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Structure and Genetic Diversity of Wild Populations of Lima Bean (*Phaseolus lunatus* L.) from the Yucatan Peninsula, Mexico

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ABSTRACT

This study was conducted to determine the genetic diversity, structure and gene flow of 11 wild populations of *Phaseolus lunatus* L. in four regions of traditional agriculture in the Yucatan Peninsula, Mexico, part of the putative domestication area of its Mesoamerican gene pool. Analyzing eight microsatellite loci, the populations showed high values of diversity: observed heterozygosity (*Ho*) 0.46 to 0.9; Nei's index of diversity (*H*) 0.35 to 0.59 and average number of alleles per locus (A) 2.37 to 3.38. Both Nei's index of populations differentiation (*G*st) and analysis of molecular variance (AMOVA) indicated strong differentiation. The Bayesian analysis of grouping and the Mantel test suggested isolation among agricultural regions as a major factor for population differentiation. Even though a low long-term gene flow (*N*m = 0.66) and low rates of recent migration among populations were observed, there were some cases where the accidental transport of seeds could be favoring a gene flow at a long distance. Data found in this study suggest a positive correlation between agricultural intensification and increase in diversity, suggesting that wild populations are favored by the intensification of disturbance in situations involving at least 3 yr of fallow. However, the opposite could be true at higher levels of intensification as has been reported in the Central Valley of Costa Rica, where the diversity is diminishing.

THERE HAS BEEN renewed interest in studying the wild ancestors of domesticated species as a plant genetic resource, given their potential value as reservoirs of genetic variation for crop improvement (Degreef et al., 2002). One species that must be included in this focus is the Lima bean, a tropical legume characterized by high levels of genetic diversity and whose primary gene pool includes both wild and domesticated forms (Baudet, 1977). This pool is divided into two main groups (Mesoamerican and Andean) and a smaller group of intermediate genotypes (Debouck et al., 1987; Maquet et al., 1990; Gutiérrez-Salgado et al., 1995; Fofana et al., 1997; Caicedo et al., 1999; Lioi and Galasso, 2002). The Lima bean is an annual or short-lived perennial species, with a mixed mating system that is predominantly autogamous but with outcrossing levels up to 48% (Baudoin et al., 1998). Wild individuals are characterized by indeterminate climbing growth, a prolonged flowering period, and production of a large number of pods (Zoro Bi et al., 2003).

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The Peninsula of Yucatan is part of the putative domestication area of the Mesoamerican gene pool of *P. lunatus* (Gutiérrez-Salgado et al., 1995). It possesses the largest diversity of domesticated forms of the species in Mexico (Ballesteros, 1999), a diversity that is possibly being generated and maintained, in part, by their sympatric growth with wild populations. Domesticated forms receive the Mayan name of *ib*, whereas the wild forms are called *ib-cho* (mouse ib). Lima bean is one of the most important crops associated with maize (*Zea mays* L.) in the traditional Mesoamerican agricultural system known as the "milpa." The milpa system is based on the use of human energy, where the vegetation is cyclically slashed and burned to plant crops in the area during a period of 2 to 4 yr and then left fallow for the next 5 to 15 yr when a new cycle can be initiated (Pérez-Toro, 1945; Hernández-Xolocotzi, 1959). This management strategy allows the conservation of vegetation patches, which in turn favors the milpa productivity, ensuring both the recovery of soil fertility and the availability of the wild genetic resources such as the wild relatives of the domesticated species, which are also part of this agricultural system (Zizumbo-Villarreal and Simá, 1988; Colunga-GarcíaMarín and May-Pat, 1992; Hernández-Xolocotzi, 1992; Zizumbo-Villarreal, 1992). In this region, during the last few decades, the milpa has undergone a series of transformations associated, in part, with the growth of the rural population, which has doubled in the last 30 yr (Cuanalo and Arias, 1997). Among the most notable changes are (i) a shortening of the fallow period, (ii) an increased use of and dependency on agrochemicals, (iii) a greater integration of the producers to an external marketing system, (iv) a reduction in the diversity of cultivated species, and (v) a reduction of the areas of vegetation bordering the milpas, where the wild populations usually grow (Reyes and Aguilar, 1992; Lazos, 1995; Ku-Naal, 1995; Remmers and Ucán, 1996).

The genetic diversity of Lima bean from the Yucatan Peninsula has mainly been studied with accessions included in germplasm banks on the basis of morphophenological characters and focusing essentially on the domesticated gene pool. Results have indicated high genetic diversity for this gene pool (Debouck, 1979; Ballesteros, 1999; Martínez-Castillo et al., 2004). However, there is little information available on the wild gene pool of the region. Until 1999, only a few collections of

Abbreviations: SSR, simple sequence repeat; CEQROO, central east Quintana Roo; SEYUC, southeast Yucatan; NECAMP, northeast Campeche; SYUC, south of Yucatan; AMOVA, analysis of molecular variance; ANOVA, one-way analysis of variance; UPGMA, unweighted pair group method with arithmetic mean; *A*, average number of alleles; *Ho*, observed heterozygosity; *H*, Nei's index of diversity; *G*st, Neí's index of populations differentiation.

wild seed had been obtained in the state of Campeche. These were characterized morphologically (seed color pattern and seed weight) and biochemically (phaseolin type and HCN content) (Debouck, 1979; Maquet, 1991; Ballesteros, 1999). For the whole Mesoamerican gene pool, only the wild populations of the Central Valley of Costa Rica have been studied (Maquet et al., 2001; Vargas et al., 2001; Ouédraogo and Baudoin, 2002; Zoro Bi et al., 2003); this is a region in which the domesticated forms of *P. lunatus* have little importance as a crop.

