patterns by artificial selection particularly towards increased levels of self-fertility (Jain, 1984). Less genetic data are available from natural plant populations, although both major gene and quantitative inheritance of mating system modification have been documented.

Population differentiation in floral traits such as flower size, style length, stamen length, and degree of herkogamy and dichogamy are among the commonest types of genetic variation influencing outcrossing rates. Unfortunately, few workers have conducted genetic studies to determine their inheritance in wild species. This approach has been used in Lycopersicon pimpinellifolium (Rick et al. 1978), Mimulus spp. (MacNair and Cumbes, 1989; C. B. Fenster and K. Ritland, unpubl.), and Turnera ulmifolia (Shore and Barrett, 1990). In each case quantitative control of variation was revealed. Few attempts, however, have been made to go beyond the simple demonstration of polygenic control. Preliminary work in Mimulus and Turnera involved estimating the minimum number of genes controlling floral traits using methods developed by Lande (1981) and Cockerham (1986). Since non-additive gene action was also detected in these studies, however, the validity of these approaches is questionable.

Major gene control of floral traits that influence outcrossing is easier to detect and is particularly evident in polymorphic sexual systems such as gynodioecism (e.g. Sun, 1987) and heterostyly (Piper et al., 1984), the flower color polymorphism in Ipomoea purpurea (Schoen and Clegg, 1985), and the ray and disc floret polymorphism in Senecio vulgaris (Marshall and Abbott, 1982). The ease with which major gene systems can be modelled and studied experimentally has enabled microevolutionary studies of these polymorphisms, and provided some of the best evidence for the mechanisms responsible for mating system modification (see Clegg and Epperson, 1988).

This approach has been employed in studies of the self-compatible, tristylous, aquatic Eichhornia paniculata (Pontederiaceae). Outcrossing rates in this species are among the most wide-ranging reported from flowering plants (Fig. 2A). The variation largely results from the evolutionary breakdown of tristyly and the spread and fixation of self-pollinating homostylous variants. While mating system modification is under clear genetic control in this species, this does not preclude a role for both environmental and demographic factors. An analysis of the effects of population size and plant density on mating patterns demonstrated that both variables account for a significant proportion of the variation in t among the populations surveyed (Barrett and Husband, 1990). In addition, comparisons of the seed fertility of selfing and outcrossing morphs and the geographical distribution of homostylous populations provide strong support for the role of reproductive assurance as a selective mechanism for the evolution of self-fertilization (Barrett et al., 1989).

Self-compatible heterostylous species may often provide opportunities for studying mating system variation in natural populations (Barrett, 1989). A recent geographical survey of style morph frequencies in the self-compatible, tristylous, clonal aquatic Decodon verticillatus (Lythraceae) revealed wide variation in both morph structure (Fig. 3) and the occurrence of floral variants with stamens and styles in close proximity (C. G. Eckert and S. C. H. Barrett, unpubl.). Evidence from other heterostylous species (e.g. Ganders et al., 1985; Glover and Barrett, 1986) would suggest that this variation is likely to have a direct bearing on the levels of self-fertilization occurring within populations.

In *D. verticillatus* populations at the margins of the range, in Michigan and Ontario, are more likely to be dimorphic or monomorphic for style length than populations in more southerly areas such as Florida and Georgia. This difference may be associated with restricted opportunities for sexual recruitment and greater reliance on clonal reproduction in northern populations. Increased asexual reproduction has often been suggested in geographically or ecologically marginal portions of a species' range because of unfavorable conditions for seed germination and seedling establishment (Barrett, 1980). These effects may directly influence outcrossing rates because higher levels of self and geitonogamous pollination

