Plant Syst Evol (2013) 299:1899–1910
DOI 10.1007/s00606-013-0846-2

ORIGINAL ARTICLE

Genetic diversity and population genetic structure of wild banana *Musa ornata* (Musaceae) in Mexico

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Received: 29 June 2012 / Accepted: 11 May 2013 / Published online: 26 May 2013

Abstract The wild banana *Musa ornata* is an inhabitant of the tropical regions of Mexico characterized by patches of tropical rainforest. The overexploitation of its habitat has caused the extinction of several populations affecting diversity and population genetic structure of remaining ones. We used microsatellite markers to determine the genetic diversity and the population's genetic structure of all extant populations. The thirty-two microsatellite loci previously characterized for *M. acuminata* and *M. balbisiana* were tested in *M. ornata*. Only twelve amplified. From these seven were polymorphic and were used for genetic analyses. The Nei's diversity estimator shows low levels of genetic diversity ($H_e = 0.263$) with a mean of 4.40 alleles per locus. Excess homozygosity was evident in all populations indicating high levels of inbreeding. F_{ST} pairwise analyses and AMOVA indicated low genetic differentiation. However, 28 % of private alleles were registered, suggesting limited gene flow. Genetic distances, Jaccard's coefficient and principal component analysis showed a good correspondence to geographical locations. The Mantel test performed was not significant. The results support the hypothesis of recent fragmentation events; therefore, not enough time has passed to detect differences between populations. However, it is also likely that results are caused by factors such as bottleneck, decline in pollinator populations, self-pollination and/or a tendency towards clonal reproduction. It is proposed that the preservation strategy focuses on maintaining all the remaining populations and ensuring their connectivity, so as to maintain gene flow and increase the genetic diversity of this species.

Keywords Conservation · Gene flow · Genetic diversity · Microsatellites · *Musa*

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Introduction

The genus *Musa* L. (Musaceae Juss.) commonly known as the banana group represents a major staple food for at least 400 millions of people in the tropics and subtropics. Furthermore, banana is the fourth most important food crop and the number one fruit crop in the world (Ortiz and Vuylsteke 1996). It is also an important source of income for many tropical countries that are home to the edible and ornamental varieties of the species (Frison and Sharrock 2000). *Musa ornata* Robx. is also widely distributed in cultivation in the tropics as an ornamental plant due to its bright purple bracts, and represents a potential gene pool for the improvement of edible varieties. This species inhabits tropical regions with Asian and Mexican representatives. In Asia, it spreads across northeast India, Bangladesh, Myanmar and northern Thailand. In Mexico, it is distributed in small populations in the states of Veracruz, Tabasco and Oaxaca. There was also evidence of distribution in the states of Puebla and Chiapas (Matuda 1950; Gutiérrez and Burgos-Hernández 2012; Fig. 1). However, there is no current record of any of the populations from Puebla or Chiapas suggesting a probable extinction in those

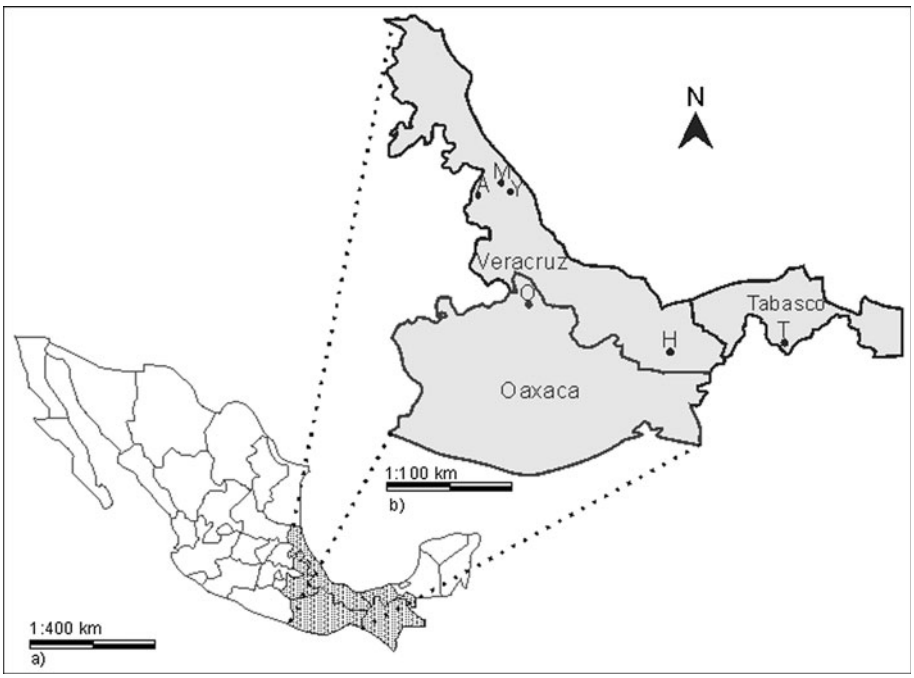

Fig. 1 **a** Historical distribution of *Musa ornata* in Mexico, **b** current distribution of populations and collection sites of *M. ornata*. *A* Atzalan, *M* Misantla, *Y* Yecuatla, *O* Oaxaca, *H* Hidalgotitlán, *T* Tabasco

areas. *M. ornata* had probably dispersed several years before it was botanically described as has been proposed for edible bananas (Bassler 1926; Cheesman 1949). In America, it is believed that was introduced by the Spanish and Portuguese and later became naturalized. However, there are studies on plants and fossils from North and South America and documents of early explorers in México that suggest the presence of *Musa* in America long before the arrival of the Spanish (e.g., Humboldt 1810; Bassler 1926; Cheesman 1949; Acosta 1950; Raven and Axelrod 1974; Manchester and Kress 1993). However, this hypothesis has not been tested.

