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Spatial distribution of genetic diversity in wild populations of *Phaseolus vulgaris* L. from Guanajuato and Michoacán, México

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Abstract

The diversity, genetic structure, and genetic flow of wild populations of *Phaseolus vulgaris* L. within its Mesoamerican area of domestication, were analyzed by means of morphological and inter-simple sequence repeat molecular markers. Overall, 89% of the loci studied were polymorphic, 35% in the least diverse population and 65% in the most diverse. Genetic diversity in the populations was high, between $h = 0.14$ and 0.29, as was the maximum distance between populations ($D = 0.3$). Between 40% and 45% of the diversity was explained by the differences among populations, indicating that a large number of populations is necessary to represent the wild gene pool in the germplasm collections. We found uniformity in allele frequencies among the populations, suggesting presence of outcrossing. We did not find correlation between genetic and geographic distances, but the dendrogram topology suggests geographical isolation due to the mountainous topography. Negative correlations were observed between the coefficient of variation of seed size and the distance between wild populations and fields . We obtained a highly negative correlation between percentage of polymorphic loci and distance to the nearest crop field, which also suggests gene flow from the domesticated populations. These observations suggest that genetic flow is taking place from domesticated toward wild populations and that the farmer, through his agricultural activities, could be influencing the magnitude and the characteristics of the gene flow, and along with this, the differentiation of wild populations. New approaches should be established for conservation *in situ* and maintaining bio-safety, given the risk of introducing genotypes from the Andes and transgenic varieties and causing genetic assimilation.

Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the 10 most important crops in the world, with a production of almost 8 million tons per year on 13 million hectares. It is calculated that more than 60% of world production derives from domesticates of Mesoamerican origin (Tohme et al. 1996; Beebe et al. 2000). In México, the common bean is the main source of protein for the human population. Current yield is estimated at around 650 kg/ha/harvest, while its potential yield is estimated between 4000 and 5000 kg/ha/harvest (Gepts 1993). The low yields at present are partly a result of a lack of knowledge and poor exploitation of the genetic diversity of the wild gene pool of the species (Gepts and Debouck 1991).

Wild and domesticated populations constitute the primary gene pool of the species, within which genetic compatibility has been reported (Debouck

and Smart 1995). Comparisons of wild and domesticated populations indicate two main gene pools from which domestication took place in the Americas (Koenig and Gepts 1989; Debouck and Smart 1995). One center of domestication is located in Mesoamerica, in mesic habitats, between 700 and 2000 m above sea level, where some wild populations present small seeds with type "S" phaseolin and large bracteoles. These populations gave rise through domestication to plants with increased seed size and with the same type of phaseolin and large bracteoles. The other center of domestication is located in the Andes, where the wild populations present larger seeds, with predominantly type "T" phaseolin and small bracteoles. Through domestication, these populations gave rise to plants with large seeds and with the same type of phaseolin (Koenig and Gepts 1989; Gepts and Debouck 1991). The existence of these two gene pools was further confirmed with isozyme, RFLP, and AFLP markers (Koenig and Gepts 1989; Becerra-Velásquez and Gepts 1994; Tohme et al. 1996).

Domestication in the Mesoamerican area could have been carried out in the area to the west of the center of México, in the states of Guanajuato, Jalisco and Michoacán, where wild populations can be found at present with the ancestral "S" type of phaseolin (Gepts 1988; Gepts and Debouck 1991). It has been suggested that primitive domesticates with "S" phaseolin were dispersed to other regions, becoming the dominant type of phaseolin in all the Mesoamerican domesticated populations. The dispersal of the "S" phaseolin type may have been favored because phenotypic characters such as seed size and color genes are linked to the phaseolin locus (Motto et al. 1978; Johnson et al. 1996; Koinange et al. 1996). Thus, the genome of most of present-day Mesoamerican landraces is derived from a single region within México (Beebe et al. 2000).

