FOSSIL AND GENETIC HISTORY OF A
PINYON PINE (*PINUS EDULIS*) ISOLATE[1]

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Abstract. The most isolated northern stand of Colorado pinyon pine (*Pinus edulis*) at Owl Canyon, Colorado, USA has a broad and flat size class distribution common to population expansions, with the largest and oldest trees near the center of the grove. Analyses of fossil packrat (*Neotoma* sp.) middens within the grove indicate that the stand originated by long-distance dispersal rather than by vicariance, a distinction seldom possible because of the unknown or incomplete histories of such isolated populations. Our 5000-yr pollen and macrofossil record suggests that pinyon pine colonized the site sometime between 1290 and 420 yr BP, the latter age corresponding to the oldest tree in the stand. Electrophoretic data from living trees show that this colonization was not attended by typical founder effects predicted by theory or observed for other known founder events. Despite its isolation, recent founding, and probable long-distance origin, the Owl Canyon stand has not suffered significant losses in genetic variation relative to likely source populations. Large initial population size, multiple founding events, rapid population growth, or selection favoring heterozygous genotypes could all explain the high heterozygosity and only slightly reduced polymorphism and number of alleles per locus found in the Owl Canyon stand. These results demonstrate the genetic complexity of founder events and the utility of blending complementary approaches such as paleoecology and genetics to better understand the population biology of peripheral isolates.

Key words: allozymes; biogeography; demography; founder effects; genetic diversity; Great Plains; packrat middens; paleoecology; Pinus edulis; population biology.

INTRODUCTION

Populations near the center of a species' range are thought to be contiguous, dense, and genetically diverse, whereas marginal populations are isolated, sparse and genetically less variable (Hamrick et al. 1989). Traditional explanations for reduced genetic diversity in peripheral isolates include random drift due to small population size and severe selection in ecologically marginal habitats. More recently, it has been suggested that reduced allelic diversity in peripheral isolates may result from the stochastic effects of repeated long-distance founding events during species migration (Cwynar and MacDonald 1987, Levin 1988). Unfortunately, the age and origin (long-distance dispersal vs. range fragmentation or vicariance) of marginal populations seldom can be determined through demographic, genetic, or biochemical studies alone. Ultimate proof may

lie with the fossil record, which all too often is unavailable, or when available, may lack adequate taxonomic, temporal, or spatial resolution.

In the American Southwest, Colorado pinyon pine (*Pinus edulis* Engelm.) exhibits the typical pattern of widely ranging and common species, i.e., a continuously inhabited central area with a patchy distribution on the periphery (Fig. 1). Isolates of this pine are ideal for population studies. The demography of individual stands can be determined from routine tree ring counts, and genetic variation can be evaluated readily by electrophoretic techniques. Wind dispersal of pine pollen enhances long-distance gene flow and prevents differentiation within the primary, large populations; hence, genetic drift in pine populations is more likely to occur in the most distant isolates (Hamrick 1987). A fine-scale fossil record for the distribution of pinyon pines during the past 40 000 yr is available from packrat (*Neotoma*) midden analysis and ^{14}C dating (Lanner and Van Devender 1981, Betancourt 1987, Van Devender 1987, Wells 1987, Betancourt et al. 1990). Packrat be-
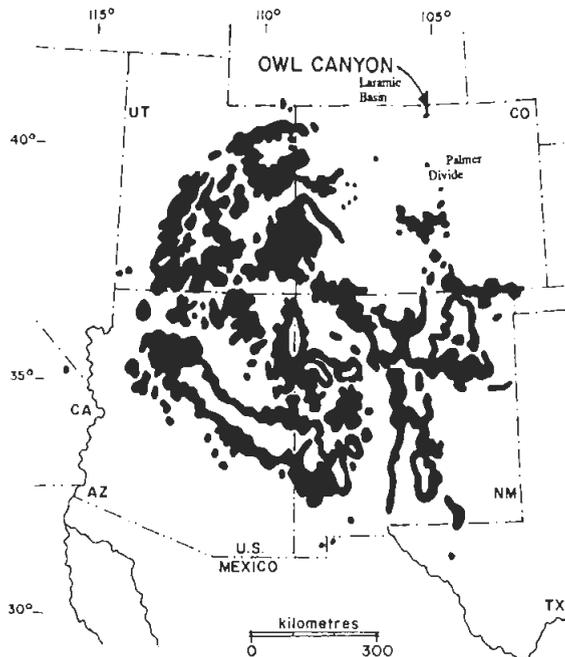
FIG. 1. Distribution of Colorado pinyon (*Pinus edulis*) showing location of Owl Canyon isolate (after Little 1971).

havior ensures a sample of plant material from within 100 m of the midden; precision of ^{14}C dating of this material or other organic debris in the midden usually is better than ±100 yr.

There has been considerable speculation about northern outposts of pinyon pines (Wright 1952, Beidleman 1953, Weber 1965, Lanner and Hutchison 1972, Quinn 1974). For example, Lanner and Hutchison (1972) suggested that "relict" stands of *P. edulis,* single-needle pinyon (*P. monophylla* Torr. and Frem.), and their hybrids in northern Utah developed by vicariance when northward expansion during the middle Holocene warming (8000–4000 yr BP) was followed by retreat during late Holocene cooling (4000 yr BP to present). They inferred the same for the most disjunct stand of *P. edulis* at Owl Canyon (Figs. 1 and 2), northern Colorado, though this was later recanted by Lanner (1975).

In an increment core survey, Wright (1952) determined that the Owl Canyon stand radiates from a few trees, now >400 yr old, in a ravine at the northwest end of the site (Area A in Fig. 2). The population has a broad and flat size class distribution (Floyd 1986), indicating constant recruitment through growth into the next age class, a characteristic of population expansions. The growth of the population is evident in the increase in crown cover shown by 1988 repetition of a 1950 photograph (Fig. 3A and B). Accurate demographic surveys are now obstructed by continued mining of a limestone cliff that traverses the long axis of the pinyon grove (Figs. 2 and 3). Unaffected parts

of this cliff are riddled with crevices containing numerous packrat middens. To determine the age and origin of the pinyon isolate, we collected and analyzed 15 middens and subjected modern needles from 150 living trees to horizontal starch gel electrophoresis. Appearance of pinyon pollen or macrofossils in the local middens during much of the Holocene would rule out a recent origin, whatever the demographic pattern. The electrophoretic analysis was intended to reveal whether or not the Owl Canyon grove experienced a reduction in genetic variation associated with a founder event, and if so, to document spatial variations in allelic and genotypic frequencies that resulted with subsequent expansion.

