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Genetic considerations for plant population restoration and conservation

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Introduction

Successful restoration policy involves three basic criteria. First, sufficient habitat must be protected for the continued persistence of a species (Gilpin & Soulé 1986). Second, demographic information must be collected to determine which life history stages are most critical to survival, reproduction, and long-term population vigor (Marcot & Holthausen 1987, Lande 1988). Third, once these fundamental criteria for population survival are met, genetic variation can be considered as an issue in restoration and conservation policy. Overall, we believe that genetic issues may be more pertinent to population restoration than to population conservation. In attempting to conserve taxa, one is initially interested in saving numbers of individuals regardless of their relatedness. Given that natural areas managers will have the opportunity to reintroduce populations that have been extirpated in nature, it seems reasonable that any genetic manipulations that may help restore a population's vigor *in situ* for the short or long term may be beneficial.

Rare and endangered taxa often exist as a few relatively small populations (Holsinger & Gottlieb 1989) subject to population bottlenecks. Thus, genetic drift and mating among relatives contributes to the loss of genetic variation and reduction in the population's overall vigor through inbreeding depression (Lacy 1987, Polans & Allard 1989). A short-term conservation goal should be to ensure that the vigor of a population is maintained or restored in the face of inbreeding by appropriate manipulation of the remaining genetic variation (Ledig 1986). Little is known of the consequences of mixing distant gene pools in natural populations in order to increase genetic variation (Templeton 1986). A long-term goal of any conservation or restoration policy must be to preserve the evolutionary potential of a species by maintaining its genetic variation either by partitioning genetic variation among individuals within a population or by partitioning genetic variation between populations (Riggs 1990). In the

past few decades there has been an explosion of techniques to document genetic variation at the molecular level (Cohn 1990). There is a need to synthesize information gathered from molecular studies with patterns of morphological variation to determine the role of molecular markers as indicators of important subsets of genetic variation.

In contrast to animals, many plant species are asexual, and upwards of 20% of all plant species are primarily selfing; the rest exhibit varying degrees of outcrossing (Fryxell 1957, Jain 1976). This great range in mating systems may dictate alternative restoration strategies.

The primary objective of this chapter is to outline experiments to address genetic issues in plant population restoration and conservation using common species as *model systems*. This approach allows us to test the consequences of genetic manipulation to restore or maintain population vigor, as well as to determine appropriate techniques for identifying important subsets of genetic variation. General principles learned from common species may be applied to the restoration and conservation of endangered taxa. This chapter focuses on methods to restore a population's vigor following extreme inbreeding by determining 1) the genetic basis of inbreeding depression in natural populations, 2) whether it is possible to purge a population of inbreeding depression with a selected breeding program, 3) the consequences of mixing distant gene pools (i.e. outbreeding depression), and 4) whether the genetic variation of our remaining source populations influences their potential colonizing ability. In addition, methods are described to identify important subsets of genetic variation using phylogenetic approaches based on molecular data. We propose to complement the molecular phylogenetic approach with quantitative genetic techniques to assess whether different lineages within a species have different evolutionary potentials. The relevance of mating system is addressed for each of the issues raised above.

Genetic manipulation to restore population vigor

Inbreeding depression

Inbreeding depression occurs when selfing or mating between relatives results in a general decline in the vigor of the resulting progeny. Deterioration of the gene pool through inbreeding has been documented in both crops (e.g. Neal 1935, Wright 1977) and natural populations (e.g. Schemske 1983, Schoen 1983, Ritland & Ganders 1987, Dudash 1990, Fenster 1991b). Detection of inbreeding depression is often dependent upon the environment in which it is examined (Dudash 1990). Progeny of *Sabatia angularis* grown in benign (i.e. greenhouse and garden) environments exhibited less inbreeding depression

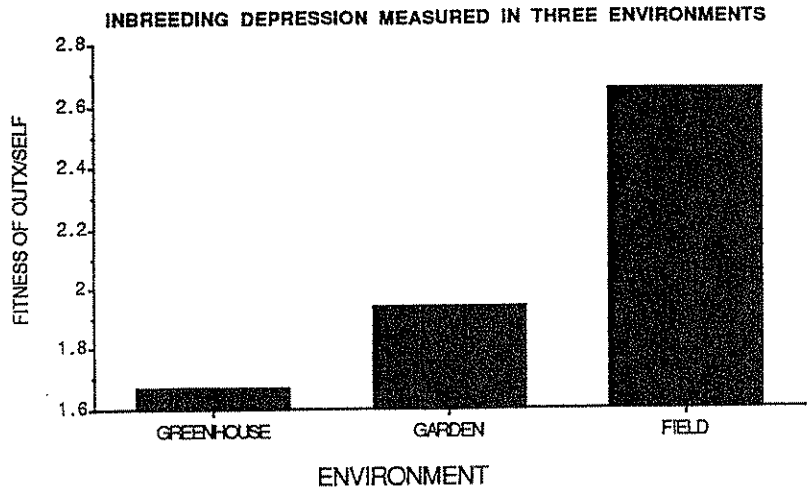

Fig. 2.1. Relative fitness of outcrossed progeny to selfed progeny of *Sabatia angularis* grown in the greenhouse, garden, and field (from Dudash 1990).

than progeny grown in the natural field habitat (Fig. 2.1; Dudash 1990). Thus, studies contrasting performance of inbred and outbred progeny in the greenhouse (e.g. Schemske 1983, Waller 1984, Mitchell-Olds & Waller 1985, Sakai, Karoly & Weller 1989) are probably conservative estimates of the effects of inbreeding depression. Many studies of progeny performance (i.e. fitness) have not been conducted throughout the entire life cycle of plants. A more robust estimate of progeny performance can be attained if it is assessed at as many points in the life cycle as possible. Although annual plants can be followed throughout their entire life cycle (i.e. seed to seed), many endangered species have longer lifespans, and thus cannot be studied at this level of completeness. Genetic manipulations of populations of long-lived organisms will require decisions about how to quantify and measure the resulting progeny's fitness. Ritland (1990) has developed a method to estimate inbreeding depression from electrophoretic data alone that may be helpful in assessing inbreeding depression for long-lived organisms. Although inbreeding depression is a generally accepted phenomenon in crops (Simmonds 1981, Hallauer & Miranda 1985), there are still only a few published studies that clearly document its importance in natural plant populations measured *in situ* (e.g. Schoen 1983, Kohn 1988, Dudash 1990, Fenster 1991b). These studies document only the short-term (one generation) consequences of extreme inbreeding. As we discuss below, the genetic basis of inbreeding depression, i.e. the role of dominance vs. overdominance in heterosis and epistasis in inbreeding depression, will determine the long-term consequences of inbreeding.

