

Genetics AND Conservation OF Rare Plants

Edited by

DONALD A. FALK
KENT E. HOLSINGER

C E N T E R F O R P L A N T C O N S E R V A T I O N



New York Oxford
OXFORD UNIVERSITY PRESS
1991

1

Genetic and Evolutionary Consequences of Small Population Size in Plants: Implications for Conservation

SPENCER C. H. BARRETT and JOSHUA R. KOHN

Plant population sizes vary in space and time both within and among species. This variability is the result of complex interactions among the life-history features of populations, local environmental conditions, and the historical ecology of particular species. Populations range in size from many thousands, as in some forest trees and savannah grasses, to small colonies of a few plants in rare orchids and certain parasitic plants. Considering the sessile habit of plants and the ease with which they can be counted, data on population sizes are surprisingly sparse in the botanical literature.

Several features of the population biology of plants may account for the limited census data on population size, in comparison with many animal groups. Accurate estimates can be complicated by subterranean seed banks and because some plants exhibit prolific clonal growth (Harper 1977). These features lead to problems in defining what constitutes a plant population (Crawford 1984). Whereas the ramet may be the unit of interest to ecologists, the genet (or genotype) is of more significance to geneticists and evolutionary biologists. Determination of the number of genets in a population requires many genetic markers, and the number is therefore difficult to obtain for most clonal species (Silander 1985a).

The significance of the size of populations to their breeding structure, genetics, and evolutionary dynamics was first recognized by Wright (1931, 1938, 1946) and is today a central focus of concern in conservation biology. One of the goals of many conservation programs, in addition to habitat preservation, is to maintain existing levels of genetic variation in species that are rare or threatened (Frankel and Soulé 1981; Simberloff 1988). Loss of variation is thought to reduce the ability of populations to adapt to changing environments and increase their susceptibility to pest and disease pressures (Fisher 1930; Hamilton 1982; Beardmore 1983).

The total genetic variation maintained within a species can be partitioned in a hierarchical manner, according to the way it is distributed among regions, populations, and individuals within populations. Four evolutionary forces—mutation, natural selection, migration, and random genetic drift—interacting with an organism's recombination system, account for the manner in which variation is distributed among levels in the hierarchy. The relative importance of these factors differs among species and

ecological groups. Where populations are small and isolated from one another, genetic drift will have a dominant influence on population genetic structure. Populations in which drift predominates lose variation more readily than do populations in which drift is not a factor, and are thus particularly prone to local extinction. Conservation practices require an understanding of the effects of stochastic forces on genetic variability, since populations conserved in reserves, and samples maintained in zoos and botanical gardens, are usually restricted in size (Berry 1971; Franklin 1980; Lande and Barrowclough 1987).

Another consequence of small population size is the occurrence of inbreeding or mating between close relatives. The loss of fitness upon inbreeding is known as *inbreeding depression*. Although the genetic mechanisms underlying inbreeding depression and its converse, *heterosis*, are still not completely understood (see below), it is generally agreed that inbreeding reduces reproductive performance in most animals, particularly mammals (Ralls et al. 1986, 1988). These effects can ultimately lead to the loss of valuable genetic stocks if intense inbreeding is practiced. Because of the occurrence of inbreeding depression in small populations of captive animals, a variety of breeding protocols have been developed in recent years to overcome its debilitating effects (Chesser et al. 1980; Templeton and Read 1983).

The effects of inbreeding on fitness in plants are likely to be a good deal more variable than in animals because plants have such diverse reproductive systems and population structures (Charlesworth and Charlesworth 1987). Generalizations on the harmful effects of inbreeding, and recommendations on the number of individuals required to maintain viable populations, may need to be modified from those available for most animals because many plant species regularly inbreed. In some cases it may be more important to determine whether *outbreeding depression*, the fitness decline that can result from hybridization, occurs (Templeton 1986). Information on outbreeding depression in plants will be of particular importance to *ex situ* conservation strategies if they involve controlled breeding of samples collected from different populations.

A burgeoning literature on conservation biology of rare and endangered species has developed during the past decade, with several volumes devoted solely to biological aspects of rare plant conservation (Simmons et al. 1976; Syngé 1981; Bramwell et al. 1987). In contrast to works on animal conservation, the botanical literature concerned with nondomesticated species has largely neglected genetic considerations in devising strategies for rare plant conservation, focusing instead on ecological and demographic issues. Here we attempt to redress the balance by examining the genetic and evolutionary consequences of small population sizes in wild plants and their implications for conservation biology. We consider the problem from both theoretical and empirical standpoints, using data from natural plant populations wherever possible, and attempt to distinguish those features of plants that make generalizations from the animal literature unsound.

We begin by reviewing some of the genetic consequences of small population size in plants, paying particular attention to the role of genetic bottlenecks in reducing diversity. This is followed by a consideration of mating patterns in small populations and an assessment of the significance of inbreeding depression and heterosis to fitness. We conclude by discussing the implications of what is known about the genetics of

studies that could be profitably undertaken on rare plants both to enhance the chances for success of the conservation effort and to increase understanding of the role of population genetic structure for species survival.

ECOLOGICAL CAUSES OF SMALL POPULATION SIZE

Despite the paucity of data on population size variation in plants, natural history observations indicate that populations of some species are large, continuous, and stable, some are regularly small and sparsely distributed, whereas others fluctuate dramatically from season to season. These differences are usually associated with contrasting life-history strategies of individual species. However, population sizes can also vary considerably within species, particularly in those subject to frequent cycles of colonization and extinction. Figure 1.1 illustrates the range of population sizes that occur in *Eichhornia paniculata*, a colonizer of ephemeral aquatic habitats in northeastern Brazil that are often short-lived as a result of drought, floods, and human disturbance. There is a large variance in population size within each of the two years that populations were sampled. In addition, 32% and 51% of populations sampled in 1987 and 1988, respectively, contained fewer than 100 individuals. This suggests that populations of *E. paniculata* are likely to be especially subject to genetic drift. Indeed, evidence is available to support this suggestion (Barrett et al. 1989).

Populations may be small for a variety of reasons. The major reasons, which have been summarized by Harper (1977), are as follows.

1. The available sites are few and separated by distances beyond a species' normal dispersal ability.
2. The carrying capacity of the site is low.
3. The habitability of the site is of short duration because of successional displacement.
4. Colonization is in its early stages, and full exploitation of the site has not occurred.

To these may be added the diverse factors associated with environmental catastrophes, including fires, grazing, droughts, floods, and insect and disease outbreaks. Because of the variety of ecological factors that limit population size, patterns of genetic variation in small populations that originate in contrasting ways are likely to differ. As we shall see, populations that remain small for long periods are likely to be considerably less diverse than those that have only recently become small.

It is often assumed that rare and endangered species inevitably occur in small populations that are geographically isolated from one another. The ecological and evolutionary processes that give rise to rarity are, however, sufficiently complex that we cannot assume that all rare plants exhibit these patterns (Kruckeberg and Rabinowitz 1985). Emphasizing the ecological heterogeneity of rare species, Rabinowitz (1981) constructed a classification of rarity based on local population size, habitat specificity, and geographical range. For each situation two categories (large vs. small, wide vs. narrow) were erected. Since species with large population sizes, wide ranges, and broad habitat preferences are unlikely to be rare, the classification scheme results

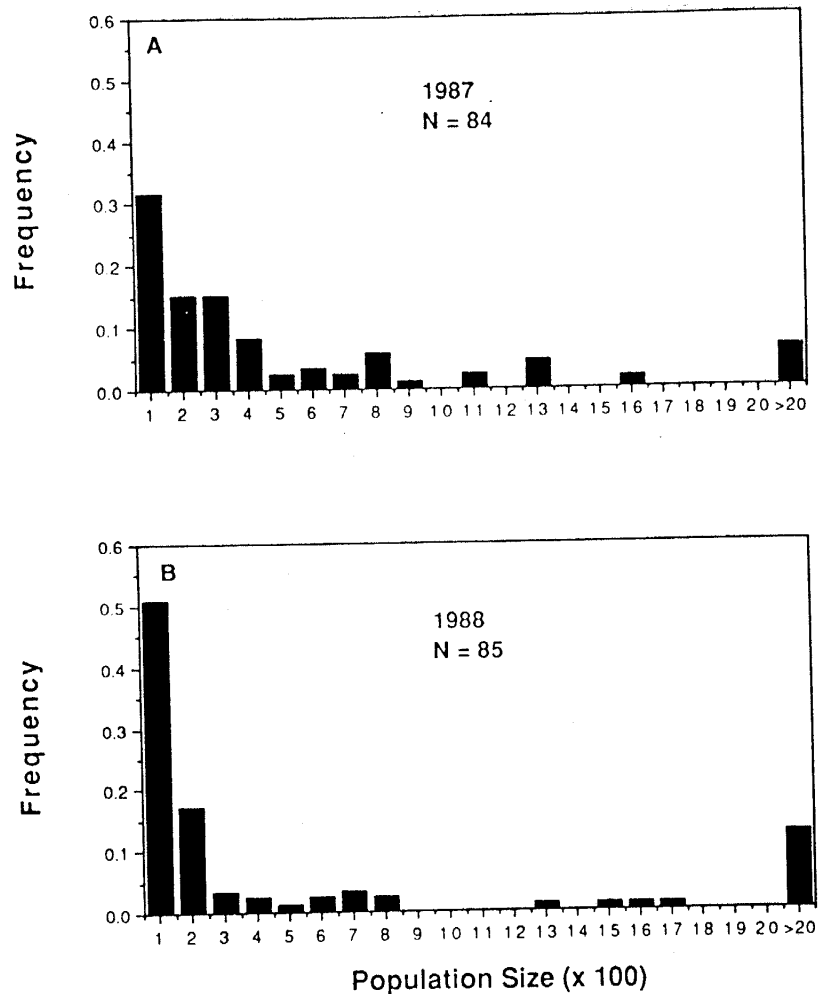


Figure 1.1. Distributions of population size in *Eichhornia paniculata* from northeastern Brazil during 1987 and 1988. Sample sizes in A and B refer to the number of populations censused in each year. (B. C. Husband and S. C. H. Barrett unpubl. data)

seven forms of rarity, only four of which were characterized by small local populations. On theoretical grounds, these differences in the spatial distribution and size of populations are likely to result in contrasting patterns of population genetic structure. Although several rare plants surveyed for genetic variation appear to be genetically depauperate because of small population size effects (see below), it may be premature to assume that this is a universal feature of rare species (Stebbins 1980; Griggs and Jain 1983; Nickrent and Wiens 1989).

