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Diversity and structure of landraces of *Agave* grown for spirits under traditional agriculture: A comparison with wild populations of *A. angustifolia* (Agavaceae) and commercial plantations of *A. tequilana*[1]

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Traditional farming communities frequently maintain high levels of agrobiodiversity, so understanding their agricultural practices is a priority for biodiversity conservation. The cultural origin of agave spirits (mezcals) from west-central Mexico is in the southern part of the state of Jalisco where traditional farmers cultivate more than 20 landraces of *Agave angustifolia* Haw. in agroecosystems that include in situ management of wild populations. These systems, rooted in a 9000-year-old tradition of using agaves as food in Mesoamerica, are endangered by the expansion of commercial monoculture plantations of the blue agave variety (*A. tequilana* Weber var. Azul), the only agave certified for sale as tequila, the best-known mezcal. Using intersimple sequence repeats and Bayesian estimators of diversity and structure, we found that *A. angustifolia* traditional landraces had a genetic diversity ($H_{BT} = 0.442$) similar to its wild populations ($H_{BT} = 0.428$) and a higher genetic structure ($\theta^B = 0.405$; $\theta^B = 0.212$). In contrast, the genetic diversity in the blue agave commercial system ($H_B = 0.118$) was 73% lower. Changes to agave spirits certification laws to allow the conservation of current genetic, ecological and cultural diversity can play a key role in the preservation of the traditional agroecosystems.

Key words: *Agave angustifolia*; agave spirits; Bayesian analysis; genetic diversity; genetic structure; germplasm conservation; in situ management; mezcal; tequila; traditional landraces.

Human population pressure and the demands of economic development are related to the general and accelerated loss in the world's biodiversity (Wilson, 1988). However, Mesoamerican farming communities that use traditional management techniques frequently maintain high levels of diversity at different biological levels, so understanding their agricultural practices has been a priority in the design of biodiversity conservation strategies (Zizumbo-Villarreal and Colunga-GarcíaMarín, 1993; Brush and Stabinsky, 1996; Toledo, 2001; Jarvis et al., 2008). The current study is part of continuing efforts to address this priority.

Traditional agriculture in Mexico has been developed under ecological, economic, and cultural conditions that have favored the conservation of its native ancient crops. This traditional agricultural rationale is characterized by the maintenance of diversity at different levels and has favored the development of new crops, new landraces, and the continuous improvement of existing germplasm (Hernández-Xolocotzi, 1973, 1993). This maintenance of diversity under the traditional systems has been documented for several perennial species: columnar cactus, *Opuntia* spp., and *Agave* spp. (Colunga-GarcíaMarín et al., 1986; Casas et al., 1997, 2007; Vargas-Ponce et al., 2007).

Mexico is the center of origin and diversity of the genus *Agave* L. (Agavaceae), in both the biological and agricultural sense (Álvarez-de Zayas, 1989; Gentry, 1982). With its multiple uses, especially as a food, fiber, and alcoholic beverage, agave has held and still holds great economic and cultural importance for the society (Gentry, 1982). For at least 9000 years, Mesoamericans have roasted and consumed agave stems and leaf bases due to their high sugar content (Callen, 1965). The most common names for the species traditionally used as food are based on the word mezcal (from the Náhuatl *metl* = agave and *ixcalli* = cooked or baked). The same word is used for the agave spirits that have been produced in Mexico since the 16th century, based on the pre-Hispanic tradition of creating fermented beverages made with agave juice (Bruman, 1940).

Agaves are semelparous rosettes. Most species cross-pollinate and frequently hybridize, and many can propagate vegetatively through bulbils and shoot roots (Gentry, 1982). When the inflorescence peduncles start to develop, farmers cut the leaves and harvest the stem with the leaf bases attached. The stem and leaf bases, jointly called heads, have abundant storage carbohydrates that provide the energy for inflorescence growth. These heads are traditionally pit-baked to transform the carbohydrates into palatable sugars.

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According to Bruman (1940, 1944), the cultural origin of agave spirits from west-central Mexico is in the foothills of the Colima volcanoes (Fig. 1), where the native people began distilling agave at the end of the 16th century. This practice adapted the use of an Asian-type still introduced by the Filipino people, who had been brought by the Spanish colonialists to the Colima coast. The Asian still was spread inland, facilitated by pre-Hispanic commercial trade routes along the Ayuquila-Tuxcacuesco-Armería (ATAR) and Tuxpan-Naranjo-Coahuayana (TNCR) rivers. These routes were cultural and biological corridors along which the associated *Agave* germplasm was dispersed (Zizumbo-Villarreal and Colunga-GarcíaMarín, 2008). From this area (the southern part of the actual Mexican state of Jalisco), the technology moved northward to the Tequila-Amatitán valley in central Jalisco (Fig. 1), where one of the drinks was named after the town of Tequila (Walton, 1977). Agave spirits production in central and southern Jalisco remains an economically and culturally important activity.

