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Genetics of wild and managed populations of *Leucaena esculenta* subsp. *esculenta* (Fabaceae; Mimosoideae) in La Montaña of Guerrero, Mexico

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Abstract

Genetic consequences of silvicultural management of *Leucaena esculenta* subsp. *esculenta* were analyzed from eight allozyme loci in half-sib families of one wild and one managed *in situ* (selectively cleared) population from La Montaña de Guerrero region, Central Mexico. A reference sample (including wild, feral and cultivated individual plants) from the states of Morelos, Puebla and Guerrero, Mexico was also analyzed. Genetic variation, population structure and mating system were analyzed. All loci showed high variation (75–87.5% polymorphic loci at 95% level; 2.4–2.8 mean number of alleles per locus). All progenies showed heterozygous deficiency, but both wild and managed parental inbreeding coefficients were negative, suggesting heterosis. Progenies of managed populations differed from those of the wild and reference samples (Nei's unbiased identities 0.874–0.934). Biparental inbreeding is suggested by Wright's-statistics ($f = 0.313$), and by outcrossing rate estimates: $t_m = 0.644$ (SE 0.094), and 0.645 (SE 0.193); $t_s = 0.576$ (SE 0.189), and 0.523 (SE 0.182), for managed and wild samples respectively. Population differentiation is significant ($\Theta = 0.210$). The species is self-incompatible and deviations from the mixed mating model were found. Indirect estimates of products of effective population size (N_e) by the proportion of migrants (N_m) were moderate, as were the N_e values. Variation due to ecotypic differentiation (related to altitude), prolonged artificial selection, and introduction from other areas is supported. A model of domestication of seed-propagated trees is suggested, based on extensive and *in situ* selection of locally adapted populations, and their diffusion to other areas.

Introduction

The *guaje rojo* (red guaje) *Leucaena esculenta* (Mociño et Sessé ex A.DC.) Benth. subsp. *esculenta* (Zárate 1994) (*L. e. esculenta*), is a dry-tropical forest tree that is widely represented in human cultures of Central Mexico since ancient times (Zárate 1997, 1998). People use its young leaves, floral buds, immature legumes and seeds – either green or dried – as food. Leaves, pods and bark are medicinal for humans and domestic animals. Wood is valued as construction material and as firewood. Within its natural geographic range in Central Mexico it is cultivated in yards, cornfield borders and in pure stands. Wild populations are distributed throughout the Balsas river basin (Zárate 1994; Casas and Caballero 1996; Hughes 1998) (Figure 1).

Previous studies in La Montaña of Guerrero (Casas and Caballero 1996) documented that

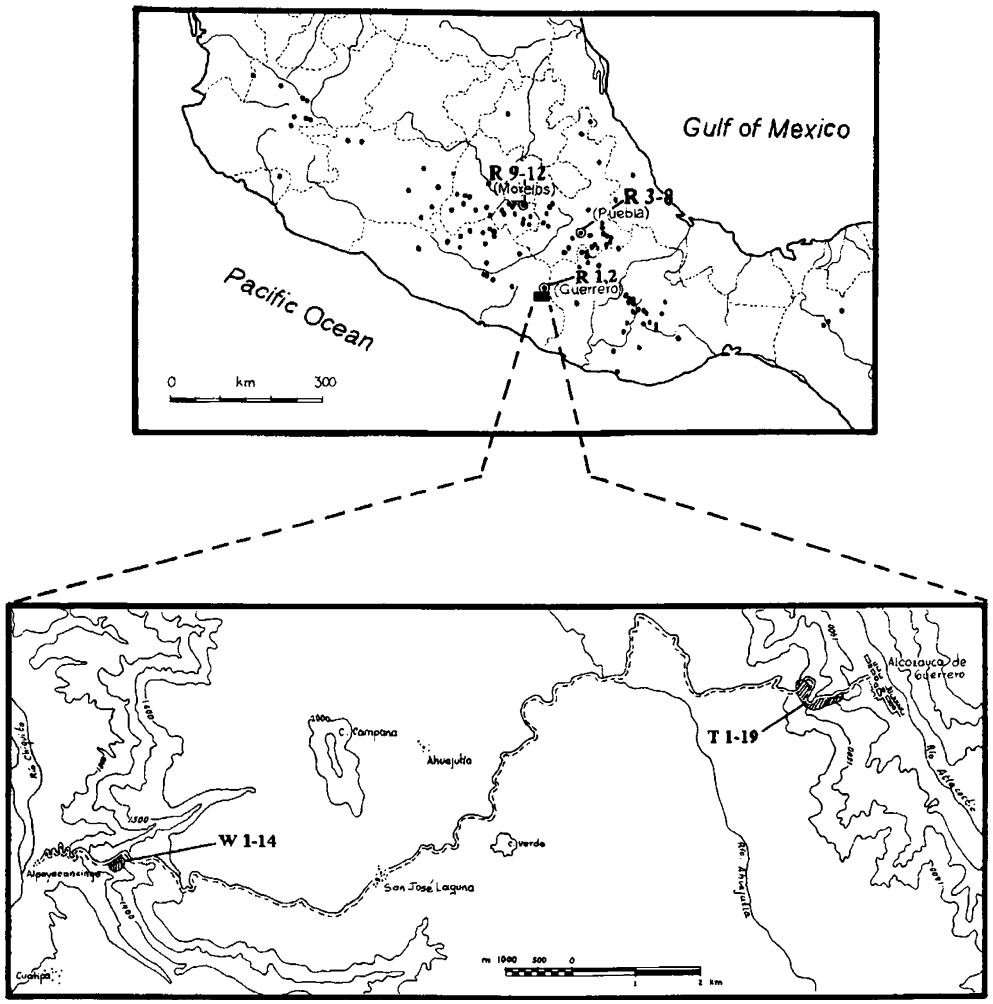

Figure 1. Known distribution of *Leucaena esculenta* subsp. *esculenta* and location of collection sites. The families sampled are identified as in Appendix 1. Sources: Zárate 1994:132, Figure 6; Hughes 1998:146, Figure 50; INEGI. 1984. Xalpatlahuac. 1:50 000 Chart E14D32).

indigenous people practice three forms of interactions with *L. e. esculenta*: (1) gathering of useful products in wild populations, practice that is mediated by the recognition and classification of variation of trees according to the quality of pods and seeds. People recognize trees producing larger pods and seeds with "sweeter" flavor (distinguished from bitter and mildly toxic variants), and selectively gather products from these trees; (2) management *in situ* of wild populations which is associated to clearance of land for agriculture. People tolerate *guaje rojo* trees while eliminating other elements of the vegetation in order to make space to maize cultivation. This tolerance is selective, people letting stand only those trees presenting the best attributes for consumption as food but eliminating others, and occurs in a disturbed forest area recurrently cleared for agriculture, and tolerated trees have the advantage over other phenotypes of leaving their seeds in the area; and (3) cultivation, which occurs in the traditional orchards, in which people sow seeds from desirable phenotypes from wild populations, other orchards within the village, and from cultivated stands of other villages or regions of Mexico.

