Genetic Resources and Crop Evolution (2005) 52: 525–538
DOI 10.1007/s10722-003-6012-x

Population genetics of *Escontria chiotilla* in wild and silvicultural managed populations in the Tehuacán Valley, Central Mexico

Alma Tinoco, Alejandro Casas*, Rosaura Luna and Ken Oyama
*Instituto de Ecología, UNAM (Campus Morelia), Apartado Postal 27–3 (Xangari) 59084, Morelia, Michoacán, Mexico; *Author for correspondence (e-mail: acasas@oikos.unam.mx)*

Received 29 November 2002; Accepted in revised form 28 June 2003

Key words: Columnar cacti, Crop evolution, Domestication, *Escontria chiotilla*, Genetic resources, Population genetics, Tehuacán Valley

Abstract

Escontria chiotilla is a columnar cactus that grows in the arid and semiarid lands of Central Mexico and produces edible fruit with economic. In the wild, this plant species is distributed as part of thorn-scrub and tropical deciduous forests, but in the Tehuacán Valley also occurs in silvicultural managed *in situ* populations, in which people practise artificial selection enhancing phenotypes with larger fruits. The population genetics of wild and managed populations was studied to analyse the effects of management on genetic structure of *E. chiotilla*. A total of 150 individuals from six populations were studied, analysing 13 loci for eight enzymes by starch gel electrophoresis. The genetic variation in wild populations was significantly higher than in managed populations (H_o = 0.079, H_e = 0.134, H_T = 0.370, and H_o = 0.052, H_e = 0.110, H_T = 0.298, respectively), indicating that silvicultural management has caused a reduction of the genetic variation in populations. Most of the genetic variation in both wild and managed populations occurs within populations (D_{ST} = 0.027 in the wild and 0.018 in managed populations). The genetic distance coefficients were slightly different for silvicultural managed populations than in wild ones, illustrating an incipient effect of management on the genetic structure of populations. However, values of Nm_{GST} = 3.845 and Nm_{FST} = 3.848 indicate that a high gene flow counteracts the effects of human selection on the differentiation of populations.

Introduction

Domestication is an evolutionary process through which humans model morphological, physiological and behavioural variation in populations of organisms for economic and cultural purposes (see Harlan 1992; Casas and Barbera 2002), so that these manipulated diverge genetically from non-manipulated wild populations. Domestication is commonly carried out in anthropogenic environments seperate from the parental wild populations of a given species (management *ex situ*), but in some regions of Mexico indigenous peoples practise forms of silvicultural management or management *in situ*. Such practices involve manipulation of plant populations within their wild environment, and may even include processes of domestication (Colunga et al. 1986; Casas and Caballero 1996; Casas et al. 1997a, 1999b). Processes of this type have been recently documented in some species of columnar cacti (Casas et al. 1997b, 1999a,b,c; Luna and Aguirre 2001; Rojas-Aréchiga et al. 2001; Hammer 2001; Cruz and Casas 2002; Arellano and Casas 2003; Carmona and Casas 2003, in press; Otero-Arnaiz et al. 2003) and these plants appear to be interesting cases for to study in order to understand such processes.

The South-Pacific Drainage of Mexico, which comprises part of the basins of the Balsas and Papaloapan rivers, has been identified as the area with the greatest species variety of columnar cacti in the world (Valiente-Banuet et al. 1996), containing 40 of the 75 species of this group of plants existing in Mexico. All species of columnar cacti are used by indigenous peoples as food, fodder and medicine, as well as for construction materials, fuel, and living fences (Casas et al. 1999c; Hammer 2001). Among these species *Escontria chiotilla* (F.A.C. Weber) Rose is one of the most economically important, since its fruits are widely consumed by the people of the region where it occurs and have important commercial value in the regional markets (Arellano and Casas 2003).

Arias et al. (1997) described *E. chiotilla* as an arboreal columnar cactus that attains a 7-m height, has numerous branches twisted when old, is dark green in color, and possesses 7–8 ribs, and areoles with 10–15 spines. Flowers are yellow, at the top of the branches, funnel form, 3 cm long including the ovary. The pericarpel and flower tube have papiraceous trans-lucid scales. Fruits, called 'jiotilla', are brownish red with sweet edible pulp and black seeds with rouge test. *E. chiotilla* forms part of the plant associations called 'jiotillales' or 'quiotillales' (Rzedowski 1978; Valiente-Banuet et al. 2000), which are thorn-scrub and tropical deciduous forests characterised by high densities of this species, possibly comprising more than 300 individuals per hectare (Valiente-Banuet et al. 2000). *E. chiotilla* occurs in arid and semiarid areas of the states of Guerrero, Oaxaca, Morelos and Michoacán (Bravo-Hollis 1978).

In the Tehuacán-Cuicatlan Valley, our study area in the states of Puebla and Oaxaca, 'jiotillales' may include other columnar cacti such as *Pachycereus weberi* (J. Coulter) Backeb., *P. hollianus* (F.A.C. Weber) F. Buxb., *Myrtillocactus geometrizans* (Mar-tius) Console, *Neobuxbaumia tetetzo* (F.A.C. Weber) F. Buxb., and *Stenocereus stellatus* (Pfeiffer) Riccob. *S. pruinosus* (Otto) F. Buxb. (Valiente-Banuet et al. 2000). In this region *E. chiotilla* is under management *in situ*, carried out on sites originally occupied by wild populations and involing the sparing and enhancing of individuals growing in perturbed areas. This form of management has been characterised as silvicultural management and commonly involves artificial selection favouring survival and reproduction of 'better' phenotypes as defined by people according to utilitarian values (Casas et al. 1997a,b; Arellano and Casas 2003). Considering such forms of management, we have hypothesised that *E. chiotilla* is undergoing a process of domestication.

