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Experimental Studies of Mating-System Evolution: The Marriage of Marker Genes and Floral Biology

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Introduction

Analysis of the causes and consequences of mating-system evolution is a major focus of research in plant population biology since shifts in mating pattern have important consequences for population genetic structure (Brown, 1979; Hamrick and Godt, 1990) and patterns of evolutionary diversification (Stebbins, 1974; Jain, 1976; Charlesworth, 1992). Empirical studies of the genetic consequences of contrasting mating systems have provided clear evidence of their role as primary determinants of the amounts and organization of genetic diversity within and among populations (e.g., Layton and Ganders, 1984; Holtsford and Ellstrand, 1989; Costich and Meagher, 1992). The evolutionary fate of genes that alter the mating system can therefore have large consequences for the future evolution of the species. Predicting the course of mating-system evolution is a complex problem, however, because of the numerous genetic, environmental, demographic, and historical factors that can influence mating patterns (Schemske and Lande, 1985; Brown et al., 1990; Barrett and Eckert, 1990).

During the past 15 years, a rich theoretical literature on the evolution of plant mating systems has developed. A variety of genetic and phenotypic models incorporating variation in mating, fertility, allocation patterns, differences in fitness between selfed and outcrossed offspring, and contrasting demographic, life history, and environmental variables have been used to explore the conditions that might favor the evolution of different levels of self- and cross-fertilization (e.g., Lloyd, 1979a; Wells, 1979; Ross and Gregorius, 1983; Holsinger et al., 1984; Lande and Schemske, 1985; Campbell, 1986; Uyenoyama, 1986; Charlesworth and Charlesworth, 1990; Iwasa, 1990; Holsinger, 1991; Yahara, 1992). The formulation of theoretical models of mating-system evolution has benefited from a recognition tha floral

traits influencing mating patterns may be subject to sexual selection and the optimal allocation of resources to female and male function (Charnov, 1982; Willson and Burley, 1983; Queller, 1983; Bell, 1985; Lyons et al., 1989). In addition, models have been developed to account for the evolution of particular reproductive systems such as heterostyly (Charlesworth and Charlesworth, 1979a; Muenchow, 1982; Lloyd and Webb, 1992a,b), self-incompatibility systems (Charlesworth and Charlesworth, 1979b; Uyenoyama, 1988), gynodioecy and dioecy (Lloyd, 1975; Charlesworth and Charlesworth, 1978; Givnish, 1982; Ross, 1982; Muenchow and Grebus, 1989), cleistogamy (Schoen and Lloyd, 1984), and more generally the evolution of combined versus separate sexes (Charlesworth and Charlesworth, 1981; Charnov, 1982; Lloyd, 1982). This work has provided the necessary conceptual framework for microevolutionary studies of mating systems based on models of individual selection. The approach contrasts with earlier largely intuitive group selection arguments involving "immediate fitness" and "long-term flexibility" that were proposed to account for the evolution of mating systems (Darlington, 1939; Stebbins, 1958; Grant, 1958).

Although these conceptual developments represent considerable progress for the study of mating-system evolution, it is clear that theoretical models have advanced more rapidly than the collection of relevant empirical data. For many of the important parameters in models of mating-system evolution (e.g., inbreeding depression, pollen discounting, biparental inbreeding, allocation patterns), few data are available from natural populations. As a result, their relative importance as factors influencing mating-system evolution are often difficult to evaluate.

Many generalizations concerning the evolutionary forces influencing mating-system evolution have been inferred from comparative studies of closely related taxa (Stebbins, 1957; Raven, 1979; Wyatt, 1988). In addition, a variety of ecological and geographical correlates have been identified as important for understanding particular mating-system shifts such as the evolution of self-fertilization (Baker, 1959; Jain, 1976; Lloyd, 1980; Barrett, 1989) and dioecy (Bawa, 1980; Thomson and Brunet, 1990; Weller and Sakai, 1990; Barrett, 1992). Paradoxically, similar conditions, such as bottlenecks, low density, and uncertain or altered pollinator regimes, have often been invoked to account for the evolution of selfing as well as mechanisms that promote outcrossing (e.g., dioecy, gynodioecy, and heterostyly). Because of the complex interplay between ecological, genetic, and historical factors, a strictly correlative approach is unlikely to enable isolation of the specific selective forces governing mating-system change. Moreover, for particular mating systems (e.g., predominant self-fertilization) the evolutionary processes responsible for the establishment of genes modifying mating patterns may be difficult to infer from present-day pro-

cesses, because the genetic and ecological conditions that favored such changes are transient (Uyenoyama and Antonovics, 1987). To prevent the gulf between theoretical and empirical work on mating-system evolution from widening further, more experimental studies are required, particularly on species with broad variation in mating patterns.

A prerequisite for the development of biologically realistic models of mating-system evolution is accurate information on the mating process, the ecological and demographic context in which it occurs, and the fitness consequences of various mating strategies. During the past decade, considerable progress has been made in the use of isozyme markers for measuring a variety of mating-system parameters (Clegg, 1980; Ritland, 1983; Brown, 1990). Quantitative estimates' of outcrossing rates using single loci and the mixed-mating model have given way to new estimation procedures based on multilocus approaches including the effective selfing model, the analysis of correlated matings, estimates of male fertility, and the paternity of outcrossed progeny. Electrophoretic techniques provide the reproductive biologist with a powerful tool for obtaining quantitative information on mating patterns in plant populations. The marriage of marker gene studies with detailed ecological observations on floral biology is required to determine how floral morphology and physiology influence the frequency of various classes of mating events. For example, information on ecological influences causing different modes of self- and cross-pollination in natural populations is needed to evaluate particular models of the evolution of self-fertilization (Lloyd, 1979a; Schoen and Brown, 1992). Future marker gene studies at increasingly more refined scales (e.g., at the population, individual, and flower levels) are likely to provide the background information for evolutionary investigations on the selective forces maintaining mating-system variation.

Experimental studies using genetic markers can be particularly revealing in plant species that are polymorphic for floral traits. Fitness comparisons of genotypes with similar genetic backgrounds, but that differ in traits influencing reproductive success, provide opportunities for investigating the potential selective forces operating on mating systems. For example, work on flower color polymorphisms in experimental populations of *Ipomoea purpurea* (L.) Roth. (Clegg and Epperson, 1988) and *Raphanus raphanistrum* L. (Stanton et al., 1989) have provided important information on the spread of mating-system modifier genes and on variation in male reproductive success, respectively. Unfortunately, relatively few species are known with discrete genetic polymorphisms for mating behavior within natural populations (although see Abbott, 1985). The best known involve the outcrossing and selfing morphs in mixed populations of heterostylous and homostylous plants (Crosby, 1949; Bodmer, 1960; Ganders, 1975; Barrett, 1979; Piper et al., 1984) and the coexistence of female (unisexual)

and hermaphroditic (bisexual) morphs in gynodioecious species (Lloyd, 1976; Gouyon and Couvet, 1987; Kohn, 1989). These polymorphic sexual systems provide a rare opportunity to investigate the relative fitness of different morphs under various ecological conditions. In addition, manipulative studies of the floral biology of polymorphic populations provide data that can be used in a predictive manner to understand the evolution and maintenance of these complex sexual systems.

In this chapter, we review experimental approaches used to study the genetic and environmental factors influencing mating-system variation in tristylous *Eichhornia paniculata* (Spreng.) Solms-Laubach (Pontederiaceae). We begin by outlining the features of this species that have made it a useful experimental system for analyzing mating-system problems and then describe what is known about variation in mating patterns of natural populations. The results from a series of experimental studies are presented next to illustrate the complex forces acting on the mating system and how the interaction of genetic and environmental factors can modify mating patterns, even in a species with major-gene control of floral traits governing its breeding system.

The Experimental System

Eichhornia paniculata is an emergent aquatic of seasonal pools, marshes, ditches, and low-lying pastures in N.E. Brazil and the Caribbean islands of Jamaica and Cuba. Single isolated localities are also reported from W. Brazil, Ecuador, and Nicaragua (Barrett, 1988). When moisture is available for extended periods, plants of *E. paniculata* behave as perennials; however, most populations are annual owing to uncertain moisture levels and desicc: on of habitats. Populations range in size from isolated individuals to mo specific stands containing several thousand plants. Most commonly however, they are small (<100 individuals) and short-lived due to drougl.. and habitat destruction (Barrett et al., 1989). Genetic estimates of effective population size (N_e) in 10 populations from N.E. Brazil ranged from 3.4 to 70.6 with a mean of 15.8, a value approximately 10% of the census number (Husband and Barrett, 1992a). Individuals display considerable phenotypic plasticity depending on site conditions with plants varying in height from 10 to 150 cm. *Eichhornia paniculata* does not regenerate by clonal propagation under field conditions, but plants can be cloned under glasshouse culture by severing young axillary shoots from the main shoot.

Flowers of *E. paniculata* are showy, mauve-blue, zygomorphic, and borne on inflorescences that usually contain 5–100 flowers that last for up to 12 days (Richards and Barrett, 1984). The anthesis period of individual flowers

is 6–8 hr, with the major pollinators in N.E. Brazil being long-tongued solitary bees (*Florilegus festivus* Smith and *Ancyloscelis* spp.) and butterflies (Husband and Barrett, 1992b). Other floral visitors include pollen-collecting Meliponid bees and, in Jamaica, *Apis mellifera* L. and occasional syrphids. Seeds of *E. paniculata* are small (0.15 mg), produced in large numbers per capsule (50–120), and shed 10–14 days after pollination. They are dispersed locally by water, over longer distances apparently by waterfowl, and germinate synchronously when rains follow the marked dry season that is typical of the parts of Brazil and Jamaica in which the species occurs.