A morphometric analysis by Casas and Caballero (1996) showed that preferred phenotypes were more

abundant in the managed *in situ* and cultivated populations, compared with the unmanaged wild population studied. These authors suggested that such long-term selective tolerance of trees in the managed *in situ* population involves an *in situ* process of domestication. Trees cultivated in orchards showed a broader range of variation than both managed *in situ* and unmanaged wild populations, a finding which was interpreted as a consequence of the diversity of seed provenance. Because similar phenotypes were found in all three management conditions, the authors considered it more probable that the observed differences were determined mainly genetically rather than environmentally.

Considering the above, *in situ* selection might have operated speeding-up the otherwise slow process of character fixation typical of outbreeding trees such as *L. e. esculenta*. The possible occurrence of gene flow among populations under different management type was considered by Casas and Caballero (1996) as a force counteracting effects of human selection; however, the consequences of the mating system prevailing in cultivated stands remained to be investigated – i.e. the effects of genetic segregation in cultivated trees, which are mostly first generation, or descendants from a relatively low number of planted generations. Nevertheless, in cultivated populations the effect of gene flow may also be diminished due to the fact that progeny from orchards is not necessarily sown or recruited. While both the cultivated and the managed *in situ* populations have been under artificial selective pressure, the length of the life cycle, the effects of segregation, and the fact that seeds from selected trees do not necessarily express the same quality of the mother contribute to suggest that the differences observed were due to the duration of the period of selection.

This paper addresses the possible effect of differential management histories in the genetic composition and structure of the same *in situ* managed and unmanaged populations from Guerrero, Mexico, studied by Casas and Caballero (1996), also including a reference sample from the Mexican states of Morelos, Guerrero and Puebla. Our hypotheses were: (1) in relation with wild populations, artificial selection operating in managed *in situ* populations by selective tolerance of preferred phenotypes and by the decrease in density due to the clearing of land for maize cultivation, has resulted, respectively, in an increased genetic disequilibrium (due to a change in the frequencies of genotypes), and a decrease in the frequency of crosses among related trees, i.e. biparental inbreeding; (2) the length of the time period during which selective clearing has been practiced in the managed *in situ* population has determined the genetic differentiation of the managed *in situ* population; and (3) selection of desired phenotypes was aided by the natural variation of ecotypes along the distribution of the species either by selecting already existing favorable variants (in higher altitudes) or by the introduction of seeds from such places.

Materials and methods

Plant material

Seed progenies (half-sib families) were sampled from single mother-trees, which had been previously marked in the field, and, for which morphology had been assessed (Casas and Caballero 1996). Part of these half-sib families was collected in a managed *in situ* population in the hills surrounding the village of Alcozauca, Guerrero. This population has been under several centuries of management by local people (Casas and Caballero 1996) and is characterized by patches of disturbed vegetation at different stages of regeneration, most of them presenting signs of previous agricultural work. Another part of the half-sib families was collected in an unmanaged wild population near San José Laguna, a neighboring village of Alcozauca. This population forms part of a tropical deciduous forest, apparently undisturbed, which, according to memory of old local people has not been used for agriculture. Seeds of trees from other localities were studied as reference samples (Appendix 1 and Figure 1).

Seeds were hand-scarified and left in water overnight to soften the tissues. After this, a small transverse section (1–2 mm) of seed was cut from the extreme opposite to the embryo, and the green cotyledon tissue was ground as described below.

Enzyme extraction and electrophoresis

Seed cotyledons were ground in weighing boats over ice, in a few drops (2–5) of chilled grinding buffer, using a test tube, a Plexiglas rod, or a glass

rod. The Tris–HCl grinding buffer was prepared according to Soltis et al. (1983). The grindate was soaked into Whatman 3 mm chromatographic paper wicks, which were used fresh. Preliminary tests were conducted with leaflets from plants obtained by sowing seeds, which were ground in the same buffer, but this process was more laborious, and enzyme expression was similar in both types of tissue.

Starch gel and electrode buffers used were system I of Shaw and Prasad (1970), modified according to system 2 of Soltis et al. (1983). Electrode buffer: 0.135 M Tris (16.35 g), 0.032 M citric acid (6.10 g, anhydrous) in 1 L, pH adjusted to 8. Gel buffer, 0.009 M Tris, 0.001 M citric acid, prepared diluting 67 mL of electrode buffer to 1 L of distilled water, adjusting pH to 8. Starch concentration was 13.2% (for thick gels, 59.4 g in 450 mL of gel buffer). Protein grindate in wicks was extracted by running during 12 min at 60 mA and 200 V, after which current was turned off, wicks were removed, and gels continued to run at 35–50 mA and 200–250 V during 7–8 h. Slices were cut from gels, and each one was stained following Soltis et al. (1983) staining schedules for aconitase E.C. 4.2.1.3 (ACO), phosphoglucoisomerase E.C. 5.3.1.9 (PGI), and phosphoglucomutase E.C. 2.7.5.1 (PGM).

Chloroplastic isozyme loci were identified experimentally by differential centrifugation following Gastony and Darrow's (1983) method. Other isozymes were assigned by determining gel zones where putative isozyme loci migrate. These zones expressed a number of putative alleles, except for that corresponding to chloroplastic *Pgi1*, which did not resolve satisfactorily and was not interpreted. These electrophoretic zones behaved as alleles in a consistent way because they segregated in the half-sib families, and because they had a maximum of two staining bands (alleles) per zone. Exceptions to this were consistent with gene silencing, i.e. the observation of variation in activity by means of variable staining intensity of allozyme bands, which express either as normal, partially, or totally inactive forms. Presence–absence of putative allelic bands was used to perform a phenetic analysis of individual seed (seedling) electrophenotypes using the computer program STATISTICA 6 (StatSoft Inc. 2001) with the unweighted pair group method with arithmetic average (UPGMA) procedure and Euclidean distances.