Note that limited gene flow resulting in population structure will cause an eventual loss of heterozygosity and increase in inbreeding (Crow & Kimura 1970). Most plant populations have considerable population subdivision (Levin 1981, Loveless & Hamrick 1984). Thus, even if random mating occurs within the limits of realized gene flow, there will be considerable mating among relatives. Consequently, it may be common for progeny from random mating within populations to incur a cost of inbreeding depression compared with longer-distance crosses (Fenster 1991b).

Genetic basis of inbreeding depression

Two models of the genetic basis of inbreeding depression are reviewed by Charlesworth & Charlesworth (1987). Inbreeding depression is due to 1) the expression of recessive or partly recessive deleterious alleles expressed in the homozygous state (partial dominance model), or 2) heterozygote superiority in which the heterozygote is more fit than either homozygote (overdominance model). These two mechanisms are not mutually exclusive, and they may occur at the level of a single locus or many loci, complicating the empirical determination of the underlying genetic mechanisms of inbreeding depression (Moll, Lindsey & Robinson 1964). Several studies using crop species have suggested that the expression of recessive deleterious alleles explains the observed inbreeding depression better than does overdominance (Comstock & Robinson 1948, Robinson, Comstock & Harvey 1949, Gardner 1962, Moll *et al.* 1964). As we demonstrate below, determining the genetic basis of inbreeding depression in natural populations facilitates the success of a program designed to rid a population of inbreeding depression.

One approach to determining the genetic basis of inbreeding depression has recently been summarized by Bulmer (1985). First, inbred lines must be generated from as many families within a population as possible, because the family attrition rate may be high (80–95%) following enforced inbreeding (Frankel & Soulé 1981). Plant breeders commonly refer to inbred lines as those having undergone five generations of selfing (Simmonds 1981). This breeding program would result in a 32-fold reduction of heterozygosity. Once inbred lines are generated, a diallel crossing program is used to generate F1, F2, and backcross progeny that eventually are grown together to assess the consequences of the crossing program and calculate the degree of dominance expressed. This approach cannot account for epistasis and linkage, which must be assessed independently (see Bulmer 1985).

Selective purging of inbreeding depression

The genetic basis of inbreeding depression has broad implications for restoration policy. If dominance is responsible for inbreeding depression, then the

genetic load can be purged through a controlled breeding program. However, if overdominance is the cause, a breeding program will not as readily rid a population of inbreeding depression. In either case, if the traits responsible for the expression of inbreeding depression are polygenic, they will not respond to artificial selection as rapidly as will traits controlled by a few genes (Lande & Schemske 1985).

Thorough studies of non-crop species are needed to determine whether enforced inbreeding can reduce inbreeding depression by selecting for vigorous inbred lines in plants. Templeton & Read (1984) were able to reduce the inbreeding depression of a captive population of Speke's gazelle using a controlled inbreeding program. However, they have not yet tried to reintroduce Speke's gazelle from this program into the wild. Testing the relative vigor of inbred lines selected for high fitness under natural conditions should be easier for plants than for animals, and may become a paradigm for both animal and plant population restorations.

To assess the possibility of purging a population of inbreeding depression, the performance of inbred lines of plants selected for high vigor could be compared with that of progeny from open-pollinated seed in native and novel habitats. In a glasshouse experiment on a highly outcrossing population of tristylous *Eichhornia paniculata*, Barrett & Charlesworth (1991) demonstrated that enforced selfing purged the population of deleterious recessive alleles. No additional decline in fitness resulted from five generations of selfing compared with one generation of selfing. Additionally, when inbred lines were crossed, they resulted in progeny of superior fitness compared with the original outbred parental population. Thus, during the five generations of selfing there appears to have been selection against different homozygous alleles among the inbred families, resulting in greater heterozygosity among the intercrossed lines compared with the base population. Their empirical and theoretical results are in agreement with a partial dominance- based model of inbreeding depression.

Populations exposed to bottlenecks frequently experience a reduction in the mean of quantitative characters due to inbreeding depression (Bryant, McCommas & Combs 1986; Polans & Allard 1989). It might be expected that populations exposed to frequent bottlenecks would be purged of inbreeding depression due to mating with close relatives (Lande & Schemske 1985). Brewer *et al.* (1990) found that peripheral populations of *Peromyscus* mice had lower genetic diversity than central populations, suggesting that they had been exposed to bottlenecks. However, no correlation was observed between genetic diversity in mice populations and the degree of inbreeding depression, suggesting a role for overdominance in the observed heterosis of this obligatory outcrossing species. In contrast, as discussed below, there is frequently a correlation between the degree of selfing and inbreeding depression, with self-

ers generally experiencing far less heterosis than outcrossers when individuals are outcrossed.

Mating system and inbreeding depression

Inbreeding depression is expected to be greater in outcrossing than in selfing populations, because there is a greater opportunity to purge deleterious alleles (Wright 1977, Falconer 1981). Recent theoretical considerations of mating system evolution (Charlesworth & Charlesworth 1979, 1987, 1990, Holsinger 1988, Charlesworth, Morgan & Charlesworth 1990) have made clear predictions regarding changes in inbreeding depression with changes in selfing rates, but the expectations differ depending on the genetic basis of inbreeding depression. If heterosis is dominance-based, deleterious recessive alleles are expected to be absent in selfers. In contrast, selfers may exhibit heterosis if there is heterozygote advantage due to overdominance.

Inbreeding depression has been examined in primarily selfing species. but either the magnitude is much less (Wright 1977 and references therein, Svensson 1988, Griffing 1989, Holtsford & Ellstrand 1990) than that of primarily outcrossing species, or inbreeding depression is not detected (Levin 1989) compared with outcrossing species. These empirical and theoretical results suggest that the maintenance of high levels of heterozygosity and avoidance of inbreeding depression may not be important issues in selfing species. However, because much of the observation of inbreeding depression in selfers has been confined to crop species, there is a need to examine the role of contrasting mating system on levels of inbreeding depression in natural populations of congeners.