PHYSICAL SETTING

The Owl Canyon grove covers ≈5 km^2 on a hogback ridge between 1770 and 1900 m elevation, ≈25 km northwest of Fort Collins and 200 km north of more

▲   Fossil packrat middens   Contour interval 60 m
☐   Areas of electrophoretic survey   kilometres
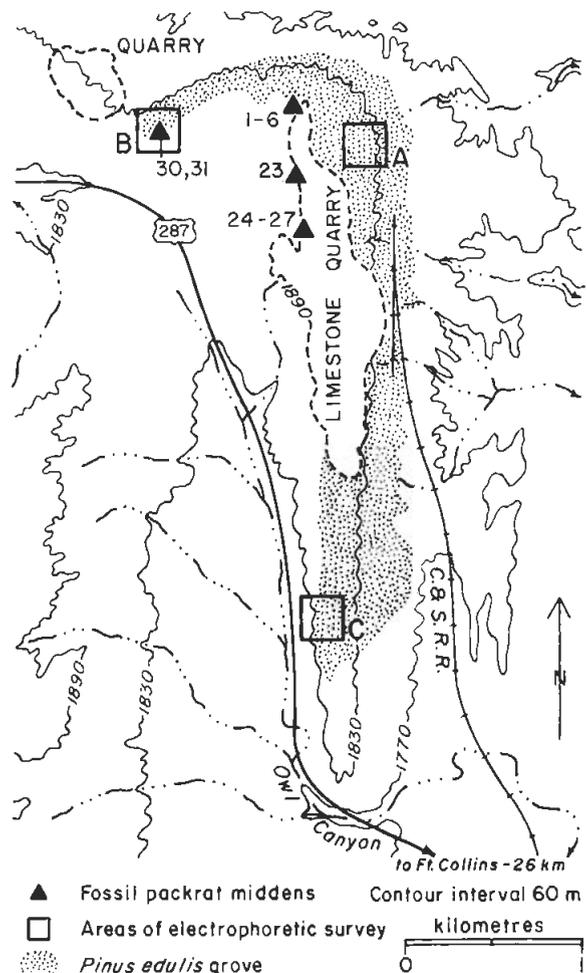⠿   *Pinus edulis* grove   0 ——————— 1

FIG. 2. Map of Owl Canyon pinyon isolate, showing location of fossil packrat middens and areas of the grove that were sampled for electrophoresis.

continuous populations near Colorado Springs, Colorado (Figs. 1 and 2). The hogback is composed mostly of limestone from the Ingleside Formation of Permian age and arkosic sandstone from the Fountain Formation of Pennsylvanian age. Limestone outcrops are rare in the foothills of the Front Range, except in the vicinity of Fort Collins. At Owl Canyon, a gentle, eastern slope or cuesta defines the tilting of sedimentary rock, with a steep escarpment 15–20 m high forming the western edge of the hogback. Exposed along the cliff are cross-bedded sandstones capped by a thin layer of limestone that is almost pure calcium carbonate. The limestone has been quarried extensively since 1930 with extensive damage to portions of the grove (Fig. 2).

Regional vegetation is grassland, with trees and shrubs restricted to breaks in topography. Rocky Mountain juniper (*Juniperus scopulorum* Sarg.) and ponderosa pine (*Pinus ponderosa* Laws.) form scarp woodlands in the narrow transition between Rocky Mountain conifer forests and Great Plains grasslands. Thickets of mountain mahogany (*Cercocarpus montanus* Raf.) and squawbush (*Rhus trilobata* Nutt.) alternate on steep, rocky slopes. At Owl Canyon pinyon pine accounts for over 85% of all trees and 40% of average crown cover for the site (Wright 1952). Based on Wright's data, the six largest trees in the stand, all within Area A (Fig. 2), are from 355 to 410 yr old (as of 1990); a smaller tree (Owl8N, Laboratory of Tree-Ring Research, University of Arizona) may be the oldest individual at 418 yr old. Rocky Mountain juniper is most common at the southern end of the grove, where the pinyons are young and sparse. Scattered individuals of ponderosa pine occur primarily along the cliff face. Excluding urban plantings at Fort Collins, only a few, scattered pinyons are known from six other localities within 26 km, thought to have resulted from dispersal of seeds by birds foraging at Owl Canyon (Hawksworth 1979).

MATERIALS AND METHODS

Midden analysis

Fifteen packrat middens (Fig. 2) were collected from limestone crevices and overhangs; discrete samples were chosen by chiseling along discernible bedding planes. Average indurated mass of the samples was ≈500 g. The crystallized urine is water soluble; dilute HCl speeds the release of plant remains, pollen, and other debris, but should be avoided because of potential damage to degraded DNA in the fossil plant tissue.

After thorough mixing of the dissolved midden material, we extracted a 1% aliquot (12–15 mL; sensu Thompson 1985, Davis and Anderson 1987), which then was processed chemically for pollen using a modified Faegri and Iversen (1975) technique (10% KOH and HCl, HF, acetolysis). *Lycopodium* tracer tablets (Stockmarr 1972) were added in the initial preparation to permit calculation of pollen concentration. The pollen-rich residues were mounted on microscope slides
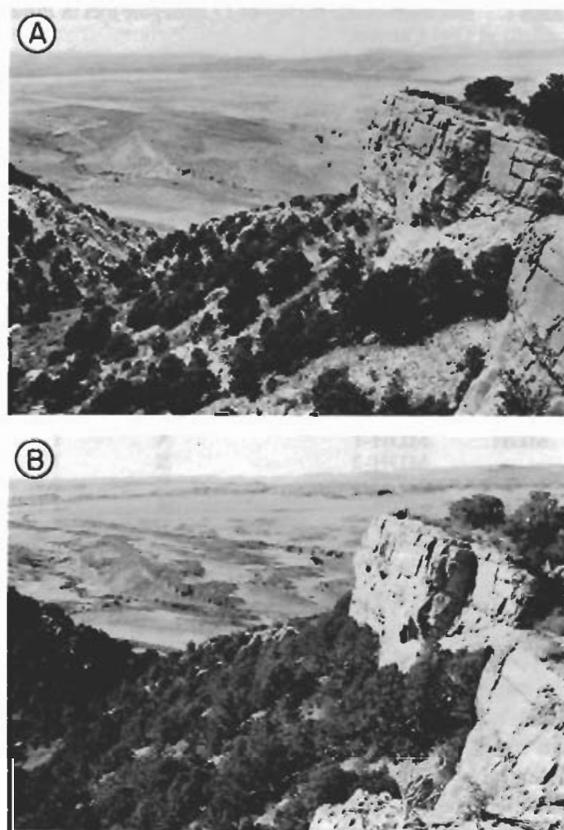