It has been argued that wild populations present greater genetic variability than domesticated beans, since the arcelin seed protein and some types of phaseolin are only found in wild populations (Romero and Bliss 1985; Gepts et al. 1986; Romero et al. 1986; Debouck and Tohme 1989; Koenig et al. 1990; Acosta-Gallegos et al. 1998). This suggests that, during the domestication process, a founder effect could have taken place, which may have excluded valuable genetic variability from the domesticates, in relation to adaptive characteristics, such as resistance to insects during storage and *Rhizobium* strain specificity (Kipe-Nolt et al. 1992; Tohme et al. 1996; Acosta-Gallegos et al. 1998).

Various reasons justify the study of genetic diversity present in wild populations, including their contribution to the genetic diversity of landraces and a better definition of the founder effect associated with domestication or, conversely, their possible genetic assimilation by landraces. For example, analysis of gene flow from domesticated to wild populations may allow us to clarify the significance that the incorporation of genes from domesticated populations has had on the wild populations during the process of domestication and the expansion of agriculture. For example, Papa and Gepts (2003) have documented that gene flow from domesticated to wild beans is three-fold higher than gene flow in the opposite direction. In addition, a better understanding of the wild gene pool could lead to the establishment of improvement programs that would increase the yield of domesticates, improve their tolerance to pathogens, diseases and environmental stress, and exploit the role of beneficial micro-organisms, thereby facilitating the establishment of sustainable productive systems through methods of conservation *in situ* and *ex situ* (Gepts et al. 1999).

The aim of this study was to analyze the levels of diversity and genetic structure of wild populations of *P. vulgaris* L. from a region bordering Guanajuato, Jalisco and Michoacán (México) in the Mesoamerican area of domestication, and to indirectly detect possible gene flow from domesticated toward wild populations, using both morphological and molecular inter-simple sequence repeat (ISSR) molecular markers.

Materials and methods

Study region

The area where the domestication of common bean is proposed to have taken place (Gepts 1988) corresponds to the old frontier between Mesoamerica and Aridoamerica, on the borders of the present day states of Guanajuato, Jalisco and Michoacán. This area borders the southern portion of an

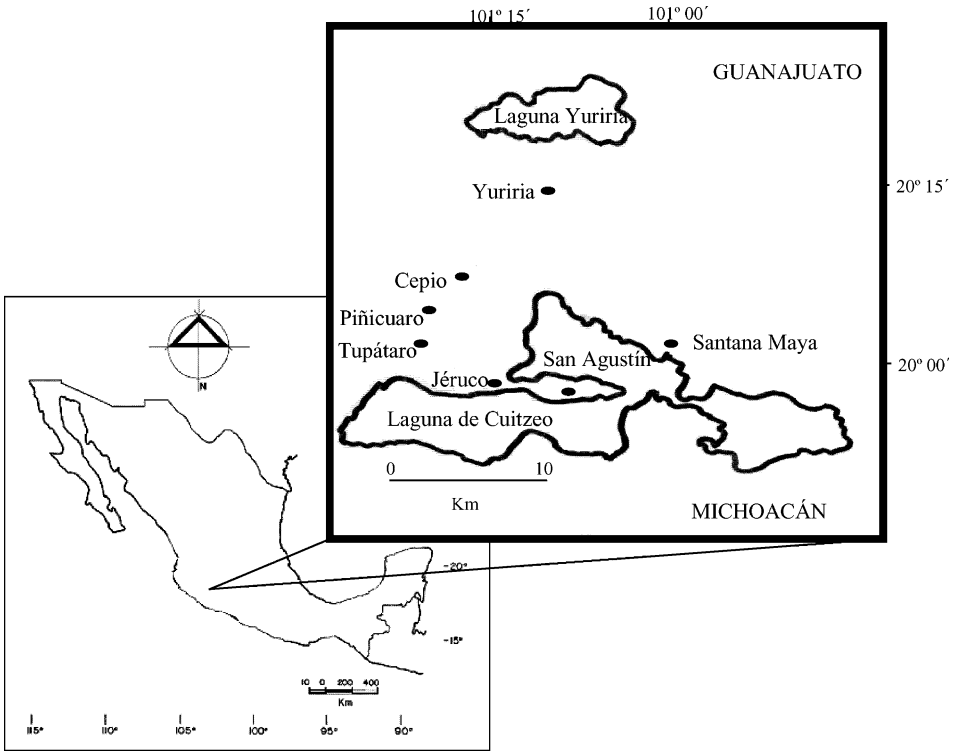

Figure 1. Locations of the seven wild bean populations studied.