Outbreeding depression

Outbreeding depression occurs when the mating of individuals from distant source populations results in progeny having reduced vigor. This phenomenon can be observed in the F1, F2, backcross, or later generations of crosses between gene pools. The consequences of backcrosses are especially relevant when F1s are introduced into areas where the parental gene pool is still extant, as in the case of the restoration of Mead's milkweed (M. Bowles, personal communication). The consequences of mixing distant gene pools will depend on both the mode of gene action and the selective agents responsible for adaptive differentiation. Thus, it is important to have an understanding of the genetic basis and selective agents of outbreeding depression. In addition, we need to know at what spatial scale crosses between gene pools result in outbreeding depression, and the magnitude of hybrid breakdown.

Causes of outbreeding depression

The genetic basis of outbreeding depression may be associated with two modes of gene action. First, outbreeding depression may be a consequence of alleles selected for their individual effect, and the hybrid offspring are adapted to neither parental environment. An example may be the observation that when hybrids between species are formed, they commonly occur in habitats intermediate to those of the two parents, but cannot survive in either parental environment (Stebbins 1977). Another mechanism for outbreeding depression is the breakup of coadapted gene complexes (*sensu* Wright 1969), resulting from epistatic interaction in fitness of alleles across loci, or genes selected for their joint effects on fitness (Falconer 1981). This is most likely observed in the F2 generation, because additional opportunities for recombination occur during meiosis of the F1, and there is loss of heterozygosity in the F2 compared with the F1 (Falconer 1981, Lynch 1991). Both types of gene action may contribute to outbreeding depression, but to date we have little empirical evidence indicating the genetic basis of outbreeding depression. However, whether outbreeding depression is the consequence of the breakdown of epistatic or coadapted gene complexes has important implications for restoration following the mixing of gene pools. If genes have been selected for their additive effect, it is much more likely that heterosis following long-distance crosses will balance or outweigh any outbreeding depression. If populations are locally adapted through the evolution of coadapted gene complexes, then progeny vigor following the mixing of gene pools may be greatly suppressed.

Templeton (1986) reviews two major selective agents that may result in the expression of outbreeding depression when gene pools are mixed. First, hybrid offspring may have lower fitness when the parental populations have differentiated to local environmental selection pressures, resulting in ecotypic differentiation (Gregor 1930, 1938; Turreson 1922). Note that the role of epistasis in the formation of coadapted gene complexes associated with ecotypic differentiation is largely untested. Under the combined influence of drift and selection, different populations may have different genetic solutions to the same selective pressures (Cohan 1984). Although populations may appear to be phenotypically similar, crosses among them may lead to a breakdown of coadapted gene complexes if there are epistatic interactions on fitness. Second, coadapted gene complexes may confer intrinsic adaptation, where genes adapt primarily to the genetic environment defined by other genes, as in cases in which populations of the same species may exhibit different chromosomal races (Templeton, Sing & Brokaw 1976; Cicmanec & Campbell 1977) or in which modifiers appear to have evolved to ameliorate the genetic load specific to a population (reviewed in Dobzhansky 1970). Coadapted gene complexes may

include both cytoplasmic (maternally inherited) and nuclear genes. Cytoplasmically inherited genes are known to have large effects on plant traits (Grant 1975, Roach & Wulff 1987). Important interactions between nuclear and cytoplasmic genes have been documented, e.g. male sterility (Meagher 1988) where nuclear genes act on particular cytoplasmic sterility genes to restore pollen production (reviewed in Frank 1989). Crosses between species and subspecies often result in an increasing role for maternal inheritance compared with crosses between more related taxa (Wright 1968). Presumably, these examples demonstrate that many traits are an expression of coadapted nuclear and non-nuclear genomes.

Scale for detection of outbreeding depression

Although there is often a genetic basis for striking morphological differences among plant populations, there are often no postzygotic reproductive isolating mechanisms associated with these differences (see Clausen & Heisey 1958). Therefore, plants that are genetically and phenotypically distinct may be crossed to produce viable F1 progeny. Outbreeding depression has historically been associated with crossing events of very long distances at the level of populations, races, or subspecies (e.g. Kruckenberg 1957, Dobzhansky 1970, Sobrevila 1988).

The experiments with maize by Moll *et al.* (1965) demonstrated a classic case in which heterosis does not increase uniformly with genetic divergence. Moll *et al. a priori* postulated a relative rank of divergence among different maize cultivars based on probable ancestry, geographical separation, and adaptation to different environments. Crosses were performed between cultivars from four regions of North America (southeast USA, midwest USA, Puerto Rico, and Mexico). They observed an outcrossing depression in progeny performance in traits correlated with fecundity, while still detecting heterosis among all of the long-distance crosses (see Fig. 2.2 for details). It may be insufficient to examine the fitness of F1 progeny resulting from crosses between genetically divergent or geographically separated populations. Outbreeding depression due to placing genes with epistatic effects on fitness in different genetic backgrounds may occur to a greater extent in the F2 progeny (Fig. 2.2), because recombination in the F1 would lead to a greater breakdown of coadapted gene complexes. By contrasting F1 and F2 fitness, Moll *et al.* (1965) were able to examine the role of coadapted gene complexes in the success of cultivars in their native environments. In the absence of epistasis, the F2 is expected to decline in fitness or yield to half the difference between the F1 and average of the two parents (midparents, or MP), or $F2 = (F1 + MP)/2$ (Wright 1968, Falconer 1981). This is because the heterozygosity of the F2 is

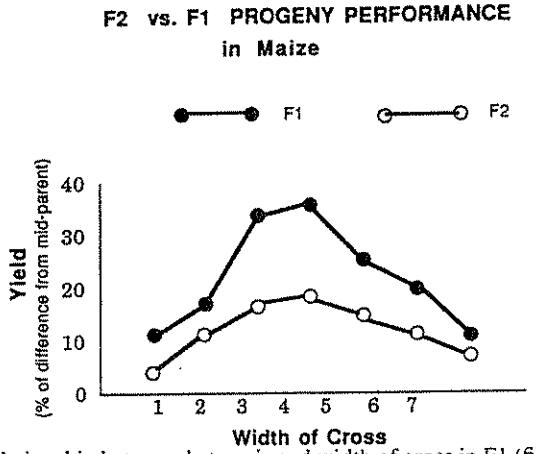

Fig. 2.2. The relationship between heterosis and width of cross in F1 (filled circles) and F2 (open circles) progeny of maize. The heterosis is expressed as the percentage of difference from the midparent. Width 1 = crosses within a region, width 2 = southeast × midwest, width 3 = southeast × Puerto Rico, width 4 = midwest × Puerto Rico, width 5 = southeast × Mexico, width 6 = midwest × Mexico, and width 7 = Puerto Rico × Mexico (from Moll *et al.* 1965).