FIG. 3.   (A) Photograph of the north end of the Owl Canyon pinyon isolate taken in 1951 (from Wright 1952). This northeastern view shows one of the younger portions of the grove. In 1951, the oldest tree in the photograph was 86 yr old. Neither pinyon pollen nor macrofossils appear in packrat middens older than ≈400 yr that were collected from limestone cliff at right of photograph. Pinyon pollen occurs in all of the younger middens, but despite pinyon's present dominance at the site, pinyon macrofossils were not found in middens dated at 210 ± 60 and 300 ± 50 yr BP. (B) Same view taken on 3 September 1988 (Courtesy of R. M. Turner, United States Geological Survey Stake Number 1330). Note the general increase in crown cover since 1951.

in silicone oil (5000 mm²/s [centistokes]). A minimum of 250 pollen grains was counted in each sample at 400× magnification. Pollen identifications were routine except for *Pinus*, which we separated according to general morphology and size. Haploxylon pines (*Pinus aristata* Engelm., *P. flexilis* James, and *P. edulis*) have verrucae or scabrae on the furrow membrane in an areolate or semi-areolate pattern, whereas diploxylon pines (*P. ponderosa* and *P. contorta* Loud.) have a furrow membrane that is psilate or bearing only scattered scabrae. We further segregated the haploxylon group by size; pollen grains with a corpus length <70 μm were assigned to *Pinus edulis*. These morphological and size separations are based on exhaustive comparisons among modern pollen samples from western pines (Hansen and Cushing 1972, Fine-Jacobs 1985).

TABLE 1. Electrophoretic survey of 17 allozyme loci in *Pinus edulis* at Owl Canyon.

Enzyme system	Locus	Variable*	Invariant	Number of alleles
GLY	GLY	X		3
PGI	PGI-1		X	1
	PGI-2	X		3
IDH	IDH	X		2
SDH	SDH	X		4
PGM	PGM-1	X		2
	PGM-2	X		2
UDP	UDP-1	X		3
	UDP-2	X		2
6PGD	6PGD	X		2
PER	PER-1	X		2
	PER-2		X	1
ME	ME		X	1
MDH	MDH-1		X	1
	MDH-2		X	1
	MDH-3		X	1
GDH	GDH		X	1

* Frequency of most common allele <99%.

Plant macrofossils were extracted by wet-screening the disaggregated, organic residue through a 0.84-mm (20-mesh) soil sieve and drying at 70°C. Fecal pellets, which normally constitute 20–50% of the washed midden mass, were removed and submitted for radiocarbon analyses. The remaining organic fraction, mostly plant fragments, was sorted, identified, and weighed by species. Species abundance was computed as the percent mass of the organic fraction without the pellets. Species identifications were done by comparison with modern voucher samples and keys to regional floras. Methods and assumptions in packrat midden studies are discussed in greater detail elsewhere (Betancourt et al. 1990).

Electrophoresis

Modern needle tissue was sampled for electrophoresis at three locations referred to as northwest (B), central (A), and south (C) subpopulations (Fig. 2). We use the terms population (all the trees at Owl Canyon) and subpopulation (each of the three sample localities) for convenience in this analysis without implying a specific population structure. Fifty-one trees were sampled in the central subpopulation, which includes the oldest trees in the stand. We sampled the 13 largest diameter trees and 38 individuals chosen at random in the surrounding 2 ha. Fifty trees were sampled randomly over a similar area near both the northwest (B) and southern (C) extremes of the stand. Trees less than ≈3 cm diameter at breast height (dbh) were not sampled. Needles were ground in liquid nitrogen with the grinding buffer specified by Mitton et al. (1979).

Horizontal starch gel electrophoresis was used to survey these samples for 11 enzyme systems encoding 17 putative enzyme loci (Table 1). Several of these loci were known to be polymorphic elsewhere in the range of Colorado pinyon (Mopper et al. 1991, J. Hamrick,

personal communication), and thus, they do not represent a true random sample of available electrophoretic loci. Tests of the inheritance patterns of these loci were not performed. However, we assumed Mendelian inheritance based on patterns previously demonstrated for nearly all of these loci in several other conifers (Linhart et al. 1981, Neale and Adams 1981, Cheliak and Pitel 1984, Shea 1988, Schuster et al. 1989). Multiple loci and alleles were coded according to speed of migration, i.e., the fastest migrating MDH locus was designated MDH-1, the next fastest was designated MDH-2, etc.

Three malate dehydrogenase loci (MDH-1, MDH-2, MDH-3) and two peroxidase loci (PER-1, PER-2) were resolved on a continuous tris-citrate buffer system (Selander et al. 1971). Two phosphoglucose isomerase loci (PGI-1, PGI-2) and one uridine diphosphoglucose pyrophosphorylase locus (UDP-1) were resolved on the discontinuous tris-citrate buffer system of Mitton et al. (1977). Two phosphoglucomutase loci (PGM-1, PGM-2), one uridine diphosphoglucose pyrophosphorylase locus (UDP-2), 6-phosphogluconate dehydrogenase (6PGD), isocitrate dehydrogenase (IDH), and malic enzyme (ME) were resolved on the histidine II buffer system of King and Dancik (1983). Shikimate dehydrogenase (SDH), glycerate dehydrogenase (GLY), glutamate dehydrogenase (GDH), and malic enzyme (ME) were resolved on the discontinuous tris-citrate buffer system of Poulik (1957). After the initial screening for variability, all trees were scored for eight of the variable loci (GLY, PGI-2, IDH, SDH, PGM-1, PGM-2, UDP-1, UDP-2), chosen on the basis of clear and reliable resolution. Several precautions were taken to assure that the allozyme genotypes were correct. Wherever justified, standards of known genotypes were run along with the new samples. In addition, 6% of all samples were run on duplicate gels to test for consistency. Finally, because the alleles of SDH had similar mobilities, all individuals were run 3 times to be certain of the SDH genotypes.