Eichhornia paniculata exhibits a wide range of mating patterns at are associated with the evolutionary breakdown of tristyly to semiho ostyly (Barrett, 1985a,b). It was the discovery of this variation, and its as ciated diversity in floral morphology, that motivated our interest in u...ng the species as an experimental system for mating-system analysis. Clearly, a major prerequisite for studies of this type is evidence that the mating system varies within and between populations and that this variation is associated with heritable differences in floral traits. Populations of *E. paniculata* often contain a mixture of outcrossing and selfing variants. Differences between the floral forms have a genetic basis with variation largely under major-gene control (S.C.H. Barrett, unpublished data). The outcrossing forms represent the conventional long-, mid-, and short-styled morphs of a tristylous system (hereafter L, M, and S morphs). The selfing variants are modified plants of the M morph (hereafter selfing variants) with between 1 and 3 elongated short-level stamens (Seburn et al., 1990; Barrett and Harder, 1991). Anthers of elongated stamens lie close to mid-level stigmas, making autonomous self-pollination of flowers possible. The different floral phenotypes in *E. paniculata* are easily recognizable under field conditions, thereby enabling surveys of their relative frequency (Barrett et al., 1989).

Eichhornia paniculata has a number of other features that facilitate both observational and experimental studies. Populations are conspicuous, easily studied under field conditions, and abundant, particularly in N.E. Brazil. The plant is diploid, highly self-compatible, has low levels of seed abortion (Morgan and Barrett, 1989), is easily crossed and cultured, flowers in 2–3 months from seed, blooms year-round under glasshouse conditions, and is polymorphic at 10–15 isozyme loci (Glover and Barrett, 1987), several of which can be assayed at the seed stage.

One of our major objectives in studying *E. paniculata* has been to obtain a comprehensive picture of the variation in mating systems of populations from different parts of the geographical range (Glover and Barrett, 1986; Barrett and Husband, 1990; Husband and Barrett, 1991b). This has been achieved by sampling open-pollinated families from a large number of populations from throughout the range of the species in N.E. Brazil and

on the island of Jamaica. Estimates of population size, plant density, and frequencies of floral morphs have also been made in each population. Multilocus outcrossing rates (*t*) were estimated for each population using isozyme markers and the estimation procedure of Ritland (1990). Figure 9.1 illustrates the range of outcrossing rates among populations of varying size and morph structure. Estimates of *t* range from 0.002 to 1.0, the widest range of values reported for any plant species (reviewed by Barrett and Eckert, 1990). Populations from Jamaica are predominantly selfing (mean *t* = 0.21, range 0.002–0.68, *N* = 11), whereas those from N.E. Brazil are more highly outcrossing, although several populations exhibit selfing rates comparable to those measured in Jamaica (mean *t* = 0.74, range 0.19–1.00, *N* = 43). Multiple regression and partial correlation analyses revealed that 60% of variation in *t* can be explained by style morph diversity and the frequency of selfing variants within populations (Barrett and Husband, 1990).
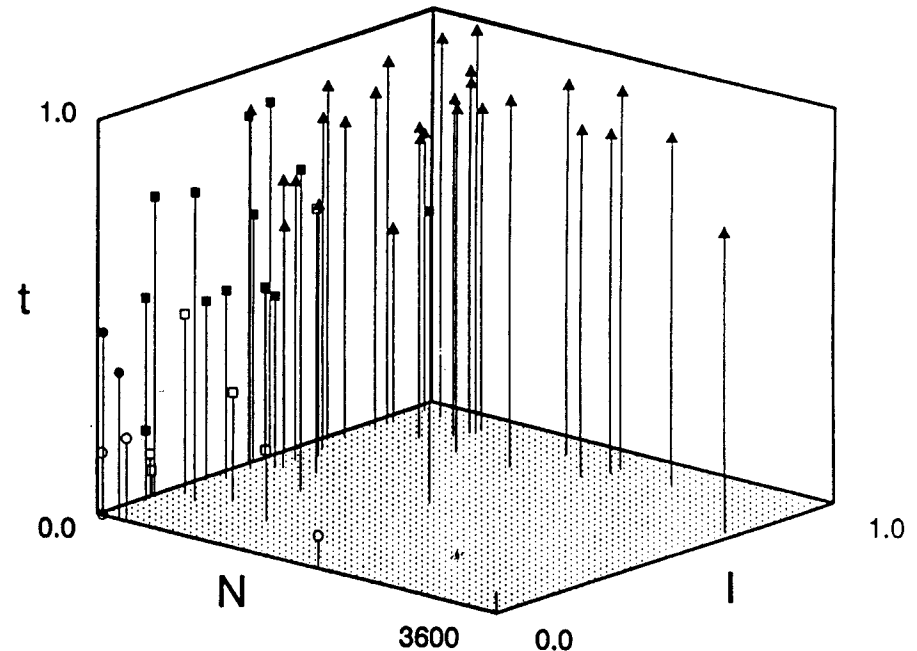

Figure 9.1. Variation in multilocus outcrossing rate (*t*) among 54 populations of *Eichhornia paniculata* in N.E. Brazil (closed symbols) and in Jamaica (open symbols) in relation to their size (*N*) and style morph diversity (*I*). Trimorphic, dimorphic, and monomorphic populations are represented by triangles, squares, and circles, respectively. Values of *I* are based on a normalized index of diversity and range from 1.0, equal frequencies of the floral morphs, to 0, floral monomorphism (after Barrett and Husband, 1990, and unpublished data).

1990). Population size and plant density also account for a significant portion of variation in t, particularly in Jamaica, where style morph diversity is low owing to a predominance of selfing variants and absence of the S morph from the island.

The wide range of population-level outcrossing rates found in *E. paniculata* raises a number of questions concerning the details of the mating process within populations and the ecological and evolutionary processes producing mating-system variation. What factors account for high outcrossing rates in some populations and the occurrence of predominant self-fertilization in others? Which aspects of the floral biology of populations have an important impact on mating patterns? How does population morph structure influence mating patterns and how important is the ecological and demographic context in which mating occurs for mating-system evolution? To address these issues, we have undertaken a series of investigations involving field, garden, and glasshouse experiments. The underlying approach in these studies has been largely reductionistic, involving an attempt to decompose the mating process into its elementary causative agents by experimental means. Although this mechanistic approach suffers from the valid criticism that ecological realism may be sacrificed, we believe that progress in reproductive ecology requires a broader understanding of the specific details of floral biology and its influence on mating patterns. This is more likely to be achieved by adopting an experimental approach in which particular stages of the mating process are analyzed to determine the possible mechanisms governing the dynamics of mating-system change.

Mating in Trimorphic Populations

Trimorphic populations of *Eichhornia paniculata* exhibit moderate to high levels of outcrossing (Fig. 9.1: mean t = 0.83, range 0.5–0.96, N = 27). Nevertheless, some self-fertilization is evident in most populations, particularly those of small size. This is consistent with the view that demographic factors are likely to influence mating patterns in self-compatible plants (Ganders, 1975; Lloyd, 1980; Schemske and Lande, 1985; Barrett and Eckert, 1990; Holsinger, 1991). Small populations may receive less reliable pollinator service resulting in increased levels of self-pollination. Attempts to correlate pollinator levels and mating patterns have proven difficult (Lloyd, 1965; Rick et al., 1977; Wyatt, 1986) and an investigation of pollinator visitation to 16 populations of *E. paniculata* in N.E. Brazil failed to detect a positive relationship between pollinator densities and population size (Husband and Barrett, 1992b). Visitation levels were, however, more variable among small populations, a pattern consistent with the hypothesis that small populations are more likely to experience un-

certain pollinator service. The difficulties in establishing relationships between ecological factors and mating patterns by correlative approaches illustrate the need for experimental studies in this area.

Floral Trimorphism and the Maintenance of Outcrossing

The high outcrossing rates measured in most trimorphic populations of *E. paniculata* are unusual for a predominantly annual, self-compatible species and are more characteristic of long-lived species (Barrett and Eckert, 1990). Controlled pollinations of *E. paniculata* have demonstrated that the species is highly self- and intramorph compatible (Barrett, 1985a; Barrett et al., 1989; Kohn and Barrett, 1992a). Moreover, individuals usually produce many flowers that open synchronously, permitting both intrafloral and geitonogamous pollen transfer. What factors counteract these effects to maintain high outcrossing rates? Several reproductive mechanisms could potentially be involved: (1) the reciprocal arrangement of stamens and styles (reciprocal herkogamy) in the floral morphs of this heterostylous species, (2) differential pollen germination and pollen tube growth of self versus outcross pollen, and (3) differential survivorship of selfed versus outcrossed embryos or plants. Studies of embryo abortion in *E. paniculata* (Morgan and Barrett, 1989; Toppings, 1989) found low levels of ovule abortion (<10%), and a glasshouse study found no differences in germination, seedling growth, and survival of selfed and outcrossed progeny (Toppings, 1989). Hence, none of these factors seems likely to have inflated our measures of outcrossing rate to any great extent. To investigate the relative importance of the remaining two factors, several experimental studies were undertaken.