Genetic analysis

Genetic analyses were made using the computer programs BIOSYS (Swofford and Selander 1981), Tool for Population Genetics Analysis (TFPGA; Miller 1997), and, for mating system analyses, the Multilocus Estimation Program (MLT; Ritland 1990) was used to calculate single and multilocus outcrossing rates (respectively, t_s and t_m). Also, some statistical analyses were computed using the algorithms quoted in each case. Estimations were made of: (1) allelic frequencies for seeds; (2) fixation index per locus $F_I = 1 - (H_o/H_e)$ and average over loci (F_M); (3) unbiased genetic identities (I) (Nei 1978); (4) hierarchical analysis at the levels of population (managed *in situ*, wild, reference) and of family (considered as subpopulations) (Wright 1978); and (5) Wright's F-statistics estimated by TFPGA using Weir and Cockerham's (1984) algorithms, in which F_{IT} corresponds to F, F_{IS} to f, and F_{ST} to Θ. Significance of fixation indexes was determined using the formulas of Li and Horvitz (1953) (F_I, F, f), and of Workman and Niswander (1970) (Θ). Using TFPGA, jackknifing was performed to calculate the average over loci Wright's-statistics and the 95% confidence interval was computed from the standard deviation of this estimate. The analyzed sample comprised 14 families (W1–W14) represented by 140 individuals from the unmanaged wild population near San José Laguna; 19 families (M1–M19) represented by 277 individuals from the managed *in situ* population near Alcozauca; and, 12 families (R1–R12) represented by 51 individuals from the reference regional population from the states of Puebla (R3–R8), Morelos (R9–R12) and Guerrero (R1, R2) (Appendix 1 and Figure 1).

The results of the genetic interpretation of zymograms of eight putative loci (*Aco1*, *Aco2*, *Aco3*, *Pgi2*, *Pgi3*, *Pgm1*, *Pgm2*, and *Pgm3*) were analyzed. Half-sib families were pooled per population and the following estimates were computed: genetic variation per locus, Hardy–Weinberg equilibrium tests, F_I and F_M, I and the corresponding UPGMA phenogram, Wright's-statistics. Other analyses were made using 12 families from the managed *in situ* population (M2–M4, M6, M10–M14, M16, M17, and M19), eight families from the unmanaged wild population (W1, W2, W4, W6–8, W10, and W11), and the families from elsewhere

Guerrero – merging all individuals as one family (R1 + R2). In these sample, a hierarchical analysis (by means of I and the corresponding UPGMA phenograms) was ran at two levels – i.e. by either pooling the individual half-sib families by population, or considering these as subpopulations (Wright 1978) (Appendix 1).

Five loci (*Pgi2*, *Pgi3*, *Pgm1*, *Pgm2*, and *Pgm3*) were used for the analysis of the mating-system. This analysis included subsamples of 11 families from the managed *in situ* population (M1–4, M6, M10–12, M14, M16, M17, M19) and nine families from the wild population (W1–5, W7, W8, W10, W11), which are designated as samples wild (t) and managed *in situ* (t) (Appendix 1). Estimates were obtained of t_s and t_m; average F_I values across loci of parental population samples; and the maternal genotypes for these families, which were inferred by Brown and Allard's (1970) method using MLT (Ritland 1990). Deviations from the mixed-mating model were calculated following Ritland (1983). Additionally, biparental inbreeding was assessed by the statistical significance of the difference $t_m - t_s$, and by the comparison of the average value of F_I across loci – from BIOSYS – with the fixation index predicted at inbreeding equilibrium by the formula: $F_{\text{eq}} = (1 - T_{\text{eq}})/(1 + T_{\text{eq}})$ (Hedrick 1983). The same formula was used to estimate the average values of the outcrossing rate at inbreeding equilibrium, in the form: $t_{\text{eq}} = (1 - F_M)/(1 + F_M)$, where t_{eq} is the estimate of the average outcrossing rate at equilibrium, and F_M is the average fixation index across loci. Also, weighted average of F_I per sample F_W was similarly used to estimate outbreeding rate at inbreeding equilibrium (t_{meq}).

The effect of gene flow on population differentiation was analyzed using the stepping-stone model formula of Crow and Aoki (1984) which indirectly estimates the product of the effective population size by the proportion of migrants (N_m) from Θ values by, $\Theta = 1/(4\ a\ N_m - 1)$, and, $N_m = (1/\Theta) - 1/4a$; where $a = [n/(n-1)]^2$ and n = number of subpopulations. It is assumed that values of N_m greater than 1 would constrain genetic differentiation of subdivisions within populations (Eguiarte 1990). Effective population size (N_e) was estimated indirectly by Slatkin and Barton's (1989) formula: $N_e = 2¶\ N_m$; were ¶ = 3.14162, and N_m = indirect estimate of gene flow of Crow and Aoki (Eguiarte 1990).

Results

Genetic interpretation of enzyme bands

Leucaena e. esculenta displays duplication of isozyme loci. Aconitase expresses three, seemingly cytosolic loci (*Aco1*, *Aco2*, *Aco3*); PGI and PGM, two cytosolic loci (*Pgi2*, *Pgi3*; *Pgm2*, *Pgm3*), and one chloroplastic locus each (*Pgi1*, not analyzed; *Pgm1*). Gene silencing was observed in dimeric PGI by the presence of heterodimeric bands, but not of one of the putative allelic homodimers. Furthermore, these null forms grade from fully to partially inactive as has been observed in other plants (Goodman et al. 1980; Wendel et al. 1986). Because these heterodimers migrate to the same position in the presence of active, partially active and null forms, it may be assumed that all of these activity variants co-migrate with observed putative alleles, i.e. they should have identical electrophoretic mobility. Thus, in a strict sense, co-migrant enzymes may not be electrophoretically assigned to a different allele with respect to normal staining forms. In theory, gene silencing in the monomeric enzymes PGM and ACO may only be detected when one of the expected isozymes is absent. Yet, these cases may be either due to monoallelic null isozymes or to double nulls, thus underestimating genetic diversity. When noticeable, these activity variants were pooled together and scored as the corresponding active allele. Appendix 2 indicates allelic frequencies per locus per population sample, and the number of individuals per locus for each population sample.

Genetic variation

All eight enzyme loci studied were highly variable (Table 1); $p = 75$–87.5% (using 95% criterion), $A_p = 2.4$–2.8. Observed heterozygosity was less than expected for all populations. The largest difference was observed in the managed *in situ* sample ($H_o = 0.227$, $H_e = 0.335$), followed by those in the reference ($H_o = 0.141$, $H_e = 0.227$), and the wild ($H_o = 0.203$, $H_e = 0.264$) samples. Differences observed in the managed *in situ* sample were statistically significant.

Table 1. Genetic variability for eigth loci in three populations of *Leucaena esculenta*. Standard errors in parentheses.

Population	Families[a]	Individuals[b]	P (%)[c]	A_p[d]	H_e[e]	H_o[f]
Managed *in situ*	19	178.8 (19.9)	87.5	2.8 (0.3)	0.335 (0.043)	0.227 (0.028)
Wild	12	88.5 (10.2)	75.0	2.4 (0.3)	0.264 (0.056)	0.203 (0.052)
Reference	14	32.9 (0.76)	75.0	2.4 (0.4)	0.227 (0.068)	0.141 (0.043)

[a] Total number of families.
[b] Mean number of individuals per locus.
[c] A locus is considered polymorphic when the frequency of the most common allele does not exceed 0.95.
[d] Mean number of alleles per locus.
[e] Expected heterozygosity (Nei 1978) (unbiased estimate).
[f] Observed heterozygosity (direct count).