Human activities such as agriculture, farming and urbanization are causing the dramatic decline of this species and its habitat in México. Guevara and Lira-Noriega (2004) and Díaz-Gallegos et al. (2010) have mentioned that the tropical regions of Mexico are characterized by large-scale jungle fragmentation that causes degradation and loss of available habitat for the species. These changes can affect the genetic diversity of species by direct loss of genotypes, reduction in population size, isolation and limitation of gene flow (White et al. 2002). Consequently, they can reduce genetic variation within the population due to genetic drift if population sizes are very small, altering also the geographic structure of the population. In populations with high levels of gene flow, these changes can be difficult to detect because populations have low levels of genetic differentiation (Waples 1998). Species might also undergo erosion in its genetic variability by inbreeding depression. This phenomenon is particularly common in small and genetically depauperate populations, where the combination of inbreeding, accumulation of deleterious recessive alleles and low levels of genetic diversity may be detrimental to fitness (Hale and Briskie 2007; Leberg and Firmin 2008). As a result, the plants are left with little capacity to adapt to environmental changes, affecting directly the viability and survival of the species (Willi et al. 2006; Frankham et al. 2010).

Genetic criteria have been used to guide conservation efforts for endangered species. Populations are not equivalent in terms of their capacity to respond adaptively to future environmental conditions. Therefore, genetic data could ensure a better use of the available resources by maximizing the evolutionary-response potential of a collection or a set of conserved populations (Petit et al. 1998; Ouborg et al. 2010). Consequently to evaluate the genetic diversity of endangered species for conservation and management purposes, it has become a priority to obtain information on the natural levels and distribution of genetic variation in populations. Until recently, biodiversity indicators were limited to ecological parameters such as population dynamics and species richness. However, the use of molecular tools in population studies has opened a new chapter in conservation at the genetic level, spanning a wide range of species in order to promote their conservation and management (Haig 1998; Ouborg et al. 2010). In this regard, the microsatellites markers have been considered as a powerful tool for studies of population genetics and genetic conservation because of their high mutation rate and high resolving power (Chase et al. 1996; Ouborg et al. 2010; Jones and Gibson 2011). The variability observed at microsatellite loci has provided estimates of

inbreeding, heterozygosity, and gene flow, all of which are important measures for assessing the conservation and management status of populations under pressure.

Although the genus *Musa* is one of the most important worldwide, there are no studies in Mexico on the only wild representative of the genus. Consequently, genetic diversity and differentiation within and among *M. ornata* populations remain unknown. This is especially noteworthy given that their habitat is highly fragmented and the species is in danger of disappearing. In this study, we used microsatellite markers to (a) know the genetic diversity and genetic population structure of *M. ornata*, (b) detect the existence of genetic differentiation among remnant populations of the species, and (c) generate basic information so as to design strategies for the conservation and sustainable management of the species in Mexico.

Materials and methods

Study site and sampling

Leaf tissue samples were collected for all extant populations of *M. ornata* from Mexico. Four populations were from Veracruz, one from Tabasco and one from Oaxaca (Fig. 1; Table 1). All are small populations, and only the one from Tabasco was found in an undisturbed patch of rainforest. About one-third of the individuals from each population were randomly collected (20 individual per population). Leaf samples were taken from individuals that were 5–10 m apart from each other, depending on the size of the population and its accessibility. In total, 120 individuals were sampled for estimating genetic diversity and population genetic structure of this species. One individual from each population was designated as voucher specimen. Vouchers were deposited at the XAL herbarium Instituto de Ecología AC, with numbers 742–747 and 24,960. All specimens collected were identified as *M. ornata* following taxonomic keys from taxonomists of the genus as follows: inflorescence erect, glabrous, orange-yellow flowers, anthers purple with white pollen, purple-pink bracts usually two, small fruits (greenish-yellow bananas) with warty black seeds, dark green petiole grooved, green to blue-green leaves with midrib grooved with reddish coloration on the abaxial surface of a leaf.

Table 1 Location and sample size (*N*) of all six extant populations of *Musa ornata* in Mexico

	Populations	Location		*N*	VT
A	Atzalan, Veracruz	N 19° 51′02.9″	W 96° 45′47.8″	20	TRF
Y	Yecuatla, Veracruz	N 19° 51′11.9″	W 96° 46′36.9″	20	SFV
M	Misantla, Veracruz	N 19° 58′14.8″	W 96° 54′18.6″	20	SFV
H	Hidalgotitlán, Veracruz	N 17° 37′ 26.6″	W 94° 37′24.3″	20	SFV
T	Teapa, Tabasco	N 17° 31′30.5″	W 92° 53′42.8″	20	TRF
O	Jalapa de Díaz, Oaxaca	N 28° 03′25.5″	W 96° 36′46.5″	20	SFV

N sample size, *VT* vegetation type where populations were located according to Rzedowski (2006), *TRF* tropical rain forest, *SFV* secondary forest vegetation associated with urbanization, agricultural and pastoral areas

DNA extraction, amplification and microsatellite analysis

DNA extraction was performed with 60 mg of dry leaf tissue using the DNeasy plant mini kit (QIAGEN. 69104), following the manufacturer's protocol. For this study, a preliminary test was conducted with thirty-four microsatellite loci previously characterized for *M. acuminata* and *M. balbisiana* (Creste et al. 2005; Ge et al. 2005; Miller et al. 2010; Jing-yi et al. 2011). From these, twelve amplified and seven showed polymorphism, which were used for this study (Table 2).

