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Patch connectivity and genetic diversity conservation in the federally endangered and narrowly endemic plant species *Astragalus albens* (Fabaceae)

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ABSTRACT

To evaluate the sufficiency of US federal critical habitat designations and a proposed conservation plan in promoting the long-term persistence of the endangered plant *Astragalus albens*, patterns of genetic diversity and landscape connectivity were examined. *A. albens* harbors substantial genetic variation and shows no evidence of historic bottlenecks, suggesting little risk of extinction due to genetic homogeneity ($A = 2.40$; $P = 0.50$) or inbreeding ($f = -0.08$) within occurrences. Low genetic differentiation among occurrences ($\theta_p = 0.01$) indicates relatively high gene flow or little genetic drift. The 91 patches of *A. albens* were connected into a single network at a distance of 2100 m; 94% of patches were <1000 m from at least one other patch. Managing ecological conditions that maintain large population sizes and connectivity among populations throughout the species' ecological and geographic ranges will most likely conserve existing diversity. Both reserve networks partially accomplish these goals by including most extant occurrences and >89% of the aerial extent of the species, including the largest populations, and capturing all detected alleles. However, both conservation networks fail to conserve occurrences from one portion of the species' range, possibly speeding loss of unique local adaptations. In addition, connectivity of the whole network is reduced with the 65 patches designated as critical habitat being connected at a distance of 6200 m and the proposed reserve sites being connected at a distance of 9500 m. Although total network connectivity would be reduced, connectivity at scales most relevant to gene flow (e.g., <1000 m) remains sufficiently in tact to provide a relatively promising outlook for species persistence.

1. Introduction

There is a general agreement that conserving genetic diversity within species is important for species persistence (e.g., Hughes and Stachowicz, 2004), for ecosystem functioning (e.g., Luck et al., 2003), and for future evolution (Ellstrand and Elam, 1993; Frankham, 2005a). Individual fitness within populations is affected by relative rates of inbreeding (Dudash, 1990; Fenster and Dudash, 1994; Öostermeijer et al., 1994), diversity among individuals (Hufford and Mazer, 2003; e.g., numbers of alleles, genotypes, and phenotypes; Williams and Davis, 1996; Williams and Orth, 1998), and degree of local adaptation (e.g., Hammerli and Reusch, 2002; Hufford and Mazer, 2003; Joshi et al., 2001; Montalvo and Ellstrand, 2000, 2001). Genetic diversity also confers population-level resilience to ecological perturbation (Hughes and Stachowicz,

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2004). In addition to these immediate effects, genetic diversity provides the raw material for evolutionary change and thus, is essential for adaptation to new environmental conditions that invariably arise due to natural and anthropogenic changes (e.g., Frankham, 2005b). Thus, it is prudent to prevent as much within-species diversity loss as possible even though the ultimate consequences of specific genetic losses are rarely understood. However, the need to manage genetic resources directly and which measures of diversity should be managed is routinely disputed (e.g., Holsinger and Vitt, 1997; Milligan et al., 1994; Schemske et al., 1994). Focus on ecological characteristics in conservation is often justified because ecological and anthropogenic factors typically pose more immediate extinction threats to species than do genetic factors, and populations that are ecologically secure are usually also genetically secure (Lande, 1988; Schemske et al., 1994; Soulé and Simberloff, 1986).

Here, I use both ecological and genetic data to evaluate the potential for ecologically based conservation efforts to maintain genetic diversity in the federally endangered plant species *Astragalus albens* E. Greene (Fabaceae). This research quantifies genetic diversity patterns in the species, relates those patterns to the current spatial distribution of populations, and evaluates the potential for conserving genetic diversity through implementation of two conservation strategies: protection of critical habitat designated by the US Fish and Wildlife Service and implementation of a conservation strategy proposed by the USDA Forest Service. Conservation potential was evaluated first by quantifying the proportion of existing genetic diversity that would be included in occurrences protected under the two conservation approaches, and second by assessing the potential for maintaining diversity over time by quantifying changes in sizes, numbers, geographic and ecological ranges, and connectivity of occurrences due to loss of locations not proposed for conservation. Connectivity was quantified using patterns of genetic differentiation and a graph theoretic assessment of the spatial distributions of *A. albens* occurrences. Merging techniques and data from landscape ecology and population genetics provide an extremely effective way to quantify the patterns and processes of conservation interest so that they can be incorporated into conservation efforts (Holderegger and Wagner, 2006; Storfer et al., 2007).

Preventing losses of genetic diversity requires conserving existing diversity and maintaining ecological and evolutionary processes that generate and promote its persistence of it (Cowling et al., 1999; Crandall et al., 2000; Margules and Pressey, 2000). Genetic losses can occur incrementally through decreases in allele richness and heterozygosity in small, isolated populations or more abruptly through loss of whole populations. Within-population processes of inbreeding and genetic drift are well understood and have long been a major focus of theoretical and empirical population genetic research (e.g., Kimura, 1962; Nei, 1973; Wright, 1931) and conservation genetics (e.g., Allendorf, 1986; Ellstrand and Elam, 1993; Frankham, 1995, 1996). In all but extreme cases, genetic losses due to drift and inbreeding within populations can be limited by keeping population sizes large relative to their historical sizes, which does not require explicit knowledge or manipulation of genetic diversity. Comparatively little attention has been paid to genetic consequences of whole population losses (Neel and Cummings, 2003a,b), despite the fact that ~80% of US endangered plant species for which data are available ($n = 404$) have lost whole populations (Neel, in press). Such losses will reduce species-level allele diversity as a function of the level of differentiation among populations. Beyond direct losses of alleles in extirpated populations, indirect effects on remaining populations will occur if newly created distributional gaps among remaining populations exceed pollen and seed dispersal distances and thus reduce connectivity among populations.

Although, conserving markers alone is not an ultimate conservation goal (e.g., Milligan et al., 1994; Storfer, 1996), considering both the diversity quantified by genetic markers and the ecological and geographic ranges of a taxon is thought be an effective and practical way to include evolutionarily significant genetic variation in conserved areas (Faith, 2003; Faith et al., 2004; Ferrier, 2002; Neel and Cummings, 2003b; Petit et al., 1998; Raabová et al., 2007). Putatively neutral markers also provide important insight into demographic and evolutionary processes such as bottlenecks within populations (e.g., Cole and Biesboer, 1992; Godt et al., 1997; Lesica et al., 1988; Luikart et al., 1998, 1999) or the relative degree of migration among different populations that can guide conservation decisions.

Connectivity is a function of both the extensiveness of individual habitat patches and the distribution of distances among those patches (Neel et al., 2004; Pascual-Hortal and Saura, 2006; Tischendorf and Fahrig, 2000). Habitat loss and fragmentation affect both these aspects of landscape pattern (Fahrig, 1997; McGarigal and Cushman, 2002). Documenting actual connectivity requires habitat suitability, mark-recapture, or experimental removal-recolonization studies that can be so data intensive that there is little practical application for conservation activities for most species (e.g., Calabrese and Fagan, 2004; Urban, 2005). Patterns of genetic variation among populations can be used to assess connections, or lack thereof, resulting from pollen and seed dispersal (Coates and Atkins, 2001; Coates et al., 2003; Diniz-Filho and De Campos Telles, 2002; Dyer and Nason, 2004). Unfortunately, conclusions regarding the degree of connectivity based on traditional genetic analyses (e.g., F_{st} and R_{st}) are limited because gene flow estimates integrate over evolutionary time making it impossible to separate current from historical processes based on pattern alone. In the absence of specific knowledge regarding actual connectivity, we can attempt to perpetuate among-population processes by maintaining current or restoring historical spatial patterns of populations or patches of suitable habitat.

Patterns of potential connectivity among habitat patches can be quantified using an array of landscape pattern analysis techniques (McGarigal et al., 2002; McRae, 2006; Neel et al., 2004; Urban and Keitt, 2001). Graph theory provides an especially effective approach in that it combines the structural attributes of landscape pattern with actual or potential dispersal distances (e.g., Bunn et al., 2000; D'Eon et al., 2002; Keitt et al., 1997; Urban and Keitt, 2001). Potential connectivity among discrete habitat patches is determined by specifying critical threshold distances below which patches are considered connected through dispersal or other movement (Urban

and Keitt, 2001). Even in the absence of species-specific data, it is possible to quantify the spatial scale at which connections manifest in a particular network by examining a range of critical distances. Traditionally, graph theory has focused only on connections among patches, but recently several statistics that measure both patch extensiveness and patch isolation have been developed (Ferrari et al., 2007; Pascual-Hortal and Saura, 2006; Saura and Pascual-Hortal, 2007b). Connectivity estimates from graph theory can be incorporated into conservation planning by specifying desired distances for population separation or by prioritizing sites that contribute the most to network integrity (Ferrari et al., 2007; Pascual-Hortal and Saura, 2006).

In this study I use graph theory to evaluate changes to the current levels of potential connectivity that would result if *A. albens* occurrences excluded from the proposed Carbonate Habitat Management Strategy and the existing critical habitat designations were lost. Combining data on genetic diversity and landscape patterns from existing populations with potential future losses can highlight where changes in patterns and processes are likely to occur, and thus focuses further monitoring and risk assessment on the most vulnerable spatial scales and geographic locations. This approach brings genetic diversity conservation into a context that is relevant to ecological conservation planning.

2. Methods

2.1. Species conservation status

Astragalus albens is characteristic of many species for which genetic diversity is a potential conservation issue in that it is geographically and edaphically narrowly distributed and it exists in patchily distributed populations (Barrett and Kohn, 1991; Ellstrand and Elam, 1993). It is found on limestone and dolomite (collectively called carbonate) substrates that occupy ~15,175 ha in the San Bernardino Mountains of southern California (Fig. 1). Within the general distribution of the carbonate substrate, high density occurrences of *A. albens* are limited to relatively small areas totaling ~537 ha (dark areas in Fig. 1B) (data provided by S. Eliason, San Bernardino National Forest). Individual occurrences range in size from 0.09 to 132 ha with a median size of 0.9 ha and a mean size of 5.4 ha. The species occurs primarily on multiple use lands managed by the Forest Service and the Bureau of Land Management (BLM), or on private land (Spellenberg, 1993; U.S. Fish and Wildlife Service, 1994). Within the naturally narrow distributional range, the number and size of populations has been reduced by surface mining activities allowed under the United States General Mining Law of 1872 (30 USC 29) and, to a lesser degree, by recreational activities such as off-highway vehicle use and target shooting. Wildfires, the major natural disturbance force in this landscape, also affect the spatial distribution of populations and densities of individuals. Agency discretion regarding proposed operations is limited to reducing impacts to non-mineral resource values only if it does not conflict with mineral extraction. Patenting, a process that allows claim holders to convert public land to private ownership for a nominal fee, threatens plant species because plants are not afforded Endangered Species Act (ESA) protection on private land. Existing losses combined with inadequate regulatory mechanisms to prevent ongoing losses resulted in the species being listed under the ESA (U.S. Fish and Wildlife Service, 1994).

The ultimate goal of the ESA is to recover listed taxa such that the provisions of the Act are no longer required. Despite the intention of recovery for listed species, continued impacts are regularly permitted under Section 7 of the ESA as long as such actions do not jeopardize the continued existence of the species. A draft recovery plan for *A. albens* (U.S. Fish and Wildlife Service, 1997) specifies withdrawing sufficient habitat on federal lands from mineral entry to support the largest populations of *A. albens* in an ecological context that will function naturally. This ecological context is to be provided by buffers that provide protection from extrinsic threats and maintain habitat connectivity. Specific locations or numbers of populations were not identified. Restoration of degraded sites was also included as a recovery strategy, but was secondary to protection and management of intact habitat. Although this plan was never approved, critical habitat has been designated (Fig. 2J) (U.S. Fish and Wildlife Service, 2002). Critical habitat is defined as habitat essential to the conservation of a species that provides essential life cycle needs (U.S. Congress, 1973). Federal agencies are prohibited from destroying or adversely modifying critical habitat without going through Section 7 consultation procedures with the service on effects of such actions (U.S. Congress, 1973). As such, critical habitat provides more stringent protection than listing alone and it should reflect the level of habitat conservation minimally necessary for recovery.

