
USING *MIMULUS* AS A
MODEL SYSTEM TO
UNDERSTAND THE ROLE OF
INBREEDING IN
CONSERVATION: GENETIC
AND ECOLOGICAL
APPROACHES¹

Michele R. Dudash,²
Courtney J. Murren,² and
David E. Carr³

ABSTRACT

In the preservation of plant biodiversity, there are fundamental genetic and ecological similarities involved in: (1) predicting the fate of small, isolated populations, (2) ensuring the successful reintroduction of endangered species back into natural habitats, and (3) understanding the establishment of species beyond their native ranges. In all three cases, populations small in size may experience high levels of inbreeding and subsequently the expression of inbreeding depression. The ability of these populations to persist will depend, in part, on the magnitude of inbreeding depression and the ability of selection to remove (or purge) the genetic load that causes inbreeding depression. The loss of genetic variation caused by inbreeding or drift in these small populations will restrict their evolutionary potential, placing a high premium on the ability of plants to respond plastically to environmental variation. When plants make appropriate phenotypic adjustments in truly novel environments while maintaining high fitness, we suggest the use of a new term, "opportunistic plasticity," for this attribute. Opportunistic plasticity may be particularly critical and advantageous in the case of plant introductions in ecological restoration where habitats resemble but are not identical to seed collection sites, or in the initial establishment phase of exotic species in non-native locations where many aspects of the environment differ from the native range. Overall phenotypic plasticity may also be an important means of dealing with environmental heterogeneity in many small populations, but unfortunately we have limited data on the interaction between inbreeding and plasticity in plants. The science and practice of conservation biology has generally appreciated the importance of inbreeding, but we feel that it will benefit greatly by considering the potential interactions between inbreeding and phenotypic plasticity and their effects in the establishment and persistence of small populations.

Key words: endangered species, inbreeding, inbreeding depression, invasive species, *Mimulus*, opportunistic plasticity, phenotypic plasticity, restoration.

A common ecological goal is the preservation of global biodiversity. The first urgent needs are habitat preservation and protection, followed by habitat restoration (e.g., Fenster & Dudash, 1994; Dudash & Fenster, 2000; Mack et al., 2000). To complement these fundamental first steps, we need creative, multifaceted research approaches that include basic biology, ecology, and genetics to provide informed predictions on how to preserve and hopefully restore native biodiversity (e.g., Dudash & Fenster, 2000; White & Boshier, 2000; Murren, 2002, 2003). Here we focus our discussion on the role of inbreeding and phenotypic plasticity in the conservation genetics of plants, and highlight the genetic and ecological obstacles facing small populations.

Newly established small populations of invasive exotics, small populations of rare plants, and new populations of native taxa utilized in restoration attempts share numerous genetic and ecological parameters (e.g., Holsinger, 1992; Adkison, 1995; Allendorf & Lundquist, 2003; Fig. 1). Genetic consequences of being in a small population potentially include inbreeding, low genetic diversity (which may represent only a subset of the genetic variation found in the entire species' range) (e.g., Nei et al., 1975; Barton & Charlesworth, 1984), limited gene flow among isolated populations (e.g., Groom, 1998; Parker & Haubensak, 2002), and rapid evolution via fixation of alleles through selection or genetic drift (Falk & Holsinger, 1991). Ecological challenges for the maintenance of repro-

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² Department of Biology, University of Maryland, College Park, Maryland 20742, U.S.A. mdudash@umd.edu

³ University of Virginia, Blandy Experimental Farm, 400 Blandy Farm Lane, Boyce, Virginia 22620, U.S.A.

When an initially large population goes through a bottleneck, some ecological-genetic consequences:

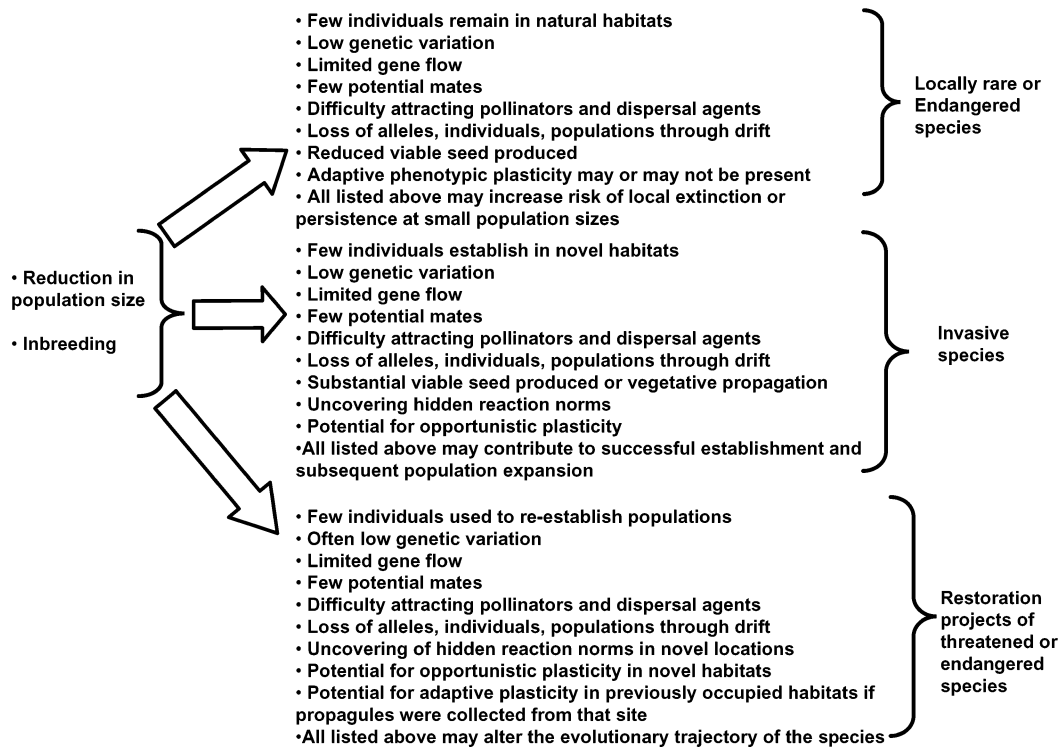


Figure 1. A flow chart that illustrates both ecological and genetic consequences of small populations following bottleneck. We draw attention to the parallelism in ecological and genetic obstacles of three conservation categories: locally rare or endangered species, invasive species, and restoration projects involving threatened or endangered species.

ductive success in small populations include attracting pollinators (Murren, 2002), selecting mates, attracting dispersal agents, and finding appropriate or open habitats for germination. Additionally, small populations must contend with both local natural and human-influenced environmental changes (e.g., Mack & Erneberg, 2002). In Figure 1 we outline the interactions between the genetic and ecological factors and how their relative importance will influence small population establishment, persistence, and risks of local population extinction (e.g., Sexton et al., 2002).

The phase in a small population's ecology on which we will focus here is the *initial* establishment, introduction, or colonization period (Williamson, 1996). In the areas of conservation and restoration, we advocate that initial colonization is a critical stage for which additional data are needed to effectively plan and manage species of concern (e.g., Elton, 1958). One ecological factor that may play an important role during the initial colonization phase of a population's history is phenotypic plasticity [changes in plant phenotypes in response

to changing environmental conditions] (Schlichting & Pigliucci, 1998; Proffitt et al., 2003). Phenotypic plasticity may enable plants to respond to variation in abiotic and biotic regimes associated with their native environment and expand into new areas (Kaufman & Smouse, 2001).