The polymerase chain reaction was carried out in a total volume of 20 µl (approximately 24 ng of genomic DNA, 5 µl Buffer 5X (20 mM Tris–HCl (pH 8.3), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 % Tween 20®, 0.5 % NP40, 50 % glicerol), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.1 µM of each primer, 1.25 U Taq polymerase (Apex, 42-800B1) and 2.75 µl distilled water). The amplifications were carried out in a BioRad thermocycler, following the conditions described by Miller et al. (2010) for primers Mbg01, Mbg06, MaSSR01 and MABN17 and those described by Creste et al. (2005) for primers MaOCEN01, MaOCEN13 and MaOCEN14.

The detection of microsatellites was performed on 6 % denaturing polyacrylamide gels, prepared and stained using the procedure described by Benbouza et al. (2006). Electrophoresis was carried out for 2.5 h to 1,680 V, 60 mA and 80 W in a vertical chamber (Thermo Scientific Owl P10DS Dual Gel System) with TBE buffer 1X. Prior to electrophoresis, the DNA was denatured for 2 min at 92 °C. Between each 10 samples, a fragment size marker was placed consisting of 52 fragments between 25 and 1,300 in 25 bp increments (ladder-Promega). After electrophoresis, gels were stained with silver nitrate to proceed with the revealing of PCR products. Finally of the 120 individuals sampled, only 108 amplified, so only these were considered for this study.

Table 2 Microsatellite loci of *Musa ornata* used in this study

Locus	Primers (5′–3′)	Rep	PIC	References
MaOCEN01	TCTCAGGAAGGGCAACAATC GGACCAAAGGGAAAGAAACC	$(CT)_{17}$	0.68	Creste et al. (2005)
MaOCEN13	GCTGCTATTTTGTCCTTGGTG CTTGATGCTGGGATTCTGG	$(TC)_{16}$	0.72	Creste et al. (2005)
MaOCEN14	TCTTTTGCGTGAGTTTTTGG CGTGGGAGGAACAGTGAA	$(CT)_{10}$	0.72	Creste et al. (2005)
MABN17	CCCATGCAACTACAACAACG GGAACCACGTGTCCTGATCT	$(TCT)_{14}$	0.65	Miller et al. (2010)
MaSSR-01	TGAGGCGGGGAATCGGTA GGCGGGAGACAGATGGAGTT	-	-	Ge et al. (2005)
Mbg06	AGCAACCCGTGGATAAAGAGC TCCCTCTCGCTCCTCTTCTTCC	$(GAA)_8$	-	Jing-yi et al. (2011)
Mbg01	GAGAGAGAGAGATCGTTTAGCA AGAGGCTCGTGATTCATGTGGT	$(GA)_6$	-	Jing-yi et al. (2011)

Rep repeat motif, PIC polymorphic information content, - information not provided for the authors

Data analysis

The gel image was captured with a Kodak Digital Science®. To know the allele size in base pairs (bp) of each individual of the different loci, the ID image Analysis software v. 3.0 was used, taking as a reference the size of the fragments of the marker (25 bp).

Genetic diversity

To assess genetic diversity, we estimated the mean number of alleles (*n*), effective number of alleles (*ne*) per locus and population, and percentage of polymorphic loci with POPGENE v. 1.31 (Yeh et al. 1999). Nei's (1973) diversity estimators such as observed (H_o) and expected (H_e) heterozygosity and fixation index (F_{IS}) were calculated using the Arlequin software v. 3.5 (Excoffier and Lischer 2010). The polymorphic index content (PIC) for each locus was obtained using the complement for Excel MICROSAT v. 2007, following the formula $PIC = 1 - \Sigma Pi$, where *Pi* is the frequency of allele *i* in the genotypes examined (Weir 1990). Levels of significance from deviations of Hardy–Weinberg (HWE) were estimated using a Markov chain method with 1,000 randomizations as described by Guo and Thompson (1992), and implemented in GenePop software v. 4.0. (Rousset 2008). This program was also used to determine whether deviations from HWE were due to the presence of null alleles. The null allele frequencies (F_{null}) were estimated using the maximum likelihood (ML) estimator based on the expectation–maximization (EM) algorithm of Dempster et al. (1977).

Population genetic structure

To assess genetic structure and to examine genetic differentiation in *M. ornata,* a F_{ST} pairwise distance and a hierarchical Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) with 1,000 and 2,000 permutations were carried out using Arlequin software v. 3.5 (Excoffier and Lischer 2010). Relationships among populations were investigated with the standard genetic distance (Nei 1972) and the chord genetic distance (Cavalli-Sforza and Edwards 1967) with 500 permutations using Phylip v. 3.68 (Felsenstein 2005). Chord genetic distance has been proposed to have a superior performance in obtaining the correct tree topology when the divergences are recent (Takezaki and Nei 1996). Phylip v. 3.68 was also used to reconstruct unrooted trees by the neighbor-joining (NJ) algorithm (Saitou and Nei 1987). Support of branches was estimated with 1,000 bootstrap iterations. We assumed infinite allele model (IAM) to calculate distances and AMOVA. This model implies that each new mutation originates with equal probability a new allele that differs from the already existing (Balloux and Lugon-Moulin 2002).

In order to estimate similarities between genotypes a binary matrix of presence–absence of alleles was constructed. Pairwise similarities were calculated using Jaccard's coefficient, and the resulting matrix was used to construct a dendrogram using the unweighted pair group method average (UPGMA) clustering procedure. A principal component analysis (PCA) was also performed on the covariance matrix of allele data to investigate spatial patterns of genetic variation. Both analyses were carried out

with SYSTAT software v. 1.3. Finally, to infer possible patterns of isolation by distance, we performed a Mantel test (Mantel 1967) using TFPGA software v. 1.3 with 1,000 random permutations.

