Ex Situ Conservation of Wild Plant Species: Time to Reassess the Genetic Assumptions and Implications of Seed Banks

MATTHEW B. HAMILTON

Graduate Program in Ecology and Evolutionary Biology
Brown University, Box G-W
Providence, RI 02912, U.S.A.

Abstract: *Ex situ conservation of wild plant species through seed banking is currently being recommended as a conservation strategy to help preserve the biological and genetic diversity of wild plants. Here I argue that ex situ collections may be ineffective at preserving genetic diversity and the evolutionary potential of populations for adaptive or neutral evolution. Treating the collection of genetic variation for seed banks as simply a problem in efficient sampling of neutral, allelic genetic polymorphism is a limited view of the types and organization of genetic variation present in wild plant species. Perspectives on genetic variation from neutral alleles to quantitative variation are necessary when considering evolutionary change. Quantitative genetic variation and genetic correlations determine the degree and form of response to natural selection on polygenic traits. Population variation in the amount of quantitative genetic variation or structure of genetic correlations argues that different populations will respond differently to the action of natural selection and are therefore unique evolutionary entities. Unavoidable selection on single traits will cause indirect selection on genetically correlated traits, possibly resulting in phenotypic changes and a reduction of genetic variation. Genotype-by-environment interactions demonstrate that the success of releasing seed bank genotypes in natural populations is dependent on the likelihood that seed bank material contains genotypes of high relative fitness in introduction habitats. Such actions can cause introduction of nonadaptive genotypes that will depress population fitness. Because not all types of genetic variation are highly positively correlated, sampling methods based on the neutral theory of alleles or allelic data will not necessarily capture representative quantitative genetic variation. More research on the*

Paper submitted August 25, 1992; revised manuscript accepted March 8, 1993.

Conservacion ex situ de especies de plantas silvestres: Tiempo de reevaluar los supuestos genéticos y las implicaciones de los bancos de semillas

Resumen: *La conservación ex situ de especies de plantas silvestres por medio de bancos de semillas esta siendo recommendada en la actualidad como una estrategia de conservación para ayudar a preservar la diversidad biológica y genética de las plantas silvestres. En el presente trabajo yo argumento que las colecciones ex situ podrían ser inefectivas en preservar la diversidad genética y el potencial evolutivo de las poblaciones para la evolución adaptativa o neutral. El tratar la colección de la variabilidad genética para los bancos de semillas simplemente como un problema de muestreo eficiente del polimorfismo genético neutral de alelos es una visión limitada del tipo y organización de la variabilidad genética presente en especies de plantas silvestres. Cuando se consideran cambios evolutivos, las perspectivas sobre la variabilidad genética de alelos neutrales para la variación cuantitativa son necesarias. La variabilidad genética cuantitativa y las correlaciones genéticas determinan el grado y forma de las respuesta a la selección neutral sobre caracteres poligénicos. La variabilidad poblacional en la cantidad de variación genética cuantitativa o en la estructura de las correlaciones genotípicas argumenta que las diferentes poblaciones van a responder diferentemente a la acción de la selección natural y que por lo tanto son unidades evolutivas únicas. La inevitable selección sobre caracteres simples va a causar selección indirecta sobre caracteres correlacionados genéticamente, resultando, posiblemente, en cambios fenotípicos y en una reducción de la variación genética. Las interacciones genotipo-ambiente demuestran que el éxito de la suelta de genotipos de bancos de semillas en poblaciones naturales depende de la posibilidad de que el material de los bancos de semillas contenga geno-*

relationship and evolutionary significance of allelic and quantitative genetic variation in collections and wild populations is needed. Initial and ongoing data on the fate of different types of genetic variation are required to determine the relative success or failure of ex situ conservation methods and to test assumptions of current programs. In situ conservation of ecosystems may offer distinct advantages for many plant species by preserving both genetic and ecological information.

tipos con alto fitness relativo en los hábitats de introducción. Tales acciones pueden causar la introducción de genotipos no adaptativos que deprimirán el fitness de la población. Dado que no todos los tipos de variación genética están altamente correlacionados en forma positiva, métodos de muestreos basados en la teoría neutral de alelos o en datos de alelos no capturarán necesariamente la variación genética cuantitativa representativa. Se necesitan más investigaciones sobre la relación y significación evolutiva de la variacion genética de alelos y cuantitativa en poblaciones silvestres. Se necesitan datos iniciales y continuos sobre la suerte de diferentes tipos de variación genéticas para determinar el éxito relativo o el fracaso de los métodos de conservación ex situ *y para testear los supuestos de los programas corrientes. La conservación* in situ *de ecosistemas puede ofrecer ventajas claras para muchas especies de plantas al preservar tanto la información genética como la ecológica.*

Introduction

The present rates of habitat loss, landscape alteration, and extinction—at the species, community, and even ecosystem level—have sent conservation biologists scrambling to devise methods and tools for species protection and preservation. Conservation biologists have recognized that preservation efforts must include levels of biological organization from ecosystems and communities to genes and genomes (see Frankel & Soulé 1981; Falk 1987, 1990). Recent discussions of plant species diversity and genetic variation in the wild have suggested that comprehensive plant conservation strategies include a variety of methods and have advocated the use of ex situ collections and germplasm banks as an integral part of any such effort (see, for example, National Research Council 1978; Frankel & Soulé 1981; Falk 1990; Given 1990; Brown & Briggs 1991; Heywood 1992; but see Ashton 1987). This shift in emphasis toward "integrated strategies" (Falk 1987, 1990) of plant conservation has changed the role of ex situ methods from a last resort to a necessary element of any comprehensive conservation effort, and several regional plant conservation programs presently employ ex situ methods (New England Plant Conservation Program [New England Wild Flower Society 1992] and Center for Plant Conservation 1991). Discussions of ex situ genetics and recommended sampling plans emphasize neutral, allelic variation as the standard measure of genetic variation present in populations (see Brown & Briggs 1991; Hamrick et al. 1991]. The exclusive use of allelic models and measures of genetic diversity, however, ignores several important issues in evolutionary genetics. Quantitative genetic variation, genetic correlations, and genotype-by-environment interactions influence the evolutionary trajectory of populations and are therefore important issues in population genetics. Careful consideration of these factors should convince us that successfully preserving genetic diversity and evolutionary potential with ex situ plant conservation programs is more difficult than neutral models indicate.