expected to be half that of the F1, and thus the F2 would have half the expression of heterosis. Note that with F3 and later generations, heterozygosity should be maintained at half the level of the F1 if there is random mating. Deviations of the F2 from (F1 + MP)/2 will be due to additive × additive and dominant × dominant epistasis (Lynch 1991). Because the F2 were nearly intermediate between the F1 and midparents in yield, there is little evidence for a role of epistasis for fitness or coadaptation for the ecotypic differentiation observed among the maize cultivars. At all crossing distances except the lowest, the F2 were slightly higher in fitness than (F1 + MP)/2, suggesting that less-related varieties of maize harbored loci that acted favorably in combination. This is contrary to the expectation that local populations consist of coadapted gene complexes (Lynch 1991). Instead, the observed optimum in yield for the F1 and F2 at intermediate crossing distances suggest that a balance between heterosis and non-epistatic hybrid breakdown has been reached. Non-epistatic hybrid breakdown refers to the phenomenon in which alleles are selected for their individual additive effects and hybrid offspring are adapted to neither parental environment. It is important to emphasize that only by creating F2 or backcrosses can one partition the effects of dominance vs. epistatic interactions. Tight linkage among epistatically interacting loci will minimize F2 breakdown. However, a comparison of the increased amount of epistatic breakdown observed in the F3 vs. the F2 will allow a more precise estimate of the formation of coadapted gene combinations. Also note in the maize example

that even with a decrease in heterosis at all widths of crosses, all of the F2 progeny were still experiencing a degree of heterosis compared with their mid-parents. *Thus, even if we have no other choice but to mix distant gene pools, heterosis may outweigh any deleterious consequences of breaking up co-adapted gene complexes.*

Unfortunately, the experimental evidence of scale-dependent heterosis and outbreeding depression carried through to the F2 generation is largely confined to organisms (such as maize) that have been the objects of artificial selection acting on additive genes. It is much more time-efficient to artificially select on genes with additive effects. Crosses between divergent lines, cultivars, or populations would not be expected to exhibit a breakdown of coadapted gene complexes if selection was not acting on interacting genes. We are more likely to observe the breakdown of coadapted gene complexes in crosses among natural populations, in which selection has had the opportunity to act on both the additive and interactive effects of genes. A greater proportional decrease in F2 progeny vigor than expected based on inbreeding depression alone was observed in laboratory populations of some *Drosophila* species (Brnic 1954, Wallace & Vetukhiv 1955). Burton (1987, 1990) observed F2 hybrid breakdown in the laboratory following crosses between populations of the marine copepod *Tigriopus californicus*, and demonstrated that hybrid breakdown is also scale-dependent. Fenster (1991b) did not detect any evidence of outbreeding depression in *Chamaecrista fasciculata* after comparing the performance of progeny generated from parental crossing distances ranging from 1 to 1000 m. There was a decelerating gain in F1 progeny fitness as the distance between the gene pools increased (Fig. 2.3A). Fenster (1988) also calculated genetic similarity among parents and found that genetic similarity was inversely related to progeny performance throughout the parental crossing distance range (Fig. 2.3B). The plateau of genetic similarity with distance corresponds to the observation that gene flow in *C. fasciculata* is limited (Fenster 1991a). In contrast, Waser & Price (1989 and earlier references cited therein) have demonstrated outbreeding depression in *Ipomopsis aggregata* on a local scale in the F1 generation. In their work, the progeny of parents separated by 10 m had a higher probability of surviving than did the progeny of parents separated by 1 and 100 m. Determining the relationship between progeny fitness and genetic similarity is important in devising management strategies for other species. For example, progeny vigor may increase up to some degree of genetic distance, reach a plateau, and then decline, as observed by Moll *et al.* (1965). If baseline studies are performed, some predictions of the consequences of crossing genetically differentiated populations can be made.

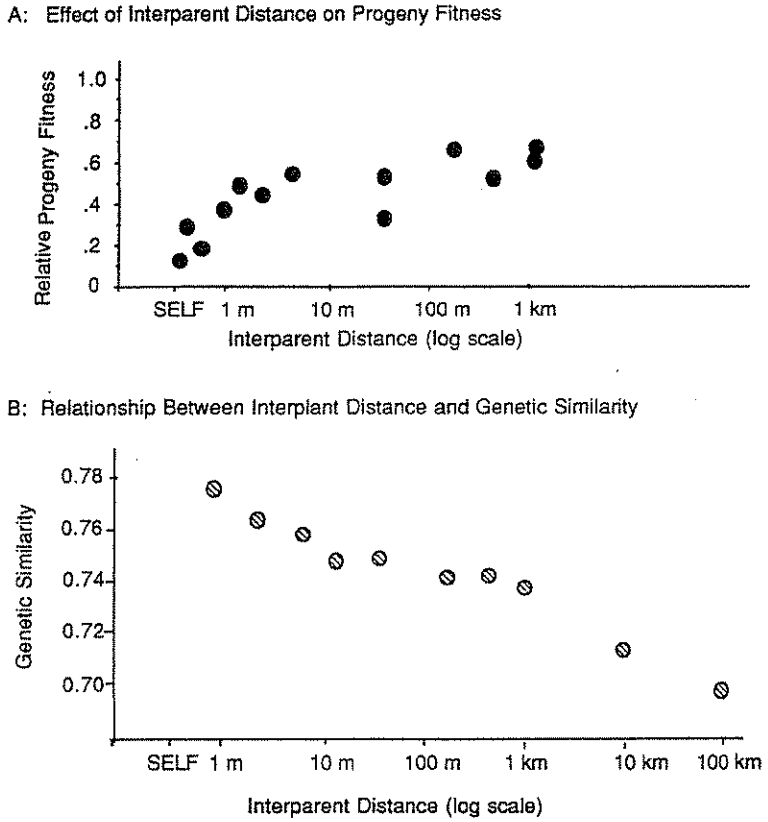

Fig. 2.3. Relative progeny fitness (A) and genetic similarity (B) for *Chamaecrista fasciculata* calculated from isozyme analyses comparing progeny generated from a range of interparent distances, from self-pollination to interparent distances of 1 m to 100 km (from Fenster 1988, 1991).