Results

Genetic diversity

We used seven microsatellite loci to characterize genetic diversity in six populations of *M. ornata*. The smallest allele was 103 bp for locus Mbg06 and the largest was 562 bp for locus MaOCEN13. In total, there were 46 alleles for all populations. The highest degree of polymorphism and genetic diversity was shown by the locus MaSSR01 (PIC = 0.753, $n = 5.33$, $H_e = 0.3525$), while the lowest was presented by the locus Mbg06 (PIC = 0.250, $n = 1.66$, $H_e = 0.1631$) (Table 3).

Genetic parameters for each population are given in Table 4. For the six populations, the percentage of polymorphic loci was 100 % and the expected heterozygosity (H_e) was 0.2637, with a mean of 4.40 alleles per locus. The highest values of H_e were for the populations of Oaxaca and Tabasco 0.2705 and 0.2641, respectively, while the lowest was for Atzalan ($H_e = 0.2601$). In contrast, the observed heterozygosity (H_o) was low, and ranged from 0.0084 (Oaxaca) to 0.0408 (Misantla).

Private alleles (alleles unique to each population) recorded for all populations represent 28 % of the total number of alleles detected (13/46). Hidalgotitlan and Yecuatla populations showed the greatest number of private alleles, 5 and 4, respectively (Table 4). The mean value of F_{IS} (0.9066) was significant ($P < 0.05$), indicating a deficiency of heterozygotes ($H_o = 0.0250$, $H_e = 0.2637$). Consequently, the HWE was rejected for all loci, which showed a significant excess of homozygosity ($P < 0.01$). Meanwhile the frequency of null alleles for all loci was low ($F_{null} < 0.40$), with an average frequency of null alleles of 0.3458 ± 0.0606 (Table 3).

Population genetic structure

F_{ST} pairwise analysis showed no significant genetic differentiation between populations ($P > 0.05$), indicating inbreeding within populations (Table 5). The AMOVA

Table 3 Values of genetic diversity per locus based on seven polymorphic loci of *Musa ornata*

Locus	PIC	*n*	*Ne*	H_e	H_o	Allelic range	F_{null}
Mbg01	0.486	2.83	2.69	0.2427	0.0158	212–259	0.3370
Mbg06	0.250	1.66	1.66	0.1631	0.0000	103–208	0.4267
MaSSR01	0.753	5.33	4.79	0.3525	0.0759	224–476	0.2482
MABN17	0.576	3.16	2.97	0.2508	0.0251	330–374	0.3000
MaOCEN01	0.575	3.16	3.03	0.2686	0.0251	265–435	0.3334
MaOCEN13	0.707	4.66	4.45	0.3757	0.0330	194–562	0.3800
MaOCEN14	0.375	2.00	2.00	0.1925	0.0000	316–332	0.3956
Mean	0.532	3.26	3.08	0.2637	0.0250		0.3458
SD	±0.178	±1.32	±1.16	±0.0777	±0.0257		±0.0606

PIC polymorphic information content, *n* mean number of alleles, *ne* effective number of alleles, H_e expected heterozygosity, H_o observed heterozygosity, F_{null} null allele frequencies estimated with GenePop v. 4

Table 4 Values of genetic diversity per population based on seven microsatellite loci and significance tests of deviation from Hardy–Weinberg equilibrium

Population	*N*	*n*	*Ne*	H_e	H_o	HWE	F_{IS}	AP
Atzalan	18	4.57	3.34	0.2601	0.0272	**	0.8977*	1
Hidalgotitlán	18	4.28	3.19	0.2624	0.0136	**	0.9493*	4
Misantla	20	4.71	3.21	0.2611	0.0408	**	0.8469*	2
Yecuatla	18	4.42	3.24	0.2639	0.0204	**	0.9244*	5
Tabasco	16	4.28	3.02	0.2641	0.0396	**	0.8534*	1
Oaxaca	18	3.57	2.51	0.2705	0.0084	**	0.9681*	0
Mean		4.40	3.08	0.2637	0.0250	**	0.9066*	
SD		±0.45	±1.63	±0.0036	±0.0133		±0.0497	

N sample size, *n* mean number of alleles, *ne* effective number of alleles, H_e expected heterozygosity, H_o observed heterozygosity, *HWE* global significance tests of deviation from Hardy–Weinberg equilibrium summed over all loci, F_{IS} inbreeding coefficient, *AP* number of private alleles

* Level of significance $P < 0.05$

** Level of significance $P < 0.01$

Table 5 F_{ST} pairwise comparison of populations (above the diagonal) and F_{ST} *P*-values (below the diagonal) based on number of different alleles

	Atzalan	Hidalgotitlán	Misantla	Oaxaca	Tabasco	Yecuatla
Atzalan	*	0.00607	0.00104	−0.00154	0.00216	0.00534
Hidalgotitlán	0.94321	*	0.00735	0.00478	0.00429	0.00487
Misantla	0.99951	0.93383	*	0.00222	0.00620	0.00346
Oaxaca	0.99951	0.98519	0.99951	*	0.00035	0.00280
Tabasco	0.98222	0.97975	0.94667	0.99951	*	−0.00028
Yecuatla	0.97580	0.98321	0.98272	0.99852	0.99506	*