Loci were considered variable only if the frequency of the most common allele was <99%. The mean number of alleles (A) was calculated over all 17 loci, and for the eight polymorphic loci scored for all trees (A_p). Observed heterozygosity (H) and expected heterozygosity under the assumptions of the Hardy-Weinberg Law (H_T) were calculated for each locus and each subpopulation, and over all loci. Inbreeding coefficients (F) were calculated as $[F = 1 - (H/H_T)]$ for each locus, and the significance of these values was examined using a chi-square test. Allele frequencies were compared among subpopulations for each locus and over all loci using the method of Workman and Niswander (1970; see also Hedrick 1985). Nei's genetic identity (I) was calculated between members of each pair of subpopulations (Nei 1972). Genetic structure was also examined using Nei's (1973) gene diversity statistics, where H_T = gene diversity in the total population, H_S = mean
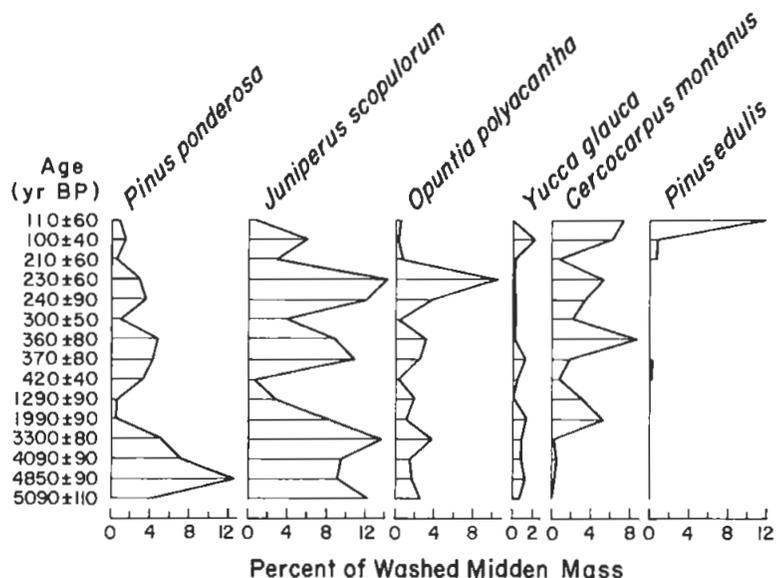
FIG. 4. Abundance of plant macrofossils in Owl Canyon packrat middens. All ages are expressed as means ± 1 SD. Abundance was obtained by dividing the mass of the plant macrofossils by the total mass of the organic debris in the sample, then multiplying by 100.

gene diversity within subpopulations, and $G_{ST} = (H_T - H_S)/H_T$, a measure of the relative differentiation of subpopulations.

RESULTS

The midden record

The midden record from Owl Canyon spans the last 5000 yr. Table 2 includes both conventional ^{14}C ages and corrected dates based on high-precision calibrations of the radiocarbon time scale from tree ring assays (Stuiver and Kra 1986). Note that multiple calendric intervals are possible for a single conventional ^{14}C age

and that resolution is seriously impaired during the last few centuries. The source of error in recent dates is commonly known as the Suess effect; it results from release of radiocarbon-free CO_2 with combustion of fossil fuels after the Industrial Revolution. Even when dates are given as $\bar{X} \pm 1$ SD (as they are throughout this paper) there is considerable overlap among all of the samples younger than 420 ± 40 yr BP; neither conventional nor calibrated dates may reflect the true chronological order of these samples.

Fig. 4 depicts macrofossil abundance for selected plant taxa in Owl Canyon middens. Pinyon macrofos-

TABLE 2. List of ^{14}C dates from fossil packrat middens at Owl Canyon, Larimer County, Colorado. All assays were provided by the Laboratory of Isotope Geochemistry, University of Arizona, Tucson. Uncalibrated dates are in ^{14}C years before present. Calibrations to ^{14}C-dated tree rings follow Stuiver (1986). All dates are means ± 1 SD.

Midden designation	^{14}C lab. no.	Uncalibrated ^{14}C age (yr BP)	Calibrated ^{14}C age
OC5	A-4092	100 ± 40	AD 1685–1729, 1808–1931, *1955
OC2	A-4109	100 ± 60	AD 1674–1743, 1802–1941, *1955
OC1	A-4110	210 ± 60	AD 1644–1680, 1736–1805, 1937–1955*
OC24	A-4403	230 ± 60	AD 1639–1677, 1739–1804, 1939–1955*
OC27	A-4406	240 ± 90	AD 1522–1585, 1624–1680, 1736–1805, 1936–1955*
OC3	A-4091	300 ± 50	AD 1493–1651
OC25	A-4404	360 ± 80	AD 1438–1643
OC26	A-4402	370 ± 80	AD 1437–1639
OC4	A-4093	420 ± 40	AD 1433–1486
OC30	A-5410	1290 ± 50	AD 662–774
OC23	A-4405	1990 ± 90	105 BC–AD 88
OC31	A-5411	3300 ± 80	1685–1514 BC
OC6A	A-4098	4090 ± 90	2875–2801, 2778–2715, 2706–2563, 2542–2498 BC
OC6B	A-4112	4850 ± 90	3775–3748, 3707–3614, 3580–3525 BC
OC6C	A-4111	5090 ± 120	4032–4026, 4000–3780, 3732–3725 BC

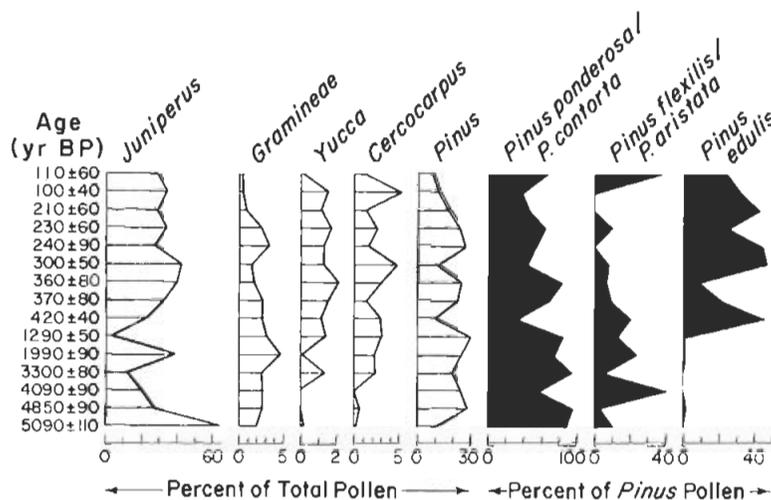* 1955 denotes influence of nuclear test (bomb) ^{14}C.