Table 2. Fixation indexes of progenies of *Leucaena esculenta* per locus (F_I) per population and average over loci per population (F_M).

	Managed *in situ*		Wild		
F_I	(whole sample)	(mating subsample)	(whole sample)	(mating subsample)	Reference
Aco1	0.491***	–	−0.016	–	−0.077
Aco2	0.107	–	0.808***	–	–
Aco3	0.651***	–	−0.038	–	–
Pgi2	0.136*	0.223**	0.054	0.006	0.292*
Pgi3	0.288***	0.180*	−0.032	0.209*	0.443***
Pgm1	0.230*	0.061	0.494***	0.446***	0.800***
Pgm2	0.270***	0.239**	−0.151	−0.090	0.224
Pgm3	−0.040***	−0.043	0.308***	−0.013	−0.044
F_M	0.267 (0.077)	0.132 (0.054)	0.178 (0.117)	0.112 (0.097)	0.273 (0.133)

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
Estimates were made for eight loci, except in the subsamples analyzed for outcrossing rates in which five loci were used (see text for details). SE of means, in parentheses. Chi-square tests for significance of difference from 0 follow Li and Horwitz (1953).

Progeny and parental fixation index

Table 2 shows the values of F_I and F_M for progenies (seeds and seedlings) estimated for samples with half-sib families pooled over population, and the per locus chi-square analyses of the significance of the difference from 0 of fixation indexes. Shown also in Table 2 are the F_I, and F_M values obtained for the subsample used for mating system analyses. In all cases, the average of the fixation indexes over loci indicates an excess of homozygous individuals in all the progenies. However, small negative values are observed for some loci. Averages across loci of the fixation index estimates were not significantly different from 0, except for sample managed *in situ* (Table 2). Not all F_I estimates were significantly different from 0; noticeably, only one negative value was significant (*Pgm3* for managed *in situ* sample; Table 2). The wild population sample has small negative values for *Aco1*, *Aco3*, *Pgi3*, and *Pgm2*, but these are not significant. Accordingly, the average deviation from Hardy-Weinberg equilibrium in the managed *in situ* population is larger than in the wild population. In contrast, the F_M values estimated for five loci for parents of those families analyzed for the mating system – whose genotypes were inferred by MLT (Ritland 1990) – were negative – $F_M = -0.254$ (0.061) in the wild population; $F_M = -0.226$ (0.103) in the managed *in situ* population.

Genetic identities among populations and among families

For the total sample group, the range of genetic identities is from 0.922 to 0.874, which is comparable

to identities measured in other cases of wild-crop populations (Doebley 1989; Gepts 1993). The maximum value for the genetic identity is between the regional and the wild population samples ($I = 0.934$), followed by that between the regional and the managed *in situ* population samples ($I = 0.922$). The less similar are the managed *in situ* and the wild population samples ($I = 0.874$). The corresponding UPGMA phenogram is shown in Figure 2A. When the families from Morelos and Puebla were excluded from the regional sample the same relation of identity was observed. The least similar samples were the managed *in situ* and wild populations ($I = 0.874$), followed by the managed *in situ* and Guerrero samples ($I = 0.879$). The most similar were the wild and the Guerrero samples ($I = 0.893$).

The family phenogram (Figure 2B) shows two main groups. One of these includes all the managed *in situ* families (12) for which data for all loci were available and one wild family (W7). The second main branch includes the remaining wild families (from a total of eight wild families for which data for all loci were available), and pooled individuals from the half-sib families from elsewhere in the state of Guerrero as a reference.

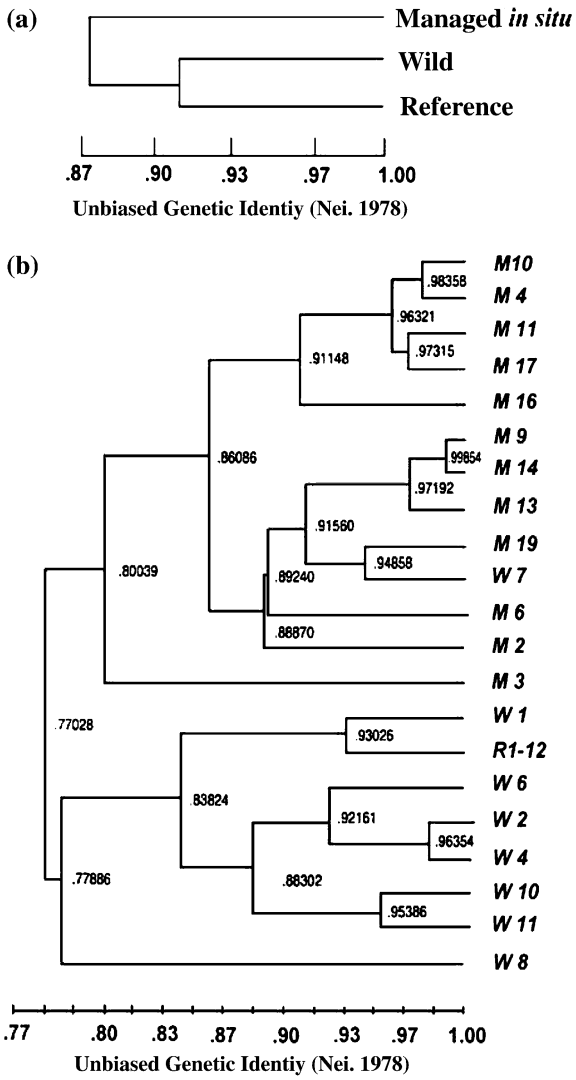

Figure 2. UPGMA phenograms generated from Nei's (1978) unbiased genetic identity based on eight loci. (A) Cladogram for all sampled half-sib families grouped per population. (B) Cladogram for a sample of half-sib families grouped per family. Linkage distances are given above each branch (see text and Appendix 1).

Mating system

Estimated outcrossing rates (Table 3) are similar in both managed *in situ* ($t_s = 0.576$; $t_m = 0.644$) and wild ($t_s = 0.523$; $t_m = 0.645$) population samples analyzed. The SE of t_m and t_s of the managed *in situ* and wild samples indicate that, at 95% confidence limit, both estimates are not significantly different from 0 but the t_m of the managed *in situ* sample is significantly different from 1. Observed estimates would indicate mixed mating with slight predominance for outcrossing (52–64%) over selfing (46–48%) (Table 3). The SE of the differences $t_m - t_s$ indicate that these are significantly different from 0 in both the managed ($t_m - t_s = 0.068 \pm 0.022$), and the wild ($t_m - t_s = 0.122 \pm 0.053$) population samples. A further test for biparental inbreeding was made based on the comparison of the observed F_M values (Table 2) with the expected value of F_{eq}, which was estimated by means of t_m values (from MLT) (Hedrick 1983). For both managed *in situ* and wild samples $F_{eq} = 0.216$. This value is greater than the average across loci for both the managed *in situ* ($F_M = 0.132 \pm 0.053$) and the wild ($F_M = 0.112 \pm 0.097$) samples (Table 2), i.e. less heterozygous deficiency is observed than would be expected if all inbreeding were caused by the mating system alone. In contrast with the above, the values of the fixation indexes for the parental populations that were inferred by MLT were significant and negative in both managed *in situ* and wild subsamples analyzed, which suggests that there is an excess of heterozygous individuals.