Another biological process that lies at the intersection of ecology, genetics, and conservation is inbreeding, which may influence new population success as its effects vary among environments (e.g., Schemske, 1983; Dudash, 1990; Norman et al., 1995). Plants are unique in that many species are hermaphroditic and exhibit the potential to self-pollinate: approximately 70% of all species have selfing rates in excess of 20% (Vogler & Kalisz, 2001). Selfing is the most severe form of inbreeding because genetic heterozygosity is reduced by 50% with each generation of selfing (e.g., Charlesworth & Charlesworth, 1987). As it is likely that only a limited number of individuals colonize new habitats, relatedness among individuals within newly founded populations will increase quickly over the first few generations, making biparental inbreeding

(i.e., matings between relatives) likely. Biparental inbreeding reduces heterozygosity more slowly than selfing because relatedness is reduced (e.g., Ritland, 1989). Inbreeding creates two formidable challenges in the early establishment of populations. First, a common outcome is the manifestation of inbreeding depression in the resulting progeny (e.g., Charlesworth & Charlesworth, 1987). Second, inbreeding reduces the overall quantitative genetic variation and subsequently could reduce the ability of a population to respond to selection as well as reducing the mean population fitness. On the other hand, in small colonizing populations, inbreeding may confer important reproductive assurance when mates are scarce (Dole, 1992). There has been a tremendous amount of basic research on the effects of inbreeding but the links to conservation and management would benefit from further exploration of how these effects vary among environments (e.g., Keller et al., 1994; Mills & Smouse, 1994; Keller, 1998; Saccheri et al., 1998; Crnokrak & Roff, 1999). A novel next step would be to explore the interactions between inbreeding and phenotypic plasticity, which may be vital to making knowledgeable predictions as to which of the myriad of potential trajectories a given small population could follow in our human-altered global landscape.

The goal of this symposium contribution is both to review some of the ecological–genetic work to date on inbreeding and phenotypic plasticity in the model system *Mimulus guttatus* DC. (Scrophulariaceae) and use it as a starting point to illustrate how these data could be extended to inform conservation and restoration projects. In the opening section we will discuss results from fundamental biological studies on the genetic basis of inbreeding and suggest ways to incorporate knowledge about inbreeding into biodiversity preservation. How populations initially establish and persist in light of environmental changes *and* in the presence of inbreeding is the focus of the second section. Data on this interaction are rare at the moment, but we will review the pertinent work and propose a conceptual framework for future study. We will close by drawing attention to the need to explore this interaction between inbreeding and phenotypic plasticity to further our understanding of the management strategies required for small populations of invasive exotics, small populations of rare plants, and newly established populations utilized in restoration projects.

MODEL ORGANISM IN EVOLUTIONARY ECOLOGY

Mimulus guttatus DC. (Scrophulariaceae, and recently assigned to the Phrymaceae; Beardsley &

Olmstead, 2002) naturally occurs from Alaska to Mexico and is common in moist habitats throughout the western United States. Populations in the natural range are often spatially isolated and vary dramatically in size from very few plants to many thousands in one area (Vickery, 1974, 1999). From field observations, moisture appears to be a defining feature in the location of *M. guttatus* populations (Vickery, 1974). In its native range where moisture is seasonally ephemeral, *M. guttatus* acts as a common annual. However, in areas where water is continuously available particularly in coastal areas, perennial individuals can be found. *Mimulus guttatus* has naturalized in the eastern United States and Canada and around the globe in such localities as the British Isles, Western Europe, New Zealand, Tasmania, and Russia (Vickery, 1974).

Mimulus guttatus has large flowers that are visited by *Bombus*, *Apis mellifera*, *Dialictus*, and other small invertebrates in its native range (Dudash & Ritland, 1991; Gardner & Macnair, 2000). Often hundreds to thousands of seed per fruit are set. Seed dispersal by birds, wind, and stream transport suggest that continuous invasion or re-colonization of open patches and gene flow among patches occurs throughout the natural range of *M. guttatus* (Lindsay, 1964; Vickery, 1999). In natural drainages in Utah, Vickery (1999) demonstrated that over 30 years population sizes greatly fluctuate, local populations frequently go extinct, and populations re-appear at a variety of sizes. *Mimulus guttatus* also commonly exhibits opportunistic colonization, through seed dispersal by roadside mowing crews and other human-mediated influences. Individuals vary in many components of vegetative and life history traits in response to variable environments (Vickery, 1974, 1983; Galloway, 1995). Additionally, phenotypic plasticity may influence its success as a naturalized exotic in other portions of the globe as well as its success in native habitats.

The *Mimulus guttatus* complex is quite variable in its reproductive dynamics since many closely related sympatric species are able to mate successfully with each other in nature (Macnair & Cumbe, 1989). Members of the genus range from highly selfing to highly outcrossing (Vickery, 1964; Ritland & Ritland, 1989; Fenster et al., 1995). *Mimulus guttatus* has a mixed-mating system with population-level outcrossing rates estimated from allozymes ranging from 0.25 to 1.0 (Ritland, 1989, 1990; Dudash & Ritland, 1991; Willis, 1993b). Inbreeding depression in *Mimulus guttatus* was first noted by Darwin (1876), and many others have been inspired by his early observations of this phenomenon and

have built upon the growing knowledge of this taxon (e.g., Ritland & Ritland, 1989; Dole, 1992; Willis, 1993a, b; Fu & Ritland, 1994; Carr & Dudash, 1996, 1997; Carr et al., 1997; Dudash et al., 1997; Dudash & Carr, 1998; Willis, 1999a, b).

INBREEDING DEPRESSION AND ITS GENETIC BASIS

One enduring question related to understanding the enormous diversity among plant species is: Why do we observe selfing taxa evolving repeatedly throughout the plant kingdom (Stebbins, 1950; Grant, 1981)? The discourse surrounding this question has been primarily in the context of understanding how floral diversity in plants has evolved, as well as the evolution of plant mating and pollination systems, yet this topic has obvious links to conservation and restoration. The phenomenon of inbreeding depression, often quantified as the reduction in progeny fitness following selfing relative to progeny produced from random matings within a local population, is thought to be primarily responsible for the maintenance of outcrossing in most plant taxa (e.g., Charlesworth & Charlesworth, 1987), and is thought to potentially negatively influence populations of threatened or endangered species (e.g., Dudash & Fenster, 2000). However, following generations of selfing in the presence of inbreeding depression, a population may have purged or reduced its genetic load (eliminated deleterious or lethal alleles from the population), which may be a benefit to both endangered species and invasive taxa. Critical topics involving inbreeding that still need to be addressed in the area of conservation genetics include: (1) how inbreeding and subsequently inbreeding depression influence population establishment and persistence and, (2) whether populations can naturally purge their genetic load. The likelihood that a population is able to purge its genetic load is dependent on many factors, including the genetic basis of the inbreeding depression expressed, its mating system (outcrossing rate, t), abiotic and biotic selection pressures, conditional environmental effects, as well as genetic and structural incompatibilities associated with its breeding system.