Arellano and Casas (2003) documented that local people perceive morphological variation in the populations of this species and manage such variation, differentially favouring a number of desirable phenotypes under silvicultural management, as this species is not cultivated. These authors performed biometric studies to evaluate the effect of artificial selection on managed populations, and found that there are significant differences among wild and *in situ* managed populations, with individuals in the latter group having larger fruits with more pulp and more and larger seeds. Morphometric studies strongly suggest that artificial selection under *in situ* management has a significant consequence in the phenotypic structure of populations (Arellano and Casas 2003).

Oaxaca (2003) studied if artificial selection has caused any changes in the pollination mechanisms and/or breeding systems of *E. chiotilla* and if such changes have erected any barriers to pollen flow among wild and managed populations. This author found that, in both wild and managed populations, the breeding system is self-incompatible. Also, the author found that in all populations studied pollination is conducted by bees (*Bombus pensylvanicus* De Geer, *Xylocopa mexicanorum* Cockerell, *Plabeia mexicana* Ayala, *Apis mellifera* L.), and hummingbirds (*Amazilia violiceps* Gould, *Cinanthus sordidus* Gould, and *C. latirostris* Gould). This information indicates that artificial selection has not changed the reproductive biology and suggests that there are not any spatial barriers to pollen flow among wild and managed population, since distances between the populations studied were well within the ratios of movement of the pollinators. Oaxaca (2003) also found that blooming periods overlap in all populations and that, therefore, temporal barriers to pollen flow are also unlikely. Therefore, any effects of artificial selection could be strongly counteracted by gene flow among populations.

In this study, we analysed population genetics in wild and *in situ* managed populations in order to examine the effects of human manipulation on

the genetic structure of populations and the dissimilarity between wild and managed populations, as well as to estimate the extent of gene flow among those types of populations. The main hypothesis of our study was that, if artificial selection favouring abundance of particular phenotypes was significant, it would reduce genetic diversity and alter allele frequencies in managed populations, causing genetic differentiation among wild and managed populations. However, since gene flow among populations can be expected to be high, genetic differentiation between wild and managed populations was expected to be relatively low.

Materials and methods

Study area

The Tehuacán-Cuicatlán Valley is located at the southeast corner of the state of Puebla and in the northeast part of the state of Oaxaca (Figure 1). Surface area of the region is nearly 10000 km^2 with an elevation range from 500 to 3200 m, mostly having an arid and semi arid climate. Annual mean precipitation is from 300 to 900 mm whereas average temperature varies from 14 to 26°C per year (García 1981). Valiente-Banuet et al. (2000) describe 29 types of plant associations for the region, and Dávila et al. (2002) report nearly 3000 plant species. These authors consider the Tehuacán-Cuicatlán Valley as one of the most important reservoirs of biodiversity for the arid and semiarid areas of Mexico.

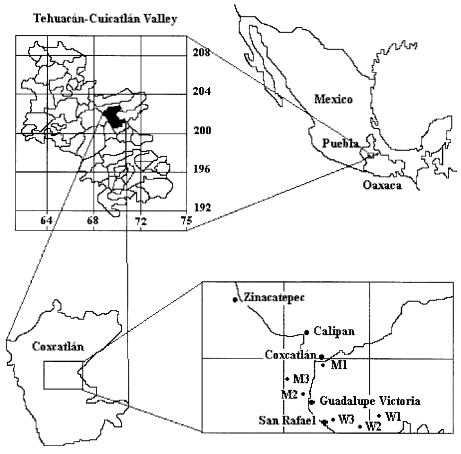

Figure 1 The study area. The Tehuacán-Cuicatlán Valley and the studied wild (Wl, W2 and W3) and managed *in situ* (M1, M2 and M3) populations of *Escontria chiotilla*.

Populations of Escontria chiotilla being studied

The study populations are located within the territories of the villages of San Rafael, Guadalupe Victoria and Coxcatlán, in the municipality of Coxcatlán, Puebla (Figure 1). Three wild populations were studied within the communal land of the village of San Rafael, about 10 km southeast of Coxcatlán, in the alluvial valley in front of the 'Maize Cave', the important archaeological site explored by MacNeish (1967). Wild populations consist of patches of vegetation, settled on soils derived from sandy stones and with elements characteristic of the tropical deciduous and thornscrub forests. *Escontria chiotilla* is one of the dominant components of the vegetation, along with *Bursera morelensis* Ramírez and *B. arida* (Rose) Standley (Burseraceae), the columnar cacti *Myrtil-locactus geometrizans, Pachycereus weberi*, and *P. hollianus, Gyrocarpus mocinoii* Espejo (Hernan-diaceae), *Acacia cochliacantha* Humb. and Bonpl. Ex Willd., *A. constricta* Benth., and *Mimosa luisana* Brandegee (Mimosaceae), *Ipomoea arborescens* G. Donn (Convolvulaceae), *Agave macroacantha* (Agavaceae), as well as *Stenocereus stellatus* and *S. pruinosus*.

Wild population 1 (Wl) is located just in front of the 'Maize Cave', wild population 2 (W2) is 2 km south of Wl, in the direction of the village of San Rafael, and wild population 3 (W3) is 3 km south of W2 and 5 km south of Wl, near the village of San Rafael (Table 1).

In addition, three silvicultural managed populations were studied within the territory of the villages of Coxcatlán, Guadalupe Victoria and San Rafael. These *in situ* managed populations are found in areas of maize cultivation, subject to cycles of use and fallow, where *Escontria chiotilla* and *Stenocereus stellatus* are spared during clearing. Other abundant species are *Opuntia pilifera* F.A.C. Weber, *Mimosa luisana*, and *Acacia cochliacantha*. Managed population 1 (M1) is 6 km south of the village of Coxcatlán, whereas managed population 2 (M2) is 4 km southwest of M1, and managed population 3 (M3) is 4 km east of M2 and 7 km southeast of M1 (Table 1).