Reciprocal Herkogamy

We used experimental garden populations of different floral morph structure to investigate the role of reciprocal herkogamy in promoting outcrossing in *E. paniculata*. Our goal was to determine whether mating patterns and measures of fertility differed between trimorphic populations and those containing a single morph. Controlled crosses were used to produce plants that were homozygous for alternative alleles at the triallelic *Aat-3* locus and polymorphic at two additional electrophoretic marker loci (*Pgi-2* and *Acp-1*). Trimorphic populations composed of 12 plants of each morph were assembled in which style morphs were homozygous for different alleles at the *Aat-3* locus. This allowed joint estimation of the levels of intermorph and intramorph mating and outcrossing rate (Kohn and Barrett, 1992a). For logistical reasons, these manipulations could not be undertaken under field conditions in N.E. Brazil. Instead the experiments were conducted in Ontario where populations were serviced by novel pollinators

[*Bombus fervidus* (Fabricius), *B. impatiens* Cresson, and *B. vagans* F. Smith]. Thus, an important issue in interpreting the results of these studies was the extent to which the use of alien pollinators may have modified mating patterns from those occurring in the native habitat.

Both outcrossing rates and seed-set were higher for all three morphs in trimorphic than in monomorphic populations (Fig. 9.2), but the magnitude of the differences varied significantly among the morphs. The higher seed-sets were apparently due to increased pollen deposition on stigmas in trimorphic populations. Outcrossing rates in experimental trimorphic populations did not vary among style morphs or replicates. The mean outcrossing rate (0.81) was similar to that obtained for the population from which experimental plants originated (t = 0.87; M.R. Dudash and S.C.H.
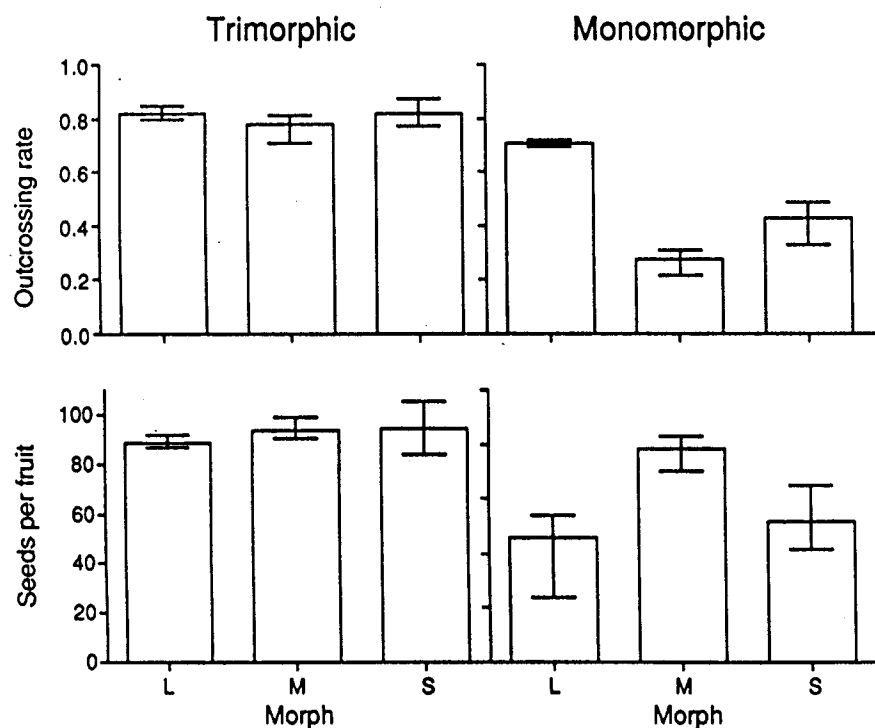

Figure 9.2. Mean morph-specific outcrossing rate (t) and seed-set per fruit in experimental trimorphic and monomorphic garden populations of *Eichhornia paniculata*. Bars represent the range of mean values from three replicates of each treatment. Trimorphic populations were composed of 12 plants per morph and monomorphic populations contained 36 plants of a single morph (after Kohn and Barrett, 1992a).

Barrett, unpublished data). This indicates that, despite the artificial conditions of our experimental garden, the influence of floral trimorphism on outcrossing rates does not appear to have been altered to any great extent. The similar outcrossing rates observed in field and garden populations challenge the widely held assumption that a high degree of pollinator specificity is required for the functioning of heterostylous systems.

The observation that seed-set increased in trimorphic populations may be of general significance for models of the evolution of heterostyly (reviewed by Barrett, 1990). If the ancestral condition in heterostylous groups resembled the L morph, as suggested by Lloyd and Webb (1992a), then the large increase in seed-set compared to the relatively small increase in outcrossing rate (Fig. 9.2), suggests that the selective basis for the evolution of reciprocal herkogamy may have been increased pollen transfer, rather than higher levels of outcrossing. The validity of this interpretation depends, however, on which morph most closely resembles the ancestral condition, as the other two morphs displayed large increases in outcrossing rate when in trimorphic populations.

If outcrossing events in experimental trimorphic populations were random, expected rates of intermorph mating would be 69% because 24 of the 35 non-self pollen donors in each array were of a different morph than the recipient. Of the outcrossed seeds assayed from experimental trimorphic populations, however, 95% resulted from intermorph fertilizations (Fig. 9.3). Rates of intermorph mating, estimated from a different trimorphic population in N.E. Brazil, indicated that a large fraction (92%) of outcrossed progeny also resulted from intermorph mating (Fig. 9.3). The high rates of intermorph mating found in both experimental and natural populations indicate that mechanisms favoring intermorph over intramorph mating occur in *E. paniculata*. Further experiments were conducted to investigate what factors might be involved.

Postpollination Mechanisms

In the garden experiments described above, the high levels of intermorph mating could result from both high levels of intermorph pollen transfer and postpollination discrimination among pollen types. Reciprocal herkogamy alone can increase the frequency of intermorph pollen transfer (Ganders, 1974; Barrett and Glover, 1985; Lloyd and Webb, 1992b); however, in species studied to date, illegitimate pollen grains typically outnumber legitimate grains in most stigmatic pollen loads (Ganders, 1979). A preliminary study (Glover and Barrett, 1986) indicated that, although there may be the potential for discrimination between selfed and outcrossed pollen, there was little evidence of discrimination among outcross pollen types. Such discrimination would be a prerequisite for postpollination pro-
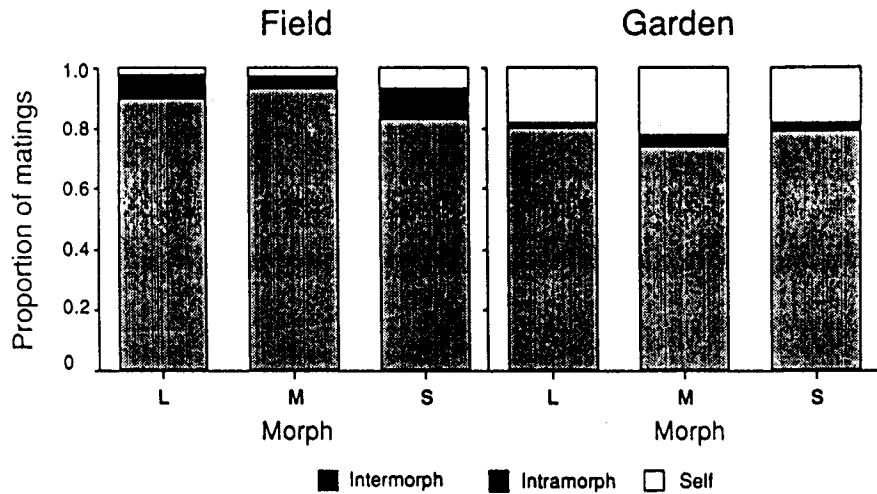
Figure 9.3. Partitioning of mating events in field and garden populations of
Eichhornia paniculata. For each floral morph (L, M, and S), intermorph,
intramorph, and self matings were estimated from open-pollinated progeny
arrays using genetic markers. Field population data are from Barrett et al.
(1987), and garden population data are from Kohn and Barrett (1992a).

cesses to affect levels of intermorph mating. To assess the potential for
postpollination discrimination among pollen types, experiments were con-
ducted using plants derived from the same population in Brazil as the
plants used in the experimental garden populations (Cruzan and Barrett,
1992 and unpublished data). The following questions were addressed: (1)
Does pollen from different anther levels germinate or grow at different
rates when deposited on each of the three style morphs? (2) When a mixture
of pollen is deposited on stigmas, does the siring ability of pollen types in
the mixture depend on style morph?

If postpollination processes contribute to the observed levels of inter-
morph mating, we might predict that legitimate pollen (i.e., pollen from
anthers equivalent in height to the stigma) should germinate or grow faster
than illegitimate pollen (i.e., pollen from the other two anther levels) in
all three style morphs. This expectation was not upheld for pollen ger-
mination: there was little difference in the germination of pollen from
different anther levels on any of the three style morphs. On the other hand,
pollen tube growth did depend on style morph. In each morph the number
of legitimate pollen tubes reaching the ovary after 3 hr was significantly
higher than for both illegitimate types. These patterns could be due to
either differential growth rate or failure of pollen tubes (i.e., attrition: see
Cruzan, 1989) in the style.

