

GENETIC CONSEQUENCES OF PRE-COLUMBIAN CULTIVATION FOR *AGAVE MURPHEYI* AND *A. DELAMATERI* (AGAVACEAE)¹

KATHLEEN C. PARKER,² J. L. HAMRICK,³ WENDY C. HODGSON,⁴ DORSET W. TRAPNELL,⁵ ALBERT J. PARKER,²
AND ROBERT K. KUZOFF⁶

²Department of Geography, University of Georgia, Athens, Georgia 30602 USA; ³Department of Plant Biology and Genetics, Athens, Georgia 30602 USA; ⁴Desert Botanical Garden, Phoenix, Arizona 85008 USA;

⁵Department of Plant Biology and the Institute of Ecology, University of Georgia, Athens, Georgia 30602 USA; and

⁶Department of Biology, University of Wisconsin, Whitewater, Wisconsin 53190 USA

Pre-Columbian farmers cultivated several species of agave in central Arizona from ca. A.D. 600–1350. Because of the longevity and primarily asexual reproduction of these species, relict agave clones remain in the landscape and provide insights into pre-Columbian agricultural practices. We analyzed variation in allozyme allele frequencies to infer genetic effects of prehistoric cultivation on *Agave murpheyi* and *A. delamateri*, specifically to estimate genetic diversity and structure, to determine whether cultivated populations descended from a single clone, and to examine regional-scale genetic variation. *Agave murpheyi* maintained more genetic diversity at the species and population levels than *A. delamateri*, and *A. murpheyi* populations typically included more multilocus genotypes. Relict plants from prehistoric fields reflect a more complex history than descent from a single clone; *A. murpheyi* populations may have included more diversity initially because bulbils (produced routinely in *A. murpheyi* but not *A. delamateri*) and possibly seed would have facilitated transport of genetically diverse planting stock. Genetic variation in both cultigens was lower than in most contemporary commercial crops but similar to that observed in modern traditional agricultural systems.

Key words: Agavaceae; agave cultivation; *Agave delamateri*; *Agave murpheyi*; pre-Columbian agriculture; Sonoran Desert.

The genus *Agave* is a group of succulent plants that have been important sources of food and fiber for humans in Mesoamerica since at least 9000 BP. (Callen, 1965; Gentry, 1982). Uses of agave in this region and farther north during pre-Columbian and historic times include fiber, paper, soaps, shampoos, medicines, armed fences, fermented beverages, and food from the starchy meristem, flowers, or young inflorescence (Castetter et al., 1938; Gentry, 1982; Hodgson, 2001a). Agave likely played an important role in the early development of agriculture in Mesoamerica (Gentry, 1982). Many agave species reproduce vegetatively, which allowed offsets to be collected easily from the wild and transplanted to sites near villages, with a higher survival than plants started from seed (Sauer, 1972; Arizaga and Ezcurra, 2002). In pre-Columbian agricultural communities, agave was often planted in marginal fields to supplement annual crops grown under irrigation and to provide food in adverse years (Fish, 2000).

Although its ethnobotanical importance in Mesoamerica had long been recognized, agave was originally considered to be of minor importance to indigenous peoples north of the modern international border. Minnis and Plog (1976) attributed the western part of the modern range in Arizona of *Agave parryi* Engelm to pre-Columbian anthropogenic range expansion, although they were uncertain whether the agave in their study sites resulted from cultivation or merely disposal of unused plants gathered from wild populations. Water-manipulation

features that often accompany agave cultivation in the northern Sonoran Desert had been described and mapped by archaeologists (e.g., Gumerman et al., 1975) for at least a decade before they were linked to agave cultivation (Hodgson, 2001a). The first conclusive evidence of pre-Columbian agave cultivation in Arizona came with reports of abundant agave remains found at archaeological sites (Miksicek, 1984), coupled with reports of tools, rock piles, and other structures indicative of agave cultivation and processing (Fish et al., 1985). Since then, at least 556 agave cultivation sites have been documented in central Arizona, mostly dating from the Classic period (A.D. 1150–1450) of the Hohokam culture (Fish, 2000). Evidence now exists for pre-Columbian cultivation of at least four agave species in Arizona (Minnis and Plog, 1976; Gentry, 1982; Hodgson and Slauson, 1995; Hodgson, 2001b), and several unclassified putative hybrids. Three species (*A. murpheyi* Gibson, *A. delamateri* Hodgson and Slauson, and *A. phillipsiana* Hodgson) exist only in association with archaeological sites and are, therefore, considered to be cultigens.

Genetic comparisons between cultivated plant populations and closely related wild populations have been used to address several evolutionary concerns, including identification of wild progenitors of individual crops (Doebley, 1989, 1992) and determination of the number of independent domestication events within individual cultigens (e.g., European potatoes [Hosaka and Hanneman, 1988], sunflower [Rieseberg and Seiler, 1990], maize [Doebley, 1990], lima beans [Salgado et al., 1995]). The vast majority of research that has examined the relationship between cultivated and wild populations has focused on modern crops that are part of either commercial or traditional agricultural systems. Although many cultigens were domesticated in prehistoric times, they have continued to evolve over subsequent millennia as cultures have developed and human selective pressures have changed. Consequently,

¹ Manuscript received 28 August 2006; revision accepted 3 July 2007. The authors thank C. Deen and the Hamrick lab personnel for laboratory assistance; J. Clark, S. Fish, P. Fish, and P. Lyons for valuable discussions about the archaeological context; S. Markwith and R. Rodgers for help with calculation of interpopulation distances; T. Jordan for assistance with ArcView; K. Haag from Research Media at the University of Georgia for figure drafting; and the National Science Foundation for financial support (#BCS-0216832).

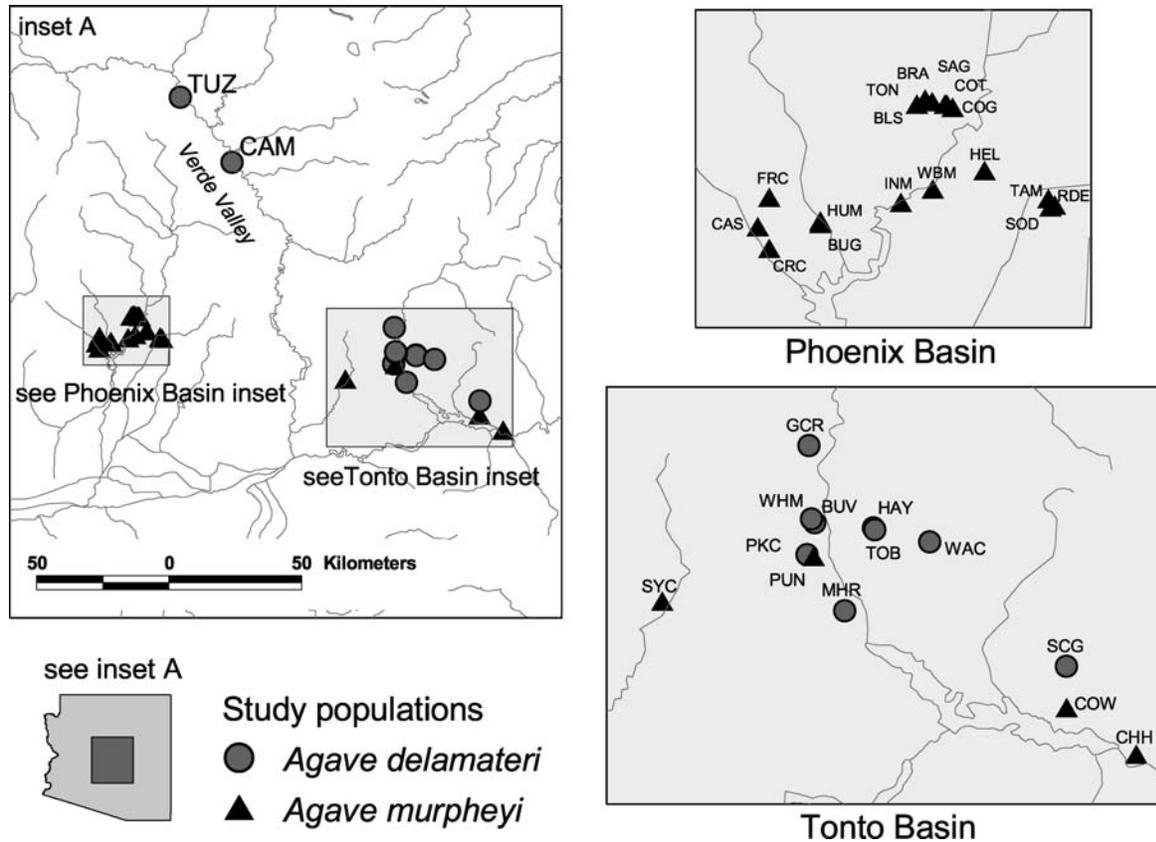


Fig. 1. Location of the *Agave murpheyi* and *A. delamateri* study populations in central Arizona.

little opportunity exists to examine the effects of cultivation within a prehistoric cultural context, and this represents a virtually unexplored area of crop evolution. Widespread demographic change in the northern Sonoran Desert after ca. A.D. 1450, followed by the absence of agrarian cultures from parts of this area until after European contact, left many prehistorically cultivated agave populations untended. Because of their predominantly vegetative reproduction, many populations still exist in the modern landscape. These relicts of pre-Columbian agave cultivation in the northern Sonoran Desert give us a narrow window into the past. Although potentially affected by differential survival over the intervening centuries, they have likely experienced only minimal alteration through continuing human selection. The information they provide about the genetic effects of prehistoric agave cultivation in the northern Sonoran Desert is the focus of this paper.