In addition to the critical habitat described above, the Forest Service has led an arduous stakeholder-based conservation planning process since 1992. These efforts have resulted in a draft Carbonate Habitat Management Strategy (CHMS) (Fig. 2O) that proposes a network of core reserve areas on National Forest land and Areas of Critical Environmental Concern (ACEC) on BLM land (S. Eliason, San Bernardino National Forest, personal communication). For the purposes of this analysis I assume that all existing critical habitat and all proposed CHMS sites will be conserved and that all other locations will be extirpated.

2.2. Species biology

A. albens most often occupies ridge tops and relatively gentle slopes (1–32° slope angles) between the elevations of ~1170 and ~2013 m (Gonella and Neel, 1995; Neel, 2000). Environments vary over this elevational range, extending from desert to montane conditions. At lower elevations annual precipitation averages 14 cm, and temperature extremes vary from −17 to 43 °C (U.S. Department of Commerce, 2006). Precipitation is higher and temperatures are lower at higher elevations but climate data are not available for these areas. Specific habitats in which this taxon occurs vary along this environmental gradient (Gonella and Neel, 1995; Neel, 2000). Lower elevations support shrub-dominated creosote bush scrub-blackbush scrub and black bush scrub communities. Higher elevations are dominated by various phases of Singleleaf Pinyon-Utah Juniper Woodland and Singleleaf Pinyon Woodland. Despite

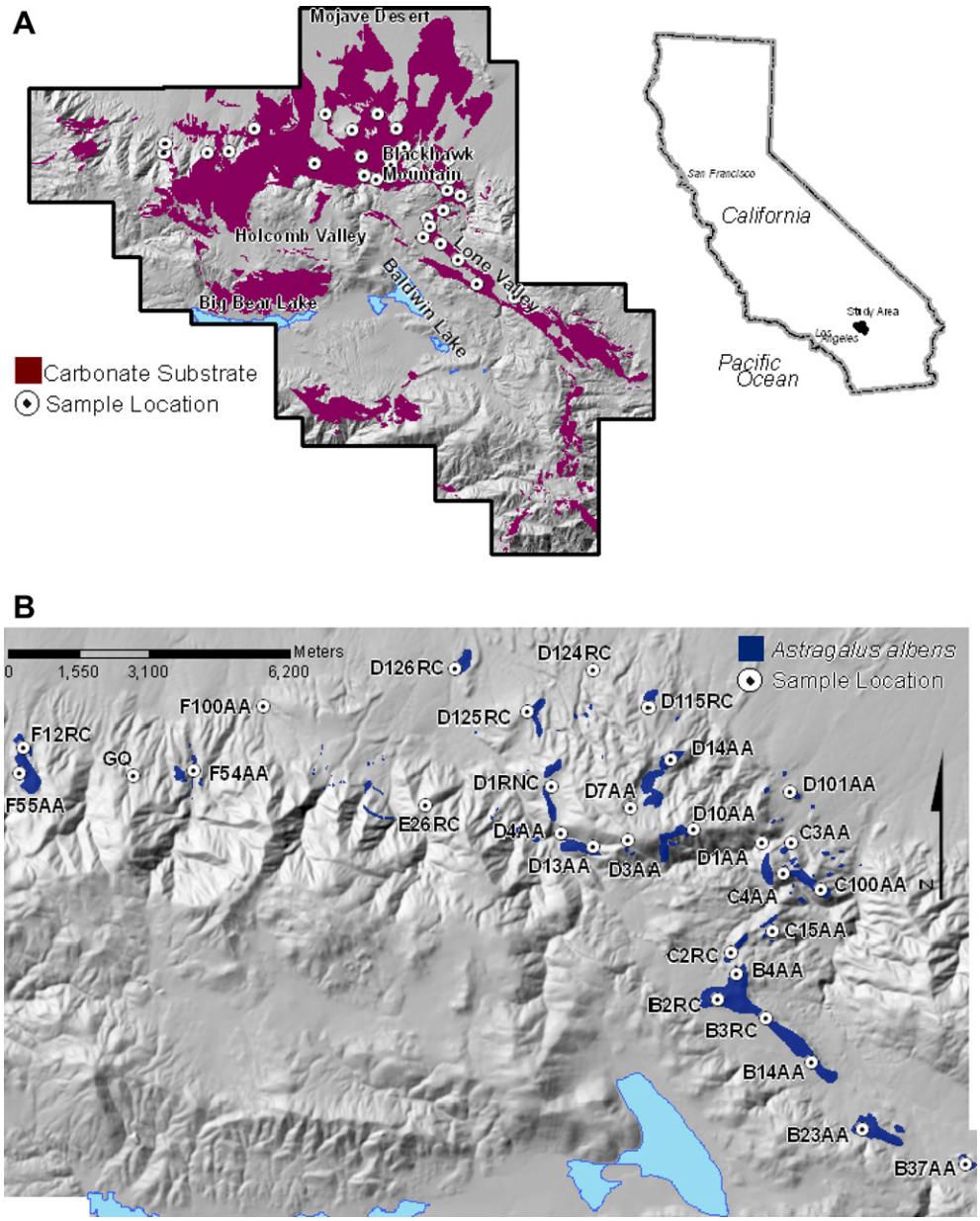

Fig. 1 – Study area showing carbonate substrate (panel A) and distribution of high density *Astragalus albens* habitat that were used as patches in graph analysis (dark areas) (panel B) and genetic sampling locations (bulls eyes).

this ecological variation, habitats supporting *A. albens* share the following features: low overstory and shrub canopy cover, high soil pH, and high percentage of soil calcium (Neel, 2000). Although most individuals occur at relatively high densities (e.g., 100–1000/ha) in limited areas, widely scattered and isolated individuals have been documented during stratified random vegetation sampling efforts. These individuals occurred at extremely low densities (e.g., <1 individual/10 ha) primarily in black bush scrub habitat at lower elevations in the study area on the northern slopes of Blackhawk Mountain (Fig. 1A).

Members of this species are typically short-lived perennial plants with a prostrate growth habit (Spellenberg, 1993), but they can grow as annuals. They have a tap root and show no evidence of vegetative reproduction. The purple and white corollas have a typical papilionaceous form with the stigma and anther complex concealed within a keel; floral length measures <9.5 mm and there are 5–14 flowers in each inflorescence (Spellenberg, 1993). Nothing is known of the mating system or of relative timing of maturity of male and female parts. Both self-compatible and self-incompatible species are known from the genus (Karron, 1989). Even self-compatible papilionaceous flowers often require insect visitors to trip the keel to transfer pollen to the stigma within a flower. Large numbers of flowers on a plant can be opened simultaneously, providing ample opportunities for geitonogamous pollination if the plants are self-compatible. Fruits and seeds of this species have no specialized dispersal mechanism.

2.3. *Data collection*

Thirty occurrences of *A. albens* were sampled for genetic diversity using allozymes (Fig. 1). Occurrences were selected

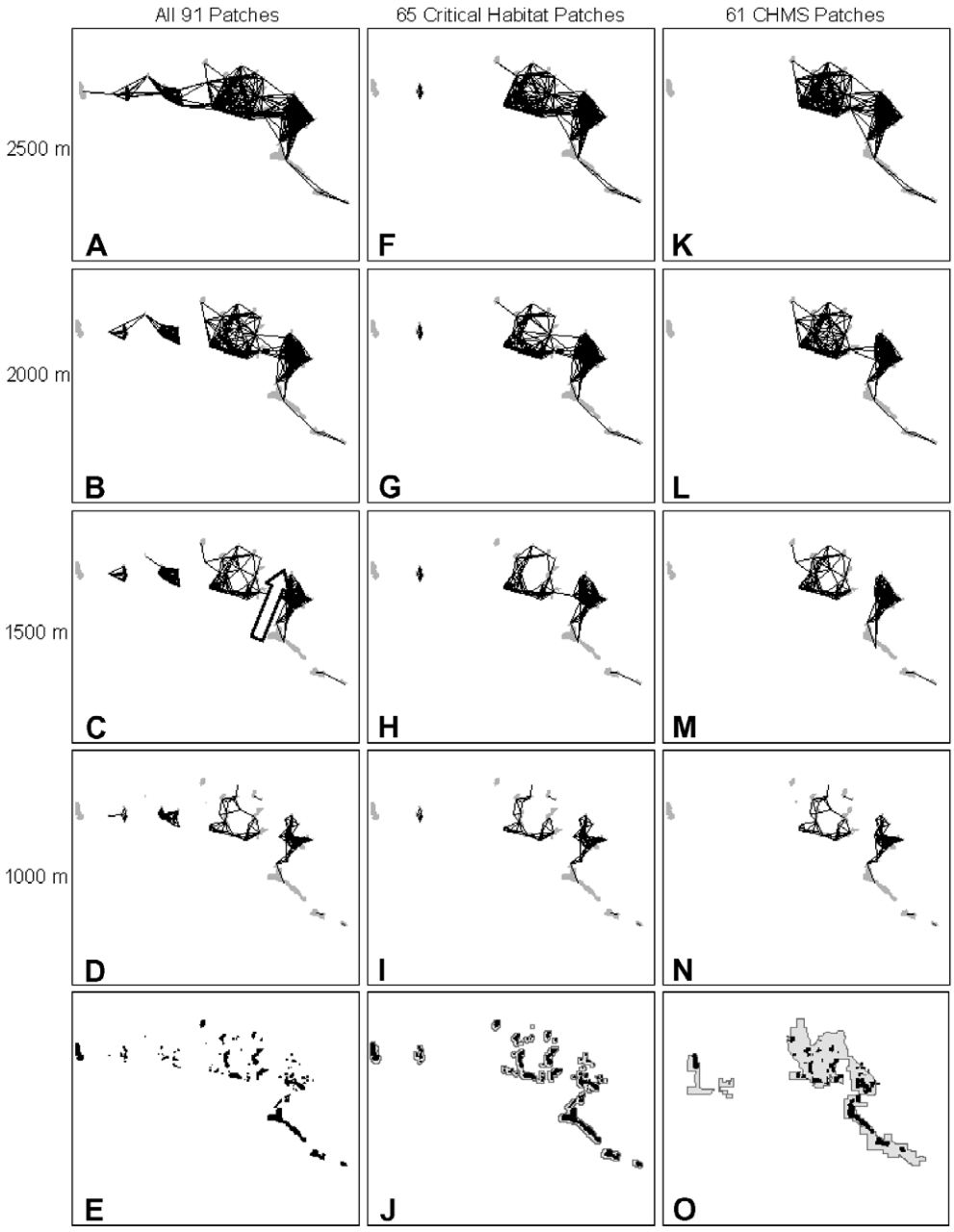

Fig. 2 – Potential connectivity shown as edges among high density patches of *Astragalus albens* that fall within critical distances of 1000, 1500, 2000, and 2500 m for all extant patches (panel E), patches designated as critical habitat (gray background in panel J), and patches included within proposed Carbonate Habitat Management Strategy reserves (gray background in panel O). The entire network is connected at a critical distance of <2500 m (panel A) but neither conservation network is completely connected at this critical distance (panels F and K); smaller distances yield increasingly disconnected networks for all data sets.

to represent the ecological range of the taxon as follows. Sampling sites were primarily chosen from a subset of plots sampled for vegetation description in a related project (Neel, 2000). Each vegetation plot supporting *A. albens* was classified according to the vegetation series in which it occurred. These plots occurred within the black bush series, the Singleleaf Pinyon-Utah Juniper Series, and the Singleleaf Pinyon Series (Table 1). A provisional classification to the association level was developed to guide selecting plots that represented the range of ecological diversity within each series (Table 1; Neel, 2000). Sampling sites were randomly selected within each vegetation association roughly in proportion to the number of populations within that association, with the exception that types with very few occurrences were over-represented in the sample. One sampled population occurred at an abandoned mining quarry (Gordon Quarry) and was not associated with a vegetation plot. This location was sampled because it was one of the westernmost occurrences for this taxon.