The distribution and amounts of genetic diversity within and among populations of rare plants are likely to depend on whether a species has always been rare or whether it has recently become so as a result of human influences. Species that occur naturally

in sparsely distributed, small populations may possess genetic systems adjusted to close inbreeding, as well as adaptations that offset the disadvantage of rarity—for instance, during mating (e.g., bird pollination in *Eucalyptus stoatei* [Hopper and Moran 1981]). In contrast, species that have experienced severe reductions in population numbers owing to habitat destruction or grazing (e.g., *Gossypium mustelinum*, [Pickersgill et al. 1976]) may be more susceptible to genetic stresses imposed by small population size. These considerations highlight the importance of considering the ecology of rare species and the diverse ways that rarity can arise (e.g., Stebbins and Major 1965; Wiens et al. 1989). These differences will have important implications for population genetic structure, the sampling of genetic material, and the specific types of breeding programs that are planned.

GENETIC CONSEQUENCES OF SMALL POPULATION SIZE

Although some rare plants maintain large population sizes, a majority occur in small populations, often with decreasing numbers (Ashton 1987). It is therefore important to assess whether significant genetic deterioration can arise through sudden or gradual decreases in numbers. Harmful genetic effects may result from inbreeding in small populations (see below). We concentrate in this section, however, on how genetic variation is affected by small population size. We consider how stochastic forces such as bottlenecks, founder events, and genetic drift influence different classes of genetic variation, and assess how important these processes are likely to be in wild populations of plants and those that are to be conserved. Before we do this, however, it is necessary to clarify concepts of population size since, from the genetical perspective, natural populations often behave as if they are smaller than a direct count of individuals would suggest.

Effective Population Size

Population sizes of relevance to conservation genetics may not be equivalent to the number of individuals in a population. The reason is that the size of the breeding population is rarely equal to the total population size (N) of most models concerned with the genetics of finite populations. Factors that cause this disparity include temporal fluctuations in numbers, nonrandom mating, differential fertility, unequal sex ratios, age and size structure, and gene flow among populations (Kimura and Crow 1963; Crawford 1984; Heywood 1986). These factors violate the assumption that each of the N individuals has an equal probability of contributing gametes to the next generation. A more useful parameter, first introduced by Wright (1931), is N_e , the effective population size. This represents the size of an idealized population in which individuals contribute equally to the gamete pool and have the same variation in allele frequencies and levels of inbreeding as the observed population. The effective size can be estimated from the actual number of breeding individuals, when factors such as mating system, sex ratio, and variation in fertility are known. Alternatively, N_e can be inferred from the between-year variance of allele frequencies at neutral loci (Kimura and Crow 1963; Crow and Denniston 1988). Estimates of N_e for a variety of animal

groups are summarized by Crawford (1984), who draws attention to the lack of data from plant populations. The few examples that are available suggest that effective number for populations of plant species may be considerably smaller than the census number (Jain and Rai 1974; MacKay 1981).

Genetic Bottlenecks and Founder Events

Theoretical Considerations

A *bottleneck* is a sharp reduction in the number of individuals of a species in a particular place or time. If the restriction in number is accompanied by significant loss of genetic diversity, the term *genetic bottleneck* is often used. *Population bottlenecks* usually refer to a collapse in the number of individuals within a single population as a result of some environmental catastrophe. Population bottlenecks may or may not be associated with genetic bottlenecks; this depends on a variety of demographic and genetic factors that are discussed below. Bottlenecks are often associated with colonizing events, if one or a few individuals establish populations in previously unoccupied territory. Irrespective of whether bottlenecks occur within a single population or through the founding of new populations, they usually involve sampling error; small samples are rarely representative of the populations from which they are drawn. The theoretical analysis of bottlenecks is of importance for the development of nonequilibrium models in population genetics as well as in studies of speciation (Carson 1968; Barton and Charlesworth 1984; Carson and Templeton 1984). In addition, the study of bottlenecks can have practical applications for genetic resource conservation in cultivated plants and animals as well as endangered species (Maruyama and Fuerst 1984).

A genetic bottleneck is a single event in time. When populations remain small for any extended period, the sampling effects become cumulative. This gives rise to random changes in gene frequency because of the sampling of gametes from generation to generation. This process is referred to as *random genetic drift*. In large populations, on average, only small random changes in gene frequency occur as a result of drift; however, where population sizes are small (e.g., <100), gene frequencies can undergo large fluctuations in different generations, leading to loss of alleles. Therefore, large populations should maintain higher levels of genetic variability than do small populations (Wright 1931; Kimura and Crow 1964). Owing to the paucity of population size data for plants, support for the theory is limited, although what data are available are in accord with the prediction that small populations are more likely to lose polymorphism at neutral loci through stochastic forces (Figure 1.2).

The term *founder effect* was coined by Mayr (1963) to refer to "the establishment of a new population by a few original founders . . . which carry only a small fraction of the total genetic variation of the parental population." Random processes of this type are central to the theories of speciation proposed by Mayr (1963). He invoked founder events as the mechanisms by which genetic revolutions, resulting in reproductive isolation, are triggered. As originally conceived by Mayr, the founder principle was thought to reduce genetic variation affecting both quantitative traits and levels of heterozygosity. However, as pointed out by Lewontin (1965), and more recently reviewed by Lande (1980), measures of genetic variation that weigh genotypes by their frequencies, such as heterozygosity or quantitative genetic variation, are not particu-

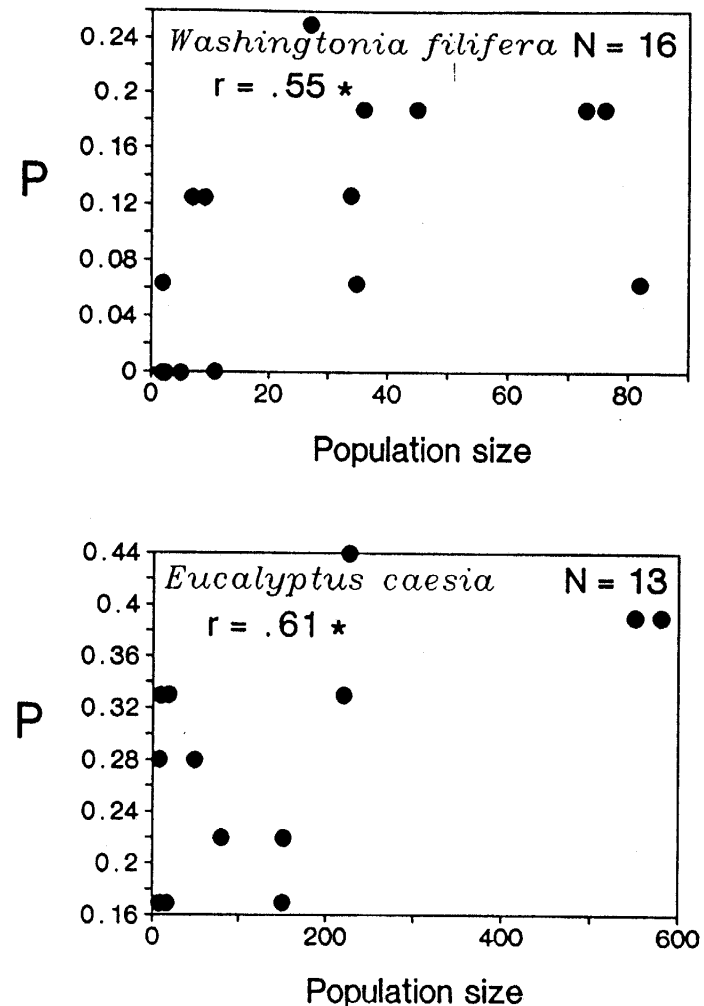


Figure 1.2. The relationship between population size and the proportion of isozyme loci that are polymorphic (P) in *Washingtonia filifera* (McClenaghan and Beauchamp 1986) and *Eucalyptus caesia* (Moran and Hopper 1983). Sample sizes (N) refer to the number of populations examined. In neither species was there a significant relationship between heterozygosity and population size. Both species are rare and maintain small population sizes. A total of 16 and 18 loci were screened in *W. filifera* and *E. caesia*, respectively.

larly sensitive to sampling error because only rare alleles tend to be lost each generation. Thus founder events in outcrossing organisms can be less effective at reducing genetic variability than is generally believed. Refinement of this point was later made by Nei et al. (1975) in their theoretical analysis of the effects of bottlenecks on genetic variation.

The loss of genetic variability concomitant with a bottleneck has both qualitative and quantitative aspects. A reduction in the number of alleles, especially rare ones, is

much greater than the loss of heterozygosity and genetic variance per se (Figure 1.2). Nei et al. (1975) used a model in which a severe size reduction occurred suddenly in a population that was in equilibrium between mutation and genetic drift. They found that the amount of reduction in average heterozygosity per locus depends not only on the size of the bottleneck, but also on the subsequent rate of population growth. If population sizes increase rapidly, reduction in average heterozygosity is minimal, even if the number of founders is small. In contrast, the loss in the average number of alleles per locus is profoundly affected by bottleneck size, but little by the rate of population growth following the bottleneck. This difference occurs mainly because genetic drift eliminates many low-frequency alleles. However, the average number of alleles per locus increases faster than average heterozygosity when large population sizes are restored.

