



Genetic consequences of ecological reserve design guidelines: An empirical investigation

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Abstract

We assessed the genetic diversity consequences of applying ecological reserve design guidelines to four federally-listed globally-rare plant species. Consequences were measured using two metrics: proportion of all alleles and of common alleles included in reserves. Common alleles were defined as those alleles having a frequency of ≥ 0.05 in at least one population. Four conservation professionals applied ecological reserve guidelines to choose specific populations of each species for inclusion in reserves of size 1 to $N - 1$, where N is the total number of populations of each species. Information regarding genetic diversity was not used in selecting populations. The resulting reserve designs were compared to random designs, and the agreement among experts was assessed using Kendall's coefficient of concordance. Application of ecological reserve design guidelines proved mostly ineffective in capturing more genetic diversity than is captured selecting populations randomly. Meeting established targets for genetic diversity, such as one advocated by the Center for Plant Conservation, required larger numbers of populations than are suggested to be sufficient. Relative performance of expert designs differed among species and was dependent on whether the proportion of all alleles or of common alleles was used as a measure of diversity. Furthermore there was no significant concordance among experts in order in which populations were incorporated into reserves as experts differed in priority they placed on individual guidelines.

Introduction

A major objective of conservation is to maintain biodiversity by promoting long-term persistence of species as part of native ecosystems (e.g., Harrison et al. 1984; Falk 1992). Because probability of species persistence increases in large blocks of habitat, much attention has focused on establishing reserves and reserve networks to maintain habitat and to slow rates of habitat loss and fragmentation (e.g., Noss 1983; Soulé and Simberloff 1986; Murphy and Noon 1992; Sætersdal and Birks 1993; Nantel et al. 1998; Soulé and Terborgh 1999). Because it is most often not

possible to commit all potential sites to conservation purposes it is necessary for conservation practitioners to decide both how many and which sites are necessary to conserve biological diversity. Increasingly, representing the amount and patterns of diversity in a system is advocated as an important conservation objective and as a means to conserve this diversity efficiently (e.g., Csuti et al. 1997; Margules and Pressey 2000). Such efficiency is important due to conflicts between committing areas to conservation and using them to meet other societal needs and desires. Historically, lands have been selected for conservation for a variety of reasons including

aesthetic value, presence of habitat for particular organisms, valuable resources for society (e.g., watersheds for domestic water supply), and availability. In many cases the resulting networks of protected areas do not conserve all biological diversity in an area; some diversity elements are over-represented while others remain underrepresented or are not represented at all (reviewed in Scott et al. 1993; Rodrigues et al. 1999; Scott et al. 2001). Although reserves can be an effective way to maintain native biodiversity, our ability to establish new reserves is frequently limited. Therefore it is also important to develop land use strategies (i.e., in the context of multiple use management) that will allow native species to persist in the face of some level of human activity.

Representative reserve networks for communities include examples of the range of vegetation types (e.g., Scott et al. 1993) or landscape features (Poiani et al. 2000) in an area in order to have a high probability of representing the largest range of taxa, while efforts focused on species richness include at least one occurrence of each species of interest (e.g., Csuti et al. 1997). Although habitat loss and fragmentation are often more immediate threats, representing levels and patterns of within-species genetic diversity in viable populations is important because of the relationship between genetic diversity and probability of persistence as well as maintaining future evolutionary potential (Barrett and Kohn 1991; Ellstrand and Elam 1993; Newman and Pilson 1997). Unquestionably, the best way to represent genetic diversity in a subset of populations is to base conservation decisions on known levels of diversity within and distribution of diversity among populations. Unfortunately, such data are lacking for most species; due to limited time and funding it is not possible to characterize diversity patterns in most taxa. Further, when conservation decisions are based on biological data, they tend to focus on ecological rather than genetic characteristics (e.g., Schemske et al. 1994; Noss et al. 1997; Soulé and Terborgh 1999). It is hoped or assumed that genetic diversity within species is also represented when conservation decisions are based on ecological characteristics. Our purpose is to examine the validity of this assumption.

A number of ecological reserve selection and design principles derived from theoretical island biogeography, community ecology, population genetics, and population biology are commonly advocated to guide selecting sites for conservation (e.g., Noss et al. 1997; Margules and Pressey 2000):

1. Conserve as large an area as possible to maintain redundancy in populations or habitat patches, to support large population sizes and habitat (community) mosaics. Redundancy is thought to buffer populations against stochastic and catastrophic events (Diamond 1975; Simberloff and Abele 1982; Noss 1987; Murphy and Noon 1992; Opdam et al. 1994).
2. Favor large populations of target species and patches of target communities over small ones (Templeton et al. 1990; Murphy and Noon 1992).
3. Represent the natural geographic and ecological range of the target taxon or community in protected sites (Austin and Margules 1986; Noss et al. 1997; Margules and Pressey 2000). Such representation increases the likelihood that the range of genetic variation within species will be represented (Namkoong 1993; Holsinger and Vitt 1997), that a whole species will not be exposed to the same environmental conditions (Stacey and Taper 1992), and that species interactions that likely vary among locations will be maintained (Thompson 1996).
4. Favor sites that contain more than one rare taxon or community when all other criteria are equivalent.
5. Minimize edge-to-area ratio and internal fragmentation of reserves to reduce risk of invasion by non-native taxa and maintain ecosystem integrity (Murcia 1995).
6. Maintain connections among historically connected populations or habitat patches via corridors (Simberloff 1988; Templeton et al. 1990).
7. Represent as much as possible the range of natural features present in an area (Bedward et al. 1992).