ancient lagoon system in the center of México formed by the river Lerma. Some of the lakes comprising the system were drained by the Europeans during the first hydraulic works of drainage and irrigation carried out in America around 1548, allowing the establishment of one of the most important commercial agricultural regions in México, called "El Bajío" (Zizumbo-Villarreal 1985). The physiography of the area includes valleys and mountains. The climate presents a transition between sub-humid, semi-arid and temperate environments. The soil is volcanic in origin, of the pelic vertisol type ranging from deep to thin and from stony to extremely stony, with a tropical deciduous forest vegetation, forest of *Prosopis laevigata* and forests of *Quercus* spp. (Rzedowski 1978). The average annual rainfall is around 700 mm, with a high coefficient of variation (close to 25%) between years, with high variation in the initiation and establishment of the rains, and with a summer dry season which is variable in intensity, amplitude and date of initiation (Wallen 1955). The conditions of precipitation, therefore, make agriculture possible, but with a high risk factor. Thus, the production rationale of the farmer is centered on securing the harvests and making good use of natural resources available given the uncertainty regarding the quantity and distribution of the rains (Zizumbo-Villarreal et al. 1988). There is evidence in this area of agricultural villages along the shore of Lake Cuitzeo since the pre-classic period (800–100 BC) (Branniff 1975; Oliveros 1975).

Plant materials

Seven wild populations of common bean were selected: Cepio (CE); Jeruco (JE), Piñícuaro (PI), San Agustín (SA), Santana Maya (SM), Tupátaro (TU) and Yuriria (YU), located in different valleys of the old Mesoamerican frontier in the states of Guanajuato and Michoacán, on the southern border of "El Bajío" (Figure 1, Table 1). In this area, the traditional "milpa" agriculture (maize, beans and squash) in which landraces of beans are involved is still practiced. In each population,

Table 1. Name of population, code, location (state, latitude, longitude) altitude and distance from crop fields of seven wild populations of *P. vulgaris* L. studied.

Population	Code	State	Latitude	Longitude	Altitude	Distance to field (m)
San Agustín	SA	Michoacán	19°58′	101°04′	1900	110
Yuriria	YU	Guanajuato	20°11′	101°08′	2000	60
Tupátaro	TU	Michoacán	20°01′	101°51′	2100	25
Cepio	CE	Guanajuato	20°05′	101°12′	1850	15
Jeruco	JE	Michoacán	19°57′	101°10′	1850	10
Piñicuaro	PI	Guanajuato	20°03′	101°14′	2000	20
Santana Maya	SM	Michoacán	20°01′	101°00′	1900	20

an average of 29 plants was collected, with the participation of local farmers. The ecological conditions of the collection sites were registered, as well as the traditional uses and values of the populations collected. The populations grew in areas with disturbed vegetation on the edge of seasonal streams or small ravines at different distances from the crop fields containing domesticated beans. The size and color of the seed were used as morphological markers, as these characters are highly heritable (Motto et al. 1978; Johnson et al. 1996; Koinange et al. 1996) and easy to measure in the field and in the laboratory. For each plant, the weight of 100 seeds was registered and the color patterns of the same following as described in Beebe et al. (1997). The mean values (M) and coefficients of variation (CV) of the seed weight per population were estimated using the Statistical Analysis System Software Release 6.03 (SAS 1992). A one-way analysis of variance and a Bonferroni means separation test for multiple comparisons were carried out in order to estimate the differences in seed size among the populations. The same software was used to estimate the correlation between distance to the nearest cultivated plot and (1) the average size of seed, and (2) its coefficient of variation.