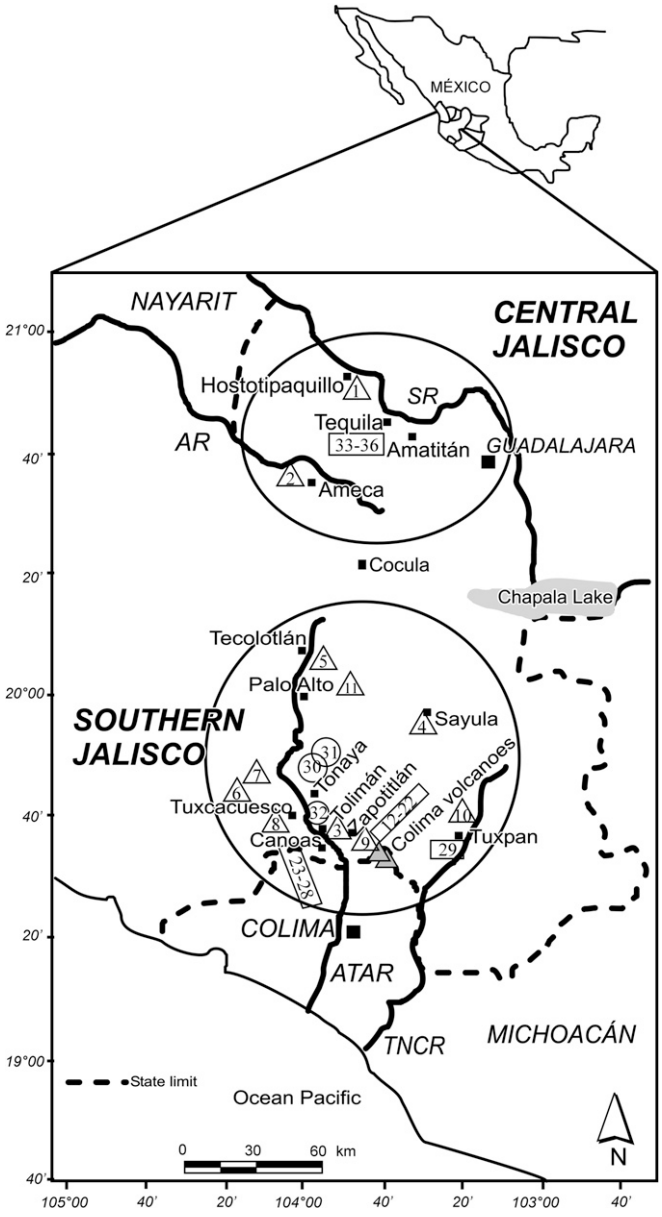

Fig. 1. Map of study area in west-central Mexico. Populations of *Agave* studied in central and southern Jalisco, capital cities of the municipalities included (square), wild population (triangle), traditional and commercial cultivated populations (circles and rectangles), Santiago River (SR), Tuxpan-Naranjo-Coahuayana River (TNCR), Ayuquila-Tuxcacuesco-Armería River (ATAR). Numerical code of populations are as in Table 1; 10 = SVA (Tuxpan), 16 = IA (Ixtero Amarillo), 13 = VR (Verde rápido), 33 = BE (Bermejo), 34 = CHA (Chato), 35 = S (Sigüin).

Ethnobotanical studies of the production systems for agave spirits in the foothills of the Colima volcanoes and the environs of the ATAR and TNCR rivers (Colunga-GarcíaMarín and Zizumbo-Villarreal, 2007) and an analysis of the morphological variation in local *Agave* germplasm (Vargas-Ponce et al., 2007) indicated that this region (southern Jalisco) is not only the origin of agave spirits in west-central Mexico, but also the area with the greatest landrace richness. These studies showed that traditional farmers from southern Jalisco cultivated different combinations of at least 24 *Agave* landraces in two traditional complex agroecosystems called mezcalera and milpa. The mezcalera system consists of agave plantations that include cattle grazing, whereas the milpa system consists of the cultivation of maize and other traditional food crops during the rainy season and cattle grazing during the dry season. These agroecosystems are part of a traditional management system that also includes the in situ management of wild plant populations by the tolerance and encouragement of desired plants. The landrace richness of these systems is the result of the continuous introduction of wild germplasm and landraces from other local farmers into cultivation, along with the continuous selection of old local landraces and new gene pools, under criteria that favors diversity. The managed gene pools are selectively propagated by farmers through vegetative propagules and by seedlings that are cultivated ex situ or tolerated in situ, from wild or from cultivated plants. The main purpose of plant production in the traditional system is the creation of spirits by the grower or the sale of the heads to local manufacturers. The primary wild genetic pool for selection of germplasm has been *A. angustifolia* Haw., an evolutionary complex sensu Gentry (1982), followed by *A. rhodacantha* Trel., a species that likely hybridizes with it (Gentry, 1982). Morphological data suggests that the cultivated pool is genetically diverse and that landraces recognized by farmers can be morphologically differentiated.

In contrast, agave spirits production in central Jalisco rests on the extensive commercial monoculture of the clone *A. tequilana* Weber var. Azul, known as blue agave. *Agave tequilana*, according to Gentry (1982), belongs to the *A. angustifolia* complex, but Gentry decided to maintain the specific name *tequilana* because of the commercial importance of blue agave. It is propagated exclusively by vegetative means, including in vitro clonal propagation by some tequila companies, with strict selective criteria for homogeneity. Its genetic diversity has been reported to be very low (Gil-Vega et al., 2001, 2006). A small number of other landraces are grown by very scattered growers because the Mexican Official Norm (NOM) of tequila defined in 1949 (DOF, 1949) establishes that this liquor can only be called tequila if made from blue agave. The quick growth of the tequila industry is threatening the diversity of these agave landraces in the foothills of the Colima volcanoes as the cultivation of blue agave expands into southern Jalisco. It also endangers the wild agave populations that are intensely harvested for tequila production when blue agave is scarce, in spite of the NOM regulations. The blue agave variety is also displacing staple food crops and causing soil erosion because it is

commonly planted along the direction of the slope on many hillsides (Colunga-GarcíaMarín and Zizumbo-Villarreal, 2007; Martínez-Rivera et al., 2007).

The purpose of this research was to document the genetic diversity and structure of the landraces of *A. angustifolia* grown for spirits under traditional agriculture in southern Jalisco and to compare these parameters with those of wild populations of *A. angustifolia* and with those of commercial plantations of *A. tequilana* var. Azul (a species belonging to the same evolutionary complex). We then discuss the results in terms of the characteristics of the traditional management system. Because the traditional management system for agave in southern Jalisco has its roots in a 9000-year-old tradition, we hypothesized that the traditional system would have a much higher genetic diversity than the commercial system, similar to that of the wild populations. In addition, we predicted that the wild gene pool would have a lower level of genetic structure with respect to the traditional landrace pools because of the prevalence of sexual reproduction and the lack of human selection in the wild gene pool and the preponderance of vegetative propagation and human selection in the landrace pools. We also present and discuss data for two very important and abundant landraces of *A. rhodacantha* and one of its wild-tolerated populations from southern Jalisco. Data for three landraces found in central Jalisco, now discontinued for use in tequila production, are discussed, too. In both cases, we also hypothesized that the traditional landraces would have a higher diversity than the commercial blue agave.

MATERIALS AND METHODS

Collection sites and plant materials—Plant populations were sampled in the ATAR and TNCR basins (southern Jalisco) and the environs of the Tequila-Amatitán valley between the Ameca and Santiago rivers (central Jalisco), based on previous findings (Fig. 1). Plants were collected along the following gradient of human management intensity: (1) wild populations, those that grow without human intervention but may be harvested for spirits production or be a source of propagules to be cultivated; (2) wild-tolerated populations, some wild plants are tolerated while undesirables are culled from the plots to be cultivated; (3) wild-encouraged populations, those tolerated wild plants and their offspring that receive attention from growers (4) traditional landraces, those recognized and named by farmers because of their morphological and agronomical characters; and (5) commercial blue agave plantations of *A. tequilana* Weber var. Azul. Voucher specimens of the taxa studied were deposited in the following Mexican scientific collections: Centro de Investigación Científica de Yucatán Herbarium (CICY), Instituto de Botánica–Universidad de Guadalajara Herbarium (IBUG), Universidad Nacional Autónoma de México–National Herbarium (MEXU), Centro de Investigación Científica de Yucatán living *Agave* plants germplasm collection (CICY-AGC) (Appendix 1). Populations belonging to the *Agave angustifolia* complex were sampled as follows. (1) Wild populations were sampled at 10 sites, 15–37 individuals per site. At each site, plants were sampled from an area of 0.5–0.8 km^2, according to site topography and population size and density. We avoided individuals from the same clonal batch and tried to include all morphological variation in the population. One of these populations consisting of wild-encouraged individuals in a milpa. (2) Landraces from southern Jalisco were sampled at six sites: one milpa and five mezcaleras. A total of 16 landraces with 19 populations (9–26 individuals per population) were collected in the company of the parcel owners, who identified the landraces. In these agroecosystems, farmers arranged plants in rows that commonly included two or more landraces, with several individuals from each landrace planted adjacent to each other. We thus did not collect adjacent plants and sampled each landrace, collecting individuals from the whole parcel. A very common and regionally appreciated landrace, named lineño, was separately collected in the milpa and in three mezcaleras, two of which focused on lineño cultivation. All 10 landraces growing in the milpa (Zapotitlán) were sampled. (3) Commercial blue agave from central Jalisco was sampled in eight different sites to obtain a representative sample of the commercial lots of this variety, reported to have very low levels of genetic variation (Gil-Vega et al., 2001; Cuevas-Figueroa and Flores-Berrios, 2006). The 22 individuals collected were pooled and analyzed as a single population.