Both genetic and ecological factors contribute to the prevalence of selfing species. If a plant species is able to mate with itself it provides reproductive assurance, i.e., independence from an external pollination agent for fruit and seed set (e.g., Schoen et al., 1996). The ability to self-pollinate increases the probability of survival at low population densities since pollinators may not be attracted to these small populations (e.g., Groom, 1998). Selfing permits a

greater potential colonizing ability, since theoretically it could take just one individual to establish a new population or maintain an existing population across generations (e.g., Baker & Stebbins, 1965). Selfing contributes to local adaptation because of reduced pollen flow and subsequent localized seed dispersal (e.g., Antonovics, 1968). Selfing species may exhibit a lower cost of reproduction if cleistogamous flowers are smaller than chasmogamous flowers that are dependent on an external agent (e.g., Carr & Dudash, 1996), allowing selfing plants to shift resources that would normally go into larger flower production to another stage in their life history.

We have discussed many evolutionary advantages of selfing, raising the question of whether selfing is, in fact, an important ecological-genetic strategy for persistence of populations of small size. Inbreeding depression, however, can counteract both the ecological and genetic advantages to selfing in many plant taxa. Fisher (1941) first noted that when an individual in a population is able to readily both self and outcross, a selfing allele could invade an outcrossing population in the absence of inbreeding depression when ample pollen is also available to both self and outcross, i.e., pollen discounting (Nagyaki, 1976; Lloyd, 1979; Lande & Schemske, 1985). Models based on population-level inbreeding depression predict that if self progeny fitness is less than half of the fitness of outbred progeny (i.e., inbreeding depression > 50%) a selfing allele cannot invade an outcrossing population (Charlesworth & Charlesworth, 1987). These theoretical arguments have been made at the population level; however, theoretical (e.g., Holsinger, 1991; Uyenoyama et al., 1993) and empirical work (e.g., Dudash et al., 1997) at the family level have demonstrated that a selfing allele may invade more easily through a population because of maternal line variation in inbreeding depression. Therefore, we recommend whenever possible that the assessment of inbreeding depression be done at both the family and population levels to provide more comprehensive insights into the inbreeding dynamics in the population of concern.

A population or family's ability to purge its genetic load is dependent upon both the presence of inbreeding depression *and* the genetic basis of the traits expressing the inbreeding depression. The genetic basis of inbreeding depression includes quantifying the number of contributing loci and the level of dominance of alleles at these loci. There are two genetic mechanisms thought to be responsible for inbreeding depression; however, they are not mutually exclusive (Charlesworth & Charlesworth,

1987). The first is dominance (or partial dominance)-based inbreeding depression in which loss of fitness is due to increased expression of recessive or partially recessive alleles as homozygosity increases (Wright, 1921). This type of inbreeding depression is maintained via a selection-mutation balance. Dominance-based inbreeding depression predicts that the frequencies of alleles that are strongly recessive and/or strongly deleterious will be greatly reduced by increased levels of selfing. On the other hand, equilibrium frequencies of more weakly recessive or mildly deleterious alleles will not be greatly affected by increased inbreeding. Selfing taxa are more likely than outcrossing taxa to purge their load if inbreeding depression is dominance based (e.g., Husband & Schemske, 1996; Dudash & Carr, 1998; Byers & Waller, 1999; Crnokrak & Barrett, 2002). Thus small populations that are primarily inbreeding have a greater probability of purging their genetic load, but their success will rest on whether the random changes in allele frequencies due to drift or the selective effects of purging prevail in the focal population. This combination of population attributes may further complicate the ability of small populations to cope with changing or unpredictable environmental regimes (but see below). In determining the role of inbreeding in conservation, an understanding of the genetic basis of inbreeding depression better enables one to predict the breadth of potential outcomes of a conservation breeding-program and provides additional insights for risk assessment models.

The second genetic mechanism thought to be responsible for the expression of inbreeding depression is overdominance or heterozygote advantage, where the heterozygote is more fit than either homozygote (Charlesworth & Charlesworth, 1987; Uyenoyama & Waller, 1991). Heterozygote advantage in this case is thought to be maintained by balancing selection. Since heterozygotes do not breed true, inferior homozygote genotypes are produced each generation, and these homozygotes will reduce the overall population fitness (i.e., segregational load). With overdominance there are no expected differences in a population's ability to purge its genetic load based on population size or mating system (selfing vs. outcrossing). However, because of the increased role of genetic drift relative to selection in small populations, we do not expect to observe the maintenance of polymorphisms at overdominant loci, which would reduce mean population fitness. Furthermore, a reduced ability of natural selection to offset the effects of genetic drift will also make it more difficult for

small populations to respond to changing environmental regimes via genetic variation alone.

In conservation genetics, knowledge of the mating system of the taxon of concern is required to effectively conduct a breeding program via hand-pollinations that produces the greatest amount of viable seed. Decreases in population size promote biparental inbreeding and often selfing, but will these shifts in mating opportunities result in a purging of the genetic load? Recent detailed reviews of the genetic basis of inbreeding depression in plants support the prevalence of dominance-based inbreeding depression (e.g., Husband & Schemske, 1996; Carr & Dudash, 2003). However, there has been some debate on the relative importance of purging in natural populations (see Byers & Waller, 1999; Crnokrak & Barrett, 2002). The debate has been fueled in part by the plethora of approaches (e.g., hand-pollination experiments vs. related selfing and outcrossing taxa assessments, variation in traits quantified, attempts at generalities among taxa) used to assess purging and the omission of important ecological and genetic parameters that will influence the likelihood of purging.

INBREEDING: INSIGHTS FOR CONSERVATION

The role of inbreeding and the likelihood of purging in small populations of conservation concern are central to understanding long-term population dynamics. In restoration attempts, one might undermine local population success by utilizing close relatives that subsequently inbreed, and compromise their ability to maximally reproduce. In fragmented habitats with isolated small populations of threatened species, we are concerned with whether inbreeding may reduce seed output, and we frequently debate whether it is necessary to import pollen from other distant populations. It is also well known that one can invoke the expression of outbreeding depression following hand long-distance crosses between two populations, which results in a reduction in progeny performance and vigor, further compromising the population of concern in the long-term (e.g., Fenster & Galloway, 2000). Monitoring invasive taxa at the initial colonization event may allow a unique opportunity to directly observe a suite of traits and how they vary among individuals that together promote a successful invasion into a non-native habitat. All of the management situations mentioned above would benefit from knowledge of the mating system (who mates with whom) and breeding system (morphological and temporal variation both within and among flowers on a plant) of the taxa of concern.