Table 1. Spatial distances (in km) between the studied populations of *Escontria chiotilla.*

Population	Wild 1	Wild 2	Wild 3	Managed 1	Managed 2	Managed 3
Wild 1	0					
Wild 2	2	0				
Wild 3	5	3	0			
Managed 1	12	11	10	0		
Managed 2	13	12	10	4	0	
Managed 3	8	7	5	7	4	0

Sampling of populations

All the populations were sampled along quadrants 10 m wide and 50 m or more long. The length of the quadrants was decided in each population in order to include at least 25 individuals per population. Each individual plant was labelled to easily identify them in further visits, since the same individuals were analysed in morphometric (Arellano and Casas 2003) and reproductive biology (Oaxaca 2003) studies.

Plant tissue for allozyme analysis was obtained from flower buds, since preliminary tests demonstrated that these plant structures have lower amounts of mucilage while manifesting the appropriate enzyme activity. An average of three flower buds were collected per individual sampled, frozen in liquid nitrogen, and then stored in a REVCO freezer at -80°C.

Allozyme analysis

For extraction of enzymes, a solution composed of three parts of the extraction buffer developed by Cheliak and Pitel (1984) per one of the extraction buffer developed by Yeh and O'Malley (1980) was used. Plant tissue was ground in frozen mortars immersed in ice, and three to five drops of extraction buffer were added. Samples were absorbed in filter paper wicks, and stored in eppendorf tubes at -4 °C.

The gel and electrode buffers systems used are shown in Table 2. The gels were prepared using 60 g of potato starch (Starch art) and 15 g of sucrose in 500 ml of gel buffer. Electric current (a constant 40 mA current, while a record was kept of the corresponding voltage) was applied for 30 min before removing wicks from the gels. Then, current was applied for 5 h. Enzymes analysed in the corresponding buffer system are shown in Table 2. Interpretation of the gels was conducted as soon as the optimum staining was achieved. Stained gels were finally washed with distilled water and fixed with 50% ethanol for 24 h.

Statistical analyses

Levels of genetic variation within and among populations of *Escontria chiotilla* were estimated from allelic frequencies, according to individual genotypes recorded (Appendix 1), using the programmes POPGENE version 1.21 (Yeh et al. 1997), TFPGA version 1.3 (Miller 1997), and Biosys-1 (Swofford and Selander 1981). This method provided information on the number of alleles per population, allele frequencies, the number of alleles per locus (A), the percentage of polymorphic loci (P%), the observed heterozygosity by counting (H_o), and the expected heterozygosity (H_e) calculated as $H_e = 1 - \Sigma p_i^2$ where p_i is the frequency of each allele in a locus. A polymorphic locus was considered when frequency of the most common allele was ≤ 0.95 (Swofford and Selander 1981). Fixation indexes (F) were estimated for polymorphic loci, as well as the significance of deviations between observed and expected frequencies. Fixation index (F) estimates the proportion of increas-

Table 2. Gel and electrode buffers systems and the corresponding analysed enzymes.

Buffers system	Enzymes
Mayze C system (Stuber et al. 1988)	G-6PD Glucose 6-phosphate dehydrogenase MNR Menadione reductase PGI Phospho-glucose isomerase PGM Phospho-gluco-mutase
Morfolin-Citrate system (Wendel and Weeden 1989)	ACPH Acid phosphatase IDH Isocitrate dehydrogenase 6-PGD 6-Phosphogluconate dehydrogenase

ing or decreasing heterozygosity in a population in relation to the expected heterozygosity. Therefore, these indexes were calculated in order to analyse excess of homozygous individuals due to non random mating or excess of heterozygous individuals due to assortative mating. The genetic diversity or the proportion of heterozygous loci expected in individuals randomly chosen within and between populations of *E. chiotilla* was estimated at three levels: (1) total genetic diversity, H_T; (2) genetic diversity within populations, H_S; and (3) genetic diversity between populations D_{ST}. The genetic differentiation coefficient G_{ST} was calculated among all populations and among wild and *in situ* managed populations. Wright's F statistics were also calculated for polymorphic loci in all populations. The relation between these statistics is given by the equation $1 - F_{IT} = (1 - F_{IS})(l - F_{ST})$, where F_{IS} and F_{IT} are the correlations among pairs of alleles in an individual, in relation to subpopulations and the total population, respectively. Both indexes estimate the reduction in the number of heterozygous individuals due to non-random mating among individuals within subpopulations and in the total population, respectively. Parameter F_{ST} estimates the correlation between two alleles randomly chosen from each population and accounts the extent of genetic differentiation. F_{IS} and F_{IT} may have negative values, whereas F_{ST} always has a positive value. Matrices of dissimilarity among populations were calculated by Nei's minimum distance index (Nei 1972) and Nei's unbiased minimum distance (Nei 1978). UPGMA dendrograms were also obtained to visualize patterns of dissimilarity among populations. Indirect estimations of gene flow were conducted by the parameters Nm_{GST} and Nm_{FST}, assuming that $Nm \geq 1$ indicate a high gene flow (Slatkin 1985).

Results

Genetic variation

A total of 13 loci for eight enzymes (6-PGD, PGI, PGM, IDH, MDH, ACPH, MNR, and G-6PD) were analysed, five of them being polymorphic in all populations, and eight being monomorphic in all populations. Table 3 summarizes the information on the analysed loci and allele frequencies recorded in the studied populations.

Table 3. Allele frequencies for the 13 loci analysed in wild and managed *in situ* populations of *Escontria chiotilla* in the Tehuacán Valley.