Table 3. Mating system estimates for subsamples [designated as (*t*)] of half-sib families from the managed *in situ* and the wild population samples.

Population	t_m	t_s	$t_m - t_s$
Managed *in situ* (*t*)	0.644 (0.094)	0.576 (0.189)	0.068 (0.022)
Wild (*t*)	0.645 (0.193)	0.523 (0.182)	0.122 (0.053)

Single (t_s) and multilocus (t_m) outcrossing rates. Estimates for five loci (*Pgi2*, *Pgi3*, *Pgm1*, *Pgm2*, *Pgm3*). Standard errors (in parentheses) based on 1000 bootstraps (Ritland 1990).

Some loci show single locus t_{eq} values above 1 (Table 4) – meaning an excess of heterozygous individuals, perhaps due to selection or to negative assortative mating – while other loci and samples show low t_{eq} values – compatible with near fixation due to either drift, selection or positive assortative mating. Average t_{eq} values are above the estimated t_m, except in the managed *in situ* sample (Table 4). All samples have values above, but not significantly off, inbreeding equilibrium, suggesting more homozygous genotypes than expected.

Genetic structure and gene flow

Average values of Θ for individual loci range from 0.007 for *Pgm2* to 0.429 for *Aco3* (Table 5). These estimates indicate that a small to moderate amount of variation is due to subpopulation differentiation (0.7–42.9%), the remaining variation being found within subpopulations (99.3 – 57.1%). The average over loci differentiation between the managed *in situ*, wild and reference populations is 21%. Most estimates for individual loci were highly significant ($p < 0.001$), except f for *Pgm2* that was significant at $p = 0.05$. Positive values of both f and F indicate an overall excess of homozygous individuals in the loci examined, which may be due to selection, inbreeding or other causes.

The indirect estimate of N_m from the average over loci Θ value is 0.94 (Table 6). For individual loci, the N_m values range from 0.31 to 80.40. The greater values of correspond to loci *Pgm2* (80.40) and *Pgm1* (11.11). The values of N_m for the remaining loci are, in decreasing order: *Pgi2* (8.58), *Aco2* (6.35), *Aco1* (2.07), *Pgm3* (1.45), *Pgi3* (0.4) and *Aco3* (0.31) (Table 6). The indirect estimate of N_e from the mean N_m value was of 5.91 (Table 6).

Discussion

Genetic interpretation

This is the first report of allozyme markers in *L. e. esculenta*. Ideally, genetic interpretation requires crosses, but in some cases progeny segregation analysis may be sufficient (Stebbins 1989), and was the strategy followed here.

The finding of consistent duplication in all enzyme systems and in all *Leucaena* species studied (data to be published elsewhere) strongly suggests polyploidy to be the cause of duplications (Weeden and Wendel 1989), which is consistent with observed chromosome numbers (see Zárate 1994; Palomino et al. 1995). The apparent disomic inheritance observed suggests allopolyploidy (Weeden and Wendel 1989).

Including null forms with their corresponding active forms as a single allele should have no effect in the genetic analysis. This is because an individual heterozygous for the inactive or partially active form is heterozygous when this activity form is coded as active. Thus, the number of heterozygous individuals should not vary in either case. However, the classes of heterozygous–homozygous individuals would be greater if activity variants were recorded, i.e. N would increase. But, as discussed above, assigning these activity variants to different alleles would be unwarranted by the technique used.

Genetic variation

The proportion of polymorphic loci found in *L. e. esculenta* is biased by the enzymes recorded, all being polymorphic systems.

The estimate of the average number of alleles per locus is also biased because only polymorphic systems were analyzed, thus it is larger than that previously reported for other plants (Weeden and Wendel 1989; Eguiarte 1990). Estimation of H_e is affected by the number and polymorphism of enzyme systems used, and by sample size (Eguiarte 1990). In this study, H_e was calculated only for the polymorphic enzymes employed, and is higher than that reported previously for other plants (Eguiarte 1990). In general, H_e is larger as the sample size increases (managed *in situ* > wild > reference > Guerrero), except for that of the wild

Table 4. Values of outcrossing rate at inbreeding equilibrium (Hedrick 1983), per locus and estimated by the arithmetic mean (t_{eq}), for full samples and for subsamples [designated as (*t*)] used for estimation of t_m and t_s (see Appendix 1).

Sample	*Aco1*	*Aco2*	*Aco3*	*Pgi2*	*Pgi3*	*Pgm1*	*Pgm2*	*Pgm3*	t_{eq}	t_{meq}
Managed *in situ*	0.341	0.807	0.211	0.761	0.553	0.626	0.575	1.083	0.619 (0.090)	0.578
Wild	1.032	0.106	1.079	0.897	1.066	0.339	1.355	0.529	0.800 (0.142)	0.698
Reference	1.166	–	–	0.548	0.386	0.111	0.634	1.092	0.656 (0.152)	0.571
Managed *in situ* (*t*)	–	–	–	0.635	0.695	0.885	0.614	1.090	0.784 (0.081)	0.767
Wild (*t*)	–	–	–	0.988	0.654	0.383	1.198	1.026	0.850 (0.131)	0.798

The weighted average of *F* was used in calculation of t_{meq}. In parentheses, standard error of means.

Table 5. Wright's *F*-statistics (Weir and Cockerham 1984) for wild, managed *in situ* and reference samples estimated by program TFPG per locus (see Appendix 1).

Locus	*f*	*F*	Θ
Aco1	0.458***	0.347***	0.066***
Aco2	0.267***	0.432***	0.088***
Aco3	0.615***	0.753***	0.429***
Pgi2	0.130*	0.161**	0.027***
Pgi3	0.214***	0.455***	0.297***
Pgm1	0.414***	0.564***	0.021***
Pgm2	0.161**	0.133**	0.007*
Pgm3	0.180***	0.201***	0.061***
Average	0.313	0.460	0.210
95% C.I.	0.254–0.371	0.370–0.551	0.133–0.287

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
Means were calculated by Jacknifing over loci. Chi-square analyses for significance of difference from 0 of F_{IS} and F_{IT}, according to Li and Horvitz (1953), the same of F_{ST}, according to Workman and Niswander (1970).

