RESEARCH ARTICLE

Wholesale replacement of lima bean (*Phaseolus lunatus* L.) landraces over the last 30 years in northeastern Campeche, Mexico

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Abstract Genetic erosion has been evaluated at the landrace level in the past, principally because the loss of landraces is believed to generate erosion at the allelic level; however, few studies had tested this hypothesis in the crop's centers of diversity and domestication. Using microsatellite markers, we analyzed for genetic erosion in lima bean (*Phaseolus lunatus*) landraces over time in samples collected in 1979 and in 2007 in northeast Campeche, in the Yucatan peninsula, Mexico, an important diversity center and part of the putative domestication area for this crop. We found that the lima bean genetic pool from 1979 had a higher genetic diversity than the one for the 2007 pool (Nei's diversity, $H = 0.18$ and 0.05, respectively). Although this result could not to be explained using a bottleneck analysis, a cluster analysis showed that the alleles present in 1979 were not the same as those found in 2007, indicating an allelic displacement in the genetic pool of the lima bean landraces in the last 30 years. This displacement could be due to the introduction of improved varieties or landraces, resulting in a displacement of local varieties or to changes in the Mayan criteria for selection of germplasm or both. This study showed

that the loss of landraces can generate both quantitative and qualitative changes in the genetic pool of the domesticated species. Such changes are very important to consider when planning *ex situ* and *in situ* programs to conserve crop diversity in their domestication areas.

Keywords Bottleneck · Genetic erosion · Lima bean landraces · Microsatellite DNA markers · *Phaseolus lunatus* · Yucatan peninsula

Introduction

Central to the establishment of germplasm banks and other strategies to conserve plant genetic resources has been the concept of genetic erosion (van de Wouw et al. 2010). Although Baur (1914) warned of the consequences of the disappearance of traditional landraces for the future of plant breeding, Frankel and Bennett (1970) introduced the concept of genetic erosion. Stating that many genetic reservoirs for crop plants were disappearing rapidly, he detailed the following five principles: (1) diversity in crops exists because of adaptation by localized populations; (2) traditional agriculture that continues in centers of diversity maintains high, stable diversity; (3) modern agricultural technology, including modern varieties, is a recent phenomenon and leads to instability; (4) competition between local and introduced varieties results in displacement of local varieties; and (5)

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displacement of local varieties reduces the genetic variability of the entire crop gene pool. Genetic erosion is presently defined as the loss or reduction of genetic diversity between and within populations of the same species over time (Jarvis et al. 2000), most often as a result of agricultural, economic and social changes (FAO 1996).

Assessing changes in genetic diversity within crops can be based on different measures of diversity. We can consider three types of diversity at the infraspecific level: (1) varietal diversity is easy to measure but does not accurately portray the genetic diversity, especially due to inconsistencies in local variety names; (2) agro-morphological diversity provides an indication of the diversity that could be tapped for agricultural uses, but this is hard to measure in large samples collected under different agro-ecological conditions; and (3) molecular markers can generate repeatable data that is free from environmental interference on genetic diversity, but markers currently available for most species are neutral and do not reflect diversity associated with adaptation (Barry et al. 2008; van de Wuow et al. 2010). In recent years, however, DNA-marker techniques have provided tools to directly measure genetic diversity and hence test for genetic erosion at the allelic level (Almanza-Pinzón et al. 2003).

Another important aspect in studies of genetic erosion is sampling. We must consider the geographic source, the number of parent plants, the number of seeds from each plant and their distance from one another, and the site where the seed is obtained (barn, plot of land or seed bank) (Guerrant 1992; Sherwin and Moritz 2000). Seed banks, preserving large amounts of genetic material in a small space, are the most practical source. But paradoxically, seed banking practices can induce high levels of internal genetic erosion, starting with the sampling process and continuing through the rest of the processes such as initial or periodic germination tests, distribution, and regeneration (Gómez-Campo 2006; Parzies et al. 2000).

Genetic erosion has been evaluated at the landrace level using demographic approaches, primarily through the study of phenotypes (Hammer and Laghetti 2005; Tsegaye and Berg 2006), which serve as the primary genetic pool in genetic improvement programs (Harlan and de Wit 1971). Indeed, the loss of landraces is believed to generate erosion at the

allelic level (Upadhyay and Sthapit 1998). Several studies to test this hypothesis using molecular data have been carried out in important world crops such as wheat (Donini et al. 2000; Khlestkina et al. 2004; Xiu-Qiang et al. 2007), maize (Le Clerc et al. 2005; Lu and Bernardo 2001), and rice (Mantegazza et al. 2008). In short, these studies have shown that plant breeding and displacement of landraces have resulted in a qualitative, rather than quantitative, change in genetic diversity over time. In contrast, comparable studies of barley have confirmed that differences in genetic diversity between landraces and cultivars appear not to be as dramatic as has often been assumed (Petersen et al. 1994; Struss and Plieske 1998; Backes et al. 2003) and, more surprisingly, that the level of genetic diversity in modern varieties of durum wheat has been increasing over time (Maccaferi et al. 2003).