Table 1 – Summary of sample sizes averaged over loci (n), genetic diversity statistics, and P values for the Wilcoxon tests for heterozygosity excess for 12 loci in 30 samples of Astragalus albens

Population	Vegetation association	Plant density	*n*	TA	*P*	*A*	A_p	H_e	H_o	*f*	Wilcoxon *P*
B4AA	Pinyon-Utah Juniper/Mt. Mahogany Woodland[a]	41	30.0	31	0.58	2.58	3.43	0.15	0.16	−0.03	0.997
B14AA	Pinyon-Utah Juniper/Mt. Mahogany Woodland [a]	39	30.0	29	0.42	2.42	3.60	0.15	0.15	0.00	0.973
B23AA	Pinyon-Utah Juniper/Yucca Woodland [a]	20	30.0	29	0.50	2.42	3.33	0.14	0.15	−0.12	0.990
B37AA	Pinyon-Utah Juniper/Mt. Mahogany Woodland [a]	55	30.0	29	0.50	2.42	3.50	0.15	0.16	−0.07	0.986
B2RC	Pinyon-Utah Juniper/Yucca Woodland [a]	14	30.0	31	0.50	2.58	3.83	0.16	0.17	−0.11	0.996
B3RC	Pinyon-Utah Juniper/Yucca Woodland [a]	243	29.8	28	0.50	2.33	3.33	0.13	0.15	−0.13	0.998
C3AA	Pinyon-Utah Juniper/Blackbush Scrub [a]	25	29.7	26	0.50	2.17	3.17	0.13	0.14	−0.02	0.992
C4AA	Pinyon-Utah Juniper/Blackbush Scrub [a]	37	29.9	32	0.50	2.67	3.67	0.15	0.16	−0.07	0.999
C15AA	Pinyon-Utah Juniper/Blackbush Scrub[a]	53	30.0	29	0.42	2.42	3.60	0.11	0.13	−0.18	1
C100AA	Black Bush Scrub[b]	91	30.0	31	0.42	2.58	4.20	0.12	0.13	−0.01	1
C2RC	Pinyon-Utah Juniper/Yucca Woodland[a]	48	29.6	33	0.50	2.75	4.00	0.13	0.15	−0.09	1
D1AA	Black Bush Scrub[b]	51	29.8	33	0.58	2.75	4.00	0.16	0.16	−0.03	0.996
D3AA	Pinyon-Utah Juniper/Mt. Mahogany Woodland[a]	66	29.9	31	0.50	2.58	3.83	0.17	0.18	−0.10	0.998
D4AA	Pinyon-Utah Juniper/Blackbush Scrub[a]	214	29.9	30	0.42	2.50	3.40	0.13	0.15	−0.12	0.997
D7AA	Pinyon-Utah Juniper/Blackbush Scrub[a]	167	8.6	17	0.42	1.42	2.00	0.11	0.11	0.01	0.891
D10AA	Black Bush Scrub[b]	90	29.3	31	0.50	2.58	3.83	0.12	0.12	−0.02	1
D13AA	Pinyon/Purshia-Yucca Woodland[c]	299	30.0	25	0.50	2.08	3.00	0.11	0.13	−0.14	0.996
D14AA	Pinyon/Blackbush Scrub[c]	92	29.9	30	0.58	2.50	3.43	0.14	0.17	−0.22	0.996
D101AA	Black Bush Scrub[b]	27	30.0	30	0.50	2.50	3.83	0.15	0.15	−0.01	1
D115RC	Black Bush Scrub[b]	8	29.9	27	0.50	2.25	3.17	0.13	0.13	−0.03	0.990
D124RC	Black Bush Scrub[b]	1	30.0	27	0.58	2.25	3.00	0.14	0.15	−0.09	0.996
D125RC	Black Bush Scrub[b]	32	29.7	25	0.50	2.08	3.00	0.12	0.14	−0.16	0.988
D126RC	Creosote Bush Scrub-Blackbush Scrub[b]	20	30.0	28	0.50	2.33	3.17	0.13	0.15	−0.10	1
D1RNC	Pinyon-Utah Juniper/Blackbush Scrub[a]	6	30.0	37	0.58	3.08	4.29	0.19	0.21	−0.09	1
E26RC	Pinyon-Utah Juniper/Blackbush Scrub[a]	24	29.8	24	0.50	2.00	2.83	0.11	0.12	−0.12	0.996
F54AA	Pinyon/Purshia-Yucca Woodland[c]	14	30.0	30	0.42	2.50	4.20	0.14	0.16	−0.18	0.984
F55AA	Pinyon-Utah Juniper/Yucca Woodland[a]	67	30.0	28	0.58	2.33	3.14	0.14	0.14	0.03	0.980
F100AA	Pinyon-Utah Juniper/Blackbush Scrub[a]	39	29.6	30	0.42	2.50	4.00	0.11	0.12	−0.08	0.992
F12RC	Pinyon/Purshia-Yucca Woodland[c]	12	29.9	27	0.58	2.25	3.14	0.15	0.13	0.14	0.945
GQ[d]	Pinyon-Utah Juniper/Mt. Mahogany Woodland[a]	ND	30.0	25	0.42	2.08	3.2	0.14	0.15	−0.13	0.976
Mean		65.3	29.2	28.8	0.50	2.40	3.47	0.14	0.15	−0.08	NA
SD		74.0	3.40	3.6	0.06	0.30	0.49	0.02	0.02	0.07	NA

Loci were considered polymorphic if the most common allele was less frequent than 0.95.
Plant density is the number of *A. albens* individuals in the 0.04 ha vegetation sampling plot from each location.
a Singleleaf Pinyon-Utah Juniper Series.
b Black Bush Scrub Series.
c Singleleaf Pinyon Series.
d Not associated with a vegetation plot, vegetation association assigned based on vegetation surrounding the quarry.

Densities of *A. albens* in the 29 sampling locations associated with vegetation plots were crudely estimated using densities recorded previously from those plots (Table 1).

Up to 10 mg of leaf tissue were collected from up to 30 individuals per location. Leaves were kept on ice during transport to the laboratory, at which time they were refrigerated at 8 °C. All samples were extracted initially within 5 days of arrival in the laboratory and refrigerated samples could be used for up to 1.5 months after collection with no loss in activity. Approximately 45 mg of leaf tissue was extracted in 7 drops of a 0.1 M, pH 8.0, Tris–HCl buffer containing 1.5 mg/ml of dithiothreitol. The resulting extract was soaked onto chromatography paper wicks and run immediately. Individuals from one location were run with a subset of individuals from other locations to calibrate alleles across gels. Additionally, individuals with alleles that were difficult to distinguish, or with alleles that had not otherwise been calibrated, were run together on the same gel to ensure equivalent scoring across individuals and sampling locations. Although this procedure required large numbers of runs for some samples, it allowed me to distinguish closely migrating alleles with high confidence and repeatability.

The following enzyme systems were assayed in 9% potato starch gels: aspartate amino transaminase (AAT, E.C. 2.6.1.1), leucyl aminopeptidase (LAP, 3.4.11.1), malate dehydrogenase (MDH, 1.1.1.37), glucose-6-phosphate isomerase (PGI, 5.3.1.9), phosphoglucomutase (PGM, 5.4.2.2), triose-phosphate isomerase (TPI, 5.3.1.1), and uridine diphosphoglucose pyrophosphorylase (UDP, 2.7.7.9). Acid phosphatase (ACP3.1.3.2), aconitase (ACO, 4.2.1.3), aldolase (ALD, 4.1.2.3), diaphorase (DIA, E.C. 1.6.4.3), hexokinase (HEX 2.7.1.1), isocitrate dehydrogenase (IDH, 1.1.1.42), mannose-6-phosphate isomerase (M-6PI, 5.3.1.8), mallate enzyme (ME, 1.1.1.44), 6-phosphogluconate dehydrogenase (6PGD, 1.1.1.25), and shikimate dehydrogenase (SKDH) were examined but could not be resolved sufficiently to score.

A total of 12 loci were resolved, although it was not possible to assess genotypes at all loci for all individuals. Numbers

of independent loci for each resolved system are as follows: AAT (2 present, 1 scored), LAP (1), MDH (3), PGI (2 present, 1 scored) PGM (1), TPI (4), and UDP (1). AAT and PGI were resolved in a discontinuous, pH 8.3, lithium hydroxide-borate gel (Heywood, 1980; Scandalios and Espiritu, 1969). This gel was run for approximately 5 h; current was kept at 75 mA until voltage reached 200 V. This voltage was maintained for the duration of the run and current ranged between 25 and 50 mA. LAP, PGD, PGM, and TPI were resolved in a pH 6 histidine–citrate gel system run at 25 mA and 150 V for 7 h (Stuber et al., 1977). MDH and UDP were resolved on a pH 7 Tris–citrate gel system run at 30 mA for 5.5 h (Shaw and Prasad, 1970).

2.4. Data analysis

2.4.1. Population genetic analyses

Total number of alleles (TA), proportion of polymorphic loci (*P*), number of alleles per locus (*A*), number of alleles per polymorphic locus (A_p), observed heterozygosity (H_o), and expected heterozygosity (H_e) were calculated for the whole species (i.e., all sample sites combined) and for each sampling site separately using the computer program Genetic Data Analysis (GDA) (Lewis and Zaykin, 2001). A locus was considered polymorphic if the most common allele had a frequency <0.95. The number of private alleles within locations (i.e., alleles that were only found in a single sample) was also assessed using GDA. Population genetic structure at polymorphic loci was assessed using f ($\approx F_{IS}$), F ($\approx F_{IT}$) and θ_p ($\approx F_{ST}$) following methods of Weir (1996) as implemented in GDA (Lewis and Zaykin, 2001). The statistic f represents departures from Hardy–Weinberg equilibrium within individual locations. F represents deviations from Hardy–Weinberg equilibrium expectations over all locations. θ_p represents the correlation between two genes drawn at random from two different subpopulations and describes the proportion of genetic variation partitioned among populations relative to the total variation present, treating both loci and populations as samples. Standard deviations of individual estimates of f, F, and θ_p were calculated by jackknifing over populations. Bounds of 95% confidence intervals for overall estimates were calculated from 5000 bootstrap replicates across loci. The number of migrants per generation among populations (N_m) was estimated as $N_m = 0.25(1 - \theta_p)/\theta_p$ (Weir, 1996) and using the private allele method (Slatkin, 1985).

I tested each population for a recent bottleneck using a heterozygosity excess test as implemented by the program BOTTLENECK (Piry et al., 1999). Populations that have recently gone through a severe bottleneck will lose alleles at a faster rate than they lose expected heterozygosity (H_e) (Allendorf, 1986) and thus H_e will be larger than heterozygosity expected at equilibrium (H_{eq}) (Cornuet and Luikart, 1996). Significance of the difference between H_e and H_{eq} was tested using a one-tailed Wilcoxon's signed rank test under an Infinite Allele Model as recommended by Piry et al., 1999) for samples of <20 allozyme loci. The null hypothesis of this test is that there is no significant H_e excess within samples averaged across loci; rejection of the null ($p < 0.05$) indicates H_e excess.

Nei's (1978) unbiased genetic distance was computed for all pairwise combinations of samples. Euclidean geographic distances among these same sampling locations were calculated using ArcView version 3.2 (Environmental Systems Research Institute, 1996). The strength of the relationship between the genetic and geographic distance matrices was evaluated with a standardized Mantel statistic (Sokal and Rohlf, 1995) using PC-ORD (McCune and Mefford, 1999). The significance of the Mantel statistic was assessed through a randomization test using 1000 Monte Carlo simulations.

The amount of diversity that would be conserved under the two conservation plans for this species was calculated by overlaying the boundaries of critical habitat (Fig. 2J), the CHMS (including Forest Service Core Reserves and the BLM ACEC) (Fig. 2O) with the genetic sample locations. I then recalculated the species-level diversity statistics *P*, TA, and population-level *P*, *A*, A_p, H_e, and H_o using only the sampled locations that were included in these areas. I also calculated the proportion of high density occurrences (number and area) included in the reserve areas and qualitatively assessed representation of the species' geographic and ecological range.