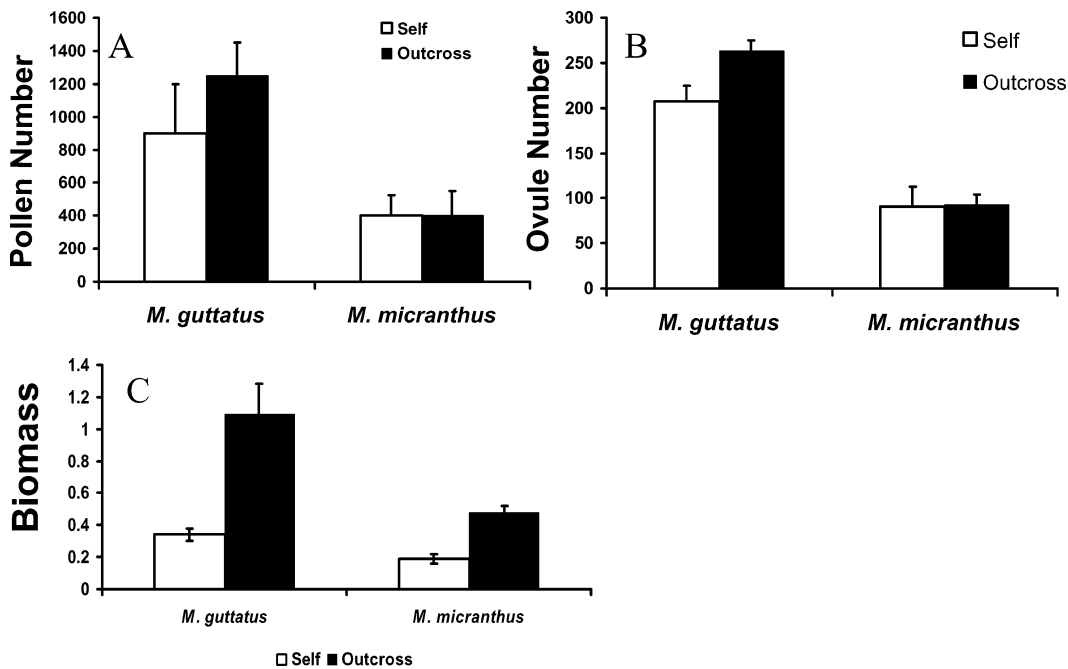


Figure 2. Comparison of progeny performance in the glasshouse following one generation of selfing and outcrossing populations in both *Mimulus guttatus* and *M. micranthus*.—A. Pollen production. —B. Ovule production. —C. Above-ground biomass. The 95% confidence intervals (indicated by vertical lines) on all three panels reflect the variation among maternal families.

By examining the relationship between the plant mating system and the expression of inbreeding depression whenever possible, we can begin to build predictions of which taxa will be more vulnerable to the consequences of small population size. To this end, we assessed whether natural selection was more effective in purging the genetic load of the selfing *M. micranthus* A. Heller versus the mixed-mating *M. guttatus*. *Mimulus micranthus* is a strict annual that produces flowers that are three to four times smaller than *M. guttatus*. This taxon has a distribution limited to the coastal range of central California, but is often found sympatrically with *M. guttatus*. Furthermore, we quantified the stage and magnitude of inbreeding depression expressed in the progeny after performing hand-self and outcross pollinations in the glasshouse. The mixed-mating *M. guttatus* progeny demonstrated significantly greater inbreeding depression in adult above-ground biomass, ovule production, and pollen production per flower than the progeny of selfing *M. micranthus* (Carr & Dudash, 1996; Fig. 2A, B, C). *Mimulus micranthus* self progeny (Fig. 2A, B, C) exhibited significant inbreeding depression in adult above-ground biomass compared to their companion outcrossed progeny (Fig. 2C), but no inbreeding depression for either pollen or ovule production per

flower, two traits closely linked to fitness (Fig. 2A, B). Overall it appears that many uncorrelated loci of small effect are responsible for inbreeding depression in both species (Carr & Dudash, 1997; Dudash et al., 1997; Dudash & Carr, 1998). As theory predicts when assuming dominance-based inbreeding depression, the magnitude of inbreeding depression when present was much greater in the mixed-mating taxon versus the selfing taxon. Additionally, purging of genetic load for pollen and ovule production in the selfing taxon has been much more efficient through its mating system.

Our work and that of others (e.g., Husband & Schemske, 1996) demonstrate the important role of mating system in the opportunity to purge genetic load. Quantifying the mating system (using allozymes if plant tissue and funds are available) as well as breeding system (using detailed field observations to determine the timing of pollen dehiscence, stigma receptivity, whether the plant is monoecious, dioecious, etc., degree of herkogamy or dichogamy of perfect flowers, and overall floral display) would be helpful as a first approximation of the likelihood that a species may be capable of purging its genetic load. For example, if a taxon of concern readily self-pollinates, it may naturally have a reduced genetic load and may be an excel-

lent candidate to use in a restoration effort; its reproductive assurance mechanism increases the chances of success of the project. On the other hand, the reintroduction or establishment of a typically outcrossing species may require a much greater propagule number in order to minimize the likelihood of matings between relatives and any subsequent expression of inbreeding depression. These scenarios call attention to the importance of knowing the reproductive biology of organisms slated for conservation management initiatives.

An approach that has been utilized in designing some conservation efforts of endangered species is hand-pollination experiments to artificially inbreed a taxon in an attempt to reduce its genetic load (e.g., Hedrick, 1994; Kalinowski et al., 2000). We performed this approach for two populations of the mixed-mating *M. guttatus* to examine how the expression of inbreeding depression varied among traits, maternal lines, and between populations, across five generations of serial inbreeding and outcrossing. The crossing program revealed that the magnitude of inbreeding depression was relatively constant in both populations for total flower production, adult above-ground biomass, and pollen and ovule production (Carr & Dudash, 1997; Dudash et al., 1997). At the population level, our results were consistent with dominance-based inbreeding depression with weak selection on partially deleterious alleles; however, we could not rule out overdominance. At the family level, maternal lines varied dramatically in their responses in their trait expression: some increased, some decreased, and some were unaffected by inbreeding. The families that exhibited purging of their genetic load (reflected by the inbred lines outperforming the outcrossed lines) also suggest dominance-based inbreeding depression. However, whether weak selection or drift was responsible for the increased fitness is not known, and both may play important roles in nature. In a greenhouse study Willis (1999b) enforced strong selection by serial inbreeding 1200 maternal lines and saw great improvement in inbred performance of many maternal lines relative to outcrossed relatives, again suggesting evidence of purging. Small populations, such as the experimental populations described above, are prone to genetic drift; therefore the fixation of alleles may have either positive or negative effects on overall fitness. Many conservation efforts would benefit greatly by gathering data on inbreeding depression to assess the consequences of long-term persistence at small population sizes across generations. However, it is not necessarily practical for

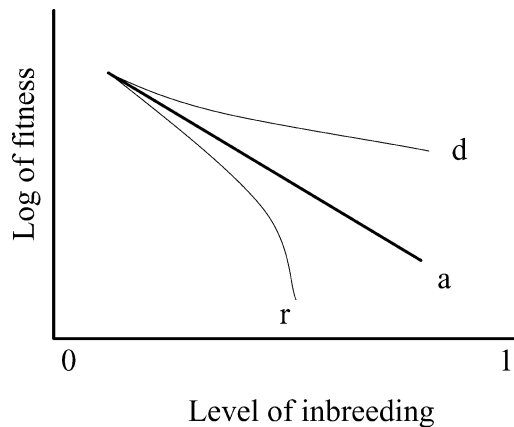


Figure 3. Logarithmic relationship of inbreeding level and fitness, with and without epistasis. Inbreeding level ranges from zero to one, with one being completely inbred. Letter **a** denotes a log-linear relationship, in the absence of epistasis, where the effect of homozygosity at a given locus is independent of the genotype at other loci. Letter **d** describes diminishing epistasis, where the interactions among loci are non-independent, making the slope of the relationship between inbreeding and fitness more shallow. Letter **r** describes reinforcing epistasis where interactions among loci are non-independent, making the slope of the relationship between inbreeding and fitness drop off sharply at intermediate levels of inbreeding. Extending this graph across environments may be an important exercise for predicting the maintenance of fitness of species of conservation concern.

many threatened species; therefore, extrapolation from other native species may be warranted.