For five of the seven populations: CE, JE, SA, TU and YU (DNA of the two other populations was inadvertently lost during transportation), diversity and genetic structure were evaluated using ISSR markers (Zietkiewicz et al. 1994; Wolfe et al. 1998; Camacho and Liston 2001). An average of 20 individuals was used per population, the same individuals that were evaluated morphologically. The genomic DNA was extracted from young leaves by the CTAB method and three ISSR primers reported to be highly polymorphic by the same authors were used: $(GACA)_3$ RG; $(GACAC)_2$; $(GA)_8$ RG (González et al. 1998). Each 20 μl amplification reaction consisted of 10 mM Tris–HC1 (pH 9.0), 50 mM KCI, 0.1% Triton X-100, 2 mM $MgC1_2$, 200 μM each dNTPs, 1 μM of primer, 1 unit of *Taq* polymerase (Promega, Madison, Wisconsin, USA) and 50 ng of template DNA. Amplification was performed in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, USA), following the conditions established by González et al. (1998). The fragments of DNA generated were separated in an electrophoresis chamber 320 mm × 380 mm × 0.4 mm (SQ3 Sequence Hoeffer), in non-denaturing 5% acrylamide–bisacrylamide (29 : 1) gels (González et al. 1998). Visualization of the fragments was carried out by means of silver nitrate staining with the modifications reported by Bassam et al. (1991) and Creste et al. (2001).

Data were scored as presence and absence of bands. Percentage of polymorphic loci, observed number of alleles, effective number of allele, Nei's genetic diversity, Shannon's information index, measures of population differentiation: *Ht* (total diversity), *Hs* (intra population diversity) and *Gst* (population differentiation), Nei's genetic distance, the number of migrant individuals (*Nm*) and dendograms based on Nei's distance using UPGMA were computed with POPGENE 1.31 (Yeh et al. 1999). The correlation between the distance to the nearest cultivated plot and (1) percentage of polymorphic loci; (2) Nei's genetic diversity and (3) Shannon's information index was estimated, as well as the correlation between Nei's genetic distance and geographic distances between populations.

Table 2. Population name, number of plants (*n*), mean seed mass (mass of 100 seeds in gram) (M), coefficient of variation of seed mass (CV), level of significance of differences among mean seed mass (s) and colors of seeds in seven wild populations of *P. vulgaris* L.

Population	*n*	M	CV	s*	Seed colors
San Agustín	33	3.9	11.6	A	Speckled
Yuriria	24	6	27.3	BC	Black, brown, cream, grey, olive and speckled
Tupataro	33	4.9	24.2	AB	Black, brown, cream, olive, pink and speckled
Cepio	39	5.1	30.2	AB	Black, brown, cream, olive and speckled
Jeruco	24	7.4	50.4	C	Black, brown, cream, olive, pink, purple and red
Piñicuaro	25	6.6	51.7	BC	Black, brown, cream, olive, pink, red speckled
Santana Maya	24	6.3	50.8	BC	Black, brown, cream, olive and speckled

* Different letter means significant difference ($P < 0.05$).

Results

The wild populations were found to be distributed only in areas of disturbed natural vegetation (deforestation, overgrazing by goats and trampling by humans) with high levels of incident light, in non-arable land near temporary streams and small ravines with extremely stony and rocky soil, on shrubs and trees of the mesquite, tropical deciduous or oak forest, where they can escape goats, sheep and horses, both in land close to and distant from cultivated areas. The wild plants showed an indeterminate, climbing growth habit, with purple flowers and short, dehiscent pods. Seeds showed different color patterns (speckled, striped, mottled, pinto and uniform) and colors (including black, brown, cream, grey, olive, purple pink, red, etc.) (Table 2). Wild beans are commonly called "frijol coyote" or "frijol cimarrón" by the producers and are sometimes harvested. When the pods do not mature, they are consumed fresh *in situ* because of their sweet taste and are considered survival or poor people's food.