Locus	Allele	Population					
		W1	W2	W3	M1	M2	M3
6-pgd2	*A*	0.259	0.226	0.422	0.133	0.103	0.040
	B	0.707	0.774	0.578	0.733	0.828	0.900
	C	0.034	0.000	0.000	0.133	0.069	0.060
Pgil	*A*	1.000	1.000	1.000	1.000	1.000	1.000
Pgi2	*A*	0.397	0.387	0.547	0.267	0.155	0.580
	B	0.603	0.613	0.453	0.733	0.845	0.420
Pgm1	*A*	1.000	1.000	1.000	1.000	1.000	1.000
Pgm2	*A*	0.086	0.016	0.453	0.267	0.155	0.140
	B	0.914	0.984	0.547	0.733	0.845	0.860
Idh1	*A*	1.000	1.000	1.000	1.000	1.000	1.000
Mdh1	*A*	1.000	1.000	1.000	1.000	1.000	1.000
Mdh3	*A*	1.000	1.000	1.000	1.000	1.000	1.000
Mdh4	*A*	1.000	1.000	1.000	1.000	1.000	1.000
Mdh5	*A*	1.000	1.000	1.000	1.000	1.000	1.000
Acph1	*A*	0.914	0.903	0.625	0.983	0.828	0.900
	B	0.086	0.097	0.375	0.017	0.172	0.100
Mnr2	*A*	0.879	0.806	0.750	0.927	0.793	0.840
	B	0.121	0.161	0.172	0.083	0.207	0.140
	C	0.000	0.032	0.078	0.000	0.000	0.020
G-6pd1	*A*	1.000	1.000	1.000	1.000	1.000	1.000

A total of 20 alleles were recorded in the 13 analysed loci, and all of them were found in all studied populations, although their frequencies varied in each population. The average number of alleles observed per locus (A) in all populations was 1.5 (Table 4). The percentage of polymorphic loci (P) was the same for wild and managed populations (35.9%). The average observed heterozygosity (H_o) in all populations was 0.065, but it was higher in wild (0.079) than in managed populations (0.052). The average expected heterozygosity was (H_e) 0.122 in all populations, but it was also higher in the wild (0.134) than in the managed groups (0.110). H_e exceeded H_o in all populations, a fact that indicates that there was a general deficiency in heterozygous individual plants.

Fixation indexes (F) significantly higher than zero indicate a deficiency of heterozygosity, whereas those significantly lower than zero indicate excess of heterozygosity. Nearly 79% of estimations of F in the polymorphic loci of all populations were significantly positive, and nearly 21% were not significantly different to zero. These data were similar in both wild and managed *in situ* populations (Table 5).

Differentiation among populations

Total genetic diversity (H_T) was 0.339, but this parameter was significantly higher in wild populations (0.370) than in managed ones (0.298) (Table 6). In wild populations, the highest values of H_T were in loci *PGI2* and 6-*PGD2*, whereas the lowest values were in locus *PGM2*. In the managed *in situ* populations the highest values of total diversity were in locus PGI2 and the lowest in locus *ACPH1*.

In wild populations, average H_S was 0.343, whereas in the managed populations it was 0.279, which indicates that a higher proportion of genetic diversity occurs within populations in both population types. In wild populations the highest H_S value was 0.483 in locus *PGI2*, whereas the lowest value was recorded in locus *PGM2* (0.228). In the managed populations the highest H_S value was 0.380 in *PGI2* locus, and the lowest was recorded in *ACPH1* locus (0.166). The extent of genetic diversity between populations (D_{ST}) was generally low (Table 6), but was significantly higher in wild populations (0.0273) than in the managed ones (0.0182) (Table 6).

Table 4. Parameters to estimate genetic variation in wild and managed *in situ* populations of *Escontria chiotilla* in the Tehuacán Valley. N = sample size; A = number of alleles per locus; P = percentage of polymorphic loci; H_o = observed heterozygosity; H_e = expected heterozygosity. Numbers in parenthesis are standard errors.

Population	N	A	P(%)	H_0	H_e
W-l	29.0(0.0)	1.5(0.2)	38.46	0.061(0.035)	0.113(0.048)
W-2	31.0(0.0)	1.5(0.2)	30.77	0.067(0.035)	0.106(0.048)
W-3	32.0(0.0)	1.5(0.2)	38.46	0.108(0.047)	0.184(0.067)
Average	**30.7**	**1.5**	**35.9**	**0.079**	**0.134**
M-l	30.0(0.0)	1.5(0.2)	30.77	0.046(0.024)	0.111(0.049)
M-2	29.0(0.0)	1.5(0.2)	38.46	0.047(0.031)	0.113(0.041)
M-3	25.0(0.0)	1.5(0.2)	38.46	0.062(0.031)	0.107(0.044)
Average	**28.0**	**1.5**	**35.9**	**0.052**	**0.110**
Total average	**29.3**	**1.5**	**35.9**	**0.065**	**0.122**

Table 5. Fixation indexes (F) for polymorphic loci in wild and managed populations of *Escontria chiotilla*. Significant deviations between observed and expected frequencies are indicated as $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

Locus	Population					
	S1	S2	S3	M1	M2	M3
6pgd 2	−0.037	0.263	0.295	0.375*	0.770***	0.351
Pgi 2	0.641***	0.728***	0.559***	0.829***	0.870***	0.425*
Pgm 2	0.785***	0.000	0.938***	0.660***	0.870***	0.834***
Acph 1	0.785***	0.629***	0.200	0.000	0.758***	0.778***
Mnr 2	0.514**	−0.198	−0.090	−0.092	−0.262	−0.168

The genetic differentiation coefficient (G_{ST}) was higher in wild populations than in the managed ones (0.738 and 0.611, respectively), meaning that in wild populations nearly 7.4% of genetic variation occurs among populations and 92.6% occurs within populations. Similarly, in the managed *in situ* populations 6.1% of genetic variation occurs between populations and 93.9% within populations.