Table 6. Values of indirect estimates of gene flow (N_m) from F_{ST} values (Crow and Aoki 1984) and effective population size (N_e) (Slatkin and Barton 1989) for samples from the wild, managed *in situ* and reference populations (see Appendix 1).

Loci	*Nm* (Θ)
Aco1	2.07
Aco2	6.35
Aco3	0.31
Pgi2	8.58
Pgi3	0.40
Pgm1	11.11
Pgm2	80.40
Pgm3	1.45
Mean N_M	0.94
N_e	5.91

All Θ values used in calculations are highly significant at $p = 0.001$, except for *Pgm2* significant at $p = 0.05$ sample group Montaña.

subsample used for mating system analysis. A similar trend is observed in the SE, which increases as the sample size decreases, except in the case mentioned. The observed number of heterozygous individuals (H_o) is smaller than the corresponding H_e values in all samples, except in the Guerrero reference sample, once more, this may be a function of sample size. The sample that deviates most from Hardy–Weinberg equilibrium is that from the managed *in situ* population.

Fixation indexes

The sample size and the number of loci analyzed were seen to bias the estimates of fixation indexes. This fact is seen in the comparison of the F_M values derived from the whole sample (Table 2) with that for the subsamples analyzed for mating system (managed *in situ*, 0.132 ± 0.054, wild, 0.112 ± 0.097; cf. Table 2). The average over loci fixation index estimates for progenies in all samples analyzed are positive, thus indicating an excess of homozygous plants; however inferred average over loci fixation

indexes for the parents are negative. A similar situation was found in *Bertholletia excelsa* Humb. et Bonpl. by O'Malley et al. (1988). Such differences may be due to selection favoring heterozygous trees, acting in post-germination stages of the life cycle, thus constraining the survival of homozygous individuals, i.e. heterosis. The action of selection over individual loci is also suggested by negative F_I values in certain enzyme loci. Other possible factors involved may relate to the mating system, such as negative assortative mating.

Genetic identities

Phenogram in Figure 2A shows the genetic identity estimated between the managed *in situ*, wild and regional population samples. This result is consistent with Θ values, family and individual grouping, and inferred maternal genotype differences between managed *in situ* and wild samples. As mentioned above, the managed *in situ* and the wild samples appear to be more similar to the reference sample group when the samples from Puebla and Morelos are included in the reference sample. The low number of individuals in the families from this latter group may explain this fact.

When the phenogram is plotted using the same estimates but pooling the individuals per family instead of per population, most families from the managed *in situ* and wild populations group together, except one wild family (W7), which clusters among the managed *in situ* families (Figure 2B). These results agree with those obtained by Casas and Caballero (1996) based on morphology.

Ecotypic differentiation

Since it is usually assumed that genetic markers used are independent from phenotypic variables selected by people, this differentiation may be interpreted as the result of ecotypic differentiation upon which artificial *in situ* selection could have acted during the prolonged management history. Alternatively, the assumed lengthy management process in the study site (Casas and Caballero 1996) may have, by itself, caused differentiation. One ecological difference between the managed *in situ* and the wild populations is altitude. This result is consistent with previous ethnobotanical and morphological data suggesting that high quality *guaje rojo* trees originate in the higher altitude regions within the geographic range for the taxon (Zárate 1994, 1998). Thus, while the fact that morphological change in the populations may be due to prolonged selection as suggested by Casas and Caballero (1996), this selection may have been aided by – and acted upon – ecotypic differentiation related to altitude. Consequently, families from the managed *in situ* managed *in situ* and unmanaged wild populations differ in their putative genetic composition in a similar way as they were shown to differ in their morphology (Casas and Caballero 1996). Furthermore, if ecotypic differentiation is relevant for the domestication process of *Leucaena*, dispersal of locally selected high-quality trees must have been frequent.

Mating system

The results are, in general, consistent with sexual mating, and were expected from the observed allelic segregation. Furthermore, since diploid species of *Leucaena* are known to be self-incompatible (Sorensson and Brewbaker 1994), departures from the mixed-mating model (Ritland 1983) are expected. The significant difference $t_m - t_s$ indicate biparental inbreeding, i.e. the crossing of related plants. According to these results, the biparental inbreeding is larger in the wild population sample. However, the F_M value (Table 2) is larger in the managed *in situ* sample, which may be due to selection, drift or both. It is likely that these estimates relate more to biparental inbreeding than to selfing.

Similarities of t in both managed *in situ* and wild samples may be interpreted to suggest that the mating system is little affected by either the management history or the ecotypic differences. However, the most critical effect of management (shift-cultivation of ancient clearings, gathering and selective tolerance) is on the population density (15 individuals/ha in the wild and 0.8 individuals/ha in the managed *in situ* populations). However, despite that outcrossing estimates are similar at both densities, inbreeding seems to be stronger in the wild population.

Genetic structure

The observed positive values of *f* indicate differentiation due to local inbreeding, which is also suggested by the analysis of the mating system. The observed range of *f*-values is considerably higher than that known for other tropical trees (Eguiarte 1990), except for the fig tree (*Ficus carica* L.) ($F_{IS} = 0.28$) (Valizadeh 1977). If any, the outstanding resemblance between *Leucaena* and the fig tree are the numerous minute florets and, most likely in the former (Zárate 1994), pollination by small insects. Values of *F* observed indicate about 23–40% differentiation due to either genetic drift or inbreeding. Per locus variation of Θ indicates that selection is contributing to local differentiation, rather than drift, particularly at *Aco3* and *Pgi3*.

The genetic structure observed in the *guaje rojo* agrees with that found in other tropical trees such as *Pithecellobium pedicellare* (DC.) Benth. (O'Malley and Bawa 1987) and *Bertholletia excelsa* (Buckley et al. 1988; O'Malley et al. 1988). These tropical trees are typically outcrossing, have low densities, high genetic variation and a marked population structure, mainly due to inbreeding. Self-incompatibility, dispersion, mechanisms such as negative assortative mating, and heterosis compensate inbreeding and low density. In olive trees Ouazzani et al. (1993) found a correlation between vigor and heterozygosity in a locus of esterase, which probably is common to other enzyme systems and tree species. Genetic structure of human-dispersed trees may be lower than that expected from values of *t*, as is suspected in *Bertholletia excelsa* in Brazil (Buckley et al. 1988).

In the case studied here, the finding of genetic differences between populations subject to a long history of distinct forms of management is consistent with *in situ* selection (elimination–toleration), migration, and *in situ* dispersal of favored genotypes. The genetic differences of the managed *in situ* sample respect to the wild and reference samples may also suggest migration of high-quality trees. This is in agreement with the hypothesis that preferred cultigens of *L. e. esculenta* come from certain regions along the upper fringes of the Balsas River Basin, and stresses the known correlation of altitude with high-quality *guaje rojo* (Zárate 1994). The genetic structure observed is consistent with known reproductive biology of *Leucaena*, characterized in natural populations by slow dispersal – aided by gravity and runoff, and – in diploids – by self-incompatibility. In people-dispersed and selected trees, inbreeding should increase considerably in both managed *in situ* stands as in seed-propagated tree groves due to the reduction of the population effective number.