Martínez-Castillo et al. (2004) performed a study of the intraspecific diversity of *P. lunatus* of the Yucatan Peninsula on the basis of ethnobotanical and morphophenological information, involving both the domesticated and wild gene pools. These authors collected 14 wild populations growing in four areas where the milpa system is still practiced, finding that (i) there are populations containing one or two plants with morphological characteristics (in seed, pod, and flower) similar to those of some landraces and (ii) although farmers occasionally tolerated the growth of wild populations, in general they controlled them through hand-weeding and herbicides. Some farmers indicated that the wild populations disappeared after the seventh or eighth year in the fallow period, and reappeared in the new agricultural cycle, suggesting the existence of seed banks, such as those reported for the Central Valley of Costa Rica (Degreef et al., 2002). This observation also suggested that the fallow period may be important in the population dynamics of wild *P. lunatus*. The changes that have taken place during the last few decades in the traditional agriculture of the Yucatan Peninsula could have important repercussions on the presence of wild populations of Lima bean as well as on its genetic structure, diversity, and gene flow. For the study of 11 wild populations of Lima bean in four important regions of traditional agriculture in the Yucatan Peninsula, the following objectives were established: (i) estimate genetic diversity; (ii) determine genetic differentiation; and (iii) estimate gene flow. The estimates were based on eight microsatellite (SSR) loci identified by Gaitán-Solís et al. (2002).

MATERIALS AND METHODS

Regions and Populations Studied

Four regions of the Yucatan Peninsula, where traditional agriculture is practiced, were considered in this study. Different degrees of agricultural intensification, defined as different fallow periods and levels of use of agrochemical products, can be observed: (i) central eastern part of the state of Quintana Roo (CEQROO), a region with fallow periods of approximately 15 yr and a low level of use of agrochemical products,; (ii) southeast of the state of Yucatan (SEYUC), a region with fallow periods of approximately 10 yr and a low use of agrochemical products; (iii) northeast of the state of Campeche (NECAMP), a region with fallow periods of four to 5 yr and a medium use of agrochemical products; and (iv) south of the state of Yucatan (SYUC), a region with fallow periods of 2 to 3 yr and intensive use of agrochemical products (Fig. 1).

Eleven of the 14 wild populations studied by Martínez-Castillo et al. (2004) were analyzed in this study: three populations from each of the four regions, except for SEYUC, where only two populations were included. For each population, the following information was recorded: (i) type of habitat, (ii) approximate area covered by the wild population, (iii) size of the wild population (assuming that each individual occupied an area of approximately 4 m^2, and including only those individuals with mature pods at the moment of collection), and (iv) morphological evidence of possible wild–domesticated introgression on flower, pod, or seed characters.

Collection of Material and Extraction of DNA

Considering that Baudoin et al. (1998) reported that the size of the neighborhood in wild populations of *P. lunatus* is 1.6 m, we collected seeds of individuals separated from each other by a distance of at least 3 m, to ensure that the seeds of each individual represented a distinct family. We collected seeds of 30 individuals for each population across their entire distribution range. Of those, 20 were selected for use in the study and the seeds of the other 10 were kept as a back up. We collected from five to 10 pods of each individual. The seeds of these pods were mixed and 10 seeds were soaked in liquid nitrogen to break the latency and to germinate. Of the germinated seeds, one plant per individual was selected for the extraction of DNA, making it a total of 20 plants per population, each belonging to an individual. Of the Chunchintok and Nohca populations, only 19 and 14 plants were respectively obtained. Genomic DNA was obtained from young leaves following the CTAB method (Doyle and Doyle, 1987).

Amplification of Microsatellites and Electrophoresis

The microsatellite technique was applied in accordance with Gaitán-Solís et al. (2002), using eight pairs of primers reported as polymorphic in *P. lunatus* (Table 1). The amplification was performed in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, USA). A volume of 4 μL of formamide, containing 0.45% (w/v) of bromophenol blue and 0.25% (w/v) of xylene-cianol was added to the product of PCR before denaturation for a period of 4 min at 94°C. It was loaded with 4 μL of the reaction mixture onto denatured gels of polyacrylamide at 5% (w/v; 19:1 acrylamide-bisacrylamide) with 5 *M* urea and continuous 0.5× TBE buffer. Electrophoresis was performed at 60 W constant power for 2.0 to 2.5 h (SQ3 sequencer, Hoeffer Scientific Instruments, S. Francisco, CA). The products of the amplification were visualized by the technique of silver staining using the Promega Q4132 kit (Promega, Madison, WI) and following the instructions of the supplier.

Data Analysis

Indices of genetic diversity were estimated using POPGENE 1.31 (Yeh and Boyle, 1999), considering three levels of analysis: populations, agricultural regions and the Peninsula of Yucatan. The estimates were *A* (Hartl and Clark, 1989), the evenness of the allelic frequencies (*Ae*/*A*, where *Ae* is the effective number of alleles (Kremer et al., 1998), *H*o, which is calculated as (number of heterozygotes at a locus/total number of individuals surveyed)/total number of loci, and *H*, calculated as $1-\frac{1}{m}\sum_{i=1}^{m}\sum_{i=1}^{k}p_i^2$, where p_i is the frequency of the *i*th of *k*th alleles and *m* is the total number of loci (Nei, 1973). Using the GLM procedure of the SAS ver. 6.12 (SAS, 1997) program, a one-way analysis of variance (ANOVA) and Duncan's multiple comparison of means tests ($\alpha = 0.05$) were conducted to compare the values of allelic richness and diversity obtained at a regional level.

Wright's inbreeding coefficient [$F_{IS} = 1 - (Ho/He)$] (Wright, 1978) was obtained as an indicator of excess or deficit of