The main purpose of this research was to assess the genetic diversity consequences of applying these seven guidelines, as interpreted by conservation professionals, to four rare plant species. Consequences were assessed by comparing the amount of genetic diversity included in reserve networks selected by experts using the guidelines with the amount included in the same number of populations selected randomly (Neel and Cummings 2003). Genetic diversity was characterized as richness of all allozyme alleles and common allozyme alleles. Common alleles were defined as electrophoretically detectable alleles that occur at a frequency ≥ 0.05 in at least one population of a species. Alleles below this frequency are considered by some to be evolutionarily insignificant in that they will likely be lost to drift in relatively few generations and they contribute little to heterozygosity (e.g., Marshall and Brown 1975). The

amount of diversity included in reserve networks was also compared with an accepted standard for genetic diversity conservation developed by the Center for Plant Conservation (1991) that recommends having a 90–95% probability of including all common alleles. As such, this research contributes both to understanding the genetic diversity consequences of applying ecological guidelines and to understanding how many populations are required to represent within-species genetic diversity when diversity patterns are unknown. This approach is in contrast to maximizing genetic diversity when patterns of within and among populations variation are known (e.g., Namkoong 1993; Ceska et al. 1997; Petit et al. 1998). Although it is equally important, directly addressing maintenance of diversity over time was beyond the scope of this research. Additionally we do not address conservation of patterns of within and among population variance as estimated by hierarchical *F* statistics because these estimates were shown not to vary with the number of populations included in randomly selected reserve designs (Neel and Cummings 2003). We also do not assess the relationships between specific ecological principles and their genetic consequences. Rather we simulate real conservation decision-making processes where multiple guidelines would be used simultaneously.

The question of how many populations are necessary to capture existing genetic diversity is important because clearly more than a single example of a particular community or species is necessary to represent genetic diversity and to provide an acceptable probability of persistence. But just how many populations are necessary is not clear, and as mentioned previously, there is great pressure to commit only minimal areas to conservation. While each species and conservation situation is unique it is helpful to have some guidance for how much conservation effort to expend on a particular species in lieu of representing additional species. A range of general target conservation intensities have been suggested, only one of which is specifically for genetic diversity. Based on theoretical predictions developed by Marshall and Brown (1975) and Brown and Briggs (1991), the Center for Plant Conservation (1991) suggests that sampling from five rare plant populations is sufficient to conserve most of the evolutionarily significant genetic variation (as represented by common allozyme alleles) for *ex situ* conservation. The World Conservation Union recommends protection of 10–12% of the land area or 10–12% of the area of each ecosystem

in a nation or region in order to maintain general species diversity (cited in Noss 1996; Soulé and Sanjayan 1998). Duffy et al. (1999) considered rare plant species in Alaska to be adequately represented if 10–12% of the populations of each species were in protective status. Kiestler et al. (1996) considered three populations sufficient to represent mid-size mammalian predators in Idaho. Additionally, methods to select reserves that represent all desired elements of biodiversity in the smallest area or number of sites (minimum-set or complementarity approaches) most often include only one occurrence of individual species (e.g., Pressey and Nicholls 1989; Margules and Pressey 2000; Sætersdal et al. 1993; Csuti et al. 1997; Cowling et al. 1999).

These targets provide a range of conservation intensities ranging from one to five populations of the species studied here. Neel and Cummings (2003) demonstrated that five populations would conserve on average 67–85% and as little as 54% of all alleles if populations were selected randomly without knowledge of genetic diversity patterns. The same conservation intensity would conserve on average 85–93% and as little as 69% of all common alleles. In this study we address whether the amount of genetic diversity included in subsets of populations selected according to ecological reserve design guidelines is significantly different from random. We also examine variation among practitioners in interpretation and application of these guidelines, and provide a summary of the strategies used.

Methods

We used empirical allozyme allele richness data (for all alleles and common alleles) from multiple populations of four federally-listed plant taxa (Neel 2000; Neel and Ellstrand 2001, in press). The taxa were *Astragalus albens* E. Greene (Fabaceae), *Erigeron parishii* A. Gray (Asteraceae), *Eriogonum ovalifolium* var. *vineum* (Small) Jepson (Polygonaceae), and *Oxytheca parishii* var. *goodmaniana* B. Ertter (Polygonaceae). The four taxa are globally rare and three are endemic to limestone and dolomite substrates occurring between 1000 and 2800 meters elevation in the northeastern San Bernardino Mountains of southern California, U.S.A (Neel 2000). One taxon, *Erigeron parishii*, although primarily restricted to limestone and dolomite substrates in this mountain range, has one extant cluster of occurrences on quartz

monzonite in the same mountain range and one historic occurrence on quartz monzonite in the Little San Bernardino Mountains. These taxa have similar range sizes and overlap throughout most of their distributions. They are also all threatened throughout the majority of their ranges primarily by limestone mining operations (U.S. Fish and Wildlife Service 1994). The taxa differ, however, in the specific microhabitats they occupy (Gonella and Neel 1995; Neel 2000) and different populations of each species are associated with a range of environments and natural features including steep slopes, rock outcrops, alluvial fans, and ephemeral washes. Further, they are not closely related phylogenetically (representing three angiosperm families in three distantly related orders) and have different population genetic structures (Neel 2000; Neel and Ellstrand 2001, in press).