One wild-tolerated population (18 individuals) and two traditional landraces (17 or 18 individuals per landrace) of *A. rhodacantha* were collected in southern Jalisco with the procedures described. In the same way, we also collected three old landraces from central Jalisco (identified as *A. angustifolia, A.* cf. *angustifolia,* and *Agave* sp.) that have been discontinued by the tequila NOM for the production of this liquor. These old landraces were collected from commercial plantations and from the Tequila Sauza collection at El Indio ranch (11–19 plants per landrace) (Appendix S1, see Supplemental Data with the online version of this article).

Studied species—Species were identified (Table 1) by reviewing MEXU and IBUG herbaria and following Gentry's (1982) taxonomic keys and diagnoses and the criteria of A. García-Mendoza (National Autonomous University of Mexico, personal communication). *Agave angustifolia, A. tequilana*, and *A. rhodacantha* take at least seven years to reach reproductive maturity, can reproduce sexually, and propagate vegetatively by shoot roots and often by bulbils. Palomino et al. (2007) have reported the ploidy of *A. angustifolia* ($2n = 2x = 60$; $2n = 3x = 90$; $2n = 4x = 120$; and $2n = 6x = 180$), *A. tequilana* ($2n = 2x = 60$ and $2n = 3x = 90$), *A. rhodacantha* ($2n = 2x = 60$), and four of the landraces analyzed in the current study: bermejo (*A.* cf. *angustifolia*, $2n = 3x = 90$), chato (*Agave* sp.) ($2n = 4\times = 120$ and $2n = 5x = 150$), lineño (*A. angustifolia*, $2n = 2x = 60$), and sigüin (*A. angustifolia*, $2n = 2x \times = 60$).

Agave angustifolia has the widest distribution of agaves, from Tamaulipas and Sonora in northern Mexico to Costa Rica in Central America and from sea level to 1500 m a.s.l. It is highly polymorphic throughout its range, and it has been considered an evolutionary and taxonomic complex (Gentry, 1982). In a study in Sonora, *A. angustifolia* was found to be self-incompatible and dependent on nectar-feeding bats for its sexual reproductive success (Molina-Freaner and Eguiarte, 2003). *Agave tequilana* is only known under cultivation. Gentry (1982) pointed out that its morphological differences with *A. angustifolia* are not a distinct contrast and that it is a cultivated variety probably selected by farmers from the *A. angustifolia* wild populations growing between Cocula and Tecolotlán, Jalisco (Fig. 1). In this study, we consider it as part of the *A. angustifolia* complex. *Agave rhodacantha* grows from southern Sonora to southeast Oaxaca. It is also an outbreeding species that may hybridize with *A. angustifolia* (Gentry, 1982).

ISSR amplification—Intersimple sequence repeats (ISSR) are DNA sequences in between microsatellite repeats, abundantly distributed throughout the genome. This amplification technique, targeting multiple dominant diallelic loci, has proven to be consistent and robust to identify cultivars and closely related species (Godwin et al., 1997; Bornet and Branchard, 2001) and to estimate genetic diversity, population structure, and evolutionary processes in plants (Wolfe et al., 1998; Zizumbo-Villarreal et al., 2005). Moreover, it has been developed and used in similar studies of *Agave* (Aguirre, 2004; Rocha, 2006).

Genomic DNA was extracted from the tissue of the youngest leaf of each individual using the CTAB method described by Doyle and Doyle (1987) as modified by Aguirre (2004). A fluorometer (DyNA Quant 200, Hoefer Pharmacia, Biotech, San Francisco, California, USA) was used to quantify DNA. Six ISSR primers reported by Zizumbo-Villarreal et al. (2005) were tested. The two with the best amplification, reproducibility, and polymorphism were selected: $(GACA)_3$ RG and YR$(GACA)_3$. Each 25 μL of amplification reaction consisted of 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2 mM $MgCl_2$, 200 μM of each dNTP, 1 μM of primer, 1 unit of *Taq* polymerase (Promega, Madison, Wisconsin, USA), and 40 ng of template DNA. Amplification was done with the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California, USA) under the following conditions: 4 min at 94°C for one cycle; followed by 2 min at 94°C, 1 min at 44°C, and 2 min at 70°C for 35 cycles; and 30 min at 72°C for the final extension. The amplified products were separated by electrophoresis on 6% nondenaturalizing bisacrylamide gels (30:1) containing 3 M urea (to improve band definition) and continuous 1× TBE buffer (Zietkiewicz and Labuda, 1994). A 100-bp molecular marker standard and a negative control were included in each gel. Gels were run at 450 V constant power for 12 h (SQ3 Sequencer, Hoefer Pharmacia Biotech), and PCR products were visualized by silver nitrate staining (Promega Q4132 kit).

Of the 95 polymorphic bands generated, 69 were chosen for the analysis because of their clear, consistent amplification; 33 were generated with the $(GACA)_3$ RG primer and 36 with the YR $(GACA)_3$ primer. All bands were between 275 and 1500 bp. Bands having similar molecular mass and migration

TABLE 1. Genetic diversity of (A) populations and (B) pools of *Agave angustifolia* Haw. used for spirits production in southern (mezcals) and central (tequila) Jalisco, Mexico.