Many archaeological sites with evidence of agave cultivation are located in the Phoenix, Tonto, and Tucson Basins, areas associated with the Hohokam culture from ca. A.D. 1 until 1450, when widespread abandonment occurred. Although agave was cultivated in this area as early as A.D. 600, large-scale cultivation did not occur until 950-1150 (Gasser and Kwiatkowski, 1991; Fish, 2000; Clark and Vint, 2004), a time of significant cultural variation within the region inhabited by the Hohokam (Doelle and Wallace, 1991). Agave cultivation permitted land that was suboptimal for other crops to be brought into production (Fish et al., 1985). Agave was cultivated along streams near dwellings in some areas. In other areas, large fields were located on bajadas some distance

from dwellings, where rockpile complexes were assembled to increase soil moisture levels around individual plants. Fish and others (Bohrer, 1991; Fish et al., 1992) suggested that *A. murpheyi* was likely the primary agave species cultivated in the southern part of the Hohokam region. Altogether, *A. murpheyi* is known from more than 59 sites—at least 52 are pre-Columbian sites in southern Arizona, with the remainder in northern Sonora (Fig. 1; Hodgson, unpublished manuscript). More recently, *A. delamateri* was discovered in association with Hohokam and Salado settlements farther north in Arizona and described as a new species (Hodgson and Slauson, 1995). Since its discovery, at least 90 *A. delamateri* sites have been located, with most in the Tonto Basin (Fig. 1). On the basis of limited allozyme analyses, Nabhan (1992) and Hodgson (unpublished manuscript) speculated that *A. murpheyi* and *A. delamateri* populations still in existence are genetic clones of the original individuals cultivated in this area, although sample sizes and the number of allozyme loci resolved were too small to address this issue conclusively (R. J. Hickey, Miami University, personal communication).

The objective of this research was to examine the effects of pre-Columbian cultivation on patterns of genetic variation in relict agave populations in central Arizona. Specifically we asked (1) What levels of genetic diversity characterize *A. murpheyi* and *A. delamateri* and how is genetic diversity partitioned within these two species? (2) Based on patterns of allozyme variation evident in *A. murpheyi* and *A. delamateri*, was each species introduced into the region from only a single clone, or alternatively, was the origin of populations in central

Arizona more complex? (3) Are geographic contrasts in genetic patterns between the Phoenix and Tonto Basins evident for the two agave cultigens?

MATERIALS AND METHODS

Study area—Our analyses focused on the northern Sonoran Desert, where we sampled agave populations primarily in the Phoenix and Tonto basins, with two additional populations in the Verde Valley (Fig. 1). Central Arizona constituted our focal area for several reasons: the Phoenix and Tonto basins are relatively well studied archaeologically, they still support numerous populations of both *Agave murpheyi* and *A. delamateri*, and they differ in their physical and pre-Columbian cultural environments (Cordell et al., 1994). Although the seminal work on agave cultivation in this region was done in the Tucson Basin (Miksicek, 1984; Fish et al., 1985), few agave populations still exist there; consequently, we focused on areas farther north.

Relict *A. murpheyi* and *A. delamateri* populations occur in different habitats and were evidently associated with different cultivation practices. Throughout most of its range, *A. murpheyi* occurs at 410–900 m a.s.l. within the Lower Colorado River Valley and lower elevations of the Arizona Upland, typically on floodplains, flat benches, and lower alluvial fan surfaces where the Hohokam constructed runoff collection features to enhance soil moisture (Hodgson, unpublished manuscript). In contrast, *A. delamateri* occurs mainly in the Arizona Upland from 725–1554 m a.s.l. on sites dominated by both Arizona Upland and Interior Chaparral floral elements (Hodgson, unpublished manuscript). Unlike *A. murpheyi*, *A. delamateri* was typically grown on alluvial terraces without water-augmenting features, often on sites overlooking irrigated crops cultivated on the floodplain (Hodgson and Slauson, 1995). Soils on these sites are typically deep, gravelly, and well drained. Several *A. murpheyi* populations at the northern range limits of the species occur in habitats that are similar to those inhabited by *A. delamateri*. These populations were apparently associated with cultivation practices more typical of *A. delamateri*, although Hodgson (unpublished manuscript) called for further comparative study of *A. murpheyi* cultivation in these two habitats.

Study species—Both *Agave murpheyi* and *A. delamateri* are members of the *Ditelpalae* group of *Agave* (Gentry, 1982; Hodgson and Slauson, 1995), which comprises 10 species that were cultivated either historically or prehistorically. All are monocarpic perennial rosettes that take at least 8–20 yr to reach reproductive maturity. Subsequently, leaves wither and the plant often produces rhizomatous suckers (Gentry, 1982). Viable seed production in *A. murpheyi* is low (Gentry, 1982; Szarek et al., 1996), and the absence of seed in *A. delamateri* suggests a partially or fully sterile taxon, a common trait of agaves cultivated by historic and prehistoric traditional farmers (Reveal and Hodgson, 2002; Colunga-GarcíaMarín and Zizumbo-Villarreal, 2006). The minimal fruit production may reflect self-incompatibility and spatial isolation of populations, initial transport of individuals out of their natural range farther south and subsequent exposure to hotter, drier conditions during flowering, or selective pressures associated with cultivation (Hodgson and Slauson, 1995; Rogers, 2000). High temperatures characteristic of Arizona summers inhibit flower and seed development of a number of agaves that originated farther south in Mexico (Gentry, 1982), including *A. delamateri* (Hodgson and Slauson, 1995). *Agave murpheyi* is a prolific bulbil producer, forming >300 bulbils along the inflorescence, even without damage to the flower stalk (Gentry, 1982); these remain attached to the plant for 2–3 yr, showing both leaf elongation and root development before detachment (Szarek et al., 1996). *Agave delamateri* rarely forms bulbils, and only if the inflorescence is damaged (Hodgson, unpublished manuscript). Agave cultivation for food typically involves the removal of embryonic reproductive structures to prevent sexual reproduction and trigger offset formation (Hodgson, 2001a). *Agave murpheyi* is diploid ($2n = 60$; Pinkava and Baker, 1985); although the ploidy level of *A. delamateri* is unknown, low pollen stainability percentages and the absence of seed production led Hodgson and Slauson (1995) to suggest hybrid origins or polyploidy for the species.

Field sampling procedures—From a list of known populations documented with voucher specimens at the Desert Botanical Garden Herbarium (DES) by Hodgson (unpublished manuscript), we selected 21 *A. murpheyi* and 11 *A. delamateri* populations in primarily the Phoenix and Tonto Basins (Fig. 1). Most populations included <30 plants; in these, we collected samples from

each rosette present. In larger populations (i.e., up to 70 ramets), we limited our sample to ~48 ramets, with individuals selected broadly across the population. We recorded cluster membership of the ramets based on inter-ramet distance (within ca. 1 m) because underground rhizomes connecting parent plants with basal suckers were not visible. UTM coordinates and elevation of each population were recorded with a global positioning system (GPS, Garmin, Olathe, KS) unit. Samples consisted of a leaf tip from the inner (i.e., younger) part of the rosette. Samples were transported at room temperature to the University of Georgia within 5 d and stored at 4°C until protein extraction.

Laboratory procedures—Tissue ≥ 5 mm distant from the original leaf cut was crushed in liquid nitrogen with a mortar and pestle. A potassium phosphate buffer (Mitton et al., 1979) was used to extract proteins, which were adsorbed onto filter paper wicks and stored at -70°C .

Starch-gel electrophoresis was used to determine allozyme variation. Four electrode buffer systems and 14 enzyme systems were used to resolve 27 loci on 10% starch gels: (1) system 6: alcohol dehydrogenase (ADH), colorimetric esterase (CE), tetrazolium oxidase (TO), and acid phosphatase (ACP); (2) system 7: aspartate aminotransferase (AAT); (3) system 8: fluorenscent esterase (FE), diaphorase (DIA), and menadiolone reductase (MNR); and (4) system 11: isocitrate dehydrogenase (IDH), shikimate dehydrogenase (SKDH), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), and malate dehydrogenase (MDH; Sherman-Broyles et al., 1992). Recipes were modified from Cheliak and Pitel (1984a) for DIA, and Soltis et al. (1983) for other stains and buffers.

Statistical analyses—Five standard measures of genetic diversity were calculated for each population and for each species: percentage of polymorphic loci (P), mean number of alleles per locus (A) and per polymorphic locus (AP), the effective number of alleles per locus (A_e), and the expected heterozygosity (H_e ; Hamrick et al., 1979). Additional subscripts indicate whether these measures pertain to the population (p) or species (s) level. A locus was considered polymorphic if a second allele was evident (in effect, with a frequency > 0.01). Population-level values for these indices were calculated for each population, then averaged over all populations within species. Because one *A. delamateri* population (SCG) had unusual allele frequencies for several loci, this population was omitted from the sample and species- and population-level indices were recalculated. Both sets of values are reported.

Multilocus genotype frequencies were tabulated for each population, and the multilocus genotype diversity (D) was calculated as $D = 1 - \sum \{[n_i(n_i - 1)]/[N(N - 1)]\}$, where n_i is the number of individuals of the i th genotype and N is the sample size (Pielou, 1969; Ellstrand and Roose, 1987). Ellstrand and Roose's (1987) proportion of the sample distinguishable (G/N) was calculated as the number of multilocus genotypes detected (G) divided by the sample size. The number of unique multilocus genotypes possible (N_g) was computed as

$$N_g = \prod_{i=1}^L [a_i(a_i + 1)]/2,$$

where a_i is the number of alleles detected at the i th locus, and L is the number of loci analyzed (Cheliak and Pitel, 1984b). The evenness of multilocus genotypes within populations was calculated as $E = (D_{\text{obs}} - D_{\text{min}})/(D_{\text{max}} - D_{\text{min}})$, where $D_{\text{min}} = [(G - 1)(2N - G)]/[N(N - 1)]$ and $D_{\text{max}} = [N(G - 1)]/[G(N - 1)]$ (Fager, 1972). Differences between *A. murpheyi* and *A. delamateri* for genetic and genotypic diversity indices were analyzed with t tests; variances were estimated with a jackknife procedure (Weir and Cockerham, 1984).

Departures of observed heterozygote frequencies from those expected under Hardy-Weinberg equilibrium were calculated for each population overall and for each polymorphic locus in each population with Wright's fixation index (F_{IS}) as $F_{\text{IS}} = 1 - H_o/H_e$, where H_o is observed heterozygosity (Wright, 1965). Significance of these departures was tested with chi-square analysis, $\chi^2 = N F_{\text{IS}}^2(a - 1)$, with $df = a(a - 1)/2$, where N is the sample size and a is the number of alleles at a locus (Li and Horvitz, 1953).

