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Evolution in Action

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The Influence of Breeding Systems and Mating Systems on Conservation Genetics and Conservation Decisions

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After habitat preservation, an understanding of the reproductive biology of an endangered, threatened, or invasive plant species is one of the first research steps that should be taken to identify conservation priorities. The goals of this chapter are to explore from an ecological–genetic perspective the essential roles that the breeding system and mating system play in conservation initiatives, and to encourage further research in this area. Many excellent reviews exist on the biology of plant mating and breeding systems (see, for example, Barrett, 2003); thus, we instead describe the relevance of such systems to the conservation of threatened and endangered populations and the management of invasive taxa. We have chosen to focus on plants because they exhibit a great diversity in breeding systems and mating systems. Many of these principles apply to animals as well, and for further details we direct our readers to a recent review on animal systems by Jarne and Auld (2006).

We begin by examining methods for assessing breeding systems, mating systems, and inbreeding depression. We take a close look at plasticity of breeding and mating system traits in response to environmental heterogeneity, and discuss how these traits contribute to long-term population persistence of taxa of conservation concern. We use three case studies from our own work with plants to highlight the complexity of interactions among breeding and mating systems and the environment, and show how these interactions

affect population persistence. Lastly, we summarize information that we believe is needed to examine the possible ecological–genetic consequences of the environment-by-breeding system interaction. We then conclude with several recommendations for conservation-specific issues and discuss suggested avenues for future research.

BREEDING AND MATING SYSTEMS

The first of the two major attributes of the reproductive biology of any plant species is the breeding system. The breeding system represents characteristics of the flowers within an individual plant that may influence gamete transfer among conspecifics (Barrett, 2002). Breeding system traits are responsible for preventing interference between pollen removal from anthers and pollen deposition onto stigmas. In addition, these traits also influence the frequency of outcrossing (Barrett, 2003; Lloyd & Webb, 1986; Webb & Lloyd, 1986). Thus, the first categorization that needs to be determined is whether a plant species is self-compatible or self-incompatible. However, self-incompatibility is complex, and determining the type of incompatibility is time-consuming. Thus we often use results in the literature on closely related taxa to infer whether a taxon of interest is self-incompatible. Terminology commonly used to

TABLE 5.1 Terms Commonly Used in Plant Reproductive Biology.

Mating system	The within-population proportion of matings between related individuals or with self compared with matings between unrelated individuals
Self-compatibility	Able to mate with self
Self-incompatibility (SI)	Genetic incompatibility that prohibits self matings and mating between individuals that share the same self-incompatibility (S) alleles
Gametophytic SI	Genotype of haploid pollen grain dictates SI reaction
Sporophytic SI	Genotype of diploid parent plant dictates SI reaction
Geitonogamy	Transfer of pollen between two flowers on the same plant
Chasmogamy or xenogamy	Flowers open to external, nonself pollen sources; cross-pollination
Cleistogamy	Flowers closed to external, nonself pollen sources; self-pollination occurs within closed flower
Autogamy	Within-flower selfing of a chasmogamous flower
Apomixis	Within-flower reproduction without fertilization such that the progeny are genetically identical to their mother
Breeding system	Attributes of the flowers within an individual plant that may influence gamete transfer among conspecifics
Protandry	Pollen dehiscence prior to stigma receptivity
Protogyny	Stigmas are receptive prior to pollen dehiscence
Herkogamy	Physical separation between male and female function within a flower
Dichogamy	Temporal separation between male and female function within a flower
Hermaphrodite	Male and female function within a single flower or between flowers of an individual plant
Monoecy	Male and female flowers on the same individual
Gynodioecy	A population composed of females and monoecious individuals
Andromonoecy	A population composed of males and monoecious individuals
Dioecy	Separate male and female individuals within a population
Gynodioecy	Coexistence of hermaphrodites and female plants within a population
Androdioecy	Hermaphrodites and male plants within a population
Monostyly	A single length of style present within a population
Distyly	Two style morphs present within a population
Tristyly	Three style morphs present within a population
Monomorphic	One style morph within a population
Polymorphic	More than one style morph present within a population
Life histories	
Semelparity	Individuals reproduce no more than once and then rapidly senesce
Iteroparity	Individuals potentially reproduce multiple times before senescence
Annual	A plant population that completes its life cycle within one season
Biennial	A plant population that completes its life cycle within two seasons
Perennial	Living longer than 2 years

describe plant breeding system traits is summarized in Table 5.1.

Breeding system assessment begins with careful observation of sexual expression among flowers within an individual. The breeding system researcher typically wishes to resolve several important issues early on in the study; for instance, ascertain whether a species is hermaphroditic or dioecious, estimate the timing of stigma receptivity relative to pollen shedding (dehiscence), and establish whether stamens mature and release pollen before the stigma is receptive (protandry) or vice

versa (protogyny). Additionally, data from inquiries into these questions allow us to garner insights into the potential for within-plant versus between-plant mating opportunities, which ultimately influence the mating system (discussed later). Many plant taxa exhibit multiple breeding system traits, suggesting that independent selection pressures may favor different traits simultaneously. Long-term phenotypic selection studies show that selection acts on plant populations in multiple ways to achieve efficient gamete transfer between conspecifics (see, for example, Fenster et al., 2004). Plant sexual

expression may vary within and among species, and new variation can be introduced through hybridization between congeners (see Weller et al., 2001). Thus, variation in the breeding system can influence population persistence (Barrett, 2003).