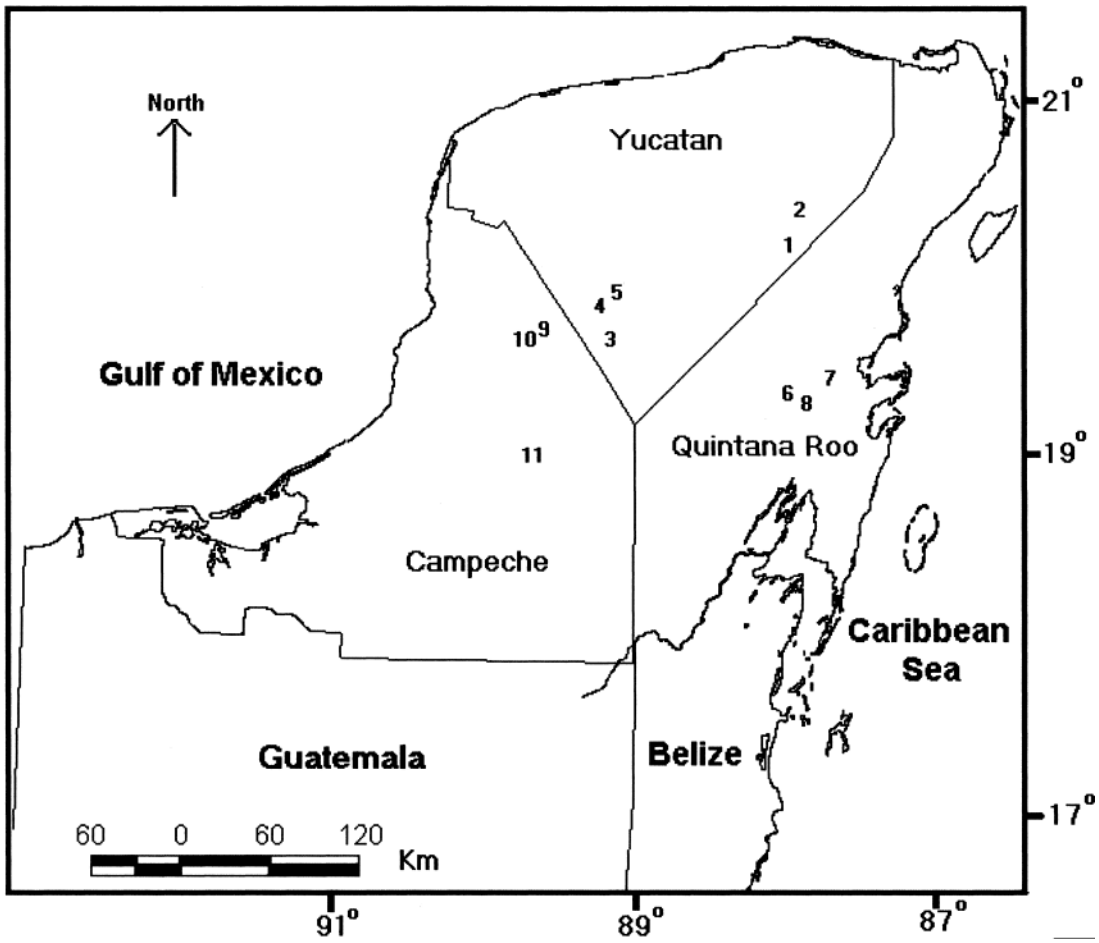

Fig. 1. Geographical location of 11 wild populations studied in four agricultural regions in the Yucatan Peninsula, Mexico. Populations of the southeast of Yucatan (SEYUC): San Fernando (1), Boje (2). Populations of the south of Yucatan (SYUC): Nohcacab (3), Xohuayán-1 (5), Xohuayán-2 (4). Populations of central eastern Quintana Roo (CEQROO): Nohcá (6), Kik (7), Holpat (8). Populations of the northeast of Campeche (NECAMP): Itzinté (9), Bolonchén (10), Chunchintok (11).

heterozygotes for each locus/population using POPGENE 1.31. We tested if these values were different from zero ($\alpha = 0.05$) with a chi-squared test, $\chi^2 = N\,(r-1)\,F_{IS}^2$ with $r\,(r-1)/2$ degrees of freedom, where N is the sample size and r is the number of alleles at the locus (Li and Horvitz, 1953). The F_{IS} values were averaged across polymorphic loci for each population by means of a jackknife procedure. To estimate if these values were significantly different from zero ($\alpha = 0.05$) we used a two-tailed Student t test based on jackknife-generated standard error values (Sokal and Rohlf, 1985).

Table 1. Characteristics of the eight pairs of microsatellite primers used in the analysis of the genetic diversity of 11 wild populations of *P. lunatus* in the Yucatan Peninsula, Mexico.

Code	SSR sequence	5′ to 3′	Primer sequence	Tm†	NoA‡	RF§
GATS91	$(GA)_{17}$	left	GAGTGCGGAAGCGAGTAGAG	53	5	218–231
		right	TCCGTGTTCCTCTGTCTGTG			
AG1	$(GA)_8GGTA(GA)_5$	left	CATGCAGAGGAAGCAGAGTG	52	7	147–155
		right	GAGCGTCGTCGTTTCGAT			
BM140	$(GA)_{30}$	left	TGCACAACACACATTTAGTGAC	55	7	162–173
		right	CCTACCAAGATTGATTTATGGG			
BM156	$(CT)_{32}$	left	CTTGTTCCACCTCCCATCATAGC	52	10	205–225
		right	TGCTTGCATCTCAGCCAGAATC			
BM160	$(GA)_{15}(GAA)_5$	left	CGTGCTTGGCGAATAGCTTTG	52	4	178–188
		right	CGCGGTTCTGATCGTGACTTC			
BM164	$(GT)_9(GA)_{21}$	left	CCACCACAAGGAGAAGCAAC	52	5	135–143
		right	ACCATTCAGGCCGATACTCC			
BM183	$(TC)_{14}$	Left	CTCAAATCTATTCACTGGTCAGC	52	5	142–148
		Right	TCTTACAGCCTTGCAGACACT			
BM211	$(CT)_{16}$	Left	ATACCCACATGCACAAGTTTGG	52	16	194–219
		Right	CCACCATGTGCTCATGAAGAT			

† Annealing temperature in °C (Tm).
‡ Number of alleles per locus (NoA).
§ Range of fragment size found in base pairs (RF).

To analyze the differentiation among populations, three statistical procedures were used: (i) the G_{ST} statistic ($G_{ST} = H_T - H_S/H_T$, where H_T is the total genetic diversity in the pooled populations and H_S is the diversity within each population; Nei, 1973) was estimated by POPGENE 1.31 considering two levels of analysis: agricultural regions and the Yucatan Peninsula, (ii) AMOVA (Excoffier et al., 1992), considering two levels of hierarchy: within and between populations, using ARLEQUIN ver 2.0 program (Schneider et al., 2000), and (iii) the Fisher's combined probability test (Raymond and Rousset, 1995) at the population level with the TFPGA program (Miller, 1997).