A. *Eichhornia paniculata*

N = 32 Populations

B. *Lupinus nanus*

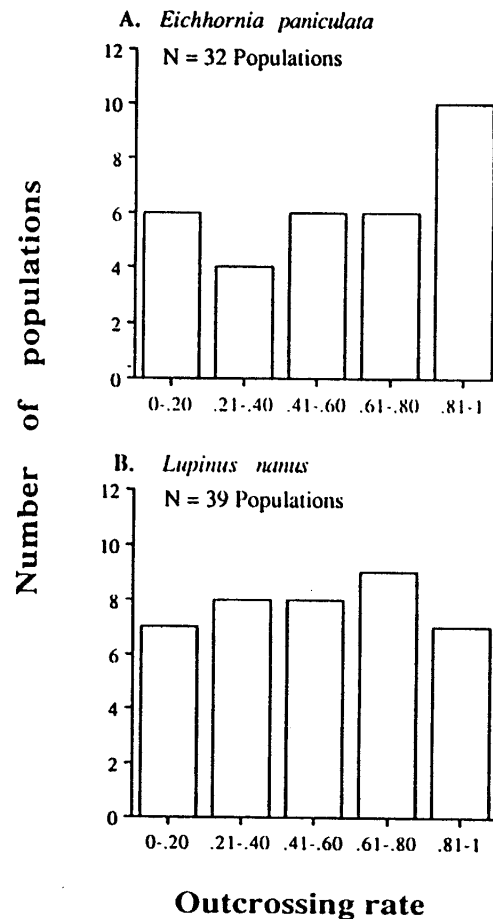N = 39 Populations

Outcrossing rate

FIG. 2 The distribution of outcrossing rates in A. Thirty-two populations of *Eichhornia paniculata* (Pontederiaceae) from northeastern Brazil and Jamaica (after Barrett and Husband, 1990); and B. Thirty-nine populations of *Lupinus nanus* (Leguminosae) from California U.S.A. (after Harding, 1970; Harding *et al.*, 1974)
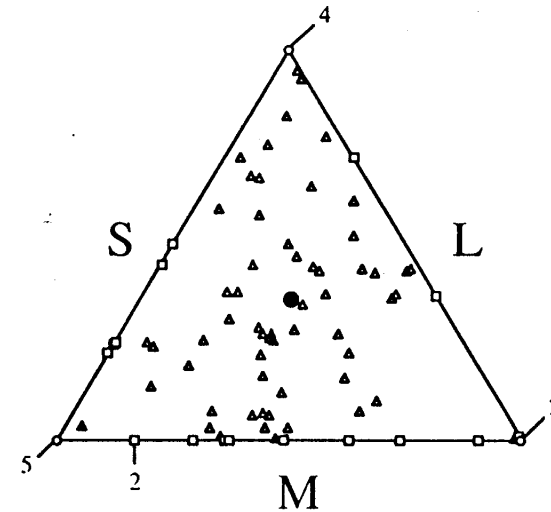
FIG. 3 Style morph frequencies in 93 populations of *Decodon verticillatus* (Lythraceae) sampled in eastern Ontario , Michigan, and southeastern USA. Each point represents morph frequencies of a single population. The distance of a population from an axis is proportional to the relative frequencies of morphs (long-, mid-, or short-styled morphs represented by L, M, S, respectively) in the population. Trimorphic, dimorphic and monomorphic populations are represented by triangles, squares and circles, respectively. Equal morph frequencies are indicated by the solid circle (C.G. Eckert and S. C. H. Barrett, unpubl.). The variation in morph structure in *D. verticillatus* is likely to be associated with considerable interpopulation variation in outcrossing rate.

would be likely in populations composed of large clones (Handel, 1985; Silander, 1985). In wide-ranging clonal species such as *D. verticillatus* this is likely to have important implications for geographical variation in mating patterns and opportunities for the evolution of self-fertilization.

Our discussion of genetic factors influencing intraspecific variation in mating systems has largely concerned models for the evolution of autogamy. Outcrossing and selfing rates are also of importance, however, for other models of mating system change, particularly those concerned with evolution of gender polymorphisms such as gynodioecism and dioecism. The selective force most commonly invoked to account for the spread of females in cosexual populations is inbreeding depression of progeny from hermaphrodites as a result of self-fertilization (Lewis, 1941; Lloyd, 1974, 1975; Charlesworth and Charlesworth, 1978). Resulting gynodioecious populations can remain evolutionarily stable, or form an intermediate stage in the evolution of dioecism (Bawa, 1980). Unfortunately, joint measures of selfing rates and inbreeding depression in gynodioecious species are unavailable (Charlesworth, 1989; Sun and Ganders, 1986; Kohn, 1988;), so it is premature to draw general conclusions on the validity of models for the evolution of sexual dimorphism based primarily on inbreeding depression .