Mating system and outbreeding depression

The amount of recombination determines the extent to which linkage disequilibrium can be formed between loci with fitness interactions (Lewontin & Kojima 1960, Lewontin 1974, Clegg 1984, Hastings 1985). With greater linkage, epistasis for fitness does not have to be as high as it does with lower linkage for the formation of epistatic gene complexes. Since inbreeding effectively reduces the level of recombination in a population (Bodmer & Parsons 1962), we expect outbreeding depression to be greatest for species with high selfing rates.

Some of the clearest evidence for epistatic selection has been documented in selfing organisms. Populations of highly selfing *Avena barbata* are monomorphic for alternative alleles at seven loci in xeric and mesic environments on a

broad (Clegg, Allard & Kahler 1972) and local scale (Hamrick & Allard 1972, Allard *et al.* 1972). Quantitative characters were also differentiated between the two ecotypes (Hamrick & Allard 1972, 1973), suggesting that the two alternative gene complexes include loci on all chromosomes. Similar results have been observed by Nevo and colleagues (Nevo *et al.* 1981, 1983, 1986) for wild populations of the selfing *Hordeum spontaneum*, recognized as the progenitor of cultivated barley. In addition, composite crosses of selfing barley (from strains collected worldwide) formed adaptive four-locus interactions through time (Clegg *et al.* 1972, Weir, Allard & Kahler 1972, Allard 1975). The results of these experiments suggest that epistatic gene complexes can be formed rapidly when recombination is limited or the approach to linkage equilibrium is decreased by selfing. Thus, in contrast to the case of inbreeding depression, selfers may experience a greater degree of outbreeding depression for any given crossing distance. Little empirical work has been directed towards documenting the role of mating system in outbreeding depression. Ideally, outbreeding depression could be compared between pairs of congeners with contrasting mating systems.

Measuring outbreeding depression

To determine the consequences of mixing distant gene pools, it is necessary to cross individuals within a population and individuals from pairs of populations of increasing geographic and genetic distance. F1 progeny should have the highest heterozygosity if inbreeding is occurring within parental source populations. F2s would be generated by random crosses between the F1s to minimize inbreeding for each cross type. The performance of the progeny would be measured by growing the progeny from the crossing array in each parental source location and in novel environments. If there is adaptation of the source populations to local environmental conditions, or intrinsic adaptation, then F2 progeny may exhibit hybrid breakdown independent of heterosis observed in the F1 generation. However, novel genotypes may arise in the F2 generation that might confer equal or higher fitness gains than those of the parental source populations.

Observation of the relationship between inbreeding and vigor may allow us to examine the role of selection to modify the genetic load specific to populations. The deleterious consequences of continued inbreeding can result from three types of interaction among homozygous loci (Crow & Kimura 1970), which are described in Fig. 2.4. Loci can act either additively (linear decline of fitness with inbreeding), with reinforcing epistasis (the homozygosity of loci reduces fitness greater than the sum of their individual effects), or with diminishing epistasis (the homozygosity of loci reduces fitness less than the sum of

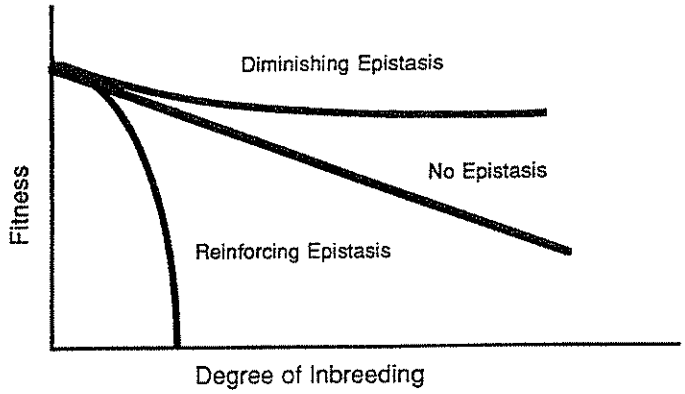

Fig. 2.4. Decline in fitness with inbreeding under no epistasis, diminishing epistasis, and reinforcing epistasis (from Crow & Kimura 1970).

their individual effects). These measures of epistasis include both dominant × dominant and additive × dominant interactions (Lynch 1991). Whether modifiers have evolved to ameliorate the genetic load has important implications for population restoration. Long-distance crosses may break up the ameliorating effects of modifiers, so that inbreeding results in a much greater loss of vigor (reinforcing epistasis) compared with inbreeding in intact populations (diminishing epistasis or linear decline).

The experimental evidence is limited, but the accumulation of non-lethal mutations in *Drosophila* stocks over time results in an accelerated decline in vigor (Mukai 1969). Deviations from linearity of fitness with degree of inbreeding were also observed in poultry (Sheridan 1980), mice and guinea pigs (Kinghorn 1982, 1987) and maize (Schnell & Becker 1986) (see also examples cited in Geiger 1988). Earlier work with maize demonstrated that yield declined linearly with degree of inbreeding (Neal 1935). Differences in the outcome of the maize experiments may have resulted from different lines tested in different environments. *Crosses between coadapted peaks may break down the modifying influence of the genetic background. Therefore, inbred lines derived from crossing widely separated parents may have a greater degree of reinforcing epistasis compared with lines derived from within natural levels of gene flow. The contributions of dominant × dominant and additive × dominant interactions to epistasis for fitness need to be quantified.*

Most examples of epistatic interactions have been restricted to quantifying non-additive interactions among nuclear loci. Like that of mitochondria, the chloroplast genome is cytoplasmically inherited and has been documented to have important interactions with the nuclear genome. Frequently, pollen and seed have different dispersal distances; e.g. Fenster (1991a), suggesting that

spatial patterns of genetic variation should differ for nuclear (pollen- and seed-dispersed) and cytoplasmic (seed-dispersed) genes. Nuclear–cytoplasmic interactions may evolve on a different spatial scale than strictly nuclear interactions. We can examine scale-dependent nuclear–cytoplasmic interactions by comparing the relative fitness of backcross progeny that have their cytoplasmic and nuclear genome from either similar or different gene pools. Thus, the role of nuclear–cytoplasmic interactions in scale-dependent epistasis for fitness can be quantified.