* Level of significance $P < 0.05$

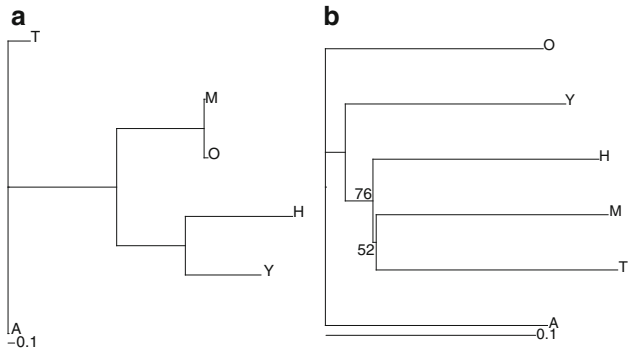

Fig. 2 Unrooted Neighbor-joining (NJ) dendrograms depicting genetic relationships among six populations of *Musa ornata:* **a** Nei's genetic distance, **b** Cavalli-Sforza and Edwards' chord distance. *A* Atzalan, *M* Misantla, *Y* Yecuatla, *T* Tabasco, *O* Oaxaca, *H* Hidalgotitlán. Numbers on branches are bootstrap support percentages

revealed that 92.47 % of molecular variance was between individuals within populations, while 9.61 % was due to genetic differences within individuals. Fixation indices F_{IS} (0.90583) and F_{IT} (0.90386) were significant ($P < 0.05$), indicating a deficit of heterozygotes. While that F_{ST} was not significantly different from 0 ($P > 0.05$), indicating the absence of genetic structure among populations of *M. ornata*.

The NJ dendrograms based upon Nei (1972) and Cavalli-Sforza and Edwards (1967) genetic distances (Fig. 2) showed similar relationships. In both analyses, Misantla, Hidalgotitlan and Yecuatla are grouped together while Atzalan appears separated from the rest. However, both genetic distances show incongruence in relationships of Tabasco and Oaxaca regarding Misantla and Atzalan. According the dendrogram obtained with Nei's distance (Fig. 2a) Atzalan is closer genetically to Tabasco and Misantla while the latter is closer to Oaxaca. However, these relationships were not supported by bootstrap. In the analysis with Cavalli-Sforza and Edwars' distance (Fig. 2b), Atzalan is closer genetically to Oaxaca, and Misantla is closer to Tabasco with bootstrap values of 95 and 52 %, respectively. The two populations that resulted genetically most distant in both analyses were Atzalan and Hidalgotitlan (5.0882 with Nei's distance and 0.2389 with Cavilla-Sforza and Edwars' chord distance).

The dendrogram obtained with Jaccard's coefficient (Fig. 3) showed two main groups. One is formed with all individuals from the population of Hidalgotitlan (group 1) and another is composed of the remaining populations (group 2). In general, populations are grouped by geographic regions. Group 1 conforms the southern part of the state of Veracruz (Hidalgotitlán), group 2 corresponds to individuals from the north-central Region of the state (Misantla, Yecuatla and Atzalan), and group 3 contains the populations from the states of Oaxaca and Tabasco. It should be noted that the Yecuatla population is sub-divided into two distinctive groups one closer to Atzalan and another separate from the rest of the populations. The same pattern is observed in the PCA performed to examine the overall pattern of population differentiation. PCA was conducted with the first two axes, which cumulatively explained 51 % of the total variance contained in the data set (Fig. 4). The over all grouping pattern of PCA corresponded well with the clustering pattern of the dendrogram. The results of the Mantel test showed no significant correlation between genetic distances and geographical distances in *M. ornata* ($r = 0.0344$, $P = 0.4260$), suggesting that distance is not the factor influencing the genetic patterns found.

Discussion

Genetic diversity

The low genetic diversity values recorded for *M. ornata* contrast with the high values reported for other species of the genus. For example, Romero and Chong (2009) obtained an average genetic diversity of $H_e = 0.7195$ in 21 genotypes of *Musa.* Oriero et al. (2006) reported a $H_e = 0.4110$ in 40 genotypes. Similarly, Ge et al. (2005) found that *M. balbisiana* showed a genetic diversity of $H_e = 0.5397$. It is likely that low levels of genetic diversity presented in populations of *M. ornata* are the result of habitat fragmentation, taking into consideration original and current distribution of populations of *M. ornata*