Long-term gene flow was indirectly estimated for both regional and the Peninsula levels from $Nm = 0.25\ (1 - G_{ST})/G_{ST}$ using also POPGENE 1.31. A Bayesian method that uses multilocus genotypes to estimate the posterior probability distributions of recent immigration rates among populations was conducted with BayesAss 1.3 (Wilson and Rannala, 2003). With this method, we also estimated the posterior probability distributions of individual immigrant ancestries (total numbers of nonimmigrants, first-generation immigrants, and second generation immigrants). The method relies on Markov chain Monte Carlo techniques to carry out estimation of posterior probabilities. The run length was of 3×10^6 iterations, discarding the first 10^6 iterations as burn-in to allow the Markov chain to reach stationarity. Samples were collected every 2000 iterations to infer posterior probability distributions. To evaluate the hypothesis of isolation by distance, a Mantel test (Mantel, 1967; Sokal, 1979) was performed using the matrixes of genetic and geographic distances of the populations using the ARLEQUIN ver 2.0.

The genetic relationships among the 11 populations were inferred by two procedures: the construction of an UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and a Bayesian clustering approach of probabilistically assigning of individuals to populations. 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 multiple loci (Nei, 1972), using the TFPGA program. Robustness of the topology was evaluated by selecting the bootstrapping option with 1000 random resamplings with replacement over loci (Felsenstein, 1985). A comparison of the topology obtained with the UPGMA method and the Neighbor-Joining method was made, as well as their goodness of fit, determined by their cophenetic correlation coefficient. These values were obtained with the NTSYS software package version 2.1 (Exeter Software, Setauket, NY). To assign individuals to populations, the Structure 2.0 program (Pritchard et al., 2000) was used, utilizing the model of ancestry with admixture and starting from the geographic localization of the populations, grouping them by agricultural region. The method relies on Markov chain Monte Carlo techniques to carry out estimation of posterior probabilities. The burn-in and the run length were both of 10^6 to allow the Markov chain to reach stationarity.

RESULTS AND DISCUSSION

Wild populations of *P. lunatus* were found in habitats generated by human activities where there was a high but variable incidence of disturbance (Table 2). The farmers tolerated the wild populations until the reproductive stage and, in many cases, the population was favored by hand-weeding that only eliminated the aerial part of the plant, allowing subsequent recovery. Many of the populations grew within crops of maize alone or in association with other crops, but were never associated with domesticated populations of *P. lunatus* to prevent wild–domesticate introgressions. However, it is possible that several of these wild populations had contact with domesticated populations of Lima bean in the past because of the migratory characteristics of the milpa. For this reason, perhaps, morphological evidence of introgression was found in the Boje and San Fernando populations, where one and three plants, respectively, were observed with morphological characters similar to those presented by some of the landraces cultivated in the peninsula, such as large white flowers and large pods with equally large seeds.

Structure and Genetic Diversity

Fifty-nine alleles were found at the eight loci studied. The locus with the greatest number of alleles was *BM211* with 16; the *BM160* locus showed the lowest number of alleles with four (Table 1). Considering the entire Yucatan Peninsula, we found the following values of allelic richness and genetic diversity: $A = 7.38$, $Ho = 0.67$, and $H = 0.69$. Even though this study considered a smaller number of populations, the obtained values were greater than those reported from isozyme analysis by Zoro Bi et al. (2003): $A = 1.1$, $Ho = 0.006$ and the

Table 2. Characterization of agricultural region for fallow interval, level of agrochemical usage, occupied habitats, approximate occupied area, estimated population size and morphological evidence of introgression, for 11 wild populations of *P. lunatus* from the Yucatan Peninsula, Mexico.

Agricultural region†	Fallow time, level of agrochemical use	Population name	Habitat	Area occupied (m²)	Estimated size‡	E-I§
CEQROO	15 yr, low use	Holpat	Milpa, hubché¶	50 000	>300	no
		Kik	Monocrop of maize	30 000	>300	no
		Noh-ca	Road-sides	6000	40–60	no
SEYUC	10 yr, low use	Boje	Monocrop of maize	20 000	100–150	yes
		San Fernando	Abandoned ranch	1600	100–150	yes
NECAMP	4–5 yr, medium use	Bolonchén	Monocrop of maize, road-side	400	50–100	no
		Chunchintok	Hubché	1600	20–30	no
		Itzinté	Hubché next to a milpa with *ib*	40 000	200–250	no
SYUC	2–3 yr, intensive use.	Nohcacab	Road-side/hubché	30 000	150–200	no
		Xohuayán-1	Monocrop of maize	1200	100–150	no
		Xohuayán-2	Milpa, hubché	800	100–150	no

† CEQROO, central eastern Quintana Roo; SEYUC, southeast Yucatan; NECAMP, northeast Campeche; SYUC, south of Yucatan.
‡ Estimated number of plants with mature pods at the moment of collection.
§ Morphological evidence of introgression in flower, pod or seed.
¶ Hubché: secondary vegetation generated by agricultural activities.

reported by Ouédraogo and Baudoin (2002): $Ho = 0.012$. These differences can be explained by the greater sensitivity of the SSR markers in the detection of polymorphisms, as indicated by Ouédraogo and Baudoin (2002). Using four microsatellite loci, these same authors reported a $Ho = 0.031$ for 10 populations from the Central Valley of Costa Rica. This value is much smaller than that found in this study.

Factors that could explain these differences may include the differential size of the populations studied. The Yucatan Peninsula study populations presented in their majority more than 100 individuals in the reproductive stage (Table 2), whereas the Central Valley of Costa Rica study populations were much smaller, as 66% of populations were no larger than 30 individuals (Maquet et al., 2001). A positive correlation between intrapopulation genetic variation and the size of the population has been reported, particularly in rare or threatened species (Godt et al., 1996; Routley et al., 1999). Zoro Bi et al. (2003) demonstrated the presence of this phenomenon in the Central Valley of Costa Rica for *P. lunatus* and suggested endogamy to be the most plausible cause.