Genetic structure was examined with Nei's (1973, 1977) genetic diversity indices. Total variation at polymorphic loci (H_T) was partitioned into within-population (H_S) and among-population components (D_{ST}), with the latter expressed as a proportion of the total variation, or $G_{\text{ST}} = D_{\text{ST}}/H_T$. Nei's statistics were also used to estimate the regional genetic variation within each species. Among-population variation in allele frequencies was tested with chi-square analysis (Workman and Niswander, 1970). Isolation of populations by distance was modeled with regression analysis relating $F_{\text{ST}}/(1 - F_{\text{ST}})$ to geographic distance between population pairs (Rousset, 1997), with significance evaluated with Mantel's tests (Manley, 1991). Values for F_{ST} were

TABLE 1. Genetic variation of (A) *Agave murpheyi* and (B) *A. delamateri* populations in central Arizona.

Population	<i>N</i>	<i>P</i>	<i>AP</i>	<i>A_e</i>	<i>H_e</i>	<i>H_o</i> ^a	<i>F_{IS}</i>
A) <i>Agave murpheyi</i>							
BLS	14	30.8	2.00	1.15	0.088 (0.032)	0.132 (0.018)**	-0.500
BRA	25	19.2	2.00	1.13	0.073 (0.031)	0.100 (0.012)**	-0.370
BUG	10	23.1	2.17	1.19	0.098 (0.039)	0.150 (0.022)**	-0.531
CAS	48	25.9	2.14	1.14	0.078 (0.032)	0.115 (0.009)**	-0.474
COG	15	23.1	2.00	1.15	0.081 (0.033)	0.133 (0.017)**	-0.642
COT	12	23.1	2.17	1.17	0.088 (0.036)	0.151 (0.020)**	-0.716
CRC	47	29.6	2.13	1.16	0.094 (0.034)	0.136 (0.010)**	-0.447
FRC	36	26.9	2.14	1.16	0.091 (0.036)	0.133 (0.011)**	-0.462
HEL	17	26.9	2.14	1.22	0.116 (0.041)	0.170 (0.018)**	-0.466
HUM	19	23.1	2.00	1.16	0.090 (0.035)	0.138 (0.016)**	-0.533
INM	27	34.6	2.11	1.21	0.106 (0.040)	0.156 (0.014)**	-0.472
RDE	13	29.6	2.00	1.13	0.078 (0.030)	0.125 (0.018)**	-0.603
SAG	20	26.9	2.14	1.17	0.095 (0.035)	0.150 (0.016)**	-0.579
SOD	16	25.9	2.00	1.15	0.087 (0.032)	0.118 (0.016)**	-0.356
TAM	40	33.3	2.22	1.14	0.080 (0.034)	0.091 (0.009)	-0.138
TON	21	23.1	2.17	1.16	0.090 (0.035)	0.139 (0.015)**	-0.544
WBM	20	30.8	2.13	1.19	0.112 (0.036)	0.140 (0.015)**	-0.250
CHH	37	29.6	2.13	1.14	0.080 (0.030)	0.118 (0.010)**	-0.475
COW	25	25.9	2.00	1.14	0.078 (0.035)	0.127 (0.013)**	-0.628
PUN	28	29.6	2.25	1.16	0.092 (0.034)	0.123 (0.012)**	-0.337
SYC	18	23.1	2.00	1.15	0.081 (0.034)	0.125 (0.015)**	-0.543
Population mean		26.9 ^{bc}	2.10 ^{bc}	1.16 ^{bc}	0.089 ^{bc} (0.008)	0.132 (0.003)	
Species level value		44.4	2.50 ^{bc}	1.16 ^{bc}	0.098 ^{bc}	—	
B) <i>Agave delamateri</i>							
BUV	53	16.0	2.25	1.13	0.068 (0.033)	0.094 (0.008)**	-0.382
GCR	15	7.7	2.00	1.07	0.038 (0.026)	0.069 (0.013)**	-0.816
HAY	16	12.0	2.33	1.08	0.043 (0.027)	0.058 (0.012)*	-0.349
MHR	27	16.0	2.25	1.13	0.064 (0.035)	0.094 (0.011)**	-0.469
PKC	34	15.4	2.00	1.08	0.043 (0.027)	0.074 (0.009)**	-0.721
SCG	8	11.1	2.00	1.06	0.037 (0.020)	0.038 (0.013)	-0.027
TOB	58	19.2	2.40	1.10	0.057 (0.029)	0.086 (0.007)**	-0.509
WAC	50	15.4	2.50	1.10	0.056 (0.028)	0.079 (0.008)**	-0.411
WHM	27	12.0	2.00	1.08	0.039 (0.028)	0.069 (0.010)**	-0.769
CAM	27	33.3	2.00	1.22	0.123 (0.048)	0.189 (0.018)**	-0.537
TUZ	48	19.2	2.20	1.09	0.051 (0.028)	0.065 (0.007)*	-0.275
Population mean (SCG included)		16.1 ^b	2.18 ^b	1.10 ^b	0.056 ^b (0.009)	0.083 (0.003)	
Population mean (SCG omitted)		16.6 ^c	2.19 ^c	1.11 ^c	0.058 ^c (0.010)	0.088 (0.003)	
Species level (SCG included)		44.4	2.25 ^b	1.11 ^b	0.067 ^b	—	
Species level (SCG omitted)		38.5	2.20 ^c	1.11 ^c	0.064 ^c	—	

Note: *P* is the percentage of polymorphic loci; *AP* is the mean number of alleles at polymorphic loci; *A_e* is the effective number of alleles; *H_e* is the expected heterozygosity under Hardy–Weinberg; *H_o* is the observed heterozygosity; *F_{IS}* is Wright's fixation index.

^a Difference between *H_o* and *H_e* significant: **P* ≤ 0.05; ***P* ≤ 0.001. Standard deviation is in parentheses.

^b Means for *Agave murpheyi* and *A. delamateri* significantly different (*P* < 0.05) with SCG included among *A. delamateri* populations.

^c Means for *Agave murpheyi* and *A. delamateri* significantly different (*P* < 0.05) with SCG omitted from *A. delamateri* populations.

estimated according to Weir and Cockerham (1984); geographic distances between GPS waypoints recorded in each population were calculated with ArcGis/ArcMap version 9.0 (ESRI, 1999–2004); and GENEPOP 3.4 (Raymond and Rousset, 1995) was used for analyses. Hillis' (1984) modification of Nei's (1972) genetic distance (*D*) was calculated for all pairs of populations within each species. Classification analysis was used to analyze relationships among populations of each species separately and for both species together. Unweighted pair-group classifications based on arithmetic averages (UPGMA) were generated with NTSYSpc 2.2 (Rohlf, 2005). Separate analyses by species produced classifications nearly identical to the combined classification; therefore, only the combined analysis is presented.

RESULTS

Genetic diversity of the two cultigens—The same 27 loci were resolved in *A. murpheyi* and *A. delamateri*, 12 of which (44.4%) were polymorphic at the species level in each cultigen (Table 1). Only six loci were variable in both species. In *A.*

murpheyi, two loci (*Adh2* and *Pgm2*) were polymorphic in every population; in *A. delamateri*, no locus was polymorphic in all populations sampled. In an average population, significantly more loci were polymorphic in *A. murpheyi* than in *A. delamateri* (26.9% vs. 16.1%). The locus *Skdh1* was fixed for a different allele in SCG relative to all other *A. delamateri* populations; therefore, this locus was polymorphic at the species level but was monomorphic within populations. Although *A. delamateri* had more alleles per polymorphic locus (*AP*) than *A. murpheyi* at the population level (2.18 vs. 2.10), the effective number of alleles (*A_e*) was significantly lower (1.10 vs. 1.16; Table 1). In *A. murpheyi*, *Pgm2* and *Adh2* were fixed heterozygotes in most populations, which contributed to the higher mean *A_e* value for that species. No locus showed such extreme heterozygosity in *A. delamateri*. Both observed and expected heterozygosities (*H_o* and *H_e*) were significantly greater at the species and population levels in *A.*

TABLE 2. Summary of multilocus genotypes^a for (A) *Agave murpheyi* and (B) *A. delamateri* populations in central Arizona based on 27 allozyme loci.

Population	<i>G</i> *	<i>N_g</i> **	<i>D</i> **	<i>E</i> *	<i>G/N</i> **	No. of clusters*	No. of clusters/genotype**	No. of genotypes/cluster*	% of genotypes restricted to one cluster**
A) <i>A. murpheyi</i>									
BLS	11	2187	0.934	0.000	0.79	3	1.1	4.0	91
BRA	10	243	0.820	0.652	0.40	5	1.7	3.4	70
BUG	9	486	0.978	0.000	0.90	1	1.0	9.0	100
CAS	18	4374	0.904	0.840	0.38	11	1.9	3.1	72
COG	8	243	0.848	0.560	0.53	1	1.0	8.0	100
COT	6	486	0.848	0.733	0.50	1	1.0	6.0	100
CRC	28	4374	0.968	0.891	0.60	15	1.5	2.8	71
FRC	20	486	0.954	0.880	0.56	10	1.5	2.9	65
HEL	17	1458	1.000	—	1.00	3	1.0	5.7	100
HUM	12	243	0.918	0.623	0.63	3	1.3	4.7	83
INM	19	4374	0.966	0.792	0.70	5	1.3	4.8	79
RDE	9	6561	0.923	0.563	0.69	3	1.2	3.7	78
SAG	9	1458	0.858	0.725	0.45	2	1.2	5.5	78
SOD	14	2187	0.975	0.000	0.88	5	1.1	3.0	93
TAM	26	65 610	0.969	0.860	0.65	40	1.5	1.0	69
TON	12	486	0.900	0.646	0.57	6	1.3	2.5	75
WBM	18	4374	0.989	0.529	0.90	6	1.1	3.3	89
CHH	16	4374	0.890	0.764	0.43	7	1.6	3.6	69
COW	8	2187	0.820	0.783	0.32	1	1.0	8.0	100
PUN	17	26 244	0.950	0.825	0.61	7	1.4	3.4	76
SYC	8	243	0.856	0.754	0.44	3	1.3	3.3	75
Population mean	14	5068	0.918	0.621	0.62	6.6	1.3	4.4	82.5
B) <i>A. delamateri</i>									
BUV	16	162	0.924	0.932	0.30	14	3.0	3.4	31
GCR	2	9	0.343	0.521	0.13	2	1.5	1.5	50
HAY	6	54	0.808	0.768	0.38	3	1.5	8.5	67
MHR	14	162	0.926	0.828	0.52	4	1.6	5.5	50
PKC	6	81	0.531	0.438	0.18	2	1.7	5.0	33
SCG	5	27	0.893	0.833	0.63	1	1.0	5.0	100
TOB	15	972	0.808	0.729	0.26	25	3.1	1.9	67
WAC	16	324	0.829	0.710	0.32	26	2.6	1.6	56
WHM	4	27	0.276	0.111	0.15	7	2.8	1.6	50
CAM	14	729	0.932	0.854	0.52	10	1.6	2.3	64
TUZ	17	486	0.917	0.889	0.35	7	1.8	5.0	47
Population mean	10	276	0.744	0.692	0.33	9.2	2.0	3.8	55.9

Note: *G* is the number of multilocus genotypes; *N_g* is the number of possible multilocus genotypes; *D* is the multilocus genotype diversity; *E* is the genotypic evenness; *N* is the sample size; clusters were identified in the field based on inter-ramet distance.