Assuming that the goals of ex situ conservation are the prevention of extinction and the preservation of the evolutionary potential (Frankel 1974) of populations through adaptive or neutral evolution, a process that requires genetic variation and the perpetuation of genetic information (Frankel & Soulé 1981; Beardmore 1983; Allendorf & Leary 1986), I will argue that ex situ collections could be largely ineffective except in applications with limited goals for the preservation of genetic diversity (for example Zobel 1977). I will discuss the genetic assumptions and conservation implications of ex situ methods, often heralded as a last resort but routinely included as a nonproblematic companion to in situ plant diversity conservation efforts. Although it clearly will not be possible to preserve all genetic variation that is present in disappearing wild plant populations, we must use the best information available to plan actions that will effectively preserve a spectrum of genetic variation into an uncertain future. Understanding the genetic dynamics of ex situ methods will permit their realistic use in conservation efforts, give a basis for evaluating competing conservation plans, and help prevent the failure to meet goals for the preservation of genetic variation. I will concentrate on ex situ conservation of wild plant species in seed banks and will not address the related but distinct issue of genetic resource conservation of crop plants, which has been dealt with recently (see Brush 1989). This topic also has obvious parallels to captive animal propagation (Templeton 1991), but the recent increase in plant-specific ex situ conservation efforts warrants exclusive consideration of plant data.

The Genetics of Seed Banks

Seed banking is a potentially attractive method to simply preserve dormant individuals until they are required to sustain or reestablish a population, at which time they are germinated and placed into wild populations or unoccupied potential habitats. Unfortunately, the genetic ramifications of this action may greatly affect the amount and distribution of genetic variation in the sample of seeds preserved in the seed bank and thus the genetic structure of any populations founded or expanded with these seeds. Several genetic points of view can be used when modeling the manner in which genetic variation is sampled, stored, and lost from ex situ collections. First I will review briefly some sampling theory of allelic genetic variation and its relationship to ex situ conservation.

Neutral Alleles and Stochastic Sampling

An understanding of seed banking and its genetic consequences requires an awareness of the effects of finite sampling, the basic mechanism behind genetic drift (Wright 1948; Lande & Barrowclough 1987), because it is a process that is repeated several (to many) times in the course of collecting, storing, and regenerating seeds in a seed bank. Field collections of seeds are usually made without the assistance of any type of quantitative measures of phenotypic or genotypic diversity, and the result is a seed collection that captures a random sample of the variation present in populations. This is the first step whereby variation present in a population can be absent from a seed bank.

The most common analogy for the effects of sampling on neutral alleles is a jar full of beads. In the jar are 50% green and 50% red beads, and we sample by drawing out handfuls of 20 beads each, obtaining on average a one-to-one proportion of colors. If we extend this concept to genetic variation, every time seeds are sampled from plant populations for storage in a seed bank, the gene frequencies represented in the sample will not necessarily be the same as those in the source population. Also, the lower the frequency of a given gene in the source population, the greater the probability it will be unrepresented in the sample. Take for example a jar of beads that is 49% red, 49% green, and 2% white. When we sample 20 beads there is a 67% (0.98^{20}) chance that a single sample will contain no white beads. If we sample 20 beads from the jar 100 times, however, there is a considerable chance ($1 - 0.98^{(20)(100)} \approx 100\%$) that at least one of the beads will be white. If the white beads represent rare genes in a plant population, we will clearly have to sample the populations many times (or take large samples) to increase the chance of representing rare genes in a seed bank. Multiple small samples instead of one large sample are often most practical and safest in rare plant populations. Populations reduced to small numbers can have yearly fecundity markedly reduced by large sample collecting for a seed bank.

The effects of sampling on genetic variation do not cease after a sample of seeds has been collected in the field. Although many authors have discussed the ways in which genetic variation is sampled and have offered sampling methodologies (Marshall & Brown 1975; Hawkes 1976; Center for Plant Conservation 1986, 1991; Falk 1991; Holsinger & Gottlieb 1991), few have discussed the realization that levels of genetic variation in a seed bank are heavily dependent on the rate at which variation is lost when seeds are stored. During storage, viability usually declines over varying periods of time. Random seed death (not correlated with genetic or phenotypic traits) will result in another bout of genetic sampling, further decreasing the probability that rare or low frequency genes will be contained in the seed bank. Seed bank managers often use the rough rule that seeds should be regenerated by germination and reproduction if viability drops by 5%. If a collection originally contained one seed from each of 1000 individuals, the probability that a gene at a frequency of 0.01 in the seed bank will not survive the viability drop is 3.9×10^{-14}, a very slight chance indeed. Predicting the results of repeated sampling by this process for this same gene over multiple regeneration cycles (assuming the gene cannot be lost through recombination) is a much more difficult problem, albeit more realistic in the case of long-term seed banks. Although a solution to such problems requires use of complex differential equations beyond the scope of this article (Crow & Kimura 1970; Roughgarden 1979), a general conclusion is that the average time of persistence of a neutral allele is a function of the size of the population and initial allele frequency (Hartl & Clark 1989). Alleles will be lost or fixed more rapidly on average for a fixed population size when they are present at low or high initial frequencies respectively, but all alleles will eventually be lost or fixed if enough time passes. This clearly establishes seed banks as temporary storehouses for genetic variation, just like real populations, but the rate of loss of many alleles will be higher than it is for natural populations because seed banks start out with only a sample of the variation in wild populations and have no source of new variation unless sampling from wild populations is repeated or mutation rates can replace variation. Obviously, an allele at a frequency of zero in a seed bank will have no chance of being preserved.

This discussion has assumed possible rates of loss of genetic variation when such variation is allelic and selectively neutral, and when the fate of polymorphism is entirely dependent on the outcome of stochastic sampling factors. For types of genetic variation that are not allelic and are less likely to be selectively neutral, our conclusions about the dynamics of ex situ plant conser-

vation methodologies and results could be different. I will now consider four nonallelic genetic perspectives on the problem of capturing and maintaining genetic variation for ex situ seed banks. These four topics will demonstrate that neutral, allelic genetic models can be a limited view of the types and organization of genetic variation present in wild plant populations, and that sampling populations using allelic models can fail to capture variation necessary to preserve a species' evolutionary potential.