2.4.2. Graph theoretic analyses

Existing patterns of potential landscape connectivity were quantified using graph theoretic measures for all mapped patches of *A. albens* shown in Fig. 1B. There is no information on the degree to which these patches function as populations or metapopulations. The term 'occurrence' is used by conservation organizations and land management agencies to avoid the connotation of a biological population. Each occurrence can be made up of multiple discrete patches that are within close proximity. Graph analysis is based on each individual patch ('nodes' in graph theory literature) and to avoid confusion with the terms population and occurrence, I use the term patches to refer to each discrete mapped polygon denoting high density *A. albens* in Fig. 1B. In graph theory, habitat patches that lie within a specified critical distance are connected by 'edges'. Components of a graph are both individual patches and networks of patches connected by edges. A completely connected network will have one component and in a completely disconnected network the number of components will equal the number of patches. Patterns and numbers of connections among patches in a network across a range of critical distances provide insight into the manner in which the network becomes functionally fragmented for species with any particular movement capabilities.

Input patch files and distance matrices for graph analyses were created with the program GENGRAPH using centroid to centroid Euclidean distance adjusted by subtracting the radius of gyration (Urban, 2003). Although landscape resistance can be incorporated into measures of distance among patches (Theobald, 2005), I used the simpler Euclidean distance because no data are available to quantify biologically meaningful resistance surfaces for *A. albens*. GENGRAPH input files for each of the three networks were created using the GRID REGIONGROUP and SAMPLE commands in ArcINFO (Environmental Systems Research Institute, 2005) to create text file representations of raster grids of *A. albens* patches. Patches were delineated using the eight neighbor rule. This process yielded 91 patches in the full data set, 65 patches in the critical habitat data set, and 61 patches in the CHMS reserve data set.

I calculated the number of components in each network and for the largest component I calculated the number of patches, number of edges, and graph diameter (a measure of network extensiveness) for critical distance thresholds from 100 to 10,000 m in 100 m increments using the program THINEDGE, part of the LANDGRAPHS package (Urban, 2003). I also used the distance matrix created by the program GENGRAPH to calculate the number of edges per patch and the proportion of patches with at least one edge for a subset of critical distances between 500 and 2500 m at 500 m increments to assess local interpatch connectivity for individual patches in the landscape. I used CONEFOR SENSINODE 2.2 (CS22) (Saura and Pascual-Hortal, 2007a) to calculate two statistics that integrate patch extensiveness and isolation into measures of habitat availability: the integral index of connectivity (IIC) and the probability of connectivity (PC) for critical distances between 500 and 2500 m at 500 m increments. IIC is calculated as

$$\mathrm{IIC} = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n} a_i a_j/(1+nl_{ij})}{A_L^2}$$

where a_i and a_j are the areas of patches i and j, nl_{ij} is the number of links in the shortest path between patches i and j, and A_L is the total landscape area (Pascual-Hortal and Saura, 2006). The PC index is calculated as

$$\mathrm{PC} = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n} a_i a_j p_{ij}^{*}}{A_L^2}$$

where a_i, a_j, and A_L are defined as above and p_{ij}^{*} is defined as the maximum value of the product of the probabilities of each of the steps in a path connecting patches i and j (Saura and Pascual-Hortal, 2007b). Thus, the two metrics differ from one another in that IIC is binary (connections either do or do not exist at a particular distance) while PC is probabilistic (there is a user defined probability of two patches being connected at a particular distance). Probability of dispersal for each distance was set to 0.5 for all PC calculations. The PC represents the probability that two organisms placed randomly in a landscape would be able to reach each other within the network of identified habitat. In addition to calculating these two indices, I use Conefor 2.2 to assess the importance of each patch in terms of the change in IIC and PC that would occur if that patch was removed from the network.

I used the non-parametric Gamma statistic to correlate the rank of each patch in which a genetic sampling location occurred in terms of amounts of genetic diversity with the rank of the same patch in terms of plant density, size, the number of edges per patch at five different critical distances (500, 1000, 1500, 2000, and 2500 m), and the importance of the patch in contributing to IIC and PC at those same spatial scales. Gamma is interpreted as the probability that the rank ordering among two lists is the same. It is preferable to Kendall's Tau because it accounts for tied ranks.

Potential for changes in connectivity among *A. albens* occurrences under the two conservation reserves was quantified by recalculating the graph theoretic metrics for only the patches included in the two sets of reserves. I graphically assessed the critical distances at which connectivity within each of the three networks changed to evaluate the spatial scales and locations at which there was greatest potential for changes in gene flow.

3. Results

3.1. Population genetic diversity

At the species level (i.e., all samples combined), 66% of the 12 loci examined were polymorphic. A total of 69 alleles were detected at all loci combined. On an average there were 5.7 (SD = 3.1) alleles per locus and 8.0 (SD = 2.76) alleles per polymorphic locus. Species-level H_e averaged 0.14 (SD = 0.14) and H_o averaged 0.15 (SD = 0.18) across loci.

The proportion of polymorphic loci within each of the 30 sampled occurrences ranged from 0.42 to 0.58 and averaged 0.50 (SD = 0.06) (Table 1). The number of alleles per occurrence averaged 28.8 (SD = 4.4) and ranged from 17 (D7AA) to 37 (D1RNC). Due to its small population size, D7AA also had the smallest sample number of individuals of any occurrence (n = 9), which likely accounts for the small number of alleles. Within occurrences, A ranged between 1.42 and 3.08 with a mean of 2.40 (SD = 0.3); A_p ranged from 2.0 to 4.29 and averaged 3.47 (SD = 0.49). H_e within occurrences ranged from 0.11 to 0.19 and averaged 0.14 (SD = 0.02) (Table 1). H_o within occurrences was comparable in magnitude, ranging from 0.11 to 0.21, and averaging 0.15 (SD = 0.02) over all occurrences (Table 1). Coancestry coefficients indicated slight to moderate heterozygote excess in most occurrences (Table 1). Sample F12RC is an exception with an f of 0.14, indicating heterozygote deficiency.

I documented a total of 14 private alleles in nine occurrences (Table 2). More than half of these occurrences had two private alleles each. Private alleles were always at low frequency (all <0.02) in the sampled occurrence (Table 2). Occurrences with private alleles were located in a limited portion within the study area from Blackhawk Mountain south and east to Lone Valley; private alleles were lacking in the extreme western and southeastern regions. In addition to the strictly private alleles, a large proportion of the alleles in this species were limited to a relatively few occurrences with 30 of the 69

Table 2 – Distribution of 14 private alleles among 12 loci in 30 occurrences of A. albens

Locus	Allele	Frequency	Population
AAT	F	0.017	C4AA
LAP	D	0.019	D10AA
PGM	A	0.017	B4AA
PGM	D	0.017	D10AA
TPI-1	A	0.017	C100AA
TPI-1	H	0.017	D1RNC
TPI-3	B	0.017	D126RC
TPI-4	A	0.017	D1AA
TPI-4	C	0.017	C15AA
TPI-4	E	0.017	D126RC
MDH-1	A	0.017	C15AA
MDH-2	B	0.017	D4AA
MDH-3	B	0.017	C4AA
UDP	A	0.017	D4AA

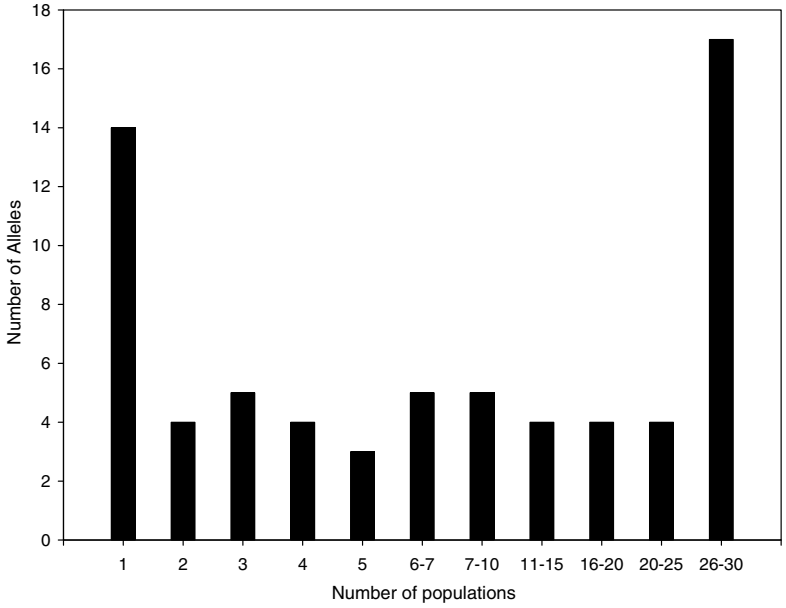

Fig. 3 – Frequency distribution of the number of occurrences in which alleles occur. A large proportion of the alleles in *A. albens* occur in relatively few occurrences.

alleles recorded for the species being found in 5 or fewer occurrences (Fig. 3).

The coancestry coefficient, f, averaged −0.08 across all loci and occurrences; based on confidence intervals derived from bootstrap estimates f was not significantly different from zero; however, individual loci did differ significantly from 0 (Table 3). F averaged −0.06, but also was not significantly different from zero (Table 3) indicating no deviation in heterozygosity in individuals relative to the all occurrences combined. Population differentiation, as assessed using θ_p, was small, averaging only 0.01 over all loci and occurrences; however, it was significantly different from zero (Table 3). Based on this level of differentiation, the number of migrants among occurrences per generation was estimated to be 24.7. The private allele method yielded a similar result of 25.4 migrants per generation.

None of the 30 samples showed any evidence of heterozygosity excess (Table 1) and thus there was no evidence of recent bottlenecks in the populations from which these samples come.

Nei's (1978) genetic distance between pairs of sampling locations averaged 0.002 (SD = 0.003) and ranged from −0.00293 to 0.013. Geographic distances between these same locations averaged 7.27 km (SD = 4.85) and ranged from 0.48 to 22.81 km. A Mantel test and associated permutation test indicated significant correlation between genetic and geographic distances ($r = 0.399$, $p = 0.003$).

Table 3 – Hierarchical F-statistics for 12 loci in 30 occurrences of A. albens calculated using methods of Weir (1996)

Locus	f	F	θ_p
AAT	0.12 (0.04)	0.14 (0.04)	0.02 (0.01)
PGI2	−0.06 (0.04)	−0.04 (0.04)	0.01 (0.005)
LAP	0.17 (0.12)	0.17 (0.12)	0.00 (0.003)
PGM	−0.08 (0.02)	−0.07 (0.02)	0.02 (0.004)
TPI-1	−0.02 (0.03)	−0.02 (0.03)	0.01 (0.006)
TPI-2	0.59 (0.14)	0.59 (0.14)	0.00 (0.003)
TPI-3	0.00 (0.0004)	0.00 (0.0009)	0.00 (0.0009)
TPI-4	0.11 (0.03)	0.12 (0.03)	0.01 (0.007)
MDH-1	−0.01 (0.003)	−0.01 (0.002)	0.00 (0.003)
MDH-2	0.00 (0.0004)	0.00 (0.00001)	0.00 (0.0004)
MDH-3	0.00 (0.0004)	0.00 (0.00)	0.00 (0.0004)
UDP	−0.35 (0.02)	−0.33 (0.02)	0.01 (0.01)
Overall	−0.08	−0.06	0.01
Upper bound	0.082	0.095	0.016
Lower bound	−0.221	−0.207	0.007

Standard deviations at each locus are in parentheses and were calculated jackknifing over occurrences.