Data came from up to 30 individuals in each of 14–31 populations for each taxon. Populations were chosen to represent the ecological and geographic range of each species. Numbers of sampled populations, loci, and total and common alleles for each taxon were as follows: *A. albens*, 30 populations, 12 loci, and 69 alleles (36 common); *E. parishii*, 31 populations, 14 loci, and 60 alleles (49 common); *E. ovalifolium* var. *vineum*, 31 populations, 11 loci, and 60 alleles (48 common); and *O. parishii* var. *goodmaniana*, 14 populations, 12 loci, and 41 alleles (31 common) (Neel and Cummings 2003). Reserve networks of 1 to $N - 1$ populations (where N is the total number of populations of each taxon) were selected by four professional conservation practitioners using the guidelines described in the introduction. The experts were conservation professionals (a biological consultant, a plant conservation advocate, a federal agency botanist, and one of the authors [M.C.N.]) who have considerable knowledge of these taxa and who have been active in planning for their conservation. In addition to the guidelines, experts were provided distribution maps for populations of each species, a description of the vegetation type in which each population occurred, and the elevation of each population. They were provided no additional direction on interpretation of the guidelines or information on patterns of genetic diversity within the taxa. Thus, this exercise closely resembles actual conservation situations and requires no simplifying assumptions associated with simulations.

For each species/expert combination, the proportion of the total number of all alleles and common alleles captured by each design strategy was calcu-

lated separately for each number of populations from 1 to $N - 1$ using the computer program Genetic Data Analysis (GDA) (Lewis and Zaykin 2001). For each number of populations we then calculated the difference in proportion of total and common alleles between expert-selected reserves and the mean of the 1000 random samples from Neel and Cummings (2003) as

$$D = (X_i - \bar{Y}_i)$$

where i = the number of populations, X_i = the proportion of alleles included in an expert design of size i and \bar{Y}_i the mean proportion of alleles included in 1000 replicate random samples of size i . Expert reserve designs of specific population numbers were considered significantly different from random if the probability of D was <0.05 (i.e., fell in the upper or lower 0.025 intervals of the 1000 replicate random samples). Deviation of overall expert reserve design strategies from random was defined as

$$\sum |D| = \sum_{i=1}^{N-1} |(X_i - \bar{Y}_i)|$$

for each taxon. This deviation was considered significantly different from random if the probability of $\sum |D|$ was <0.05 based on the 1000 replicate random samples. The sign of $\sum D$ defined as

$$\sum D = \sum_{i=1}^{N-1} (X_i - \bar{Y}_i)$$

is the overall direction of the differences over all numbers of populations.

The proportion of all alleles and common alleles included in five expert-selected populations was examined and compared with random samples of five populations to assess how well currently suggested conservation intensities succeeded at representing genetic diversity. We also determined the smallest number of populations required to include all alleles and common alleles in expert reserve selections. These numbers were compared to the smallest number of randomly-selected populations that included all alleles and all common alleles. Additionally, we compared the number of expert-selected populations needed to include all common alleles with the number of randomly selected populations needed to have a 0.90–0.95 probability of including all common alleles [i.e., the Center for Plant Conservation (1991) standard].

Thus, we assessed differences between expert and random reserve selections in three ways. The first was to compare each individual selection of each number of populations for each expert and species. The second was to examine the overall design strategy over all numbers of populations for each expert and each species. Third we examined the diversity included at specific sampling intensities (e.g., five populations, which is the level suggested by the Center for Plant Conservation (1991), and the minimum number of populations chosen by experts and random sampling containing all alleles or all common alleles). Together, these comparisons assessed the potential for general reserve design guidelines based on ecological characteristics to conserve genetic diversity when patterns of such diversity are unknown.

Many of our comparisons focused on including all alleles or common alleles not because we feel this is always a realistic conservation goal, but rather because it provides unambiguous and objective measures of genetic diversity with which to compare reserve selection methods across species. Additionally, while there is general agreement that reduced genetic diversity decreases evolutionary potential and has a negative impact on short-term viability (Holsinger and Gottlieb 1991; Ellstrand and Elam 1993), there is no information on exactly how much genetic diversity can be lost before these impacts are realized and therefore there is no biological justification for an acceptable level of loss.

To examine variation among experts in the effects of their interpretation and application of the guidelines for each species, we recorded the order in which each expert selected populations for inclusion in a reserve network. Overall agreement in the order of inclusion of populations among experts was evaluated using Kendall's coefficient of concordance (W). To determine the underlying structure of the concordance/discordance we used Kendall's coefficient of rank correlation (Kendall's τ) to assess correspondence between all pairs of experts within each species. W and τ range from 0 (no concordance/correlation in the order of population selection) to 1 (perfect concordance).

Results

All alleles

Individual selections. *A priori* we have a null expectation that out of a possible 424 specific expert/species/population number comparisons approximately 21.5 would be significantly different from random at $\alpha = 0.05$; however, only 5 (0.0118) were actually significantly different from random at this α (Figure 1). Three of these differences were above expectations and two were below (Figure 1).

Overall design strategy. Of 16 overall expert strategies (patterns across all numbers of populations), four had significantly higher allele richness (both Experts B and D for both *A. albens* and *E. ovalifolium* var. *vineum*) and none had significantly lower allele richness than randomly selected populations (Figure 1). By chance we would expect ~ 1 strategy to be significantly different than random. Thus, while reserve designs of specific numbers of populations did not deviate from random more often than expected by chance, overall reserve strategies of two experts did for two taxa.

Specific conservation intensities. Experts included between 0.65 (Expert D, *E. parishii*) and 0.92 (Expert B, *O. parishii* var. *goodmaniana*) of all alleles in five populations (Figure 2) compared with an average of 0.67–0.83 of all alleles in five randomly selected populations (Neel and Cummings 2003). Only 14 populations of *Oxytheca parishii* var. *goodmaniana* were included in the study thus five populations represented a relatively large proportion of the total number of populations. For the other 3 species with more than twice the total number of sampled populations, 5 populations included less than 0.80 of all alleles in 11 of 12 expert-species combinations (Figure 2).