To determine the potential for differences in pollen tube growth rate to
affect siring success, mixtures of genetically marked pollen were applied
to stigmas of each style morph at different densities. The pollen density
classes (high and low) were used to explore the possibility that mating
patterns may be influenced by the total amount of pollen deposited on
stigmas. Patterns of pollen siring ability reflected the observed differences
in pollen tube growth rate; legitimate pollen always obtained more fertil-
izations than both illegitimate pollen types (Fig. 9.4). The relative com-
petitive ability of pollen in each recipient style morph did not depend on
its source (i.e., self, intramorph, or intermorph), but only on the anther
level from which it originated. The total amount of pollen applied to stigmas
had a large effect on the frequency of fertilizations by legitimate pollen
(Fig. 9.4). Higher pollen loads resulted in a significant increase in the
frequency of legitimate fertilization in the L and M morphs. In the S morph,
however, there was no increase in the frequency of legitimate fertilization
for larger stigma pollen loads.

These experiments suggest that postpollination mechanisms involving
differential pollen tube growth contribute to the high levels of outcrossing
and intermorph mating observed in *E. paniculata*. Further studies are re-
quired, however, to determine the relative roles of reciprocal herkogamy
and pollen competitive ability in promoting these mating patterns under
different demographic and environmental conditions.

The data on pollen competitive ability in *E. paniculata* closely parallel
the patterns of pollen tube growth and seed-set observed in related tris-
tylous *Pontederia* species with strong trimorphic incompatibility systems
(e.g., Barrett and Anderson, 1985; Scribailo and Barrett, 1991). This sug-
gests that the functional basis of differences in pollen competitive ability
in *E. paniculata* results from a weak (or cryptic) trimorphic incompatibility
system. The predominantly annual life cycle of *E. paniculata* and ephemeral
aquatic habitats it occupies may have favored a more flexible reproductive
system than the strong self-incompatibility observed in *Pontederia* species,
which tend to occur in more permanent aquatic habitats. The ability to
produce large numbers of seeds under a variety of ecological conditions,
while also being able to take advantage of high levels of pollinator activity
by producing mostly outcrossed progeny, was suggested as the primary
factor maintaining cryptic incompatibility in *Clarkia unguiculata* (Bowman,
1987) and has been called the "best-of-both-worlds" strategy by Becerra
and Lloyd (1992). Whereas cryptic incompatibility in *E. paniculata* may
allow for the maintenance of populations by assuring high seed output, the
lack of strong incompatibility barriers combined with uncertain pollinator
service likely contributes toward the diversity of morph frequencies and
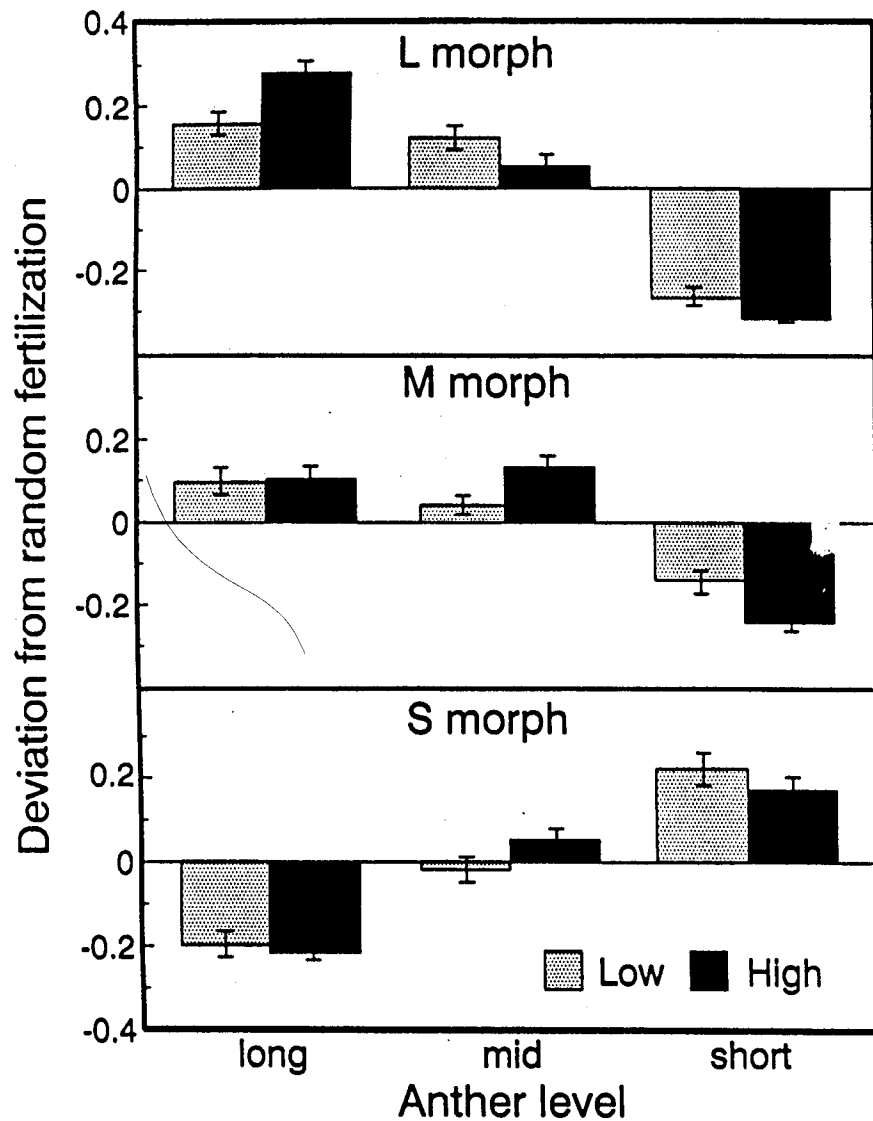selfing rates found in populations throughout N.E. Brazil (Fig. 9.1).

Figure 9.4. Fertilization success of pollen from long-, mid-, and short-level anthers when applied in equal mixtures to stigmas of the L, M, and S morphs of *Eichhornia paniculata*. Pollen mixtures were applied at two densities (high and low), and genetic markers were used to determine the fertilization success of the different pollen types. A value of zero indicates random fertilization. Error bars indicate two standard errors of the mean (M.B. Cruzan and S.C.H. Barrett, unpublished data).

Pollen Precedence and Correlated Mating

Field observations of pollinator visitation indicate that flowers of *E. paniculata* commonly receive multiple visits from insect pollinators. Because flowers are short-lived and pollen types differ in competitive ability, it was of interest to determine whether the probability of fertilization depends on the sequential order of pollination (Epperson and Clegg, 1987a) and whether pollen precedence differed among the morphs. To investigate these possibilities, experiments involving the application of genetically marked pollen mixtures and electrophoretic screening of seed progeny were used (Graham and Barrett, 1990 and unpublished data).

Early-arriving pollen experienced a fertilization advantage, but the magnitude of this advantage varied with floral morph and whether pollen was self or outcross (Fig. 9.5). Time delays in the order of pollination >2-3 hr virtually guaranteed that all fertilizations resulted from early-arriving pollen, irrespective of pollen type. For shorter time intervals, however, outcross pollen experienced a greater fertilization advantage than self pollen when each pollen type was applied first. This effect was most pro-
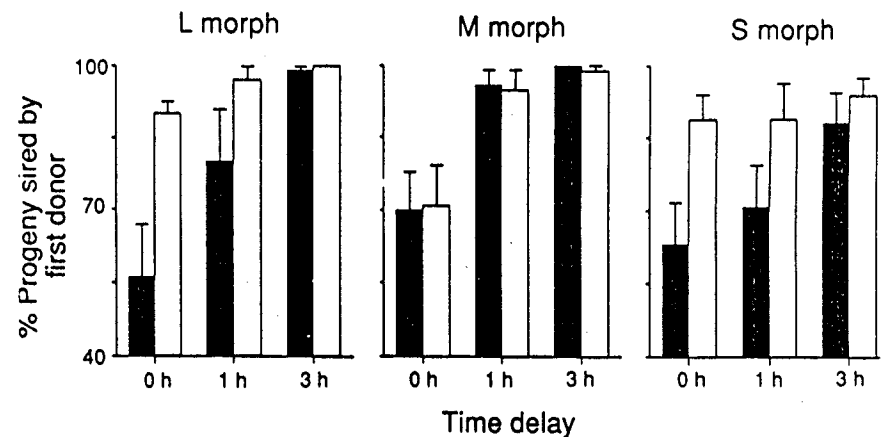

Figure 9.5. Pollen precedence in the L, M, and S morphs of *Eichhornia paniculata*. Self or outcross pollen was applied in equal amounts either 0, 1, or 3 hr after the first pollination. Hatched bars indicate treatments where self pollen was applied first and outcross pollen second; open bars represent treatments where outcross pollen was applied first and self pollen second. Self pollen types used from each morph were L morph, mid-level anthers; M morph, long-level anthers; and S morph, mid-level anthers. Outcross pollen was always from the same anther level as the stigma (legitimate pollen). The mean percentage of progeny sired by the first donor was obtained using genetic markers. Error bars indicate two standard errors of the mean (after Graham and Barrett, 1990 and unpublished data).

nounced in the L and S morphs. In the M morph, there was no significant difference between self and outcross pollen in the degree of precedence shown by early-arriving pollen. This suggests that the M morph is more likely to experience self-fertilization if outcross pollen delivery is delayed owing to reduced levels of pollinator service.