FIG. 5. Frequency of pollen types (expressed as percent of total pollen, excluding trilete spores and cf. Umbelliferae) in Owl Canyon packrat middens. All ages are expressed as means ± 1 SD. Pines are segregated into three groups: diploxylon (*Pinus ponderosa, P. contorta*), haploxylon with a corpus length >70 µm (*P. aristata, P. flexilis*), and haploxylon with a corpus length of <70 µm (*P. edulis*).

sils are missing from all of the middens except OC5 (100 ± 40 yr BP), OC2 (110 ± 60 yr BP), OC26 (370 ± 80 yr BP), and OC4 (420 ± 40 yr BP). The latter sample contained only a few chlorophyllous needles of pinyon that may reflect low levels of contamination by modern material. Note that pinyon macrofossils were not found in five middens dated from 210 ± 60 to 360 ± 80 yr BP, even though pinyon now comprises >25% of total ground cover within 100 m of each of the midden sites (e.g., Fig. 3). Variations in macrofossil abundance for each taxon may be due to site differences rather than time.

The pollen data (Fig. 5) parallel the midden record, except that high levels of pinyon pollen, relative to other pines, occur in all but one of the middens younger than 420 ± 40 yr BP. Note the 800-yr gap between 1290 and 420 yr BP that precludes better discrimination of when pinyon pine pollen increases beyond presumed contamination levels. The source of pollen in the middens lacking pinyon pine macrofossils may be the first pinyons to become established at Owl Canyon; apparently, these trees were close enough to contribute to the local pollen rain, but still beyond the foraging range of packrats. This is consistent with the relatively young age of trees along the limestone escarpment (Fig. 3; Wright 1952). Some of the older middens contained a single pollen grain of pinyon, which could easily result from contamination with occasional rehydration of crystallized urine or accidental inclusion of outer weathering rinds in the washing process. Overall, our 5000-yr record points up the absence of pinyon along limestone cliffs at Owl Canyon until its establishment and expansion within the last few centuries, when it replaces ponderosa pine and Rocky Mountain juniper as the dominant tree.

Electrophoresis

From the initial sample of 17 loci (Table 1), 10 loci were polymorphic, segregating 2 or more alleles. In our sample of 8 polymorphic loci for which all individuals were scored, 4 loci segregated 3 or 4 alleles (Table 3). Excluding mutations, loci segregating 3 or more alleles indicate that the population was not founded by a single seed. F values (Table 3) indicate no consistent pattern of inbreeding overall, or in any of the three areas sampled. Both PGM loci depart significantly from expectations due to a deficit of heterozygotes. Inbreeding probably is not the cause because this deficit is not reflected at other loci.

The values obtained for polymorphism (P), total expected heterozygosity (H_T), and alleles per locus (A) (Table 4), the three most common measures of genetic variation, are higher than average for studies of plant populations, though somewhat below average for conifers (P, H_T, and A = 34.2, 0.113, and 1.53 for all plants, and 70.9, 0.173, and 2.35 for conifers; Hamrick et al. 1979).

Seven of the eight loci exhibit no differences in allele frequency among the three areas sampled (Table 3); the overall chi-square value (26.8) also is not significant. One locus (SDH) does exhibit significant differences among these subpopulations, primarily due to the distribution of rare alleles. Nei's gene diversity and genetic identity statistics provide additional evidence of the genetic homogeneity of the population. The G_{ST} value (Table 4) indicates that only 1.2% of the total genetic diversity is found among the subpopulations at Owl Canyon, while the average genetic identity between subpopulations is close to unity (>0.996; Table 4).

TABLE 3. Allelic and genotypic frequencies, F (inbreeding) values, and χ^2 tests of geographic differences in allele frequencies for 10 polymorphic loci within three areas of the Owl Canyon pinyon pine grove.

Enzyme system	Area	(N)	Genotype frequencies							Allele frequencies				F	χ^2
			11	12	22	13	23	33	34	1	2	3	4		
GLY	B	(49)	.000	.000	.286	.000	.551	.163		.000	.562	.439		−.12	7.48
	A	(51)	.000	.039	.471	.000	.412	.078		.020	.697	.284		−.09	
	C	(50)	.000	.020	.400	.000	.360	.220		.010	.590	.400		.23	
	Overall	(150)	.000	.020	.387	.000	.440	.153		.100	.617	.373		.02	
PGI-2	B	(43)	.047	.186	.744	.000	.023	.000		.140	.849	.012		.19	0.84
	A	(46)	.000	.217	.761	.000	.022	.000		.109	.881	.011		−.12	
	C	(46)	.022	.152	.804	.000	.022	.000		.098	.890	.011		.10	
	Overall	(149)	.022	.185	.770	.000	.022	.000		.115	.874	.011		.05	
IDH	B	(49)	.388	.449	.163					.613	.388			.06	1.99
	A	(50)	.240	.560	.120					.520	.480			−.12	
	C	(50)	.320	.560	.120					.600	.400			−.16	
	Overall	(149)	.315	.523	.161					.577	.423			−.06	
SDH	B	(49)	.000	.000	.000	.000	.000	.898	.102	.000	.000	.949	.051	−.05	25.63***
	A	(49)	.000	.000	.000	.020	.000	.941	.039	.010	.000	.970	.020	−.21	
	C	(50)	.000	.000	.040	.000	.100	.860	.000	.000	.090	.910	.000	.39	
	Overall	(150)	.000	.000	.013	.007	.033	.900	.047	.003	.034	.940	.023	.12	
PGM-1	B	(45)	.267	.267	.467					.400	.600			.44**	4.52
	A	(51)	.412	.274	.314					.549	.451			.45**	
	C	(45)	.311	.311	.378					.466	.534			.37*	
	Overall	(141)	.333	.284	.383					.475	.525			.43***	
PGM-2	B	(48)	.042	.125	.833					.105	.895			.33*	3.92
	A	(51)	.078	.235	.686					.196	.804			.25	
	C	(49)	.020	.260	.720					.150	.850			−.02	
	Overall	(149)	.047	.208	.745					.151	.849			.19*	
UDP-1	B	(49)	.000	.184	.776	.000	.041	.000		.092	.888	.020		−.14	7.04
	A	(51)	.000	.020	.941	.000	.039	.000		.010	.970	.020		−.13	
	C	(49)	.020	.061	.857	.000	.061	.000		.050	.920	.030		.17	
	Overall	(149)	.007	.087	.859	.000	.047	.000		.050	.927	.023		.03	
UPD-2	B	(49)	.918	.082	.000					.959	.041			−.04	1.32
	A	(51)	.961	.039	.000					.981	.019			−.05	
	C	(50)	.900	.100	.000					.950	.050			−.05	
	Overall	(150)	.927	.073	.000					.964	.036			−.05	