The number of populations required to include all alleles varied among experts and among species, ranging from 21 (0.68; Expert D, *E. ovalifolium* var. *vineum*) to 31 (1.0; Experts A and B, *E. parishii* and Expert C, *E. ovalifolium* var. *vineum*), as well as all 14 *O. parishii* var. *goodmaniana* populations (Experts A, B, and C) (Figure 2). The corresponding minimum proportion of randomly selected populations necessary to include all alleles was substantially less, and ranged from 0.42 (*O. parishii* var. *goodmaniana*) to 0.57 (*A. albens*) (Neel and Cummings 2003). At least

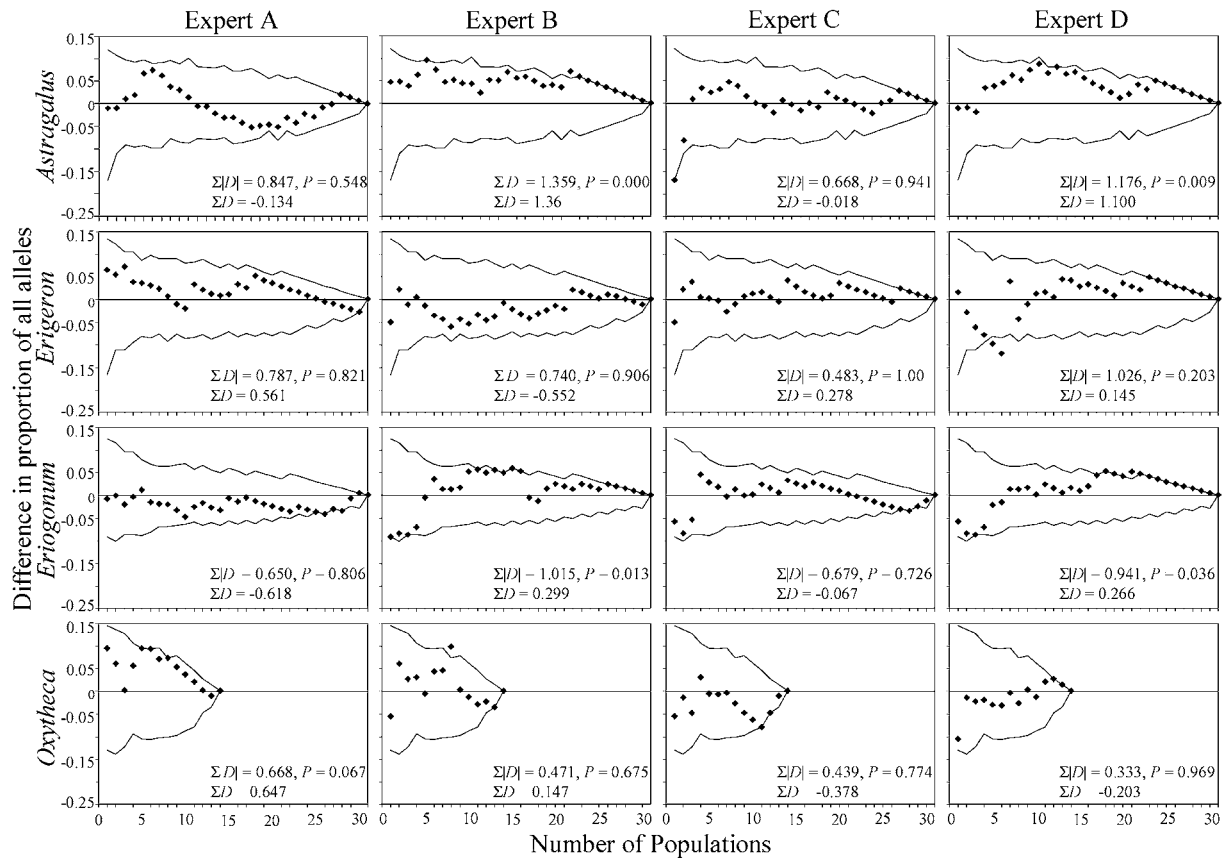


Figure 1. Differences in proportion of all alleles between expert- and random-selected populations, D , as a function of number of populations sampled; 95% confidence limits are denoted with thin lines. Data for the randomly selected populations are from Neel and Cummings (2003). The test statistic sum of absolute differences ($\sum|D|$) and its associated P -value assess the significance of differences of overall expert-selected reserve designs from random expectation. The sign of the sum of the differences ($\sum D$) indicates the direction of this difference.

0.90 of populations were required to capture all alleles in 8 of 16 expert-species combinations.

Common alleles

Individual selections. Again, *a priori* we have a null expectation that out of a possible 424 specific expert/species/population number comparisons approximately 21.5 would be significant at $\alpha = 0.05$, however only 14 (0.033) were significantly different from random at this α (Figure 3). Ten differences were below expectation and four were above.

Overall design strategy. Of 16 expert strategies, 2 had significantly lower common allele richness (Expert B, *E. parishii*; Expert C, *A. albens*) and none had significantly higher common allele richness than randomly selected populations (Figure 3).

Specific conservation intensities. Experts included between 0.77 (Expert D, *E. parishii*) and 0.97 (Expert C, *A. albens*) of all common alleles in five populations (Figure 2). At least 0.90 of common alleles were included in 5 populations for 8 of 16 expert-species combinations (Table 1, Figure 2). The number of populations that experts required to include all common alleles ranged from 7 (0.23; Expert A, *A. albens*) to 31 (1.0; Expert B, *E. parishii*) (Figures 2 and 4). For each species the proportion of populations required to capture all common alleles varied among experts and at least one expert required 0.60–1.0 of the populations (Figures 2 and 4) compared with 0.10 (3, *A. albens*) to 0.29 (9, *E. parishii*) when populations were selected randomly. More than 0.70 of the populations of a species were required to include all common alleles in 6 out of 16 cases and 0.50–0.70 of the populations were required in five additional cases.