A) *A. angustifolia* populations						
Num[a]	Pop,[b] pool	Collection site	*N*	P_S	H_B	H_B, CrI 95%
Wild						
1	AH	Hostotipaquillo	37	97.1	0.346	0.308, 0.383
2	AM	Ameca	35	95.7	0.347	0.318, 0.374
3	AP	Perempitz	22	85.5	0.327	0.303, 0.351
4	ASA	Sayula	25	95.7	0.348	0.326, 0.370
5	AT	Tecolotlán-1	22	97.1	0.404	0.386, 0.420
6	AW	Tuxcacuesco-1	15	95.7	0.353	0.329, 0.377
7	ACT	Tuxcacuesco-2	16	97.1	0.375	0.350, 0.397
8	ATX	Tuxcacuesco-3	36	92.8	0.319	0.304, 0.335
9	ADB	Zapotitlán	24	89.9	0.335	0.315, 0.355
Wild-encouraged						
11	APA	Tecolotlán-2	21	72.5	0.272	0.253, 0.291
Southern Jalisco traditional landraces						
12	B	Zapotitlán	20	92.8	0.336	0.316, 0.354
13	C	Zapotitlán	23	87.0	0.324	0.306, 0.340
14	CH	Zapotitlán	26	84.1	0.303	0.281, 0.323
15	CZ	Zapotitlán	24	78.3	0.269	0.246, 0.292
17	IV	Zapotitlán	18	76.3	0.302	0.283, 0.322
18	LZ	Zapotitlán	20	76.8	0.297	0.279, 0.315
19	P	Zapotitlán	9	78.3	0.320	0.296, 0.343
20	PE	Zapotitlán	14	63.8	0.237	0.206, 0.270
21	TE	Zapotitlán	18	81.2	0.251	0.218, 0.287
22	Z	Zapotitlán	18	18.8	0.141	0.115, 0.173
23	CN	Tolimán-1	17	75.4	0.264	0.243,0.286
24	HO	Tolimán-1	20	78.3	0.247	0.227,0.266
25	LSE	Tolimán-1	20	82.6	0.317	0.297,0.337
26	MP	Tolimán-1	20	88.4	0.322	0.301,0.345
27	SO	Tolimán-1	20	75.4	0.280	0.256,0.304
30	TC[1]	Tolimán-2	13	44.9	0.217	0.188,0.248
31	LP	Tolimán-3	19	69.6	0.259	0.235,0.285
32	LT	Tonaya	17	52.2	0.210	0.181,0.242
29	G	Tuxpan	26	91.3	0.335	0.317,0.352
Central Jalisco commercial tequila						
36	TEQ[1]	Tequila-Amatitán-1-	22	24.6	0.118	0.092,0.155

B) A. angustifolia pools	N	P_T	H_{BT}	H_B CrI 95%
Wild	232	100.0	0.428 ± 0.015	0.400, 0.450
Southern Jalisco				
Traditional landraces	362	100.0	0.442 ± 0.003	0.435, 0.447
Traditional milpa (Zapotitlán)	190	100.0	0.437 ± 0.003	0.430, 0.444

[a] Numerical codes as in Fig. 1.

[b] Alphabetical population (pop.) codes used in text and common name of cultivated populations: B = Brocha; C =Cenizo; CH = Chancuella; CZ = Cimarrón Hoja Larga; IV = Ixtero Verde; LZ = Lineño; P = Prieto de Telcruz; PE = Perempis; TE = Telcruz; Z = Prieto Presa Grande; CN = Cimarrón Negro; HO = Hojudo; LSE = Lineño; MP = Mezcal Piña; SO = Soca; TC = Azul Criollo; LP = Lineño; LT = Lineño; G = Garabato; TEQ= Blue agave.

Note: [1]*A. tequilana* Weber var. Azul (included in the *A. angustifolia* complex). *N* = number of sampled individuals; P_S = percentage of polymorphic loci assuming Hardy–Weinberg equilibrium (HWE); H_B = Bayesian expected panmictic heterozygosity not assuming HWE; P_T, H_{BT} = diversity in the entire pool; CrI = credibility interval.

distance across individuals were assumed to be homologous fragments. Whenever there was doubt about the presence of a band, the procedure was repeated for the individuals in question.

Data analysis—Presence or absence of each band was determined visually for each individual. Genetic diversity and its distribution within and among populations were analyzed with Bayesian methods for dominant markers that do not assume previous knowledge of the inbreeding coefficient *f*; in particular, they do not assume that genotypes within populations are in Hardy–Weinberg equilibrium (HWE) (Holsinger, 1999; Holsinger et al., 2002; Holsinger and Wallace, 2004). Because ISSRs are dominant genetic markers, it is not possible to directly determine the allelic frequencies within populations. An approach to estimate them assumes that genotypes are in HWE, so the frequency of the recessive allele is equal to the square root of the recessive homozygote frequency. But HWE may be an unreliable assumption for our study because the wild and cultivated populations that we analyzed propagate sexually and vegetatively, both at unknown frequencies.

The Bayesian estimator H_B of genetic diversity, comparable to Nei's (1973) genetic diversity or expected panmictic heterozygosity (*Hs*), and the Bayesian estimator of population structure θ^B, directly comparable to estimates of the Wright's (1951) F_{ST} based on the θ of Weir and Cockerham (1984), were calculated with the program Hickory version 1.1 (Holsinger and Lewis, 2007) using the *f* free model that chooses values of *f* at random, incorporating all of the uncertainty in the prior of *f* in the parameters obtained. We used the recommended default settings. To allow the convergence of the Markov chain to its stationary distribution and to obtain reliable estimates of the 95% credibility intervals (CrI, equivalent to confidence intervals), the program took samples from the chain only after discarding 50 000 iterations (*n* burn-in). Once the chain converged, each sample chain consisted of 250 000 iterations (*n* sample). Values were only retained every 50 iterations (thin) to ensure that the autocorrelation among samples was close to zero; therefore, the sample size was 5000. Monomorphic loci are not analyzed in Hickory (defined for dominant markers as those in which the dominant allele is present or not in every individual in every population). We report the mean, the standard deviation (SD), and the 95% CrI of the posterior distributions of H_B and θ^B.

We obtained the genetic diversity per population (Table 1A) and the total diversity of each pool of *A. angustifolia* complex (Table 1B). Genetic structure was also evaluated for these pools (Table 2). Data for *A. rhodacantha* and the three old landraces from central Jalisco are mentioned in the text. To test whether significant differences among the populations of each pool existed, we compared the deviance information criterion (DIC) values for the full model and the $\theta^B = 0$ model, as described in Holsinger and Lewis (2007). The correlation among populations ρ was obtained by modeling the distribution of allelic frequencies across loci (Fu et al., 2003; Holsinger and Lewis, 2007).

To allow comparisons with available literature, we calculated other estimators of genetic diversity (Appendix S1, see Supplemental Data with the online version of this article) and structure (Table 2). For genetic diversity, we calculated (1) the information index of Shannon *I*, which also does not assume HWE, using the program Popgene version 1.31 (Yeh et al., 1999); (2) the percentage of polymorphic loci *P* with respect to the overall number of examined loci that resulted polymorphic under the 99% criterion; and (3) Nei's (1973) genetic diversity (expected panmictic heterozygosity) H_s. The last two were calculated using the program TFPGA version 1.3 (Miller, 1997) assuming HWE and estimating the allelic frequencies using the Lynch and Milligan (1994) correction for dominant markers.

To estimate genetic structure, we used the following methods: (1) calculation of variance estimators for θ of Weir and Cockerham (1984), an equivalent of Wright's F_{ST}, with TFPGA version 1.3, assuming HWE, and jackknife and bootstrap procedures with 5000 iterations to generate 95% confidence intervals (CIs). (2) An analysis of molecular variance (AMOVA) with the program Arlequin version 3.1 (Excoffier and Schneider , 2005) to determine the variance among individuals, among populations, and between the wild and cultivated groups for the ISSR phenotypes. We also used AMOVA to estimate Φ_{ST}, an analogue of F_{ST} (Excoffier et al., 1992). Variance components were tested statistically by a nonparametric randomization test using 16 000 permutations. For Φ_{ST}, 95% CIs were obtained with a bootstrap approach using 20 000 iterations. (3) The exact test for population differentiation (Raymond and Rousset, 1995) as implemented in TFPGA version 1.3 (Miller, 1997) that determines whether significant differences in dominant marker frequencies exist among populations by locus and performs a global test over loci with Fisher's combined probability test, using 1000 dememorization steps, 20 batches, and 2000 permutations per batch.