Population structure and gene flow

The low values of estimated N_m observed agree with moderate (but significant) values for Θ thus suggesting limited gene flow between populations. This implies that differentiation is not due to gene flow but to drift, selection or ecotypic differences. However, a low value of N_m may imply either limited gene flow, or low effective number. High values of N_m observed for certain loci suggest that a certain amount of gene flow may be constraining population structure, thus the effective number is expected to be low. Selection may be acting to increase migrant proportion of some but not other loci. Estimations of N_e are low compared to those in other tropical trees (Eguiarte 1990).

A model of domestication of seed-propagated trees

Plant domestication, in general, is here considered as the process of interaction of people with plants during a period of time, leading to evolutionary changes in managed plant populations. This process involves several degrees of management purposefulness (inventiveness) and intensity (amount of work invested), and it is closely related with a given economic (sustenance), and cultural relevance (signification) (Zárate 1998, 1999). From the genetic perspective, tree domestication is influenced by the length of the plant life cycle, which makes intensive selection more difficult than in annual plants. Also, typically, by open pollination and high levels of genetic variation, which make seed propagation unrewarding, or at best, a slow selection procedure (Torres 1989; Casas et al. 1997). Thus, except for grafting and vegetative propagation, tree domestication should be a difficult and lengthy process (Spiegel-Roy 1985). In the

Old World, tree domestication occurred mainly through asexual propagation, until the discovery and diffusion of grafting (Zohary and Hopf 1988). However, in the New World, tree cultivation tradition makes use of seed propagation (Smith 1966, 1968; Zárate 1998).

The results presented here suggest that the inconveniences of sexual reproduction – such as segregation, crossing with wild trees and length of time needed for quality assessment – may be avoided to some extent by traditional cultivators of trees such as the *guaje rojo*. Local land races may be the result of gradual molding of populations of introduced high quality trees, and of local selected trees, in interaction with local environmental conditions. As an alternative, seed propagation may be equivalent to vegetative propagation due to agamospermy. In this latter case, fixation of characters is achieved by merely planting. Extreme cases would be those in which a fixed lineage becomes established in the seed bank, making planting totally unnecessary, such as in the *guaje colorado* [*L. esculenta* subsp. *paniculata* (Britton et Rose) Zárate] in Chapulco, Puebla (Zárate 1994). In the case of Alcozauca, high-quality trees are introduced from other regions – such as from Cuernavaca, Morelos (Casas and Caballero 1996), which seems better than planting seeds from the open pollinated good quality trees from either the managed *in situ* and the wild populations, or from other cultivated stands. In practice, all these procedures have been observed to happen and, thus are believed to contribute to land race differentiation and adaptation to local environmental and cultural conditions.

Another example of an American seed-propagated tree that seems to also conform to the domestication model assigned here to the *guaje rojo* is the avocado (*Persea americana* Mill.). While the vegetative propagation in avocado came to be practiced only since a few years before 1900, traditional cultivators in Mexico and Central America, through selection of open-pollinated seeds achieved such quality, which modern breeders have not been able to surpass. Popenoe (1919, quoted by Bergh 1975) envisioned three main processes involved in the selection of open-pollinated avocado trees: cutting of undesired trees; planting of high-quality trees; the selling of high-quality fruit, which resulted in the dissemination of their genotypes to other orchards.

The role of dispersal

In the domestication process of *L. e. esculenta* dispersal must have been, and still is, playing a definite role because of the long life cycle, the high rates of outcrossing, and segregation. Likewise, dissemination is relevant because inbreeding will cause a loss in quality of local populations due to inbreeding depression or adaptive selection processes acting together with artificial selection. However, parallel to diffusion, independent cultivation of stands along the distribution range of wild populations, and even along the range of cultivation (planting) increases genetic variation (Blumler 1992), differentiation, and adequacy of cultigens to a range of environments. This complements with gene flow across land races and even subspecies or species (Hughes and Harris 1994).

Conclusions

The multidisciplinary study of the process of domestication of the *guaje rojo* (Zárate 1994, 1997, 1998, 1999, 2000), including the present results, gives support to the occurrence of an extensive historical selection, local *in situ* intensification and dispersion of better quality trees, a model that could be extensive to other tropical seed-propagated trees. In particular, these results suggest that populations of *L. e. esculenta* are subject to adaptive selection at local level, which may be caused by polygenic structure and heterosis, aided by some gene flow. While inbreeding due to mating among relatives seems to be present, reproductive mechanisms and selection oppose it. Thus, cultivation must count with adequate founding lineages to start with, and maintain quality by selective management *in situ* and by dispersal. This evolutionary scenario agrees with findings in other tropical trees (Eguiarte et al. 1992). Overall, in the managed *in situ* population, the process of management seems to have involved several steps: (1) ecotypic differentiation; (2) decrease in density by selective clearing and (3) continued selective management *in situ* and diffusion of high-quality trees.

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Appendix 1

Collection data of mother trees whose half-sib families were sampled.

Collection number	Population number[a]	Elevation (m)	Number of seeds screened	Locality	Management
Viveros and Casas 605	W1 (W1)*	1430	12	25 km W of Alcozauca. Mun. Alcozauca, Gro.	Wild in well preserved tropical deciduous forest; gathered
Viveros and Casas 607	W2 (W2)*	1440	14	25 km W of Alcozauca. Mun. Alcozauca, Gro.	Wild in well preserved tropical deciduous forest; gathered
Viveros and Casas 610	W3 (W3)*	1400	5	25 km W of Alcozauca. Mun. Alcozauca, Gro.	Wild in well preserved tropical deciduous forest; gathered
Viveros and Casas 612	W4 (W4)*	1400	10	25 km W of Alcozauca. Mun. Alcozauca, Gro.	Wild in well preserved tropical deciduous forest; gathered
Viveros and Casas 615	W5 (W5)*	1375	5	25 km W of Alcozauca. Mun. Alcozauca, Gro.	Wild in well preserved tropical deciduous forest; gathered
Viveros and Casas 606	W6 (W10)	1425	20	25 km W of Alcozauca. Mun. Alcozauca, Gro.	Wild in well preserved tropical deciduous forest; gathered
Viveros and Casas 608	W7 (W11)*	1435	16	25 km W of Alcozauca. Mun. Alcozauca, Gro.	Wild in well preserved tropical deciduous forest; gathered
Viveros and Casas 611	W8 (W13)*	1400	10	25 km W of Alcozauca. Mun. Alcozauca, Gro.	Wild in well preserved tropical deciduous forest; gathered
Viveros and Casas 613	W9 (W14)	1380	5	25 km W of Alcozauca. Mun. Alcozauca, Gro.	Wild in well preserved tropical deciduous forest; gathered
Viveros and Casas 614	W10 (W15)*	1375	16	25 km. W of Alcozauca. Mun. Alcozauca, Gro.	Wild in well preserved tropical deciduous forest; gathered
Viveros and Casas 618	W11 (W16)*	1360	4	25 km W of Alcozauca. Mun. Alcozauca, Gro.	Wild in well preserved tropical deciduous forest; gathered
Viveros and Casas 619	W12 (W17)	1360	15	25 km. W of Alcozauca. Mun. Alcozauca, Gro.	Wild in well preserved tropical deciduous forest; gathered
Viveros and Casas 620	W13 (W18)	1360	4	25 km W of Alcozauca. Mun. Alcozauca, Gro.	Wild in well preserved tropical deciduous forest; gathered
Viveros and Casas 622	W14 (W20)	1360	4	25 km W of Alcozauca. Mun. Alcozauca, Gro.	Wild in well preserved tropical deciduous forest; gathered