Natural Selection and Quantitative Genetic Variation

It is clear that natural selection acting in a seed bank will remove certain alleles, genotypes, or phenotypes differentially and cause variation to be lost more rapidly or in different patterns than it would be removed by sampling events alone. Seed bank curators must prevent the erosion of genetic variation through selection, which will necessitate treating each plant individually by following maternal seed families to be sure that the number of seeds represented by each maternal genotype is constant over time and through regeneration cycles. In natural populations, however, natural selection is a vital part of evolutionary potential and long-term change. Endler's (1986) summary of selection data concludes that the "frequent statement that selection is usually weak in natural populations is without merit" (for empirical examples also see Scheiner 1989; Mitchell-Olds & Bergelson 1990; Jordan 1991). This is a very significant finding because it indicates that selection is an active part of wild populations and their evolutionary potential and that is must be considered in conservation plans. For example, five populations of the rare endemic *Arabis fecunda* show different patterns of selection on floral and vegetative traits, with among-population variability resulting in directional, stabilizing, or disruptive selection depending on the population (Hamilton, unpublished data). These data indicate that populations have qualitatively different selection pressures and could be undergoing unique phenotypic evolution given genetic variation to respond to such selection.

Recent theoretical work (Lande 1979; Lande & Arnold 1983) on the action of natural selection in shaping phenotypic character distributions has stressed the importance of the genetically determined component of variation in metric characters, or quantitative genetic variation. Because quantitative genetic variation, expressed as the heritability of a trait, is necessary for the mean and distribution of a phenotypic character to respond to the action selection (Lande & Arnold 1983; Falconer 1989:chapter 20), it follows that conservationists should be concerned with the amount of additive genetic variation within and among populations if a seed bank has the goal of protecting or preserving the evolutionary potential of a species. Because there may be variation in the amount and distribution of quantitative genetic variation for a given character, different populations could respond very differently to the action of natural selection (Lande 1988*b*; Falconer 1989; Venable & Burquez 1989; Schaal et al. 1991*b*).

Although quantitative genetic variation studies have been carried out on a variety of plant species (see Hallauer & Miranda 1981; Lacey 1986; Mitchell-Olds & Bergelson 1990), there are few clear correlations between levels of quantitative genetic variation and plant life history or breeding system traits. Because most quantitative traits are presumably the result of several to many genes of small effect (Falconer 1989), it will be difficult to determine levels of variation using single gene or enzyme methods. Presently there is little agreement on the nature and function of the individual genes that control the expression of quantitative traits and account for heritability differences among populations (Mather & Jinks 1977; Shrimpton & Robertson 1988; Falconer 1989), and almost no data that address the correlations between levels of quantitative genetic variation and DNA or isozyme variation. Work with crop plant species has shown that mapped isozyme loci have small but often significant associations with quantitative trait expression. Regression models show that significant individual isozyme loci explained between 0.23% and 16.3% of the phenotypic variation in 25 above-ground plant dimension and yield traits in two F_2 hybrid lines derived from inbred strains of maize, with 7 to 19 locus models explaining between 8% and 40% of the phenotypic variation in these traits (Edwards et al. 1987). More than 60% of associations between 82 quantitative traits and isozyme loci explained significant proportions of phenotypic variance in maize; however, more than 75% of these significant associations explained less than 2.0% of phenotypic trait variance (Stuber 1989). Numerous estimates of heritability for these traits range from 0.30 for grain weight to between 0.50 and 0.70 for plant height and days to flower (Hallauer & Miranda 1981). If 14 isozymes explain 40% of plant height variation and it has a heritability of 0.70, the isozymes explain at most 28% of the quantitative genetic variation for this trait. Another example is grain weight, with 14% of phenotypic variation explained by 13 isozymes and a heritability of 0.30, the isozymes explaining a maximum of 4.2% of the quantitative genetic variation. It should be noted that the maize data utilize isozyme markers that are mapped and evenly distributed throughout the genome to provide relatively tight linkage to quantitative trait loci. Isozyme surveys of wild plant species are likely to utilize enzyme loci that have random linkage associations and unknown genomic distributions, which will tend to reduce the association between isozyme genotypes and quantitative trait variance. Also, correlations between isozymes and quantitative trait variances are only available in crop plants that have been subject

to intense artificial selection for agricultural form and yield characters; such data are not available for wild species that are subject to quantitatively and qualitatively different selection pressures. These examples demonstrate that not all types of genetic variation and phenotypic variation are highly positively correlated (Lande & Barrowclough 1987), and sampling methods that are based on neutral theory of alleles or utilize only allelic data will not necessarily capture adequate quantitative genetic variation.

Genetic Correlations

Another factor determining the manner in which traits are affected by the action of selection is the genetic correlation between two traits. Such genetic correlations are caused by pleiotropic effects of genes or quantitative trait loci and indicate the degree to which two traits are influenced by the same genes and expressed together. Positive correlations mean genes affecting one trait will cause a similar change in the other, while negative correlations indicate the pleiotropic genes will tend to increase one character and reduce the other (see Falconer 1989). Genetic correlations can indicate constraints in response to selection due to nonindependent genetic expression of two traits. The presence of nonzero genetic correlations means that selection occurring in an ex situ collection may cause responses in more than just the character under direct selection pressure. Take, for example, the herbaceous annual *Heterosperma pinnatum,* which has a negative genetic correlation between number of seed heads and number of centrally placed seeds per head. This correlation suggests a negative genetic correlation between dormancy and dispersal because central seeds are less dormant and have higher dispersal ability (Venable & Burquez 1990). If this genetic relationship were true and *H. pinnatum* were collected for a seed bank, we would expect the required selection for dormancy to cause the differential loss from the seed bank of plants with larger numbers of seed heads and therefore greater dispersal abilities. In this way the unavoidable selection pressures during ex situ storage, regrowth, and introduction will result in indirect genetic changes that cannot be predicted without knowledge of genetic correlations.