Another potential explanation is that the populations of the Central Valley of Costa Rica are subject to intensive commercial agricultural management and to encroachment from urban areas. Many of the wild *P. lunatus* plants were found on the fences of the coffee plantations near secondary roads where weeding and burnings were frequent. This situation leads to repeated episodes of founder effect and bottlenecks (Zoro Bi et al., 2003). In contrast, the Yucatan Peninsula agriculture is still essentially traditional. Although there have been important changes, fallow periods from 3 to 18 yr are conserved. Farmers have reported that the majority of the Lima bean populations have existed in these places for as long as they can remember, which suggests that they have not undergone episodes of extinction and recolonization or that these episodes have not been as frequent as those in the Central Valley of Costa Rica. Another aspect suggesting the antiquity of these populations is their close distribution to ancient pre-Columbian human settlements, as in the case of the populations of Itzinté, Chunchintok, Holpat, Boje, and Kik.

Different outcrossing rates could also generate differences in diversity between the two regions. In the Central Valley of Costa Rica, the outcrossing rate was estimated between 0.027 and 0.268 (Zoro Bi, 1999). Although Baudoin et al. (1998) reported outcrossing rates of up to 48% for the wild pool, there are no estimates for the populations of the Yucatan Peninsula that would permit comparisons. During the collection of the wild germplasm, we observed a large number of insect pollinators, which suggests a potential for substantial outcrossing in the region.

In the Yucatan Peninsula, 27% of the total variation was found among populations ($G_{ST} = 0.27$). This differentiation was supported by an AMOVA, which showed that 27% of the total variation was found among the populations and 73% within the populations, and by Fisher's test of combined probability, which showed that all the populations are different from each other in their allelic frequencies (data not shown). These results can be explained by a low level of long-term gene flow found ($Nm = 0.66$) and by the low rates of recent migration presented in the majority of populations studied (Table 3). Only three pairs of populations showed high rates of migration: Xohuayán-2 ($m = 0.271$) with immigrants originating from Nohcacab; Itzinte ($m = 0.271$) with immigrants originating from Bolonchén; and Nohcacab ($m = 0.265$) with immigrants originating from Itzinté. In the three cases, the proportion of immigrants of the second generation (sons of a migrant individual and a nonmigrant individual) was between 75% and 100%. These results seem to show a relationship of source–sink, since the proportion expected of migrants in inverted sense of these three pairs of populations is a lot smaller (Table 3). For the first two cases, such relationship can be explained by the closeness of the populations and their ownership to the agricultural zone worked specifically by the farmers of one same village or of near villages, since these aspects favor the accidental transport of seeds by the producers of one population to the other. For the third case, even when the populations are found in two different agricultural regions (Nohcacab in the SYUC and Itzinté in the NECAMP), these regions are found next to each other and connected by a transited highway, which could favor the migration of farmers between both regions and with it the accidental transport of seeds.

Our results for genetic differentiation were lower than those reported in the Central Valley of Costa Rica

Table 3. Means of the posterior distribution of the migration rates (m) among 11 wild populations of *P. lunatus* from the Yucatan Peninsula, Mexico.

	Pop1†	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10	Pop11
Pop1	***0.984***	0.002	0.001	0.001	0.001	0.002	0.002	0.001	0.001	0.002	0.001
Pop2	0.002	***0.983***	0.002	0.002	0.002	0.001	0.002	0.002	0.002	0.002	0.001
Pop3	0.002	0.001	***0.984***	0.002	0.001	0.002	0.002	0.002	0.002	0.002	0.001
Pop4	0.005	0.005	0.007	***0.682***	0.011	0.005	0.005	0.005	***0.265***	0.005	0.005
Pop5	0.005	0.005	0.005	***0.271***	***0.682***	0.005	0.005	0.006	0.005	0.005	0.005
Pop6	0.003	0.002	0.002	0.003	0.003	***0.972***	0.004	0.004	0.002	0.002	0.003
Pop7	0.002	0.002	0.002	0.001	0.002	0.002	***0.984***	0.002	0.002	0.001	0.002
Pop8	0.002	0.002	0.002	0.002	0.002	0.002	0.002	***0.983***	0.002	0.002	0.002
Pop9	0.006	0.005	0.005	0.005	0.005	0.005	0.005	0.006	***0.681***	***0.271***	0.007
Pop10	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.002	0.002	***0.985***	0.001
Pop11	0.001	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	***0.980***

† The populations into which individuals are migrating are listed in the rows, while the origins of the migrants are listed in the columns. Values along the diagonal are the proportions of individuals derived from the source populations each generation. Migration rates ≥0.01 are in italics. Standard deviations for all distributions were <0.05. Populations are San Fernando (1), Boje (2), Xohuayán-1 (3), Nohcacab (4), Xohuayán-2 (5), Nohca (6), Kik (7), Holpat (8), Itzinté (9), Bolonchén (10), Chunchintok (11).

(G_{ST} = 0.56) (Ouédraogo and Baudoin, 2002). These differences may be a result of a lower level of long-term gene flow present in that region (Nm = 0.17) (Ouédraogo and Baudoin, 2002), than in the Yucatan Peninsula. Low levels of gene flow in the wild populations of *P. lunatus* in the Central Valley of Costa Rica were also reported by Hardy et al. (1997) and Baudoin et al. (1998). As determined by Baudoin et al. (1998), the horizontal transfer of pollen and seeds did not exceed a distance of 6 m, and the size of the neighborhood was 1.6 m, implying the existence of local genetic differentiation in these populations.

The value of F_{IS} for the entire Yucatan Peninsula was high ($F_{IS} = -0.31$, $P < 0.05$), indicating an excess of heterozygosity. This resulted in contrast with that reported by Zoro Bi et al. (2003) for the Central Valley of Costa Rica. These authors found that the populations of that region also deviated from the Hardy-Weinberg equilibrium; however, they found a deficit of heterozygosity in those populations. These differences between both regions appear to correspond to the difference in the size of the populations and the levels of endogamy.

Figure 2 shows the topology generated with a UPGMA of the 11 wild populations analyzed. This topology indicates a grouping in accordance with the geographical location of the populations, with the exception of the group comprising the populations of San Fernando and Boje, located in SEYUC, and Chunchintok, located in NECAMP. A possible explanation of the clustering of Chunchintok with the populations of SEYUC could be the accidental transportation of seed by Campeche farmers, who mentioned that they transport their agricultural products for sale in Valladolid, the principal town of SEYUC. The clustering patterns of the populations presented in the dendrogram (Fig. 2) agree with the results obtained from the Mantel test (Fig. 3), which indicates the existence of a geographical isolation among the wild populations of *P. lunatus*. The individual assignment test showed that a large number of individuals of the Chunchintok population presented high coefficients of ancestry of the SEYUC region (Fig. 4), supporting with this the results showed by the UPGMA. Neighbor-Joining results showed a smaller cophenetic correlation value than UPGMA (0.65 in comparison to 0.72), and they were not consistent with the Mantel test and the individuals assignment test.