A classic illustration of the interaction between population size and breeding system is the breakdown of tristylous to distylous and homostylous in heterostylous taxa (see, for example, Husband & Barrett, 1993; Weller 1979). In the tristylous annual plant *Eichhornia paniculata* (Pontederiaceae), Husband and Barrett (1993) demonstrated that populations in Brazil often consist of three female plant morphs represented by three different stigma lengths, each of which is accompanied by two whorls of stamens of dissimilar lengths that preclude self-pollination (Fig. 5.1). This arrangement of reproductive parts inhibits gamete transfer within and between flowers of the same floral plant morph. Through careful monitoring of numerous populations in Brazil and Jamaica (where the species has migrated), Husband and Barrett (1993) showed that small populations in Jamaica often lose the short morph (Fig. 5.1). One dominant allele is responsible for the presence of the short morph within the population (see, for example, Barrett, 1988). The Jamaica population thus exemplifies the ease with which an allele can be lost in a small population when the breeding system is affected by invasion into a new habitat. Studies of other tristylous taxa have also shown morph loss via drift in small populations, and demonstrated that gene flow among populations can prevent morph loss in small populations (see, for example, Andersson, 1994b; Eckert & Barrett, 1992; Eckert et al., 1996). Thus, in a fragmented native habitat with limited gene flow, drift may remain a concern.

The number of census individuals (N) often differs from the effective population size (N_e) within a population. Therefore, different breeding and mating strategies may influence the number of individuals contributing progeny to the next generation (Table 5.2). A general trend is that whenever a breeding/mating system restricts matings between individuals (for example, restriction through self-incompatibility), then a population is likely to experience a decrease in N_e . In contrast, when matings occur more easily among individuals within a population because, for instance, plants are self-compatible, then the effective population size is increased because the population of

potential mates is greater. However, inbreeding may also occur in these latter situations (discussed later).

The second major attribute of the reproductive biology of a plant species is its mating system. The mating system describes the proportion of matings between related individuals or the proportion of self-matings compared with matings between unrelated individuals within a population (Barrett, 2002). Selfing occurs when both the pollen (sperm) and the ovule (egg) are produced by the same individual. This type of hermaphroditism is more common in plants than animals, thus the selfing rate in plants is much greater (see, for example, Dudash & Fenster, 2000). However, in a recent analysis of published counts, Jarne and Auld (2006) report that the incidence of mixed mating (outcrossing estimates, t , between 0.2–0.8) in animals was 47% compared with 42% in plants.

In comparison with outbred lineages, selfing lineages exhibit a 50% decline in heterozygosity per generation. In outbred dioecious taxa, for example, the decline in heterozygosity after matings between related individuals is significantly less than for selfing organisms (Falconer, 1981), with the rate of loss of heterozygosity depending on the extent of coancestry among alleles. In natural populations, inbreeding has at least two significant consequences: a decline in number or phenotypic quality of offspring, and a decline in heterozygosity that in turn reduces a population's ability to adapt to a changing environment. In both plants and animals, there are documented declines in vigor after matings between related individuals and the expression of inbreeding depression (reviewed in Crnokrak & Roff, 1999; Dudash & Fenster, 2000; Fenster & Dudash, 1994). The effects of inbreeding depression are especially aggravated in small and fragmented populations where matings among related individuals are more likely to occur and thus precipitate the onset of population decline (Charlesworth & Charlesworth, 1987).

The mating system of a plant can be estimated (1) ecologically through observations of within-plant pollinator movements versus between-plant pollinator movements (see, for example, Dudash, 1991; Dudash & Fenster, 2001), aided by the use of fluorescent dye powders, visual inspection, and videorecorded observations (see, for example, Dudash, 1991); (2) genetically via allozymes (Soltis et al., 1983) or other molecular genetic markers (Carr & Dudash, 2005); and

(3) through the use of common-garden experiments that compare performance of progeny from selfed, outcrossed, and open-pollinated parents (Charlesworth & Charlesworth, 1987; Dudash, 1987, 1990).

Study of mating system variation within and among populations has been an area of active research since the advent of isozyme electrophoresis, from which we can readily and repeatedly obtain estimates of genetic variation for many individuals and populations (Hamrick & Schnabel, 1985). Using genetic data, the outcrossing rate parameter t can be estimated. Several alternative methods for estimation of t have been implemented (Ritland, 2002). The outcrossing rate parameter t ranges from zero to one, where a value of zero indicates that all matings result from selfing events and a value of one indicates that all matings are between totally unrelated individuals. One method to estimate t utilizes seed progeny arrays for which the maternal genotype is known and all individuals (mother and progeny) are scored at multiple isozyme loci or other codominant markers. A robust sampling protocol is 10 offspring from each of 30 maternal

plants per population of interest. However, in many biological cases, the maternal genotype may not be known, and in those cases sampling of adult tissue randomly throughout the population can be utilized (Crow & Kimura, 1970). From a sample of adult tissue, one can obtain an estimate of the population-level outcrossing rate by calculating the inbreeding coefficient F for each locus where, under random mating, $F = (1 - \text{observed heterozygosity}/\text{expected heterozygosity})$. Outcrossing rate can be estimated as $t = (1 - F)/(1 + F)$. To estimate the outcrossing rate with reasonable confidence, at least 30 individuals within a population and as many variable loci as possible should be used for an assessment (Dudash & Fenster, 2001). The mean and standard errors of F statistics can be calculated by jackknifing estimated F values across each locus used to calculate t (Sokal & Rohlf, 1985). The population-level approach, therefore, has the financial benefit of requiring fewer individuals to be genotyped in comparison with the progeny array approach. The population-level outcrossing rate via F statistics also has a biological benefit in that it