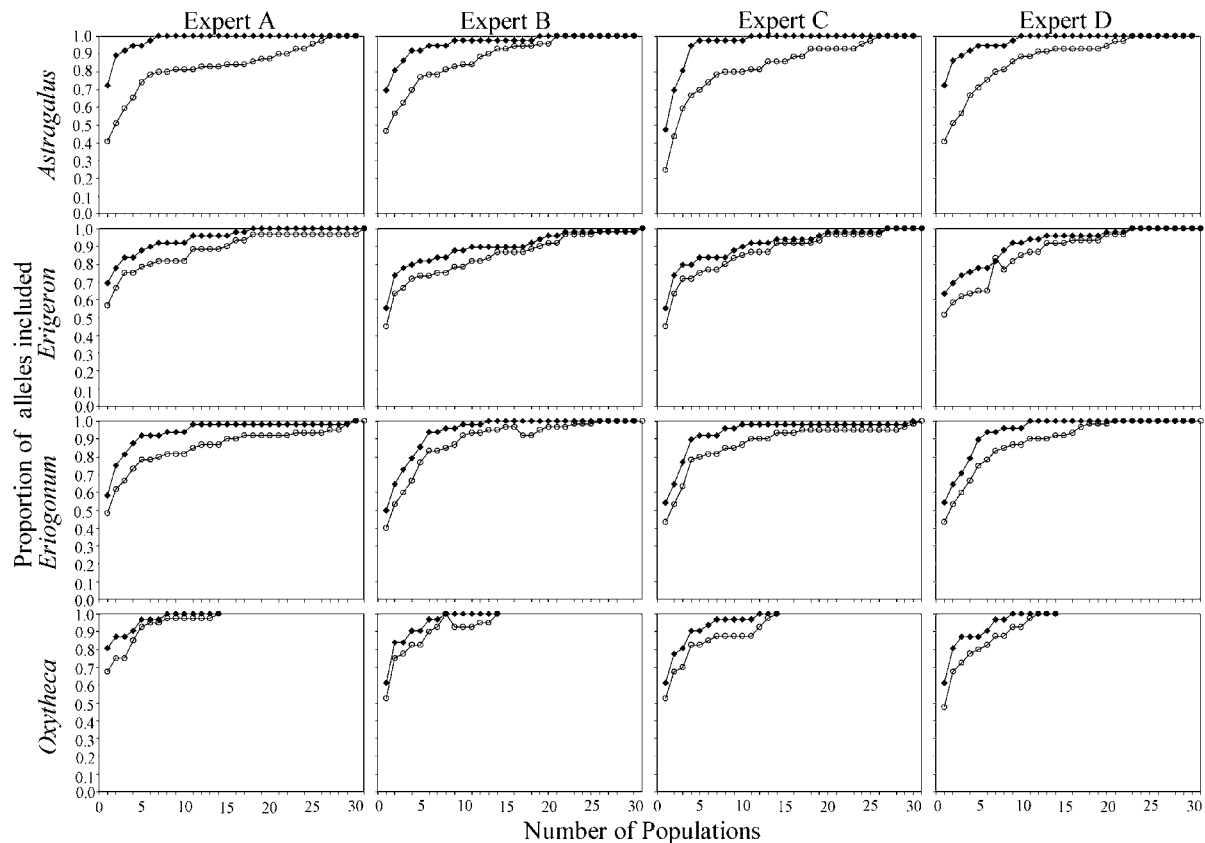


Figure 2. Accumulation curves for all alleles (open circles) and common alleles (filled diamonds) for each taxon (rows) and each expert (columns).

The number of expert-selected populations needed to capture all common alleles was equal to or greater than the number of randomly-selected populations needed to meet the 0.90 probability standard 3 of 16 times and equaled the number needed to meet the 0.95 standard 2 times (Figure 4). The number of expert-selected populations was always greater than the smallest number of randomly-selected populations included all common alleles (Figure 4).

Concordance among experts

Kendall's W indicated no significant concordance among the orders in which the four experts chose populations for any species (*A. albens*, $W = 0.0138$, $P = 0.741$; *E. parishii*, $W = 0.017$, $P = 0.658$; *E. ovalifolium* var. *vineum*, $W = 0.007$, $P = 0.888$; *O. parishii* var. *goodmaniana*, $W = 0.02$, $P = 0.749$). In pair-wise comparisons of experts using Kendall's τ , population selection by Expert B was significantly correlated with at least one other expert for each

species. These correlations were positive between Experts B and C for two of the four species (*E. parishii*, $\tau = 0.29$, $P = 0.024$; *O. parishii* var. *goodmaniana*, $\tau = 0.44$, $P = 0.026$) and between Experts B and D for three of the four species (*A. albens*, $\tau = 0.33$, $P = 0.008$; *E. ovalifolium* var. *vineum*, $\tau = 0.52$, $P = 0.00003$; *O. parishii* var. *goodmaniana*, $\tau = 0.40$, $P = 0.045$). The order in which Expert A selected populations was negatively correlated with Expert B for *E. parishii* ($\tau = -0.33$, $P = 0.008$) but was never correlated with Experts C or D.