Many of these studies cited were done outside of the crop natural distribution areas that encompass the centers of landrace diversity and the domestication areas of the crops. The agricultural, socioeconomic, cultural, and environmental dynamics that affect the crops in the studies are very different from those that impact landraces in their domestication centers. Thus, it is very important that studies about the genetic erosion and replacement of landraces be done in their centers of domestication where high levels of genetic diversity for these crops are concentrated, wild populations still exist, and genetic flow and introgression between wild and domesticated populations are common. Indeed, in these areas, farmers maintain the germplasm of the ancestral, domesticated populations and the knowledge and cultural practices that created this diversity (Bellon and Taylor 1993; Brush 1991).

A case for which we can test whether the loss of landraces leads to genetic erosion is the lima bean (*Phaseolus lunatus* L.) landraces from the Yucatan peninsula of Mexico. Lima bean is, after common bean (*P. vulgaris* L.), the second most important domesticated species of the genus *Phaseolus* (Martínez-Castillo et al. 2004). In Mexico, it is cultivated on lowland slopes adjacent to the Pacific Ocean and the Gulf of Mexico by diverse ethnic groups (Ballesteros 1999). In these areas, lima bean is a common cultivar of the milpa, an ancestral, rainfed farming system of Mesoamerica based on human energy, the cyclical slash and burn of vegetation, and a group of basic crops such as corn (*Zea mays* L.),

beans (*Phaseolus* spp.) and squash (*Cucurbita* spp.) (Hernández-Xolocotzi 1992).

The Yucatan peninsula is the most important center of lima bean landrace diversity for Mexico (Ballesteros 1999). Indeed, the Yucatan could be part of the putative domestication center of the Mesoamerican genetic pool of this crop (Gutiérrez-Salgado et al. 1995) or be very near this center (Serrano-Serrano et al. 2010; Motta-Aldana et al. 2010). In this region, the lima bean landraces, called ibes, represent the fourth most important crop of the Mayan milpa (Martínez-Castillo et al. 2004). Over the last few decades in this region, the milpa has undergone a series of changes associated, in part, with the growth of the rural population, that has doubled over the last 30 years (Cuanalo and Arias 1997). Among the most notable changes are (1) shortening of the fallow period, (2) increased use of and dependency on agrochemicals, (3) greater orientation of the Mayan farmers to an external marketing system, and (4) reduction of the areas of vegetation bordering the milpas, where the wild relatives usually grow (Ku-Naal 1995; Reyes and Aguilar 1992). These changes have led to a reduction in the diversity of cultivated species, at both the interspecific and intraspecific levels.

Another important change associated with this agricultural intensification that has led to genetic erosion in local varieties of several crops in the Yucatan peninsula is the introduction of improved varieties. Although maize, the main crop of the Mayan milpa, has been studied intensively (Arias et al. 2007), we do not have much data on lima bean, nor do we know which varieties were released or when and where these varieties were introduced. Hernández and Delgado (1992) reported that between 1942 and 1985, the Instituto Nacional de Investigaciones Agrícolas Forestales y Pecuarias (INIFAP-Mexico) released bean cultivars, including three lima bean cultivars, in the Yucatan peninsula. However, official documents of the INIFAP have never reported data on these introduced lima bean cultivars.

In spite of all the changes just mentioned, the Mayan milpa continues to be the most important agricultural system in many areas of the Yucatan (Martínez-Castillo et al. 2004). It has survived over the centuries primarily because the rocky, shallow soils have limited the introduction of other crops, agricultural machinery and broadcast sowing. Another significant factor contributing to the persistence of the milpa has been the cultural resistance offered by the Mayan people who have maintained this agricultural system as the material basis of their culture over many generations (Zizumbo-Villarreal 1992). However, in areas with flat, deep soils, agricultural intensification has been greater, and the milpa has been transformed into a monoculture of maize or replaced by other non-native crops such as sugarcane (*Saccharum officinarum* L.) or sorghum (*Sorghum bicolor* (L.) Moench s.l.). The direct consequence of this change has been the displacement of secondary crops (e.g., beans, squash, cassava, sweet potato). As an example, many lima bean landraces have disappeared from Nohalal, a Mayan town in northeastern Campeche (NECAMP) (Fig. 1), where a series of low to high hills alternate with interspersed plains that favor agricultural intensification. NECAMP represents one of the four geographic areas in the peninsula where the milpa continues to be the most important economic activity (Martínez-Castillo et al. 2004). Indeed, it is one of the 13 cultural–geographic zones determined by Adams and Culbert (1977) as the possible origin of the Mayan lowland civilization.