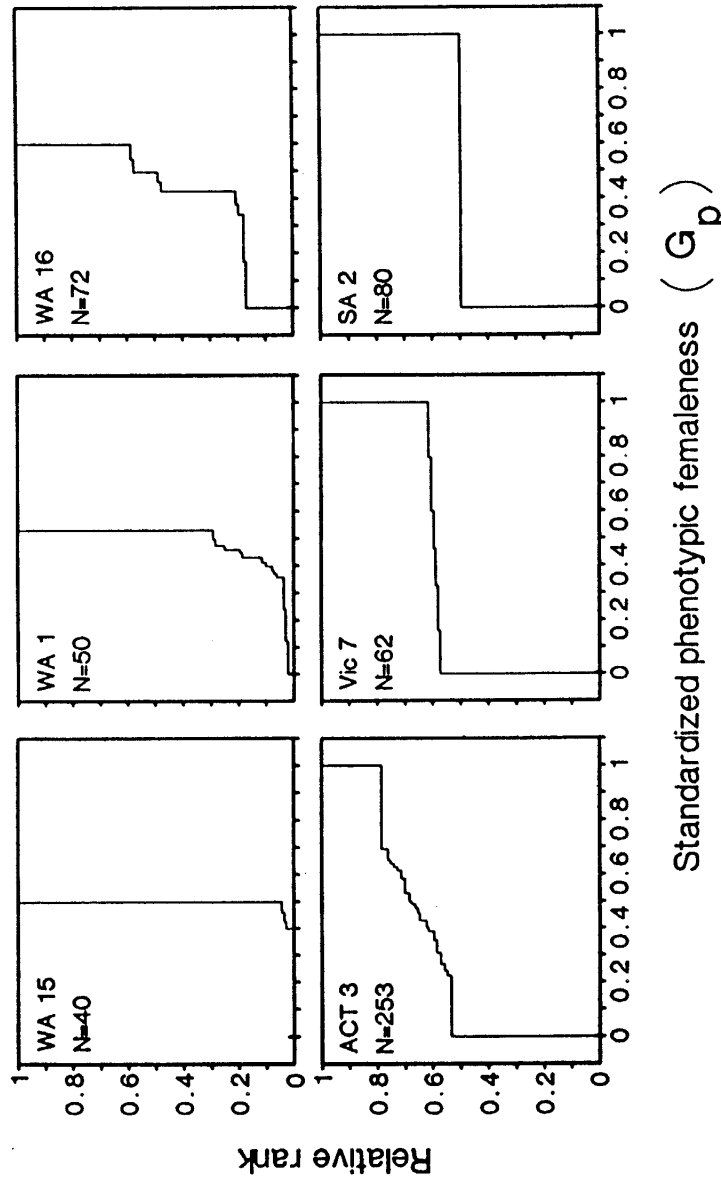A difficulty with testing models for the evolution of dioecism is that few species

FIG. 4 Variation in phenotypic gender (G_p) among six populations of *Wurmbea dioica* (Liliaceae) in Australia. Gender measures were calculated following the method of Lloyd and Bawa (1984). Plants with a G_p value of 1 or 0 are purely female or male, respectively. Sample sizes refer to the number of plants sampled in each population (S.C.H. Barrett, unpubl. MS.).

are known that possess both cosexual and dioecious populations (e.g. *Sagittaria latifolia*, Wooten, 1971; *Cotula* spp., Lloyd, 1972; *Ecballium elaterium*, Costich, 1989). It appears that once dioecism arises it is often accompanied by speciation, perhaps because of strong character divergence associated with the acquisition of sexually dimorphic flowers. While quantitative data on selfing rates, inbreeding depression and allocation of resources to male and female function can often be obtained from related species, the value of these measures for mating system models are questionable because of the confounding influences of other taxonomic differences that result from adaptive differentiation.

The Australian geophytic lily *Wurmbea dioica* provides a useful system for testing hypotheses concerned with the evolution of dioecism. In this wide ranging, fly-pollinated species of rocky woodland slopes, gender expression varies widely both within and between populations (Fig. 4). The two extremes of gender represented by cosexual and dioecious populations are linked by gynodioecious and subdioecious populations, indicating the probable evolutionary pathway to sexual dimorphism involves a gynodioecious stage. In parts of western Australia cosexual and sub-dioecious populations grow in close proximity, enabling microevolutionary investigation of the factors maintaining gender polymorphism. The estimates of phenotypic gender illustrated in Figure 4, while useful in drawing attention to the quantitative nature of sex expression, give only a rough guide to mating patterns in each population. Rigorous testing of models of mating system evolution would require outcrossing estimates for cosexual and gynodioecious populations, as well as data on inbreeding depression, male and female fertility variation, and patterns of sex allocation. Nevertheless, geographical surveys of the type conducted in *Wurmbea* and *Decodon* provide an important first step in identifying promising species for intraspecific studies of mating system evolution. Systematists, because of their wide knowledge of geographical variation in floral traits, may be in the best position to provide population biologists with this kind of information.