^a Variables with * in the column headings differed significantly between species at $P < 0.05$; with **, at $P < 0.001$.

murpheyi than *A. delamateri*. The exclusion of one outlier population (SCG) from the *A. delamateri* sample slightly elevated most genetic diversity measures at the population level, but they remained significantly different from indices for *A. murpheyi* (Table 1; discussed later).

Variation in multilocus genotypes—At the species level, *A. murpheyi* had nearly four times as many multilocus genotypes as *A. delamateri* (162, $N = 508$ vs. 46, $N = 363$). The majority of genotypes were only found in a single population. In *A. delamateri*, 15% of the genotypes occurred in >4 populations; in *A. murpheyi*, only 7% of the genotypes were that common. In both species, these more common genotypes accounted for slightly greater than half the individuals sampled. Ellstrand and Roose (1987) considered the number of different genotypes detected divided by the sample size (G/N) to be a good indication of overall clonal diversity in each population; this value was greater on average for *A. murpheyi* (0.32 vs. 0.13).

Agave murpheyi populations had 14 multilocus genotypes on average, and *A. delamateri* had 10 (Table 2). A random sample of seven populations had a mean difference of 2.41 loci among

genotypes within populations for *A. murpheyi* and 2.00 for *A. delamateri*, which suggests that processes other than somatic mutation are responsible for the genotypic variation observed. The mean index of multilocus genotype diversity (*D*) was also greater in *A. murpheyi* (0.918 vs. 0.744), as was the mean G/N value (0.62 vs. 0.33). Within each species, larger populations tended to have more multilocus genotypes (Pearson r for *A. murpheyi* = 0.72, $P < 0.001$; for *A. delamateri*, $r = 0.79$, $P < 0.05$); but *D* was not significantly related to population size (for *A. murpheyi*, $r = -0.16$, for *A. delamateri*, $r = 0.29$, $P > 0.05$ in both species). Although both species had intermediate values for the mean evenness of multilocus genotype frequencies (*E*), values for individual populations varied substantially, ranging from 0.000–0.891 (mean = 0.621) for *A. murpheyi* and from 0.111–0.932 (mean = 0.692) for *A. delamateri*. Values of *E* range from 0 (where all plants have identical genotypes; or one genotype dominates, and the other genotypes are each represented by one ramet) to 1 (where all genotypes have the same frequency; Fager, 1972; Albert et al., 2003). Genotypes represented by a single ramet accounted for a greater proportion of populations in *A. murpheyi* than in *A. delamateri*

(mean of 46% vs. 17%). The number of possible multilocus genotypes was nearly 20 times greater in *A. murpheyi* (mean $N_g = 5068$, range = 243–65 610) than in *A. delamateri* (mean $N_g = 276$, range = 9–972).

In both *A. murpheyi* and *A. delamateri*, plant clusters identified in the field based on inter-ramet distance did not correspond closely with the spatial distribution of multilocus genotypes within populations (Table 2). The majority of genotypes in populations of both species were narrowly distributed, although this tendency was more pronounced in *A. murpheyi*. On average, 82.5% of genotypes were restricted to only one cluster for *A. murpheyi*, in contrast to 55.9% for *A. delamateri*. Most spatial clusters, however, included a combination of multilocus genotypes. *Agave murpheyi* averaged 4.4 genotypes per cluster, with mean values for individual populations ranging from 1.0 to 8.0. *Agave delamateri* populations averaged 3.8 genotypes per cluster, with individual population means ranging from 1.5 to 5.0. Four *A. murpheyi* populations only comprised one cluster of plants, in contrast to one *A. delamateri* population; but all of these populations included more than one multilocus genotype.

Genetic variation among populations—Although genetic diversity was generally greater in *A. murpheyi* than in *A. delamateri*, both species varied appreciably among populations in allele frequencies and overall diversity measures. For *A. murpheyi*, the higher values for several diversity indices were found in three populations centrally located within Phoenix Basin (INM, BUG, and HEL; Tables 1 and 2), even though these were not the largest populations. Populations with the lowest values for most genetic diversity measures (CAS, COW, RDE, CHH, TAM) were outside the core area of Phoenix Basin. Five private alleles were evident in *A. murpheyi*; these were all present in populations in Tonto Basin or on the periphery of Phoenix Basin. TAM had 2 private alleles, and CAS, PUN, and CHH each had one.

The most genetically diverse *A. delamateri* populations were BUV and MHR, both on high terraces to the south of Tonto Creek (now Theodore Roosevelt Lake) in Tonto Basin. BUV was one of the largest *A. delamateri* populations sampled; MHR was intermediate in size. The least diverse population was SCG, a small isolated population high on a terrace north of Tonto Creek in the Sierra Ancha, the mountain range that forms the northern rim of Tonto Basin.

Two *A. delamateri* populations had allele frequencies for several loci that were dramatically different from other populations. SCG had three of the nine private alleles detected in this species, despite comprising only eight ramets. SCG also had allele frequencies for two loci (*SKDH1* and *PGM1*) that were markedly different from other *A. delamateri* populations. SCG was located about 5 km downslope from an unusual agave population near an archaeological site that included individuals with relatively clear *A. murpheyi*, *A. delamateri*, and *A. chrysantha* Peebles characteristics, respectively, as well as individuals with traits that were intermediate among these taxa (W. Hodgson, personal observation). This, coupled with its unusual allele frequencies, raises concerns about the taxonomic identity of SCG, despite its classical *A. delamateri* morphology (Hodgson, unpublished manuscript).

CAM was the other unusual *A. delamateri* population. CAM possessed four private alleles; CAM was fixed for 34 heterozygotes at *6PGD1* and *PGM1*, while the other populations were fixed for allele 4, or 3 in the case of SCG for *PGM1*.

CAM was located in the Verde Valley north of Tonto Basin, relatively isolated from most other *A. delamateri* populations. TUZ, the other population in the Verde Valley, had one private allele (*FE1*). The other private allele evident in this species (*AAT2*) was in TOB, a large population in Tonto Basin.

Populations were more strongly differentiated from each other in *A. delamateri* than in *A. murpheyi*. A significantly greater proportion of the total allozyme variation was distributed among populations in the former species ($G_{ST} = 0.243$ and 0.113 , respectively). The omission of SCG from the *A. delamateri* populations decreased the G_{ST} for that species to 0.144. The greater genetic differentiation among *A. delamateri* populations accords with the greater number of private alleles, as well as the unusual allele frequencies for several loci in CAM. In *A. delamateri* all polymorphic loci except one (92%; *FE1*) had significant heterogeneity among populations in allele frequencies (Table 3). Although the majority of polymorphic loci followed the same pattern for *A. murpheyi*, significant among-population heterogeneity in allele frequencies was only evident for 8 of 12 loci (67%).

Regression analysis relating $F_{ST}/(1 - F_{ST})$ to geographic distance between population pairs showed an absence of isolation by distance for both species. The mean genetic distance across all *A. murpheyi* populations was 0.015 (range of individual population means, 0.008–0.045). Individual *A. delamateri* differed appreciably in their mean genetic distance. The mean genetic distance of SCG (0.095) was nearly twice as high as the next highest value (CAM, 0.056); all other populations had mean genetic distances <0.02 . The overall mean genetic distance for *A. delamateri* was 0.027; the deletion of SCG reduced this mean to 0.012.

Partitioning of genetic variation by regions for *A. murpheyi* and *A. delamateri* revealed little regional contrast for either species. Within *A. murpheyi*, Phoenix Basin was slightly more diverse overall than Tonto Basin by most measures; but on average, each region maintained $>99\%$ of the genetic variation detected in the species. *Agave delamateri* similarly maintained most of its genetic variation within regions. The Verde Valley was slightly more diverse than Tonto Basin, despite its representation by only two populations. Nonetheless, each region maintained 95–96% of the variation evident within the species, depending on whether SCG was included.

The classification analysis further demonstrated the absence of a relationship between genetic similarity and geographic proximity of populations in either species. Many populations in the same basin were placed in different clusters in the UPGMA classification (Fig. 2). If the mean genetic distance for the species is used as a guideline for defining groups (Holmes and Recher, 1986; Parker and Hamrick, 1996), four distinct clusters are evident within the *A. murpheyi* group on the UPGMA. BLS and SYC each formed a single-population group; and HEL and WBM, the most diverse populations, formed a third group. BLS, HEL, and WBM were centrally located in Phoenix Basin. The other *A. murpheyi* populations were all classified together in a fourth group. In some cases population pairs from different basins showed lower genetic distances (e.g., CHH and RDE) than pairs within either Phoenix or Tonto Basin.

Within the *A. delamateri* group, SCG and CAM formed single-population clusters at genetic distances greater than the mean for the species. Despite its questionable taxonomic affinity, SCG joined the other *A. delamateri* populations (though at a relatively high level) rather than the *A. murpheyi* group. The other Verde Valley population (TUZ) joined most

TABLE 3. Distribution of genetic diversity within and among populations of (A) *Agave murpheyi* and (B) *A. delamateri* in central Arizona for each polymorphic locus.