* $P < .05$; ** $P < .01$; *** $P < .001$.

Spatial patterns of alleles within the site generally support the notion that Area A, where the oldest trees are located, was the site of initial establishment (Table 3; Fig. 2). Trees in this area possess nearly all of the alleles found in our survey, excluding one rare allele at SDH. The 13 largest trees sampled, growing close together in Area A and including the oldest trees in the grove, contain most but not all of the alleles present in the population, and thus cannot be the sole progenitors of the stand. Both genetic drift, especially in the beginning stages, and natural selection may be respon-

sible for some of the patterns in gene frequency across the site. At GLY, for example, the frequency of the third allele increases from 19% in the 13 largest trees, to 28% among surrounding trees in Area A, to 40% or higher in Areas B and C. Consistent patterns of microgeographic genetic differentiation have been observed in conifers (e.g., Mitton et al. 1989), revealing a role for selection in generating population structure. Furthermore, an association between GLY frequencies and soil type has been found in pinyon pine (Mopper et al., *in press*), with allelic frequencies differing be-

TABLE 4. Levels of diversity in the isolated *Pinus edulis* population at Owl Canyon, northeastern Colorado. P = polymorphism at 99% level; A = mean number of alleles per locus; A_p = mean number of alleles per polymorphic locus; H = observed heterozygosity; H_T = total gene diversity; H_S = subpopulation gene diversity; G_{ST} = gene differentiation among subpopulations; A-B-C = pairwise comparisons between oldest part of population (Area A), northwest sector (Area B), and southern sectors (Area C).

N (no. loci)	P* (99%)	No. of alleles		H (observed)	Gene diversity			Genetic identity		
		A	A_p		H_T	H_S	G_{ST}	A-B	A-C	B-C
17	.588	1.88	2.63	.145	.167	.165	.012	.994	.997	.998

* P = proportion of loci that had the frequency of their most common allele <99%. Reported polymorphism levels may be overestimates since loci expected to be polymorphic were preferentially surveyed.

tween cinder and soils derived from other parent material. However, for Owl Canyon, we do not have the data to accept or reject the most parsimonious hypothesis that the differences observed were caused by drift during population expansion.

DISCUSSION

The fossil record from Owl Canyon supports longstanding speculation that *Pinus edulis* only recently colonized Owl Canyon. Conceivably, the oldest living trees at the site were among the first colonists, though we cannot rule out colonization as early as 1290 yr BP due to the 800-yr gap in the midden chronology. Also, we cannot yet ascertain the relation between Owl Canyon and other smaller isolated stands that occur between it and more continuous populations near Colorado Springs. A regional presence for pinyon, however, would be inconsistent with: (1) its known distribution and migrational history during the past 40 000 yr, (2) the presence of a formidable topographic barrier between the current northern limits and Owl Canyon, and (3) fossil evidence for the middle-to-late Holocene expansion of conifers other than pinyon only 50 km north of Owl Canyon in the Laramie Basin, southern Wyoming.

Late Quaternary history of pinyon pines

From 40 000 to 11 000 yr ago, pinyons were widespread in the lowlands (<1700 m elevation) of the Mojave, Sonoran, and Chihuahuan Deserts. North of 35° N, about the latitude of Flagstaff, Arizona and Albuquerque, New Mexico, northern distributions were truncated by minimum lowland elevations exceeding the upper limits of pinyons (today, this physiography sets the northern limits of such thermophiles as mesquite, *Prosopis* spp., and creosote bush, *Larrea divaricata* Cav.). Postglacial expansion of pinyons northward and to higher elevations involved unexpected shifts in dominant taxa. *Pinus edulis* and *P. monophylla* Torr. & Frem., which had restricted ranges during glacial times, became widespread in the Holocene. Conversely, *P. remota* (Little) Bailey & Hawksworth and *P. californiarum* subsp. *fallax* (Little) D. K. Bailey (Syn: *Pinus edulis* Engelm. var. *fallax* Little; see Bailey 1987) were widespread during the last ice age, contracting to their present relictual distributions in postglacial times (Betancourt 1987, Van Devender 1987, Wells 1987). Late Wisconsin records of *P. edulis* are restricted to a few localities in the northern Chihuahuan Desert (Van Devender 1987), and southern Colorado Plateau (Cinnamon and Hevly 1988). After 11 000 yr ago, *P. edulis* expanded northward from these localities.

During the Holocene, there is no evidence that pinyon occurred beyond its modern range in Colorado and subsequently retreated southward, leaving behind relict populations. However, we cannot discount the possibility that, at other times during the Holocene, small populations became established in the intervening area between the northern edge of the main population near Colorado Springs and Owl Canyon. The paucity of adequate fossil sites in the foothills between Colorado Springs and Owl Canyon exposes a potential weakness in our argument against vicariance. In part, we base our argument for a long-distance origin on the presence of a topographic barrier, known as the Palmer Divide, in the eastern foothills of the Rockies between Colorado Springs and Denver. The Palmer Divide, which is a large area primarily above 1980 m in elevation, now supports the Black Forest, an expansive forest of ponderosa pine that grades directly into Plains grassland. Pinyon colonized Owl Canyon only within the last 1300 yr, probably as recently as 420 yr ago. If the Owl Canyon stand developed by vicariance, then the Black Forest and other low-elevation ponderosa stands in the foothills of the Rockies must have a recent origin. This would run counter to Wells' (1983a) fossil evidence 50 km north of Owl Canyon in the Laramie Basin, which shows that woodlands composed of ponderosa pine and Rocky Mountain juniper were more common at lower elevations until the last few hundred years. The most parsimonious explanation is that Owl Canyon represents the most recent, northward advance of *Pinus edulis,* which was accomplished by dispersal of seeds from likely source populations 200 km or more to the south.