B. Ecological influences on mating system variation

Where ecological factors play a major role in causing variation in outcrossing levels the effect of selection on the mating system is likely to be reduced. With low heritability of traits influencing mating patterns and unpredictable changes in those features of the environment that influence reproduction, the mating system may be unable to respond in an adaptive fashion. A wide array of ecological factors can influence levels of outcrossing in plant populations. Their effects may be direct such as the influence of environmental factors (e.g. temperature, humidity) on pollination and pollen-pistil interactions, or indirect such as the effect of local habitat on the size and spatial structure of populations.

In animal-pollinated plants a major source of variation appears to be the reliability of pollen vectors. Inclement weather during flowering may influence the number and types of pollinators that visit flowers. However, even where pollinator activity

is reliable, changes in the species composition and foraging behavior of pollinators may have potentially subtle effects on mating patterns and reproductive success (Schemske and Horvitz, 1984; Wolfe and Barrett, 1988). Any consistent failure of pollinators to service outcrossing populations can potentially lead to the evolution of self-fertilization. It has been suggested that this is more likely to occur in small populations, at low density, or under ecologically or geographically marginal conditions (Lloyd, 1980). However, with few exceptions (e.g. Wyatt, 1986), there has been little effort to measure visitation levels in populations with contrasting mating systems, or to determine how often pollinator limitation occurs in outcrossing taxa in which selfing populations have evolved.

The large variation in outcrossing rates displayed by populations of several *Lupinus* species (e.g. *Lupinus nanus*, Fig. 2B) may largely result from differences in pollinator abundance within and among sites (Horovitz and Harding, 1972; Schemske and Lande, 1985). While some evidence for the genetic control of floral traits influencing mating patterns has been found in *Lupinus* spp., common garden studies strongly implicate differing pollinator levels as the major cause of variation in *t* (Horovitz and Harding, 1972). Moreover, studies of year to year variation in outcrossing rate detected several cases of dramatic changes in *t* (Harding, 1970; Harding *et al.*, 1974), a result unexpected if genetic factors were largely responsible for the observed levels of outcrossing. More work of the type conducted by Harding and colleagues on *Lupinus* is required to determine to what extent pollinator levels account for mating system variation in other animal-pollinated plants. In addition, the role that different pollinators play in influencing outcrossing rates would be particularly valuable.

Several other ecological factors have been implicated as the cause of mating system variation in plant populations (Table 1). However, the effects of these parameters may be highly idiosyncratic, depending on particular attributes of the species in question. Plant density and plant size are good examples of this problem. Studies in both animal- and wind-pollinated species have produced contrasting results with respect to the effect of density on outcrossing rate (Table 1). Similarly, the predicted relationship between plant size and outcrossing rate might appear straightforward, because of the propensity of large plants to experience high levels of geitonogamous pollination, however, the limited data available provide variable results. In *Malva moschata*, the selfing rate and size of floral display were positively correlated (Crawford, 1984) , whereas in *Sabatia angularis* this relationship was only obtained in one of four subpopulations that were investigated (Dudash and Barrett, 1989).

Most studies on the effects of ecological factors on outcrossing rates are based on correlations rather than experimental approaches. Because of the complex interactions of ecological variables it can be extremely difficult to isolate important factors affecting mating patterns using a correlative approach. The ease with which many plants can be experimentally manipulated should offer broad scope for controlled investigation in this regard.

VI. Future Directions

There has been impressive growth in studies of plant mating systems, particularly procedures for measuring mating parameters and the formulation of theoretical models for evolutionary change. Despite this progress, our discussion has highlighted a number of issues concerned with the evolution of mating systems where our existing body of knowledge is limited. In this concluding section we draw attention to several areas which we believe would repay detailed investigation in the future.

A. Taxonomic and ecological surveys of outcrossing rates

The 129 species in our survey of outcrossing rates is far from a random sample. Aside from a general appeal for data from taxonomic and ecological groups not studied to date, there are several gaps in the data of particular interest to models of mating system evolution. For example, what is the true distribution of *t* among wind-pollinated species, and will estimates from groups other than conifers and grasses support the strong bimodal distribution apparent in the current data? If pollen vector reliability is a key factor in explaining outcrossing rate distributions (Aide, 1986), estimates from hydrophilous plants may be particularly interesting because of parallels between wind and water in terms of their reliability as abiotic vectors of pollen. In addition, quantitative estimates of mating system parameters in Pteridophytes and Bryophytes would also be valuable and allow comparisons to be made with the patterns observed in seed plants.