In 1979, Debouck collected Lima bean landraces in Nohalal and found more than 10 landraces planted by Mayan farmers. In 2007, we visited the same town and found only three landraces. The germplasm collected in 2007 was mainly that of sac and mulicion, landraces, that were not collected in 1979. Now, these landraces are the most important ones in the milpa on the entire peninsula, perhaps as a consequence of market preferences (Ballesteros 1999; Martínez-Castillo et al. 2004).

The Yucatan peninsula could be playing an important role in *in situ* conservation of the Mesoamerican gene pool of lima bean crop because (1) this region contains the highest morphological variation of lima bean landraces reported for all Mexico (Ballesteros 1999); (2) traditional Mayan farmers conserve ancestral landraces, along with the knowledge and cultural practices that created this diversity (Martínez-Castillo 2004); and (3) the genetic diversity of the domesticated gene pool present in this region is higher compared to other Mesoamerican regions (Martínez-Castillo et al. 2008). Indeed, many wild populations with high levels of genetic diversity grow in the peninsula (Martínez-Castillo et al. 2006) and wild-domesticated gene flow and weedy forms have been reported for this region (Martínez-Castillo et al. 2007). In addition, on the basis of recent ethnobotanical observations (J. Martínez-Castillo) in the barns of

Fig. 1 Study area where the plant material analyzed was collected
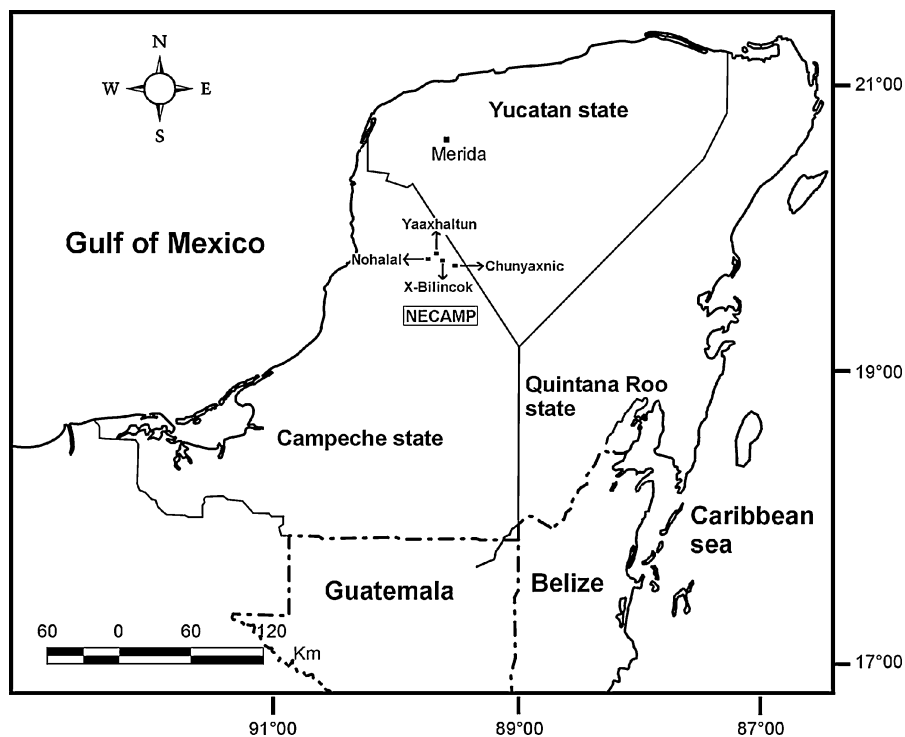
Mayan farmers, the weedy forms generated by introgression are being incorporated by farmers into their cultivated gene pools. In this regard, several authors (Altieri and Montecinos 1993; Harlan 1965; Jarvis and Hodgkin 1999; Quirós et al. 1992; Slatkin 1987; Stebbins 1959) have considered that gene flow and introgression between wild and domesticated gene pools have played a vital role in the evolution of domesticated species and continue to significantly augment genetic diversity in modern crops.

To understand the role of a geographical region in the *in situ* conservation of the genetic diversity of any domesticated species, it is necessary to study the genetic erosion affecting the crop in that region, i.e., the present conservation status of that crop and any changes in genetic diversity during the last decades. In a study of the current genetic erosion and *in situ* conservation of lima bean landraces in the Yucatan peninsula using inter-sequence simple repeats (ISSR) as DNA markers, Martínez-Castillo et al. (2008) found that total genetic diversity was high in this gene pool ($H = 0.29$). However, the three most abundant landraces (sac ib, mulicion and putsica-sutsuy) had genetic diversity values lower ($H = 0.13$) than those of the rare landraces ($H = 0.24$). They concluded that the rare landraces are at higher risk of genetic erosion due

to local extinction because only a few Mayan farmers plant them. To complement the approach of Martínez-Castillo et al. (2008), we used microsatellite (simple sequence repeats [SSR]) DNA markers to analyze any genetic erosion in lima bean landraces from NECAMP that may have occurred between 1979 and 2007. Our objectives were to (1) test the hypothesis that the loss of landraces leads to genetic erosion at the allelic level and (2) test for the existence of a recent bottleneck in the domesticated gene pool from NECAMP as a factor that could explain any genetic erosion found.