The situation modeled by Nei et al. (1975) is likely to apply most often to exotic pests and weeds, whose populations expand rapidly following introduction to a new region (Elton 1958; Parsons 1983). More frequently, however, wild populations experience periods during which size fluctuates. In the case of rare species, populations may go through repeated bottlenecks, or, where plants or animals are brought into captivity, a single bottleneck may be followed by a protracted period of small population size. Several of these more complex situations have been examined analytically by Denniston (1978), Watterson (1984), and Maruyama and Fuerst (1984, 1985a, 1985b). In general, their results confirm that the loss of alleles greatly exceeds reductions in heterozygosity, when populations remain small following a bottleneck, although protracted periods of small population size will result in a significant loss of variation.

The effects of stochastic loss of genetic variation from small managed populations have been considered by Lacy (1987). Using computer simulation, he investigated the interacting effects of genetic drift, mutation, immigration, population subdivision, and various forms of selection on the loss of heterozygosity over 100 generations in populations ranging in size from 20 to 500. He found that genetic drift was the predominant force controlling reductions in genetic variation. Mutation had no noticeable effect on populations of the size typical for many species managed in nature reserves, zoos, or botanical gardens. Immigration from a large source population can retard, halt, or even reverse the loss of genetic variation, even with only one or a few migrants per generation. He also found that unless selection is considerably stronger than is commonly observed in nature (but see Endler 1986), it is ineffective in counteracting genetic drift when population size is 100 or fewer. Subdivided populations rapidly lose variation from within each subpopulation but retain variation across subpopulations better than a single panmictic population does (although see Fuerst and Maruyama 1986). Lacey's results, some of which are illustrated in Figure 1.3, indicate that conservation biologists should be most concerned about the effects of genetic drift in reducing genetic variation in managed populations. The regular introduction of immigrants and the division of managed populations into smaller breeding groups that interchange enough migrants to prevent unacceptably high levels of inbreeding are two strategies that would ameliorate the loss of variability due to drift.

Most theoretical work on the stochastic loss of variation through bottlenecks and founder events has considered diploid, outbreeding organisms with separate sexes.

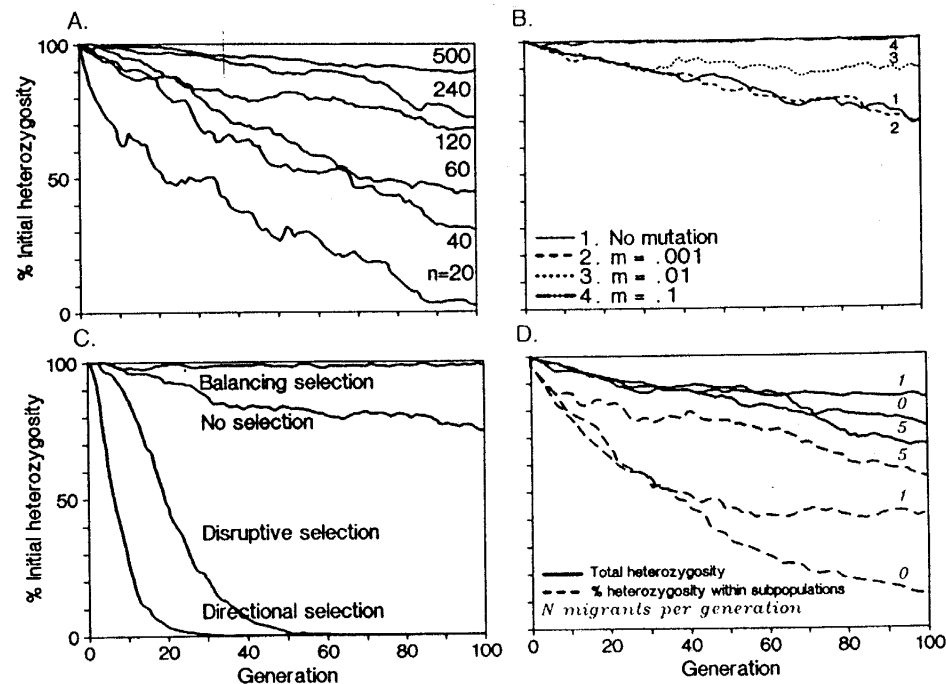


Figure 1.3. The percent of initial heterozygosity retained in populations with time (in generations) for (A) population sizes ranging from 20 to 500; (B) populations with equal forward and backward mutation rates of 0, 10^{-4} , 10^{-3} , or 10^{-2} per generation; (C) populations subjected to balancing selection, no selection, disruptive selection, and directional selection (relative fitnesses of 0.8 : 1.0 : 0.8; 1.0 : 0.8 : 1.0; and 1.0 : 0.8 : 0.6, for balancing, disruptive, and directional selection, respectively); (D) populations (solid lines) and five subpopulations (dashed lines) with an average of 0, 1, and 5 inter-subpopulation migrants per generation. Individual trajectories in each figure represent the average of 25 simulated populations. Except where indicated, all figures are based on simulations involving a population size of 120 individuals. (After Lacy 1987)

of most plant species where polyploidy, hermaphroditism, and varying degrees of self-fertilization commonly occur. These features of plant genetic systems modify in different ways the significance of stochastic influences on patterns of genetic variation within populations. For example, in autopolyploids with polysomic inheritance, population size restrictions will have less of an effect on the loss of genetic variability because high levels of segregational heterozygosity are maintained in populations (Haldane 1930; Mayo 1971). In allopolyploid species with high levels of gene duplication, loss of heterozygosity is unimportant for loci that exhibit "fixed" heterozygosity. Similarly, the diverse array of mating systems in plants will vary in the extent to which they are influenced by bottlenecks and genetic drift. For instance, in selfing species severe population restriction followed by population growth might not affect heterozygosity, but could cause severe loss of allelic variation. Theoretical work could usefully explore the effect of various mating systems and types of polyploidy on the stochastic loss of genetic variation from small populations of plants. Approaches of

librium conditions on the maintenance of variation at loci controlling the mating-system polymorphism tristylly (Heuch 1980; Morgan and Barrett 1988), and at the self-incompatibility locus in species with multiallelic homomorphic systems (Imrie et al. 1972).

Empirical Evidence

Examination of the patterns of genetic variation at isozyme loci in different groups of colonizing animals has provided some evidence for the role of bottlenecks in reducing variation (Taylor and Gorman 1975; Huettel et al. 1980; Richardson et al. 1980; Bryant et al. 1981; Berlocher 1984; Baker and Moeed 1987; Menken 1987). Although there are few comparable studies in plants, there are good reasons to believe that bottlenecks and founder events play a more significant role in governing patterns of variability, particularly in colonizing species (Barrett and Richardson 1986). One reason is that many cosmopolitan plants reproduce either through clonal propagation or by predominant self-fertilization. With uniparental reproduction and limited gene flow from the source region, genetically uniform population systems are likely in the introduced range. Examples of genetic bottlenecks in colonizing plants have recently been discussed by Barrett and Husband (1989), and isozyme surveys that have revealed extensive areas of genetic uniformity in cosmopolitan taxa are reviewed in Barrett and Shore (1989).

Not all plant isozyme studies that have revealed genetically depauperate populations involve cosmopolitan species with uniparental reproductive systems. Karron (1987a), in a literature survey, compared levels of genetic diversity at isozyme loci in 11 pairs of congeneric species that were either rare or common. Diversity was reduced in the rare species, irrespective of the reproductive system, perhaps as a consequence of bottlenecks associated with declining population numbers. Several authors have invoked bottlenecks during the evolutionary history of a species as an explanation to account for the low levels of genetic variation maintained in contemporary populations. Examples involve rare and widespread species and both inbreeders and outbreeders (Table 1.1). In most cases, restriction of populations to small refugia, owing to climatic changes associated with Pleistocene glacial events, are postulated as the causal factor resulting in genetic bottlenecks.

Inferences on the role of historical bottlenecks in reducing genetic variation can be strengthened if genetic data are available for congeneric species with similar life histories and reproductive systems. In *Pinus* spp., virtually all taxa investigated, except the locally restricted *P. torreyana* and more widespread *P. resinosa*, exhibit high levels of genetic diversity (Ledig 1986a). Although fewer data are available for Australian *Acacia* spp., several species of the genus that occupy environments similar to that of the genetically depauperate *A. mangium* possess higher levels of genetic diversity. Moran et al. (1989) suggest that these taxa were able to maintain wider distributions during interglacial floods because of broader ecological tolerance. In contrast, several geographical surveys of isozyme variability in different European and North American taxa of *Salicornia* have revealed very low levels of variability within each taxon (Jefferies and Gottlieb 1982; Wolff and Jefferies 1987a, 1987b). It is possible that destruction of coastal environments, as a result of glacial advance during the Pleistocene, affected all taxa of *Salicornia* throughout the north temperate zone. Alternatively, low

predominantly selfing habit of these species and adaptation to the highly specialized salt marsh environment with its high predictability and low biotic diversity.