While among-expert variation in population selection order was high, individual experts followed the same strategy for all four species. As inferred by their population selection patterns, experts differed in terms of which of the seven guidelines they emphasized and the order in which guidelines took precedence at a given decision point, although they all typically emphasized population size (as estimated by mapped population extents). Expert A first selected a large centrally located population and then empha-

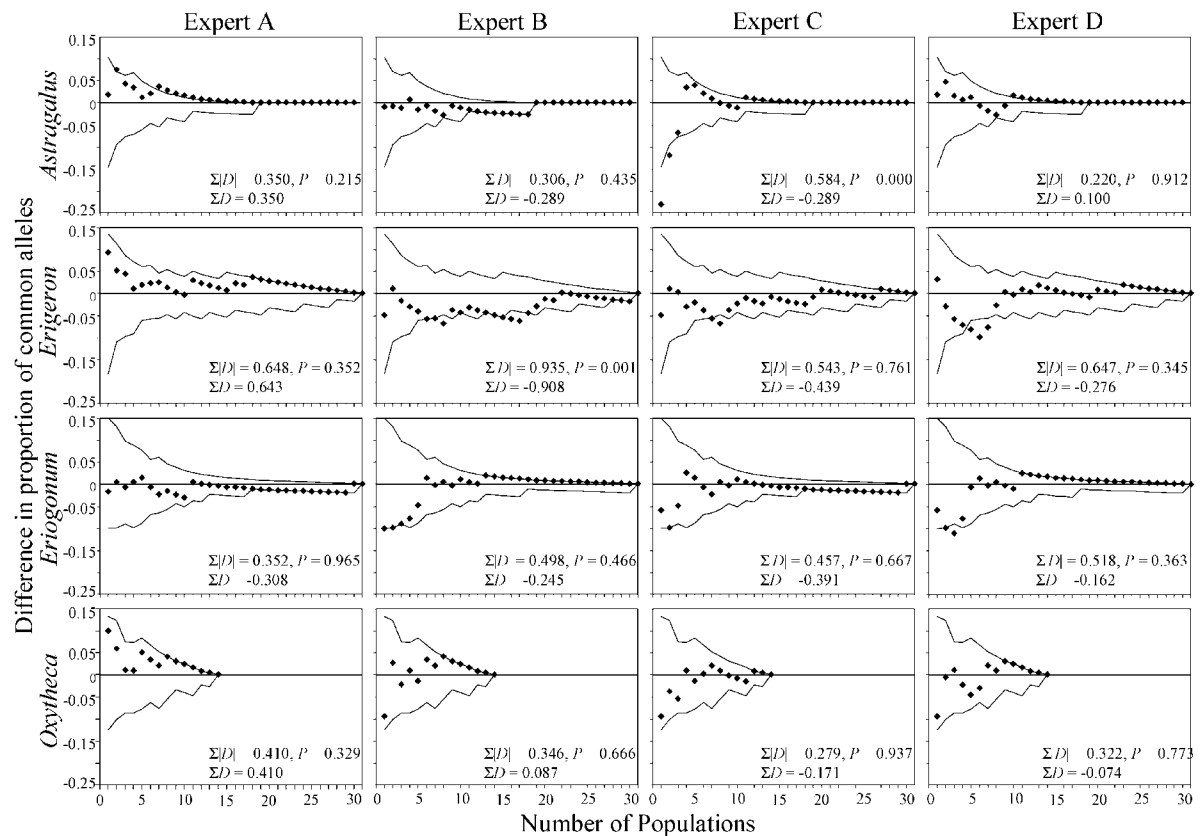


Figure 3. Differences in proportion of common alleles between expert- and random-selected populations, D , as a function of number of populations sampled; 95% confidence limits are denoted with thin lines. Data for the randomly selected populations are from Neel and Cummings (2003). The test statistic sum of absolute differences ($\sum|D|$) and its associated P -value assess the significance of differences of overall expert-selected reserve designs from random expectation. The sign of the sum of the differences ($\sum D$) indicates the direction of this difference.

sized maintaining geographic and ecological ranges of each species by selecting the largest populations from the range extremes as characterized by geographic distribution, elevation, and vegetation types. Populations adjacent to those already selected were aggregated only after the extremes were represented. In contrast, experts B, C, and D placed more emphasis on maintaining connections among existing populations, although they differed in their specific approaches. Expert B began with a large population from a geographically central portion of a species range always including adjacent populations in closest proximity to those already selected such that the resulting reserve network had a relatively high density of populations. Experts C and D identified closely distributed clusters of populations and selected all populations within a cluster before selecting from another cluster, although they differed in how they

selected clusters. Expert C first aggregated a dense cluster of relatively large populations near the center of the species distribution and then added population clusters from the perimeter of the geographic and ecological distribution. Expert D also started with a centrally located cluster, but then aggregated clusters of centrally located populations, only adding peripheral populations after including all central ones. Once they selected a population, experts A, C, and D kept that population and subsequent populations were added to the network. Thus diversity included in any specific number of populations is not independent from diversity included in smaller numbers of populations. Expert B's approach was a combination of retaining populations once they were selected and completely changing the selected populations to select the overall highest density of populations at any particular number of populations.

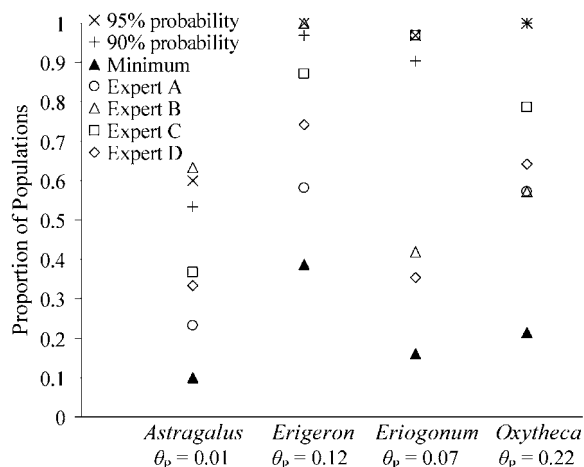


Figure 4. Minimum proportion of random- and expert-selected populations of each of the four rare plant taxa containing all common alleles and number of random-selected populations needed to have a 90% or 95% probability of including all alleles. Data from randomly selected populations come from Neel and Cummings (2003). Values of θ_p for each species are from (Neel 2000; Neel and Ellstrand 2001, in press).