Materials and methods

Plant material and extraction of DNA

Plant material was collected in NECAMP (Fig. 1). Seeds of 23 accessions, collected in 1979 by D. G. Debouck in Nohalal, were obtained in 2008 from the Genetic Resources Unit of the Centro Internacional de Agricultura Tropical (CIAT-Colombia) (Fig. 2a, Table 1). We also collected seeds of 21 accessions in 2007 in the town of Nohalal and three adjacent Mayan towns, Chunyaxnic, X-Bilincok and Yaax-haltun

Fig. 2 a Accessions from 1979 analyzed. **b** Accessions from 2007 analyzed. Letter after the accession represents the town where that accession was collected: *a* Yaaxhaltun; *b* Chunyaxnic; *c* X-Bilincok; *d* Nohalal

(Fig. 2b, Table 1). Five plants of each accession were grown from seed in a greenhouse at the Centro de Investigación Científica de Yucatán, in Merida, Mexico. Total DNA was extracted from young leaves using the CTAB (cetyltrimethyl ammonium bromide) method described by Doyle and Doyle (1987). The

Table 1 Plant material used in the temporal analysis of the genetic erosion of lima bean landraces from Northeast Campeche, Mexico

1979 collection			2007 collection		
Code no.	Landrace	Town	Code no.	Landrace	Town
G25770	Petch	Nohalal	JMC1028	Sac	Yaaxhaltun
G25771	Kolbini	Nohalal	JMC1029	Sac	Yaaxhaltun
G25772	Madza-kitam	Nohalal	JMC1030	Morado	Yaaxhaltun
G25773	Morado	Nohalal	JMC1031	Mulición	Chunyaxnic
G25774	Dzitziba	Nohalal	JMC1032	Sac	X-Bilincok
G25774-A	Dzibziba	Nohalal	JMC1033	Putsica-sutsuy	Chunyaxnic
G25774-B	No data	Nohalal	JMC1034	Mejen	Chunyaxnic
G25775	No data	Nohalal	JMC1035	Morado	Chunyaxnic
G25776	Kan	Nohalal	JMC1036	Mulicion	X-Bilincok
G25777	Huihitz	Nohalal	JMC1037	No data	X-Bilincok
G25778	Morado	Nohalal	JMC1038	No data	X-Bilincok
G25779	Dzitziba kankab	Nohalal	JMC1039	No data	X-Bilincok
G25779-A	Dzitziba kankab	Nohalal	JMC1040	No data	X-Bilincok
G25780	Dzitziba gris	Nohalal	JMC1043	Sac	Nohalal
G25781	Dzitziba gris	Nohalal	JMC1044	Sac	Nohalal
G25781-A	Dzitziba gris	Nohalal	JMC1045	Sac	Nohalal
G25783	Dzitziba guinda	Nohalal	JMC1046	Sac	Nohalal
G25784	Pinto box	Nohalal	JMC1047	Chak mejen	Nohalal
G25994	Putsica-sutsuy	Nohalal	JMC1048	Chak mejen	Nohalal
G25995	Putsica-sutsuy	Nohalal	JMC1051	Sac	Nohalal
G25996	No data	Nohalal	JMC1052	Mulición	Nohalal
G25997	Putsica-sutsuy	Nohalal	–	–	–
G25998	Madza-kitam	Nohalal	–	–	–

purified total DNA was quantified by fluorometry and its quality verified by agarose gel electrophoresis.

Amplification of microsatellites and electrophoresis

The microsatellite markers were analyzed in accordance with Martínez-Castillo et al. (2006), using nine pairs of primers reported as polymorphic in *P. lunatus* by Gaitán-Solís et al. (2002) (Table 2). The amplification was performed in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA, USA). A 4-μl volume of formamide, containing 0.45% of bromophenol blue and 0.25% of xylene-cianol was added to the product of PCR prior to denaturation for 4 min at 94°C. A 4-μl sample of the reaction mixture was then loaded onto denatured gels of polyacrylamide

at 5% (19:1 acrylamide-bisacrylamide) with 5 mol/l urea and continuous $0.5\times$ Tris-borate-EDTA (TBE) buffer. Electrophoresis was carried out at 60 W constant power for 2.0–2.5 h (SQ3 Hoeffer sequencer). The products of the amplification were visualized by silver staining (Bassam et al. 1991).