Genetic correlations can also vary among populations and environments, which indicates that each population can "store" genetic variation differently between trait pairs. For example, genetic correlations among physiological traits were different in three nutrient treatments in *Brassica campestris,* changing in sign and magnitude (Evans 1991). Similar changes in genetic correlation structure have been found in other species after transplanting or growth in a variety of experimental environments, although less data is available for wild species than for crop species (see references in Schlicting 1986). Differences in the genetic correlation matrix within or among populations of a species argue that trait pairs in each population will respond differently to the action of selection, and such populations are therefore unique evolutionary entities that should be recognized by conservation efforts.

Genotype-by-Environment Interactions

Genotype-by-environment interactions are another area of potential genetic concern for ex situ seed banks that are not evident when considering only allelic, selectively neutral polymorphism as the basis for sampling strategies. Genotype-by-environment interactions describe the relationship between genotypes and their performance in specific environments (Falconer 1989). For example, a group of genotypes may show a given order of fecundity rankings when placed in one environment and different fecundity rankings in another environment. Alternatively, the rank order may remain constant but variance in fitness-related characters will change (see a review of genotype-by-environment interactions and reaction norms by Stearns [1989]). Figure 1 demonstrates both types of genotype-by-environment interactions in eight maternal full-sib families of *Arabidopsis thaliana* that were divided into two groups and treated differently before germination. One group of seeds was imbibed and stratified in soil at 4°C for 21 days, and the other was kept in envelopes at room temperature for the same period; then both groups were grown under identical conditions until fruiting. This norm of reaction shows that stratification causes greater variance in time to first flower and that the flowering time rank of some genotypes changes between the two environmental treatments. The point is that for many plant species the fitness of a particular genotype will depend on the environmental context in which it exists. Different rank ordering of plant genotypes has been shown in environments of different planting densities (Mather & Jinks 1982), different light (Scheiner et al. 1984) and nutrient levels, and different blocks within a field plot (Schmitt & Antonovics 1986). The demonstration that genotype-by-environment interactions are present in many plant species has profound implications for ex situ seed banks because storage is essentially another specific environment where genotypes may have a unique fitness order. This means that the seed bank is likely to favor certain genotypes and that this rank order fitness could be different than that expressed in wild environments. Factors as diverse and seemingly irrelevant to the genetic diversity of banked seeds as the specific storage temperature, the garden or glasshouse where regeneration takes place, and the concentration of fertilizer used are specific environments for the expression of genotypes.

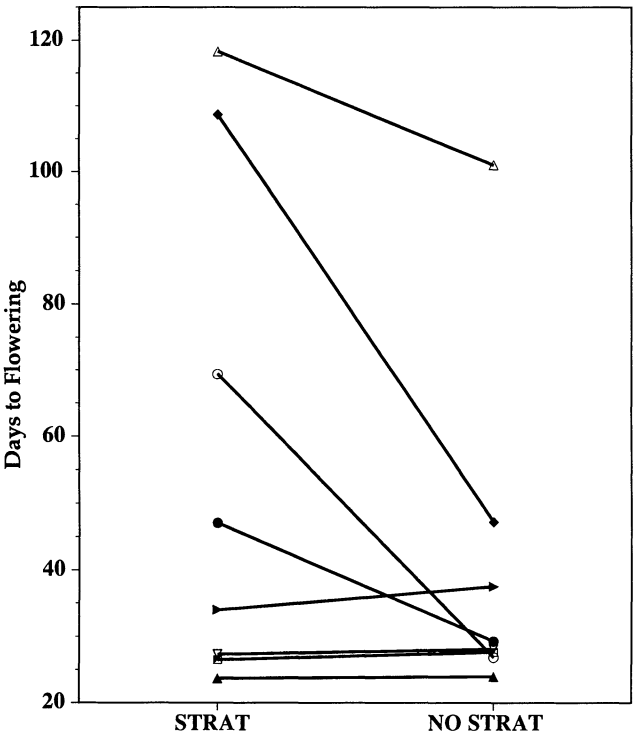

Figure 1. A norm of reaction that demonstrates significant genotype by environmental interactions in the form of changes in variance and genotype ranks of time to flowering for eight full-sib families of Arabidopsis thaliana. *Stratified (STRAT) seeds were imbibed in soil and placed at 4°C for 21 days, while those not stratified (NO STRAT) were kept in envelopes at room temperature and planted on the date that stratification ended. Time to flowering was scored as days to flowering minus days to germination to equalize plants that germinated at different times. Days to germination and days to flowering have heritabilities significantly different than zero. Data were kindly provided by Lisa Dorn.*

Genotype-by-environment interactions also have major implications for potential reintroduction efforts using seed bank material for at least two reasons. First, the success of the introduction will depend on the performance of the seed bank genotypes in the chosen habitat. The lower the diversity of genotypes in a seed bank, the higher the likelihood that seed bank material will not contain genotypes of high relative fitness in the introduction habitat, especially in novel habitats where the genotype-by-environment relationship is random and not the result of past evolutionary change. Second, introducing seed bank genotypes into an existing natural population can cause gene flow of nonadaptive genotypes into wild populations if seed bank genotypes are of low fitness in the introduction environment. This exact situation has been observed in wild populations of mosquitofish (*Gambusia affinis*), where gene flow between small fresh-water and large brackish-water populations continually introduces brackish-water genotypes and their associated genotype-by-environment interactions. This results in a phenotypic response that lowers the fitness of the fresh-water population, because the brackish-water genotypes have norms of reaction that have evolved in response to the brackish environment (Stearns 1989). In this manner, the introduction of seed bank plants collected from various populations and introduced into a declining wild population could actually cause more harm than good if seed bank genotypes have low fitness in the introduction environment.

Genotype Diversity

Genetic variation can also be approached from the point of view of genotypes or allelomorphs, where biologically important variation is contained in the combination of genes or alleles that are carried by an individual organism. Consider an organism that has 100 loci, orders of magnitude fewer loci than even the simplest prokaryotic organism, each with two alleles. There are 2^{100} (or 1.27×10^{30}) possible combinations of alleles, many more than there are individuals for many species. In some sense then, each organism can contain unique and irreplaceable genetic information in the way its genes are organized (Wilson 1988). DNA or isozyme surveys can give us a limited idea of this type of variation by estimating the number and frequency of alleles present at a locus, but present methods allow only the screening of relatively limited numbers of loci.