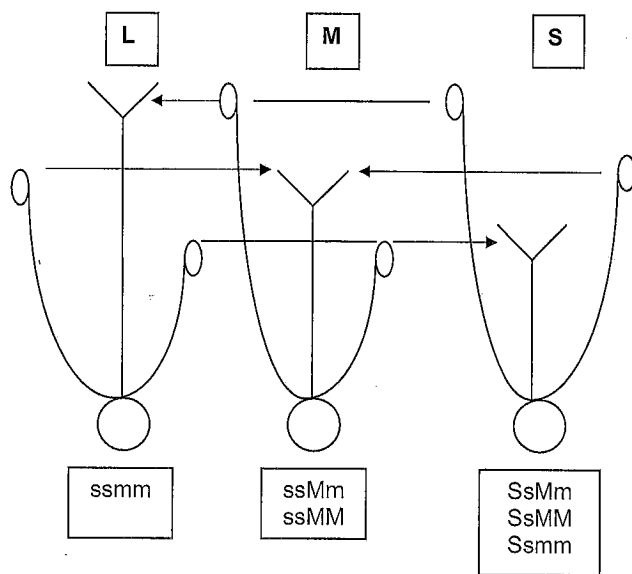


FIGURE 5.1 Schematic representation of relative positions of styles and stamens in the three floral morphs (L, long; M, mid length; S, short) of *Eicchornia paniculata*. Genotypes of the floral morphs under a two-locus model (S, M) for the inheritance of tristily are shown. Arrows represent compatible pollen transfers. (Redrawn from Barrett [1988].)

TABLE 5.2 How Breeding System and Mating System May Affect N_e (the Effective Number of Reproducing Individuals within a Population).

Type of System	Effect on Effective Population Size		
	Decrease	No change	Increase
Mating	Self-incompatibility (SI)	Self-compatibility	Chasmogamy
	Gametophytic	Selfing	Cleistogamy
	Sporophytic	Apomixis	Autogamy
Breeding	Geitonogamy		
	Tristyly (if SI)	Hermaphrodite	Protandry
	Distyly (if SI)	Monostyly	Protogyny
	Polymorphic (if SI)	Monomorphic	Herkogamy
			Dichogamy
			Monoecy
			Gynodioecy
		Andromonoecy	
		Dioecy	
		Gynodioecy	
		Androdioecy	

captures variation across the lifetime of individuals within a population and is thus less sensitive to variation among seasons in reproductive success.

Population outcrossing estimates can also be based on performance comparisons among hand-self, hand-outcross, and naturally open-pollinated progeny (described in Charlesworth & Charlesworth, 1987). Because the performance of selfed and outcrossed progeny are already being quantified with this combination of mating procedures, inbreeding depression can be evaluated as well. In our opinion, this approach has been underutilized and can have great utility in assessing the conservation status because we are able to estimate both population-level outcrossing rate and inbreeding depression through a crossing program that can be readily conducted in the field. Greenhouse or common-garden space is needed for growing progeny, and in many circumstances the method may be more cost-effective and more accessible to stewards and land managers than are techniques that use genetic markers. Obtaining estimates of mating system parameters and the magnitude of inbreeding depression can help stewards assess the vulnerability of populations of concern. In other words, one can assess the probability of a population experiencing extreme effects of inbreeding along with a decrease in population size because of habitat fragmentation

or edge effects, and whether it is more vulnerable to extinction in the presence of environmental stochasticity.

Mixed Mating

Schemske and Lande (1985) observed that in most plant populations, mating systems were either highly selfing (range in t : 0.0–0.2) or highly outcrossing (t : 0.8–1.0), with few plant species in the middle range. More recently, other authors (see, for example, Barrett & Eckert, 1990; Goodwillie et al., 2005; Vogler & Kalisz, 2001) have also noted bimodality in plant mating system estimates and consider this repeatable pattern suggestive of a biotic or abiotic (wind, water) vector for gamete transmission. However, mixed mating systems (defined broadly here as populations with t in the range of 0.2–0.8) still persist in many animal-pollinated plant taxa (see, for example, Goodwillie et al., 2005; Vogler & Kalisz, 2001) and animal fauna (Jarne & Auld, 2006). The presence of mixed mating systems in animals is just beginning to be explored and requires a more balanced phylogenetic sampling within the animal kingdom.