A comparative analysis among agricultural regions for genetic diversity and allelic richness using the population means of each region (Tables 4 and 5) indicated significantly higher *Ho* values for SYUC and NECAMP than those of CEQROO and SEYUC. The SEYUC had the lowest *A* value, but only significantly lower than CEQROO, which had the highest value. The high *A* and low *Ho* values for CEQROO are explained by the low evenness of allelic frequencies evaluated by *Ae/A* (Table 4). The SEYUC, in addition to the lowest *A* value, had a low evenness coefficient. A possible explanation for the low diversity of CEQROO and SEYUC may be the existence of gene flow from the domesticated to the wild populations. Martínez-Castillo et al. (2004) found weedy plants growing within two domesticated populations of *P. lunatus* in CEQROO, one and two weedy plants out of a total of about 1000 and 4000, respectively. These weedy plants could be hybrid forms generated by gene flow events between domesticated and wild popu-

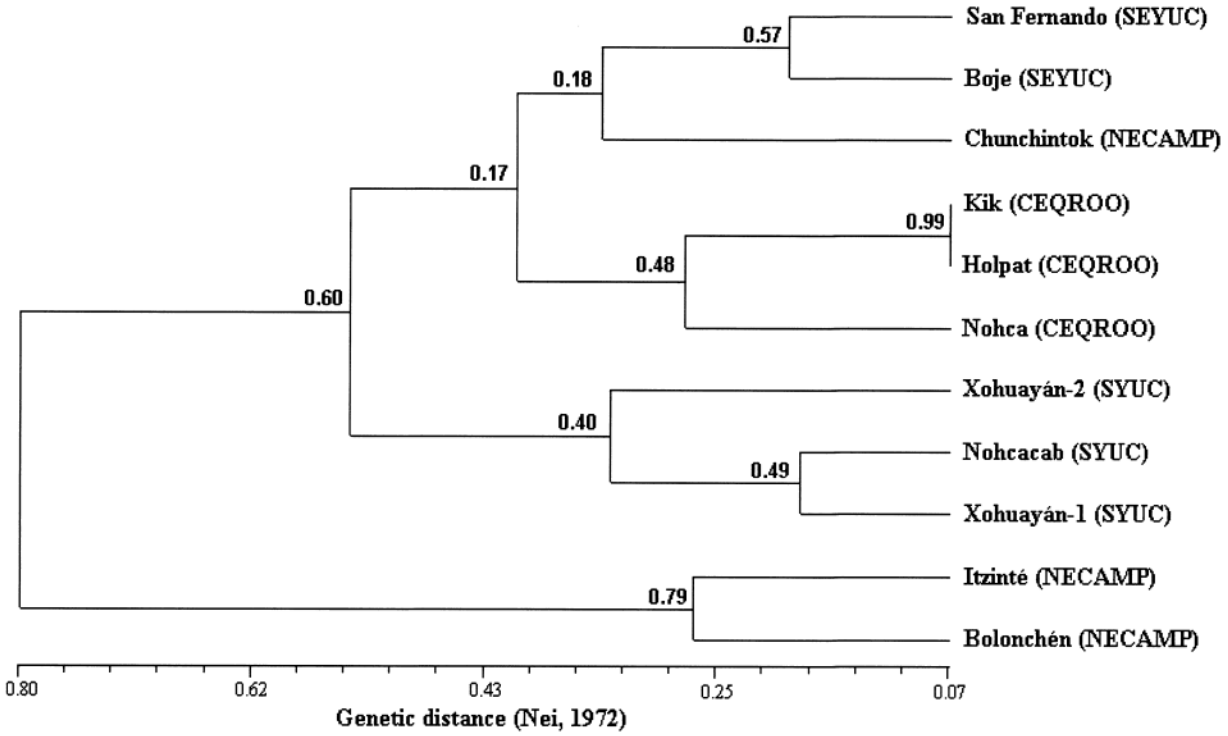

Fig. 2. Dendrogram (UPGMA) based on Nei's genetic distance (1972) of 11 wild populations of *P. lunatus* studied in four agricultural regions of the Yucatan Peninsula, Mexico. The numbers above the lines are the proportion of similar replicates supporting each node. South of Yucatan (SYUC), central eastern Quintana Roo (CEQROO), southeast of Yucatan (SEYUC), northeast of Campeche (NECAMP).