Discussion

Despite the role genetic diversity plays in persistence of populations and species, most reserve selection and design efforts focus on ecological characteristics including habitat requirements and demography of a species (e.g., Murphy and Noon 1992; Burgman et al. 2001), on species distribution patterns (e.g., Csuti et al. 1997), or on community level diversity (e.g., Scott et al. 1993). Ecological approaches are justified because ecological and anthropogenic factors typically pose more immediate extinction threats to species than do genetic factors (Lande 1988; Schemske et al. 1994). Because populations that are ecologically secure are typically also genetically secure, it is assumed that ecological approaches to species conservation are sufficient to conserve genetic diversity (Soulé and Simberloff 1986; Lande 1988). This assumption is valid when the focus is on necessary sizes of individual populations. The current results provide insight into whether this assumption is also valid when the genetic diversity conservation goal is representing diversity of a species in subsets of populations selected according to ecological criteria. Our results also contribute to the evaluation of current conservation intensities with respect to representing genetic diversity.

Effects of reserve design guidelines

Previous work has shown that reliably capturing all alleles or meeting the genetic diversity conservation standard of having a 0.90–0.95 probability of including all common alleles (Center for Plant Conservation 1991) requires a larger than expected proportion of randomly-selected populations of a species (i.e., 0.53–1.0) (Neel and Cummings 2003). Results of the present research show that selecting populations using ecological reserve design guidelines does not reduce the number of populations needed to represent genetic diversity, and that genetic consequences of ecological reserve design guidelines are strongly dependent on the genetic diversity measure examined. We compared the amount of diversity in terms proportion of all alleles and common alleles included in expert reserve design strategies with random population selection in three ways: for each individual number of populations; for all numbers of populations combined; and at particular conservation intensities (i.e., five populations and the minimum number of populations required to include all alleles). The only one of these comparisons for which expert-selected populations differed significantly from random was for reserve designs over all numbers of populations. The directions of these differences were opposite for all alleles and common alleles. Two experts captured more diversity than was captured in randomly selected populations of two species each when the measure of diversity was all alleles (Figure 1) but two expert strategies did worse than random when the measure was common alleles (Figure 3). Designs that were significantly different from random for all alleles were not different for common alleles and vice versa (Figures 1 and 3).

Reserve design guidelines also did not prove effective in capturing genetic diversity in terms of the proportion of populations required to capture all alleles or common alleles. For all species, experts exceeded the minimum proportion of random populations required to capture all alleles (0.68–1.0 for experts versus 0.42–0.57 for random) (Neel and Cummings 2003) and all common alleles (0.23–1.0 for experts versus 0.10–0.29 for random). As mentioned previously, we do not necessarily advocate conservation of all alleles as a realistic conservation target. Rather we use it as an easily quantified measure of genetic diversity that can be compared objectively across experts, taxa, and selection methods. Conserving most alleles and particularly

Table 1. Proportion of expert- and random-selected (Neel and Cummings 2003) populations required to conserve specified proportions of all alleles and common alleles for *Astragalus albens* (AA), *Erigeron parishii* (EP), *Eriogonum ovalifolium* var. *vineum* (EO), and *Oxytheca parishii* var. *goodmaniana* (OP). Values for individual taxa represent the proportion of populations for which all four experts had at least the specified proportion of alleles. Expert mean and random means are averages across the four taxa

Proportion of alleles	Proportion of populations needed											
	Common alleles						All alleles					
	AA	EP	EO	OP	Expert mean	Random mean	AA	EP	EO	OP	Expert mean	Random mean
1.00	0.63	0.87	0.97	0.78	0.812	0.957	0.90	1.00	1.00	1.00	0.975	1.00
0.95	0.30	0.65	0.35	0.50	0.450	0.378	0.83	0.71	0.87	0.86	0.817	0.750
0.90	0.13	0.35	0.19	0.43	0.275	0.235	0.70	0.61	0.48	0.86	0.662	0.535
0.85	0.13	0.29	0.16	0.28	0.215	0.150	0.60	0.45	0.35	0.50	0.475	0.385
0.80	0.10	0.22	0.16	0.21	0.172	0.110	0.30	0.35	0.22	0.36	0.307	0.295
0.75	0.10	0.10	0.13	0.14	0.118	0.102	0.23	0.22	0.16	0.28	0.225	0.207
0.70	0.10	0.10	0.10	0.14	0.110	0.55	0.17	0.22	0.16	0.23	0.195	0.168

most common alleles may be sufficient (Brown and Briggs 1991) and requires substantially fewer populations. For example, it takes approximately half as many populations to capture on average 0.90–0.95 of all or common alleles compared to the number required to capture all alleles (Table 1).

Assessing the genetic diversity consequences of guidelines further is complicated because selection strategies employed by experts had inconsistent effects across species and among measures of allelic diversity. For example, Expert A included all common alleles in the smallest number of populations for *A. albens* and *E. parishii*, but the largest proportion of populations for *E. ovalifolium* var. *vineum* and *O. parishii* var. *goodmaniana* (Figures 2 and 4). In contrast to the performance for common alleles for *A. albens*, Expert A required the largest number of populations of any expert to capture all alleles for this species (Figure 2). Similarly, Expert B included all alleles in the smallest number of populations of all experts for *A. albens*, but required the largest number of populations to include all common alleles in the same species as well as the largest numbers of populations to capture all alleles for *E. parishii* and *O. parishii* var. *goodmaniana* (Figure 2). These inconsistent effects indicate that differences among experts in the order in which populations were selected had little predictable influence on representation of diversity. These results are not due to lack of ability of experts themselves, but rather due to a lack of correlation between genetic diversity and ecological characteristics associated with the guidelines.