In relation to Wright's F statistics, Table 7 shows that the average of F_{ST} was 0.075 in wild and 0.061 in managed populations, indicating that 7.5% of the total variance in allele frequencies in wild populations is due to genetic differences among populations, whereas this value corresponds to 6.1% of the total variance in allele frequencies in managed populations.

Genetic dissimilarity

Figure 2 shows that, according to Nei's minimum distance (Nei 1972) coefficient, the level of differentiation among populations is low, suggesting that all six populations may form a single metapopulation. Wild populations W1 and W2 are genetically indistinct and slightly different from the rest of the populations. Wild population W3 is distinct from both the wild and managed populations that were studied. Managed *in situ* populations M1 and M2 were closely grouped, whereas M3 is intermediately similar to W1, W2 and the other two managed populations. This pattern was consistent in analysis involving Nei's unbiased minimum distance (Nei 1978) coefficient.

Discussion

Levels of genetic variation found in *Escontria chiotilla* can be compared with those reported for other cacti species. In general, *E. chiotilla* had 35.9% of poly-morphic loci (P), an average of 1.5 effective alleles per locus (A), an average of 0.065 of observed hetero-zygosity (H_o), an average of 0.122 of expected hetero-zygosity (H_e), an average of total genetic diversity (H_T) of 0.340. These values are similar to those reported by Parker and Hamrick (1992) for the cactus *Lophocereus schotii* (P = 34.4%, A = 1.22, H_o = 0.159, H_e = $O.U6$, and H_T = 0.326), but lower than those reported by Fleming et al. (1998) for the cardon *Pachycereus pringlei* (P = 92.17%,

Table 6. Total genetic diversity (H_T), genetic diversity within (H_s) and among (D_{ST}) populations, and genetic differentiation coefficient (G_{ST}), Wright's parameter of differentiation among subpopulations (F_{ST}), and parameters estimating gene flow ($Nm_{(GST)}$ and $Nm_{(FST)}$), for wild, managed *in situ*, and all studied populations (All) of *Escontria chiotilla*.

Parameter	Population	Locus					Average
		6pgd 2	*Pgi 2*	*Pgm 2*	*Acph 1*	*Mnr 2*	
H_S	Wild	0.423	0.483	0.229	0.267	0.312	**0.343**
	Managed	0.304	0.380	0.298	0.166	0.252	**0.280**
	All	0.364	0.432	0.263	0.217	0.282	**0.312**
D_{ST}	Wild	0.014	0.010	0.073	0.036	0.040	**0.027**
	Managed	0.006	0.065	0.007	0.008	0.005	**0.018**
	All	0.027	0.044	0.040	0.026	0.005	**0.028**
H_T	Wild	0.437	0.494	0.302	0.303	0.316	**0.370**
	Managed	0.310	0.445	0.305	0.174	0.257	**0.298**
	All	0.391	0.475	0.303	0.243	0.287	**0.340**
G_{ST}	Wild	0.032	0.021	0.242	0.117	0.013	**0.074**
	Managed	0.020	0.146	0.021	0.047	0.020	**0.061**
	All	0.070	0.092	0.131	0.106	0.018	**0.083**
$Nm_{(GST)}$	Wild	7.517	11.709	0.784	1.884	19.762	**3.136**
	Managed	12.072	1.464	11.499	5.119	11.997	**3.845**
	All	3.345	2.474	1.659	2.099	13.542	**2.752**
F_{ST}	Wild	0.033	0.022	0.243	0.118	0.014	**0.075**
	Managed	0.071	0.092	0.132	0.107	0.019	**0.084**
	All	0.023	0.145	0.021	0.047	0.020	**0.061**
$Nm_{(FST)}$	Wild	7.326	11.114	0.779	1.869	17.607	**3.083**
	Managed	10.620	1.474	11.655	5.069	12.250	**3.848**
	All	3.271	2.467	1.644	2.086	12.908	**2.726**

Table 7. Wright's *F* statistics for polymorphic loci in wild and managed populations of *Escontria chiotilla* in the Tehuacán Valley.

Locus	Populations Wild			Managed		
	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IS}	F_{ST}
6pgd 2	0.173	0.020	0.033	0.500	0.500	0.023
Pgi 2	0.641	0.649	0.022	0.666	0.666	0.145
Pgm 2	0.857	0.892	0.243	0.768	0.768	0.021
Acph 1	0.408	0.478	0.118	0.714	0.714	0.047
Mnr 2	0.009	0.023	0.014	−0.192	−0.192	0.020
Average	**0.403**	**0.447**	**0.075**	**0.503**	**0.533**	**0.061**

$A = 3$, $H_e = 0.25$) and by Nassar et al. (2001) for *Melocactus curvis-pinus* ($P = 89.5\%$, $A = 3.53$, $H_e = 0.145$). Nevertheless, it is important to consider that only five of the loci analysed were polymorphic, and that further analyses including more polymorphic loci could help to further refine the present information. In addition, further analyses including populations of *E. chiotilla* from outside the area of Coxcatlán and from outside the Tehuacán-Cuicatlán Valley would be important to explore the identification of new alleles at the existing loci.

Levels of genetic variation in wild populations were generally higher than in managed *in situ* populations ($H_o = 0.079$, $H_e = 0.134$, $H_T = 0.370$, and $H_o = 0.052$, $H_e = 0.110$, $H_T = 0.298$, respectively), suggesting that human management has caused a reduction of genetic diversity in populations of *Escontria chiotilla*. Management *in situ* of this species involves a process of selection favouring those phenotypes with better utilitarian attributes (Arellano and Casas 2003). Therefore, this process after several cycles of use and fallow of land where managed populations occur has eliminated part of the variation existing in wild un-managed populations.