The frequency of occurrence of mixed mating systems in nature is not fully understood. However, we are gaining insight into the factors that influence persistence of mixed mating systems in

plants, including factors such as variable expression of inbreeding depression, variation in life history strategy, and breeding system traits (Goodwillie et al., 2005). These factors can vary as a result of both genetic and environmental variation within and among populations (see, for example, Elle & Hare, 2002). To complicate the mix further, the use of maternally based seed or seedling progeny arrays versus population-level adult tissue approaches to estimate t can influence observed values (described earlier). Using a maternally based estimate of outcrossing rate, variation in estimates of t (in other words, among mothers) may in part reflect the fact that all or most seeds within a fruit can readily germinate in the greenhouse and then be used in the molecular genetic sample. However, in the field some seed may not germinate and therefore may not be present in the sample for the population-level adult tissue approach. Discrepancies in t observed in greenhouse and natural field populations may be the result of differential expression of inbreeding depression later in the life history (Husband & Schemske, 1996). Thus, adults that flower in any given population likely represent a fraction of those individuals that were produced initially via seed within a population. Moreover, pollinator fauna varies within and among seasons in both abundance and efficiency (see, for example, Kalisz et al., 2004), which may in turn influence seed quality and quantity, and ultimately the mating system. Data from self-compatible, hermaphroditic snails exemplify the within- and among-population variation in mating systems and expression of inbreeding depression (see, for example, Jokela et al., 2006; Trouve et al., 2003). Together these data suggest that mixed mating systems are indeed present in the field, and likely vary temporally, but additional attention is required to determine their exact nature and to find the selective influences that maintain their presence.

Population Genetics and the Mating System

The mating system also contributes to how genetic variation is partitioned within a population. In general, selfing species tend to have lower within-population genetic variation than populations of outcrossing species. Additionally, there is greater genetic differentiation between populations of selfing species than populations of outcrossing species

(see, for example, Hamrick & Godt, 1989). These patterns are explained in part by higher overall homozygosity in selfing species than outcrossing species, thus causing recombination and segregation to play a more important role in enhancing genetic variation within an outcrossing population (Falconer, 1981). Subsequently, the inbred mating system more readily allows the purging of genetic load of some traits more than others within a population (Dudash & Carr, 1998). In contrast, for populations with an outcrossing mating strategy, the genetic variation of the population is represented both within each individual family and among families within the population. Consequently, an outcrossing mating strategy reduces the opportunity to purge deleterious recessive alleles from the population, and we expect a greater genetic load (held in the heterozygous state) in the outcrossing than the selfing species. For instance, if a population of a selfing species and a population of an outcrossing congeneric species were each reduced from 500 individuals to 50 individuals, one would expect that the 50 individuals remaining in the outcrossing population would represent a greater proportion of the original population genetic variation than that remaining in the selfing congener. This prediction stems from the genetic variation both within and among maternal families in the outcrossing populations and reflects significant maternal line variation commonly detected within populations (Dudash et al., 2005). Additionally, it may turn out that phenotypic plasticity among maternal families may also be quite variable (see, for example, Peperkorn et al., 2005) and is yet another reason why it is important to track variation in the maternal line whenever feasible (see, for example, Murren et al., 2006).

Ecological Influences on the Mating System

Although the scale and aspect of environmental heterogeneity may vary widely, environmental heterogeneity is present in most plant populations (Gurevitch et al., 2002). Environmental variation can occur at both spatial and temporal scales and have differential effects on breeding and mating system traits. If environmental variation occurs at spatial scales between populations, selection may favor different breeding or mating systems in different populations. In contrast, if environmental

variation occurs within a population, selection may favor multiple characteristics of the breeding or mating system simultaneously. Fluctuations of the environment on temporal scales may contribute to mixed mating systems within populations (see, for example, Holsinger, 1993). Abiotic factors such as precipitation, temperature, and soil may directly and indirectly influence plant reproduction. For example, reproduction of the garlic mustard *Alliaria petiolata* was greatest at the edges of well-lighted, mesic forests that were more susceptible to invasion than drier, upland forest interiors (Meekins & McCarthy, 2001). From a biotic point of view, many plant species are dependent on mutualists to reproduce successfully, and the activity and efficiency of these mutualists can be heterogeneous (see, for example, Kalisz & Vogler, 2003). Antagonistic interactions with herbivores may also be heterogeneous in their mode and impact. Herbivores may reduce resources available for reproduction, thus creating reproductive variation from another source (Carr & Eubanks, 2002; Ivey et al., 2003; Steets et al., 2006). Given the biotic and abiotic variation noted among populations, it is not surprising that we have detected variation in mating system estimates among plant populations and across years (Ashman et al., 2004; Goodwillie et al., 2005).

From a biotic perspective, pollination environments can vary spatially and temporally, and this variation can influence the mating system. One particularly dramatic example includes populations in habitat fragments after landscape-level fragmentation. The isolation of patches and reduction of habitat size associated with fragmentation can lead to the reduction in size of some native plant populations, and can increase habitat for other edge or weedy species. Several challenges face small remnant populations of native flora that remain in forest fragments. In order for successful pollination and reproduction to occur, animal visitors first need to be attracted to a site, and animal attraction may also be dependent upon whether other conspecifics are flowering in the same habitat space. Small populations of native plants that remain in forest fragments may be influenced by both pollinator assemblage and visitation rate. Temporal variation of pollinator activity can further affect the reproductive success and mating system of these small populations (see, for example, Johns & Handel, 2002). On the other hand, nonnative invasive plant species are a threat to native biodiversity in forest fragments in a number of ways, and tend to have their greatest

presence in disturbed areas such as the edges of forest fragments. Both intentionally and accidentally introduced invasive plants outcompete native plant species and disrupt native plant pollinator mutualisms (see, for example, Ghazoul, 2004; Traverset & Richardson, 2006), and facilitate the spread of invasive pollinators (Hanley & Goulson, 2003). Facilitation among native and invasive taxa in attracting pollinators may also occur in some natural populations (Moeller, 2004); moreover, if invasive taxa make a particular area more attractive to both native and nonnative taxa, both may benefit in terms of enhanced reproductive success.