Locus	No. alleles	χ^2 ^a	H_T	H_S	G_{ST}
A) <i>A. murpheyi</i>					
<i>Aat2</i> ^c	2	429.21 (20)***	0.104	0.060	0.423
<i>Ce2</i> ^c	3	98.58 (40)***	0.096	0.089	0.071
<i>Fe1</i> ^c	2	89.81 (20)***	0.499	0.453	0.092
<i>Fe3</i> ^c	4	128.35 (60)***	0.142	0.128	0.099
<i>Mdh1</i> ^c	4	256.47 (60)***	0.313	0.281	0.103
<i>Pgi2</i> ^c	3	262.63 (40)***	0.171	0.145	0.151
<i>Adh2</i>	2	20.97 (20)	0.500	0.489	0.022
<i>Ce1</i>	2	14.84 (20)	0.008	0.008	0.015
<i>Fe2</i>	2	280.79 (20)***	0.252	0.182	0.276
<i>Idh2</i>	2	23.45 (20)	0.004	0.004	0.023
<i>Mnr</i>	2	79.08 (20)***	0.048	0.044	0.078
<i>Pgm2</i>	2	6.22 (20)	0.498	0.495	0.006
Species values ^b	2.50	1690.40 (360)***	0.219	0.198	0.113
B) <i>A. delamateri</i>					
<i>Aat2</i> ^c	2	21.15 (10)*	0.011	0.011	0.029
<i>Ce2</i> ^c	3	49.21 (18)***	0.178	0.167	0.062
<i>Fe1</i> ^c	2	5.93 (9)	0.003	0.003	0.009
<i>Fe3</i> ^c	2	164.98 (9)***	0.012	0.009	0.246
<i>Mdh1</i> ^c	3	199.15 (20)***	0.301	0.253	0.159
<i>Pgi2</i> ^c	2	23.96 (9)**	0.493	0.475	0.036
<i>6Pgd1</i>	2	348.98 (10)***	0.072	0.037	0.481
<i>6Pgd2</i>	2	33.87 (10)***	0.498	0.472	0.053
<i>Aat1</i>	2	24.96 (10)**	0.006	0.005	0.034
<i>Adh1</i>	2	69.23 (10)***	0.031	0.027	0.135
<i>Pgm1</i>	3	1074.65 (20)***	0.113	0.037	0.671
<i>Skdh1</i>	2	328.00 (6)***	0.093	0.000	1.000
Species values ^b	2.25	2344.07 (141)***	0.151	0.125	0.243

Note: H_T = total genetic diversity of the species; H_S = mean within-population genetic diversity; G_{ST} = the proportion of the total genetic diversity found among populations.

^a Significance of χ^2 tests: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

^b Species values are means calculated over polymorphic loci for the number of alleles, H_T , H_S , and G_{ST} ; χ^2 and its associated degrees of freedom are the sums of the respective values over all polymorphic loci.

^c Loci that are polymorphic in both species.

Tonto Basin populations at a lower genetic distance than BUV and MHR (the two diverse core Tonto Basin populations), although all these cluster agglomerations occurred at a level far below the overall mean genetic distance for the species.

Genotype frequencies within populations—Genotype frequencies for polymorphic loci in individual *A. delamateri* and *A. murpheyi* populations departed widely from Hardy–Weinberg equilibrium. For *A. delamateri*, the majority of F statistics were significantly different from zero (25 of 43 chi-square tests significant with $p < 0.05$, 58%). Most of the significant departures from Hardy–Weinberg equilibrium involved an excess of heterozygotes (76%). Nearly all populations showed a significant departure for both *6Pgd1* and *Pgi2*; virtually all departures were negative F values, indicating a heterozygote excess.

In *A. murpheyi*, the percentage of significant departures from Hardy–Weinberg expectations was lower (58 of 148, $p < 0.05$, 39%), but the number was nonetheless much greater than would be expected by chance (at $p < 0.05$). Most of these significant departures were negative F values (86%). For *Adh2* and *Pgm2*, all populations but one (SYC and HEL, respectively) had a significant heterozygote excess.

The overall F_{IS} values calculated for each population were negative in all cases (Table 1). All populations of both species

had an excess of heterozygotes; in all populations but two (TAM for *A. murpheyi* and SCG for *A. delamateri*), this excess was significant.

DISCUSSION

Genetic diversity maintained within species—Genetic diversity maintained at the population level in *A. murpheyi* and *A. delamateri* is lower than values reported previously for virtually all wild agave species (H_e ranging from 0.335 for *A. victoriae-reginae* T. Moore [Martínez-Palacios et al., 1999] to 0.394 for *A. lechuguilla* Torr. [Silva-Montellano and Eguiarte, 2003]). Although contrasting results have been reported from some comparisons of crops and their wild relatives based on different genetic markers, most authors have found lower levels of genetic diversity in crops than in their wild ancestors at the population level (e.g., barley [Neale et al., 1988], lentils [Havey and Muehbauer, 1989], sunflowers [Rieseberg and Seiler, 1990; Tang and Knapp, 2003]).

Genetic diversity of cultivated plants is often reduced relative to progenitor populations for several reasons. Early agriculturalists typically manipulate only a small percentage of the variation evident in the progenitor species, in essence causing founder effects and the associated loss of genetic

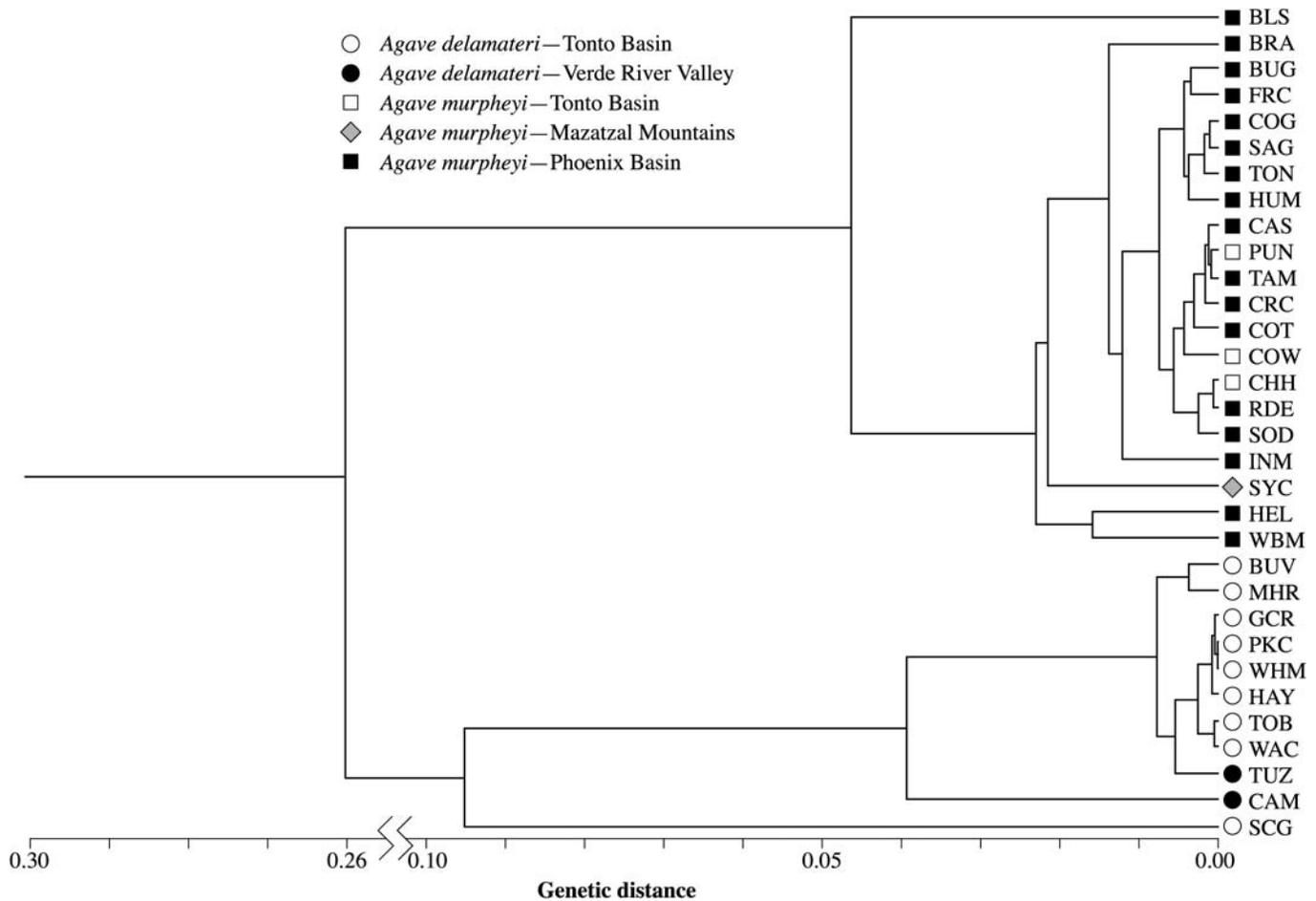


Fig. 2. UPGMA phenogram based on genetic distances (D ; Hillis, 1984) among *Agave murpheyi* and *A. delamateri* populations in central Arizona.

variation (Doebley, 1989). By saving seed or offsets from individuals with the most favored phenotypes after each harvest, farmers further narrow variation within their crops (Sauer, 1972; Doebley, 1992). Different villages, or even individual farmers, often apply different selection pressures, leading to different genes or combinations of genes becoming fixed in different landraces (Nabhan, 1985; Colunga-García-Marín and Zizumbo-Villarreal, 2006). This fosters reduced variation within cultivated populations, depending on exchange between villages, while maintaining relatively high species-level diversity (Brush et al., 1995; Hamrick and Godt, 1997). The reduced genetic variability within cultigens is particularly pronounced if the species is propagated vegetatively and transported outside its native range for cultivation (Baker, 1972). The end result may be a fully domesticated plant that has evolved in tandem with human selective pressures for a sufficiently long period that it depends on humans for survival (Harlan, 1992).