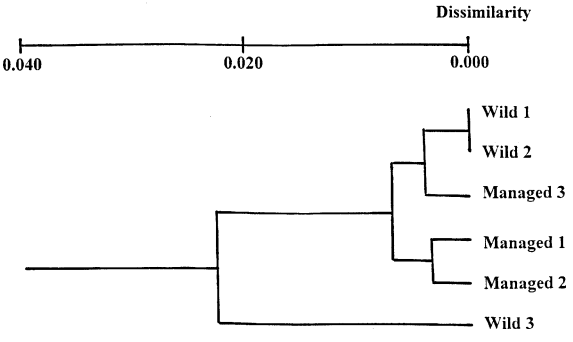

Figure 2 Dendrogram of dissimilarity between the studied wild and managed *in situ* populations of *Escontria chiotilla* according to Nei's minimum genetic distance (Nei 1972). 1, 2 and 3 correspond to wild populations W1, W2 and W3, respectively; 4, 5 and 6 correspond to managed *in situ* populations Ml, M2 and M3, respectively. A consistent pattern was obtained by Nei's unbiased minimum distance (Nei 1978) coefficient.

This information appears to support hypotheses from morphological analyses on *E. chiotilla* (Arellano and Casas 2003) and other columnar cacti (Casas et al. 1997a,b, 1999a; Luna and Aguirre 2001; Cruz and Casas 2002; Carmona and Casas 2003, submitted), suggesting that artificial selection under management *in situ* is a process involving alterations of the genetic structure of populations. In all those studies, the authors have found consistent patterns of morphological differentiation among wild and managed *in situ* populations. However, since morphological characters analysed are quantitative traits which can be influenced by both genetic and environmental factors, in all those studies there has been uncertainty about the real effect of artificial selection on the genetic structure of populations.

Levels of dissimilarity slightly distinguished among wild and managed *in situ* populations. But effects of human management are apparently counteracted by a high gene flow among wild and managed *in situ* populations which appear to conform a single meta-population, as shown by the indirect estimations of gene flow from our study. Gene flow may occur through pollen exchange among populations. Studies on reproductive biology (Oaxaca 2003) have documented that both spatial and temporal barriers to pollen exchange among the populations studied are unlikely. But gene flow may also occur through seed dispersal. Although there has not been a formal study on this topic, we have observed that fruits of *E. chiotilla* are consumed by several

species of birds, as well as by humans, which are potentially able to transport seeds within the ratio of distances among the populations studied. Such processes allow to identify that artificial selection acts as a force promoting and maintaining genetic differences among populations, whereas gene flow acts to dilute such effects. Ethnobotanical studies revealed that artificial selection practised on the same managed *in situ* populations (Arellano and Casas 2003) is of relatively low intensity, compared with artificial selection practised in other species of faster growth and managed under cultivation, such as *Stenocereus stellatus* (Casas et al. 1999b,c). However, even when artificial selection by silvicultural management is relatively weak, its absence would dilute the differences among wild and managed populations in a relatively short time. Ethnobotanical studies by Arellano and Casas (2003) revealed that, at present, nearly 60% of people of the villages studied practise artificial selection in managed *in situ* populations of *Escontria chiotilla*, whereas nearly 28% of people let stand all individuals of this plant species and 12% eliminate all of them when clearing the land. According to local people, management *in situ* and artificial selection favouring good phenotypes of this and other species were stronger in the past and, apparently, we are confronted with a process of losing an important cultural element. According to the information in this study, the complete loss of the practice of artificial selection *in situ* would eventually lead to the disappearance of morphological and genetic differences in the populations we are studying.

Summarising, our study indicated that silvicultural management of *E. chiotilla* has caused a reduction of genetic diversity and an alteration of the genetic structure of populations. There is a slight differentiation among wild and managed populations which could be related to human alteration of populations selecting in favour of better phenotypes. However, such a process appears to be strongly counteracted by a high gene flow among all populations. Silvicultural management and artificial selection related to this form of management could be an important factor influencing evolution of populations of *E. chiotilla* determining incipient processes of domestication. However, both low intensity artificial selection and high gene flow between wild and managed populations appears to be crucial factors that delay the process of domestication.

Acknowledgements

The authors thank the General Directorate Committee for support of Academic Personnel of the National Autonomous University of México (D.G.A.P.A., U.N.A.M. research project IN224799) for financial support, as well as the people of the villages of San Rafael and Coxcatlán for permitting us to work on their land. Also, we thank José Antonio Soriano and Nidia Pérez Nasser for field and laboratory work, as well as Heberto Ferreira for computer assistance.

Appendix A. Genotypes scored in individuals of the studied populations.

Individual plants	*6-PGD2*	*PGI1*	*PGI2*	*PGM1*	*PGM2*	*IDH1*	*MDH1*	*MDH3*	*MDH4*	*MDH5*	*ACPH1*	*MNR2*	*G-6PD1*
Wild population 1 (W1)													
1	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
2	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*
3	*AB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
4	*AB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
5	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AA*
6	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
7	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
8	*AA*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
9	*AA*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
10	*AB*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
11	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
12	*BC*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AA*
13	*BB*	*AA*	*AA*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*	*AA*

Appendix A. Continued.