Plant responses to environmental heterogeneity may potentially influence both the mating system and the breeding system. However, few studies in plants have examined how phenotypic plasticity and mating system are related (Dudash et al., 2005). However, in the water flea, *Daphnia*, sexual and asexual lineages exhibited differences in morphological characters, but did not differ in amounts of phenotypic plasticity (Scheiner & Yampolsky, 1998). In a study that compared populations of an inbred plant, *Sporobolus cryptandrus* (sand dropseed), with the closely related but outcrossing *Panicum virgatum* (switchgrass), researchers concluded that both species exhibited significant variation in pattern and amount of phenotypic plasticity among populations, and that *Panicum* exhibited greater plasticity than *Sporobolus* for a variety of traits (Quinn & Wetherington, 2002). This study further showed that observed patterns of phenotypic variation were consistent with the extent of environmental heterogeneity within the native ranges of the two species.

Although few studies have been conducted on the relationship between plant phenotypic plasticity and the nature of mating and breeding systems, we suggest here several ideas that we find important to consider for species of conservation concern. Because seeds of endangered or threatened species are seldom available, experimental evidence of plasticity is rarely at hand for such taxa. However, species that are able to maintain populations (even if in low census population numbers) after habitat fragmentation or other environmental disturbance may in fact be those populations that harbor the ability for many of their phenotypic traits to express plasticity when exposed to new environmental conditions and may thus express adaptive plasticity. Taxa that exhibit opportunistic plasticity are those

that can maintain maximal fitness while moving from native to novel environments or across novel environments, and at the same time have not had sufficient time to adapt across generations to either a particular habitat or a new source of heterogeneity (Dudash et al., 2005). In discussion of contrasts between expression of phenotypic plasticity in native and invasive species, researchers have hypothesized that invasive species may have greater plasticity and plasticity of a greater number of traits. In *Lythrum salicaria*, Mal and Lovett-Doust (2005) demonstrated significant phenotypic plasticity for an array of vegetative and morphological traits in response to experimental changes in soil moisture. Quite relevant to the discussion of mating and breeding systems here, *L. salicaria* exhibited a genotype-by-environment interaction in reproductive traits, suggesting that soil moisture may modify positions of anthers relative to stigmas. For this highly invasive species in North America, the continued investigation of the interaction between breeding and mating systems and response to environmental heterogeneity may lead to important insights into the invasion success of this species.

Environmental heterogeneity can influence the expression of inbreeding depression, an important component of mating system evolution (Hedrick & Kalinowski, 2000). From an abiotic perspective, Dudash (1990) detected significant variation in inbreeding depression across three environments: the field, a garden plot, and the greenhouse in the native species *Sabatia angularis*. Specifically, Dudash found a greater expression of inbreeding depression in the field environment and the least expression of inbreeding depression in the greenhouse. Furthermore, when CVs for reproduction of maternal families were assessed across environments, the inbred progeny exhibited greater values than the outcross progeny. These data support the idea of buffering via genomic heterozygosity, such that increased heterozygosity in the outcross progeny improved the ability of these plants to maintain more consistent performance across the three environments than the self progeny (Falconer, 1981; Lerner, 1954). More recently, Daehler (1999) demonstrated that competitive environments exerted greater inbreeding depression by assessing a high-nutrient environment and a low-nutrient environment for populations of *Spartina alterniflora* invading San Francisco Bay. For this species, variation in inbreeding depression

among environments and within populations may significantly influence the invasion success of this species.

CASE STUDIES

Variation in Mating System, Expression of Inbreeding Depression, and Purging of Genetic Load in *Mimulus* Congeners

Understanding the role of inbreeding depression in the evolution of mating systems remains an overarching question in our quest to explain the immense diversity of breeding and mating systems documented among the angiosperms. The feasibility of purging the genetic load from a population is an important management concern because populations purged of deleterious alleles tend to increase in fitness, thus enhancing the probability of population persistence in a relatively constant environment. Thus, to address these issues we took a multifaceted approach. First we assessed the differential performance of self and outcross progeny in each of two hermaphroditic *Mimulus* species with contrasting mating systems. Second we determined whether we could purge the genetic load in two complementary ways by first performing self-pollinations by hand ("hand-self") across five generations of selfing and outcrossing (Carr & Dudash, 1997; Dudash & Carr, 1998; Dudash et al., 1997). Next, we used the same progeny and parents in a North Carolina 3 (NC3) breeding design (Comstock & Robinson, 1952) to calculate the level of dominance responsible for the observed inbreeding depression in these two taxa. This multifaceted approach has implications for species of conservation concern when stewards are trying to assess congeners that exhibit variable breeding and mating system traits.