Genetic perspectives of genotype diversity and stochastic sampling of neutral alleles are opposite extremes—neutral theory argues minimal sample size, while genotype diversity argues maximal sample size (every extant individual). The point is that estimating sample sizes needed for preservation of evolutionary potential through ex situ conservation using either method as the sole basis will provide an extreme view that does not account for all of the observed dynamics of genetic variation in plant populations. A realistic approach to sampling theory will not base target sample sizes at the minimum size, if for no other reason than to provide room for uncertainty or unanticipated events such as storage irregularities, new research results, or a change of opinion in the scientific community about a favored theory.

Discussion

Several types of data are currently used to estimate the distribution of genetic variation within and among populations of plant species, chiefly electrophoretic mobility patterns of isozymes or restriction endonuclease digested DNA fragments, DNA nucleotide sequences, and the amount of additive genetic variation in quantitative

characters (reviewed in Schaal 1991*a*, 1991*b*). Although it is possible to estimate levels of additive genetic variation in several ways (Falconer 1989), these methods generally require greenhouse or common garden experiments, need large sample sizes for statistical power, work best with fast-growing species such as annual plants, and are computationally intensive (Mitchell-Olds & Rutledge 1986). DNA-level methods are also time- and resource-intensive, requiring permanent laboratory equipment, expensive reagents, and relatively long development times. Isozyme surveys are less dependent on large labs, generally require less time to complete, and use techniques that have been available for a longer period of time than many DNA techniques. These factors contribute to the existence of more isozyme data than DNA or quantitative genetic data. This artifact should not be taken as an indication that isozyme data are more important in the evaluation of genetic variation in plant populations. It should also be recognized that isozyme mobility and DNA sequence variation do not have a one-to-one correspondence due to the degenerate nature of the genetic code (one amino acid is often coded by several codon sequences) (Li & Graur 1991). In fact, 69% of substitution in the third codon position in DNA will result in no change in polypeptide sequence and thus no change in isozyme mobility on a gel. This generates the question of what exactly isozyme data describe relative to genetic variation in plant populations, and also the potential difficulties with interpretation of such data (see Simon & Archie 1985). Reviews of the plant isozyme literature have shown correlations between enzyme variation and breeding system, life history parameters, and taxonomic groupings (see Karron, 1991; Hamrick & Godt 1989; Hamrick et al. 1991), and there is a marked tendency to equate isozyme and correlated life-history variability with "ideal" genetic variability (see Schoen & Brown 1991). The ease of manipulating isozymes and their discrete allelic states make them attractive as marker loci, but isozymes do not always behave as ideal neutral markers and they can be a poor measure of underlying DNA sequence variation. Conservation biologists need to address the fact that "allozyme diversity may not be well correlated with other measures of genetic diversity (e.g., quantitative traits) that may be of equal or greater importance in conservation" (Hamrick et al. 1991) and to recognize that isozyme surveys are one of several techniques to reach *estimates* of the genetic variation that is available to be sampled.

Falk (1991) has argued that under neutral theory there are declining increments of genetic variation collected for increasing sample size or number of populations sampled. If rare alleles are simply less frequent but otherwise biologically equivalent to frequent alleles, the time and expense of collecting for rare alleles is not justified. Whether or not rare alleles play roles in the evolutionary dynamics of species and populations is certainly debatable. But one must avoid the tautology of describing rare alleles as evolutionarily insignificant because they are presently rare, especially if one assumes allelic neutrality. Under neutrality any allele no matter how rare can become evolutionarily significant (frequent); the fate of all alleles rests only on sampling. Gould (1989) has repeatedly stressed this theme of contingency. Some types of rare alleles may also confer large fitness advantages under conditions of frequency-dependent selection (Holsinger & Gottlieb 1991). Rare alleles for self-incompatibility, disease or herbivore resistance, and heavy-metal tolerance fit into this category. Because we cannot accurately predict the condition of habitats that will be available in the future, it seems wise to preserve as much variation as possible. At this time, more data on the fate of rare alleles (and genotypes) in plant populations is necessary before cost-benefit functions of sampling can be explicitly defined.

It is also risky to consider seed banks as "insurance" against extinction in the wild (see Falk 1987; Brown & Briggs 1991; Adams & Adams 1992:preface). As with most insurance policies, you must continually pay the premiums in order to be covered. We must avoid thinking that seed banks are a one-time collecting effort if ex situ methods are to be useful in preserving genetic variation. Such programs will require a great deal of time, personnel, money, and resources to effectively preserve the genetic variation now present in wild populations because genetic data will be necessary prior to sampling and periodically during storage. It will be necessary to store, monitor, and regenerate seeds for long periods—possibly infinite periods in cases where species and suitable habitats disappear permanently. Given that conservation resources are limited, ex situ efforts could divert resources away from in situ efforts and may provide a false sense of security because some potential genetic drawbacks of seed banks are not widely known. The starting and ending conditions of seed banks are not static like insurance policies; the genetic characteristics of the stored organisms will change with time as in natural populations. Because of this dynamic nature of seed bank material, the future needs of conservation efforts may not match the material that is contained in a seed bank. The seed bank and wild populations are likely to become genetically more and more different as generations pass, leading to the question of whether we are saving a distinct population or a representative sample of past populations.

Seed banks have additional weaknesses that are not related to the way in which they store genetic variation. The concept of ex situ conservation in seed banks is implicitly biased toward temperate plant species that have extended natural or inducable dormancy. This conservation strategy will be much less effective for

most tropical species, which have seeds that tend to be "recalcitrant" or lack dormancy (see Eberhart et al. 1991; Thornhill & Koopowitz 1992). The industrial complex requirements for seed banking (very low temperature refrigeration, liquid nitrogen, indefinite electrical service, and so forth) are also things that are taken for granted in developed nations but are not always present or planned in developing nations. This is clearly a factor in the present uneven global distribution of botanical gardens and arboreta (Given 1990). Seed banks are also limited to taxonomically described species and do not contribute to the preservation of unknown species at any biological level (National Research Council 1978:16), a large drawback in areas of the world where a significant portion of the flora is still unclassified (as in new and old world tropics). Further, ex situ collections cannot preserve the information contained in the relationships of groups of species at the community and ecosystem levels. In the event that functioning ecosystems embodied in wilderness and large reserve areas are lost, the species contained in the seed bank are just disjunct biological entities without a selective or environmental context. In order for seed banks to have any hope of successfully preserving thousands of plant species, the information about the spatial and temporal organization of a species and its relationship to other species must be stored somewhere. The most logical place to store this information is in functioning ecosystems, a level of biological organization that also includes the species of interest. If ecosystems are successfully preserved then seed banks will be redundant; if ecosystems disappear then species in seed banks will represent only part of the biological information necessary to reestablish a self-sustaining biological system. Either case argues for strong conservation efforts to be directed toward maintaining large or partial ecosystems.