These examples illustrate some of the difficulties that are associated with inferring the past occurrence of genetic bottlenecks. Comparisons among related taxa can provide indirect evidence on the role of bottlenecks; however, simple correlation analysis frequently confounds differences in evolutionary history among related species with contemporary ecological factors that also affect population genetic structure. Although intraspecific studies reduce these problems, difficulties can still arise when genetic differentiation among conspecific populations occur in traits that directly influence recombination (e.g., Glover and Barrett 1987). Clearly, the major problem in evaluating the role of past genetic bottlenecks in reducing variability is that so little is known about the evolutionary history of most plant species (but see Cwynar and MacDonald 1987). Recent migration events can provide more convincing evidence of genetic bottlenecks, particularly if the source region is known (e.g., *Echinochloa microstachya* in Australia [S. C. H. Barrett and A. H. D. Brown unpubl. data]) or the number of individuals and time of introduction have been documented (e.g., *Sarracenia purpurea* [Schwaegerle and Schaal 1979]). However, to understand fully the genetic consequences of finite population size effects in plants, experimental approaches are required. These can involve the monitoring of artificially established colonies of known size and genetic composition (Martins and Jain 1979; D. W. Schemske unpubl. data) or the investigation of natural colonization processes (Harding and Mankinen 1972; Jain and Martins 1979; Barrett and Husband 1989).

One of the few experimental studies in plants explicitly designed to investigate the effect of restrictions in population size on genetic variation was conducted by Polans and Allard (1989) using field collected genotypes of *Lolium multiflorum*, an outbreeding grass. Artificial populations composed of one, two, four, or eight plants were established for three successive generations, and changes in allele frequencies and levels of heterozygosity were monitored at three isozyme loci. The results obtained were consistent with expectations for neutral loci subject to genetic drift. The severity in loss of genetic variation increased with smaller population sizes. Restrictions in population size also resulted in significant phenotypic effects in four of six quantitative traits that were studied. Smaller populations flowered more slowly, and were shorter with fewer tillers and seeds, than populations of larger size (Figure 1.4). This indicates the potential ill effects of restrictions in population size on traits that are likely to affect fitness.

When population sizes of experimental populations of *L. multiflorum* were increased, some of the detrimental effects were still discernible. However, the expression of quantitative traits recovered to levels similar to those in the original population, after a single generation of random mating, in composite populations formed by mixing genotypes from the small inbred populations. Recovery from the debilitating effects of drift presumably results from reassociation of favorable genetic combinations in mixtures of inbred lines.

The study by Polans and Allard (1989) considered the effects of small population size on both isozyme loci and quantitative characters. This is important because species that demonstrate low variation at isozyme loci owing to bottleneck effects may not be devoid of variation at other gene loci (e.g., Moran et al. 1981; Giles 1983; Warwick

Table 1.1. Plant Taxa in Which Genetic Bottlenecks Arising from Founder Events and Drift May Account for the Low Levels of Isozyme Polymorphism

Taxon	Current distribution	Life form	Breeding system	Features of bottleneck	Source
Ancient bottlenecks					
<i>Acacia mangium</i>	Widespread Australasia	Tree	Outcrosser	Interglacial flooding in tropical Australasia	Moran et al. 1989
<i>Chrysplenium iowense</i>	Narrow endemic Iowa	Herb	Outcrosser	Pleistocene glaciation, small refugia	Schwartz 1985
<i>Clarkia franciscana</i>	Narrow endemic N. California	Herb	Selfer	Bottleneck via a rapid speciation event?	Gottlieb 1973a
<i>Eucalyptus caesia</i>	Narrow endemic W. Australia	Tree	Outcrosser	Small isolated populations on granite rocks	Moran and Hopper 1983
<i>Howellia aquatilis</i>	Narrow endemic N.W. U.S.	Herb	Selfer	Pleistocene glaciation, small refugia	Lesica et al. 1988
<i>Pedicularis furbishiae</i>	Narrow endemic Maine	Herb	Outcrosser	Postglacial colonization and frequent extinctions	Waller et al. 1987
<i>Pinus resinosa</i>	Widespread E. U.S.	Tree	Outcrosser	Pleistocene glaciation, small refugia	Fowler and Morris 1977
<i>Pinus torreyana</i>	Narrow endemic S. California	Tree	Outcrosser	Climate change during xerothermic period	Ledig and Conkle 1983
<i>Salicornia europaea</i>	Widespread N. temperate zone	Herb	Selfer	Interglacial flooding of salt marshes	Wolff and Jelleries 1987a, 1987b
<i>Stephanomeria malheurensis</i>	Narrow endemic Oregon	Herb	Selfer	Bottleneck via a rapid speciation event	Gottlieb 1977b
<i>Typha</i> spp.	Widespread N. America	Herb	Clonal	Pleistocene glaciation, small refugia?	Sharitz et al. 1980
<i>Washingtonia filifera</i>	Narrow endemic S.W. U.S.	Palm	Outcrosser	Colonization from refugial populations	McClenaghan and Beauchamp 1986
<i>Eichhornia paniculata</i>	Widespread disjunct N. Brazil and Caribbean	Herb	Variable	Bottleneck during colonization of Jamaica	Glover and Barrett 1987
<i>Turnera ulmifolia</i> ^a	Widespread neotropics	Herb	Outcrosser	Bottleneck during colonization of Caribbean	Barrett and Shore 1989
Recent bottlenecks					
<i>Avena barbata</i>	Cosmopolitan	Herb	Selfer	Bottleneck during introduction to California	Clegg and Allard 1972
<i>Bromus tectorum</i>	Europe N. America	Herb	Selfer	Bottleneck during introduction to N. America	S. Novak, unpubl. data
<i>Chondrilla juncea</i>	Mediterranean Australia	Herb	Apomict	Bottleneck during introduction to Australia	Burdon et al. 1980
<i>Echinochloa</i> spp.	Cosmopolitan	Herb	Selfer	Bottleneck during introduction to Australia	S. C. H. Barrett and A. H. D. Brown unpubl. data
<i>Eichhornia crassipes</i>	New and Old World tropics	Herb	Clonal	Bottleneck during introduction to Old World	S. C. H. Barrett unpubl. data
<i>Sarracenia purpurea</i>	N. America	Herb	Outcrosser	Bottleneck during introduction to Ohio reserve	Schwaegerle and Schaal 1979
<i>Xanthium</i> spp.	New World Australia	Herb	Selfer	Bottleneck during introduction to Australia	Moran and Marshall 1978

Note: Ancient bottlenecks refer to events that occurred prior to historic times; except where indicated, these are manifested at the species level. Recent bottlenecks refer to events occurring within historic times and are evident at the population level.

^aPopulation-level bottleneck.

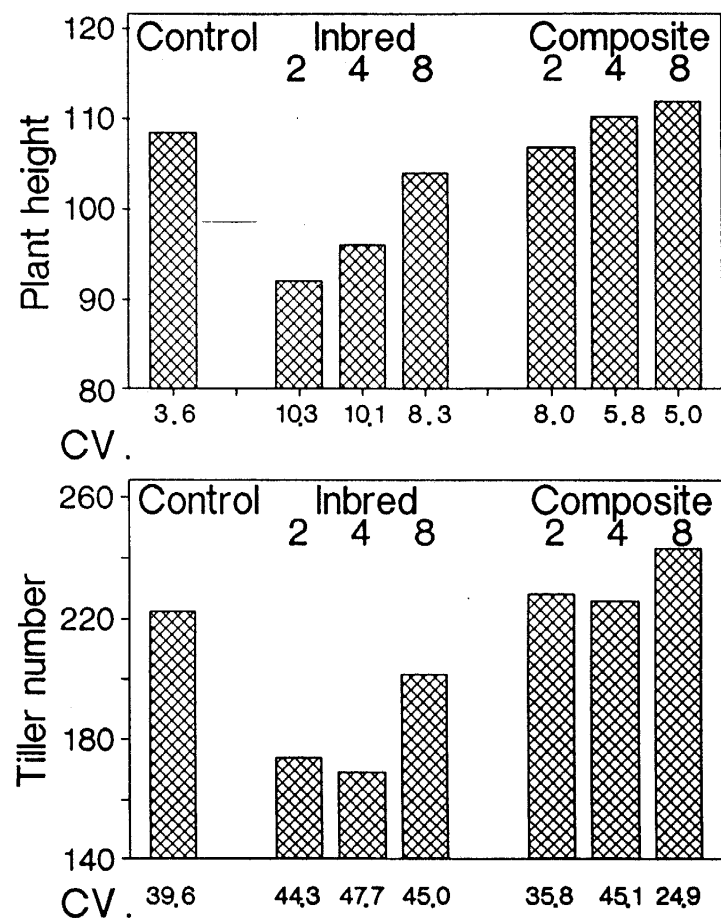


Figure 1.4. The effect of experimental bottlenecks on plant height (cm) and tiller number in *Lolium multiflorum*. Experimental populations of restricted size (2, 4, and 8) were maintained for three generations and then grown in a common garden with a control treatment and composite mixture of genotypes from the inbred treatments. The mean and coefficient of variation for each treatment are illustrated. (After Polans and Allard 1989)

populations expand rapidly following a bottleneck, the high mutability of polygenic characters can generate sufficient variability for rapid evolution into new adaptive zones. Rates of restoration of genetic variability following a bottleneck are orders of magnitude faster for quantitative characters than for heterozygosity at isozyme loci. Spontaneous mutation in metrical traits produces additive genetic variation on the order of 10^{-3} times the environmental variance (σ_e^2) per generation, whereas the usual single-locus rates for major mutants are about 10^{-6} per generation. The high mutation rate of metrical traits follows because many genes contribute to their expression. Because of the high mutability of polygenic characters, even small isolated populations that have lost most of their genetic variability through a bottleneck can generate sufficient

When genetic variation in quantitative characters is the result of additive effects, variation should decrease in proportion to $1/(2N_e)$ after a bottleneck of N_e individuals (Lande 1980). However, if nonadditive effects, such as epistasis and dominance, contribute to genetic variation, then the effects of restrictions in the size of populations will not be a simple function of N . Recent theoretical work by Goodnight (1987, 1988) indicates that additive genetic variance can increase, at least temporarily after a bottleneck, as inbreeding converts nonadditive genetic variation from the donor population to additive genetic variation in the derived population. Few studies, other than those on houseflies (e.g., Bryant et al. 1986a, 1986b), have assessed these theories, and none have been undertaken on plants. Since so little work has been conducted on the quantitative genetics of metrical traits in wild plant populations (reviewed in Mitchell-Olds and Rutledge 1986), it is difficult to evaluate how large the contribution of non-additive gene effects is likely to be for most quantitative traits.