A cautionary note

Hybridization, resulting in secondary contact between races or populations that have long been isolated from one another, may be quite common in the evolutionary history of species (Anderson 1949, Stebbins 1950, Mayr 1970, Wright 1977). The exchange of genes from distant populations may occur through rare long-distance gene dispersal or, more likely, through a series of range expansions and contractions throughout the evolutionary history of a species. Gene flow between populations that differ greatly in their genetic composition may have consequences similar to hybridization at higher levels; e.g., introgression, breakup of character correlations, and change in the mode of inheritance of traits. Artificial mixing of distant gene pools may parallel the dynamics of gene flow during the evolutionary history of a species. Breakup of coadapted gene complexes may lead to genetic systems that confer even higher adaptation to the environment. In any event, the composition of gene pools may be part of a dynamic process of isolation and mixing. *Thus, preservation of the genetic integrity of a species may be an ideal with no natural basis; therefore, it should not be used,* a priori, *as an obstacle to the mixing of gene pools.* Nevertheless, we should not take irreversible steps in our fragile existing populations. The artificial mixing of distant gene pools should be tested during species reintroductions in which we have limited options, or tested in model systems using common species.

Genetic diversity of source populations

Genetic diversity in the source gene pool may influence the colonizing ability and prolonged persistence of a population (Polans & Allard 1989, Barrett & Kohn 1991). In outcrossing species, the mean fitness of a population may correspond to levels of genetic diversity. Thus, an alternative to mixing distant gene sources to restore population heterozygosity would be to choose only populations with high levels of genetic diversity for restoration programs.

Quattro & Vrijenhoek (1989) observed that natural populations of Topminnows containing high genetic isozyme diversity had a greater probabil-

ity of survival and experienced earlier fecundity than natural populations with no detectable electrophoretic variation. Note that in selfing species isozyme diversity is often low or absent (Fenster & Ritland 1993). However, selfers may have equivalent levels of within-population quantitative genetic variation (Hillel, Feldman & Simchen 1973, Clay & Levin 1989, D. E. Carr & C. B. Fenster, unpublished data). Thus, choosing those populations of selfers that have higher than average levels of quantitative genetic variation for restoration programs may result in a lower probability of extinction.

Maintenance of the evolutionary potential of a species

Because a successful conservation policy must include the maintenance of the evolutionary potential of a species by preserving genetic diversity (e.g. Frankel & Soulé 1981, Allendorf & Leary 1986), important subsets of genetic variation within a species must be identified and preserved. Molecular markers, such as isozyme or DNA variation, may be *relatively* quick and affordable methods of identifying important subsets of genetic variation. The morphological diversity most closely associated with a species' ability to maintain its range, however, may be partitioned differently from molecular markers (Brown *et al.* 1978, Lewontin 1984). A distinction should be made between present morphological diversity among populations and the evolutionary potential of populations. Populations that are phenotypically differentiated from one another may be interchangeable if one can evolve into the other given the appropriate selective regime. However, as will be discussed below, populations with different amounts of genetic variation and patterns of covariation are not interchangeable; one cannot evolve into the other even with identical selection pressures. If different populations of a species have different sets of evolutionary constraints associated with different patterns of genetic variance and covariance, we should aim to preserve the evolutionary potential by sampling among populations. If we do not conserve the full evolutionary potential of species, we may irreversibly reduce their range.

Recent developments in molecular genetics, combined with more traditional quantitative genetic techniques, greatly increase our ability to detect different levels of genetic variation within a species. This section describes a synthetic approach that allows detection of levels of genetic variation important to the preservation and restoration of rare and endangered species. The experiments discussed involve detection of isozyme and chloroplast DNA variation within common native species, using a phylogenetic approach combined with extensive crossing programs. Results from these experiments may be used to formulate conservation and restoration policy for rare and endangered taxa.

Evolutionary lineage

Extant species represent successful evolutionary experiments. One of the roles of conservation biologists is to ensure that this unbroken genetic ancestry is preserved. Genetic differentiation at the species level is only one point in a continuum of genetic divergence beginning within the population and extending across a species. Methods are needed to detect ancestral relationships that represent subsets of genetic variation important to maintaining the evolutionary potential of a species.

One method of partitioning genetic diversity within a species is to determine independent evolutionary lineages (Avise *et al.* 1987, Templeton 1989) (Fig. 2.5). Because a lineage represents an unbroken genetic heritage, it may be the most appropriate level for conserving genetic diversity. A species is composed of many lineages, each with a unique history of selection pressures and genetic bottlenecks. The number of lineages uncovered within a species will depend largely on the methods used to detect them. For example, given a complete genetic history of a species, we could discriminate among lineages within a population using pedigree analysis. It is likely, however, that genetic variation important to conservation issues resides at or above the population level. Methods are needed to detect lineages at the level of groups of populations. The mating system will also determine the number of lineages within a species. Plants with limited recombination, such as selfers and apomicts, will exhibit a

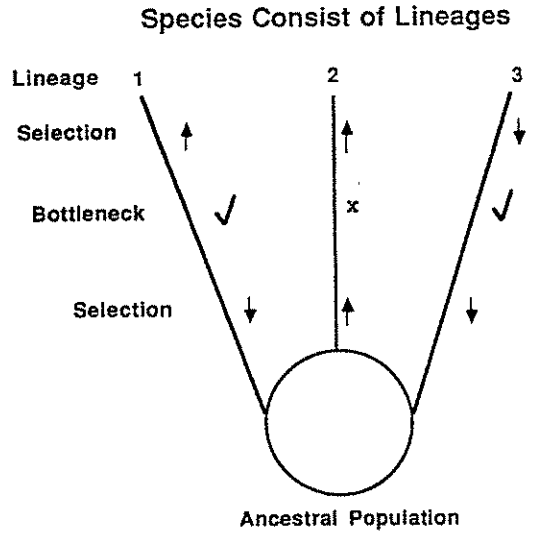

Fig. 2.5. Genetic variation can be partitioned into various lineages (different lines in the figure) that have different histories of selection (direction of selection given by arrows) and drift (presence or absence noted by check or x).

greater number of unique lineages for nuclear, biparentally inherited genes than species with higher rates of recombination. Because lineages in selfers and apomicts do not represent the free recombination found in outcrossers, the evolutionary time scale that they represent will be much smaller than it will be in outcrossers. Thus, restoration policy aimed at conserving evolutionary lineages must take into account the mating system of the target species.