With a fuller appreciation of the scope of genetic ramifications of seed banking, we can plan uses of seed banks that will have a higher likelihood of success in the short and long term (Ashton 1987). Even if seed banks make a less than ideal tool to preserve genetic variation, they may still have a potential function in an integrated plant conservation strategy. Seed banks have a great deal of potential as a reservoir of individuals that could be used to overcome detrimental demographic events—such as rates of local extinction that are greater than rates of colonization in metapopulations—to allow managers to buffer the impact of catastrophes, and to offer a potential (although temporary) escape from strongly negative population growth rates. This alternative role for seed banks is consistent with Lande's (1988*a*) argument that demographic factors are often of more immediate importance for the survival of some types of endangered species. Recall, however, the mosquitofish example demonstrating that gene flow between differentially adapted populations can have deleterious consequences. Seed banks also have a potential role in education and research similar to that of botanical gardens for making plant material accessible to researchers and allowing cultivation for educational purposes (Ashton 1988). The existence of alternative conservation roles does not mean that seed banks can automatically serve multiple functions without explicit planning and basic data on life history and genetic variation.

Conclusions and Recommendations

The points raised above strongly suggest that treating genetic variation for ex situ seed banks as simply a problem in efficient sampling of neutral, allelic genetic polymorphism is a limited view of the types and organization of genetic variation that may be present in wild populations. Genetic diversity can be present within and among populations as quantitative genetic variation for metric characters associated with patterns of genetic correlations among traits, unique fitness rank orders created by the effects of a specific environment on a group of genotypes, and many unique combinations of genes or alleles organized at the genotype level. Evolutionary change is also contingent on selective pressures and ecological context, forces that can not be preserved effectively ex situ. The recognition of these multiple levels of variation also forces the conclusion that a great deal more effort and resources will be required for sampling and monitoring of ex situ collections in order to accomplish evolutionarily significant conservation. It seems unwise to define integrated strategies of conservation in a fashion where ex situ methods are necessary for every species.

Exclusive use of isozyme markers will not provide the diversity of genetic data necessary to understand evolutionary change at multiple genetic and phenotypic levels. Techniques to examine DNA-level genetic variation are becoming more accessible and cost effective, with markers based on a polymerase chain reaction (PCR) having the potential to allow examination of genetic variation in multiple genome locations for large numbers of individuals (after characterizing the sequence basis of length or restriction site polymorphism). Although quantitative genetic designs are often avoided because of a stereotype that they are unusually labor intensive, it will be necessary to undertake such studies in a conservation context, since the added genetic information may prevent the longer-term waste of effort and resources. Fortunately, plants offer many advantages in quantitative genetic research because they are sessile, readily manipulated in controlled pollinations and environmental treatments, and have been examined in such studies previously, so established methodologies are available (Mitchell-Olds & Rutledge 1986).

Even given these severe limitations, ex situ conservation methods may be one of the only alternatives in cases where other options have been truly exhausted or it is certain that extant individuals will be extirpated (such as road building or clearcutting). If ex situ conservation is going to take place, it is imperative that seed collectors attempt to gather a variety of genetic data when accessions are initially deposited and then follow seed families through time to track the fate of genetic variation. This will require the maintenance of records indicating the population and maternal family that seeds originate from and necessitate that seeds be stored by maternal family within populations and *never* be collected or stored in bulk. Collections organized by family and population will allow seeds to be easily and accurately assayed for quantitative and allelic variation, and will provide the necessary information for breeding designs that avoid inbreeding and prevent effects of phenotype and fecundity selection in captivity. Seeds that are collected in bulk lose this vital information and therefore have limited value for estimating and maintaining quantitative genetic variation, preventing inbreeding and selection, and preserving population differentiation. Recall that many efforts to use zoo populations as a source of animals for reintroduction are hampered by a partial or complete absence of lineage information, requiring lineages to be reconstructed and thus using time and money that could be spent on wild populations. Seed bank curators must invest considerable effort in collecting and maintaining as much information about accessions as possible, much more than is commonly gathered presently, because the future genetic diversity and conservation utility of the material they are trying to preserve will be contingent on these efforts.

Active research at both the level of preserving specific species and at the level of broadening our conceptual understanding of genetic and phenotypic variation in wild populations is badly needed. In the short term we need to answer questions like, "Does seed storage cause nonrandom mortality, do seed regeneration conditions lead to differential fecundity, and what selective conditions are ex situ samples experiencing relative to wild populations?" Accurate answers will consider genetic correlation structure, which can constrain or produce indirect evolutionary change in wild populations, and the effects of genotype-by-environment interactions on the genotypic diversity of seed banks. Unfortunately, these questions may need to be addressed for many individual species, making this an unrealistic task unless generalizations emerge from conceptually based research. Plant species with naturally occurring seed banks may provide valuable models for ex situ methods and their evolutionary contexts and constraints (see Venable 1989). The recognition and empirical investigation of continuous genetic and phenotypic traits should be an area of active research in conservation biology. Data that address the broad question of the nature and number of genes affecting a quantitative trait will provide a basis to estimate the correlation between levels of quantitative genetic variation and the allelic variation of DNA and enzymes, and will increase our understanding of the relationship between polygenic traits and the individual genes that control them. Most important, all conservation efforts related to genetic diversity need to include the collection of initial data and monitoring as crucial elements of the process in order to provide a description of initial conditions that can be used periodically to assess progress toward goals and revise them if necessary. Without careful and planned data collection we can never objectively determine the relative success or failure of any type of conservation methods or strategies, ex situ or otherwise.