RESULTS

Genetic diversity—The 69 bands analyzed were polymorphic within the whole sample (100% polymorphism). Table 1

shows that the Bayesian genetic diversity estimator H_B has the highest values in the wild populations (0.319–0.404), while the wild-encouraged population has a slightly lower value (H_B= 0.272). More than half of the 19 traditional landraces populations from southern Jalisco (52%) have genetic diversity values with 95% CrIs that intersect with the CrIs of the wild populations (0.303–0.420). A large contrast was found in the genetic diversity of the commercial blue agave tequila from central Jalisco, whose value (H_B= 0.118) is 63–70% lower than those of the wild populations. The wild-tolerated *A. rhodacantha* population (SVA) has a value [H_B= 0.330; CrI (0.312, 0.348)] within the range of the wild *A. angustifolia* populations. The two landraces of *A. rhodacantha* have less genetic diversity [IA, H_B= 0.21, CrI (0.183, 0.260); VR, H_B= 0.270, CrI (0.250, 0.289)] than the wild-tolerated population (SVA) of this species. Two of the three central Jalisco traditional landraces have values [BE, H_B= 0.260, CrI (0.244, 0.295); CHA, H_B= 0.328, CrI (0.307, 0.347); SI, H_B= 0.316, CrI (0.291, 0.341)] that intersect with the range of the *A. angustifolia* wild populations.

Sixteen of the 19 traditional landraces populations of *A. angustifolia* from southern Jalisco (84%) have an H_B value that is double that of the blue agave commercial variety (TEQ). Only one landrace (Z) has a genetic diversity value with a CrI (0.115–0.173) that intersects with the TEQ CrI (0.092–0.155). The other two populations that do not have twice the value are the blue agave local gene pool (TC) and one of the lineño populations cultivated commercially for a local spirits industry (LT). The three central Jalisco traditional landraces are also two times the TEQ value, as is one of the *A. rhodacantha* landraces (VR).

The comparison of the entire sample Bayesian diversity (H_{BT}) for the *A. angustifolia* complex pools (Table 1B, Fig. 2) shows that the pool of landraces traditionally managed in southern Jalisco (H_{BT} = 0.442) and the pool of landraces managed in just one traditional plot (Zapotitlán milpa) (H_{BT} = 0.437) have the same genetic diversity as the wild populations (H_{BT} = 0.428) (95% CrIs intersect), in strong contrast with the gene pool in the commercial blue agave tequila system (TEQ) with a genetic diversity (H_B = 0.118) 73% lower than southern Jalisco traditional landraces. The values obtained for P_T, I_T, and H_T followed the same trends described for H_{BT}. For commercial blue agave (TEQ), these values expressed even lower diversity compared with the southern Jalisco traditional landraces pool: 75% P_T, 80% I_T, and 79% H_T.

***Genetic structure*—**The Bayesian genetic structure analysis of the *A. angustifolia* complex pools (Table 2), showed that the pool of landraces traditionally managed in southern Jalisco and the pool of landraces cultivated in only one traditional plot (Zapotitlán milpa) have the same genetic structure values (θ^B = 0.405); both are almost twice as large as the wild populations pool structure (θ^B = 0.212). The comparison between the DIC values for the full model and the θ^B = 0 model for each pool indicated significant differences among the populations in each pool, because full model values are two to three times lower than the values for the θ^B = 0 model (wild 3048.99 vs. 5986.86; South Jalisco landraces 4977.72 vs. 15192.30; Zapotitlán plot 2619.09 vs. 7774.99). The correlation of allelic frequencies across loci among landrace populations (ρ = 0.212; 95% CrI = 0.1476–0.287) was lower than among wild populations (ρ = 0.302; 95% CrI = 0.206–0.418).

The values of Φ_{ST} and θ are similar to θ^B and are within the 95% CrI (Table 2). The AMOVA results also indicated that in the three pools the highest variation occurs within populations, but that in the cultivated landraces and the traditional plot, the variation among populations is almost as high as the variation within populations and almost double the variation among populations of the wild pool (Table 2). All sources of variation and Φ_{ST} values were significant (P = 0). The exact differentiation test showed significant differences between populations within each pool as a result of differences in the marker frequencies.

The AMOVA of all *A. angustifolia* populations that were classified as either wild or cultivated (Table 2) showed that variation between the two groups is very low (0.92%) but significant (P = 0.037) and indicated that variation within populations (66.50%) and among populations within groups (32.59%) are both significant (P = 0).

DISCUSSION

On the basis of all diversity estimators, our prediction that the genetic diversity of the traditional management system would be much higher than in the commercial tequila system

TABLE 2. Genetic differentiation estimators, analogous to Wright's (1951) *F* statistics, for wild and cultivated *Agave angustifolia* Haw. populations used for spirits production in southern (mezcals) and central (tequila) Jalisco, Mexico.

Agave angustifolia pools	AMOVA, Excoffier et al. (1992)						Bayesian estimator, Holsinger (1999)			Weir and Cockerham (1984)	
Source of variation	df	SS	VC	%V	Φ_{ST}	CI	θ^B	SD	CrI	θ	CI
Wild pool											
Among populations	8	789.830	3.385	21.326	0.213	0.182, 0.247	0.212	0.019	0.175, 0.250	0.182	0.149, 0.215
Within populations	223	2784.791	12.488	78.674							
Southern Jalisco traditional landraces											
Among populations	18	2302.178	6.243	40.256	0.403	0.372, 0.434	0.405	0.015	0.375, 0.433	0.357	0.328, 0.389
Within populations	343	3177.811	9.265	59.744							
Southern Jalisco traditional milpa (Zapotitlán)											
Among populations	10	1315.378	6.515	41.945	0.402	0.363, 0.441	0.405	0.018	0.367, 0.439	0.366	0.331, 0.404
Within populations	197	1776.689	9.018	58.055							
Wild pool and southern Jalisco traditional landraces											
Among groups	1	180.384	0.145	0.915							
Among populations within groups	26	3092.007	5.162	32.581							
Within populations	566	5962.602	10.535	66.498							

Note: df = degrees of freedom; SS sum of squares; VC = variance components; %V = percentage of variation; CI = confidence interval, SD = standard deviation; CrI= credibility interval.

and be similar to the wild populations was proven to be correct using ISSRs markers. The total genetic diversity of the southern Jalisco traditional landrace pool of the *A. angustifolia* complex (H_{BT} = 0.442) was similar to that of wild populations (H_{BT} = 0.428) (slightly higher but in the same 95% CrI) and almost four times higher than that of the commercial blue agave system (H_B = 0.118), which consists of only one variety (Table 1, Fig. 1). Genetic structure of the pool of traditional landraces populations was higher (θ^B = 0.405) than that of the wild population pool (θ^B =0. 212) (Table 2).