Appendix 1. Continued.

Collection number	Population number[a]	Elevation (m)	Number of seeds screened	Locality	Management
Viveros and Casas 599	M1 (M2)*	1580	15	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 635	M2 (M5)*	1460	16	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 638	M3 (M6)*	1450	12	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 640	M4 (M7)*	1450	16	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 642	M5 (M14)	1420	5	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 643	M6 (M8)*	1420	30	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 595	M7 (M11)	1590	3	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 596	M8 (M12)	1580	4	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 597	M9 (M1)	1580	10	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 598	M10*	1580	15	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 601	M11*	1530	33	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 629	M12*	1530	16	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 634	M13 (M13)	1500	27	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 636	M14 (M17)	1450	16	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 637	M15	1450	2	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 639	M16 (M18)*	1440	15	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 641	M17 (M19)*	1430	27	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 644	M18 (M20)	1420	10	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered

Appendix 1. Continued.

Collection number	Population number[a]	Elevation (m)	Number of seeds screened	Locality	Management
Viveros and Casas 645	M19*	1420	5	Slopes W of Alcozauca, Mun. Alcozauca, Gro.	Managed *in situ* in shifting cultivated maize fields; gathered
Viveros and Casas 572	R1	1350	20	Tlalixtlaquilla-Tlapa Mun. Pozo Blanco, Gro.	Wild in tropical deciduous forest; gathered
Viveros and Casas 579	R2	1500	2	Temalacacingo, Gro.	Cultivated in field with no irrigation
Arellano et al. 21	R3	1700	2	5 km N Santa Cruz Nuevo. Mun. Totoltepec, Pue.	Cultivated in field; seed provenance: San Vicente Coyotepec
Arellano et al. 23	R4	1850	5	7.8 km NW Santa Cruz Nuevo. Mun. Totoltepec, Pue.	Wild, probably escaped by run-off from San Juan Ixcaquixtla
Arellano et al. 25	R5	1850	5	7.8 km NW Santa Cruz Nuevo. Mun. Totoltepec, Pue.	Wild, probably escaped by run-off from San Juan Ixcaquixtla
Arellano et al. 44	R6	1800	4	10 km NW Teloxtoc. Mun. Atexcatl, Pue.	Cultivated in maize field border; seed provenance Atexcatl
Arellano et al. 27	R7	1850	4	San Juan Ixcaquixtla, Pue.	Cultivated in dense pure stand
Arellano et al. 28	R8	1850	2	San Juan Ixcaquixtla, Pue.	Cultivated in dense pure stand
O. Dorado unnumbered (1)	R9	–	2	Road Tlayacac-Xalostoc, Mor.	–
O. Dorado unnumbered (2)	R10	–	2	Road Tlayacac-Xalostoc, Mor	
O. Dorado unnumbered (3)	R11	–	2	Road Tlayacac-Xalostoc, Mor	
O. Dorado unnumbered (4)	R12	–	1	Road Tlayacac-Xalostoc, Mor	

[a] Vouchers deposited in the Herbario Nacional de México (MEXU).
[b] Arbitrary numbers, W = wild population from San José Laguna; M = managed *in situ* population from Alcozauca de Guerrero; *R* = reference samples. In parentheses, equivalent identification numbers from Casas and Caballero (1996). Asterisks indicate the individuals included in the subsamples Wild (*t*) and Managed *in situ* (*t*), used in mating system analysis.

Appendix 2

Allelic frequencies per locus, per population sample (see text and Appendix 1). In parentheses, number of individuals per locus.

Sample	Locus Allele	*Aco1*	*Aco2*	*Aco3*	*Pgi2*	*Pgi3*	*Pgm1*	*Pgm2*	*Pgm3*
Managed *in situ*		(112)	(105)	(117)	(211)	(211)	(226)	(222)	(226)
	1	0.196	0.762	0.402	0.810	0.218	0.204	0.739	0.958
	2	0.804	0.238	0.568	0.190	0.765	0.794	0.243	0.038
	3	–	–	0.030	–	0.017	0.002	0.016	0.004
	4	–	–	–	–	–	–	0.002	–
Wild		(62)	(46)	(54)	(112)	(112)	(109)	(108)	(105)
	1	0.016	0.870	0.963	0.888	0.763	0.275	0.792	0.686
	2	0.984	0.130	–	0.112	0.232	0.725	0.208	0.157
	3	–	–	0.037	–	0.004	–	–	0.152
	4	–	–	–	–	–	–	–	0.005

Appendix 2. Continued

Sample	Locus Allele	*Aco1*	*Aco2*	*Aco3*	*Pgi2*	*Pgi3*	*Pgm1*	*Pgm2*	*Pgm3*
Reference		(7)	(7)	(7)	(51)	(50)	(47)	(49)	(45)
	1	0.071	1	1	0.941	0.130	0.309	0.776	0.811
	2	0.929	–	–	0.059	0.810	0.606	0.163	0.144
	3	–	–	–	–	0.060	0.064	–	0.044
	4	–	–	–	–	–	0.021	0.061	–
Managed *in situ* (*t*)		–	–	–	(164)	(164)	(159)	(159)	(159)
	1	–	–	–	0.805	0.238	0.201	0.761	0.956
	2	–	–	–	0.195	0.756	0.799	0.239	0.041
	3	–	–	–	–	0.006	–	–	0.003
Wild (*t*)		–	–	–	(102)	(101)	(103)	(102)	(102)
	1	–	–	–	0.863	0.693	0.301	0.789	0.446
	2	–	–	–	0.137	0.307	0.699	0.211	0.373
	3	–	–	–	–	–	–	–	0.181

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