Dispersal agents and propagule sources

Either corvids or humans could have dispersed pinyon seeds to Owl Canyon from faraway sources. Pinyon seeds are heavy, wingless, and ballistically unsuited for wind dissemination. Corvid (e.g., *Nucifraga columbiana* and *Gymnorhinus cyanocephalus*) dispersal distances of pinyon seeds may reach tens of kilometers (Vander Wall and Balda 1977, Ligon 1978), or conceivably farther (Wells 1983b). Native Americans provide another possible dispersal mechanism across long distances. Dispersal by humans already has been proposed to explain spotty distributions of limber pine on the Great Plains (Potter and Green 1964). Holocene migration of *Pinus monophylla* in the Great Basin might have been delayed until initial expansion of Archaic peoples who used it as a staple (Mehringer 1986). Pinyon nuts were highly valued by most southwestern groups, some of whom regularly availed themselves of caches in packrat dens. Plains Indians from northeastern Colorado, including Kiowas, Pawnee, and Arapaho, raided and traded with the Rio Grande Pueblos in northern and central New Mexico during historic times. Their predecessors on the Plains probably did the same. No doubt they would have coveted pinyon nuts from New Mexico as winter food and "trail mix." Wright (1952) was first to speculate that Indian dispersal of seeds may explain the isolated pinyon population at Owl Canyon, but we have no evidence to either accept or reject his hypothesis.

Biochemical data indicate unusual affinities among *Pinus edulis* populations in the southwestern United States. Zavarin et al. (1989) found that the small outlier at Hewlett Gulch, 15 km northwest of Owl Canyon, contained high levels of monoterpenoids biosynthetically related to sabinene; in a limited sample of southwestern populations, such high levels were found only in *P. edulis* populations in southeastern Arizona/southwestern New Mexico. However suggestive, the monoterpene data presently are insufficient to determine the affinity of the Hewlett Gulch stand, which may or may not have been derived from Owl Canyon, to other populations in the southwest.

Founder effects and founder events

Mayr (1963:211), in developing a model of allopatric speciation, defined the founder effect as the "establishment of a new population by a few original founders . . . which carry only a small fraction of the total genetic variation of the parental population." This loss of variation was an essential part of the model, leading to the breakup of co-adapted gene complexes and allowing formation of novel gene combinations, a "genetic revolution." He suggested founder effects were often responsible for the genetic uniformity of peripherally isolated populations. Early experimental bottlenecks with fruit fly populations, maintained at the same small size for many generations, clearly demonstrated allele frequency drifts and loss of heterozygosity (Kerr and Wright 1954, Buri 1956).

More recent theoretical results indicate that the effects of a founding event on genetic variation will depend on both the original population size and the rate of increase. The initial heterozygosity of the founder population will be:

$$H = \left(1 - \frac{1}{2N_c}\right)H_0$$

where N_c is the effective breeding size of the founder population and H_0 is the heterozygosity of the source population (Crow and Kimura 1970). Thus, if the original founder group consisted of 10 mating individuals, the initial loss in heterozygosity would only be 5%. In a random-mating population with discrete generations, heterozygosity will continue to decrease according to the above equation (substituting new values of N_c and H_0 each generation), until a minimum level is reached that will persist for many generations (Nei et al. 1975). Thus, heterozygosity should be strongly affected by rate of population increase. If the rate of increase is high, additional losses in heterozygosity after the founding event will be minimal (Nei et al. 1975).

The mean number of alleles per locus is more likely to be affected by a founder event than heterozygosity (Lewontin 1965, Nei et al. 1975, Powell 1983). The severity of the reduction will depend, once again, primarily on founding population size, because this is the

stage where many rare alleles are lost. However, populations that expand quickly should experience little loss in genetic diversity (Powell 1983, Barton and Charlesworth 1984).

Reduced genetic variation in founder populations relative to presumed source populations has been documented in pitcher plants (Schwaegerle and Schaal 1979, Taggart et al. 1990), *Anolis* lizards (Taylor and Gorman 1975), *Peromyscus* (Selander et al. 1971), *Drosophila pseudoobscura* (Prakash 1972), and the walnut husk fly, *Rhagoletis completa* (Berlocher 1984). Some studies indicate that heterozygosity was affected less than the number of alleles per locus, similar to the pattern expected in a rapidly expanding population (Bryant et al. 1981, Berlocher 1984).

Significance of Owl Canyon. —Our estimates of genetic variation at Owl Canyon are somewhat higher than average for plant populations, and much higher than most previous reports of populations known to have resulted from a founding event. However, these estimates neither preclude nor indicate a past founding event. Conifer populations typically possess high levels of electrophoretic variation, but there is a general paucity of electrophoretic data for *Pinus edulis* (Hamrick et al. 1979, Hamrick and Godt 1990).

The only comparable allozyme survey of *Pinus edulis* involves eight populations from several western states (J. L. Hamrick et al., *unpublished data*). A total of 72 trees were sampled in each population and were scored for 23 loci. While the Owl Canyon analysis did not involve all of the same loci, over half of the loci (12) overlapped in the two studies. The eight populations had an average polymorphism of 72% with a standard deviation of 3%, compared to 59% polymorphism at Owl Canyon. Among the 12 shared loci, 4 that were monomorphic at Owl Canyon were polymorphic in the eight populations, which could be construed as a founder effect. On the other hand, one locus in the Owl Canyon population, PGM-1, was highly variable at Owl Canyon, but monomorphic in all of the other populations. We found an average of 1.88 alleles per locus at Owl Canyon, compared with 2.25 alleles (SE = 0.05) in the eight other populations, indicating that some rare alleles may have been lost in the founding event. However, heterozygosity values at Owl Canyon are similar to or higher than values in the other eight populations (Owl Canyon: $H = 0.145$, $H_T = 0.167$; other populations: $H = 0.142$, $H_T = 0.150$). We have interpreted this to mean that the founding group must have been genetically diverse. This is supported by the fact that the founding occurred within the last millennium, not nearly enough time for heterozygosity to rebound (Nei et al. 1975).