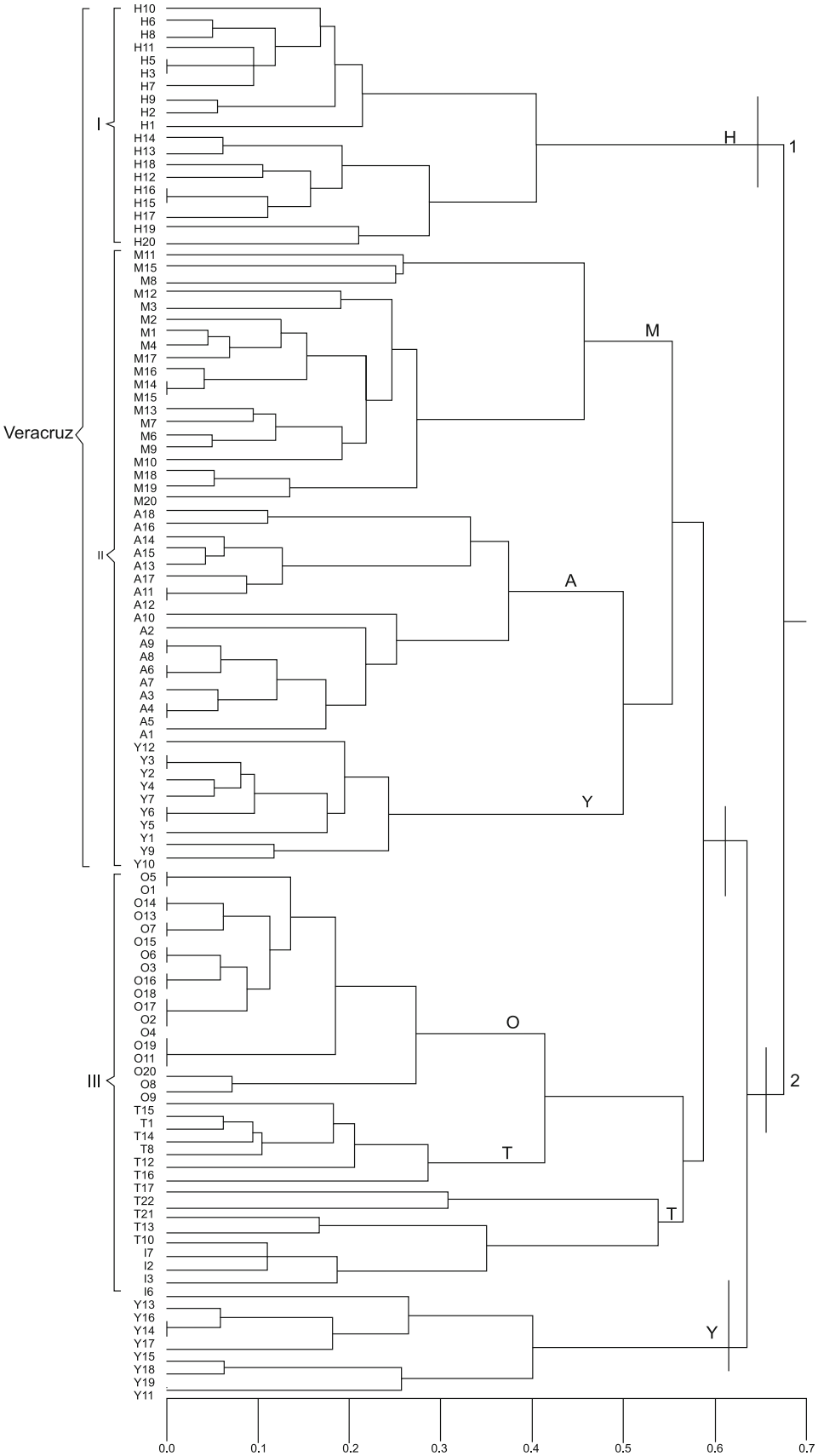

Fig. 3 UPGMA phenetic dendrogram based on Jaccard's coefficient indicating genetic similarities among genotypes of *Musa ornata* from six populations. *A* Atzalan, *M* Misantla, *Y* Yecuatla, *T* Tabasco, *O* Oaxaca, *H* Hidalgotitlán

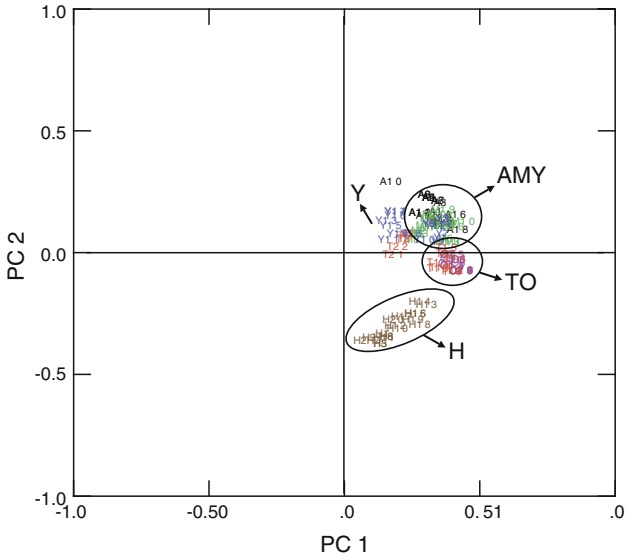

Fig. 4 Principal component analysis (PCA) of allele data from seven microsatellites loci genotyped in six populations of *Musa ornata*. Projection on axis 1 and axis 2, which cumulatively explained 51 % of the total inertia contained in the data set. *A* Atzalan, *M* Misantla, *Y* Yecuatla, *T* Tabasco, *O* Oaxaca, *H* Hidalgotitlán

(Gutiérrez and Burgos-Hernández 2012), coupled with the evidence of extensive conversion of tropical rainforest to pastures and other cultivations in these regions (Díaz-Gallegos et al. 2010). Following this rationale, it is possible that extinct and extant populations of *M. ornata* were part of a formerly more extensive single population that was subject to habitat fragmentation originating subpopulations. Several studies have shown that discontinuities in the habitat can erode the genetic diversity of populations due to a decrease in effective population size and degree of isolation (Pither et al. 2003; Reed and Frankham 2003; Frankham 2010). Therefore, the low diversity and the deficit of heterozygote obtained in this study could be explained with the increase of the mating between closely related individuals (Young et al. 1996). In this regard, Aguilar et al. (2008) reported that progenies in fragmented habitats presented significant higher means of inbreeding coefficients than progenies in non-fragmented habitats, suggesting a more frequently mating among related individuals and/or autogamous pollination mating. According to Reed and Frankham (2003) and Frankham (2010), these processes lead to loss of genetic diversity, followed by the decrease in the potential for adaptation of the species to environmental changes. Honnay and Jaquemiyn (2006) reported this phenomenon for fragmented populations of plants, proving that small populations contain significantly less genetic diversity than large populations.