3.2. *Graph theoretic landscape patterns*

The 91 high density patches of *A. albens* habitat were distributed such that they comprised a single connected network with a graph diameter of 21,216 m at a critical distance of ~2100 m (Figs. 2A and 4). The network broke into three components at a critical distance of 2000 m and into five components at approximately 1700 m (Figs. 2B and C, and 4). The graph diameter of, and number of patches in, the largest component also declined abruptly at these points (Fig. 4). Even as the overall network broke apart, however, four of the subcomponents remained internally well connected (Fig. 2B–D). This local connectivity was due to the fact that most occurrences lay in close proximity to at least some other occurrences. For example, 87.8% of mapped patches were within 500 m of at least one other patch, 94% of all mapped patches were within 1 km of at least one other patch, and 72.2% were within 500 m of at least 2 other patches (Table 5). Although there is

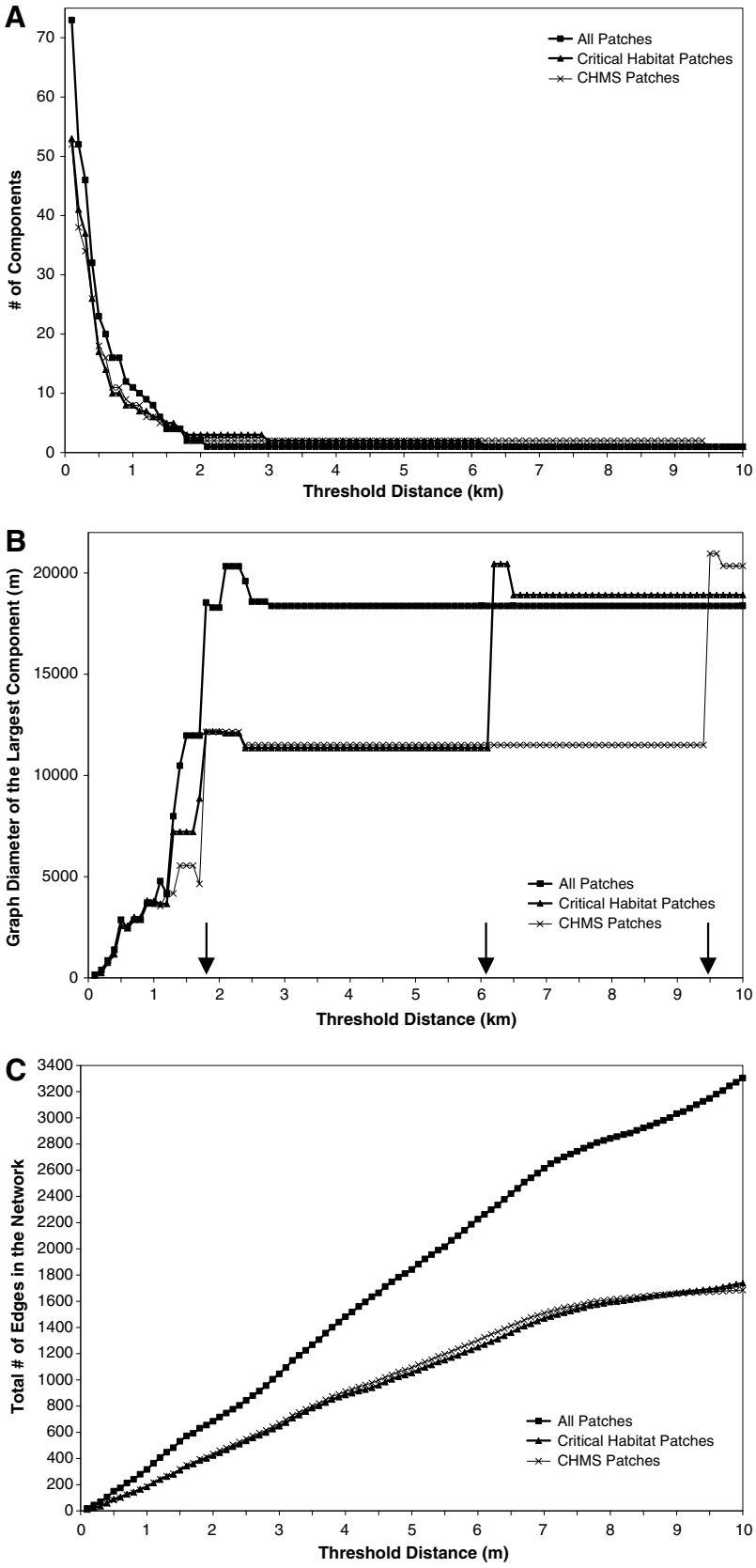

Fig. 4 – Changes in (A) the number of components, (B) the graph diameter of the largest component of the network, and (C) the total number of edges in all components in the network as a function of critical threshold distance. Arrow on panel B denotes critical distance thresholds for the total network (2100 m), the critical habitat network (6200 m), and the CHMS network (9500 m).

Table 4 – Comparison of genetic diversity values in other Astragalus species and subsets of other angiosperms

Species	Range	Species level		Population level					Citation
		P	A	P_p	A	A_p	H_o	H_e	
Astragalus albens	Restricted	0.66	5.75	0.50	2.4	3.47	0.148	0.139	This paper
Astragalus bibullatus	Restricted	0.27	1.4	0.256	NR	NR	0.064	0.63	Baskauf and Snapp (1998)
Astragalus cremnophylax var. *cremnophylax*	Restricted	NR	NR	NR	1.44	2.48	0.082	0.135	Travis et al. (1996)
Astragalus osterhoutii	Restricted	0.167	2.42	0.111	2.50	2.33	NR	NR	Karron (1989)
Astragalus linifolius	Restricted	0.33	2.22	0.250	2.25	2.37	NR	NR	Karron (1989)
Astragalus pattersoni	Widespread	0.25	2.25	0.194	2.35	2.18	NR	NR	Karron (1989)
Astragalus pectinatus	Widespread	0.33	3.00	0.330	2.73	3.00	NR	NR	Karron (1989)
Outcrossing plants	Mixed	0.501	1.99	0.359	1.54	NR	NR	0.124	Hamrick and Godt (1990)
Selfing plants	Mixed	0.418	1.69	0.200	1.31	NR	NR	0.074	Hamrick and Godt (1990)
Endemic plants	Restricted	0.400	1.80	0.263	1.39	NR	NR	0.063	Hamrick and Godt (1990)
Regionally distributed	Restricted	0.529	1.94	0.364	1.55	NR	NR	0.118	Hamrick and Godt (1990)
Widespread plants	Widespread	0.589	2.29	0.430	1.72	NR	NR	0.159	Hamrick and Godt (1990)

NR = not reported; P = proportion of polymorphic loci; A = number of alleles per locus; A_p = number of alleles per polymorphic locus; H_o = observed heterozygosity; H_e = expected heterozygosity.

Table 5 – Connectivity among high density patches of A. albens as quantified by the number of edges per node, the integral index of connectivity, and potential connectivity in the total network (91 patches), the network of sites designated as critical habitat (65 patches), and the proposed Carbonate Habitat Management Strategy reserve network (61 patches)

Threshold distance Data Set	Mean # of edges per patch	Median # of edges per patch	Maximum # of edges per patch	Proportion of patches with at least one edge	Total # of edges in the network	Integral index of connectivity (IIC)	Potential connectivity (PC)
2500 m							
All	18.53	20	31	1.00	1686	0.0000081	0.0000095
Critical habitat	16.46	18	30	0.98	1070	0.0000052	0.0000063
CHMS reserves	18.00	20	27	0.92	1098	0.0000047	0.0000056
2000 m							
All	25.03	17	27	0.99	1386	0.0000069	0.0000084
Critical habitat	13.02	11	26	0.98	846	0.0000049	0.0000055
CHMS reserves	14.23	13	26	0.92	868	0.0000042	0.0000049
1500 m							
All	11.67	10	22	0.99	1062	0.0000060	0.0000071
Critical habitat	9.57	8	21	0.96	622	0.0000036	0.0000046
CHMS reserves	10.46	9	21	0.92	638	0.0000026	0.0000040
1000 m							
All	6.94	6	16	0.96	632	0.0000041	0.0000054
Critical habitat	5.67	5	15	0.96	368	0.0000026	0.0000035
CHMS reserves	6.10	5	16	0.89	372	0.0000023	0.0000030
500 m							
All	3.27	3	12	0.86	298	0.0000026	0.0000036
Critical habitat	2.77	2	11	0.89	180	0.0000018	0.0000023
CHMS reserves	2.69	2	12	0.80	164	0.0000015	0.0000020

a high degree of local connectivity, both IIC and PC indicate that the existing network of patches has low intra- and inter-patch connectivity relative to the whole landscape in which the patches are found (Table 5).

3.3. *Relationships of genetic diversity with population and landscape characteristics*

Only two aspects of genetic diversity were consistently correlated with population or landscape characteristics, and even then the correlation coefficients were relatively small (Table 6). Patches with high allelic richness (TA, A, A_p, and number of alleles per individual) also had a larger number of edges connecting to other patches at the 500 and 1000 m scales. There was no correlation between the rank of patches in terms of any measure of genetic diversity and plant density; however, patches with a high rank for proportion of polymorphic loci were highly ranked in terms of patch size (Table 6). Graph metrics that incorporate both intra- and inter-patch connectivity had mixed relationships with genetic diversity that were difficult to interpret. Patches that contributed most to the IIC also tended to be high in TA, A, and, P at the 1000 m scale, high in P and H_e at the 500 m scale, and high in H_e at the 2500 m scale (Table 6). Plots with high connectivity as measured by PC were also high in A at the 1500 m scale, but the gamma coefficient for this relationship was low (Table 6).

Table 6 – Gamma correlation coefficients between genetic diversity statistics and plant density, patch area, and three aspects of landscape connectivity calculated across a range of critical distances from 500 to 2500 m

Variable	P	TA	A	A_p	H_e	H_o	f
Plant density	−0.255	0.018	0.018	−0.033	−0.205	−0.142	−0.007
Patch area (ha)	**0.375**	0.086	0.091	0.044	0.245	0.175	0.044
# Edges, 500 m	−0.289	**0.324**	**0.328**	**0.372**	0.061	0.200	−0.080
# Edges, 1000 m	−0.110	**0.305**	**0.309**	**0.318**	0.000	0.115	−0.043
# Edges, 1500 m	−0.114	0.141	0.145	0.135	−0.102	0.022	−0.048
# Edges, 2000 m	−0.112	0.205	0.209	0.203	−0.126	−0.002	−0.058
# Edges, 2500 m	−0.046	0.238	0.242	0.214	−0.132	−0.026	−0.026
IIC, 500 m	**0.319**	0.204	0.208	0.113	**0.269**	0.161	0.152
IIC, 1000 m	**0.333**	**0.326**	**0.329**	0.176	0.241	0.189	0.077
IIC, 1500 m	0.304	0.173	0.178	0.064	0.082	0.052	0.077
IIC, 2000 m	0.283	0.086	0.091	0.054	0.110	0.137	−0.082
IIC, 2500 m	0.290	0.076	0.071	0.054	**0.302**	0.218	−0.030
PC, 500 m	0.241	0.250	0.254	0.147	0.222	0.152	0.096
PC, 1000 m	0.269	0.250	0.254	0.118	0.213	0.152	0.077
PC, 1500 m	0.262	0.265	**0.269**	0.132	0.218	0.166	0.073
PC, 2000 m	0.269	0.260	0.264	0.147	0.208	0.185	0.026
PC, 2500 m	0.276	0.25	0.254	0.137	0.218	0.194	0.016

Number of edges measures only among-patch connectivity; the integral index of connectivity (IIC), and probability of connectivity (PC) measure both within- and among-patch connectivity.
The magnitude of the Gamma coefficient is the difference between the probability that the rank ordering of the two variables agrees minus the probability that they disagree, divided by 1 minus the probability of ties.
Coefficients significant at the $P < 0.05$ level are in bold.

3.4. *Genetic diversity in reserve networks*

Critical habitat designated for *A. albens* included 96.5% of the aerial extent of mapped high-density patches of the species and included all detected alleles at the species level. Genetic diversity patterns generally reflected those found when all populations were conserved (Table 7). All habitat types in which the species occurs were represented as was the species' elevational range. The geographic range of *A. albens* was also maintained in that the east–west and north–south distributional limits would not be decreased if the excluded populations were lost. Occurrences not designated as critical habitat were all in the west-central portion of the species range where a number of relatively small patches of occupied habitat exist (Fig. 2J). Two relatively isolated clusters of patches are found in this portion of the range; the more western occurring of these clusters was included in critical habitat. A number of quarries and associated disturbances are also found in these areas and *A. albens* occurrences here are in remnant natural habitat around the periphery of disturbed areas, suggesting that existing disturbances have already eliminated previously occupied habitat. Almost none of the low density occurrences on the north slope of Blackhawk Mountain were included in critical habitat.