Both agave species probably fit this scenario of domestication in several ways. Founder clones that were introduced into pre-Columbian fields in central Arizona were likely transported north from more southern progenitor populations (Hodgson and Slauson, 1995), initially reducing genetic diversity relative to wild source populations. In the absence of human management in modern times, populations are left to reproduce

primarily asexually, and many local extinctions of populations have already occurred (Hodgson, unpublished manuscript). Only limited sexual reproduction in *A. murpheyi* and the absence of sexual reproduction in *A. delamateri*, their existence in an environment that is different from conditions where their progenitor occurred, and the historic loss of many populations from central Arizona have probably further eroded genetic variation in both species, countering pre-Columbian management practices that may have promoted genetic diversity under active cultivation. Despite low genetic variation, both species are characterized by a heterozygote excess, and several populations are fixed for heterozygosity at certain loci. High levels of heterozygosity have also been reported for different landraces of cassava grown in traditional agricultural systems (Pujol et al., 2005). Genetic variation maintained by the two agave cultigens is much lower than values reported previously for crops; however, most of the crops reviewed by Hamrick and Godt (1997) are widespread species that are part of modern agricultural systems. *Agave murpheyi* and *A. delamateri* are currently extremely rare species with narrow distributions; such species are often associated with reduced genetic variation relative to more widespread relatives (Hamrick et al., 1979).

Although *A. murpheyi* and *A. delamateri* populations have less genetic variation than populations of wild agave species, they maintain substantially greater variation than two agave

species that have been cultivated commercially in the postcontact era (*A. fourcroydes* Lem. and *A. tequilana* Weber var. Azul). Widespread cultivation of *A. fourcroydes* developed in Yucatan, Mexico, in the 1800s, when seven different varieties and its wild progenitor, *A. angustifolia* Haw., were grown in plantations for fiber export, but by 1980, only three varieties were grown (Colunga-GarcíaMarín and May-Pat, 1993). Colunga-GarcíaMarín et al. (1999) found minor allozyme differences among the three remaining *A. fourcroydes* varieties but virtually no variation within each variety—the result of state restrictions on agave varieties accepted for fiber processing (Colunga-GarcíaMarín and May-Pat, 1993). In contrast, they reported substantial within-population variation in *A. angustifolia*. Using RAPDs, Gil Vega et al. (2001) found similarly low variation in *A. tequilana* var. Azul, which they attributed to years of state-controlled human selection, reinforced by exclusively asexual reproduction.

The few studies that have examined genetic diversity of domesticated plants in traditional farming systems have reported that farmers closely manage their plants and maintain remarkably high genetic diversity (Brush et al., 1995). Farmers typically cultivate several varieties to hedge their bets throughout weather fluctuations (Brush et al., 1995; Pujol et al., 2005) or to use for different purposes (Casas et al., 1997). Colunga-GarcíaMarín and Zizumbo-Villarreal (2006) found over 20 agave cultivars grown by traditional farmers in Jalisco, Mexico, each producing a fermented beverage with a unique taste. Even where farmers grow plants that are part of a local or regional economic system, they often grow certain noncommercial varieties for personal use (Brush et al., 1995). Indeed, door gardens of traditional farms represent a treasure trove of both floristic and genetic diversity (Coomes and Ban, 2004; Zaldivar et al., 2004). Seeds or suckers are often traded among farmers or collected from the wild in traditional systems to maintain variety in their fields (Colunga-GarcíaMarín and Zizumbo-Villarreal, 2006).

Some of the social and environmental factors that foster relatively high genetic diversity in modern traditional crops likely applied to prehistoric agave farmers in central Arizona. The existence of at least modest genetic variation within and among the agave populations studied offers little support to earlier speculation that modern *A. murpheyi* and *A. delamateri* populations were descended from a single clone for each species (Nabhan, 1992). Levels of genetic variation that characterize modern populations are greater than would be expected from somatic mutation alone and instead fit a scenario of polyphyletic population origins, with populations subsequently managed by prehistoric farmers in a way that probably maintained or promoted genetic diversity. Plants were likely traded at a local scale, as has been reported for landraces of agave cultivated for distillation in south Jalisco (Colunga-GarcíaMarín and Zizumbo-Villarreal, 2006). Additional diversity may have been introduced into fields as immigration occurred, and plants may have been traded at a regional scale. Even limited sexual reproduction in *A. murpheyi* over the centuries since importation may have helped maintain genetic diversity in this species. Individual agave species have been used for a wide array of purposes both prehistorically and in the modern era (Colunga-GarcíaMarín and May-Pat, 1993; Hodgson 2001a); therefore, early farmers may have selected for a variety of traits among the *A. murpheyi* and *A. delamateri* they cultivated. Colunga-GarcíaMarín and Zizumbo-Villarreal (2006) and Colunga-GarcíaMarín et al. (1993) presented

models for prehistoric agave cultivation in Mexico that included initial selection for superior traits of first, floral peduncles, then hearts, for food; subsequently, when annual crops developed, selection shifted to traits favored for fiber or fermented beverages, depending on species and region. Based on the roasting pits, agave knives, and other food-related archaeological artifacts found with *A. murpheyi* and *A. delamateri* populations in Arizona, there is no doubt that agave was used for food, particularly at a time of the year when annual crops were not productive (Fish et al., 1985; Gasser and Kwiatkowski, 1991). Historic uses of *A. murpheyi* by traditional farmers in Sonora included both food and alcoholic beverages (Nabhan, 1992). Both species also contain fibrous leaves, and some plants grown by prehistoric agriculturalists may have been used for fiber extraction and textile development (Fish et al., 1985).

Interspecific contrasts in genetic variation—By most measures, *A. murpheyi* was characterized by more genetic diversity than *A. delamateri*. A similar interspecific contrast was evident in terms of multilocus genotype diversity. The production of bulbils and limited seed in *A. murpheyi* may partially account for these contrasts. Although leaves of the parent plant wither after the inflorescence emerges, roots continue to take up water from the soil, which permits root development and leaf elongation in bulbils attached to the inflorescence (Szarek and Holmesley, 1996). Even when bulbils are detached and have no access to water, as many as 25% survive temperatures of 24°C for close to 3 yr (Szarek et al., 1996). Low bulbil survival has been reported for wild agave species, but the Hohokam practice of piling rocks around the base of plants likely promoted bulbil establishment. Bulbils have greater survival rates during unusually wet years and in protected microsites beneath nurse plants (Szarek and Holmesley, 1996; Arizaga and Ezcurra, 2002); rock piles promoted infiltration of rainwater and reduced evaporation (Fish et al., 1985), which would have facilitated bulbil establishment. Seeds and bulbils represented convenient packets of plant material that were likely transported long distances from a Mesoamerican source along pre-Columbian trade or migration routes, as Gentry (1982) suggested occurred with *A. appplanata* Koch ex Jacobi. Even occasional sexual reproduction in *A. murpheyi* populations during prehistoric cultivation and since abandonment would have helped maintain genetic diversity through recombination and possible gene flow. Introduction of *A. murpheyi* could have involved a pool of bulbils and seeds from several sources, with genetic variation inherent in cultivated populations from the outset. In contrast, movement of *A. delamateri* stock from its source region would have required transport of basal offsets, a more cumbersome means of anthropogenic plant dispersal. Individual events of human migration or agave trade, either initially or subsequent to field establishment, would likely have involved a lower diversity of *A. delamateri* stock, hence leading to less genetically diverse populations.

Although these populations provide some clues about pre-Columbian cultivation practices, the story they give is incomplete. Postabandonment events may have also shaped patterns of genetic diversity observed in the modern landscape. In addition to occasional recombination (and in *A. murpheyi*, sexual mating), genetic drift or nonanthropogenic selection may have caused a differential loss of genetic diversity for the two species. Most metrics of genetic diversity are greater for *A. murpheyi* at both the population and species levels; therefore, if

differential survival between *A. murpheyi* and *A. delamateri* has occurred, it may have affected patterns of genetic diversity at both the population and species levels.

Multilocus genotype diversity—Both *A. murpheyi* and *A. delamateri* had multilocus genotype diversities similar to values reported in reviews of primarily wild clonal plant species (Ellstrand and Roose, 1987), although comparisons among studies must be made cautiously, because sampling protocols, levels of polymorphism, and the type of genetic marker all influence the amount of genotypic diversity detected (Duhovnikoff and Dodd, 2003). The mean number of genotypes in *A. murpheyi* and *A. delamateri* populations ($G = 14$ and 10) were slightly lower than the mean value of 16.1 reported by Ellstrand and Roose (1987) in a review of plants based primarily on isozymes. The decay of diversity through genetic drift in these two agave and other long-lived clonal species (e.g., *Lophocereus schottii* (Engelm.) Britton & Rose [Parker and Hamrick, 1992]) has likely been slowed by their prolific vegetative propagation and longevity (Gabrielsen and Brochmann, 1998). In both *A. murpheyi* and *A. delamateri*, the number of genotypes detected in populations varied widely, as reported for other clonal species (Widén et al., 1994); but even the smallest agave populations included more than one genotype. Because allozymes are typically less variable than most other markers used to identify clones (e.g., RAPDs, AFLPs; Esselman et al., 1999), they provide a conservative estimate of the clonal diversity within populations (Duhovnikoff and Dodd, 2003).

Although most multilocus genotypes in both species were restricted to one population, a small number of genotypes were widespread across populations, collectively representing a majority of individuals of each species. The more common genotypes may have been present in individual fields since the original introduction of agave into the study region; alternatively, subsequent prehistoric immigration and trade of bulbils and offsets may have reduced initial genotypic differentiation among populations.

Although ramets of both *A. murpheyi* and *A. delamateri* had a strongly aggregated spatial arrangement in the field, genotypes failed to correspond closely to spatial clusters of plants. Intermingling of clones characterizes other long-lived plant species, including the succulents *Lophocereus schottii* (Parker and Hamrick, 1992) and *Stenocereus eruca* (Brandege) Gibson & Horak (Clark-Tapia et al., 2005), with genets often >20 m apart. In both *A. murpheyi* and *A. delamateri*, most genotypes were restricted to one or two clusters, but clusters included about four genotypes, on average, for each species. This type of clonal structure has been reported for the ericaceous shrub *Vaccinium myrtillus* L., which Albert et al. (2003) described as having a phalanx form (Lovett-Doust, 1981), with dense masses of stems comprising several genotypes in juxtaposition. Two factors, however, cause the spatial pattern to be more complex in *A. murpheyi* and *A. delamateri*. First, the wide spacing of some clones may reflect a mixing of genotypes when fields were originally planted by pre-Columbian farmers. Subsequent manipulation of plants as fields were tended, including the introduction of new plants, could have further mixed genotypes. Second, the production of bulbils in *A. murpheyi* presents an opportunity for the relatively wide dispersal of offspring. Szarek and Holmesley (1996) found that bulbils can be dispersed >3 m from the parent plant when the inflorescence eventually falls, resulting in more

distant dispersal than could be accomplished by several generations of basal offsets. Gabrielsen and Brochmann (1998) attributed intermingling of *Saxifraga cernua* L. clones in arctic environments to bulbil dispersal by both wind and animals. Occasional reproduction from seed could have a similar effect. Not surprisingly, clusters of *A. murpheyi* tended to include more genotypes than clusters of *A. delamateri*.