Variation among maternal lines provides: (1) a mechanism for the invasion of a selfing variant into an outcrossing population (Holsinger, 1991; Dudash et al., 1997), or (2) the maintenance of genetic variation among different maternal lines in alternate homozygous states. This variation can be produced from random differences in the distribution of deleterious mutations (Schultz & Willis, 1995), prior history of inbreeding (Uyenoyama & Waller, 1991), and also by epistatic interactions among loci (Goodnight, 1999). In our experiments we detected evidence of epistasis (intergenic interactions) at the maternal family level but not at the population level (Dudash et al., 1997). The data suggest diminishing epistasis, which by definition slows down the purging of genetic load, i.e., the maintenance of fitness as inbreeding increases (Fig. 3). These results have implications for breeding programs of endangered or threatened species because enforced selfing may *not* purge the genetic load in a linear fashion if maternal line variation and epistasis inhibit the purging process (Fig. 3). The presence of epistasis makes it even more difficult to readily estimate the

likelihood of purging in conservation breeding programs (Fenster et al., 1997; Fenster & Galloway, 2000). Caution should be exhibited by all researchers attempting to enforce purging as a mechanism of preserving quantitative genetic diversity of species of conservation concern.

Overdominance could not be excluded as a possible explanation for some of the patterns that we observed in our comparisons of *M. guttatus* and *M. micranthus* and the population level behavior of our inbred lines of *M. guttatus*. We therefore sought further evidence that selection was acting against recessive deleterious alleles (dominance-based inbreeding depression) with a more direct estimate of dominance from a North Carolina III quantitative genetics design (Comstock & Robinson, 1952). We showed both above-ground biomass and total flower production in *M. micranthus* were more nearly additive and expressed less dominance-based inbreeding depression than in *M. guttatus*. We directly confirmed theoretical predictions that purging had been more efficient in the selfing taxon. The significance of these results for conservation is that both indirect estimates described above and the direct estimate from the North Carolina III breeding design were consistent in demonstrating evidence for dominance-based inbreeding depression. We do not recommend replication of the North Carolina III breeding design (Comstock & Robinson, 1952) for target conservation species because of the need for a large number of individuals, the use of inbred lines, and the extensive time required to conduct the experiment. Nevertheless, we can utilize the inferences made from model systems to make knowledgeable predictions for conservation. Therefore, the numerous indirect estimates of inbreeding depression and the likelihood of purging that have been the model for native plant species can be utilized to build management plans.

In all the experiments that we have described above, maternal line variation was a critical source of genetic variation. We consider this to be an important observation that should be kept in mind when collecting seed in the field for germplasm databases. We suggest that maternal lines should be kept separate instead of bulk collected for use in breeding programs to enhance or maintain genetic diversity for restoration projects. Conservation efforts for invasive species warrant *aggressive removal of small populations* as soon as they are detected (e.g., Simberloff, 1997; Farnsworth & Ellis, 2001), as any observed maternal lines are likely those that are successful in the novel environment and may have overcome the effects of inbreeding depression, perhaps via purging. Prior to the impending pop-

ulation explosion of the non-native plants, this is the stage where control efforts may actually make a huge difference, and we consider this management practice to be under-utilized.

A notable result of direct relevance to conservation initiatives involving breeding programs is that the process of generating inbred lines of *M. guttatus* reduced the number of original maternal lines by 50% (Dudash et al., 1997). This dramatic loss of lines is an important warning when designing a hand-pollination breeding design in the greenhouse or in situ hand-pollination plan for viable seed generation for plant taxa of conservation concern for which little seed is available. A breeding design with the intent of reducing the genetic load may result in the loss of important genetic variation through the extinction of maternal lines, from male sterility or loss of seed viability, owing to the exposure of homozygous recessive lethals or partial lethals (Dudash et al., 1997; Dudash & Carr, 1998; Willis, 1999a, b; Hedrick & Kalinowski, 2000). On the other hand, for invasive species a history of purging, or purging following initial establishment, or the expression of non-additive genetic effects (e.g., epistasis) may contribute to success in a novel environment.

Data on inbreeding depression are often generated in the glasshouse environment, which is known to minimize the effects of inbreeding (e.g., Schemske, 1983; Dudash, 1990; Norman et al., 1995). We advocate that the quantification of inbreeding depression be done in the field whenever possible, and to test whether purging in nature occurs at a much slower rate across many generations than the serial inbreeding approach commonly used in greenhouse experiments. Different genetic mechanisms and interactions may be occurring to either enhance or inhibit the efficacy of purging. We re-emphasize the importance of maternal line variation discussed above, although our *M. guttatus* data were all collected in a controlled greenhouse environment. Patterns of variation among maternal families observed in the greenhouse may be significantly altered when environmental heterogeneity is experienced in the field in native and non-native habitats. As conservation efforts are focused in field settings, the exploration of the interactions between maternal line variation and habitat heterogeneity are vital data to collect (see Mazer & Leubhn, 1999).

PHENOTYPIC PLASTICITY

Together with inbreeding, changes in the environment may dramatically influence the probability