Demographic studies of the Owl Canyon pinyon population suggest that the founding population was probably small, perhaps in the range of 1–20 individuals. Genetic data clearly show that the population was not started with a single individual, because four poly-

morphisms segregate three or four alleles, and a single individual can carry, at most, only two alleles at each locus. The allele frequency data can be used to provide quantitative estimates of the number of founders. If we assume that allele frequencies have not changed significantly since the bottleneck, then the minimum number of founders needed to possess a gene at a certain frequency $f(x)$, would be

$$N = \frac{1}{2f(x)}.$$

Thus, an allele of frequency 0.05 could only result from a sample of at least 10 diploid individuals. Fully one-third of the alleles at Owl Canyon had frequencies of 0.05 or less, indicating an initial population size of at least 10. Three of the alleles were estimated to have frequencies of 0.011 or less, indicating a founder population of at least 45.

If the population started with 10 or more individuals little more than 400 yr ago, it certainly expanded quickly. The moderate population bottleneck and rapid expansion of the population help us understand the relatively high level of genetic variation within the population, but natural selection may have played a role as well. A recent study of allozyme variation in pinyon pine (Mopper et al. 1991) revealed that mortality associated with other factors favored heterozygous genotypes. Viability selection favoring heterozygous genotypes is common in conifer populations (Mitton and Jeffers 1989). If natural selection were similar in the population at Owl Canyon, that selection would preserve or perhaps enhance the level of genetic variation carried by the founders.

Without the fossil evidence, we could not have concluded safely that a founder event had occurred from slightly reduced polymorphism and/or number of alleles per locus. Part of the problem lies in the paucity of electrophoretic data from other populations. A better case might be made for examining genetic variation in other conifers for which extensive electrophoretic data are readily available, such as bristlecone pine (*Pinus longaeva* D. K. Bailey) and ponderosa pine. In the Great Basin, for example, little genetic heterogeneity among insular populations of bristlecone pine and extensive fossil evidence at basal elevations have been interpreted to mean fragmentation of a more continuous range at the end of the Pleistocene (Hiebert and Hamrick 1983, Wells 1983b). Likewise, peripheral isolates of ponderosa pine in scarp woodlands of the western Great Plains have as many polymorphic loci as massive populations in the Front Range. For some authors, this argues against long-distance dispersal and suggests instead that these scarp woodlands were once continuous across what is now prairie (Wells 1983a, Hamrick et al. 1989). For conifers, El-Kassaby (1990) summarizes other cases where genetic variation has been used to reconstruct population histories, more often than not in the absence of an adequate fossil record.

It is generally thought that plant migration occurs by the founding of small populations following long-distance dispersal (Cwynar and MacDonald 1987). Such migration may produce a cline in reduced genetic variation from central to peripheral populations through reduction of population size during founding events. Genetic variation also is expected to decrease in the direction of migration as the time since founding decreases (Cwynar and MacDonald 1987). Owl Canyon represents both the longest dispersal and most recent migration of *Pinus edulis* in the Rocky Mountain region, yet genetic diversity was generally maintained during this founding event. Owl Canyon raises serious questions about how much genetic variation can be expected after a founder event and hence, to what extent can genetic variation be used to reconstruct population history independent of fossil evidence. However, these expectations assume that the genetic variation is not substantially influenced by natural selection. Presently, we do not fully appreciate how selection would alter these expectations.

CONCLUSIONS

The 5000-yr fossil record clearly indicates the Owl Canyon pinyon grove was initiated by a founding event following long-distance dispersal between 1290 and 420 yr BP. The demographic data, along with the fact that the oldest remaining trees contain most of the genetic variation found in the population today, suggest that these trees may be remnants of the original founding group. Abundant genetic variation in the population suggests that founder effects were slight. There is scarce indication of traditional founder effects (slightly lower polymorphism and number of alleles compared to other pinyon populations), indicating substantial initial founding population size. It is apparent that some marginal populations, even those established by long-distance dispersal, can maintain high levels of genetic diversity.

This study points up the advantages of using a combination of approaches. Had the electrophoretic study not been complemented by a fine-scale fossil record, we would have lacked the evidence to conclude that a founder event had occurred. Instead, we might have sought a hypothesis of vicariance to explain the Owl Canyon population. Certainly a pattern of high heterozygosity and slightly lower than average polymorphism and number of alleles per locus can be explained by mechanisms other than a founder effect. Due to the clarity of the fossil record, however, we now have estimates of levels of genetic variation in an expanding population subsequent to a founding event.

On the basis of the demographic data and fossil evidence alone, we deduced that the Owl Canyon grove developed after long-distance dispersal; we can even estimate the timing. However, we could not have es-

timated the number of founders or the subtle founder effects that accompanied long-distance dispersal. The electrophoretic data provide insights into the details of population founding and expansion.

These empirical results also relate to contemporary issues in evolutionary theory and conservation biology. Speciation is thought to occur commonly on range margins following founder events. Controversy exists about hypothetical losses in genetic variation and whether or not such losses are a prerequisite for speciation. Several recent experiments and developments in theory indicate that short-term bottlenecks can result in increased genetic variance for quantitative characters (Lande 1980, Carson and Wisotzkey 1989). Our study shows that a species can pass through population bottlenecks with little loss in allozyme diversity. In conservation biology, it is assumed that isolated populations, particularly newly found ones, are at risk because theory predicts reduced genetic variance. Our study adds fuel to the current controversy, summarized by Lewin (1987), that this assumption may be incorrect. Though average fitness may be lowered, increased genetic variance may provide ample opportunities for selection to act on new and fitter genetic combinations.

Finally, a better understanding of genetic structure and population dynamics of central vs. peripheral populations may be necessary for realistic predictions of migrational responses to future climates (Davis 1988). Owl Canyon demonstrates that such a goal can be served best by adopting a strong historical perspective and blending approaches from paleoecology, genetics, and population ecology.

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