Data analyses

To analyze the hypothesis that the loss of landraces means genetic erosion at the allelic level, we used three approaches:

(1) *Genetic diversity present in years of collection (1979 vs. 2007)* Considering the interchange of seed between farmers from neighbouring towns, we (1) compared the genetic diversity of all

Table 2 Characteristics of nine SSR loci used to estimate the genetic erosion of lima bean accessions collected in 1979 and 2007 in northeastern Campeche, Mexico

Primer	SSR sequence	5′ to 3′	Primer sequence	Annealing temperature (°C)	Base pairs
GATS91	$(GA)_{17}$	Forward	GAGTGCGGAAGCGAGTAGAG	53	229
		Reverse	TCCGTGTTCCTCTGTCTGTG		
AG1	$(GA)_8GGTA(GA)_5$	Forward	CATGCAGAGGAAGCAGAGTG	52	132
		Reverse	GAGCGTCGTCGTTTCGAT		
BM140	$(GA)_{30}$	Forward	TGCACAACACACATTTAGTGAC	55	190
		Reverse	CCTACCAAGATTGATTTATGGG		
BM156	$(CT)_{32}$	Forwarrd	CTTGTTCCACCTCCCATCATAGC	52	267
		Reverse	TGCTTGCATCTCAGCCAGAATC		
BM211	$(CT)_{16}$	Forward	ATACCCACATGCACAAGTTTGG	52	186
		Reverse	CCACCATGTGCTCATGAAGAT		
BM202	$(GA)_9GT(GA)_4$	Forward	ATGCGAAAGAGGAACAATCG	50	156
		Reverse	CCTTTACCCACACGCCTTC		
BM170	$(CT)_5CCTT(CT)_{12}$	Forward	AGCCAGGTGCAAGACCTTAG	50	179
		Reverse	AGATAGGGAGCTGGTGGTAGC		
BM183	$(TC)_{14}$	Forward	CTCAAATCTATTCACTGGTCAGC	52	149
		Reverse	TCTTACAGCCTTGCAGACACT		
BM197	$(GT)_8$	Forward	TGGACTGGTCGATACGAAGC	54	201
		Reverse	CCCAGAAGATTGAGAACACCAC		

accessions collected in 1979 in Nohalal versus the accessions collected in the same town in 2007 and (2) compared the genetic diversity of the 1979 accessions collected only in Nohalal versus all accessions collected in 2007 (Nohalal and the other three Mayan towns).

First, Wright's (1978) fixation index (F_{IS}) as a measure of heterozygote deficiency or excess was obtained by analyzing the Hardy–Weinberg equilibrium in materials from the 2 years of collection. Then, we calculated the following estimators of genetic diversity: the percentage of polymorphic loci (%P), the average number of alleles per locus (n_a), the alleles effective (n_e); and the expected heterozygosity using Nei's genetic diversity index ($H = 1 - \frac{1}{m}\sum_{i=1}^{m}\sum_{i=1}^{k}p_i^2$ where p_i is the frequency of the ith of k alleles and m is the total number of loci) (Nei 1973). Both analyses were done using the program POPGENE 1.31 (Yeh and Boyle 1999).

(2) *Genetic differentiation between years of collection (1979 vs. 2007)* To statistically assess genetic variation within and among years of collection, we performed an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) with the software package Arlequin, version 2000 (Schneider et al. 2000).

(3) *Genetic relationships* Genetic relationships among all accessions collected in both years were analyzed using the UPGMA method (unweighted pair group method with arithmetic mean). The construction of the UPGMA was based on the standard genetic distance of Nei for various loci ($D' = -\ln I'$, where I' is the genetic identity for multiples loci (Nei 1972), using the TFPGA program (Miller 1997). The robustness of the topology was evaluated by the selection of a bootstrapping option with 1,000 random re-samplings with replacement over loci (Felsenstein 1985).

To analyze the existence of a recent bottleneck event (1979–2007) as a consequence of the displacement and/or loss of landraces in the NECAMP, we used the Bottleneck program v1.2.02 (http://www.ensam.inra.fr/URLB).

For each population sample and for each locus, the Bottleneck program computes the distribution of gene diversity expected from the observed number of alleles (k), given the sample size (n) under the assumption of mutation–drift equilibrium (Luikart and Cornuet 1997; Luikart et al. 1998). The probability distribution was established using 1,000 simulations under three models: IAM (infinite allele model), SMM (step-wise mutation model), and two-phase model of mutation TPM (two-phased model). This distribution is obtained by simulating the coalescent process of n genes under these three models, thus enabling the computation of the average (H_e), which is compared to the observed genetic diversity (H_o, or Hardy–Weinberg heterozygosity) to establish whether there is a gene diversity excess or deficit at this locus. To test for the existence of the bottleneck for the years of collection (1979, 2007, and Nohalal-2007) and for all accessions (the 44 accessions), we used the Wilcoxon sign-rank test. It provides relatively high power and can be used with as few as four polymorphic loci and any number of individuals.