B. Intraspecific studies of spatial and temporal variation of outcrossing rates

In 48% of the species in our sample *t* was estimated only once from a single population. More extensive sampling of populations is likely to detect variation, particularly in animal-pollinated, self-compatible species. For those species in which a mixture of self- and cross-fertilization occurs it would be particularly valuable to determine the extent of temporal variation in outcrossing rates both within (e.g. Burdon *et al.*, 1988) and between (e.g. Cheliak *et al.*, 1985) seasons. If outcrossing rates in populations with intermediate values are of similar magnitude over several years it would provide some evidence in support of the mixed mating system as an evolutionarily stable strategy. Perhaps the most critical gap in our knowledge concerns the pattern and extent of plant to plant variation in mating behavior within populations. To what extent do attributes such as size, spatial location and floral display influence outcrossing rates? Statistical procedures required to estimate individual outcrossing rates are limited by the need for large sample sizes. The search for a wider array of genetic markers, such as restriction fragment length polymor-

phisms, or hypervariable minisatellite sequences, may alleviate this problem to some extent.

C. Genetic analysis of mating system variation

More information is required on the genetic architecture of reproductive traits that influence mating patterns. At present we know little about the extent of heritable variation for reproductive traits within natural plant populations. Quantitative genetic approaches are required to determine the heritability of outcrossing rates and the extent to which patterns of genetic correlation among floral traits are likely to influence response to selection on the mating system. Of particular importance will be to determine how often selfing rates are under simple versus more complex modes of inheritance; this information has a direct bearing on the evolutionary dynamics of mating system modification (Lande and Schemske, 1985; Charlesworth et al., 1990). Since inbreeding depression is a critical parameter in most models of the evolution of self-fertilization more empirical work is required on its occurrence and genetic basis, particularly in populations with different mating systems. Of particular interest will be to determine whether genetic variation for inbreeding depression commonly occurs within natural populations. Such variation has important consequences for the spread of genes influencing the selfing rate (Holsinger, 1989). Because the dynamics of genetic change for more than one locus in populations with partial selfing are so complex, predicting the course of mating system evolution is likely to be particularly difficult (Weir and Cockerham, 1973; Wright, 1987). Further theoretical work on the quantitative genetics of partial selfing may help clarify some of the issues concerned with the evolution of mixed mating systems.

D. Measurement of selection on mating systems

While the measurement of selection in natural populations has advanced greatly in the last decade (Endler, 1986), there have been few attempts to measure selection on traits likely to influence plant mating systems (see Campbell, 1989; Schemske and Horvitz, 1989). Estimating the strength and nature of selection, however, is central to testing models of mating system evolution. For instance, Lande and Schemske's (1985) models predict strong directional selection on the outcrossing rate within populations. Measurement of selection on the mating system is difficult because the outcrossing rate is a complex phenotypic trait reflecting interactions between reproductive characters, demography and environmental factors (Table 1). Although this appears complicated, quantitative methods such as path analysis (Schemske and Horvitz, 1988) and multivariate selection models (Lande and Arnold, 1983) may enable us to untangle some of this biological complexity.

E. Phylogenetic analysis of mating systems

Most work on mating systems has been conducted by geneticists and evolutionary biologists working at the population or species level. There is now a need to couple these microevolutionary studies with patterns of macroevolution. This is of particular importance since mating system traits are a special class of evolutionary characters. By influencing mating patterns, they not only affect their own transmission but also the transmission of all other genes in an organism. Studies on mating system variation among related species should be conducted in conjunction with estimation of phylogenetic relationships using cladistic methods (Olmstead, 1989). Molecular approaches that use various classes of DNA now offer a source of characters which can be used for reconstructing phylogenies and are independent of the mating system. Once phylogenies have been estimated, the distribution of mating systems can be mapped onto the phylogeny enabling various evolutionary questions to be addressed (Coddington, 1988). Of particular interest will be how often and in what sequence in relation to other reproductive traits breeding systems such as dioecism, heterostyly, self-incompatibility, and autogamy have evolved within lineages. Many controversies that relate to evolutionary origins will undoubtedly be resolved by the adoption of phylogenetic approaches. Systematists could play a leading role in these investigations.

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