The results of these experiments suggest that individual maternal plants are unlikely to experience random outcrossing under field conditions. Depending on the schedule of pollen receipt and the composition of the pollen load, seeds are likely to be sired by a limited number of male parents. Analysis of mating patterns in trimorphic populations provides evidence in support of this suggestion (Barrett et al., 1987; Morgan and Barrett, 1990). Data on the segregation of both style morphs and allozymes in open-pollinated families indicated heterogeneous patterns, a result consistent with nonrandom outcrossing. Furthermore, estimates of the correlation of outcrossed paternal parentage using Ritland's (1989) correlated mating model, indicated that 32% of progeny sampled from within fruits of E. paniculata are full sibs. Studies of several other animal-pollinated species have also reported significant levels of correlated mating (Schoen and Clegg, 1984; Schoen, 1985, 1988; Brown et al., 1986; Dudash and Ritland, 1991), suggesting that it may be a general feature of such plants. Presumably, stigmatic pollen loads of animal-pollinated plants rarely contain gametes sampled from the entire population of potential male parents but instead are composed of pollen from a restricted subset of male donors owing to fertility variation, phenological differences, pollen carryover, and population substructure (Marshall, 1990). Depending on the schedule of pollen arrival, postpollination phenomena such as pollen precedence may serve to restrict further the subset of males that is successful in siring seeds.

Functional Gender

In recent years, several workers have proposed that heterostyly can be viewed from the perspective of sexual selection and sex allocation theory (Wilson, 1979; Beach and Bawa, 1980; Casper and Charnov, 1982; Casper, 1992). Following this view the floral morphs may exhibit gender specialization and gain differential reproductive success through male and female function (Lloyd, 1979b; Hicks et al., 1985; Nicholls, 1987). Unfortunately, because of the difficulties in measuring male reproductive success in hermaphroditic plant populations, there have been few reliable estimates of functional gender based on the use of genetic markers (although see Ennos and Dodson, 1987; Broyles and Wyatt, 1990).

The experimental design used in the garden studies described above has enabled us to measure the functional gender of floral morphs in E. paniculata. In trimorphic populations, plants of each morph were homozygous

for one of three alleles at the Aat-3 locus. Thus, a seed heterozygous at this locus indicated an intermorph mating and the identity of the paternal morph could be determined unambiguously. By combining data on male reproductive success with measures of seed fertility, it was possible to estimate the functional gender of each floral morph using the formula:

$$G_i = \frac{f_i}{(2 - d_i)f_i + f_j p_{ji} + f_k p_{ki}}$$

where f_i is the seed production of morph i, d_i is the proportion of seeds of morph i sired by intermorph pollen, and p_{ji} is the proportion of seeds of morph j sired by morph i. Data on functional gender revealed a significant difference between the L and S morphs in each replicate population. The L morph was more female and the S morph more male (mean $G_i \pm$ 2 standard errors: L = 0.56 ± 0.04, M = 0.51 ± 0.03, S = 0.45 ± 0.03). Although these differences may not appear large, a deviation of ±0.05 from the value of 0.5 (the value for a hermaphrodite that gains equal reproductive success through male and female function) implies that individuals of that morph gain approximately 20% more fitness through one sex function than the other. Thus, small differences in gender may have significant consequences for optimal allocation to male versus female structures. Interestingly, the difference in gender between the L and S morphs in E. ·niculata is of the type described by models of the evolution of dioecy ·om heterostyly (Beach and Bawa, 1980; but see Muenchow and Grebu   1989). That is, in dioecious taxa derived from distylous ones, it is thought that the L morph became the female and the S morph became the male.

The difference in gender between the L and S morphs was primarily the result of their strikingly different abilities to sire seeds of the M morph. In 1989, when these experiments were first conducted, the S morph was three times more likely than the L morph to sire seeds produced by the M morph. This mating asymmetry, while not as large, was also evident when experiments were repeated in 1990 (Fig. 9.6). What factors could account for the differences in male reproductive success of the L and S morphs when acting as paternal parents to the M morph? Two hypotheses could account for these differences. The first stems from Webb and Lloyd's (1986) suggestion that the female organs of approach herkogamous plants, as in the L morph, may interfere with pollen removal from flowers by pollinators. This "pollen–stigma interference" hypothesis can be tested by comparing the male reproductive success of L plants with styles intact versus removed. The second hypothesis proposes that the difference in siring success of the L and S morphs results from differences in the frequency of illegitimate mating. Pollen from long-level anthers of the S morph
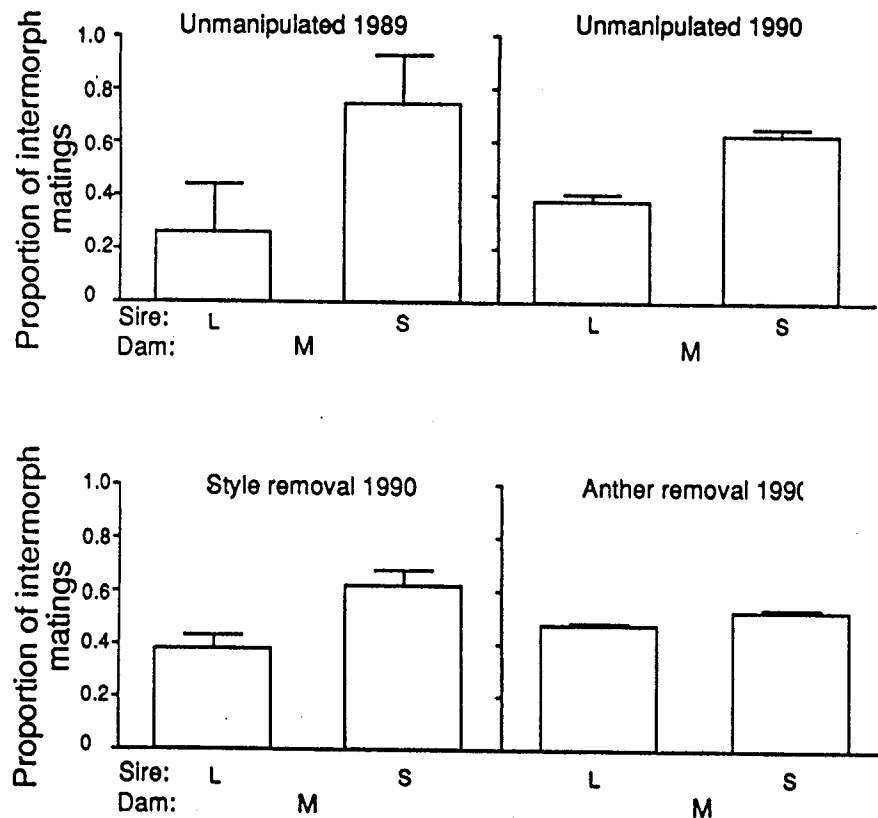
Figure 9.6. Proportion of seeds from the M morph sired through intermorph mating by the L and S morphs of *Eichhornia paniculata* in experimental garden populations in 1989 and 1990. In 1990 two experiments were conducted with trimorphic populations (see text for details); styles were intact versus removed on the L morph, and illegitimate anthers of the L and S morphs were intact or removed (after Kohn and Barrett, 1992b).

may fertilize more ovules of the M morph than pollen from short-level anthers of the L morph due to differences in transfer efficiency and/or siring ability of the two pollen types. This hypothesis can be tested by the removal of short-level anthers from the L morph and long-level anthers from the S morph, leaving only mid-level anthers on the L and S morphs. If the mating asymmetry is largely a result of illegitimate mating, then it should disappear following the anther removal treatment.

Experimental manipulations of the floral biology of trimorphic arrays were used to test the two hypotheses in 1990 (Kohn and Barrett, 1992b). The experiments gave unequivocal results (Fig. 9.6). In the first experiment

there were no significant differences in the siring ability of L plants with styles intact or removed, and the mating asymmetry between L and S plants persisted. In the second experiment there were no significant differences in the siring abilities of the L and S morphs, indicating that the asymmetries observed in previous experiments resulted from differential contribution of illegitimate pollen types to male reproductive success. The experiments on pollen competitive ability described above indicated large differences in the siring ability of pollen from long-level versus short-level anthers when present in mixtures on stigmas of the M morph (Fig. 9.4). Pollen from long-level anthers achieved more fertilizations than pollen from short-level anthers, a pattern consistent with the data on gender asymmetry. Several lines of evidence, however, also suggest that pollen transfer from long-level anthers exceeds that from short-level anthers (see Kohn and Barrett, 1992b). Further studies are therefore required to determine the relative contributions of pollen transfer and/or siring ability of illegitimate pollen to the higher male reproductive success of the L morph in comparison with the S morph.