Results and discussion

We found 27 alleles for the nine analyzed loci. Of these, 14 alleles were found in the 1979 collection and 13 in the 2007. Eleven alleles were unique for 1979 and 10 for 2007. The GATS91 and BM156 loci had the highest number of alleles (4). The AG1, BM202, BM170, BM183 and BM197 loci had the lowest number of alleles (2). These results indicate that there is a clear difference in the allelic composition for each year. Maras et al. (2006), using 14 microsatellite loci, analyzed temporal changes in genetic diversity of common bean (*Phaseolus vulgaris*) accessions cultivated between 1800 and 2000 in Slovenia and nearby regions. They demonstrated that, during a particular period, a certain number of unique alleles were initially present and that the allelic composition changed during the cultivation of common bean in that region. A similar qualitative shift in genetic diversity has been shown for other crops also (Donini et al. 2000; Koebner et al. 2003; Roussel et al. 2004).

The low number of alleles per loci found in the present study could be a consequence of the nature of

the accessions analyzed and the traditional Mayan agricultural practices (sowing different landraces in the same milpa and the exchange of seeds among farmers), which could lead to genetic homogenization of the landraces and, concomitantly, to the fixation of alleles (Martínez-Castillo et al. 2004). A different situation has been observed for wild populations of *P. lunatus* in Yucatan peninsula, where we have found up to 16 alleles per locus (Martínez-Castillo et al. 2006).

The analysis of Hardy–Weinberg equilibrium showed a disequilibrium for both years of collection, with an excess of homozygotes in both cases. Only the GATS91 locus showed equilibrium for 2007 ($F_{IS} = -0.016$). Although *P. lunatus* presents a mixed cross-breeding system with outcrossing levels up to 48%, it is a predominantly autogamous species (Baudoin et al. 1998). This selfing reproductive system and the Mayan agricultural seed practices help us understand the results found in this study.

Genetic diversity in 1979 and 2007

Nohalal-1979 versus Nohalal-2007

All genetic diversity estimators showed higher values for Nohalal-1979 (Table 3). These results could be a consequence of the number and kind of landraces collected in each year. In 1979, Dr. Debouck collected 10 landraces at least, whereas in 2007 we collected only three landraces, and many of the accessions had seeds with white testa (Fig. 2b). However, this result should be taken with caution; in the Nohalal-1979 sample included 23 accessions, whereas the Nohalal-2007 sample included only eight accessions because that year many farmers did not plant lima bean (Table 1). Now, the white seed

Table 3 Estimators of genetic diversity among the lima bean accessions collected in northeastern Campeche analyzed by collection year (1979, 2007) and location

Sample	%P	n_a (SD)	n_e (SD)	H (SD)
1979	44.44	1.55 (0.73)	1.34 (0.50)	0.18 (0.24)
2007-Nohalal	5.00	1.04 (0.01)	1.00 (0.02)	0.01 (0.01)
All-2007	44.44	1.56 (0.73)	1.05 (0.08)	0.05 (0.07)

$\%P$ = percentage of polymorphic loci, n_a = observed no. of alleles, n_e = effective no. of alleles, H = Nei's genetic diversity index, SD = standard deviation

landraces (sac ib, mulicion and x-mejen ib) dominate lima bean production in the Yucatan peninsula (Martínez-Castillo et al. 2004). A recent study using ISSR DNA markers showed that these most abundant landraces have genetic diversity values lower than those of the rare landraces (Martínez-Castillo et al. 2008). At present, there is no evidence whether these abundant landraces are actually landraces favored by the incorporation of Mayan farmers into the market (Ballesteros 1999) or are improved varieties introduced in the 1970s and 1980s (Hernández and Delgado 1992).

Nohalal-1979 versus all-2007

There were no differences in the values of %P and n_a between the two collection years, suggesting that the allelic richness is similar in both years. However, we found differences in n_e and H, whose values were higher in Nohalal-1979 (Table 2). Importantly, the inclusion of accessions collected in the other three Mayan towns adjacent to Nohalal (X-Bilincok, Chunyaxnic and Yaax-haltun) decreased the differences observed between the genetic diversity parameters in relation to the previous analysis (*Nohalal-1979 vs. Nohalal-2007*) (Table 3). The germplasm collected in 2007 in X-Bilincok, Chunyaxnic and Yaax-haltun could contain accessions of other landraces not grown in Nohalal that year. These landraces may have high levels of genetic diversity that could compensate for

the lower levels of genetic diversity present in the landraces collected in 2007 in Nohalal. These landraces are no longer planted in Nohalal, even though the four Mayan towns are neighbors. The main reason for this could be the agricultural intensification that has taken place in Nohalal over the last 30 years. The Nohalal Mayan farmers had better soils that could be used to plant improved varieties of maize and other species with a higher commercial value. In the case of the lima bean landraces, only those with commercial value such as sac ib and mulicion are considered for planting in these areas. Agricultural intensification has been the main factor associated to the genetic erosion in the crops (van de Wouw et al. 2010).