Mimulus micranthus is primarily a selfing taxon resulting from the close proximity of stigmas to dehiscing anthers, whereas *M. guttatus* exhibits spatial separation between male and female function (in other words, herkogamy) and is generally dependent on animal visitors for outcross pollination. However, in older *M. guttatus* flowers, the anthers may brush over the sensitive stigma and induce self-pollination, a process called *corolla dragging* (Dole, 1992).

In comparison with outcrossed replicates, five generations of enforced selfing caused a loss of 50% of the *M. guttatus* maternal lines and differential expression among traits in inbreeding depression (Dudash et al., 1997). Overall, the mixed mating species *M. guttatus* exhibited a greater magnitude of expression of inbreeding depression across a larger number of traits than did the selfing species *M. micranthus*. Interestingly, the traits most closely associated with reproductive fitness—ovule and pollen production—showed no inbreeding depression when self progeny were compared with outcross progeny in *M. micranthus*. In stark contrast, the larger flowered *M. guttatus* exhibited strong inbreeding depression in gamete production (Carr & Dudash, 1997). Furthermore, results of the quantitative genetic breeding experiment found that gamete production in *M. micranthus* was additive, with little evidence of dominance effects, whereas in *M. guttatus* there was evidence of dominance-based inbreeding depression (Dudash & Carr, 1998).

This study demonstrates that the investment of time and the loss of maternal lines makes purging of inbreeding depression a less-than-ideal management tool. However, a promising application of this body of research is to compare the breeding system (and infer potential mating systems when possible) between congeners without conducting extensive breeding programs. Using knowledge of the breeding and mating systems, we can infer an expected level of inbreeding depression that will help estimate population vulnerability in the face of declining habitat and unpredictable environmental heterogeneity.

This body of research also revealed the potentially important role of maternal variation in the expression of inbreeding depression, thus helping to explain the fluctuations in mating patterns among individuals observed within a population. Variation among individuals within a population in expression of inbreeding depression also contributes to the persistence of mixed mating in nature (Dudash et al., 1997). Other researchers have showed that significant maternal variation within populations is common in nature (see, for example, Goodwillie et al., 2005; Pico et al., 2004). Tremendous variation among maternal lines has conservation significance and again highlights the need to collect seed for germplasm and empirical experiments.

We also caution researchers against collecting seeds haphazardly with respect to maternal identity. Thus maternal variation may be the key to maintaining the evolutionary potential of many species in peril and deserves further investigation (Dudash et al., 2005; Fenster & Dudash, 1994).

Catasetum viridiflavum Reproduction and Gene Flow across a Fragmented Forest Landscape

Habitat fragmentation and population isolation are considered to affect pollinator visitation rate negatively and thus increase the potential for inbreeding and reduced reproductive success in habitat fragments (Laurance & Bierregaard, 1997), particularly for obligately outcrossing species. The question that one of us (Murren) set out to answer was whether a plant species with a species-specific pollination system and an epiphytic life history would show increased inbreeding and lowered reproduction in response to habitat fragmentation (Murren, 2002).

Catasetum viridiflavum is an epiphytic orchid endemic to central lowland Panama. It has an unusual breeding system that entails functional dioecy and sex switching. Female *C. viridiflavum* are present in high-resource environments. The euglossine bee *Eulaema cingulata* is its sole pollinator. Male bees visit flowers and collect volatile substances produced in both male and female flowers. When the bee hits a whisker located inside male flowers, the spring-loaded pollinia are slapped on its back, and some of them are carried by the bee to female flowers.

Islands created during construction of the Panama Canal served as replicate forest "fragments." These forest islands are advantageous for many reasons, including the fact that experimental replicates are each surrounded by a nearly identical matrix: water. For comparison, large tracts of mainland forest were also chosen. For 3 years, plants on each of 10 islands and each of five mainland sites were sampled to estimate correlates of male and female reproductive success (pollinia removal and fruit set, respectively). During 1 year of the study, male and female estimates of reproductive success were not significantly different between island and mainland sites. However, fruit set was reduced on island sites in comparison with

mainland sites in the other 2 years. Two pieces of ecological evidence suggest that plants in forest fragments and mainland sites continue to be interconnected: (1) pollinia were viable for long periods of time and (2) pollinators moved frequently among sites. Although fruit set data indicated some impact of fragmentation, among-year variation in reproductive success suggested interconnection among sites, with pollinators possibly playing a key role in maintaining connection among fragments (Murren, 2002).

In a companion genetic study, Murren (2003) detected temporal genetic structure but minimal among-site variation. However, inbreeding coefficients (in other words, the proportion of the variance in the subpopulation contained in an individual) were large in magnitude and thus consistent with a mating system that has frequent among-relative matings and high rates of seed movement. Although *C. viridiflavum* has a very specific breeding system, the observed patterns of mating seen in a heterogeneous forest fragment nevertheless exhibited patterns more similar to temperate or tropical trees with generalist pollination systems. Highly selfing species with a shorter dispersal distance would have caused a very different pattern of response to fragmentation (for example, a high level of differentiation among populations in different fragments). In summary, study of the mating and breeding systems of plant taxa that differ in pollinator specificities and life history strategies may provide novel insights into the potential responses of plants in habitats of conservation concern. These data are valuable guides when outlining the complex set of possible outcomes during the creation of management plans when a multiyear study is not feasible.