It is also likely that a recent switch from sexual reproduction to clonal reproduction coupled with one or several bottlenecks are causing the low diversity and deficit of heterozygotes in *M. ornata*. Bananas have both sexual and clonal reproduction (Cheplick 1995). Ge et al. (2005) observed in *M. balbisiana* that clonal reproduction rarely occurs in wild populations because the plants produce flowers and fruits regularly. However, when plants are in adverse conditions they may switch from sexual to clonal reproduction. Populations of *M. ornata* in México have a discontinuous flowering and fruiting, suggesting that populations are subject to strong anthropogenic pressure and environmental. According to Vallejo-Marín et al. (2010), these factors can result in the loss of sexual reproduction in clonal populations leading to genetic uniformity. Particularly, when there is a recent trend to clonal reproduction, the population structure created may lead to frequent inbreeding. Clonal growth has long ago been viewed as a mechanism to allow an individual to persist in adverse conditions, and the factors causing plants to make the switch from sexual to clonal reproduction are often correlated with suboptimal environmental conditions (Honnay and Bossuyt 2005; Silvertown 2008). Habitat fragmentation and habitat lost also induce to plant populations to allocate more resources for vegetative reproduction (Smith et al. 2003; Lhullier et al. 2006).

It has also been shown that population bottlenecks occurring during introduction of species and genetic drift in small founding populations can dramatically reduce levels of genetic diversity (Davies et al. 1999). Thus, the lack of genetic structure and the low genetic diversity in *M. ornata* may also suggest that an introduction of few founders may have occurred. In this sense, several studies of plants introduced show a reduced genetic diversity within populations (e.g., Husband and Barrett 1991; Amsellem et al. 2000). However, few studies have also demonstrated an increase in genetic variability of introduced species (DeWalt and Hamrick 2004; Bossdorf et al. 2005). Therefore, the low diversity and the deficit of heterozygotes obtained in this study show the possibility that *M. ornata* was introduced to America, probably before the sixteenth century.

Presence of null alleles is often reported as the cause of deficit of heterozygotes (Chapuise and Estoup 2007). These alleles do not amplify by various causes ranging from low DNA concentration or bad quality to the presence of mutations in the flanking regions. We are confident to discard this because we did not have problems in obtaining PCR fragments at any of the loci. In addition, we never had a sample that did not show an amplified product, which would be expected for a homozygote or heterozygote for null alleles. Besides, analysis showed low frequencies of null alleles across all loci ($F_{null} < 0.4$). Therefore, our data suggest that deviations from Hardy–Weinberg equilibrium detected are not due to the presence of null alleles.

At the population level, Tabasco, Atzalan and Misantla presented the highest values of H_o, indicating that these populations best retain their genetic diversity. This is consistent with field observations, since these populations are immersed in preserved jungles. If so, it is likely that these populations are sharing alleles that in other populations have disappeared by the degree of disturbance, which would explain that geographically distant populations (Atzalan-Oaxaca, Atzalan-Tabasco, Misantla-Oaxaca y Misantla-Tabasco) are closer genetically. The Oaxaca population was the least conserved genetically ($H_o = 0.0084$). This is also consistent with field observations, since it is the smallest population and the most disturbed one. Meanwhile, the highest number of private alleles was recorded in the Hidalgotitlan and Yecuatla populations (5 and 4, respectively). This is congruent with the degree of isolation, as shown in the Jaccard dendrogram and PCA (Figs. 3 and 4). This would suggest that these populations have been fragmented the longest, and therefore tend towards genetic differentiation. If we consider a scenario in which *M. ornata* was introduced, the number of private alleles presented by Hidalgotitlan and Yecuatla (5 and 4, respectively) suggests that these populations may have independent origins. An alternative possibility is that the genetic difference among populations arose as the lineages followed different routes of introduction before being established in their current localities. Because this process would involve multiple population bottlenecks and periods of small population size, different lineages from the same source could rapidly become genetically different through a series of founder effects and genetic drift. This process could be happening in Hidalgotitlan and Yecuatla populations.

Furthermore, the dendrogram based upon Jaccard's coefficient shows the division of Yecuatla population into two distinct groups: a group of 10 individuals related to Atzalan, and another independent from the rest of the population comprising eight individuals (Fig. 4). The introduction of some specimens from Atzalan to Yecuatla due to its geographic proximity could explain the first group, while the isolated group would correspond to native specimens of Yecuatla.

Population genetic structure

The low genetic differentiation and the absence of variation among populations of *M. ornata* suggest a high gene flow (Yang et al. 2007). This differs with the significant values of F_{ST} registered in other studies for the genus *Musa* (Romero and Chong 2009; Ge et al. 2005; Oriero et al. 2006), in which also reported an excess of heterozygotes (negative values of F_{IS}), contrasting with the positive values recorded in this study. Low genetic differentiation is consistent with studies of the effects of fragmentation on genetic diversity in plants. For example, Pither et al. (2003) found low levels of genetic differentiation for *Terminalia amazonia*. Young et al. (1999) described the same for *Rutidosis leptorrhynchoides* and Young et al. (1993) for *Acer saccharum*, who attributed this finding to increased gene flow between fragments due to their connectivity. However, this explanation is not consistent with our data, since the *M. ornata* populations are isolated. Moreover, considering that the number of unique alleles is an indirect estimate of gene flow (i.e., the lower is the gene flow, more alleles of this type arise and are fixed by genetic drift in a population) (Takahashi et al. 2005) then, the number of registered private alleles (13) indicate limited gene flow. Therefore, it is likely that our results suggest a recent fragmentation event, which does not leave a significant genetic mark in the population when compared to historical events (Lindenmayer and Peakall 2000). If fragmentation was older, we would most likely get a clear population structure, as a result of genetic drift and isolation of populations, as shown by Young et al. (1999). However, a recent fragmentation implies that not enough time has passed for significant differences between populations to accumulate, particularly if they had maintained high levels of gene flow in the past (Waples 1998). This would explain the lack of genetic differentiation in populations of *M. ornata*, although sufficient to observe the different genotypes in the Jaccard dendrogram, in Nei and Cavalli-Sforza genetic distance trees and in the PCA. This may also explain the low bootstrap support registered among population's relationships.