Similarly, the predictions that the two *A. rhodacantha* traditional landraces from southern Jalisco and that the three old landraces, no longer used to make tequila, in central Jalisco would have a higher diversity than the commercial blue agave proved to be correct. All landraces had a significantly higher genetic diversity, and four of the five had diversity that was twice that of blue agave.

These results seem to be the natural consequence of the traditional management system described by Colunga-GarcíaMarín and Zizumbo-Villarreal (2007) and Vargas-Ponce et al. (2007). Because the main use of plants produced in this system is the elaboration of traditional agave spirits by the same growers, and not for the sale of plants to the commercial tequila industry, the growers can manage the system according to their tradition, which maintains (1) high crop diversity within the milpa and mezcalera and (2) high levels of genetic diversity within each agave landrace. Through their agave germplasm selection pressures and vegetative propagation, traditional farmers have generated a bottleneck that has been considered to be inherent to human selection (Hawkes, 1983; Doebley, 1992) and have also generated a higher genetic structure in the cultivated pool than in the wild. This bottleneck, however, has not been very narrow as a result of (1) farmers using broad selection criteria related to flavors, resistance to predation and diseases, and adaptability to multispecific and multivarietal agroecosystems that include cattle; and (2) the propagation of the selected plants occasionally by seed nurseries and the toleration of seedlings from wild plant seeds. Moreover, the bottleneck has been disrupted continually, maintaining levels of diversity as high as the wild pool through the (1) introduction of selected wild germplasm into cultivation, (2) toleration of selected wild plants, (3) in situ encouragement of tolerated wild plants, and (4) introduction of landraces from other towns in the region.

These findings are important considering that the distillation of agaves for spirits, although a relatively recent use that began c. 400 years ago, grew out of a culinary tradition that started in Mesoamerica at least 9000 years ago, with the use of agave as food (Bruman, 1940; Callen, 1965). Traditional management in southern Jalisco has been able to maintain high genetic diversity during cultivation of the *A. angustifolia* complex despite it having been propagated vegetatively for centuries. The genetic diversity for the entire pool of *A. angustifolia* landraces and that for the cultivated pool in the traditional Zapotitlán milpa are similar, indicating that the systems used by these traditional farmers are highly representative of the traditional system as a whole. The differentiation values within both pools (θ^B = 0.405) are also equal and are very high according to Wright (1978). This considerable differentiation is a natural consequence of high human selection pressures to differentiate landraces. The very low differentiation between the wild and the cultivated pools (0.92% of the total variation can be found among these two groups according to the AMOVA) is coincident with the assumption that they belong to the same evolutionary complex.

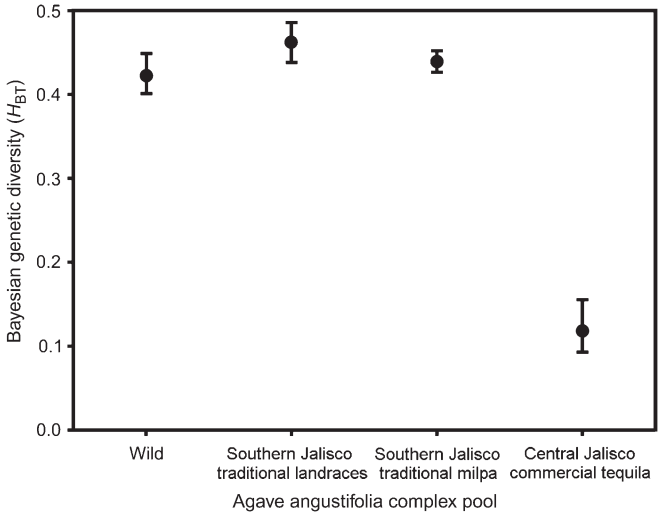

Fig. 2. Bayesian genetic diversity (H_{BT}) and 95% credibility intervals (CrI) for wild and cultivated *Agave angustifolia* complex pools used for spirits production in southern (mezcals) and central (tequila) Jalisco, Mexico.

Other authors have found this same type of traditional management for other ancient Mexican perennial food crops that are reproduced both sexually and vegetatively, whereby the farmers maintain high diversity. Colunga-GarcíaMarín et al. (1986), using morphological variation, described a traditional system of wild, tolerated, encouraged, and cultivated *Opuntia* spp. populations, in which farmers maintain a constant diversity in the *Opuntia* spp. community while significantly increasing the predominance of the most desirable variants. In addition to vegetative propagation, the toleration and even the transplantation of wild seedborne *Opuntia* plants is a traditional practice in this system. Casas et al. (1997, 2007) described a traditional system for *Stenocereus stellatus* (Pfeiffer) Riccobono in which cultivation in home gardens includes the sparing of desirable individuals that spontaneously established from seeds. With isozyme analysis, they found that managed in situ and cultivated populations had higher levels of diversity than wild populations. In both cases, the conservation of high diversity was related to the desire of farmers to cover a wide range of uses, preferences, and harvest periods. Miller and Schaal (2006), studying *Spondias purpurea* L., a fruit tree cultivated in the traditional management systems of southern Jalisco since pre-Hispanic times, also found that cultivated populations in backyards and living fences have the same genetic diversity as in wild populations, although according to these authors they are exclusively propagated by vegetative means.

In great contrast with the southern Jalisco traditional system, we found that the genetic diversity of the only variety cultivated in the commercial tequila system, the blue agave (TEQ), is 72% lower (H_B = 0.118) than that of the wild genetic pool (H_{BT} = 0.428), that is, the *A. angustifolia* complex, to which it belongs (Fig. 2). This very low diversity is consistent with the narrow selection criteria that guide its vegetative dissemination, which includes in vitro clonal propagation by several tequila companies. Its low genetic variation has already been reported in two studies that also sampled the Jalisco commercial tequila planta-

tions. Gil-Vega et al. (2001) found that only 1 of 124 RAPD products (0.8%) was polymorphic and 39 of 40 plants (sampled in four fields) were completely isogenic. Cuevas-Figueroa and Flores-Berrios (2006) using AFLPs reported a polymorphism lower than 0.4%, analyzing 80 individuals (sampled in various fields, number not specified by these authors).

The high levels of genetic diversity found in the old tequila landraces (H_B = 0.269–0.328) still cultivated by a few traditional farmers in the Tequila-Amatitán valley confirms that the tequila liquor once belonged to a traditional system that had maintained the richness of landraces with high diversity that was described by Pérez (1887). The reduction from nine traditional landraces to only one in the tequila industry during the 20th century has been documented by Valenzuela-Zapata (1997).