Role of Breeding System and Inbreeding Depression in the Maintenance of an Outcrossing Mating Strategy

Understanding the relationship among the breeding system, mating system, and expression of inbreeding depression of a long-lived perennial allows one to infer the reproductive dynamics of a species, and to examine both the causes and the consequences of selfing. Dudash and Fenster (2001) utilized this combined approach and we suggest here

that conservation initiatives could readily apply the method discussed next to assess the reproductive status and potential for population growth of many at-risk plant taxa.

Silene virginica (Caryophyllaceae) is a protandrous long-lived iteroparous species native to eastern North America. The average numbers of flowers open on any given day on an individual is two, such that geitonogamy is possible between flowers within the same plant. Each flower is first male, with the first whorl of anthers presented on day 1 of flower opening, and another set of five anthers presented on the second day. Each flower then normally spends 1 day in a neuter phase, where pollen has been removed and styles are emerging from the functional corolla tube. About 4 days after opening, the flower enters the female phase. Flowers are visited diurnally by pollinators, with the ruby-throated hummingbird as the most important and most effective pollinator of *S. virginica* (Dudash & Fenster, 1997; Fenster & Dudash, 2001; and unpublished data).

In the field, careful breeding experiments were first conducted to determine the timing of pollen presentation and stigma receptivity. Whole-plant relative sex ratios (female-to-neuter-to-male-stage flowers) were also sampled daily. Observations of pollinators were performed to assess within-plant visits (potential selfing via geitonogamy) versus between-plant visits (likely resulting in matings between less related individuals). Next, hand-self and outcross pollinations on field plants were performed to generate progeny to assess inbreeding depression in the greenhouse. Lastly, researchers estimated a population-level outcrossing rate with adult tissue (described earlier).

Strong congruence among mating system parameters was observed among the cumulative inbreeding depression estimate derived from sampling germination rate and flower production, the potential for geitonogamy, and the estimated population-level outcrossing rate of $t = 0.89$. Together these results readily explained the observed relationship among breeding system, mating system, and the expression of inbreeding depression (Dudash & Fenster, 2001). We advocate the use of this multi-prong approach to learn basic breeding and mating system information about species of concern so that knowledgeable decisions can be made when planning conservation and restoration initiatives.

MANAGEMENT AND CONSERVATION CONSIDERATIONS OF BREEDING AND MATING SYSTEMS

The conservation implications of mating and breeding systems are broad and diverse, thus we highlight only a few key issues. Attributes of breeding and mating systems have direct effects on the probability of a population persisting over a given period of time. These attributes can be of striking importance when a small population is at risk of extinction. This is because the census population size N (total number of individuals within a population independent of reproductive status) does not necessarily take into account which individuals actually survive to mate. Thus, additional information may be required when assessing populations that are in decline. The effective number of reproducing individuals, N_e , is a more appropriate measure than N as a guide to assessing the potential for a change of population size (Brook, this volume; Falconer, 1981; Reed, this volume). Additional reductions in N_e may occur when only a subset of flowering plants produce mature fruit, further compromising vulnerable small populations (Dudash and Murren, unpublished data). Study of the breeding system, the number of reproductive individuals within a focal population, and estimates of the opportunities to mate with self and others, can provide insights into the long-term likelihood of persistence of a population in question. In Table 5.2 we summarized the potential effects that variation in the breeding system and mating system may have on N_e within a plant population.

Inbreeding can lead to inbreeding depression, and reduced quantity and quality of offspring are not desirable traits for endangered species. Thus, in restoration efforts where small populations consist of genetically related individual plants, we should consider lessons from captive animal breeding programs. Maximizing genetic diversity is one of the primary goals of zoo breeding programs (see, for example, Fiumera et al., 2004). By analogy, restoration attempts in natural field conditions of plants with purged genetic load may be those with the greatest chance for success.

Management and Conservation Recommendations to Assess Mating and Breeding Systems

Our goal for this section is to highlight what we consider "best practices" with respect to breeding

and mating systems that in our view need to be compiled to assess factors influencing the persistence of endangered species, to assist in restoration efforts, and to combat spread of invasive taxa.

Breeding and Mating System Considerations

As we described earlier, the breeding system can be used as a predictor of mating system, can contribute to estimates of N_e , and can illuminate many other critical population parameters. First one needs to assess breeding system traits to understand features such as type of breeding system (for example, herkogamy, dichogamy, heterostyly, dioecy), schedule of pollen dehiscence and stigma receptivity, likelihood of pollen movement within a flower versus between flowers on the same plant, and the overall phenology for individual plants and the local population (Dudash & Fenster, 2001). The descriptive biology that emerges from the completion of these tasks will assist in determining whether and how mating system studies should be pursued. Second, perform hand-self pollinations to determine whether the species is self-compatible. If a species is self-incompatible, it may be more susceptible to population decline as a result of habitat fragmentation. If a species is self-compatible, then one can perform pollinator observations to quantify within-plant (potential for selfing) versus between-plant pollinator movements. We also suggest that researchers perform emasculation experiments to determine whether the species is apomictic (see, for example, Dupont, 2002). Apomixis results in the production of seed identical to the maternal parent, and thus new genetic variation arises in apomictic populations through mutation. The relative role of inbreeding and outbreeding depression should be considered and tested with controlled hand pollinations if possible (Dudash & Fenster, 2001), such that appropriate seed stocks can be maintained and restoration efforts can later target plant lineages that maximize the chance of success.