Regional genetic variation—The genetic variation evident in *A. murpheyi* and *A. delamateri* failed to conform to a regional pattern. Regional G_{ST} values were low for both species, indicating that each region maintains nearly all the genetic variation observed within each species (99% and 95–96%, respectively). Furthermore, clear patterns of multilocus genotypic variation and isolation by distance were not evident in either species. Populations that were in different regions were often more similar to each other than populations in the same region; clusters in the UPGMA classification often included populations from more than one region, while populations from the same region were in different clusters.

Although differential patterns of survival for the two species cannot be ruled out, the absence of isolation by distance is not surprising in view of the rich human history associated with both cultigens. Humans were the primary force to move genes and shape patterns of genetic variation across the landscape. The agave populations studied occur in areas with a long and complex pre-Columbian history (Clark, 2001). Architecture, ceramics, and other archaeological evidence indicate that the eastern Tonto Basin was settled by Hohokam immigrants from the Phoenix Basin ca. A.D. 750–950 (Clark, 2001). In addition to these types of artifacts, agave bulbils and offsets may have been regionally mobile as well. Tonto Basin, the Verde Valley, and Phoenix Basin all experienced significant immigration from the puebloan region to the east and north during the Classic period (ca. early 1200s; Ciolek-Torrello, 1997; Clark, 2001), with original inhabitants and immigrants coexisting within communities in many cases. Therefore, by late in the period of prehistoric agave cultivation, these regions each included a diversity of cultures, rather than each being homogeneous and clearly distinct from one another. Immigrant farmers may have contributed to a subregional-scale mosaic of genetic variation, if preferences for agave traits differed from those of original inhabitants, thereby weakening interregional contrasts in genetic variation.

In conclusion, *A. murpheyi* and *A. delamateri* varied in allele frequencies and multilocus genotypes both within and among populations. The formation of bulbils and limited viable seed likely contributed to the maintenance of greater genetic diversity evident in *A. murpheyi* at both the species and population levels. Bulbils and seeds from different source populations would have been easily transported from Mexico, resulting in a more diverse mixture of plants initially cultivated in central Arizona relative to *A. delamateri*. Both species maintain less genetic variation than most widely distributed crops that are part of modern commercial agricultural systems. Both species also show less genetic diversity than wild agave species. Instead, the patterns of genetic variation that characterize *A. murpheyi* and *A. delamateri* are more in keeping with traditional farming systems, where farmers grow a collection of landraces, thus maintaining diverse genotypes within their fields. Modern populations of both cultigens reflect dynamic human populations during the period of agave cultivation in the study area; prehistoric trade and immigration

have contributed to a finer-scale mosaic of genetic variation rather than a pattern of clear regional differentiation—a pattern still evident many centuries later.

LITERATURE CITED

- ALBERT, T., O. RASPÉ, AND A.-L. JACQUEMART. 2003. Clonal structure in *Vaccinium myrtillus* L. revealed by RAPD and AFLP markers. *International Journal of Plant Sciences* 164: 649–655.
- ARIZAGA, S., AND E. EZCURRA. 2002. Propagation mechanisms in *Agave macroacantha* (Agavaceae), a tropical arid-land succulent rosette. *American Journal of Botany* 89: 632–641.
- BAKER, H. G. 1972. Human influences on plant evolution. *Economic Botany* 26: 32–43.
- BOHRER, V. L. 1991. Recently recognized cultivated and encouraged plants among the Hohokam. *Kiva* 56: 227–235.
- BRUSH, S., R. KESSELL, R. ORTEGA, P. CISNEROS, K. ZIMMERER, AND C. QUIROS. 1995. Potato diversity in the Andean center of crop domestication. *Conservation Biology* 9: 1189–1198.
- CALLEN, E. O. 1965. Food habits of some pre-Columbian Mexican Indians. *Economic Botany* 19: 335–343.
- CASAS, A., B. PICKERSGILL, J. CABALLERO, AND A. VALIENTE-BANUET. 1997. Ethnobotany and domestication in xoconochtili, *Stenocereus stellatus* (Cactaceae), in the Tehuacán Valley and La Mixteca Baja, México. *Economic Botany* 51: 279–292.
- CASTETTER, E. F., W. H. BELL, AND A. R. GROVE. 1938. The early distribution and utilization of agave in the American Southwest, University of New Mexico Bulletin No. 335. University of New Mexico, Albuquerque, New Mexico, USA.
- CHELIAK, W. M., AND J. A. PITEL. 1984a. Electrophoretic identification of clones in trembling aspen. *Canadian Journal of Forest Research* 14: 740–743.
- CHELIAK, W. M., AND J. A. PITEL. 1984b. Techniques for starch gel electrophoresis of enzymes from forest tree species. Information Report PI-X-42. Petawawa National Forestry Institute, Agriculture Canada, Canadian Forestry Service, Chalk River, Ontario.
- CIOLEK-TORRELLO, R. S. 1997. Prehistoric settlement and demography in the lower Verde River region. In S. M. Whittlesey, R. Ciolek-Torrello, and J. H. Altschul [eds.], *Vanishing river: landscapes and lives of the lower Verde River valley, 531–595*. SRI Press, Tucson, Arizona, USA.
- CLARK, J. J. 2001. Tracking prehistoric migrations: Pueblo settlers among the Tonto Basin Hohokam. *Anthropological Papers of the University of Arizona* No. 65. University of Arizona Press, Tucson, Arizona, USA.
- CLARK, J. J., AND J. M. VINT. 2004. Pre-Classic and early Classic settlement and subsistence. In J. J. Clark and J. M. Vint [eds.], *2000 years of settlement in the Tonto Basin: overview and synthesis of the Tonto Creek Archaeological Project, 257–304*. Center for Desert Archaeology Anthropological Papers No. 25, Tucson, Arizona, USA.
- CLARK-TAPIA, R., C. ALFONSO-CORRADO, L. E. EGUIARTE, AND F. MOLINA-FREANER. 2005. Clonal diversity and distribution in *Stenocereus eruca* (Cactaceae), a narrow endemic cactus of the Sonoran Desert. *American Journal of Botany* 92: 272–278.
- COLUNGA-GARCÍA-MARÍN, P., J. COELLO-COELLO, L. E. EGUIARTE, AND D. PIÑERO. 1999. Isozymatic variation and phylogenetic relationships between henequén (*Agave fourcroydes*) and its wild ancestor *A. angustifolia* (Agavaceae). *American Journal of Botany* 86: 115–123.
- COLUNGA-GARCÍA-MARÍN, P., J. COELLO-COELLO, L. ESPEJO-PENICHE, AND L. FUENTE-MORENO. 1993. Agave studies in Yucatan, Mexico. II. Nutritional value of the inflorescence peduncle and incipient domestication. *Economic Botany* 47: 328–334.
- COLUNGA-GARCÍA-MARÍN, P., AND F. MAY-PAT. 1993. Agave studies in Yucatan, Mexico. I. Past and present germplasm diversity and uses. *Economic Botany* 47: 312–327.
- COLUNGA-GARCÍA-MARÍN, P., AND D. ZIZUMBO-VILLARREAL. 2006. Tequila and other agave spirits from west-central Mexico: current germplasm diversity, conservation and origin. *Biodiversity and Conservation*. DOI: 10.1007/s10531-006-9031-z. Issue: Online First. Pages: 1–15. 09 July.
- COOMES, O. T., AND N. BAN. 2004. Cultivated plant species diversity in home gardens of an Amazonian peasant village in northeastern Peru. *Economic Botany* 58: 420–434.
- CORDELL, L. S., D. E. DOYEL, AND K. W. KINTIGH. 1994. Processes of aggregation in the prehistoric Southwest. In G. J. Gumerman [ed.], *Themes in Southwest prehistory, 109–133*. School of American Research Press, Sante Fe, New Mexico, USA.
- DOEBLEY, J. 1989. Isozymic evidence and the evolution of crop plants. In D. E. Soltis and P. S. Soltis [eds.], *Isozymes in plant biology, 165–191*. Dioscorides Press, Portland, Oregon, USA.
- DOEBLEY, J. 1990. Molecular evidence and the evolution of maize. *Economic Botany* 44S: 6–27.
- DOEBLEY, J. 1992. Molecular systematics and crop evolution. In P. S. Soltis, D. E. Soltis, and J. J. Doyle [eds.], *Molecular systematics of plants, 202–222*. Chapman and Hall, New York, New York, USA.
- DOELLE, W. H., AND H. D. WALLACE. 1991. The changing role of the Tucson Basin in the Hohokam regional system. In G. J. Gumerman [ed.], *Exploring the Hohokam: prehistoric desert peoples of the American Southwest, 279–345*. University of New Mexico Press, Albuquerque, New Mexico, USA.
- DOUHOVNIKOFF, V., AND R. S. DODD. 2003. Intra-clonal variation and a similarity threshold for identification of clones: application to *Salix exigua* using AFLP molecular markers. *Theoretical and Applied Genetics* 106: 1307–1315.
- ELLSTRAND, N. C., AND M. L. ROOSE. 1987. Patterns of genotypic diversity in clonal plant species. *American Journal of Botany* 74: 123–131.
- ESRI. 1999–2004. ArcMap version 9.0. Environmental Systems Research Institute, Redlands, California.
- ESSELMAN, E. J., L. JIANQIANG, D. J. CRAWFORD, J. L. WINDUS, AND A. D. WOLFE. 1999. Clonal diversity in the rare *Calamagrostis porteri* ssp. *insperata* (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and intersimple sequence repeat (ISSR) markers. *Molecular Ecology* 8: 443–451.
- FAGER, E. W. 1972. Diversity: a sampling study. *American Naturalist* 106: 293–310.
- FISH, S. K. 2000. Hohokam impacts on Sonoran Desert environment. In D. L. Lentz [ed.], *Imperfect balance: landscape transformations in the precolumbian Americas, 251–280*. Columbia University Press, New York, New York, USA.
- FISH, S. K., P. R. FISH, AND J. H. MADSEN. 1992. Evidence for large-scale agave cultivation in the Marana Community. In S. K. Fish, P. R. Fish, and J. H. Madsen [eds.], *The Marana community in the Hohokam world, 73–117*. University of Arizona Press, Tucson, Arizona, USA.
- FISH, S. K., P. R. FISH, C. MIKSICEK, AND J. MADSEN. 1985. Prehistoric agave cultivation in southern Arizona. *Desert Plants* 7:100, 107–112.
- GABRIELSEN, T. M., AND C. BROCHMANN. 1998. Sex after all: high levels of diversity detected in the arctic clonal plant *Saxifraga cernua* using RAPD markers. *Molecular Ecology* 7: 1701–1708.
- GASSER, R. E., AND S. M. KWIAKOWSKI. 1991. Regional signatures of Hohokam plant use. *Kiva* 56: 207–226.
- GENTRY, H. S. 1982. *Agaves of continental North America*. University of Arizona Press, Tucson, Arizona, USA.
- GIL VEGA, K., M. GONZÁLEZ CHAVIRA, O. MARTÍNEZ DE LA VEGA, J. SIMPSON, AND G. VANDEMARK. 2001. Analysis of genetic diversity in *Agave tequilana* var. *Azul* using RAPD markers. *Euphytica* 119: 335–341.
- GUMERMAN, G., J. HANSON, D. BREW, K. TOMOFF, AND C. WEED. 1975. The hydrology of prehistoric farming systems in a central Arizona ecotone. Lyndon B. Johnson Space Center, Houston, Texas, USA.
- HAMRICK, J. L., AND M. J. W. GODT. 1997. Allozyme diversity in cultivated crops. *Crop Science* 37: 26–30.
- HAMRICK, J. L., Y. B. LINHART, AND J. B. MITTON. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Annual Review of Ecology and Systematics* 10: 173–200.
- HARLAN, J. R. 1992. *Crops and man*. American Society of Agronomy and Crop Science Society of America, Madison, Wisconsin, USA.