A very similar, but even more drastic loss of genetic diversity has been documented for another *Agave* species that belongs to the *A. angustifolia* complex: henequén (*A. fourcroydes* Lem. also known as sisal hemp), domesticated by the Yucatan Maya in pre-Hispanic times for its fiber. The loss of at least seven of eight traditional varieties, as a consequence of the development of an exportation plantation system during the 19th and 20th centuries was documented by Colunga-GarcíaMarín et al. (1999). Current genetic diversity (estimated through isozyme analysis) in wild ancestral populations of *A. angustifolia* is I_T = 3.06, whereas in sack ki, the only commercial variety used for exportation, is I = 0. The traditional system and its varieties are now extinct (Colunga-GarcíaMarín et al., 1999).

The high diversity described here for the entire traditional landraces pool in southern Jalisco and for plots like the Zapotitlán milpa is similarly threatened by the rapid expansion of the tequila industry, with the quadrupling of blue agave cultivation in 12 years to more than 1 million tons, that produced 285 million liters of tequila in 2007 (CRT, 2008). In contrast to the system for henequén, the original traditional system still continues in southern Jalisco and can be preserved. The most important way to preserve the system is by supporting and stimulating the interest of traditional farmers to conserve and use their landraces and to continue to generate new germplasm. The entire traditional system, including the in situ management of wild populations and the various components such as the inclusion of staple food crops and cattle within the agroecosystem, must be supported, because this complex system has been proven to preserve diversity on many levels. This complexity contributes to the sustainability of the entire system.

To benefit the farmers and their communities, this encouragement should also include legal protection of the germplasm and products derived. At present, these farmers, like many other Mexican native peoples, germplasm, and traditional processes, have been excluded from the Appellation of Origin Tequila and Mezcal (Colunga-GarcíaMarín and Zizumbo-Villarreal, 2007). A change in the Appellation of Origin and/or the certification of a new legal product designed to help preserve genetic, ecological, and cultural diversity is needed. Informing local and national markets is essential to these conservation efforts. The globalization of markets has accelerated the loss of diversity because half of the tequila production is exported, mainly to the United States of America, but global markets represent an opportunity to conserve germplasm and culture if interest in local products and their cultural dimensions can be increased.

The characteristics of the traditional and the commercial agave cultivation systems to create spirits and the contrasts between the two can also be illustrated with the population diversity values for the landraces. With the exception of Z, all the traditional landraces had significantly higher levels of genetic diversity than the commercial blue agave. Five of the six southern Jalisco traditional landraces that farmers considered to be the older ones (C, IV, SO, G, and lineño) are among the nine landraces with higher diversity values (H_B), indicating that their high diversity is probably in a steady state. Only three landraces did not have double the diversity of the blue agave commercial variety (TEQ): Z, population LT, and TC. The low diversity of Z (H_B = 0.141) may be explained by the fact that it was recently introduced to cultivation with the shoot roots of only four individuals and illustrates the bottleneck generated when the selection of a new landrace is started by vegetative propagation. Lineño population LT, one of the four lineño populations studied, is cultivated commercially for a local spirits industry developed around this landrace. Its low diversity (H_B = 0.210), compared with the two lineño populations that are cultivated for autoconsumption (LZ, H_B = 0.297; LSE, H_B = 0.317) illustrates the effects of narrow criteria for commercial selection and the abandonment of traditional practices. The same occurs, but to a lesser extent, with the lineño population LP (H_B = 0.259), an abandoned commercial plantation. TC is the local gene pool of blue agave. Even though it is also grown for commercial purposes, its diversity (H_B = 0.217) is significantly higher than the commercial blue agave (H_B = 0.118). The diversity values for LT and TC illustrate that under traditional management, even the landraces that have had great commercial importance have conserved a relatively high diversity, compared with the commercial blue agave used by the tequila industry. Another case of high diversity under traditional management is the case of the *A. rhodacantha* landrace IA, a highly important fiber source in the past.

The high diversity value of population LSE illustrates the effect of sexual reproduction in breaking the bottleneck that originated from selection via vegetative propagation, because the LSE individuals resulted from the sexual reproduction of several plants and were initially grown in a seed nursery. The effect of seed propagation in recovering diversity has been documented by Colunga-GarcíaMarín (1996) for *A. fourcroydes.* While populations propagated vegetatively had I = 0, those derived from seeds had I = 2.17, as high as some populations of the wild ancestor (*A. angustifolia*). The practice of tolerating and encouraging seedborne plants that are later vegetatively propagated, even sporadically practiced, may have a very important effect on diversity. Pujol et al. (2005) demonstrated in Guyane, using eight microsatellite loci, that the traditional farming system for *Manihot esculenta* Crantz can maintain high levels of observed heterozygosity (0.50–0.82) by allowing the growth of seedlings that appear spontaneously in fields and using them later as cuttings, the more frequent mode of propagation. Our values of total genetic diversity for the *A. angustifolia* wild pool, estimated with Nei's (1973) genetic diversity ($H_T = 0.370 \pm 0.092$) (Appendix S1, see Supplemental Data with the online version of this article), are similar to values reported for other agaves used for spirits that were based on the same estimator and program using other dominant markers. For instance, Barraza-Morales et al. (2006) reported $H_T = 0.313 \pm 0.037$ for three wild populations of the same species in Sonora using 353 AFLP polymorphic loci. Despite the high number of loci they used, we obtained slightly higher diversity values, probably because we analyzed more populations (9 vs. their 3). Our estimations were also similar to values reported by Aguirre (2004) for 39 ISSR loci from five wild populations of *A. cupreata* ($H_T = 0.294 \pm 0.133$) and *A. potatorum* ($H_T = 0.269$

± 0.149) also in subgenus *Agave*. Although both species reproduce only sexually while *A. angustifolia* also reproduces vegetatively, we had slightly higher values, probably because again we studied more populations (9 vs. their 5) and analyzed more loci (69 vs. their 39).

The Bayesian diversity estimates (H_B, H_{BT}) obtained in the current study (Table 1) were always higher than Nei's 1973 genetic diversity (H_S, H_T) (online Appendix S1), for two reasons: (1) If $f > 0$, the program TFPGA will overestimate the frequency of the recessive allele because it takes the square root of the recessive homozygote frequency. For most possible allelic frequencies, the TFPGA overestimate will yield lower values of expected heterozygosity. (2) Even if $f = 0$, the allelic frequency estimates from TFPGA will tend to be more extreme, because Bayesian estimates are "smoothed" toward 0.5, which produces the maximum possible value (K. Holsinger, University of Connecticut, personal communication). The SD of each H_T value is within the 95% CrI of the H_{BT} values.