Within-Population Considerations

Whenever possible, seed collection initiatives should maintain maternal-line independence (Dudash et al., 2005). This consideration is important because maternal lines are often found to contain significant amounts of genetic variation within a population. Thus, maintenance of independent

maternal lines allows the researcher to examine among-family variation in traits to assess local adaptation and variation among populations in a quantitative way. The information can later be ignored if it is uninformative, but after bulk collection occurs, one cannot recover this information unless new seed is collected.

Efforts to determine the number of maternal lines that initiated populations of invasive species are useful to guide management and eradication efforts. All else being equal, populations of nonnative species initiated from multiple maternal lines should be eradicated first because they likely house greater quantitative genetic variation (including genetic variation for plasticity) that can respond more rapidly to selection, and thus harbor greater potential for invasion success. When seed are available to conduct restoration efforts, we could simultaneously gather valuable information regarding phenotypic plasticity among maternal lines for a variety of ecologically important traits. Estimates of variation in phenotypic expression across microenvironments may guide choice of sites for restoration efforts, some of which may be beyond the region where the population previously resided. Such advance planning for restoration projects on particular rare or endangered species may shed light on the relatively little studied interaction among mating system, breeding system, and phenotypic plasticity.

Population-Level Considerations

Whenever possible, conservation managers should use a number of different source populations for separate reintroduction projects to maximize genetic variation in the system for rare species (Murren et al., 2006). This bet-hedging approach also minimizes the risk of mixing distant gene pools that may result in outbreeding depression of native taxa (see, for example, Dudash & Fenster, 2000). By contrast, the goal for management of invasive taxa should be to minimize mixing of seed sources, a process that may otherwise maximize genetic variation and contribute to a heterozygote advantage for invasives.

Repeated introductions of new genetic material in a restoration project may be needed to maintain populations for the long term at the restoration site. Repeated introduction of new material would maximize both genetic diversity and the potential

for establishing families that exhibit complementary phenotypic plasticity over the environmental heterogeneity of the restoration site. These same reasons that could contribute to predicting the success of a restoration effort are attributes that are widely described as important in nonnative species establishment. Furthermore, assessment of differential performance in native and nonnative areas may also provide novel insights into potential interactions between phenotypic plasticity, and the mating and breeding systems (Dudash et al., 2005; Murren et al., 2006) that contribute to the success of both invasive and native species in novel field restoration sites.

The quantification of abiotic habitat heterogeneity (for example, water level and light) of source and target populations (Murren et al., 2006) of species of concern may provide additional insights into the interaction between abiotic pressures and the expression of the breeding and mating systems. These abiotic attributes may directly contribute to variation in breeding and mating system estimates in populations of endangered species. We recommend, therefore, that, if possible, more than one population of a threatened species or an invasive be monitored. Using detailed and replicated monitoring programs we can assay variation in population responses and highlight whether gene-by-environment interactions contribute to population success (Murren et al., 2006).

FUTURE DIRECTIONS

Although the conceptual links between mating and breeding systems and conservation have been discussed for some time, we find that there are numerous future directions that would clarify our understanding and strengthen our predictions regarding population persistence. Basic research on breeding and mating systems and phenotypic plasticity are needed to allow informed assessments of how their interactions influence plant population persistence. Surveys that document pollinator abundance on native and nonnative sympatric species can alert land managers to the need for replacing the invasive species with sufficient numbers of native taxa that flower at similar times to ensure adequate pollinator visitation to the natives (and perhaps outcompete the invasive). For highly invasive species, the continued investigation of the interaction between breeding/mating

systems and response to environmental heterogeneity may lead to important insights into invasion success and thus assist efforts to thwart continued population expansion. Further work on the effects of fragmentation on species persistence is also desirable, and researchers should focus on quantifying the species' breeding system and mating system (if feasible) along with other abiotic and biotic attributes of these vulnerable populations.

Too often policy recommendations are made with little or no data on the species in question. We could benefit greatly from a universal database that allows researchers around the globe to share information through a data network. Bioinformatics modeling approaches will only become more robust with the compilation of more empirical data. Further communication efforts are needed between academicians and land managers, such that exchange of perspective, expertise, and insights may lead to successful conservation strategy. We need to advocate for funding nationally and internationally to protect biodiversity and natural resources.

SUGGESTIONS FOR FURTHER READING

Barrett (2003) provides an overview of the variation in mating strategies in flowering plants. Goodwillie and colleagues (2005) explore the evolution of selfing and the persistence of mixed mating in plants, and Jarne and Auld (2006) review the presence of selfing among hermaphroditic animals; both papers discuss how biotic and abiotic ecological factors likely contribute to the persistence of mixed mating systems in nature. Dudash and Fenster (2000)

and Dudash and colleagues (2005) provide the link between basic research on mating system evolution and how these studies can provide insights to current obstacles facing conservation and restoration initiatives.

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