Many conservation biologists have commented that theoretical considerations must yield rapidly to action if species are to be saved, even if those actions are not ideal and may not achieve all of the goals that conservationists would like to address. It is clear that ex situ methods provide conspicuous measures of "success" for conservation programs (seeds *do* get collected and stored) in light of often snarled efforts to preserve ecosystem-level diversity through land purchase and management reform. It is not clear, however, that ex situ methods will result in significant conservation of genetic variation and evolutionary potential without a great expansion of the scope of biological information used to establish and monitor such programs. Accepting these limitations can allow ex situ efforts to be more realistically focused and resources to be channeled into in situ conservation strategies that will be more effective in preserving not only present genetic variation but also the dynamic forces that produce and shape past, present, and future genetic variation.

Acknowledgments

I thank three anonymous reviewers, as well as A. Brown, B. Brumback, S. Dudley, S. Gaines, C. Purrington, and A. Schmitt for helpful discussion and comments that greatly improved the form and content of earlier drafts of the manuscript. I am grateful to L. Dorn for helpful comments and permission to use unpublished data.

Literature Cited

Adams, R. P., and J. E. Adams. 1992. Conservation of plant genes. Academic Press, San Diego, California.

Allendorf, F. W., and R. F. Leary. 1986. Heterozygosity and fitness in natural populations of animals. Pages 57–76 in M. E. Soulé, editor. Conservation biology. Sinauer, Sunderland, Massachusetts.

Ashton, P. S. 1987. Biological considerations in *in situ* vs *ex situ* plant conservation. Pages 117–130 in D. Bramwell, O. Hamann, V. Heywood, and H. Synge, editors. Botanic gardens and the world conservation strategy. Academic Press, London, England.

Ashton, P. S. 1988. Conservation of biological diversity in botanical gardens. Pages 269–278 in E. O. Wilson, editor. Biodiversity. National Academy Press, Washington, D.C.

Beardmore, J. A. 1983. Extinction, survival and genetic variation. Pages 125–151 in C. M. Schoenwald-Cox, S. M. Chambers, B. MacBryde, and L. Thomas, editors. Genetics and conservation. Benjamin-Cummings, Menlo Park, California.

Brown, A. H. D., and J. D. Briggs. 1991. Sampling strategies for genetic variation in *ex situ* collections of endangered plant species. Pages 99–119 in D. A. Falk and K. E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, New York, New York.

Brush, S. B. 1989. Rethinking crop genetic resource conservation. Conservation Biology **3**:19–29.

Center for Plant Conservation. 1986. Recommendations for the collection and *ex situ* management of germplasm resources from wild plants. Jamaica Plain, Massachusetts.

Center for Plant Conservation. 1991. Genetic sampling guidelines for conservation collections of endangered plants. Pages 225–238 in D. A. Falk and K. E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, New York, New York.

Crow, J. F., and M. Kimura. 1970. An introduction to population genetic theory. Harper and Row, New York.

Eberhart, S. A., E. E. Roos, and L. E. Towill. 1991. Strategies for long-term management of germplasm collections. Pages 135–145 in D. A. Falk and K. E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, New York, New York.

Edwards, M. D., C. W. Stuber, and J. F. Wendel. 1987. Molecular-marker-facilitated investigations of quantitative trait loci in maize. I. Numbers, genomic distribution and types of gene action. Genetics **116**:113–125.

Endler, J. A. 1986. Natural selection in the wild. Princeton University Press, Princeton, New Jersey.

Evans, A. S. 1991. Leaf physiological aspects of nitrogen-use efficiency in *Brassica campestris* L.: Quantitative genetic variation across nutrient treatments. Theoretical and Applied Genetics **81**:64–70.

Falconer, D. S. 1989. Introduction to quantitative genetics. 3rd edition. Longman, London, England.

Falk, D. A. 1987. Integrated conservation strategies for endangered plants. Natural Areas Journal **7**:118–123.

Falk, D. A. 1990. The theory of integrated conservation strategies for biological conservation. Pages 5–10 in R. S. Mitchell, C. J. Sheviak, and D. J. Leopold, editors. Ecosystem management: Rare species and significant habitats, Proceedings of the 15th Natural Areas Conference. New York State Museum, Albany, New York.

Falk, D. A. 1991. Joining biological and economic models for conserving plant genetic diversity. Pages 209–223 in D. A. Falk and K. E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, New York, New York.

Frankel, O. H. 1974. Genetic conservation: Our evolutionary responsibility. Genetics **78**:53–65.

Frankel, O. H., and M. E. Soulé. 1981. Conservation and evolution. Cambridge University Press, Cambridge, England.

Given, D. 1990. Conserving botanical diversity on a global scale. Annals of the Missouri Botanical Garden **77**:48–62.

Gould, S. J. 1989. Wonderful life: The Burgess Shale and the nature of history. W. W. Norton, New York, New York.

Hallauer, A. R., and J. B. Miranda. 1981. Quantitative genetics in maize breeding. Iowa State University, Ames, Iowa.

Hamrick, J. L., and M. J. W. Godt. 1989. Allozyme diversity in plant species. Pages 43–63 in A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir, editors. Plant population genetics, breeding, and genetic resources. Sinauer, Sunderland, Massachusetts.

Hamrick, J. L., M. J. W. Godt, D. A. Murawski, and M. D. Loveless. 1991. Correlations between species traits and allozyme diversity: Implications for conservations biology. Pages 75–86 in D. A. Falk and K. E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, New York, New York.

Hartl, D. L., and A. G. Clark. 1989. Principles of population genetics. Sinauer, Sunderland, Massachusetts.

Hawkes, J. G. 1976. Sampling gene pools. Pages 145–154 in J. B. Simmons, R. I. Beyer, P. E. Brandham, G. LI. Lucas, and V. T. H. Parry, editors. Conservation of threatened plants. Plenum Press, New York, New York.

Heywood, V. H. 1992. Efforts to conserve tropical plants—a global perspective. Pages 1–14 in R. P. Adams, and J. E. Adams, editors. Conservation of plant genes. Academic Press, San Diego, California.