Genetic differentiation between the 2 years of collection

Analysis of molecular variance (AMOVA) revealed that a great proportion (82.2%) of the total variation can be explained by differentiation among two temporal groups of accessions (1979 vs. all-2007), with only 12.9% of the total variation found among accessions within years. This result is expected, considering the high differences in the allelic composition of each year. Due to the high frequencies of specific alleles in each year, such high differentiation was expected because the AMOVA takes into account both allelic richness and abundance.

Fig. 3 Dendrogram (UPGMA) based on Nei's genetic distance (1978) of 44 accessions analyzed using nine SSR loci. The numbers at the nodes are the proportion of similar replicates supporting each node
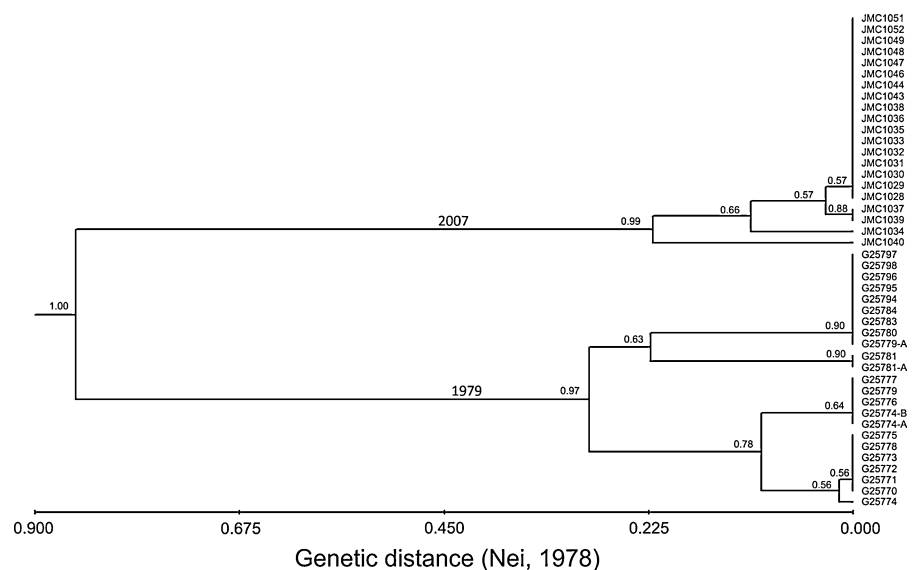
Genetic relationships

Figure 3 shows the topology of a tree generated with a UPGMA of the 44 lima bean accessions analyzed. This topology indicates a grouping of the accessions in accordance with the years of collection, with high bootstrap values supporting each group. This result indicates that the genetic erosion is not only quantitative as described in the earlier sections, but also qualitative, meaning that over the last 30 years, the genetic make-up of this crop in NECAMP has shifted. This result is also similar to other studies that indicated that plant breeding can generate a qualitative change in the genetic diversity of the crops (Donini et al. 2000; Khlestkina et al. 2004; Xiu-Qiang et al. 2007; Le Clerc et al. 2005; Lu and Bernardo 2001; Mantegazza et al. 2008).

Indeed, our result supports the hypothesis proposed by Martínez-Castillo et al. (2004) that the sac ib and mulicion landraces could be improved varieties or landraces introduced to the Yucatan peninsula (probably between 1970 and 1980), that has displaced the local varieties of lima bean. However, this point is not clear because there is not much data about this introduction (Hernández and Delgado 1992) and its effect on the displacement of local lima bean varieties. Gao (2003), using ethnobotanical data, found that the abandonment of traditional agricultural practices and the adoption of highly productive rice varieties has led to the loss of local rice varieties in China, thus driving serious genetic erosion for this crop. Similar results were found for Colunga-GarcíaMarín and Zizumbo-Villarreal (2007) and Vargas-Ponce et al. (2007), who reported that the current germplasm diversity used in the production of Agave spirits in west-central Mexico is in danger of erosion due to an expansion in the cultivation of the clone *A. tequilana* Weber var. azul, used for the elaboration of the Tequila.

Bottleneck analysis

For the bottleneck analysis of genetic diversity for the years of collection, the Wilcoxon sign-rank tests were not significant, indicating there is no excess in gene diversity under all three models. Thus, there was no bottleneck event for these lima bean gene pools. For Nohalal-1979, SMM, TPM, and IAM, the *P*-values were the same (0.06250). For all-2007, the *P*-values

were also the same for IAM (0.9687), TPM (1.000) and SMM (1.000). For Nohalal-2007, the *P*-values were the same for the three models (1.000). Of all these *P*-values, only the Lima bean gene pool from 1979 had values close to $\alpha = 0.05$. It is important to remember that the accessions of Nohalal-1979 were obtained from the CIAT seed bank where the preservation process of the seed can lead to genetic erosion (Gómez-Campo 2006; Parzies et al. 2000). Even with this, Nohalal-1979 gene pool had higher genetic diversity than the all-2007 gene pool ($H = 0.18$ vs. $H = 0.05$, respectively). In the bottleneck analysis considering all accessions, the Wilcoxon sign-rank tests indicate no significant differences. For SMM, TPM, and IAM, the *P*-values were the same (1.000), indicating there is no excess in gene diversity under all three models.