Another possible explanation for the low genetic differentiation is that the habitat disturbance has caused the decline of pollinator populations, thereby limiting gene flow in populations of *M. ornata.* Liu et al. (2002, 2004) mentioned that the viability of *Musa* populations depends on the connectivity between fragments, which are mediated by pollinators and seed dispersers. In this context, several studies have reported that bats and birds play an important role as pollinators of the genus *Musa* (Itino et al. 1991; Liu et al. 2002). Moreover, the maintenance of genetic diversity among and within populations has been attributed to these organisms in several studies (Ge et al. 2005; Anderson et al. 2011). However, both types of pollinators are sensitive to human disturbance, causing their populations to decrease (Chávez and Ceballos 1998; Anderson et al. 2011). According to Wilcock and Neiland (2002), increased fragmentation directly affects the richness and activity of these pollinators, and therefore pollination itself. In plants, the decrease in pollination may have direct repercussion on the production of fruits and seeds (Aguilar and Galetto 2004). Consequently, sexual reproduction may be adversely affected.

Field observations suggest that *M. ornata* does not produce viable seeds when subjected to strong environmental and anthropogenic pressure. According to Cheplick (1995), in species like bananas that have both sexual and asexual reproduction, the reproduction type is determined by seed production and pollination. If this is so, *M. ornata* populations would have a tendency to clonal reproduction as a response to habitat fragmentation, which would explain the heterozygote deficiencies and low levels of genetic diversity. In contrast, Ge et al. (2005) mention that clonality prolongs the life of wild bananas, reducing the effects of genetic drift and contributing to the maintenance of genetic variation. However, this would happen only if clonal reproduction lasted for several generations, allowing the accumulation of somatic mutations. In this regard, Birky (1996) mentions that heterozygosity should increase with an increasing age of asexual lineages, and Klekowski (1997) that the evidence of somatic clonal mutations in wild species is minimal and occurs infrequently. Considering events of fragmentation and a tendency to recent clonal reproduction, probably not enough time has passed for changes to be detectable.

Moreover, the excess of homozygotes observed in *M. ornata* can also be the result of self-pollination, particularly given the small numbers of individuals in most populations. Nur (1976) showed that species with an erect inflorescence such as *M. velutina* can present self-pollinating. This could be the case of *M. ornata,* as it presents an erect inflorescence. However, there are no specific studies on this topic for this species. Our results could also represent a combination of genetic drift and population's bottlenecks, if the wild populations were introduced to America, coupled with clonal reproduction and inbreeding if the wild populations also represent escapes from cultivation. But to date, no formal work has been done to demonstrate that this happened in *M. ornata*.

Implications for conservation

Our results show that populations of *M. ornata* in Mexico have low levels of genetic diversity, significant deficit of heterozygotes and high levels of inbreeding. This may be related to high fragmentation and overexploitation of tropical ecosystems, genetic drift, bottleneck, self-pollination, tendency to recent clonal reproduction, as well as the decline in pollinator populations. Furthermore, it is likely that the low genetic differentiation between populations still reflects historical gene flow as opposed to the more recent (given that not enough time has passed to detect changes). This may compromise the species' ability to respond to selective pressures (Keller and Waller 2002). Thus, one can argue that the conservation strategy for the populations of *M. ornata* in Mexico should be concentrated on keeping the six remaining populations, and ensuring the connectivity between these fragments, allowing the maintenance of gene flow between populations and therefore an increase in genetic diversity. If preservation of all populations of the species is not possible, the most viable population for conservation is Tabasco, because of its genetic diversity, inaccessibility, larger size and best state of preservation. However, Yecuatla and Hidalgotitlan are also important, because they contain the largest amount of private alleles and are in the process of genetic differentiation.

It should be emphasized that there is evidence that *M. ornata* populations existed in other regions of southern Mexico, such as in the states of Chiapas and Puebla. According to the local populace, these populations have become extinct by the use of herbicides, the clearing for crop-growing areas and urbanization. Because of this, it is necessary to implement a program of in situ conservation to preserve most populations that are near populated areas, crop areas and livestock, where the number of individuals is most likely progressively decreasing. This strategy must be implemented in the short term, as there is danger that several populations could disappear, particularly the ones in Hidalgotitlan and Oaxaca.

Another alternative is ex-situ conservation, by collecting seeds for propagation and preservation, and their eventual reintroduction into restoration programs. Finally, it would be interesting to complement our results with demographic and/or ecological studies, which allow for more information so as to implement conservation programs and management of this species as an ornamental plant.

This study reveals the importance of preserving all *M. ornata* populations and provides a foundation for future conservation planning to ensure their permanence. It is also important to pay special attention to what happens with the ecosystems they inhabit, as well as pollinators and seed dispersers that play an important role in maintaining the species.

Acknowledgments We thank Macotulio Soto Hernández for support in image editing and Javier Barrientos Villalobos for fieldwork. We also acknowledge Joaquin Murguía González and Andrew Peter Vovides for comments on the early stage of this study. The authors express their gratitude to two anonymous reviewers for their valuable comments to improve this manuscript.

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