Evolution of Self-Fertilization

Many populations of *E. paniculata* experience moderate to high levels of self-fertilization (Fig. 9.1). The primary cause of increased rates of selfing is the spread of mating-system modifier genes among plants of the M morph in dimorphic and monomorphic populations. Selfing M variants predominate in Jamaica and occur in varying frequencies in nontrimorphic populations from different parts of the geographical range in N.E. Brazil. A survey of the frequency of selfing variants in populations of different morph structure in N.E. Brazil clearly indicates their nonrandom distribution (Table 9.1). The variants occur only rarely in trimorphic populations, whereas they are abundant in many dimorphic and monomorphic populations. Morph-

Table 9.1. The Frequency of Selfing Variants of the M Morph in *Eichhornia paniculata* Populations of Contrasting Morph Structure in N.E. Brazil[a]

Measure	Trimorphic	Dimorphic	Monomorphic
N (populations)	118	42	7
Percentage with selfing variants	8.5	51.2	85.7
Mean frequency of variants in pupulations occupied	0.05	0.37	1.00
Mean population size	146.3	38.2	10.2

[a]The average population sizes are based on the geometric means of population samples (after Barrett et al., 1989 and Husband and Barrett, 1992b).

specific estimates of outcrossing rate in dimorphic populations of *E. paniculata* have demonstrated dramatic differences in levels of selfing between the L and variant M morphs. For example, in three Jamaican populations the mean value of *t* for the L and variant M morphs was 0.82 and 0.12, respectively (Barrett et al., 1989). This suggests that selfing variants are likely to experience a significant transmission advantage in comparison with the L morph.

The potential transmission advantage of selfing variants of the M morph may be further augmented in some ecological circumstances by maternal differences in seed fertility. Where pollinator service is unreliable, the facility for autonomous self-pollination in variant M plants can potentially provide them with reproductive assurance. The geographically marginal distribution of selfing variants, and their occurrence in populations that tend to be smaller and less dense than trimorphic populations, suggests that founder events and periods of uncertain pollinator service may have been of primary importance for the evolution of self-fertilization in *E. paniculata* (Barrett et al., 1989). The association between selfing and low-density, marginal conditions is a recurrent theme in the mating-system literature (Baker, 1955; Jain, 1976; Lloyd, 1980), but few empirical studies have provided clear evidence bearing on this relationship. This is because selfing and outcrossing plants are usually geographically or ecologically segregated. Consequently, their performances in particular environments are difficult to compare. Because heterostylous and homostylous plants can coexist within populations, however, comparisons of seed fertility can provide direct evidence in support of the reproductive assurance hypothesis (Barrett, 1979; Piper et al., 1986). Comparisons of this type in dimorphic populations of *E. paniculata* have demonstrated large differences in the maternal fertility of selfing and outcrossing morphs, particularly in Jamaican populations (Barrett et al., 1989). Hence, depending on the ecological and demographic conditions in which the variant M morph occurs (see below), it can benefit from increased genetic transmission through both high selfing rates and elevated seed fertility. These advantages may help explain the predominance of selfing variants in Jamaica and their occurrence in many nontrimorphic populations in N.E. Brazil.

Floral Instability and Modes of Self-Fertilization

Patterns of floral variation in selfing variants of the M morph of *E. paniculata* are complex. The variation results from both genetic and non-genetic causes and is manifested at a number of levels, including between populations, between genotypes within populations, and between flowers of individual plants (Richards and Barrett, 1992). The most conspicuous variation involves the elongation of filament length in short-level stamens.

An unusual feature of this modification is the discontinuous nature of the elongation patterns. The most common variant, particularly in N.E. Brazil, has a single stamen in the mid-level position, with the remaining two stamens largely unmodified. Another variant occurring in Jamaica and only rarely in N.E. Brazil has all three "short-level stamens" adjacent to mid-level stigmas. This type of phenotype is referred to as a "semihomostyle" in the literature on heterostyly (Ornduff, 1972). Inheritance studies of the modified M variants indicate recessive gene control of filament elongation. Crosses between variants from different parts of the geographical range in N.E. Brazil give rise to progenies composed of M plants lacking stamen modifications. This indicates complementary gene action resulting from the occurrence of different recessive modifiers in the populations (C.B. Fenster and S.C.H. Barrett, unpublished data).

A curious feature of the genetic modifications to stamen position in the M morph is that in some plants not all flowers within inflorescences exhibit elongated filaments (Barrett, 1985a; Seburn et al., 1990). As a result, inflorescences are frequently composed of both unmodified and modified flowers. Because the former are incapable of autonomous self-pollination, genotypes displaying floral instability are likely to produce both selfed and outcrossed progeny, if plants are visited by pollinators. This type of variation within an inflorescence is particularly evident in dimorphic Brazilian populations that contain both unmodified and variant M plants and presumably represents an early stage in the evolution of self-fertilization. Unmodified flowers occur very rarely in variant M plants from Jamaica, where selfing rates are considerably higher and populations have probably experienced a much longer history of inbreeding (Barrett, 1985b; Husband and Barrett, 1991).

To investigate the influence of genetic, developmental and environmental factors on stamen modification in *E. paniculata*, experimental studies were undertaken of cloned genotypes of the M morph grown under various environmental conditions (Barrett and Harder, 1991). Significant position effects were detected among genotypes with variant flowers. Flowers with modified short-level stamens were most frequently produced on later inflorescence branches in the flowering sequence and at proximal flower positions within an inflorescence branch. These patterns, however, were complex and varied among populations, genotypes, and experimental treatments. Stamen modification increased in clones grown under water stress or at high temperature, demonstrating a significant environmental component to floral instability.

The occurrence of floral instability in *E. paniculata* may have ecological and evolutionary significance. Genotypes producing modified and unmodified flowers are likely to display mixed mating systems. In this respect they resemble taxa that produce cleistogamous and chasmogamous flowers,

but the position effects are far more subtle and the contrast between the flower types in terms of selfing rate may not be as extreme (Lord, 1981; Ellstrand et al., 1984). Another parallel with cleistogamous taxa concerns the strong environmental component to the formation of selfing flowers (Waller, 1980). Stressful growing conditions increase the frequency of selfing flowers. Because the habitats in which the species occurs in N.E. Brazil are subject to frequent droughts and desiccation, the ability of selfing variants of the M morph to adjust the frequency of flowers capable of autonomous self-pollination may have contributed to the spread of this morph in populations, particularly those in marginal habitats.

The mode of self-pollination (sensu Lloyd, 1979a) in variant M plants displaying floral instability is of relevance to models concerned with the evolution of predominant self-fertilization. Models that focus primarily on the joint evolution of selfing and inbreeding depression generally predict that mixed mating systems should be evolutionarily unstable, with selection favoring either complete outcrossing or complete selfing (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1990). On th other hand, models that incorporate details of floral biology suggest th intermediate levels of self-fertilization can be evolutionarily stable, part ularly if pollination conditions limit seed-set and progeny from selfing ar /iable (Lloyd, 1979a; Schoen and Lloyd, 1984; Schoen and Brown, 1992). Under conditions of uncertain pollinator service, the mode of self-fertilization in unstable variant M plants is likely to vary from flower-to-flower on a plant. Unmodified flowers may primarily experience competing selfing, in which a mixture of selfed and outcrossed offspring is produced. In contrast, in flowers with stamen modification all ovules within a flower may be self-fertilized. This is particularly likely where pollinators are infrequent and time delays in the delivery of outcross pollen give a competitive advantage to self pollen already deposited on stigmas through prior contact with anthers (see Fig. 9.5). A mixture of whole-flower and part-flower selfing will result in intermediate levels of self-fertilization (see Schoen and Brown, 1991).

Whether mixed mating remains evolutionarily stable or not will depend on levels of pollinator service. Schoen and Brown (1992) have shown that whenever flowering seasons contain a mixture of temporal phases, both favorable and unfavorable for pollinator activity, and where whole-flower and part-flower selfing of the type found in E. paniculata occurs, stable mixed mating systems should evolve. The rarity of predominantly selfing populations in N.E. Brazil and their abundance in Jamaica may reflect contrasting levels of pollinator activity and differences in the modes of self-fertilization in the two regions (Barrett and Husband, 1990).

Constraints on the Spread of Selfing Variants

Whereas selfing variants of E. paniculata occur commonly in dimorphic and monomorphic populations, they occur infrequently in trimorphic populations (Table 9.1). This nonrandom distribution with respect to morph structure raises the question: What evolutionary forces constrain the spread of selfing variants of the M morph in trimorphic populations? Three hypotheses could potentially explain the overall rarity of selfing variants in trimorphic populations: (1) the recessive genes governing mating-system modification are unlikely to spread in large outcrossing populations since they remain unexpressed in the heterozygous condition (Haldane's Sieve Hypothesis); (2) inbreeding depression in the progeny of selfing variants is large enough to prevent the "automatic selection" of selfing genes (Inbreeding Depression Hypothesis); and (3) the selfing variants have no transmission advantage in trimorphic populations due to the influence of morph structure on mating-system parameters (Context-Dependent Transmission Hypothesis). To evaluate the relative importance of these three hypotheses, several lines of inquiry were pursued. Below, we summarize observations and experiments that we have conducted to address why selfing variants rarely occur in tristylous populations.

Haldane's Sieve

In large outcrossing populations the chance of a new favorable recessive gene (as opposed to one with dominant expression) spreading is very small. Such mutations are more likely to be lost through drift than to reach high enough frequencies to occur in the homozygous state and be exposed to selection (Haldane, 1924, 1927). This principle, known as "Haldane's sieve" (Turner, 1981; Charlesworth, 1992), results in a strong bias against the evolution of newly evolved traits governed by recessive genes, despite the fact that most mutations that arise in the laboratory with large phenotypic effects are recessive (Fisher, 1931). Because mating-system modification in E. paniculata is largely governed by recessive genes, the overall rarity of selfing variants in trimorphic populations may be partly explained by the operation of Haldane's sieve.