MATING IN SMALL POPULATIONS

The frequency and intensity of inbreeding are often far greater in plants than in most animal groups. A variety of factors account for this difference, including the sessile habit of plants, restricted gene dispersal through pollen and seeds, small population sizes, and high levels of self-fertilization and sib-mating. These factors not only influence the levels and organization of genetic variation in plant populations, but also reduce effective population size, and hence increase inbreeding.

The level of inbreeding (F) in a population increases over time at a rate dependent on the effective population size (N_e) such that $\Delta F = 1/(2N_e)$ per generation (Wright 1922; Falconer 1981). Therefore, populations become inbred more rapidly when they are of small size (Figure 1.5). For very small populations, the influence of size on inbreeding may overwhelm effects brought about by the system of mating alone. Nevertheless, mating patterns are prime determinants of the levels of inbreeding in both large and small populations.

In this section, we begin by discussing the measurement of inbreeding and its relationship to the mating system of plants. We then consider how inbreeding influences offspring fitness by reviewing both theoretical models and empirical data on inbreeding depression in wild plant populations. Finally, we review evidence for the occurrence of outbreeding depression in plant populations and its relationship to mating systems.

Types of Inbreeding in Plants

Plants display a wide variety of breeding systems that differ in their influence on mating patterns and population genetic structure (Richards 1986). The mating parameter with the largest influence on genetic structure is the *selfing rate* (s), the proportion of matings that result from self-fertilization. Although self-incompatible and dioecious species are prevented from self-fertilization, inbreeding may still occur through matings between related individuals. Biparental inbreeding is particularly likely in plants, since nearby individuals are often closely related, owing to restricted gene dispersal.

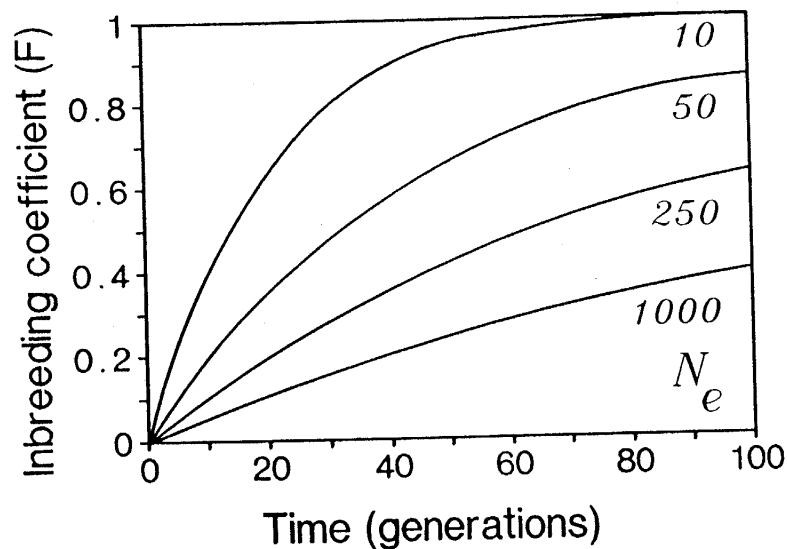


Figure 1.5. Changes in the inbreeding coefficient (F) with time (in generations) for a pair of alleles at a neutral locus in four populations of various sizes. Note the rapid increases in F for populations of small effective size (N_e). (After Parkin 1979)

local environmental conditions. Some species are predominantly selfing, others exhibit mixed mating systems, whereas some are highly outcrossing (reviewed in Schemske and Lande 1985; Barrett and Eckert 1990).

Inbreeding in any form (selfing, sib-mating, etc.) increases homozygosity in a population above levels expected under random mating. Wright (1933, 1977) defined the coefficient of inbreeding (F) as a measure of this increase in homozygosity, resulting from the correlation of uniting gametes. With inbreeding, genotype frequencies in a population for a single locus are

$$(p^2 + pqF) + (2pq - 2pqF) + (q^2 + pqF) = 1$$

Thus Hardy-Weinberg frequencies obtain when $F = 0$, and all individuals are homozygous when $F = 1$. Given genotype and allele frequencies, F is estimated as

$$F = 1 - \frac{H}{2pq}$$

where H is the proportion of heterozygotes. The F referred to here is a within-population measure. The mean of F over all populations is termed F_{is} . The related measure F_v is the amount of species-level deviation from Hardy-Weinberg expectations associated with between-population differences in allele frequencies. Detailed treatments of F statistics and the theory of inbreeding in general are available in Wright (1922, 1977), Fisher (1949), Cockerham (1969, 1973), Malécot (1975), and Weir and Cockerham (1984).

If s is the proportion of self-fertilized progeny, then s and F are related by

$$F' = \frac{s(1+F)}{2-s}$$

where F and F' are the inbreeding coefficients of the parents and progeny before selection, respectively. In the absence of inbreeding depression, an equilibrium value of F is reached when

$$F = \frac{s}{2-s}$$

Measurement of F and s in plant populations has been greatly facilitated by the use of gel electrophoresis and the development of statistical procedures for the analysis of electrophoretic data (reviewed in Brown and Weir 1983; Ritland 1983; Brown 1989c). F is calculated from allele and genotype frequencies, whereas s is estimated from the segregation of marker loci in open-pollinated progeny arrays from maternal parents whose genotypes are either assayed directly or inferred from progeny genotypes. The most commonly employed model to estimate selfing and outcrossing ($t = t - s$) rates is the mixed-mating model (Fyfe and Bailey 1951; Clegg 1980). The model assumes that matings are of two types: self-fertilizations and random matings. In most natural plant populations, however, outcrossing between related individuals will commonly occur. The single-locus selfing rate, s , estimates the homozygosity in progeny that is due to the combined effects of self-fertilization and all forms of biparental inbreeding. Thus s gives a measure of what the selfing rate would be if self-fertilization were the only form of consanguineous mating practiced by a population.

Procedures for estimating selfing rates have been extended to make use of the greater information provided by multilocus data (Ritland and Jain 1981; Shaw et al. 1981). Multilocus models give a more accurate estimate of the true selfing rate because outcrossing events are more reliably detected, even if they are consanguineous. Hence the multilocus estimate of s will often be lower than estimates based on single loci. In self-compatible populations, the difference between the multilocus estimate and the mean of the single-locus estimates of s can be used to assess the degree of biparental inbreeding (Ritland 1984; Brown 1989c).

In plants that cannot self, owing to male sterility or self-incompatibility, single-locus estimates of s can be used to assess the degree of biparental inbreeding. For instance, male-sterile individuals in seven populations of gynodioecious *Bidens* spp. in Hawaii had average "selfing" rates of 15% (range 0–25% [Sun and Ganders 1988]). This is equivalent to saying that the average degree of relatedness between male-steriles and their mates was about that of first cousins. The rate of apparent selfing in self-incompatible *Helianthus annuus* varied from 9% to 46% among populations and years (Ellstrand et al. 1978) and was positively correlated with plant density. Not all studies of outcrossing levels in self-incompatible or sexually dimorphic species have revealed significant values of s (e.g., Ennos 1985a; Barrett and Shore 1987).

Measures of Inbreeding Depression

The major effect on fitness of self-fertilization in plants is *inbreeding depression*, which can be defined as the relative reduction in fitness of selfed offspring compared with outcrossed offspring, or

$$1 - \frac{\text{fitness of selfed offspring}}{\text{fitness of outcrossed offspring}}$$

Inbreeding depression can be measured in plants, as originally undertaken by Darwin (1876), in experiments that compare the relative fitness of progeny obtained from artificial self- and cross-pollinations. A growing body of empirical data (reviewed below) from experiments that have employed this approach is now available for both cultivated and wild plant populations.

The extent of inbreeding depression can also be assessed in natural populations without resorting to experimental studies. One approach recently developed by Ritland (1989, 1990) involves the use of electrophoretic data. If the inbreeding coefficient and the selfing rate are measured in a given generation, and the former is remeasured when progeny reach adulthood, then inbreeding depression is expressed as

$$1 - \frac{(1-s)F'}{(F' - F'') + (1-s)F''}$$

where F' and F'' are the inbreeding coefficients of the progeny generation before and after selection, respectively. This method would be difficult to use for long-lived plants, especially those with overlapping generations, but could be profitably employed in studies of annual species.

An additional method by Ritland (1989, 1990) assumes that the population under study maintains an equilibrium F value in each adult generation. Thus if inbreeding depression is present, selfing will increase F values measured in juveniles (seeds, seedlings) over those values for adults. Selection against selfed progeny then returns the population to equilibrium. In this case, the inbreeding coefficient of the parental generation and its selfing rate are sufficient to estimate inbreeding depression as

$$1 - \frac{2F(1-s)}{s(1-F)}$$

In the absence of electrophoretic information, comparison of the relative performance of hand-selfed and -outcrossed seed with that of open-pollinated seed has been suggested as a way to estimate the selfing rate of populations (Charlesworth 1988). By this approach, the selfing rate is

$$s = \frac{\text{outcrossed performance} - \text{open-pollinated performance}}{\text{outcrossed performance} - \text{selfed performance}}$$

Thus inbreeding depression and the selfing rate can be estimated with electrophoretic data if experimental studies are impractical; alternatively, both can be estimated from field data when electrophoretic techniques are not available.