Individual plants	*6-PGD2*	*PGI1*	*PGI2*	*PGM1*	*PGM2*	*IDH1*	*MDH1*	*MDH3*	*MDH4*	*MDH5*	*ACPH1*	*MNR2*	*G-6PD1*
14	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
15	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*
16	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
17	*AB*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
18	*AB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
19	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
20	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
21	*BC*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
22	*BB*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
23	*AB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
24	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
25	*AB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
26	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
27	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
28	*AB*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
29	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
Wild population 2 (W2)													
1	*AA*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*
2	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
3	*AB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AB*	*AA*
4	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
5	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
6	*BB*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
7	*AA*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AC*	*AA*
8	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
9	*AB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
10	*BB*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
11	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
12	*BB*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
13	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
14	*BB*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
15	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*	*AA*
16	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
17	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
18	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
19	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AB*	*AA*
20	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AC*	*AA*
21	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
22	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
23	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
24	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
25	*AA*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
26	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
27	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
28	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
29	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
30	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
31	*BB*	*AA*	*BB*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
Wild population 3 (W3)													
1	*BB*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AC*	*AA*
2	*BB*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*	*AA*
3	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
4	*BB*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
5	*AA*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AC*	*AA*
6	*AB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AB*	*AA*
7	*AB*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*	*AA*

Appendix A. Continued.

Individual plants	*6-PGD2*	*PGI1*	*PGI2*	*PGM1*	*PGM2*	*IDH1*	*MDH1*	*MDH3*	*MDH4*	*MDH5*	*ACPH1*	*MNR2*	*G-6PD1*
8	*AB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AB*	*AA*
9	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*	*AA*
10	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*	*AA*
11	*AA*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AB*	*AA*
12	*BB*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*BB*	*AA*
13	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
14	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
15	*AA*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
16	*AA*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AC*	*AA*
17	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
18	*AB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
19	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*	*AA*
20	*AB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*
21	*AA*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
22	*AA*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
23	*AA*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
24	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
25	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*	*AA*
26	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AC*	*AA*
27	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
28	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AC*	*AA*
29	*BB*	*AA*	*BB*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*
30	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
31	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*	*AA*
32	*BB*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AB*	*AA*
Managed *in situ* population 1 (M1)													
1	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
2	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
3	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
4	*CC*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
5	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
6	*BB*	*AA*	*AB*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
7	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
8	*AC*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
9	*AB*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
10	*AB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
11	*CC*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
12	*AC*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
13	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
14	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
15	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
16	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
17	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
18	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
19	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
20	*BB*	*AA*	*BB*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
21	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
22	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
23	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
24	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*	*AA*
25	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
26	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
27	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
28	*BB*	*AA*	*BB*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
29	*CC*	*AA*	*AA*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
30	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*

Appendix A. Continued.

Individual plants	*6-PGD2*	*PGI1*	*PGI2*	*PGM1*	*PGM2*	*IDH1*	*MDH1*	*MDH3*	*MDH4*	*MDH5*	*ACPH1*	*MNR2*	*G-6PD1*
Managed *in situ* population 2 (M2)													
1	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
2	*AA*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AB*	*AA*
3	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*
4	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
5	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
6	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
7	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
8	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
9	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
10	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
11	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
12	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
13	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
14	*AA*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*	*AA*
15	*CC*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
16	*CC*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AB*	*AA*
17	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
18	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
19	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
20	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
21	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
22	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
23	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
24	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AB*	*AA*
25	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AB*	*AA*
26	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
27	*BB*	*AA*	*BB*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
28	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
29	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
Managed *in situ* population 3 (M3)													
1	*BB*	*AA*	*AA*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
2	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
3	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
4	*AB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
5	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
6	*BC*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
7	*BB*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
8	*BB*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AB*	*AA*
9	*BB*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
10	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
11	*BB*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
12	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
13	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
14	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*
15	*BB*	*AA*	*AB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
16	*BB*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AC*	*AA*
17	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
18	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*BB*	*AB*	*AA*
19	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
20	*AB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
21	*BB*	*AA*	*AA*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
22	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
23	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*
24	*BB*	*AA*	*BB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AB*	*AA*
25	*CC*	*AA*	*AB*	*AA*	*BB*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*	*AA*

References

Arellano E. and Casas A. 2003. Morphological variation and domestication of *Escontria chiotilla* (Cactaceae) under silvicultural management the Tehuacán Valley, Central México. Genet. Resour. Crop Evol. 50: 439–453.

Arias S., Gama S. and Guzmán U. 1997. Flora del Valle de Tehuacán-Cuicatlán. Fascículo 14. Cacteceae A. L. Juss. Universidad Nacional Autónoma de México, México.

Bravo-Hollis H. 1978. Las Cactáceas de México. Volumen 1: Universidad Nacional Autónoma de México, México.

Carmona A. and Casas A. in press. Management and morphological variation in *Polaskia chichipe* (Cactaceae) under domestication in the Tehuacan Valley, Central Mexico. Arid Environ.

Casas A. and Caballero J. 1996. Traditional management and morphological variation in *Leucaena esculenta* (Fabaceae: Mimosoideae) in the Mixtec region of Guerrero, Mexico. Econ. Bot. 50: 167–181.

Casas A., Caballero J., Mapes C. and Zárate S. 1997a. Manejo de la vegetación, domesticación de plantas y origen de la agricultura en Mesoamérica. Boletín de la Sociedad Botánica de México 61: 31–47.

Casas A., Pickersgill B., Caballero J. and Valiente-Banuet A. 1997b. Ethnobotany and domestication in xoconochtli, *Stenocereus stellatus* (Cactaceae) in the Tehuacán Valley and La Mixteca Baja, México. Econ. Bot. 51: 279–292.

Casas A., Caballero J. and Valiente-Banuet A. 1999a. Use, management and domestication of columnar cacti in south-central Mexico: a historical perspective. J. Ethnobiol. 19: 71–95.

Casas A., Caballero J., Valiente-Banuet A., Soriano J.A. and Dávila P. 1999b. Morphological variation and the process of domestication of *Stenocereu stellatus* (Cactaceae) in central Mexico. Am. J. Bot. 86: 522–533.

Casas A., Valiente-Banuet A., Rojas-Martínez A. and Dávila P. 1999c. Reproductive biology and the columnar cactus *Stenocereus stellatus* in central de México. Am. J. Bot. 86: 534–542.