- HAVEY, M. J., AND F. J. MUEHLBAUER. 1989. Variability for restriction fragment lengths and phylogenies in lentil. *Theoretical and Applied Genetics* 77: 839–843.
- HILLIS, D. M. 1984. Misuse and modification of Nei's genetic distance. *Systematic Zoology* 33: 238–240.
- HODGSON, W. C. 2001a. Food plants of the Sonoran Desert. University of Arizona Press, Tucson, Arizona, USA.
- HODGSON, W. C. 2001b. Taxonomic novelties in American Agave (Agavaceae). *Novon* 11: 410–416.
- HODGSON, W. C., AND L. SLAUSON. 1995. *Agave delamateri* (Agavaceae) and its role in the subsistence patterns of pre-Columbian cultures in Arizona. *Haseltonia* 3: 130–140.
- HOLMES, R. T., AND H. F. RECHER. 1986. Determinants of guild structure in forest bird communities: an intercontinental comparison. *Condor* 88: 427–439.
- HOSAKA, K., AND R. E. HANNEMAN JR. 1988. The origin of the cultivated tetraploid potato based on chloroplast DNA. *Theoretical and Applied Genetics* 76: 172–176.
- LI, C. C., AND D. G. HORVITZ. 1953. Some methods of estimating the inbreeding coefficient. *American Journal of Human Genetics* 5: 107–117.
- LOVETT DOUST, L. 1981. Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*). I. The dynamics of ramets in contrasting habitats. *Journal of Ecology* 69: 743–755.
- MANLY, B. F. J. 1991. Randomization and Monte Carlo methods in biology. Chapman and Hall, London, UK.
- MARTÍNEZ-PALACIOS, A., L. E. EGUIARTE, AND G. R. FURNIER. 1999. Genetic diversity of the endangered endemic *Agave victoriae-reginae* (Agavaceae) in the Chihuahuan Desert. *American Journal of Botany* 86: 1093–1098.
- MIKSICEK, C. 1984. Historic desertification, prehistoric vegetation change, and Hohokam subsistence in the Salt-Gila Basin. In L. S. Teague and P. L. Crown [eds.], Hohokam archaeology along the Salt-Gila Aqueduct, Central Arizona Project, 53–80. Arizona State Museum Archaeological Series 150, Tucson, Arizona, USA.
- MINNIS, P. E., AND S. E. PLOG. 1976. A study of the site specific distribution of *Agave parryi* in east central Arizona. *Kiva* 41: 299–308.
- MITTON, J. B., Y. B. LINHART, K. B. STURGEON, AND J. L. HAMRICK. 1979. Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. *Journal of Heredity* 70: 86–89.
- NABHAN, G. 1985. Native crop diversity in Aridoamerica: conservation of regional gene pools. *Economic Botany* 39: 387–399.
- NABHAN, G. P. 1992. Threatened Native American plants. *Endangered Species Update* 9: 1–4, 9.
- NEALE, D. B., M. A. SAGHAI-MAROOF, R. W. ALLARD, Q. ZHANG, AND R. A. JORGENSEN. 1988. Chloroplast DNA diversity in populations of wild and cultivated barley. *Genetics* 120: 1105–1110.
- NEI, M. 1972. Genetic distance between populations. *American Naturalist* 106: 283–292.
- NEI, M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA* 70: 3321–3323.
- NEI, M. 1977. *F*-statistics and analysis of gene diversity in subdivided populations. *Annals of Human Genetics* 41: 225–233.
- PARKER, K. C., AND J. L. HAMRICK. 1992. Genetic diversity and clonal structure in a columnar cactus, *Lophocereus schottii*. *American Journal of Botany* 79: 86–96.
- PARKER, K. C., AND J. L. HAMRICK. 1996. Genetic variation in sand pine (*Pinus clausa*). *Canadian Journal of Forest Research* 26: 244–254.
- PIELOU, E. C. 1969. An introduction to mathematical ecology. Wiley-Interscience, New York, New York, USA.
- PINKAVA, D. J., AND M. A. BAKER. 1985. Chromosome and hybridization studies of agaves. *Desert Plants* 7: 93–100.
- PUJOL, B., P. DAVID, AND D. MCKEY. 2005. Microevolution in agricultural environments: how a traditional Amerindian farming practice favours heterozygosity in cassava (*Manihot esculenta*) Crantz, Euphorbiaceae). *Ecology Letters* 8: 138–147.
- RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248–249.
- RIESEBERG, L. H., AND G. J. SEILER. 1990. Molecular evidence and the origin and development of the domesticated sunflower (*Helianthus annuus*, Asteraceae). *Economic Botany* 44S: 79–91.
- ROGERS, G. K. 2000. A taxonomic revision of the genus *Agave* (Agavaceae) in the Lesser Antilles, with an ethnobotanical hypothesis. *Brittonia* 52: 218–233.
- ROHLF, F. J. 2005. NTSYSpc: numerical taxonomy and multivariate analysis system, version 2.20b. Applied Biostatistics, Port Jefferson, New York, USA.
- ROUSSET, F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* 145: 1219–1228.
- SALGADO, A. G., P. GEPTS, AND D. G. DEBOUCK. 1995. Evidence for two gene pools of the lima-bean, *Phaseolus lunatus* L., in the Americas. *Genetic Resources and Crop Evolution* 42: 15–28.
- SAUER, C. 1972. Seeds, spades, hearths, and herds. The domestication of animals and foodstuffs. MIT Press, Cambridge, Massachusetts, USA.
- SHERMAN-BROYLES, S. L., J. P. GIBSON, J. L. HAMRICK, M. A. BUCHER, AND M. J. GIBSON. 1992. Comparisons of allozyme diversity among rare and widespread *Rhus* species. *Systematic Botany* 17: 551–559.
- SILVA-MONTELLANO, A., AND L. E. EGUIARTE. 2003. Geographic patterns in the reproductive ecology of *Agave lechuguilla* (Agavaceae) in the Chihuahuan Desert. II. Genetic variation, differentiation, and inbreeding estimates. *American Journal of Botany* 90: 700–706.
- SLATKIN, M. 1985. Rare alleles as indicators of gene flow. *Evolution* 39: 53–65.
- SOLTIS, D. E., C. H. HAUFLE, D. C. DARROW, AND G. J. GASTONY. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *American Fern Journal* 73: 9–27.
- SZAREK, S. R., B. DRISCOLL, C. SHOHET, AND S. PRIEBE. 1996. Bulbil production in *Agave* (Agavaceae) and related genera. *Southwestern Naturalist* 41: 465–469.
- SZAREK, S. R., AND G. E. HOLMESLEY. 1996. Physiological activity in persistent bulbils of *Agave vilmoriniana* (Agavaceae). *American Journal of Botany* 83: 903–909.
- TANG, S., AND S. J. KNAPP. 2003. Microsatellites uncover extraordinary diversity in native American land races and wild populations of cultivated sunflower. *Theoretical and Applied Genetics* 106: 990–1003.
- WEIR, B. S., AND C. C. COCKERHAM. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* 38: 1358–1370.
- WIDÉN, B., N. CRONBERG, AND M. WIDÉN. 1994. Genotypic diversity, molecular markers and spatial distribution of genets in clonal plants, a literature survey. *Folia Geobotanica and Phytotaxonomica* 29: 245–263.
- WORKMAN, P. L., AND J. D. NISWANDER. 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. *American Journal of Human Genetics* 22: 24–49.
- WRIGHT, S. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution* 19: 395–420.
- ZALDIVAR, M. E., O. J. ROCHA, G. AGUILAR, L. CASTRO, E. CASTRO, AND R. BARRANTES. 2004. Genetic variation of cassava (*Manihot esculenta* Crantz) cultivated by Chibchan Amerindians of Costa Rica. *Economic Botany* 58: 204–213.