Patches of *A. albens* included in the proposed CHMS reserves included 89.8% of the total aerial extent of the high density locations including the extents of the geographic and ecological ranges (Fig. 2O). As was the case with critical habitat, all sampled alleles would be captured if the core reserves were protected, and patterns of genetic diversity were similar to the full data set (Table 7). The primary difference from critical habitat was that the CHMS network excluded both of the clusters of *A. albens* that were found in the west-central portion of the range (Fig. 2J and O). The westernmost population cluster primarily occurred in a vegetation association that was not typical of the rest of the range of *A. albens* (singleaf pinyon/*Purshia-Yucca* woodland). This vegetation type dominated the two westernmost components of habitat, only one of which was included in the CHMS reserve network. Although this network had fewer high density patches than did the locations designated as critical habitat, it included larger blocks of habitat and would also include the north slope of Blackhawk Mountain.

Table 7 – Comparison of amounts of sampled genetic diversity that would be protected by the various protective mechanisms proposed by the US Fish and Wildlife Service, the Forest Service, and the Bureau of Land Management

Conservation status	Species level		Population level				
	P	A	P	A	A_p	H_o	H_e
Astragalus albens in critical habitat	0.5	5.75	0.50	2.40	3.46	0.149	0.139
Astragalus albens populations in Forest Service CHMS reserves	0.5	5.58	0.50	2.43	3.49	0.150	0.142
Astragalus albens populations in BLM ACECs	0.52	2.33	0.52	2.33	3.29	0.145	0.137
Astragalus albens in Forest Service Reserves and BLM ACEC combined (complete CHMS)	0.50	5.74	0.51	2.41	3.45	0.149	0.139

3.5. Connectivity of reserve networks

The 65 patches designated as critical habitat for *A. albens* were not connected into a single component until the critical distance was 6200 m. Connectedness of the network as measured by the total number of edges is 58–63% of that of the total network at scales of 500–2500 m (Table 5 and Fig. 4C). Connectedness measured by IIC dropped to 60–71% of the complete network over this range of critical distances and PC dropped to 64–66% of the total (Table 5). The 61 patches proposed for inclusion in the CHMS network required a critical distance of 9500 m to become a single component. The number of edges was 55–65% of the number found in the total network at scales of 500–2500 m (Table 5 and Fig. 3C). Connectedness measured by IIC dropped to 43–61% of that of the complete network over this range of critical distances and PC drops to 56–59% of the existing network. Despite drastic overall decreases in network connectivity, local connectivity, as measured by the mean number of edges per patch and the proportion of patches with at least one edge, remains similar to current conditions in both conservation networks at all distances between 500 and 2500 m (Table 5). For example, the mean number of edges per patch in the critical habitat and CHMS networks is >81% of that of the total network over all critical distances (Table 5). Thus, changes in connectivity were due more to isolation of western occurrences from eastern occurrences and due to decreases in redundancies of connections among patches within clusters than to complete isolation of patches within clusters.

4. Discussion

Given its degree of endemism, *A. albens* supports a surprisingly large amount of genetic variation. Values for *P* and H_e at the species level were comparable to values for regionally distributed species (Hamrick and Godt, 1990, 1996). Species-level *A* exceeded average values for all distributional categories of plant species (Godt et al., 1996; Hamrick and Godt, 1990; Loveless and Hamrick, 1989) (Table 4). Population-level values for most diversity measures exceeded averages for widespread species. These high levels of genetic diversity contrast with other studies in the genus *Astragalus* that have shown reduced levels of variation in restricted species (Baskauf and Snapp, 1998; Karron, 1989; Travis et al., 1996) (Table 4). Although there are trends for reduced diversity in rare species, similarly high levels of diversity have been noted for other rare taxa (Archibald et al., 2001; Cruzan, 2001; Gitzendanner and Soltis, 2000; Prober and Brown, 1994; Young et al., 1996), including unrelated taxa that co-occur with *A. albens* (*Eriogonum ovalifolium* var. *vineum*, *Erigeron parishii*, and *Oxytheca parishii* var. *goodmaniana*) (Neel, 2000; Neel and Ellstrand, 2001, 2003).

Potential explanations for high levels of genetic diversity include evolutionary origin, consistently large population sizes, a predominantly outcrossing mating system, a persistent seed bank, and high levels of gene flow among populations. It is possible that the ancestor to *A. albens* was genetically diverse and current diversity retains that historical signature. There is no information on phylogenetic affinities of *A. albens* beyond its inclusion in section Leptocarpi (Barneby, 1964). Without such information it is not possible to compare diversity levels in closely related taxa. Hybrid origin is known to yield highly diverse taxa (e.g., Arft and Ranker, 1998; Hegde et al., 2006; Sun, 1996). Although it was not specifically examined, there is no evidence of hybrid origin for *A. albens* in that it is not similar to or intermediate between other sympatric species. Population size certainly plays a role in the observed high levels of diversity because small population size would have caused reduction in diversity over few generations regardless of the starting levels of diversity. Thus, it is likely that populations have been relatively large for most of the evolutionary history of the species. Mating system and the resulting mating patterns are also important factors because of the role outcrossing versus selfing and other nonrandom mating play in structuring genetic diversity within and among populations and determining rates of loss of that diversity across generations (Brown, 1989; Hamrick et al., 1979). Nothing is known of the mating system in *A. albens* and both highly selfing and highly outcrossing species are known from the genus (Karron, 1989). Levels of heterozygosity and genetic diversity patterns in *A. albens* are consistent with those of highly outcrossed species in general (Hamrick and Godt, 1996; Karron, 1991) and outcrossed species of *Astragalus* specifically (Karron, 1987).

Levels of diversity can also be enhanced by high rates of gene flow among populations because such gene flow increases effective population size and facilitates exchange of alleles. Levels of differentiation based on θ_p were lower than is typical for most other plant species including other Fabaceae (Table 4) indicating high levels of gene flow, at least historically. The correlation between allelic richness and connectedness at the scale of 500–1000 m was suggestive that gene flow occurring at this scale has contributed to allelic diversity within populations (Table 6). On the other hand the large number of private or otherwise restricted alleles indicated some degree of isolation (Table 1; Fig. 3). Statistics related to F_{ST} such as θ_p and G_{ST} are most sensitive to alleles that are at moderate to high frequency thus the low frequency geographically restricted alleles were not reflected in the θ_p value. Values of F_{ST}-related statistics are also known to be depressed by high allelic diversity (Hedrick, 2005) because the maximum possible value of within-population heterozygosity (H_S) decreases as the number of alleles per locus increases. Because $G_{ST} = 1 - H_S/H_T$, depression of H_S ultimately limits the range of possible values of the ratio of within- to among-population variation. Allelic diversity was not the cause of low θ_p in this case and applying the correction factor suggested by Hedrick (2005) to account for the maximum possible H_S did not affect estimates of differentiation.

Regardless of the mechanisms that have generated them, the observed genetic diversity patterns can be used to evaluate and inform existing and proposed conservation measures. The amounts and patterns of diversity indicate no imminent genetic threats to this species that require specific management actions such as genetic rescue. In fact, the data indicate an optimistic view of the biological potential for recovering this species in that there are likely to be few intrinsic genetic impediments to species persistence. As such, recovery can likely be realized through actions that manage ecological

conditions, remove the threat of habitat destruction, and restore sites that have already been degraded by human activities. The probability of maintaining diversity in a particular species is a result of complex interactions among population sizes, life history characteristics, dispersal ability, and mating systems. Despite these idiosyncrasies, the key elements to successful recovery are retaining or increasing numbers of populations, keeping the sizes of those populations large relative to historical levels, and maintaining the spatial structure of populations that facilitate historical levels of seed dispersal and pollen movement. The lack of relationship between plant density and genetic diversity supports the inference that populations of *A. albens* have historically been large, at least larger than sizes at which genetic risks manifest. Thus, it is especially important to prevent severe reductions in population size to prevent alteration of mating structure within populations that would increase selfing or mating among relatives (Holsinger and Vitt, 1997; Husband and Schemske, 1996). The risk of inbreeding depression due to increased inbreeding is of particular concern when genetic load (in terms of deleterious recessive alleles) is high. The large number of private alleles is a circumstantial evidence that genetic load might be high in this species. Similarly, the high levels of gene flow as measured by θ_p (Table 3) provide evidence that isolation under anthropogenic fragmentation would reduce natural gene flow levels among populations which could then exacerbate loss of diversity through drift by decreasing effective population sizes in the remaining isolated occurrences (Templeton et al., 1990; Young et al., 1996). Estimates of gene flow based on hierarchical *F*-statistics are known to be imprecise (Bohonak, 1999) and thus the specific number of migrants estimated based on θ_p needs to be interpreted with caution. Still, the lack of differentiation documented and correlation with connectivity indicates a relatively high degree of connectivity combined with little genetic drift within populations over evolutionary time.

The graph metrics illustrate that connectivity is not a simple concept: the degree of connectivity construed is a function of which metric is examined. For example, all patches were connected into a single network only at a critical distance >2500 m, a distance beyond what is reasonable to expect to be typical for pollen and seed dispersal of most plant species that lack specific mechanisms. Even species with wind dispersed pollen show spatial autocorrelation of genetic diversity at scales from 500 to 1700 m (Williams et al., 2007). IIC and PC also indicate low levels of connectivity, but the small values for these indices are due to the inclusion of the total landscape area in calculation of these two values. Their dependence on the ratio between area of the focal habitat and total landscape extent makes these indices potentially problematic for situations in which patches are very small relative to the total landscape. As with all landscape pattern metrics, however, the absolute values are less useful to interpret than are relative values such as the magnitude of change resulting from losing patches that are excluded from the conservation or the relative importance of individual patches in maintaining connectivity as measured by these statistics.

Although some metrics indicate low connectivity in the network as a whole, the fact that more than 86% of *A. albens* patches lie within 500–1000 m of at least some other patches (Table 5) indicates local connectivity. Most gene flow in plants takes place on the scale of meters, but distances <1000 m are close enough to allow substantial gene flow (Ellstrand, 2003). Because the majority of patches are within this distance of at least one other patch, there is a high potential for gene flow directly among adjacent patches and in a stepping stone fashion across many patches throughout most but not all of the existing network of occurrences. Maintaining existing clusters of populations will be important for facilitating ongoing gene flow. The widely scattered individuals on the north slope of Blackhawk Mountain provide an additional potential mechanism by which occurrences in that portion of the range may remain connected. The higher density occurrences may occur in habitat that is more suitable under a wider range of annual weather conditions and that scattered individuals germinate from a seedbank in years with sufficient precipitation. These scattered individuals may intermittently increase connectivity among the high density occurrences. This hypothesis needs to be tested by quantifying the abundance and spatial distribution of these individuals, the annual frequency with which they appear and environmental correlates of their appearance. Their role in providing connectivity needs to be assessed by quantifying gene flow through these individuals among higher density occurrences using paternity-based approaches (e.g., Smouse et al., 2001; Sork et al., 1999; Sork and Smouse, 2006).

Both critical habitat and the CHMS networks include >89% of the patches of high density occurrences the species, including the most extensive ones. All the sampled alleles (Table 7) and all habitat types that support *A. albens* are represented as are the geographic and much of the ecological ranges of the species. The primary concern with both networks is that they include fewer populations than existed at the time the species was listed. Further, the excluded occurrences are all in one part of the species range. Any unique adaptations to local environmental conditions in those areas would be lost if those occurrences were eliminated. This concern is greater for the CHMS reserves due to exclusion of most of the occurrences in the west-central portion of the range (Fig. 2O), some of which are designated as critical habitat (Fig. 2J). Spatial bias in conservation is not uncommon because threats to species due to conflicts with other resource uses or values are often themselves spatially correlated. In this case the priority conservation areas in both plans exclude the highest quality limestone ore and as such minimize conflict with mining activities to facilitate both biodiversity conservation and continued economic benefits.