Two related factors, however, can reduce the bias against the selection of recessive genes. In small populations genetic drift may occasionally lead to the fixation of recessive genes, even those with detrimental effects (Pollack, 1987). More importantly, for populations experiencing inbreeding, through partial self-fertilization or biparental inbreeding, recessive genes are more likely to occur in the homozygous condition. If the genes increase fitness, then they are likely to increase in frequency and become fixed. Recent models by Charlesworth (1992) on the rate of fixation of favorable

and deleterious mutations in partially self-fertilizing populations predict that moderate rates of selfing will greatly increase the probability of fixation of favorable recessive mutations, even in large populations. Favorable recessive genes are much less likely to become fixed than dominant genes in outcrossing populations, but there is little difference between the two in highly selfing populations.

Episodes of small population size and inbreeding may account for the irregular occurrence of selfing variants in trimorphic populations of *E. paniculata*. Whereas population sizes tend to be larger in trimorphic than nontrimorphic populations (Table 9.1), annual censuses indicate dramatic fluctuations in numbers, irrespective of morph structure. Crashes of population size in trimorphic populations may provide occasional opportunities for the exposure and spread of recessive mating-system modifier genes as a result of inbreeding. Moderate levels of self-fertilization occur in many small trimorphic populations (Fig. 9.1) and estimates of biparental inbreeding [$(t_{ML} - t_{SL})/t_{ML}$; Waller and Knight, 1989] averaged 0.30, suggesting that matings between related individuals are common (Barrett and Husband, 1990 and unpubl. data). Haldane's sieve may explain the rarity of selfing variants in certain parts of the geographical range of *E. paniculata* in N.E. Brazil (e.g., Ceará and Bahia States), where historically large, highly outcrossed populations may have been maintained because of more extensive habitats suitable for the species. In other areas of N.E. Brazil (e.g., Pernambuco and Alagoas States), however, habitat fragmentation and small population sizes may have provided greater opportunities for the exposure and spread of mating-system modifier genes.

Inbreeding Depression

Most models of mating-system evolution in plants incorporate inbreeding depression, the reduced fitness of selfed offspring in comparison with outcrossed offspring, as the major force opposing the spread and fixation of selfing variants (e.g., Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987 and references therein). The maintenance of high genetic loads in historically large, outcrossing populations serves to restrict the spread of selfing variants because deleterious recessive genes, normally sheltered in the heterozygous condition, reduce the fitness of selfed progeny. Where genetic loads are reduced by bottlenecks, or pollinator failure, however, selfing variants can spread because fitness differences between selfed and outcrossed offspring are not of sufficient magnitude to prevent the automatic selection of selfing genes. Models of the joint evolution of mating systems and inbreeding depression generally predict that levels of inbreeding depression should decline with increasing levels of self-fertilization. However, few empirical studies have investigated the relationships

between mating patterns and inbreeding depression (see Holtsford and Ellstrand, 1990) and little is known about the genetic basis of inbreeding depression (reviewed by Charlesworth and Charlesworth, 1987; Barrett and Kohn, 1991).

What are the relationships between mating patterns and inbreeding depression in *E. paniculata* and could fitness differences between selfed and outcrossed offspring account for the failure of selfing variants to spread in trimorphic populations? To investigate the relationships between mating patterns and inbreeding depression, fitness comparisons were undertaken of selfed and outcrossed progeny grown under a variety of experimental conditions. Eleven populations of *E. paniculata*, with outcrossing rates spanning the full range encountered in the species, were used in these studies (Toppings, 1989). Fitness differences between selfed and outcrossed offspring were largest in trimorphic populations with high outcrossing rates, intermediate in nontrimorphic populations with mixed mating systems, and absent altogether in Jamaican populations with high levels of self-fertilization. This overall pattern was confirmed in a second experiment involving a comparison of the effects of continued inbreeding on two populations representing the extremes of mating-system variation in the species (Barrett and Charlesworth, 1991). Five generations of selfing followed by random outcrossing had no effects on fitness components in a monomorphic selfing population from Jamaica. In contrast, in an outcrossing trimorphic population from N.E. Brazil, fitness components declined on selfing but showed a dramatic increase when plants in the S_5 generation were randomly intercrossed (Fig. 9.7). The large difference in flower production between the S_5 and C_5 generations in comparison to the difference between the S_1 and C_1 generations (Fig. 9.7) was most likely the result of purging of recessive or partially recessive deleterious alleles within the inbred lines. The experimental results were in close agreement with theoretical expectations of a model of inbreeding depression based on mutation-selection balance at many unlinked loci with a high mutation rate to moderately deleterious, partially recessive alleles (Barrett and Charlesworth, 1991). The results support the partial dominance (mutational) hypothesis for genetic load (see Charlesworth and Charlesworth, 1987) and suggest that purging of partially recessive mutations accounts for a significant component of heterosis.

Although these experimental results provide useful comparative data on the relative patterns of inbreeding depression in populations with contrasting mating systems, they cannot determine whether inbreeding depression limits the spread of selfing variants in trimorphic populations. First, because the studies were conducted under glasshouse rather than field conditions, they may provide little information about the magnitude of inbreeding depression under field conditions (reviewed by Charlesworth and Charles-
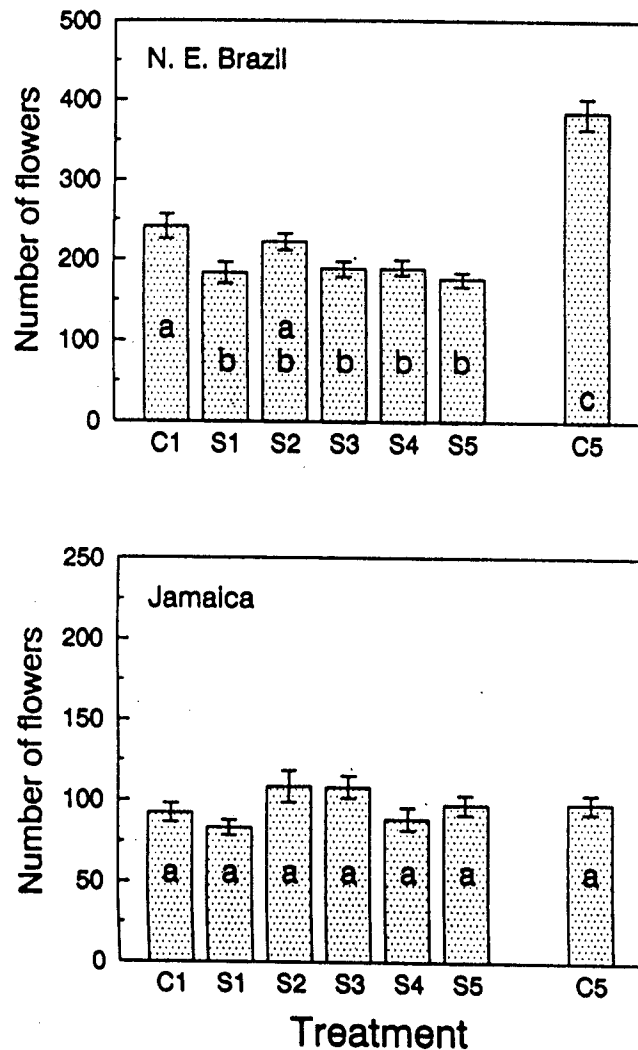
Figure 9.7. The effects of five generations of selfing on flower production in populations of *Eichhornia paniculata* with contrasting mating systems. The population from N.E. Brazil was trimorphic and highly outcrossing, whereas the population from Jamaica was monomorphic and predominantly selfing. Selfed (S_1–S_5) and outcrossed generations (C_1 and C_5) were compared in a single multigeneration experiment under uniform greenhouse conditions. The Brazilian population was represented by 30 lines and the Jamaican population by 10 lines. Error bars represent two standard errors of the mean, and letters indicate statistically significant ($p < 0.05$) differences (after Barrett and Charlesworth, 1991).

worth, 1987; and see Schoen, 1983; Schemske, 1983; Kohn, 1988; Dudash, 1990). Such information is required to evaluate whether selfing variants would be likely to experience a transmission advantage in trimorphic populations. Second, even though inbreeding depression was found to be lower in more inbred populations (Toppings, 1989), it cannot be determined whether the loss of load (e.g., due to small population size) preceded the spread of selfing variants or whether load was purged as a consequence of the spread of selfing variants.

Transmission Characteristics of Selfing Variants

The effects of population morph structure on gamete transmission by variant M plants was investigated by J.R. Kohn and S.C.H. Barrett (unpublished data) using genetic markers and garden populations that were trimorphic, dimorphic (S morph absent), or monomorphic (L and S morphs absent). In each replicated population male and female reproductive success of unmodified and variant M plants were measured. Male reproductive success was partitioned into self and outcross pollen components. It was hypothesized that, in trimorphic populations, the elongated short-level stamen of variant M plants might result in a reduced ability to fertilize plants of the S morph. This effect could counter the increased transmission through selfing caused by the stamen modification. In dimorphic and monomorphic populations, the absence of the S morph would eliminate this cost.