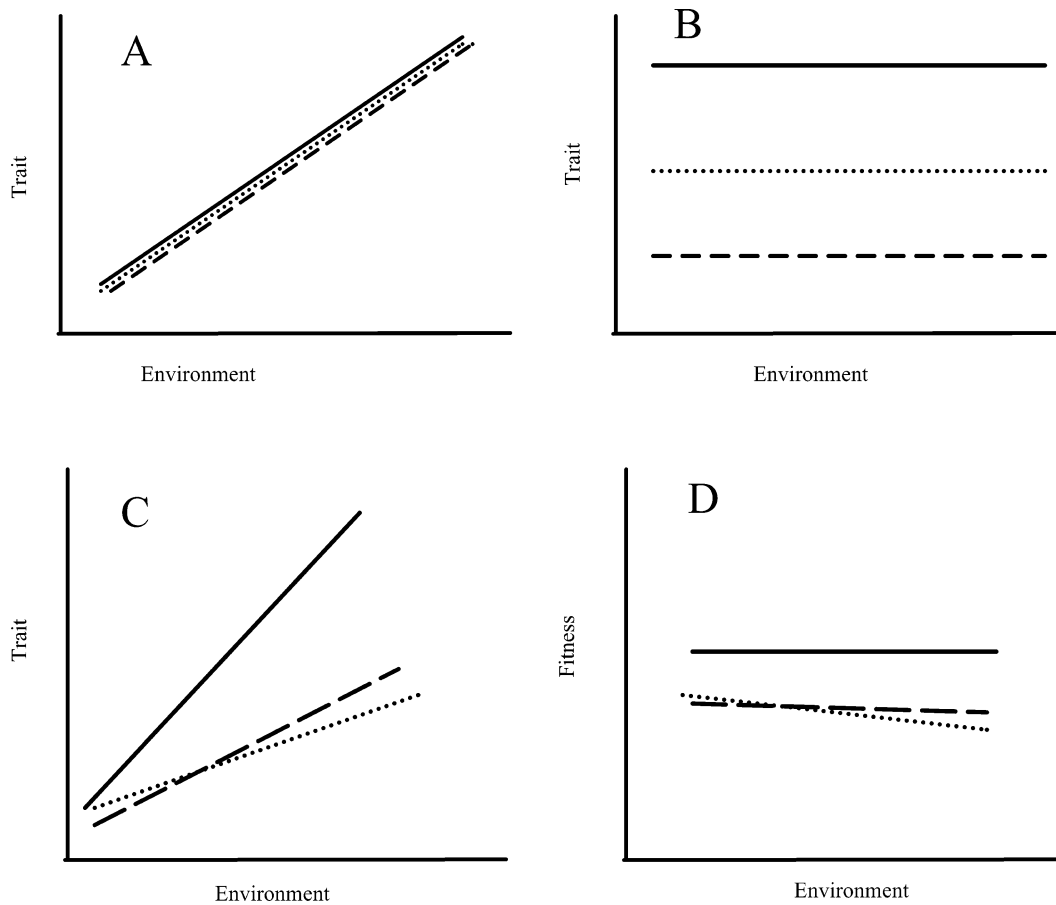


Figure 4. Each line represents a genetic unit (genotype, family, or population: depending on the level of analysis). —A. All three genotypes respond in the same way to the environmental gradient, thus all genotypes are plastic (significant treatment main effect in ANOVA model). —B. All genotypes have flat reaction norms, in other words, they are not plastic because they do not respond to the environmental gradient. However, the genotypes do differ in their mean trait response (significant genotype, family or population main effect in ANOVA model). —C. This is a common reaction norm diagram. All three genotypes are responding differently to the environmental gradient by both changes in slope and changes in rank order of genotypes from one environment to another (crossing reaction norms). This indicates plasticity (trait value is higher as environmental unit increases) and genotype by environment interactions. (Significant effects in ANOVA model, treatment, treatment*genotype, and genotype.)—D. This panel is different in that now the Y-axis represents fitness (fitness reaction norm). Two genotypes have flat reaction norms for fitness (solid and dashed line). The genotype with the dotted line is plastic for fitness. *It is important to note that the solid and dashed line genotypes may be plastic for morphological, physiological, life history, and other traits, which may contribute to the maintenance of a flat reaction norm for fitness.* If this is the case, then genotypes represented by the solid line and dashed line may be indicative of adaptive phenotypic plasticity.

of small population establishment and subsequent persistence of threatened and endangered species as well as newly established populations of non-native taxa. How a genotype alters or maintains its phenotype in response to a range of environments can be explored graphically by examining patterns of a reaction norm diagram (see Fig. 4 for an explanation of common patterns). The maintenance of a phenotype by a genotype across environmental gradients describes a non-plastic genotype for the phenotypic trait of interest (a flat reaction norm),

whereas a plastic response is characterized by variation in the phenotype across environments (a sloped reaction norm).

Examining the reaction norm diagrams for fitness-related traits together with the patterns of plasticity of other phenotypic traits may suggest how plasticity of other phenotypic traits influences fitness across environments. Adaptive phenotypic plasticity describes the capability of organisms to maintain high fitness across different native environmental conditions by exhibiting plasticity in oth-

er non-fitness traits (which broadly include morphological, architectural, anatomical, physiological, behavioral, and life history traits, all of which can vary across development) in response to environmental heterogeneity (Sultan, 1995; Pigliucci, 2001). Because selection favors high fitness of genotypes in all environments, it will also favor plastic responses of non-fitness traits across environments that achieve this fitness effect. However, phenotypic changes may not be sufficient to adequately buffer against environmental changes, leading to variation in fitness levels across environments (Schlichting & Pigliucci, 1998). Different genotypes or different populations may respond plastically to the environmental gradient by changing *alternative* quantitative traits (e.g., leaf size, time to flowering, number of branches, leaf thickening) to maintain or maximize fitness (Marshall et al., 1986; Sandquist & Ehleringer, 1997; Ottenheim et al., 1998; Sultan et al., 1998). The correlations of reaction norms of vegetative and fitness traits may suggest adaptive phenotypic plasticity; however, explicit tests are required to confirm the hypothesis (Schlichting & Pigliucci, 1998).

We predict that small populations, which express genetic variation for plasticity, may increase their chances of survival in light of human disturbance of native habitats (Fischer et al., 2000; Paschke et al., 2003). Similar patterns to those described above might be observed for invasive species outside their native range or for populations established as restoration projects in putative suitable habitats (e.g., Schweitzer & Larson, 1999). In these cases, as selection may or may not have had time to directly shape these responses in the novel non-native or previously unoccupied environment (Mack et al., 2000; Heath et al., 2003), we suggest that the term adaptive phenotypic plasticity may not be entirely appropriate. Instead, here, we coin a new term, *opportunistic plasticity*.

We define *opportunistic phenotypic plasticity* as (1) the maintenance of fitness across the heterogeneous habitats of the novel environment, or (2) the increase in fitness when moving from the native to non-native environment, through the modification of non-fitness traits (e.g., vegetative and life history characters). In the case of invasive taxa, we predict that mean performance frequently will increase in the novel environment in comparison to the native environment (Fig. 5), associated with ecological release from predators, parasites, herbivores, competitors, etc., of the native habitat (e.g., Williamson, 1996; Keane & Crawley, 2002; Quinn & Wetherington, 2002; Conner et al., 2003), as resources can be reallocated to optimize growth and reproduction.

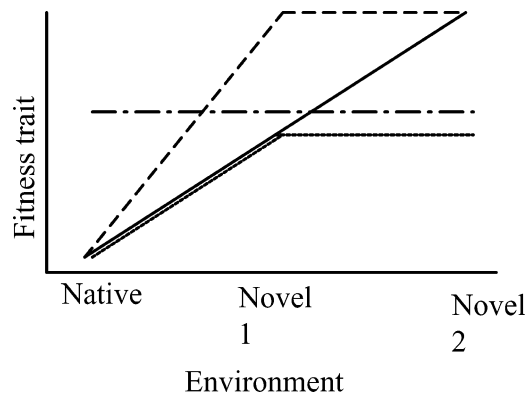


Figure 5. Fitness-related traits in response to environmental variation. The graph illustrates the transition from native to novel environments, and two different habitats in the novel environment. We characterize several potential fitness responses that may be expressed by invasive taxa. The dash-dot line represents a genotype that expresses no variation in fitness-related traits across environments. The solid line represents a genotype that shows a linear increase in fitness across environments. The dotted line represents a genotype that shows a marked increase from native to novel environments, but a flat reaction norm to fitness across the novel environments. The dashed line shows a similar pattern for the genotype as the dotted line genotype, with the exception that the plasticity of fitness is much greater in the initial transition from native to novel environment.