Data on morphological variation are shown in Table 2. The populations showed a range in seed size from 3.9 to 6.6 g/100 seeds, similar to the results obtained by Delgado-Salinas et al. (1988), who reported a range between 3.6 and 5.8 g/100 seeds for populations in the states of Morelos and Puebla. Tohme et al. (1996) reported values between 3.4 and 5.2 g/100 seeds in populations in the states of Guanajuato and Michoacán. Significant differences were found between populations in relation to seed size. Seeds of the SA population were significantly smaller than those of the PI, YU, SM and JE populations (Table 2). The CE and TU populations had medium-sized seeds. These differences were associated with different color patterns in the seeds. The SA population presented only a speckled color pattern, whereas the others presented different patterns (Table 2). Domesticated seeds generally have a seed size above 20 g/100 seeds (Koinange et al. 1996).

CVs for seed weight ranged between 11.6% and 51.7%. The SA population showed the lowest values, while the PI, JE and SM populations showed values almost five times higher, suggesting the presence of greater genetic variability in these populations. We did not observe a statistically significant correlation between average seed size and the distance to the nearest crop field (Figure 2, $r = -0.68$; $P = 0.10$) but we found a significant negative correlation between the coefficient of variation of seed size and the distance from the nearest crop field (Figure 2: $r = -0.76$; $P = 0.045$). This observation suggest the existence of gene flow from the domesticated plants to the wild populations as the increase in the variability is correlated with the proximity of domesticated populations. This phenomenon was also suggested by a greater number of color patterns among the wild populations in proximity to crop fields (Table 2).

For the molecular analysis, we studied 37 bands (putative loci), which were obtained with three ISSR primers, 7 with the primer $(GACA)_3$ RG, 21 with the primer $(GACAC)_2$ and 9 with the primer $(GA)_8$ RG (Figure 3, Table 3). In the five populations, 89% of the loci were polymorphic, with a range from 35% to 64% (Table 4). Because studies of *P. vulgaris* demonstrated high degrees of inbreeding but also some instances of outcrossing (Brunner and Beaver 1989; Triana et al. 1994), the diversity statistics were calculated twice. Once with the assumption of Hardy–Weinberg equilibrium ($Fis = 0$) and once with the assumption that the populations are mostly selfing ($Fis = 0.95$). The genetic diversity statistics were similar under both of these assumptions except for the YU population

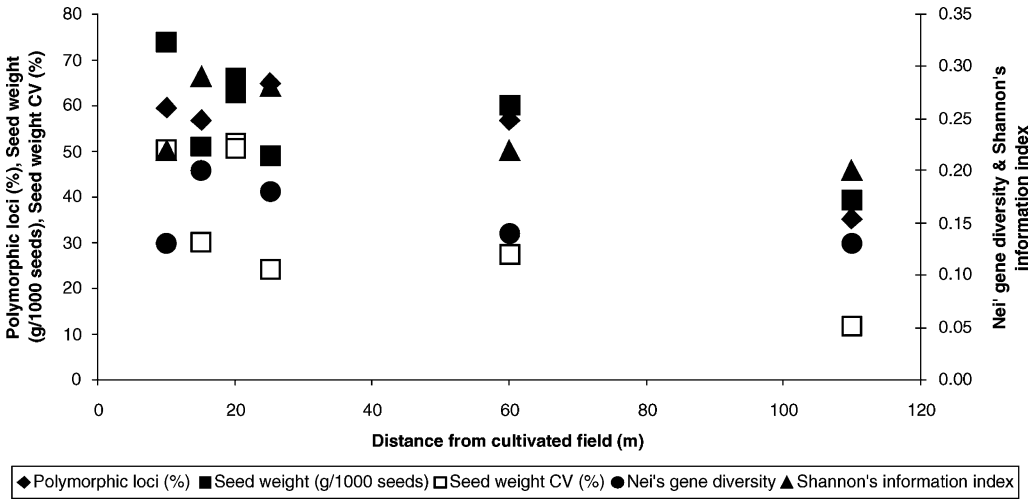

Figure 2. Relationship between distance to a cultivated field (independent variable) and seed weight, seed weight coefficient of variation (CV), polymorphic loci frequency, Nei's genetic diversity, and Shannon's information index.