Genetic differentiation values for the wild pool of *A. angustifolia* are high according to Wright (1978). Our values, estimated with Weir and Cockerham (1984) θ, obtained with TFPGA version 1.3, assuming HWE, shows that its structure is similar (θ = 0.182, 95% CI = 0.149, 0.215) to that recorded by Barraza-Morales et al. (2006) for *A. angustifolia* populations of Sonora using AFLPs (θ = 0.165). Using isozymes and a different procedure to estimate F_{ST}, Colunga-GarcíaMarín (1996) found a much higher structure in *A. angustifolia* populations growing in the Yucatan Peninsula (F_{ST} = 0.39), perhaps as a result of its highly fragmented natural habitat (P. Colunga-GarcíaMarín, personal observation). In Jalisco, *A. angustifolia* has a wide continuous distribution, and in the study area, the ATAR and TNCR basins are biological corridors where its potential pollinator (*Leptonycteris curasoae*) occurs (Rojas-Martínez et al., 1999), probably favoring gene flow by long-distance movement as suggested for other *Agave* species by Eguiarte and Souza (2007). The lower correlation values of alleleic frequencies across loci (ρ) among landraces populations compared with wild populations suggest that wild populations are more connected by gene flow than are cultivated populations, as was expected. The values for *A. cupreata* (θ = 0.145) and *A. potatorum* (θ = 0.084) reported by Aguirre (2004) using ISSR are smaller than *A. angustifolia* wild populations in this study, probably because they only reproduce sexually and gene flow between populations may be higher than in *A. angustifolia*.

The ISSR phenotypes were different for all individuals, indicating that they are genetically distinct, without apparent clonality. This may be a product of occasional events of sexual reproduction or also of somatic mutations. Asexual genetic variability has been observed in various species of wild and cultivated agaves (Infante et al., 2003, 2006).

In conclusion, the landraces of *A. angustifolia* complex grown for spirits under traditional agriculture in southern Jalisco have a genetic diversity similar to wild populations in the region. At the same time, its genetic structure is more distinct. In great contrast, the genetic diversity of the commercial tequila system, consisting only of the blue agave variety, a clone that belongs to the same evolutionary complex, is reduced by 73%. The differences between the traditional and the commercial system are explained for their contrasting principles. The traditional agave system from southern Jalisco is based on the Mesoamerican pre-Hispanic tradition of maintaining diversity at different biological levels and maintaining genetic contact between wild and cultivated gene pools through their cultivation in complex agroecosystems that include the in situ management of wild, wild-tolerated, and wild-encouraged populations. The commercial tequila system is based on an industrial tradition of homogeneity at all levels. Conservation of the wild and cultivated germplasm, endangered by the commercial expansion of blue agave, must be linked to the preservation of the entire traditional system. Understanding the traditional management system in the center of agave genetic diversity in west-central Mexico has been essential in the design of a current on-farm conservation project with southern Jalisco traditional farmers. Changes to all agave spirits (mezcals) certification laws to allow the conservation of current genetic, ecological, and cultural diversity can play a key role in the preservation of the traditional agroecosystems.

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APPENDIX 1. Voucher specimens of the taxa studied deposited in the following Mexican scientific collections: Centro de Investigación Científica de Yucatán Herbarium (CICY), Instituto de Botánica–Universidad de Guadalajara Herbarium (IBUG), Universidad Nacional Autónoma de México–National Herbarium (MEXU), Centro de Investigación Científica de Yucatán living *Agave* plants germplasm collection (CICY-AGC).

Taxon. Human management intensity (wild, wild-tolerated or wild-encouraged population, traditional landrace, commercial blue agave); Code,[a] Collection site, *Voucher specimen*; Herbarium or CICY-AGC.

Agave angustifolia Haw. evolutionary complex. **Wild populations**; AH; Hostotipaquillo; *Vargas 1786*; CICY, IBUG, MEXU. AM; Ameca; *Vargas 1751*; CICY, IBUG, MEXU. ASA; Sayula; *Vargas 1608*; CICY, IBUG, MEXU. AT; Tecolotlán; *PC-043-01*; CICY-AGC. AW; Tuxcacuesco-1;, *Vargas 1025* to *Vargas 1027*; CICY-AGC. Wild-e**ncouraged populations**; APA; Tecolotlán-2; *Vargas 1619*; CICY, IBUG, MEXU. **Southern Jalisco traditional landraces**; B; Zapotitlán; *Vargas 1603*; CICY, IBUG, MEXU. C; Zapotitlán; *Vargas 1602*; CICY, IBUG, MEXU. CH; Zapotitlán; *Vargas 1604*; CICY, IBUG, MEXU. IV; Zapotitlán; *Vargas 1607*; CICY, IBUG, MEXU. LZ; Zapotitlán; *PC-03211*; CICY, IBUG, MEXU. P; Zapotitlán; *Vargas 1606*; CICY, IBUG, MEXU. PE; Zapotitlán; *Vargas 1599*; CICY, IBUG, MEXU. TE; Zapotitlán; *Vargas 1605*; CICY, IBUG, MEXU. Z; Zapotitlán; *Vargas 1600*; CICY, IBUG, MEXU. CN; Tolimán-1; *Vargas 1547*; CICY, IBUG, MEXU. HO; Tolimán-1; *Vargas 1515*; CICY, IBUG, MEXU. LSE; Tolimán-1; *PC-04461* to *PC-04518*; CICY-AGC. MP; Tolimán-1; *Vargas 1568*; CICY, IBUG, MEXU. SO; Tolimán-1; *Vargas 1525*; CICY, IBUG, MEXU. TC[2]; Tolimán-2; *PC-04461* to *PC-04518*; CICY-AGC. LP; Tolimán-3; *Vargas 1401*; CICY, IBUG, MEXU. LT; Tonaya; *PC-03183-1* to *PC-03183-20*; CICY-AGC. G; Tuxpan; *Vargas 1189*; CICY, IBUG, MEXU. **Central Jalisco traditional landraces**; BE[1]; Tequila-Amatitán-1 & 2; *PC-0370* & *PC-0373*; CICY, IBUG, MEXU. SI; Tequila-Amatitán-1 to 5; *PC-0341*; CICY, IBUG, MEXU. **Central Jalisco commercial blue agave**; TEQ[2]; Tequila-Amatitán; *PC-0343* & *PC-0350-1*; CICY-AGC.

Agave rhodacantha **Trel. Wild-tolerated populations**; SVA; Tuxpan; *Vargas 1181*; CICY, IBUG, MEXU. **Southern Jalisco traditional landraces**; IA; Zapotitlán; *Vargas 1601*; CICY, IBUG, MEXU. VR; Tolimán-1; *Vargas 1591*; CICY, IBUG, MEXU.

Agave **spp. Central Jalisco traditional landraces**; CHA; Tequila-Amatitán-1 & 2; *PC-0360* & *PC-0343-1*; CICY, IBUG, MEXU.

[a]Alphabetical code used in text (Table 1) and common name of cultivated populations: B = Brocha; BE= Bermejo; C =Cenizo; CH = Chancuella; CHA = Chato; CN = Cimarrón Negro; CZ = Cimarrón hoja larga; G = Garabato; HO = Hojudo; IA= Ixtero Amarillo; IV = Ixtero verde; LP = Lineño; LSE = Lineño; LT = Lineño; LZ = Lineño; MP = Mezcal Piña; P = Prieto de Telcruz; PE = Perempis; SI = Sigüin; SO = Soca; TC = Azul criollo; TE = Telcruz; TEQ = Blue agave; VR = Verde rápido; Z = Prieto Presa Grande.

Notes: [1]*A.* cf. *angustifolia.* [2]*A. tequilana* Weber var. Azul (included in the *A. angustifolia* complex).