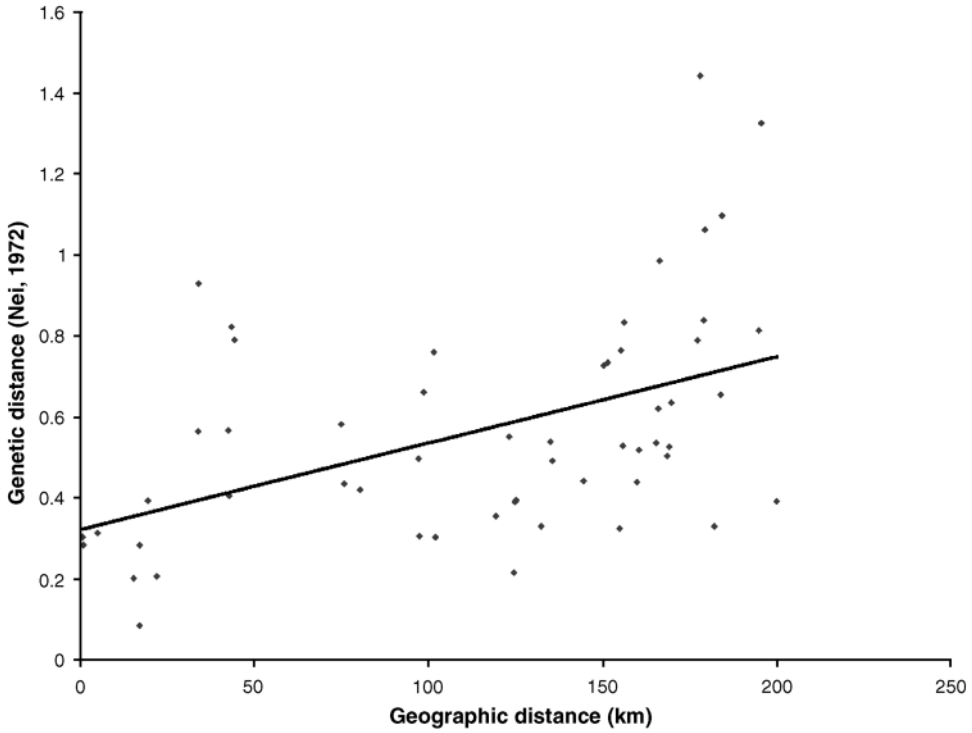

Fig. 3. Correlation between the genetic distance (Nei, 1972) and the geographical distance of the 11 wild populations of *P. lunatus* studied in the Yucatan Peninsula, Mexico. $r = 0.47$, $p < 0.000001$.

lations. These authors also found a weedy population growing beside a domesticated population in SEYUC. In this weedy population, plants were found with wild-type seeds and others with domesticated-type seeds. Also, they found two wild populations in SEYUC with wild–domesticated introgression characteristics (Table 2). Several studies have indicated that the gene flow from the domesticated populations can diminish the genetic diversity of the wild populations through the displacement of wild alleles (Gepts et al., 1999).

At the population level, Xohuayán-2 exhibited the highest value of *H*o (Table 4). This population was not sizable, nor did it occupy a large area, and it was also subjected to episodes of extinction because of agricultural management. However, the agricultural management of this region may explain the high level of diversity observed. Xohuayán-2 is located in SYUC, a region where the farmers plant a local variety of *P. lunatus* called *xmejen-ib*, which has a high market value. Other local varieties of secondary commercial importance are also

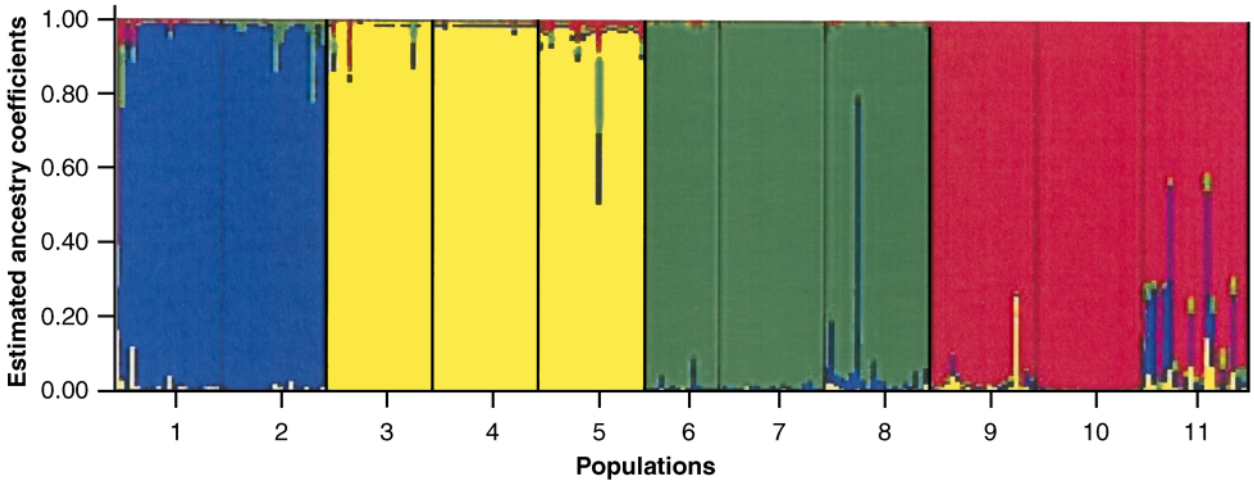

Fig. 4. Coefficients of estimated ancestry per individual grouped by population and region. Each individual is represented by a single vertical line broken into four colored segments, with lengths proportional to the individual's estimated ancestry fraction from each of the four regions: SEYUC (blue), SYUC (yellow), CEQROO (green), NECAMP (red). Populations: San Fernando (1), Boje (2), Xohuayán-1 (3), Nohcacab (4), Xohuayán-2 (5), Nohca (6), Kik (7), Holpat (8), Itzinté (9), Bolonchén (10), Chunchintok (11).

Table 4. Estimators of the genetic diversity of 11 wild populations of *P. lunatus* from the Yucatan Peninsula, Mexico.

Agricultural region†	Population name	*n*‡	*A*§	*Ae*/*A*¶	*Ho*#	*H*††
CEQROO	Holpat	20	3.38	0.70	0.46	0.54
	Kik	20	3.38	0.64	0.60	0.48
	Nohcá	14	3.38	0.67	0.52	0.51
	Mean	44.7	3.38	0.67	0.53	0.51
SEYUC	Boje	20	2.87	0.72	0.51	0.47
	San Fernando	20	2.38	0.70	0.49	0.35
	Mean	20	2.63	0.71	0.50	0.41
NECAMP	Bolonchén	20	2.37	0.81	0.67	0.41
	Chunchintok	19	3.13	0.83	0.67	0.57
	Itzinté	20	3.25	0.85	0.82	0.59
	Mean	19.7	2.92	0.83	0.72	0.52
SYUC	Nohcacab	20	3.13	0.73	0.87	0.53
	Xohuayán-1	20	3.25	0.77	0.82	0.55
	Xohuayán-2	20	3.01	0.81	0.90	0.57
	Mean	20	3.13	0.77	0.86	0.55

† CEQROO, central eastern Quintana Roo; SEYUC, southeast Yucatan; NECAMP, northeast Campeche; SYUC, south of Yucatan.
‡ Number of plants (*n*).
§ Average number of alleles (*A*).
¶ Allelic frequencies evenness (*Ae*/*A*).
# Observed heterozygosity (*Ho*).
†† Nei's index of diversity (*H*).

Table 5. Duncan's test for comparison of means for the values of observed heterozygosity (*Ho*) and average number of observed alleles (*A*) found in 11 wild populations of *P. lunatus* from four agricultural regions in the Yucatan Peninsula, Mexico.