In other words, we consider plasticity *opportunistic* when initial colonists are not adapted to these novel conditions, yet their subsequent generations could become adapted as selection and drift operate in the *new* environment. Opportunistic plasticity as defined here may play an important role in the establishment and persistence of new populations.

HIDDEN REACTION NORMS

Invasive species may express phenotypic plasticity of a multiplicity of traits in response to the new environmental conditions of their non-native range to maximize fitness. The response to novel environments may represent an extension of the responses to environmental conditions experienced in their native environment, particularly if there are similarities among habitat characteristics and gradients between the native and non-native environment (Schlichting & Pigliucci, 1998; Schlichting & Smith, 2002; Schlichting, 2003, 2004; Schlichting & Murren, 2004; Fig. 6). On the other hand, completely different reaction norms could be uncovered (Schlichting, 2003, 2004). Examining phenotypes or phenotypic responses only in the native range may limit our ability to predict vegetative and fitness-related responses to non-native environments.

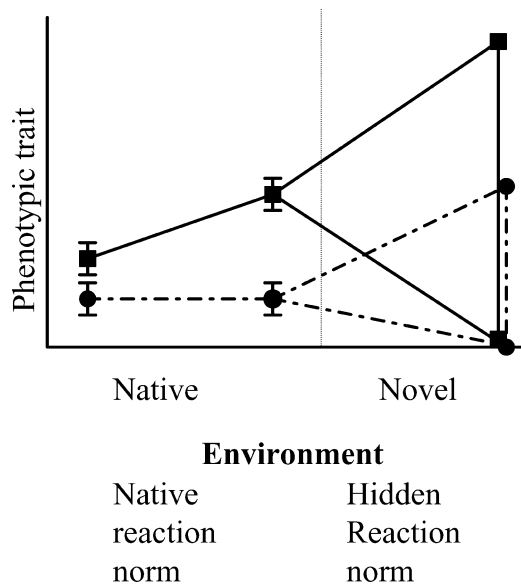


Figure 6. A conceptual hidden reaction norm diagram of a phenotypic trait across two native and one novel environment. The vertical dotted line demarcates the transition from native to novel environments. Here we illustrate two genotypes. The dash-dot line genotype has a flat and canalized (represented by tiny error bars around trait means) reaction norm in the native environment. The solid-line genotype has a plastic and canalized reaction norm in the native environment. For both genotypes, the response to the novel environment may not be predictable given the shape of the reaction norm in the native environment. In both cases, the trait value can increase, decrease, or remain the same. The varieties of potential responses are indicated through triangles across the transition from the native to the novel environment.

For example, novel patterns of phenotypic plasticity may be expressed in the new environment associated with the non-native geographic location (Fig. 6). Many studies that examine plasticity under stressful or novel environmental conditions have shown that the phenotypic variance around a trait mean is increased in the new environment, in comparison with the native environment (e.g., Windig et al., 1994; Holloway et al., 1997). Uncovering these hidden reaction norms (Schlichting & Pigliucci, 1998; Schlichting & Smith, 2002; Schlichting, 2003, 2004; Schlichting & Murren, 2004) could reveal a potential storehouse of genetic and phenotypic variation that could be selected upon and expressed in the new geographic location (Fig. 6).

In regard to invasive species and traits associated with invasion success, examination of genetic variation for plasticity has just begun. We will briefly discuss a few published examples of invasives, while keeping in mind that the same issues may be

relevant for restoration projects (e.g., Montalvo & Ellstrand, 2000), where we are not aware of any studies where plasticity has been explicitly quantified. In some cases invasives may have more rapid growth rates, express physiological plasticity (e.g., Baruch et al., 2000), or may be superior competitors in low-nutrient conditions (e.g., Milberg et al., 1999). Population persistence of an invasive species necessitates that the individuals exhibit phenotypic responses appropriate to novel environmental attributes in the non-native range. For example, Byers and Quinn (1998) compared responses in demographic and reproductive characteristics of *Allyria petiolata* (M. Bieb.) Cavara & Grande (garlic mustard, Brassicaceae) to four microhabitats in an invasive location. They conclude that although survivorship differed among habitats, there is a positive correlation between the observed phenotypic variation in demographic and reproductive characteristics and success in the novel environment (see also: Luken & Goessling, 1995; Stewart & Potvin, 1996; Kaufman & Smouse, 2001; Stratton & Goldstein, 2001). These studies serve as a starting point to understand the relative importance of genotype by environment interactions for particular traits that may act as vital predictors of invasiveness.

Returning to the model organism, *Mimulus guttatus*, genetic variation for phenotypic plasticity is known to be present for several vegetative and life history traits from glasshouse and field experiments (Vickery, 1974, 1983; Galloway, 1995). The presence of phenotypic variation in the field persuaded Vickery (1974) to test whether tolerance to a broad range of temperatures and light intensities might contribute to the success of *M. guttatus* in colonizing a wide variety of habitats in native and non-native locations. Vickery studied an array of native populations, which originated from Alaska south to Utah, and exposed them to combinations of light and temperature treatments reflecting their range in natural environments. Vickery found significant variation in responses to artificial temperature environments among clones of different populations and among genotypes within populations for plant height, plant dry weight, days to first flower, leaf length and width, and node of first flower. Overall, plants at the extreme temperatures were smallest. Increased light availability resulted in significant increases in plant biomass and flower production. Vickery's graphs provide evidence for genotype by environment interactions (see Fig. 7 drawn from data in Vickery, 1974). Yet, Vickery did not find direct concordance between their optimal growth conditions in the glasshouse and their native con-