Assessing conservation value of the excluded occurrences is not straightforward. They are mostly relatively small and many are interspersed with existing mining activity. Larger, less fragmented blocks of habitat that do not have to be micromanaged in the midst of an industrial milieu are clearly more valuable for conservation. An additional concern with critical habitat is that it is focused immediately around occupied habitat and thus it does not provide any ecological context or area for dynamic ecological processes around sites that currently support *A. albens*. In contrast, the CHMS network includes larger blocks of habitat that could continue to support large-scale ecological processes. The two sets of conservation areas are, of course, not mutually exclusive and are potentially highly complementary. Critical habitat protects

most of the highest density populations from all threatening activities while the CHMS reserves would also protect buffer zones around critical habitat from mining activities but not from other uses. Such a pattern of zones with varying degrees of restriction would implement a model of conservation long suggested in the literature (e.g., Noss and Harris, 1986).

Loss of populations excluded from critical habitat or CHMS reserves would change patterns of connectivity by increasing distances required for a fully connected network from 2100 to 6200 m or to 9500 m, respectively The biological effects of these increases depend on the dispersal distances of seed and pollen. Although nothing is known of dispersal distances for *A. albens* specifically, seed dispersal distances >2 km are unlikely for a species with no specialized dispersal mechanism (Cain et al., 1998) and pollen flow distances for insect pollinated plants are typically <1 km (Ellstrand, 2003). However, current genetic diversity distributions indicate a highly connected network of populations. If natural dispersal distances are primarily <1 km, the loss of the populations excluded from CHMS reserves would not affect gene flow among the majority of remaining populations because distances within the remaining networks are already <1 km. Additional individuals between the high density mapped populations, such as those in the Blackhawk Mountain area, could provide stepping stones of connectivity between mapped occurrences that would lessen the apparent losses of connectivity documented here. It is not possible to completely assess the effect of changes in network but this research points out the data we need.

In summary, *A. albens* currently supports a substantial amount of genetic variation at the species and population levels indicating that detrimental effects due to lack of genetic diversity are not of immediate concern. As such, managing ecological conditions that maintain large population sizes and low levels of among-population isolation and that represent the ecological range of the species is likely sufficient to maintain genetic diversity. The areas designated as critical habitat and proposed for CHMS reserves have high potential to meet these goals. The primary concern with these conservation networks is the exclusion of all occurrences in a distinct portion of the species range. Because of the potential for local adaptation and the effect their loss would have on connectivity among populations, it is important to maintain at least some of these occurrences or at the very least conserve germplasm from them for use in mine reclamation efforts. The data presented here serve as a valuable baseline for monitoring effectiveness of these management actions in maintaining genetic diversity, evaluating consequences of further fragmentation and population loss, and prioritizing locations for restoration that will contribute to maintaining or increasing network connectivity. Quantifying actual connectivity among populations is especially important for verifying assumptions that maintaining current spatial population distributions will maintain movement among populations.

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REFERENCES

30 USC 29, 1872. The General Mining Law of 1872. U.S. Code, pp. 22–42.

Allendorf, F.W., 1986. Genetic drift and the loss of alleles versus heterozygosity. Zoo Biology 5, 181–190.

Archibald, J.K., Wolf, P.G., Tepedino, V.J., Bair, J., 2001. Genetic relationships and population structure of the endangered steamboat buckwheat *Eriogonum ovalifolium* var. *williamsiae*. American Journal of Botany 88, 608–615.

Arft, A.M., Ranker, T.A., 1998. Allopolyploid origin and population genetics of the rare orchid *Spiranthes diluvialis*. American Journal of Botany 85, 110–122.

Barneby, R.C., 1964. Atlas of North American *Astragalus*. Memoirs of the New York Botanical Garden 13, 1–1188.

Barrett, S.C.H., Kohn, J.R., 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk, D.A., Holsinger, K.E. (Eds.), Genetics and Conservation of Rare Plants. Oxford University Press, New York, pp. 3–30.

Baskauf, C.J., Snapp, S., 1998. Population genetics of the cedar-glade endemic *Astragalus bibullatus* (Fabaceae) using isozymes. Annals of the Missouri Botanical Garden 85, 90–96.

Bohonak, A.J., 1999. Dispersal, gene flow, and population structure. Quarterly Review of Biology 74, 21–45.

Brown, A.H.D., 1989. Genetic characterization of plant mating systems. In: Brown, A.H.D., Clegg, M.T., Kahler, A.L., Weir, B.S. (Eds.), Plant Population Genetics, Breeding, and Genetic Resources. Sinauer, Sunderland, MA, USA, pp. 145–162.

Bunn, A.G., Urban, D.L., Keitt, T.H., 2000. Landscape connectivity: a conservation application of graph theory. Journal of Environmental Management 59, 265–278.

Cain, M.L., Damman, H., Muir, A., 1998. Seed dispersal and the Holocene migration of woodland herbs. Ecological Monographs 68, 325–347.

Calabrese, J.M., Fagan, W.F., 2004. A comparison shopper's guide to connectivity metrics; trading off between data requirements and information content. Frontiers in Ecology and the Environment 2, 529–536.

Coates, D.J., Atkins, K.A., 2001. Priority setting and the conservation of Western Australia's diverse and highly endemic flora. Biological Conservation 97, 251–263.

Coates, D.J., Carstairs, S., Hamley, V.L., 2003. Evolutionary patterns and genetic structure in localized and widespread species in the *Stylidium caricifolium* complex (Stylidiaceae). American Journal of Botany 90, 997–1008.
Cole, C.T., Biesboer, D.D., 1992. Monomorphism, reduced gene flow, and cleistogamy in rare and common species of *Lespedeza* (Fabaceae). American Journal of Botany 79, 567–575.
Cornuet, J.M., Luikart, G., 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144, 2001–2014.
Cowling, R.M., Pressey, R.L., Lombard, A.T., Desmet, P.G., Ellis, A.G., 1999. From representation to persistence: requirements for a sustainable system of conservation areas in the species-rich Mediterranean-climate desert of southern Africa. Diversity and Distributions 5, 51–71.
Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M., Wayne, R.K., 2000. Considering evolutionary processes in conservation biology. Trends in Ecology & Evolution 15, 290–295.
Cruzan, M.B., 2001. Population size and fragmentation thresholds for the maintenance of genetic diversity in the herbaceous endemic *Scutellaria montana* (Lamiaceae). Evolution 55, 1569–1580.
D'Eon, R.G., Glenn, S.M., Parfitt, I., Fortin, M.-J., 2002. Landscape connectivity as a function of scale and organism vagility in a real forested landscape. Conservation Ecology 6, 10 <http://www.consecol.org/vol16/iss12/art10>.
Diniz-Filho, J.A., De Campos Telles, M.P., 2002. Spatial autocorrelation analysis and the identification of operational units for conservation in continuous populations. Conservation Biology 16, 924–935.
Dudash, M.R., 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. Evolution 44, 1129–1139.
Dyer, R.J., Nason, J.D., 2004. Population graphs: the graph theoretic shape of genetic structure. Molecular Ecology 13, 1713–1727.
Ellstrand, N.C., 2003. Current knowledge of gene flow in plants: implications for transgene flow. Philosophical Transactions of the Royal Society of London Series B 358, 1163–1170.
Ellstrand, N.C., Elam, D.R., 1993. Population genetic consequences of small population size: implications for plant conservation. Annual Review of Ecology and Systematics 24, 217–242.
Environmental Systems Research Institute, 1996. ArcView 3.2. Environmental Systems Research Institute.
Environmental Systems Research Institute, 2005. ArcInfo 9.1. Copyright 1982–2005, Redlands, CA.
Fahrig, L., 1997. Relative effects of habitat loss and fragmentation on population extinction. Journal of Wildlife Management 61, 603–610.
Faith, D.P., 2003. Environmental diversity (ED) as surrogate information for species-level biodiversity. Ecography 26, 374–379.
Faith, D.P., Ferrier, S., Walker, P.A., 2004. The ED strategy: how species-level surrogates indicate general biodiversity patterns through an'environmental diversity' perspective. Journal of Biogeography 31, 1207–1217.
Fenster, C.B., Dudash, M.R., 1994. Genetic considerations for plant population restoration and conservation. In: Bowles, M.L., Whelan, C.J. (Eds.), Restoration of Endangered Species. Cambridge University Press, London, pp. 34–61.
Ferrari, J.R., Lookingbill, T.R., Neel, M.C., 2007. Two measures of landscape-graph connectivity: assessment across gradients in area and configuration. Landscape Ecology 22, 1315–1323.
Ferrier, S., 2002. Mapping spatial pattern in biodiversity for regional conservation planning: where to from here? Systematic Biology 51, 331–363.
Frankham, R., 1995. Inbreeding and extinction – a threshold effect. Conservation Biology 9, 792–799.
Frankham, R., 1996. Relationship of genetic variation to population size in wildlife. Conservation Biology 10, 1500–1508.
Frankham, R., 2005a. Conservation biology – ecosystem recovery enhanced by genotypic diversity. Heredity 95, 183.
Frankham, R., 2005b. Genetics and extinction. Biological Conservation 126, 131–140.
Gitzendanner, M.A., Soltis, P.S., 2000. Patterns of genetic variation in rare and widespread congeners. American Journal of Botany 87, 783–792.
Godt, M.J.W., Johnson, B.R., Hamrick, J.L., 1996. Genetic diversity and population size in four rare southern Appalachian plant species. Conservation Biology 10, 796–805.
Godt, M.J.W., Walker, J., Hamrick, J.L., 1997. Genetic diversity in the endangered lily *Harperocallis flava* and a close relative, *Tofieldia racemosa*. Conservation Biology 11, 361–366.
Gonella, M.P., Neel, M., 1995. Characterization of rare plant habitat for restoration in the San Bernardino National Forest. In: Roundy, B.A., McArther, E.D., Haley, J.S, Mann, D.K. (Eds.), Proceedings: Wildland Shrub and Arid Land Restoration Symposium. General Technical Report INT-GTR-315. USDA Forest Service, Intermountain Research Station, Ogden, UT, pp. 81–93.
Hammerli, A., Reusch, T.B.H., 2002. Local adaptation and transplant dominance in genets of the marine clonal plant *Zostera marina*. Marine Ecology Progress Series 242, 111–118.
Hamrick, J.L., Godt, M.J.W., 1990. Allozyme diversity in plant species. In: Brown, A.H.D., Clegg, M.T., Kahler, A.L., Weir, B.S. (Eds.), Plant Population Genetics, Breeding, and Genetic Resources. Sinauer, Sunderland, MA, pp. 43–63.
Hamrick, J.L., Godt, M.J.W., 1996. Conservation genetics of endemic plant species. In: Avise, J.C., Hamrick, J.L. (Eds.), Conservation Genetics: Case Histories From Nature. Chapman & Hall, New York, pp. 281–304.
Hamrick, J.L., Linhart, Y.B., Mitton, J.B., 1979. Relationship between life history characteristics and electrophoretically detectable genetic variation in plants. Annual Review of Ecology and Systematics 10, 173–200.
Hedrick, P.W., 2005. A standardized genetic differentiation measure. Evolution 59, 1633–1638.
Hegde, S.G., Nason, J.D., Clegg, J.M., Ellstrand, N.C., 2006. The evolution of California's wild radish has resulted in the extinction of its progenitors. Evolution 60, 1187–1197.
Heywood, J.S., 1980. Genetic correlates of edaphic differentiation and endemism in *Gaillardia*. Ph.D. Dissertation, University of Texas, Austin.
Holderegger, R., Wagner, H.H., 2006. A brief guide to landscape genetics. Landscape Ecology 21, 793–796.
Holsinger, K.E., Vitt, P., 1997. The future of conservation biology: what's a geneticist to do? In: Pickett, S.T.A., Ostfeld, R.S., Shachak, M., Likens, G.E. (Eds.), Enhancing the Ecological Basis of Conservation: Heterogeneity, Ecosystem Function, and Biodiversity. Chapman & Hall, New York, pp. 206–216.
Hufford, K.M., Mazer, S.J., 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends in Ecology & Evolution 18, 147–155.
Hughes, A.R., Stachowicz, J.J., 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. Proceedings of the National Academy of Sciences of the United States of America 101, 8998–9002.
Husband, B.C., Schemske, D.W., 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50, 54–70.
Joshi, J., Schmid, B., Caldeira, M.C., Dimitrakopoulos, P.G., Good, J., Harris, R., Hector, A., Huss-Danell, K., Jumpponen, A., Minns, A., Mulder, C.P.H., Pereira, J.S., Prinz, A., Scherer-Lorenzen, M., Siamantziouras, A.S.D., Terry, A.C., Troumbis, A.Y., Lawton,