The lack of difference between expert-selected and random populations could be a result of a lack of correlation between neutral or nearly-neutral allozyme variation and environmental gradients. This raises the question of how well these results inform conservation activities given that generally agreed upon genetic diversity conservation goals include maintaining adaptive variation that affects current fitness of individuals and populations, providing for ongoing processes, and maintaining variation for adaptation to future environmental change (e.g., Namkoong 1993; Storfer 1996). While, adaptive traits are ultimately of interest, they are difficult to define, identify, and measure, especially in endangered species. Levels of genetic diversity as described by number of alleles or levels of heterozygosity at marker loci are used as surrogates for estimating total diversity and patterns of gene flow among populations. Opinion varies as to how well primarily neutral markers reflect putatively adaptive quantitative traits (e.g., Marshall and Brown 1975; Milligan et al. 1994; Storfer 1996). Regardless, maintenance of identifiable marker alleles is advocated due to their indirect value to conservation as indicators of levels of genomic variation (Namkoong 1993; Schoen and Brown 1993; Petit et al. 1998) and has often been advocated as a conservation goal (e.g., Haig et al. 1996; Ceska et al. 1997; Daniels et al. 1997; Shaanker and Ganeshiah 1997; Petit et al. 1998; Kark et al. 1999). Thus, we use allelic diversity as an estimate of genetic diversity within species. Although we recognize the limitation of this estimate it has the strength of providing an

objective measure with which to evaluate different methods of selecting populations for conservation. Further, allelic diversity should be relatively easy to capture compared with more complex, fitness-related quantitative traits because while individual traits will respond to environmental gradients, different traits will likely be responding to different environmental gradients across heterogeneous landscapes. Thus, it is unlikely that more directly adaptive variation would be captured in fewer populations than the allelic variation addressed here. As such, allelic diversity potentially serves as a reasonable lower estimate of the number of populations needed to represent genetic diversity in general. As for molecular markers with larger numbers of alleles that each occur at lower frequencies (e.g., microsatellites) no conservation standards have been established, but the number of populations required to capture all alleles will increase with the total number of alleles.

Clearly the idea that genetic diversity is easy to capture in small numbers of populations of species (e.g., Falk 1991) is not supported by the results of the present study. Large numbers of populations were required to capture all alleles or common alleles even for species with little differentiation among populations as indicated by a low θ_p value (Figures 2 and 4, Table 1). As discussed in Neel and Cummings (2003) this discrepancy between the number of populations and level of differentiation is likely due to alleles that are at low to moderate frequency in populations but that occur in only a limited number of populations. These alleles require a large amount of sampling effort to capture and yet they have little influence on θ_p . The effect of these alleles can be seen most clearly in *A. albens* where only 36 of the 69 alleles were common. When only common alleles are included in the analysis the number of populations required for this species drops considerably. Marshall and Brown (1975) suggest that alleles that are limited to subsets of populations but that are moderately common are most important for conservation efforts because alleles occurring at the high frequencies ($\gg 0.05$) will be captured regardless of the specific sampling strategy and alleles at very low frequencies (< 0.05) are less likely to be evolutionarily significant and may even be slightly deleterious (Holsinger and Gottlieb 1991).

Adequacy of current conservation intensities

Whether or not currently recommended conservation intensities discussed in the introduction are sufficient

to represent genetic diversity depends on how much and what type of diversity one considers appropriate to target for conservation. For example, 0.10–0.12 of populations never included all or all common alleles but would conserve approximately 0.70–0.75 of common alleles and < 0.70 of all alleles on average across species and experts (Figure 2, Table 1). All alleles also were never captured by experts in five populations. All common alleles were captured in six populations by one expert for one species (Expert A, *A. albens*) (Figure 4). Conserving 0.45 of populations, which is more comparable to intensities recommended by Sanjayan and Soulé (1998) and Noss (1996) on average included 0.95 of common alleles and 0.80–0.85 of all alleles. However, even at this intensity there is a high variance and up to 0.30 of all alleles could be lost, which is likely beyond a level acceptable to practitioners who consider all alleles to be the more relevant measure of diversity.

In summary, selecting populations according to ecological reserve design guidelines generally did not capture more genetic diversity than selecting populations at random. The number of populations selected is much more important than how those populations were selected. Thus, focusing on ecological features for selecting sites for conservation will ensure representation of genetic diversity only when sufficient numbers of populations are included in reserves. The proportion of sites needed to capture all alleles can be substantially larger than the 0.10–0.12 or five sites that are currently advocated; and is more similar to intensities suggested by Sanjayan and Soulé (1998) and Noss (1996). Of course, if losing some potentially substantial proportion of the existing genetic diversity is acceptable and one is concerned only with diversity represented by common alleles, then lower conservation targets may be sufficient. It is not our purpose to criticize the reserve design guidelines, but rather to point out that capturing one type of diversity does not necessarily capture other types. Other merits of the guidelines are well documented (see introduction for citations) and their application can yield appropriate configurations of conserved populations that will increase the likelihood of maintaining genetic diversity over time. Furthermore, emphasizing conservation of the geographic and ecological ranges of species provides some measure of buffering against threats from environmental and human-mediated stochasticity. For these reasons, we do not argue for abandoning the guidelines. Rather we emphasize that large proportions or numbers of

populations are necessary for these guidelines to be effective in capturing genetic diversity.

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