Inbreeding Depression and Mating Patterns

Theoretical Considerations

Fisher (1941) first pointed out that a selfing individual has an automatic advantage over an outcrossing individual owing to the greater number of genes that it contributes to its own seeds. In a population of outcrossers, each individual, on average, passes

one haploid copy of its genome through each of its own seeds and one such copy each time it fertilizes the seeds of another plant. A variant that can self-fertilize, without losing the ability to donate pollen to others, will pass on two haploid copies of its genome in each selfed seed as well as one copy through pollen donation. This automatic advantage will result in the spread of the variant to fixation if inbreeding depression is <0.5 . If inbreeding depression is >0.5 , complete outcrossing is favored (Lloyd 1979; Lande and Schemske 1985). Since this model gives a quantitative prediction of the amount of inbreeding depression necessary to favor selfing or outcrossing, it has stimulated much empirical work on the relationships between the selfing rate and inbreeding depression in natural populations (see below). The prediction that at equilibrium plant populations should practice either complete selfing or complete outcrossing conflicts with the frequent observation of mixed mating patterns in nature (Schemske and Lande 1985; Aide 1986; Waller 1986; Barrett and Eckert 1990). This discrepancy has motivated theoretical work that attempts to find conditions under which mixed mating systems are stable and how changes in the rate of inbreeding affect the level of inbreeding depression (Campbell 1986; Holsinger 1986, 1988a, 1988b; Uyenoyama 1986; Charlesworth and Charlesworth 1987, 1990).

The most commonly assumed genetic basis for inbreeding depression is the presence in a gene pool of lethal or highly deleterious recessive alleles. In an outcrossing population, these are maintained by the balance between mutation and selection. When an individual self-fertilizes (or mates with a relative), these alleles are often made homozygous, resulting in inbreeding depression. If lethal or highly deleterious recessive alleles are the primary cause of inbreeding depression, even relatively low rates of selfing (e.g., 10–20%) should rapidly purge a population of most of its genetic load (Figure 1.6A; Lande and Schemske 1985; Charlesworth and Charlesworth 1987). Thus, according to this model, populations with a long history of inbreeding would be expected to show little inbreeding depression. Controlled inbreeding to reduce genetic load has been proposed as a conservation strategy for mammalian zoo populations, and a test of this approach in Speke's gazelle did indeed lead to a significant reduction in inbreeding depression in a few generations (Templeton and Read 1984).

If inbreeding depression is modeled as a quantitative trait (Lande and Schemske 1985) or, similarly, as being due to many partially recessive genes, each of small deleterious effect (Charlesworth and Charlesworth 1987), then selfing also reduces genetic load. However, modeled in this way, selfing reduces inbreeding depression much more slowly than under the recessive lethal model, and even under complete selfing, populations may still exhibit significant inbreeding depression (Figure 1.6B).

Finally, if overdominance is the basis of inbreeding depression, selfing can actually increase the severity of inbreeding depression. The reason is that selfing increases homozygosity, whereas random mating always restores Hardy-Weinberg genotype frequencies. Thus the difference between the average heterozygosity of selfed and outcrossed progeny increases with the selfing rate (Charlesworth and Charlesworth 1987, 1990). In the case of balanced overdominance, where each homozygote is equally fit, inbreeding depression will always increase with increased selfing (Figure 1.6C). With asymmetrical overdominance, inbreeding depression increases with the selfing rate until selfing reaches a level too high to maintain the polymorphism. Above this level, one of the alleles is lost and inbreeding depression falls to zero (Figure 1.6D).

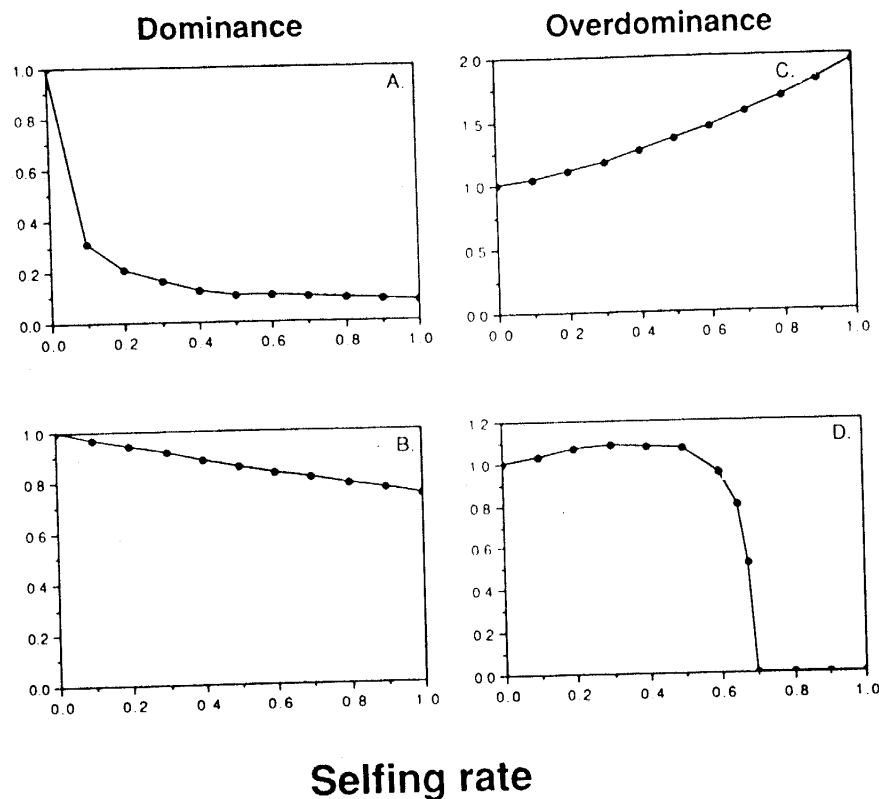


Figure 1.6. Relationships between inbreeding depression and the selfing rate under models assuming different genetic bases for inbreeding depression (redrawn from Charlesworth and Charlesworth 1987). Inbreeding depression values are expressed relative to those of an outcrossing population. The assumed bases of inbreeding depression are: (A) lethal or highly deleterious recessive alleles; (B) many genes, each of small deleterious effect; (C) symmetrical overdominance; (D) asymmetrical overdominance. The shapes of the curves change somewhat with parameter values, but these are representative. See Charlesworth and Charlesworth (1987) for model details.

Empirical Evidence

Prior to the review by Charlesworth and Charlesworth (1987), summaries of work on inbreeding depression in plants were made by Darwin (1876), Crumpacker (1967), and Wright (1977). Many earlier studies were conducted on cultivated species and involved only measurements of yield components. Despite these limitations, the results in general indicated that inbreeding depression usually occurs in outcrossing plants, is less severe in species that are partially self-fertilizing, and may be absent altogether in species that are highly selfing (Wright 1977). During the past decade, more sophisticated work that has involved joint estimates of both the selfing rate and inbreeding depression have provided more extensive data in which to assess the relationships between inbreeding depression and mating systems in natural populations of plants.

Figure 1.7 summarizes recent work on inbreeding depression in wild plant species.

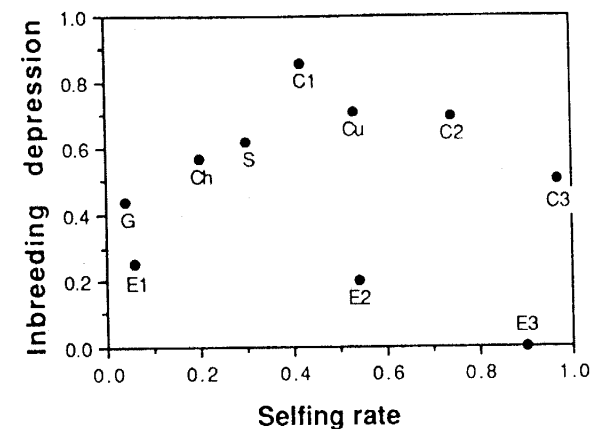


Figure 1.7. Relationship between inbreeding depression and the selfing rate in several species of herbaceous angiosperms. Species are: G = *Gilia achilleifolia* (Schoen 1983); Ch = *Chamaecrista fasciculata* (Fenster 1991a, 1991b); S = *Sabatia angularis* (Dudash 1990, unpubl. data); Cu = *Cucurbita foetidissima* (Kohn 1988, unpubl. data); C = *Clarkia tembloriensis* (Holtsford 1989); E = *Eichhornia paniculata* (P. Ippings and S. C. H. Barrett unpubl. data). For *Clarkia* and *Eichhornia*, data from three populations are plotted. Inbreeding depression for *Clarkia* and *Eichhornia* was measured in the lath house and glasshouse, respectively; all others were measured in the field.

where an attempt has been made to estimate mating system parameters. As can be seen, values of inbreeding depression can be quite high, often exceeding 0.5. The relationship between inbreeding depression and rates of selfing varies considerably. In several cases (e.g., *Clarkia tembloriensis*, *Cucurbita foetidissima*, *Sabatia angularis*), species with moderate to high selfing rates exhibit strong inbreeding depression, indicating that the common perception that these species will suffer little from inbreeding effects (e.g., Templeton and Read 1984) may be largely unfounded. The occurrence of inbreeding depression in species that frequently self-fertilize supports the idea that recessive lethals are not the sole cause of inbreeding depression in nature.