Fruit- and seed-set by unmodified and variant M plants were not significantly different among any of the morph structure treatments. Variant M plants had higher mean selfing rates than unmodified M plants in each treatment, and mean selfing rates of unmodified and variant M plants increased with the progressive loss of morphs (Fig. 9.8). In trimorphic populations, variant M plants suffered reduced transmission through outcross pollen donation relative to unmodified M plants. This effect was largely due to an approximate 50% reduction in the ability of variant M plants to sire seeds of the S morph relative to unmodified M plants. Thus, in experimental trimorphic populations, the cost of increased selfing was reduced reproductive success through outcross pollen donation. As a result, total transmission of gametes by unmodified and variant M plants were similar in trimorphic populations.

In dimorphic and monomorphic populations, the modification increased both self and outcross pollen donation. Apparently, the occurrence of a single anther at the mid-level caused variant M plants to sire a greater proportion of seeds of unmodified M plants than vice versa. Thus, in dimorphic and monomorphic populations, variant M plants were favored over unmodified M plants through a large increase in gamete transmission
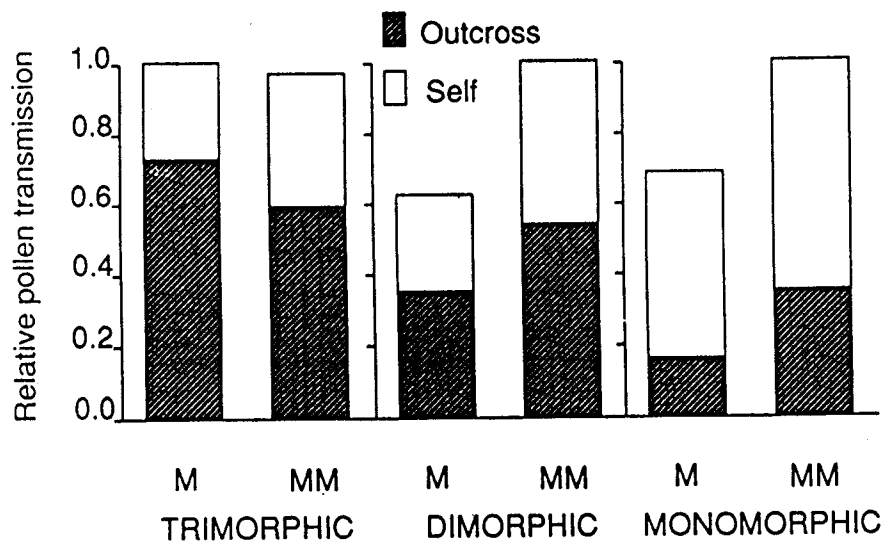
Figure 9.8. Relative transmission of gametes through pollen by unmodified (M) and variant (MM) plants of the mid-styled morph in experimental trimorphic, dimorphic (S morph absent), and monomorphic (L and S morphs absent) garden populations of *Eichhornia paniculata*. All populations contained a total of 36 plants. Estimates of male fertility through self and cross pollen donation are based on electrophretic assays of five seeds from each of two fruits per plant. In trimorphic populations there was no difference in transmission by unmodified (M) and variant (MM) plants of the mid-styled morph. In populations lacking the S morph, transmission by variant M plants was significantly greater ($F_{1,8}$ = 22.2, $p < 0.01$) than transmission by unmodified plants (J.R. Kohn and S.C.H. Barrett, unpublished data).

by pollen (Fig. 9.8). These results demonstrate the large role that the morph structure of populations can play in altering the effects that a given floral morphology has on the mating system.

In trimorphic populations in N.E. Brazil, variant M plants may not benefit from the transmission advantage normally associated with selfing. In addition to possible reduced transmission through cross pollen donation, the altered morphology of M variants may not increase their selfing rate relative to unmodified M plants as much in trimorphic as in nontrimorphic populations. Since trimorphic populations tend to be larger and contain plants at higher density, M variants may experience less increase in selfing due to greater availability of outcross pollen. A study of outcrossing rates in two trimorphic populations containing variant and unmodified M plants provided data that are consistent with this view (M.R. Dudash and S.C.H.

Barrett, unpublished data). Outcrossing rates of the two types of M plants were high and not significantly different within each population (B46: M morph, $t = 0.79$; variant M, $t = 0.72$; B58: M morph, $t = 1.0$; variant M, $t = 0.95$). The high outcrossing rates may result from delivery of large amounts of legitimate pollen early in the anthesis period of flowers, allowing little opportunity for precedence of self pollen (Fig. 9.5). Such an effect, combined with effective postpollination discrimination among pollen types, would serve to maintain high levels of outcrossing even in a selfing variant capable of autonomous self-pollination. Thus, the ecological context may alter the transmission of genes for floral modification by affecting relative female function (seed-set) and relative male function through both self and outcross pollen donation.

The available data from experiments with *E. paniculata* do not enable us to reject any of the three hypotheses proposed to explain the overall rarity of selfing variants in trimorphic populations. The hypotheses are not mutual exclusive, and it seems likely that some elements of each hypothes (i.e., genetic basis of selfing genes, inbreeding depression, transmission characteristics of selfing variants) will determine whether selfing variants spread in populations. Perhaps the only way to identify unequivocally the selective forces influencing the frequencies of selfing variants in trimorphic populations is to introduce variants into populations occurring under different ecological circumstances and then follow their fate using demographic genetic approaches. Field experiments have been used to investigate both local adaptation in plant populations (Antonovics, 1976; Schemske, 1984; Waser and Price, 1985) and the fitness consequences of different mating patterns (Clay and Antonovics, 1985; Waser and Price, 1989; Schmitt and Gamble, 1990), but no field studies to date have followed the fate of mating-system variants introduced into natural populations. Such experiments, while logistically demanding, would provide powerful insights into the mechanisms governing mating-system change. The major drawback of using this approach in *E. paniculata* is the short-lived nature of many populations, which makes meaningful chronological data on changes in floral morph frequency difficult to obtain. Whereas colonizing species with short life cycles can provide excellent experimental material for answering certain types of questions, the ephermeral nature of many of the habitats they occupy can limit opportunities for long-term studies of the ecological basis of evolutionary change.

Conclusions

Populations of *Eichhornia paniculata* exhibit a wide range of outcrossing rates associated with the dissolution of the tristylous genetic polymorphism

and the evolution of self-fertilization. By coupling marker gene analysis of mating-system parameters with information on the demography and floral biology of populations, we have made some progress in unraveling the complex forces influencing mating-system evolution in this species. We have been able to identify the stages of the mating process on which selection is most likely to operate and have determined how pre- and post-pollination mechanisms influence mating behavior. Outcrossing rates in *E. paniculata* are strongly influenced by various aspects of the pollination biology of populations (e.g., levels of pollinator service, timing of self and outcross pollen arrival, and size and composition of pollen loads). Moreover, mating patterns in *E. paniculata* are strongly correlated with the morph structure of populations and, in particular, the frequency of selfing variants.

An important finding from our experimental manipulations of morph structure in garden populations is that the mating patterns of floral morphs are strongly influenced by the morph composition of the array. The transmission characteristics of selfing variants varied with population structure, and outcrossing rates and seed-set in unmodified plants (L, M, S morphs) differed between trimorphic and monomorphic populations. This context-dependent mating behavior has also been demonstrated in experimental populations of *Ipomea purpurea* polymorphic for flower color (Epperson and Clegg, 1987b and unpublished data). Collectively the two studies provide empirical support for mating-system models that assume fre·iency-dependent influences on selfing rate (Charlesworth and Charleswort 1981; Gregorius et al., 1987; Holsinger, 1991). Not only were selfing rat influenced by morph structure, but male reproductive success also va:._d, depending on the frequency and occurrence of other floral phenotypes within populations. Empirical evidence for frequency-dependent male reproductive success has not been previously demonstrated in plant populations, although it has been inferred from studies of temporal variation in phenotypic gender of diclinous taxa (Thomson and Barrett, 1981; Ross, 1990).

Although our experimental studies of *E. paniculata* have provided useful insights into the potential selective mechanisms governing mating-system evolution, they cannot by themselves provide definitive answers about the causes of mating-system change. This can be achieved only by long-term field studies of the ecological genetics of natural populations (Ford, 1964; Endler, 1986). Field studies can be particularly revealing if they involve experimental manipulations of both the population and the environment in which it occurs. Using such an approach, it is possible to identify not only how selection occurs, but also why it operates in the way it does (Wade and Kalisz, 1990). There have been few attempts to measure selection on floral traits influencing mating patterns in plant populations (but see Campbell, 1989; Schemske and Horvitz, 1989) and only limited work

involving the experimental manipulation of floral traits and their reproductive consequences (Ganders, 1974; Queller, 1983; Barrett and Glover, 1985; Bell, 1985; Cruzan et al., 1988). Future work concerned with the selective basis of mating-system evolution could profitably focus on experimental field studies of taxa with wide intraspecific variation in outcrossing rates.

The relative roles of floral morphology, pollen–pistil interactions, and ecological factors in determining mating patterns in populations need to be determined for individual species. If the mating system is primarily controlled by traits under genetic control, then genetic models of mating-system evolution can guide our experimental efforts. On the other hand, data from field and experimental populations reported here indicate that ecological variation can have large effects on mating patterns. To the extent that this is true, more attention will need to be paid to the development and testing of models that incorporate both ecological and genetic factors.

Acknowledgments

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