Holsinger, K. E., and L. D. Gottlieb. 1991. Conservation of rare and endangered plants: principles and prospects. Pages 195–208 in D. A. Falk and K. E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, New York.

Jordan, N. 1991. Multivariate analysis of selection in experimental populations derived from hybridization of two ecotypes of the annual plant *Diodoa teres* W. (Rubiaceae). Evolution **45**:1760–1772.

Karron, J. D. 1991. Patterns of genetic variation and breeding systems in rare plant species. Pages 87–98 in D. A. Falk and K. E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, New York.

Lacey, E. P. 1986. The genetic and environmental control of reproductive timing in a short-lived monocarpic species *Daucus carota* (Umbelliferae). Journal of Ecology **74**:73–86.

Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. Evolution **37**:402–416.

Lande, R. 1988*a*. Genetics and demography in biological conservation. Science **241**:1455–1460.

Lande, R. 1988*b*. Quantitative genetics and evolutionary theory. Pages 71–84 in B. S. Weir, E. J. Eisen, M. M. Goodman, and G. Nankoong, editors. Proceedings of the Second International Conference on Quantitative Genetics. Sinauer Associates, Sunderland, Massachusetts.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution **37**:1210–1227.

Lande, R., and G. F. Barrowclough. 1987. Effective population size, genetic variation, and their use in population management. Pages 87–124 in M. E. Soulé, editor. Viable populations for management. Cambridge University Press, Cambridge, England.

Li, W. H., and D. Graur. 1991. Fundamentals of molecular evolution. Sinauer Associates, Sunderland, Massachusetts.

Marshall, D. R., and A. H. D. Brown. 1975. Optimum sampling strategies in genetic conservation. Pages 53–80 in O. H. Frankel, and J. G. Hawkes, editors. Crop genetic resources for today and tomorrow. Cambridge University Press, Cambridge, England.

Mather, K., and J. L. Jinks. 1977. Introduction to biometrical genetics. Chapman and Hall, London, England.

Mather, K., and J. L. Jinks. 1982. Biometrical genetics. Chapman and Hall, London, England.

Mitchell-Olds, T., and J. Bergelson. 1990. Statistical genetics of an annual plant, *Impatiens capensis*. II. Natural selection. Genetics **124**:417–421.

Mitchell-Olds, T., and J. J. Rutledge. 1986. Quantitative genetics in natural plant populations: A review of the theory. American Naturalist **127**:379–402.

National Research Council. 1978. Conservation of germplasm resources, an imperative. National Academy of Sciences, Washington, D.C.

New England Wild Flower Society. 1992. New England plant conservation program. Wild Flower Notes **7**:1–79.

Roughgarden. 1979. Theory of population genetics and evolutionary ecology: An introduction. MacMillian Publishing, New York, New York.

Schaal, B. A., S. L. O'Kane, Jr., and S. H. Rogstad. 1991*a*. DNA variation in plant populations. Trends in Ecology and Evolution **6**:329–333.

Schaal, B. A., W. J. Leverich, and S. H. Rogstad. 1991*b*. A comparison of methods for assessing genetic variation in plant conservation biology. Pages 123–134 in D. A. Falk, and K. E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, New York, New York.

Scheiner, S. M. 1989. Variable selection along a successional gradient. Evolution **43**:548–562.

Scheiner, S. M., J. Gurevitch, and J. Teeri. 1984. A genetic analysis of the photosynthetic properties of *Danthonia spicata* that have different growth responses to light level. Oecologia **64**:74–77.

Schlicting, C. D. 1986. The evolution of phenotypic plasticity. Annual Reviews of Ecology and Systematics **17**:667–693.

Schmitt, J., and J. Antonovics. 1986. Experimental studies of the evolutionary significance of sexual reproduction. III. Maternal and paternal effects during seedling establishment. Evolution **40**:817–829.

Schoen, D. J., and H. D. Brown. 1991. Intraspecific variation in population gene diversity and effective populations size correlates with the mating system in plants. Proceedings of the National Academy of Sciences U.S.A. **88**:4494–4497.

Shrimpton, A. E., and A. Robertson. 1988. The isolation of polygenic factors controlling bristle score in *Drosophila melanogaster*. I. Allocation of third chromosome sternopleural bristle effects to chromosome sections. Genetics **118**:437–443.

Simon, C., and J. Archie. 1985. An empirical demonstration of the liability of heterozygosity estimates. Evolution **39**:463–467.

Stearns, S. C. 1989. The evolutionary significance of phenotypic plasticity. BioScience **39**:436–445.

Stuber, C. W. 1989. Isozymes as markers for studying and manipulating quantitative traits. Pages 206–220 in D. E. Soltis and P. S. Soltis, editors. Isozymes in plant biology. Dioscorides Press, Portland, Oregon.

Templeton, A. R. 1991. Off-site breeding of animals and implications for plant conservation strategies. Pages 182–194 in D. A. Falk and K. E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, New York, New York.

Thornhill, A., and H. Koopowitz. 1992. Viability of *Disa uniflora* Berg (Orchidaceae) seeds under variable storage conditions: Is orchid gene-banking possible? Biological Conservation 21–27.

Venable, D. L. 1989. Modeling the evolutionary ecology of seed banks. Pages 67–87 in M. A. Leck, V. T. Parker, and R. L. Simpson, editors. Ecology of soil seed banks. Academic Press, San Diego, California.

Venable, D. L., and A. Burquez M. 1989. Quantitative genetics of size, shape, life-history, and fruit characteristics of the seed-heteromorphic composite *Heterosperma pinnatum*. I. Variation within and among populations. Evolution **43**:113–124.

Venable, D. L., and A. Burquez M. 1990. Quantitative genetics of size, shape, life-history, and fruit characteristics of the seed-heteromorphic composite *Heterosperma pinnatum*. II. Correlation structure. Evolution **44**:1748–1763.

Wilson, E. O. 1988. The current state of biological diversity. Pages 3–18 in E. O. Wilson, editor. Biodiversity. National Academy Press, Washington, D.C.

Wright, S. 1948. On the roles of directed and random changes in gene frequency in the genetics of populations. Evolution **2**:279–294.

Zobel, B. 1977. Gene conservation—as viewed by a forest tree breeder. Forest Ecology and Management **1**:339–344.