In two studies that examine the relationship between selfing rates and inbreeding depression within species, evidence was obtained indicating the importance of mating patterns in influencing levels of inbreeding depression. In *Eichhornia paniculata*, levels of inbreeding depression for most life-history characters were weak. However, differences in flower production of outcrossed and selfed progeny were largest in tristylous populations with little selfing, smaller in populations with mixed mating systems, and absent altogether from a highly selfing population from Jamaica (Figure 1.8). The relatively low levels of inbreeding depression observed in this study may have occurred because comparisons were conducted under glasshouse conditions (see below) or because natural populations of *E. paniculata* are often small and experience frequent bottlenecks (Figure 1.1; Barrett et al. 1989); these effects reduce genetic load (Lande and Schemske 1985). Studies by Holtsford (1989) on inbreeding depression in three populations of *Clarkia tembloriensis* with different selfing rates revealed strong inbreeding depression for survivorship and fertility in each population (Figure 1.7). The highest levels were found in the most outcrossing population ($t = 0.58$; inbreeding

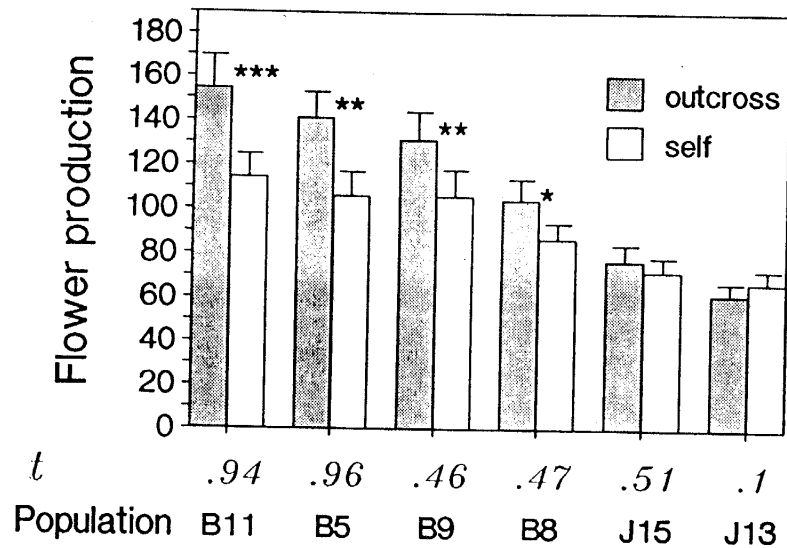


Figure 1.8. Flower production ($\bar{x} \pm 2 SE$) of selfed and outcrossed offspring in populations of *Eichhornia paniculata* with different outcrossing rates (t). Four populations from Brazil (B) and two populations from Jamaica (J) were compared under uniform glasshouse conditions. (From Toppings 1989)

depression = 0.77) and the lowest levels were evident in the most selfing population ($t = 0.03$; inbreeding depression = 0.51). Comparisons were conducted in a lath house, indicating that high levels of inbreeding depression can be detected in some selfing populations, even under artificial conditions.

Levels of inbreeding depression can depend on the environment in which it is measured. In *Sabatia angularis*, for example, values of inbreeding depression increased when comparisons were conducted in the glasshouse, common garden, and field, respectively (Figure 1.9). Similarly, higher levels of inbreeding depression in *Clarkia tembloriensis* were found in garden plots as compared with lath-house conditions (Holtsford 1989). Overall, measures of inbreeding depression in the field tend to be higher than those in glasshouse experiments (Charlesworth and Charlesworth 1987), suggesting that conditions in the field, where plants are exposed to pests, diseases, drought, and competitive interactions, are likely to be more stressful. Kohn (1988) found only 1% survival after one year for seeds of *Cucurbita foetidissima* planted in the field, but outcrossed seeds were three times as likely to establish as selfed seeds. Such low rates of establishment are likely to be common in plants with high fecundity, yet few investigators would tolerate such severe mortality in controlled glasshouse studies, even under stress conditions.

Another common finding of inbreeding depression studies in herbaceous plants is that inbreeding effects are often manifest most strongly late in the plants' life cycle. Studies in which a multiplicative fitness function (e.g., seed set \times germination \times survival \times flower or fruit production) was employed to compare selfed and outcrossed progeny (e.g., Toppings 1989; Dudash 1990; Fenster 1991b) found the largest relative differences in flower or fruit production. Schoen (1983) and Kohn (1988) found small

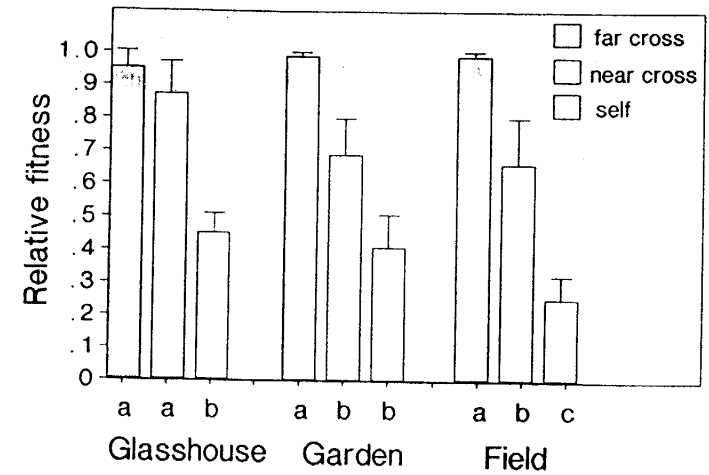


Figure 1.9. Relative fitness of offspring of the herbaceous biennial *Sabatia angularis* resulting from hand self-pollinations, cross-pollinations within a subpopulation (near cross), and cross-pollinations between subpopulations (far cross) measured under glasshouse, garden, and field conditions. Seed set did not differ among pollination types. All seeds were germinated in the glasshouse and transplanted as seedlings into the appropriate environment. Relative fitness ($\pm 2 SE$) was estimated from the product of germination, survival, and seed set (see Dudash [1990] for details).

differences between selfed and outcrossed seeds in their ability to germinate in the field, but strong effects on the probability of postestablishment survival. These findings support the idea that in these groups, inbreeding depression does not result solely from a few genes with major effects. Rather than being highly dysfunctional, most selfed offspring that germinate appear to be somewhat less vigorous than outcrossed offspring. This small effect, however, becomes compounded during growth and reproduction and can ultimately result in large reductions in fitness. On the other hand, differences in seed set following selfing and outcrossing suggest that inbreeding depression is commonly expressed during embryo development in certain plants, particularly woody groups such as the gymnosperms (Charlesworth and Charlesworth 1987). Thus inbreeding depression may result from the combined effects of a few lethal recessive genes, and perhaps many other genes of small effect. As yet there are no genetic models of inbreeding depression that take into account a mixture of causes.

Outbreeding Depression

Outbreeding depression usually refers to the reduction in fitness that occurs following intraspecific hybridization between individuals from spatially separated genetic sources (demes, subpopulations, populations). Outbreeding depression may occur when populations are adapted to local environmental conditions and hybrids are less fit in either location, or when combinations of genes are *intrinsically coadapted*; that is, they are selected to perform well in the internal genetic environment defined by other interacting genes in the population (Toppings 1989; Weller

different populations are crossed, the coadapted complexes of genes are broken apart, particularly in later generations, and most new hybrid combinations perform poorly (Templeton et al. 1986).

The genetic and mating structure of plant populations is particularly favorable for the development of locally adapted races as well as intrinsic coadaptation. Subpopulation structure, small effective population size, limited gene dispersal, and inbreeding favor restricted recombination and facilitate the evolution of locally adapted gene complexes (Levin 1978). A considerable literature beginning with the genecological studies of Turesson (1922) and Clausen et al. (1940) documents examples of local adaptation by the use of transplant studies, and, in some cases, genetic differentiation has been demonstrated over distances of a few meters in response to small-scale changes in soil conditions (Snaydon 1962; Snaydon and Davies 1976), local competitors (Turkington and Harper 1979), and pathogens (Parker 1985). These considerations would suggest that many plant species would be likely candidates to exhibit outbreeding depression when crosses between plants from different locations are made. However, the limited data currently available provide no clear answer to this question.

Price and Waser (1979) suggested that because of limited gene dispersal and local environmental heterogeneity there would be an optimal outcrossing distance for many plant species. Crosses between near neighbors would suffer from inbreeding depression because the plants would likely be related, whereas crosses from distant plants would suffer from outbreeding depression through the disruption of favorable gene complexes. Thus crosses at some intermediate distance would result in the fittest offspring. Experimental field studies of *Delphinium nelsonii* and *Ipomopsis aggregata* have confirmed some of these predictions. Measurements of pollen and seed dispersal were consistent with the idea that neighborhood areas for both species were small, since dispersal distances were mostly less than a meter. Local adaptation would thus be favored because of restricted gene flow and environmental heterogeneity. Evidence for local adaptation was obtained for both species by using reciprocal transplants of seeds in the case of *D. nelsonii* (Waser and Price 1985), or by planting seeds near and distant from their maternal parent in the case of *I. aggregata* (Waser and Price 1989). In both species, crosses between plants from intermediate distances (3–10 m) set more seed than crosses between nearby (1 m) or more distant (100–1000 m) plants (Waser and Price 1983, 1989). Furthermore, seeds produced by crosses between plants separated by 10 m performed better than seeds from crosses between plants occurring in close proximity to each other, or from more distant pairs of plants (Waser et al. 1987; Waser and Price 1989). In *D. nelsonii*, pollen tube growth rates are apparently faster for pollen grains from plants at the optimal distance (10 m) than for pollen from 1 or 100 m. This raises the possibility that pollen from the optimal distance may preferentially fertilize ovules when in competition with pollen from other distances.