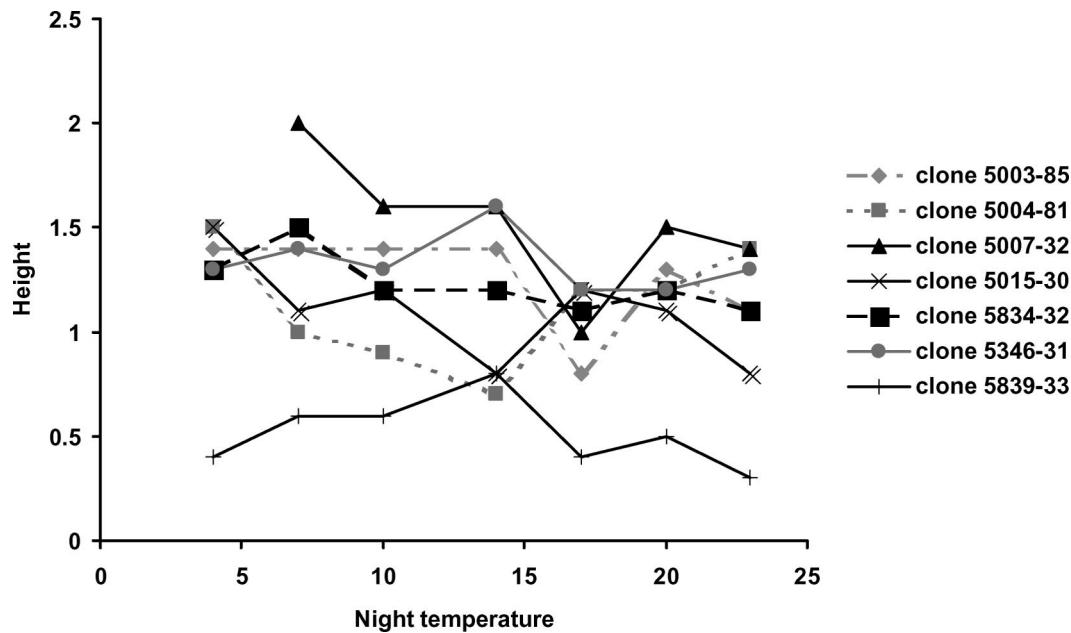


Figure 7. The reaction norms of seven *Mimulus guttatus* clones from different geographic areas drawn from data in Vickery (1974). The height of each clone was measured in seven night temperature environments. The diagram depicts phenotypic plasticity, genotype by environment interactions, and genetic variation for height within environments. What is not shown on this diagram but reported by Vickery (1974) is that optimal fitness and non-fitness trait expression does not necessarily correspond to the home environmental conditions.

ditions. Several cloned genotypes of *M. guttatus* showed both the maintenance of seed production across environmental treatments and plasticity of vegetative traits. These two components provide evidence for the expression of opportunistic plasticity of this species. Since these populations often grew best in conditions outside their native habitat, Vickery concluded that these responses might contribute to the success of *M. guttatus* as a colonist both in its native habitat and in non-native habitats. Observation of patterns such as these in other species could be used as evidence to predict colonization success, persistence, and expansion of populations outside their native range and may serve as an indicator of invasive potential.

Galloway (1995) examined phenotypic plasticity of *M. guttatus* in naturally heterogeneous field conditions using clonally propagated individuals, and found significant genotype by environment interactions for a number of traits. She went on to demonstrate that maternal environment affected the strength of genotype by environment interactions of the offspring in two ways: (1) greater mean flower production and less plasticity for vegetative traits in the offspring of maternal plants from more stressful sites, and, in contrast, (2) decreased flower production and greater plasticity in a variety of vege-

tative traits in the offspring of maternal plants from less stressful sites. These data are evidence that maternal effects, genotype by environment interactions, and environmental variation at macro and micro scales contribute to the maintenance of genetic variation for vegetative and life history characters and their plasticities in populations of this species. This study illustrates the importance of collecting data on habitat heterogeneity and genetic variation of seed source populations, and such data could serve as indicators of which populations may be successful in novel environments.

LINKING INBREEDING AND PHENOTYPIC PLASTICITY IN CONSERVATION

Very few researchers have explored the link between inbreeding and phenotypic plasticity within the basic research arena; therefore, we have limited predictions of how these two important ecological and genetic phenomena interact and influence a population's evolutionary trajectory (Lewis, 1954; Pederson, 1968; Schlichting & Levin, 1986). Are phenotypic plasticity and inbreeding positively correlated, negatively correlated, or not correlated at all? And if correlated, are fitness-related traits positively or negatively impacted? We contend that in-

dependently both inbreeding and phenotypic plasticity have important implications for conservation as we have discussed above. Furthermore, the interaction between inbreeding and phenotypic plasticity may directly impact whether small populations are maintained, whether new small populations successfully establish, and whether these new populations persist. Thus understanding the interaction between inbreeding and phenotypic plasticity has the potential of providing important insights into conservation and management of threatened or endangered species, restoration projects of species of concern, and invasive species (e.g., Quinn & Wetherington, 2002).

We are aware of a few published studies that directly examined plasticity of vegetative traits among inbred and outcrossed lines. Earlier work on *Lycopersicon* (*Solanum* in Solanaceae) and *Arabidopsis* (Brassicaceae) has demonstrated positive correlations between degree of inbreeding and plasticity (Lewis, 1954; Pederson, 1968). Schlichting and Levin (1986) examined the effects of inbreeding on genotype by environment interactions of a self-compatible cultivar, *Phlox drummondii* Hook. (Polemoniaceae). Members of the genus *Phlox* are normally obligately outcrossing. The authors found significant genetic effects (outcrossing and inbreeding lines differed in trait means) and treatment effects. In 75% of the cases inbred and outcrossed lines responded similarly to treatments (similarity in plasticity); conversely, 25% of the traits responded differentially to inbreeding levels (significant variation in plasticity). Overall there was no correlation between increased inbreeding and greater expression of phenotypic plasticity. However, in order to draw inferences to natural settings, as this work was completed on a *Phlox* cultivar, additional studies of native species are required. These three published studies together suggest that the interaction between inbreeding and plasticity is a crucial ecological-genetic interaction that warrants further empirical investigation.

A logical extension of the above studies in a conservation framework is to compare taxa or populations that vary naturally in their levels of inbreeding and habitat heterogeneity and measure phenotypic plasticity of families across environments. For non-native taxa, does a positive correlation between inbreeding and *opportunistic* phenotypic plasticity act as an indicator of potential invasiveness? For restoration projects, selecting native species with: (1) a history of genetic variation for plasticity, (2) possibly *adaptive* phenotypic plasticity across naturally occurring environmental gradients, and (3) a history of inbreeding may be the

most appropriate approaches to ensure success, as their chances of establishment may be greatest.

SUMMARY

Some of the more pressing issues in conservation biology center around: (1) predicting the fate of small, isolated populations (e.g., Menges, 1998), (2) ensuring the successful reintroduction of endangered species back into natural habitats, and (3) understanding the establishment of species beyond their native ranges (Chornesky & Randall, 2003; Denslow, 2003). In this paper we recognize important similarities in the genetic and ecological challenges that face each of these types of populations. In all three cases small population sizes will necessarily lead to increased levels of inbreeding and the likely expression of inbreeding depression. The ability of these populations to persist will depend, in part, on the magnitude of inbreeding depression, variation in the response of families to inbreeding, and the ability of selection to remove (or purge) the genetic load that causes inbreeding depression. These, in turn, will depend on the genetic basis of inbreeding depression (overdominance or dominance, epistasis, and the number of loci involved) and the historic mating system of the species. The loss of genetic variation caused by inbreeding and drift in these small populations will restrict their evolutionary potential, placing a high premium on the ability of plants to respond plastically to environmental variation. The ability of plants to make appropriate phenotypic adjustments in truly novel environments, an ability we refer to as *opportunistic plasticity*, may be particularly critical in the case of introductions in ecological restoration work where habitats resemble, but may not be identical to, seed collection sites or in the establishment of exotic species in non-native locations where habitats may differ dramatically from native sites. Whenever plants are not growing in their native environment, they have by default not had the opportunity to adapt specifically to this new environment. Although plasticity may be an important means of dealing with environmental heterogeneity in small populations, unfortunately we have almost no data on the interaction between inbreeding and plasticity in plants. The science and practice of conservation biology has generally appreciated the importance of inbreeding, but we feel that it will benefit greatly by consideration of its potential interactions with plasticity and their role in the establishment phase of small populations. The uniquely rich literature on inbreeding and plasticity in *Mimulus guttatus* make this plant an attrac-

tive model system for studying how these issues act separately and in concert in small, inbreeding populations.

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