Detection of evolutionary lineages

Taxonomies based on morphological data alone may not be adequate to identify subsets of genetic variation within a species (Avise 1989a). Recent surveys have demonstrated that mitochondrial DNA (mt-DNA) variation often exists within animal species (Avise & Nelson 1989). Since mt-DNA is maternally inherited, each of these maternal lineages represents its own history of past selection and genetic drift (Avise 1989b). Application of lineage analysis using mt-DNA variation to conservation biology is exemplified by the case of the dusky seaside sparrow, *Ammodramus maritimus nigrescens*. Avise (1989a) demonstrated that the endangered dusky seaside sparrow was part of a lineage that included seaside sparrows from the Atlantic coast. Conservation efforts aimed at preserving the gene pool of the dusky seaside sparrow had been directed towards crossing the endangered taxon with a different lineage from the Gulf coast. In this case, traditional taxonomies were unable to detect the important divergence of seaside sparrows between Atlantic and Gulf coastal populations, but the divergence was detected by molecular techniques.

Plant mt-DNA is too conservative in its primary sequence, and evolves too rapidly in terms of its size, to be useful for detection of major genetic differences within species (Palmer 1987, Sederoff 1987). However, restriction fragment length polymorphisms in maternally transmitted chloroplast DNA (cp-DNA) evolve slowly enough to provide resolution of genetic variation from above the population level to the species level for some species (Palmer 1987). Thus, analysis of cp-DNA variation may reveal independent and anciently derived maternal lineages within a plant species consisting of groups of populations. There have been few studies attempting to document cp-DNA variation within a plant species. When it has been studied, different lineages have often been identified (Clegg, Brown & Whitfield 1984a, Banks & Birky 1985, Soltis, Soltis & Ness 1989, Fenster & Ritland 1992, but see Clegg, Rawson & Thomas 1984b, Clegg 1987).

Fig. 2.6 shows a within-species phylogeny using cp-DNA of *Mimulus guttatus* (Fenster & Ritland 1992). Although the number of cp-DNA mutations detected is limited, they do allow us to make some inferences about the detection of different lineages. For example, it appears that populations 7 and 8 are derived from an ancestral population with a genotype similar to that of population 6, and that pop-

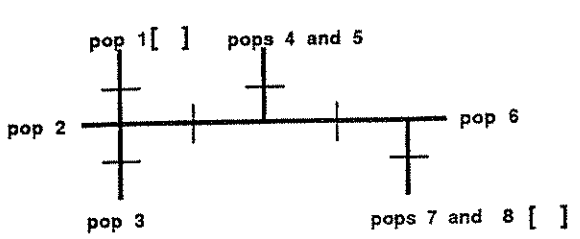

Fig. 2.6. An unrooted Wagner tree showing the relationships among populations of *Mimulus guttatus* using cp-DNA variation. Each hash mark represents a different cp-DNA mutation. For example, population 1 has diverged from populations 7 and 8 by 4 mutations. Brackets represent genetic variance–covariance matrices (from Fenster & Ritland 1992).

ulations 6, 7, and 8 are more closely related to one another than they are to population 1. The lineages detected by cp-DNA variation may identify populations with different evolutionary potentials, since each lineage represents a different history of selection and drift. Maintenance of the full evolutionary potential may depend on the preservation of the genetic diversity from each of these lineages.

Isozyme analysis is a common approach for partitioning genetic variation within a species. Isozyme variation can be used to group populations based on their genetic similarity. This is often done using dendrograms constructed from Nei's genetic distances (Nei 1972). Thus, subsets of populations that represent the range of isozyme diversity within a species can be identified. The advantages of using cp-DNA variation over isozymes are that cp-DNA is maternally inherited with limited recombination, and that it represents a gene lineage. Synapomorphies, or shared derived characters used to infer ancestral relationships, are more apparent with cp-DNA than with isozymes. Thus, it is easier to resolve intraspecific phylogenetic patterns and detect lineages using cp-DNA (Moritz & Hillis 1990). Nevertheless, there are a number of approaches for using continuous data (e.g. isozyme frequencies) in the construction of phylogenies (Felsenstein 1981, 1985a,b, Rogers 1986, Swofford & Berlocher 1987). For some taxa, cp-DNA may be too conservative to determine gene lineages (Clegg 1987). In such cases, isozyme variation may be relied upon to estimate genetic relationships among populations. In addition, because so many studies using isozyme variation have been conducted, there is fairly good agreement on taxonomic affinities based on genetic distances. Local races of species generally have genetic distances of approximately 0 to 0.05, while subspecies have genetic distances of 0.02 to 0.20 (Nei 1976). In addition, in species that are highly selfing, clonal, or apomictic, isozyme variation may be in linkage disequilibrium with important ecological variation (Allard *et al.* 1972, Hamrick &

Allard 1972, Nevo *et al.* 1986). Isozyme variation may thus be an indicator of important ecological variation in species with limited recombination.

Role of evolutionary lineages in conservation biology

Grouping populations on the basis of their cp-DNA or isozyme affinities is only the first step in determining major subsets of genetic variation. We need to know whether the subsets of genetic variation identified by cp-DNA or isozyme affinities are actually groups with different evolutionary potentials. Ideally, lineages that have unique evolutionary and ecological roles would be identified. Fortunately, animal and plant breeders have used quantitative genetic techniques with great success to predict responses to artificial selection (Falconer 1981). These same methods can be used to predict the ability of populations to respond to natural selection, thus providing a gauge of the evolutionary potential of a population.

First, consider single trait selection. The response to selection (R), or the change in the mean of a trait in a population from $\bar{z}$ to $\bar{z}^*$, is proportional to the amount of genetic variation for that trait (Var_G) and the selection differential (S) (Falconer 1981):

$$R=\bar{z}^*-\bar{z} \propto Var_G S \qquad \text{Eq. 2.1}$$

For outcrossing populations, only the additive component of genetic variation (loci segregating alleles with additive effects) will respond to selection acting on individuals. Note that for apomicts, clonal species, and selfers, the response to selection will also depend on non-additive genetic effects, such as dominance or epistasis.