Most other experimental studies in which investigators have conducted crosses between plants at different spatial scales have failed to detect the occurrence of an optimal outcrossing distance or evidence for outbreeding depression (see Table 9 in Sobrevilla 1988; Newport 1989). In *Chamaecrista fasciculata*, seed set and the progeny performance of field transplants increased with interparent distance and did not decline

neighborhood areas were estimated to have a radius of 3.1 m (Fenster 1991a, 1991b). Studies of pollen tube growth in crosses involving maternal and paternal parents of *C. fasciculata* from different spatial scales also failed to detect any location effects (Fenster and Sork 1988). Similarly, in *Sabatia angularis*, the total relative fitness of progeny transplanted back into the field was higher for between-subpopulation crosses than within-subpopulation crosses (Dudash 1990). In *Phlox drummondii*, seed set increased and levels of abortion decreased with interparent distances up to 200 m (Levin 1984), whereas in *Espeletia*, distance showed no clear effect on patterns of seed set (Sobrevilla 1988).

The complex relationships between proximity and fitness in plants are exemplified by studies of *Mimulus guttatus* by Ritland and Ganders (1987a, 1987b). Different fitness components showed varying relationships with distance, depending on the local population genetic structure and degree of relatedness of colonies. The variable responses they observed indicate some of the difficulties in predicting whether outbreeding depression is likely to occur in particular populations or species. Many factors—including the magnitude and scale of local selection pressures, the extent of genetic drift, and the mating system—will influence genetic differentiation; thus the lack of consistent results is not particularly surprising. Since all species mentioned above are animal pollinated and primarily outcrossing, it appears, however, that the mating system alone does not reliably predict whether outbreeding depression will occur.

Few data are available bearing on outbreeding depression in predominantly selfing species (but see Svensson 1988). In a study of *Impatiens pallida*, D. W. Schemske (pers. commun.) made crosses using pollen donors from within the same transect (0–18 m distance) and donors from a second transect (>32 m distance). He then planted these two types of seeds, and selfed seeds from cleistogamous flowers, back into the shaded habitat of maternal plants, as well as into a treefall gap some 500 m away. In the maternal environment, there were no fitness differences among the three seed types. In the treefall, however, the relative fitness of seeds from the most distant crosses was 60% higher than for either of the other seed types. Thus neither inbreeding nor outbreeding depression was evident when seeds were planted in their maternal environment, but wide crosses appeared to provide an advantage for exploitation of a novel environment.

The degree of genetic divergence among populations of some selfing species can complicate attempts to predict the outcome of interpopulation crosses. This problem was encountered in a study of the tropical disjunct *Eichhornia paradoxa* (Cole and Barrett 1989). Fewer than a dozen isolated collections of this rare species have been made, ranging from Guatemala to southern Brazil. Two morphologically differentiated populations from northeastern Brazil located some 500 km apart were artificially hybridized, resulting in F_1 progenies with a high degree of heterosis for vegetative traits. The hybrids were highly sterile, however, producing no viable pollen or seeds. Electrophoretic analysis of the two populations revealed that they were nearly uniform, but fixed for alternative alleles at 14 of 27 loci screened. Hence, the genetic identity of populations was 0.657, a value normally associated with congeneric species. Clearly, the two populations are distinct biological species, raising the difficult issue of taxonomic delimitation in highly selfing groups and the problem of how to sample variation

IMPLICATIONS FOR CONSERVATION

Despite strong genetic foundations to conservation practices employed for domesticated plants and their wild relatives (e.g., Frankel and Bennett 1970; Frankel and Hawkes 1975), the role of population genetics in rare plant conservation has only recently been appreciated. Most management practices have been directed toward habitat preservation, particularly understanding the demographic features of endangered species, such as the size of local populations and their growth rates. However, if the long-term survival of rare plant populations is to be considered, strategies that maintain genetic variation must be attempted. Fortunately, since the demography and genetics of populations are not independent properties, management practices that aim to maintain large population sizes will tend to conserve genetic diversity. Loss of genetic variation through stochastic forces and the deleterious effects of inbreeding in small populations are the major threats that compromise attempts to maintain the long-term viability of populations of rare plant species.

Where habitats are in immediate danger of destruction, the collection from the wild of plant material (e.g., seeds, clonal fragments) and its maintenance in botanical gardens become necessary. Here the input from genetics becomes even more important both at the sampling stage (Brown and Briggs, Chapter 7) and where attempts are made at controlled breeding. If manipulation of genetic resources is considered important to enhance the chances of survival of an endangered species, this should be undertaken only after some understanding of the breeding biology and population genetic structure has been obtained. The availability at botanical gardens of glasshouses and other facilities, where studies of plant reproductive biology can be conducted, should enable basic information to be obtained on the breeding systems of rare plants. Data on population genetic structure require more sophisticated approaches, although the use of electrophoretic methods and various molecular techniques is now routinely employed in many studies in systematics and population biology. Collaboration with university research departments, or the installation in botanical gardens, of laboratory facilities equipped with biochemical and molecular tools, would greatly assist the monitoring of genetic variation in rare plant species.

The plant conservation biologist faces a series of decisions when developing a plan for the preservation of a species *ex situ*, or for its reestablishment into the wild. It is of importance to determine to what extent local populations are genetically differentiated, whether such differences have adaptive value, and whether the mixing of gene pools from different populations will increase or decrease successful establishment and long-term survival. There is abundant evidence that most plant populations are genetically differentiated from one another (Antonovics 1976). However, population biologists are far from being able to make general predictions on whether such differences will be of adaptive value under new environmental conditions, or how novel genetic combinations are likely to fare in nature. Because of our overall ignorance of the population biology and genetics of rare plants, it seems likely that conservation biology will be a "learn as we go" endeavor. We therefore advocate the use of experimental approaches to speed up the learning process. We believe that conservation plans that use experimental approaches are more likely to succeed and will increase our overall

In addition, work of this type is more likely to increase potential funding sources for conservation over that which would be obtained from descriptive studies and the simple monitoring of rare plant populations.

For instance, if a few small populations of a rare plant species remain, and the goal is to reestablish new wild populations in sites that are conserved, experimental approaches are required. One might establish pure samples from extant populations, as well as composite mixtures with greater genetic variation, even at the expense of breaking up coadapted gene complexes. Despite considerable theoretical work on the relationship between environmental heterogeneity and genetic variability, few experiments with plants have investigated whether colonization and persistence are related to genetic variability (Martins and Jain 1979; Bell 1985). We have little knowledge of whether genotypes found in local environments are finely adapted or whether they represent chance combinations of genes resulting from founder effects and genetic drift. Well-planned experimental reintroductions could increase the likelihood of success, particularly if multiple seed stocks are used. Such experiments would require only that hybrids be produced and that different seed sources be involved. Experiments of this type could encourage greater interchange of views between conservation biologists and experimental population biologists, and may potentially attract funds not normally directed at either discipline.

A recent conservation program in Illinois illustrates how experimental approaches may be required to enable reintroduction of rare plants into the wild (De Mauro 1989 and pers. commun.). The last two remaining populations of *Hymenoxys acaulis* var. *glabra* were recently extirpated from the state as a result of habitat destruction. Although material was collected from the last remaining population of 30 plants, no seeds could be synthesized because of strong self-incompatibility and the fact that all plants were apparently of the same mating group. However, controlled crosses between Illinois plants and material from the only known population in Ohio resulted in abundant viable seed, owing to the presence of different mating groups in the Ohio population. The hybrid seed has been used in experimental plantings in Illinois in an effort to reestablish populations in the state. Although, in this case, interpopulation hybridization was undertaken out of necessity in order to restore fertility to the Illinois material, carefully controlled studies, similar to those conducted by Templeton and associates in Missouri Ozark populations of collared lizards (Templeton 1986), would be likely to provide valuable information on the extent of local adaptation in rare plants, as well as furthering conservation efforts.

Genetic manipulations of rare plants will obviously lead to the disruption of locally adapted gene combinations. This may be of concern when local races of a species are morphologically or ecologically distinct and are recognized taxonomically. In some cases, the attempt to preserve population differentiation may conflict with practices aimed at species preservation. Such cases require individual judgment, but we believe that, in the long-term, species conservation is, in general, of greater importance. Attempts to preserve population distinctiveness should be undertaken only when they do not endanger species conservation. Should reestablishment practices be successful at a number of sites, population differentiation will almost inevitably follow.

It should be clear from the evidence presented above that knowledge of a species' selfing rate or inbreeding coefficient does not necessarily allow us to predict with accuracy the level of inbreeding depression that it is likely to suffer. Thus inbreeding

depression might occur even in species with a history of inbreeding. In addition, the level of inbreeding depression will usually depend on the environment in which it is assessed. Thus although little or no inbreeding depression may be evident when a plant is grown under the benign conditions of a glasshouse or botanical garden, its effects may be more severe when seeds are reintroduced back into the wild. Seeds produced under uncompetitive conditions may have lower "ecological value" than their numbers might suggest. Small samples of rare plants maintained in botanical gardens may be inbred and of inferior genetic quality, complicating attempts at reintroduction. Thus conservation of wild plants in botanical gardens may lead to unconscious "domestication," particularly if many sexual generations under benign conditions are allowed to occur. Occasionally challenging seed stocks to natural or seminatural conditions could aid in assessing the durability of species to the rigors of nature, even at the expense of losing some genetic diversity. A major challenge of *ex situ* conservation will be to ensure that sexually propagated samples of rare plants do not become museum specimens incapable of surviving under natural conditions.

ACKNOWLEDGMENTS

We thank Deborah Charlesworth, Michele Dudash, Marcy De Mauro, Charles Fenster, Tim Holtsford, Brian Husband, Doug Schemske, and Peter Toppings for providing unpublished data and manuscripts. Brian Husband and Bill Cole helped with the figures. The manuscript was prepared while S.C.H.B. was a recipient of an E.W.R. Steacie Memorial Fellowship funded by the Natural Sciences and Engineering Research Council of Canada, and J.R.K. was supported by an NSERC of Canada Postdoctoral Fellowship.