	Ho		*A*	
Agricultural region†	Mean	Duncan test‡	Mean	Duncan test‡
SYUC	0.86	A	3.13	AB
NECAMP	0.72	B	2.92	AB
CEQROO	0.53	C	3.38	A
SEYUC	0.50	C	2.63	B

† SYUC, south of Yucatan; NECAMP, northeast Campeche; CEQROO, central east Quintana Roo; SEYUC, southeast Yucatan.
‡ Regions with the same letter are not different significantly ($\alpha = 0.05$).

planted. For marketing reasons, the farmers take great care in maintaining purity of this germplasm by ensuring that local varieties do not crossbreed with the wild populations. Thus, they plant this germplasm in sites where wild populations are not found or, if found, they eliminate them with herbicides. In this way, by protecting the purity of this germplasm, the farmers are indirectly maintaining the identity and diversity of the wild populations still existing in the region.

In regard to allelic richness, the Holpat, Kik, and Nohca populations presented the highest values (Table 4), which could be a result, in the case of the two first populations, of their larger population size. In contrast, Bolonchén presented the lowest value, a consequence, perhaps, of its small population size and recent events of extinction–recolonization, which would have caused a process of genetic drift in this population (Table 2). Theoretical work on the effects of genetic drift suggest that allelic frequencies fluctuating in small populations will produce a reduction in *H*o (Wright, 1931; Kimura, 1955; quoted by Cole, 1998). It has also been noted that genetic drift must have a more immediate effect on the loss of rare alleles, thus causing a reduction of *A* (Cole, 1998).

Apart from the Bolonchén population, the San Fernando and Boje populations showed the lowest allelic richness of all wild populations analyzed. These populations had some plants with morphological characteristics of flowers, pods, and seeds very similar to those of the domesticated germplasm, which is suggestive of past introgression events with domesticated germplasm, causing a reduction in genetic diversity.

The excess or deficit of heterozygosity tests indicated that 40.5% of the locus–population analyzed have an excess of heterozygotes, 11.9% a deficit, and 47.6% are in Hardy-Weinberg equilibrium. When the average values of F_{IS} are obtained per population for all the loci studied, the tests show that the 11 populations are in Hardy-Weinberg equilibrium, even though Xohuayán-1, Nohcacab, and Xohuayán-2 had a high number of loci with heterozygote excess (each with five) (Table 6). At the level of loci for the entire peninsula, the tests indicated that four of the eight loci studied presented an excess of heterozygotes (AG1, BM140, BM156, and BM160), one locus showed a deficit (GATS91), and three loci were in Hardy-Weinberg equilibrium (BM164, BM183, and BM211). These results at the locus–population and the loci–Peninsula levels show evidence of a tendency toward an excess of heterozygotes in the wild pool of *P. lunatus*, an excess, perhaps, as a consequence of natural selection favoring heterozygosity and/or of a Wahlund effect inside the populations.

Table 6. Coefficients of Inbreeding (F_{IS}) of 11 wild populations of *P. lunatus* studied in the Yucatan Peninsula, Mexico, based on the mean of the F_{IS} values for the polymorphic loci studied.

Agricultural region†	Population name	*n*‡	F_{IS}§	Number of polymorphic loci	Loci with excess of heterozygotes¶	Loci with deficit of heterozygotes#
CEQROO	Holpat	20	0.16	8	2	2
	Kik	20	−0.23	7	2	0
	Nohcá	14	0.05	8	1	1
SEYUC	Boje	20	−0.04	8	1	0
	San Fernando	20	−0.15	7	3	1
NECAMP	Bolonchén	20	−0.34	6	4	1
	Chunchintok	19	−0.20	8	2	1
	Itzinté	20	−0.40	8	4	0
SYUC	Nohcacab	20	−0.38	8	5	2
	Xohuayán-1	20	−0.44	8	5	1
	Xohuayán-2	20	−0.36	8	5	1

† CEQROO, central eastern Quintana Roo; SEYUC, southeast Yucatan; NECAMP, northeast Campeche; SYUC, south of Yucatan.
‡ Number of plants (*n*).
§ Values are not different significantly from zero ($\alpha = 0.05$).
¶ $P < 0.05$.
# $P < 0.05$.

CONCLUSIONS

Results indicate a high intrapopulation genetic diversity and a structure that results from processes of geographic isolation and low levels of gene flow. Even though a low gene flow was observed, high rates of recent migration were found among several populations, as well as gene flow at a great distance. Both aspects are consequences, perhaps, of accidental carrying of seeds by the producers. Data found in this study suggest a positive correlation between agricultural intensification and increase in diversity, as greater values of *H*o were recorded in the areas with greater intensification (Tables 2 and 5). These results suggest that wild populations of *P. lunatus* are actually favored by the intensification of disturbance in situations involving at least 3 yr of fallow. However, the opposite could be true at higher levels of intensification, as can be observed in the Central Valley of Costa Rica, where the diversity is diminishing. Two aspects could explain these results: (i) long fallow periods, combined with the existence of a soil seed bank, favors the existence of gene flow from domesticated toward the wild pool, diminishing with this its genetic diversity, as it could be the case of the SEYUC (fallow of 10 yr): and (ii) longer fallow periods determine stronger bottlenecks and genetic drift on the soil seed bank, diminishing with this also the genetic diversity of the wild populations of *P. lunatus.* The importance of the soil seed bank in the dynamics of the genetic diversity of *P. lunatus* and its role in the in situ conservation of this species has been indicated recently (Degreef et al., 2002).

Therefore, it would be reasonable to assume that the wild pool of *P. lunatus* presents a response curve to intensification: at low disturbance levels, low diversity is observed; at medium levels, high diversity is observed; and at high disturbance levels, low diversity is observed once again. These results suggest that the degree of intensification is a key factor for the design of in situ conservation. Martínez-Castillo et al. (2004) have pointed out that in the two areas with the highest levels of diversity, populations are under great pressure from agricultural intensification, which could lead to a severe reduction in size or to their disappearance. In turn, this situation increases the necessity of establishing detailed agroecological studies that could help to implement immediate plans for in situ conservation of the wild pool of *P. lunatus* of this region of Mexico.

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