However, traits do not evolve independently. For example, there is often genetic variation for yield components in crops, but different yield components are often negatively correlated with one another (Adams 1967). Thus, breeders can select for greater seed number, but often at the cost of lower protein content per seed (Simmonds 1981). We can model multitrait selection by expanding Eq. 2.1 above to the multivariate equivalent:

$$\begin{vmatrix} \bar{z}_1^* \\ \bar{z}_2^* \\ \cdot \\ \cdot \\ \cdot \end{vmatrix} - \begin{vmatrix} \bar{z}_1 \\ \bar{z}_2 \\ \cdot \\ \cdot \\ \cdot \end{vmatrix} \propto \begin{bmatrix} Var_G & & \\ & Cov_G & \\ & & \cdot \\ & & & \cdot \end{bmatrix} \begin{vmatrix} S_1 \\ S_2 \\ \cdot \\ \cdot \\ \cdot \end{vmatrix} \qquad \text{Eq. 2.2}$$

Thus, the change in mean of suites of characters represented by the two vectors on the left side of the equation will depend not only on the genetic variance and selection on a particular trait, but also on how these traits genetically covary (Lande & Arnold 1983). In other words, a character can evolve in response to both direct selection on a trait and indirect selection on genetically correlated traits.

The following scenario might help to clarify the importance and consequences of two populations sharing similar vs. dissimilar genetic variance–covariance matrices. Imagine two populations that have genetically differentiated from one another owing to locally divergent selection pressures. Perhaps one population is adapted to a coastal climate and the other to alpine conditions, such as observed by Clausen & Heisey (1958) in their classic studies of ecotypic differentiation along the altitudinal transect from Stanford to Timberline in California. If the two populations have identical genetic variance–covariance matrices, then, given time and similar selection pressures, the coastal population may evolve the same suite of characters that confer adaptation to the alpine environment as the alpine population and vice versa. However, if the two populations differ in their genetic variance-covariance matrices, then selection for adaptation to the alpine environment on the genotypes from the coastal population will not result in the same suite of characters. The latter scenario has two implications. First, if we preserve only one population, we will have lost a unique subset of genetic variation that defines the species. Second, we may irreversibly reduce the potential range of the species if there is no genetic variation in either population allowing for the potential to evolve adaptations to either environment.

Both theoretical and empirical studies suggest that genetic variances and covariances may be altered by either strong selection (Crow & Kimura 1970, Sheridan & Barker 1974, Falconer 1981, Istock 1983, Lofsvold 1986, Billington, Mortimer & McNeilly 1988) or drift due to founder events and inbreeding in small populations (Lande 1976, Avery & Hill 1977). Among-population variation in the genetic covariance structure implies that populations will respond differently to the same selection pressures and that each population represents an important lineage with its own potential to evolve. Consequently, it is essential to determine if the genetic variance–covariance matrix is stable across populations. Since cp-DNA lineages represent ancient divergence events within a species, it seems likely that if populations have evolved different variance–covariance matrices, these differences would occur at the level of cp-DNA lineages. Similarly, populations with greater genetic distance between them should share fewer historical processes, and should be good starting points in determining the stability of the genetic variance–covariance matrix.

There are a number of techniques for determining the genetic variance–covariance matrix using quantitative genetics, which involve the analysis of families or parent–offspring regressions (Falconer 1981). These methods are often time-consuming and impossible to accomplish for organisms with long lifespans. However, there is some empirical evidence that the phenotypic variance–covariance matrix is similar to the genetic variance–covariance matrix (Cheverud 1988, but see Willis, Coyne & Kirkpatrick 1991). This means that traits of individuals in natural populations can be measured in the field and used to estimate a population's evolutionary potential. Perhaps of greater promise is the technique derived by Ritland (1989) to estimate genetic variances and covariances for a population by using genetic markers (such as isozymes or DNA variation) to infer genetic relatedness among individuals. Once the relationships among individuals are known, one can estimate how quantitative genetic variation is partitioned among or within families, or even among full and half-sibs. These methods have added power because measurements of genetic parameters are made in the most appropriate environments, i.e. natural populations. These methods suggest that estimation of a population's evolutionary potential may be accomplished without conducting crosses.

There are at least two approaches to determining the stability of the genetic variance–covariance matrix. Returning to the within-species phylogeny of eight populations of *Mimulus guttatus* (Fig. 2.6), it might be asked to what extent the genetic architecture differs among the different lineages. To answer would require testing whether different evolutionary lineages have different genetic variance–covariance matrices and hence different evolutionary potentials. For example, does the genetic variance–covariance matrix of population 1 differ from the matrix of population 8? Knowledge of evolutionary lineages may allow determination of the consequences of mixing gene pools from within vs. between lineages. Perhaps the consequences of crosses between populations 7 and 8 would be very different from those of crosses between 1 and 7 or 1 and 8.

Another approach is to try to disrupt the genetic variance–covariance matrix. Different selection intensities to disrupt character correlations could be tested to determine the stability of the matrix in natural populations. For example, different lines could be exposed to different levels, in terms of magnitude and sign, of two-trait selection for several generations. Once the lines have diverged, the genetic correlations could be tested for any differences among the lines. In addition, the effect of bottlenecks on the stability of the matrix could be tested by taking replicate populations through a series of population reductions and comparing the genetic variances and covariances before and after the bottlenecks.

Summary

We do not propose that all of the experiments described here are necessary for the formulation of restoration policy for every species. However, we do contend that baseline data collected from a number of *model systems* would provide insight for the formulation of restoration policy. Although the use of common species is advocated, studies using rare species would also allow resource managers to make educated decisions (as in the case of Lakeside daisy; see DeMauro, Chapter 12 of this volume). The consequences of manipulating genetic variation to restore or increase the vigor of a small population must be determined. In addition, markers that delineate genetic diversity important to the maintenance of a species' evolutionary potential are needed. Chloroplast DNA and isozyme variation may serve as markers of significant evolutionary events (i.e. selection, gene flow, and drift) that might alter the evolutionary potentials of populations. It is important to note that often there is no opportunity for defining significant lineages within species. Most rare species occur in fewer than 10 populations, and the vast majority of threatened species occur in fewer than 20 populations (Holsinger & Gottlieb 1989). However, we certainly have a greater opportunity to save species before they become endangered. Identification of important lineages may be a necessary part of the blueprint for the survival of species that are now widespread, but could soon become fragmented and endangered. In the absence of such information, resource managers must rely on their knowledge of the demography and ecology of the species. The preservation of ecotypes or populations representing the extremes of the range of the species should be paramount. This is the necessary approach to making critical decisions regarding the restoration of populations and the conservation of genetic diversity until more baseline data are collected.

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