J.H., 2001. Local adaptation enhances performance of common plant species. Ecology Letters 4, 536–544.
Karron, J.D., 1987. A comparison of levels of genetic polymorphism and self-compatibility in geographically restricted and widespread plant congeners. Evolutionary Ecology 1, 47–58.
Karron, J.D., 1989. Breeding systems and levels of inbreeding depression in geographically restricted and widespread species of *Astragalus* (Fabaceae). American Journal of Botany 76, 331–340.
Karron, J.D., 1991. Patterns of genetic variation and breeding systems in rare plant species. In: Falk, D.A., Holsinger, K.E. (Eds.), Genetics and Conservation of Rare Plants. Oxford University Press, New York, pp. 87–98.
Keitt, T.H., Urban, D., Milne, B.T., 1997. Detecting critical scales in fragmented landscapes. Conservation Ecology 1, 4.
Kimura, M., 1962. On the probability of fixation of mutant genes in a population. Genetics 47, 713–719.
Lande, R., 1988. Genetics and demography in biological conservation. Science 241, 1455–1460.
Lesica, P., Leary, R.F., Allendorf, F.W., Bilderback, D.E., 1988. Lack of genic diversity within and among populations of an endangered plant, *Howellia aquatilis*. Conservation Biology 2, 275–283.
Lewis, P.O., Zaykin, D., 2001. Genetic data analysis: computer program for the analysis of allelic data. Version 1.0 (d16c). <http://alleyn.eeb.uconn.edu/gda/>.
Loveless, M.D., Hamrick, J.L., 1989. Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics 15, 65–95.
Luck, G.W., Daily, G.C., Ehrlich, P.R., 2003. Population diversity and ecosystem services. Trends in Ecology & Evolution 18, 331–336.
Luikart, G., Allendorf, F.W., Cornuet, J.M., Sherwin, W.B., 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity 89, 238–247.
Luikart, G., Cornuet, J.M., Allendorf, F.W., 1999. Temporal changes in allele frequencies provide estimates of population bottleneck size. Conservation Biology 13, 523–530.
Margules, C.R., Pressey, R.L., 2000. Systematic conservation planning. Nature 405, 243–253.
McCune, B., Mefford, M.J., 1999. PC-ORD. Multivariate Analysis of Ecological Data. Version 4.0. MJM Software Design, Gleneden Beach, OR, USA.
McGarigal, K., Cushman, S.A., 2002. Comparative evaluation of experimental approaches to the study of habitat fragmentation effects. Ecological Applications 12, 335–345.
McGarigal, K., Cushman, S.A., Neel, M.C., Ene., E., 2002. FRAGSTATS: Spatial Pattern Analysis Program for Categorical Maps. Computer software program produced by the authors at the University of Massachusetts, Amherst. <www.umass.edu/landeco/research/fragstats/fragstats.html>.
McRae, B.H., 2006. Isolation by resistance. Evolution 60, 1551–1561.
Milligan, B.G., Leebens-Mack, J., Strand, A.E., 1994. Conservation genetics: beyond the maintenance of marker diversity. Molecular Ecology 3, 423–435.
Montalvo, A.M., Ellstrand, N.C., 2000. Transplantation of the subshrub *Lotus scoparius*: testing the home-site advantage hypothesis. Conservation Biology 14, 1034–1045.
Montalvo, A.M., Ellstrand, N.C., 2001. Nonlocal transplantation and outbreeding depression in the subshrub *Lotus scoparius* (Fabaceae). American Journal of Botany 88, 258–269.
Neel, M.C., 2000. The structure of diversity: implications for reserve design. Ph.D. Thesis, University of California, Riverside, USA.
Neel, M.C., in press. Conservation planning and genetic diversity. In: Carroll, S.P., Fox, C.W. (Eds.), Conservation Biology: Evolution in Action. Oxford University Press, New York, NY.
Neel, M.C., Cummings, M.P., 2003a. Effectiveness of conservation targets in capturing genetic diversity. Conservation Biology 17, 219–229.
Neel, M.C., Cummings, M.P., 2003b. Genetic consequences of ecological reserve design guidelines: an empirical investigation. Conservation Genetics 4, 427–439.
Neel, M.C., Ellstrand, N.C., 2001. Patterns of allozyme diversity in the threatened plant *Erigeron parishii* (Asteraceae). American Journal of Botany 88, 810–818.
Neel, M.C., Ellstrand, N.C., 2003. Conservation of genetic diversity in the endangered plant *Eriogonum ovalifolium* var. *vineum* (Polygonaceae). Conservation Genetics 4, 337–352.
Neel, M.C., McGarigal, K., Cushman, S.A., 2004. Behavior of class-level landscape metrics across gradients of class aggregation and area. Landscape Ecology 19, 435–455.
Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences of the United States of America 70, 3321–3323.
Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.
Noss, R.F., Harris, L.D., 1986. Nodes, networks, and MUMs: preserving diversity at all scales. Environmental Management 10, 299–309.
Öostermeijer, J.G.B., van Eijck, M.W., den Nijs, J.C.M., 1994. Offspring fitness in relation to population size and genetic variation in the rare perennial plant species *Gentiana pneumonanthe* (Gentianaceae). Oecologia, Berlin 97, 289–296.
Pascual-Hortal, L., Saura, S., 2006. Comparison and development of new graph-based landscape connectivity indices: towards the prioritization of habitat patches and corridors for conservation. Landscape Ecology 21, 959–967.
Petit, R.J., El Mousadik, A., Pons, O., 1998. Identifying populations for conservation on the basis of genetic markers. Conservation Biology 12, 844–855.
Piry, S., Luikart, G., Cornuet, J.M., 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity 90, 502–503.
Prober, S.M., Brown, A.H.D., 1994. Conservation of the grassy white box woodlands: population genetics and fragmentation of *Eucalyptus albens*. Conservation Biology 8, 1003–1013.
Raabová, J., Münzbergová, Z., Fischer, M., 2007. Ecological rather than geographic or genetic distance affects local adaptation of the rare perennial herb, *Aster amellus*. Biological Conservation 139, 348–357.
Saura, S., Pascual-Hortal, L., 2007a. CONEFOR SENSINODE 2.2 User's Manual: Software for Quantifying the Importance of Habitat Patches for Maintaining Landscape Connectivity through Graphs and Habitat Availability Indices. University of Leida, Leida, Spain.
Saura, S., Pascual-Hortal, L., 2007b. A new habitat availability index to integrate connectivity in landscape conservation planning: comparisons with existing indices and application to a case study. Landscape and Urban Planning 83, 91–103.
Scandalios, J.G., Espiritu, L.G., 1969. Mutant aminopeptidases in *Pisum sativum*. I. Developmental genetics and chemical characteristics. Molecular and General Genetics 105, 101–112.
Schemske, D.W., Husband, B.C., Ruckelshaus, M.H., Goodwillie, C., Parker, I.M., Bishop, J.G., 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology 75, 584–606.
Shaw, C.R., Prasad, R., 1970. Starch gel electrophoresis of enzymes – a compilation of recipes. Biochemical Genetics 4, 297–320.
Slatkin, M., 1985. Rare alleles as indicators of gene flow. Evolution 39, 53–65.

Smouse, P.E., Dyer, R.J., Westfall, R.D., Sork, V.L., 2001. Two-generation analysis of pollen flow across a landscape. I. Male gamete heterogeneity among females. Evolution 55, 260–271.
Sokal, R.R., Rohlf, F.J., 1995. Biometry, third ed. W.H. Freeman, San Francisco, CA, USA.
Sork, V.L., Nason, J., Campbell, D.R., Fernandez, J.F., 1999. Landscape approaches to historical and contemporary gene flow in plants. Trends in Ecology & Evolution 14, 219–223.
Sork, V.L., Smouse, P.E., 2006. Genetic analysis of landscape connectivity in tree populations. Landscape Ecology 21, 821–836.
Soulé, M.E., Simberloff, D., 1986. What do genetics and ecology tell us about the design of nature reserves? Biological Conservation 35, 19–40.
Spellenberg, R., 1993. *Astragalus*. In: Hickman, J. (Ed.), The Jepson Manual: Higher Plants of California. University of California Press, Berkeley, pp. 583–605.
Storfer, A., 1996. Quantitative genetics: a promising approach for the assessment of genetic variation in endangered species. Trends in Ecology & Evolution 11, 343–348.
Storfer, A., Murphy, M.A., Evans, J.S., Goldberg, C.S., Robinson, S., Spear, S.F., Dezzani, R., Delmelle, E., Vierling, L., Waits, L.P., 2007. Putting the 'landscape' in landscape genetics. Heredity 98, 128–142.
Stuber, C.W., Goodman, M.M., Johnson, F.M., 1977. Genetic control and racial variation of beta-glucosidase in maize (*Zea mays* L.). Biochemical Genetics 15, 383–394.
Sun, M., 1996. Effects of population size, mating system, and evolutionary origin on genetic diversity in *Spiranthes sinensis* and *S. hongkongensis*. Conservation Biology 10, 785–795.
Templeton, A.R., Shaw, K., Routman, E., Davis, S.K., 1990. The genetic consequences of habitat fragmentation. Annals of the Missouri Botanical Garden 77, 13–27.
Theobald, D.M., 2005. A note on creating robust resistance surfaces for computing functional landscape connectivity. Ecology and Society, 10.
Tischendorf, L., Fahrig, L., 2000. How should we measure landscape connectivity? Landscape Ecology 15, 633–641.
Travis, S.E., Maschinski, J., Keim, P., 1996. An analysis of genetic variation in *Astragalus cremnophylax* var. *cremnophylax* a critically endangered plant, using AFLP markers. Molecular Ecology 5, 735–745.
U.S. Fish and Wildlife Service, 1994. Final Rule. Endangered and threatened wildlife and plants: five plants from the San Bernardino Mountains in southern California determined to be threatened or endangered. 50 CFR Part 17. Federal Register 59, 43652–43664.
U.S. Fish and Wildlife Service, 1997. San Bernardino Mountains carbonate endemic plants draft recovery plan. Region 1, Portland, OR, USA.
U.S. Fish and Wildlife Service, 2002. Designation of critical habitat for five carbonate plants from the San Bernardino Mountains in Southern California. Federal Register 67, 78569–78610.
U.S. Congress, 1973. Endangered Species Act. 16 US Code §1531–§1544 et seq.
U.S. Department of Commerce, 2006. Climate Records for Victorville, California (station 49325) and Big Bear Lake, California (station 40741). National Climatic Data Center. <http://www.wrcc.dri.edu>.
Urban, D., Keitt, T., 2001. Landscape connectivity: a graph-theoretic perspective. Ecology 82, 1205–1218.
Urban, D.L., 2003. LandGraphs: A Package for Graph-Theoretic Analyses of Landscapes. Landscape Ecology Laboratory, Nicholas School of the Environment and Earth Sciences, Duke University.
Weir, B.S., 1996. Genetic Data Analysis II. Sinauer, Sunderland, MA, USA.
Williams, D.A., Wang, Y.Q., Borchetta, M., Gaines, M.S., 2007. Genetic diversity and spatial structure of a keystone species in fragmented pine rockland habitat. Biological Conservation 138, 256–268.
Williams, S.L., Davis, C.A., 1996. Population genetic analyses of transplanted eelgrass (*Zostera marina*) beds reveal reduced genetic diversity in southern California. Restoration Ecology 4, 163–180.
Williams, S.L., Orth, R.J., 1998. Genetic diversity and structure of natural and transplanted eelgrass populations in the Chesapeake and Chincoteague bays. Estuaries 21, 118–128.
Wright, S., 1931. Evolution in Mendelian populations. Genetics 16, 97–159.
Young, A.G., Boyle, T., Brown, T., 1996. The population genetic consequences of habitat fragmentation for plants. Trends in Ecology & Evolution 11, 413–418.