Implications for conservation

As mentioned earlier, the *P. lunatus* landraces are the fourth most important crop in traditional Mayan agriculture in the Yucatan peninsula. Martínez-Castillo et al. (2008) showed that the present genetic diversity of these landraces is at risk of being eroded because the three most abundant landraces in this region (mulicion, sac ib, putsica-sutsuy) had lower levels of genetic diversity in comparison to the rare landraces (at least 12 landraces). Unfortunately, these rare landraces could soon disappear because they are only planted by a few Mayan farmers in this region (Martínez-Castillo et al. 2004). The present results showed a decrease in the genetic diversity between 1979 and 2007 and seem to confirm the high risk of genetic erosion faced by this crop predicted by Martínez-Castillo et al. (2004, 2008). Thus, with fewer landraces planted, the genetic diversity in the crop will be lower.

The present study also showed a great shift in the allelic composition. Similar results were reported for many other crops (Khlestkina et al. 2004; Xiu-Qiang et al. 2007; Le Clerc et al. 2005; Mantegazza et al. 2008). This genetic shift may be a consequence of the introduction of improved varieties or landraces of *P. lunatus* (Hernández and Delgado 1992). Unfortunately, again we do not have enough data about the introduction of lima bean germplasm in this part of Mexico to understand this shift. The shift could also be due to changes in Mayan selection criteria of

germplasm or responses to market preference (Ballesteros 1999). Studies considering these aspects in relation to traditional agricultural intensification showed similar results for rice landraces in China (Gao 2003), wheat landraces in Ethiopia (Tsegaye and Berg 2006), and agave spirits landraces in Mexico (Colunga-GarcíaMarín and Zizumbo-Villarreal 2007; Vargas-Ponce et al. 2007).

Similar to the case in Nohalal town, the loss of landraces has occurred in many others Mayan towns in the Yucatan peninsula. This loss is not only a consequence of factors associated with agricultural intensification or the incorporation of the Mayan farmers into an external market system, but is also due to a series of environmental, socioeconomic and cultural factors. The passage of hurricane Dean in 2007 in the south central region of the state of Quintana Roo can be cited as an example. During a collection trip in 2008, we talked with Mayan farmers of this region who reported that Dean caused the seed loss of many cultivated species, among them, many rare landraces of *P. lunatus*. At present, we have been unable to collect several of these landraces for *ex situ* conservation. A similar case was reported when the hurricane Mitch struck Central America in 1998, threatened the existence of landraces of different crops (Haugen 2001).

An important aspect in studies of genetic erosion in crops is sampling strategy (Gómez-Campo 2006; Guerrant 1992; Parzies et al. 2000; Sherwin and Moritz 2000). Even though the seed samples for the 2 years of collection in this study differed (seed origin, sample size), we consider that the results obtained for allelic composition, genetic diversity estimators, AMOVA, and cluster analysis are consistent: a great proportion of the genetic diversity present in 1979 has been lost; however, this loss cannot be explained by the existence of recent bottleneck events. Two plausible explanations are that the allelic composition from 1979 has been replaced with another one in just 30 years due to the switching of the Mayan farmers to an external market, which favors just a few landraces, mainly white seed landraces (compare Fig. 2a, b), and the introduction of improved varieties or landraces of lima bean that have decreased the genetic diversity in the landraces planted in this part of Mexico.

The results of this study stress the need for establishing an appropriate strategy to manage genetic resources. In relation to this, laudable efforts had been made by nongovernmental institutions to promote agricultural diversity and seed interchange, for example, through seed fairs, among the Mayan farmers in the Yucatan. However, these efforts have not reached the few Mayan farmers that plant rare landraces. Many of these farmers live in marginal agricultural towns and do not possess the economic resources to participate in these events. An example of this was the seed fair organized in Felipe Carrillo Puerto, Quintana Roo, a region where our research group has collected up to 14 landraces (Martínez-Castillo et al. 2004), but this landrace richness was not evident at the seed fair. On one hand, this lack could confirm the high genetic erosion risk for *P. lunatus* landraces; on the other hand, this observation shows the urgent need for the participation of the different societal sectors to conserve the genetic diversity of our crops. With this in mind, our research group is actively collecting the landraces and wild populations of *P. lunatus* present in the Yucatán peninsula to create a core collection for this important crop in traditional Mayan agriculture of Mexico.

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