Casas A. and Barbera G. 2002. Mesoamerican domestication and diffusion of cacti. In: Nobel P.S. (ed.), Cacti: Biology and Uses. University of California Press, Los Angeles, CA, pp. 143–162.

Cheliak W.M. and Pitel J.A. 1984. Techniques for Starch Gel Elctrophoresis of Enzymes from Forest Tree Species. Information Report PI-X-42. Petawa National Forestry Institute, Berkeley, CA.

Colunga P., Hernández-Xolocotzi E. and Castillo A. 1986. Variación morfológica, manejo agrícola tradicional y grado de domesticación de *Opuntia* spp. en el Bajío Guanajuatense. Agrociencia 65: 7–44.

Cruz M. and Casas A. 2002. Morphological variation and reproductive biology of *Polaskia chende* (Cactaceae) under domestication in Central Mexico. J. Arid Environ. 51: 561–576.

Dávila P., Arizmendi M.C., Valiente-Banuet A., Villaseñor J.L., Casas A. and Lira R. 2002. Biological diversity in the Tehuacán-Cuicatlán Valley, México. Biodiversity and Conservation 11: 421–442.

Fleming T., Sandrine M. and Hamrick J.L. 1998. Geographic variation in the breeding system and the evolutionary stability of trioecy in *Pachycereus pringlei* (Cactaceae). Evolut. Ecol. 12: 279–289.

García E. 1981. Modificaciones al sistema de clasificación climática de Kopper para adaptarlo a las condiciones de la República Mexicana. Instituto de Geografía. Universidad Nacional Autónoma de México, México.

Hammer K. 2001. Cactaceae. In: Hanelt P. (ed.), Institute of Plant Genetics and Crop Plant Research (eds.), Mansfeld's Encyclopedia of Agricultural and Horticultural Crops. Springer-Verlag, Berlin, pp. 198–222.

Harlan J.R. 1992. Origins and processes of domestication. In: Chapman G.P. (ed.), Grass Evolution and Domestication. Cambridge University Press, Cambridge, MA, pp. 159–175.

Luna C. and Aguirre R. 2001. Clasificación tradicional, aprovechamiento y distribución ecológica de la pitaya Mixteca en México. Interciencia 26: 18–24.

MacNeish R.S. 1967. A summary of the subsistence. In: Byers D.S. (ed.), The Prehistory of the Tehuacan Valley. Vol. 1. Environment and Subsistence. University of Texas Press, Austin TX, pp. 290–231.

Miller M.P. 1997. Tools for genetic population analysis (TFPGA) version 1.3. A windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author.

Nassar J.M., Hamrick J.L. and Fleming T. 2001. Genetic variation and population structure of the mixed-mating cactus, *Melocactus curvispinus* (Cactaceae). Heredity 87: 69–79.

Nei M. 1972. Genetic distance between populations. Am. Nat. 106: 283–292.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590.

Oaxaca B. 2003. Biología reproductive de Escontria chiotilla (Weber) Rose (Cactaceae) en el Valle de Tehuacán, Puebla, México. BSc Dissertation, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, México.

Otero-Arnaiz A., Casas A., Bartolo C., Perez-Negrón E. and Valiente-Banuet A. 2003. Evolution of *Polaskia chichipe* (cac-taceae) under domestication in the Tehuacan Valley, Central Mexico. Reproductive biology. Am. J. Bot. 90: 593–602.

Parker K.C. and Hamrick J.L. 1992. Genetic diversity and clonal structure in a columnar cactus, *Lophocereus schotii*. Am. J. Bot. 79: 89–96.

Rojas-Aréchiga M., Casas A. and Vázquez-Yanes C. 2001. Seed germination of wild and cultivated *Stenocereus stellatus* (Cactaceae) from the Tehuacán-Cuicatlán Valley, Central Mexico. J. Arid Environ. 49: 279–287.

Rzedowski J. 1978. Vegetación de México. Limusa, México.

Slatkin M. 1985. Gene flow in natural populations. Ann. Rev. Ecol. Syst. 16: 393–430.

Stuber C.W., Wendel J.M. and Goodman M.M. 1988. Techniques and scoring procedures for starch gel electrophoresis of enzyme from maize (*Zea mays*). Technical Bulletin 286. North Caroline State University, USA.

Swofford D.L. and Selander R.K. 1981. BIOSYS-1 (release 1.7): a computer program for the analysis of allelic variation in population genetics and biochemical systematics. User's manual. Illinois Natural History Survey, EUA.

Valiente-Banuet A., Arizmendi M.C., Rojas Martínez A. and Domínguez-Canseco L. 1996. Ecological relationships between columnar cacti and nectar feeding bats in Mexico. J. Trop. Ecol. 12: 103–119.

Valiente-Banuet A., Casas A., Alcántara A., Dávila P., Flores N., Arizmendi M.C., Villaseñor J.L., Ortega Ramírez J. and y Soriano J.A. 2000. La Vegetación del Valla de Tehuacán-Cuicatlán. Boletin de la Sociedad Botánica de México.

Wendel J. and Weeden N. 1989. Visualization and interpretation of plant isozyme. In: Soltis D.E. and Soltis P.S. (eds), Isozymes in Plant Biology. Dioscorides Press, Portland, Oregon, pp. 5–45.

Yeh F.C. and O'Malley D. 1980. Enzyme variations in natural populations of Douglas fir, Pseudotsuga menziesii (Mub.) Franco, from British Columbia. 1. Genetic variation patterns in coastal populations. Silvae Genetica 29: 83–92.

Yeh F.C., Yang R.C.Y. and Boyle T. 1997. POPGENE version 1.21. Microsoft windows-based freeware for population genetic analysis. University of Alberta/Centre for International Forestry Research.