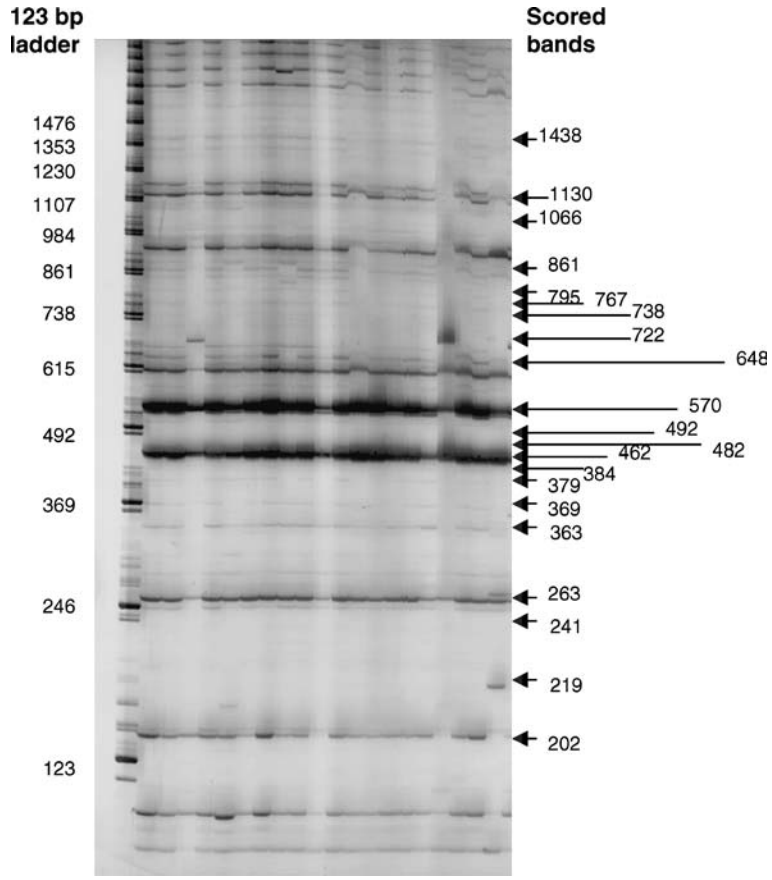

Figure 3. Sample of ISSR profiles for the $(GACAC)_2$ primer of the Yuriria wild *P. vulgaris* population. Arrows on the right indicate bands scored with size in bp.

Table 3. Primers used in ISSR analyses of five wild populations of *P. vulgaris* L. and size of the bands they produced.

Primer	Primer sequence	ISSR band sizes in base pairs
15	$(GACA)_3$ RG*	146, 183, 232, 257, 369, 480, 502
17	$(GACAC)_2$	202, 219, 241, 263, 363, 369, 379, 384, 462, 482, 492, 570, 648, 722, 738, 767, 795, 861, 1066, 1130, 1438
21	$(GA)_8$ RG*	289, 409, 466, 492, 547, 608, 615, 738, 779

* R = A,G; Y = C,T.

Table 4. Genetic diversity of five wild populations of *P. vulgaris* L.

			na		ne		*h*		*I*	
Population	*n*	%	(*Fis* = 0)	(*Fis* = 0.95)	(*Fis* = 0)	(*Fis* = 0.95)	(*Fis* = 0)	(*Fis* = 0.95)	(*Fis* = 0)	(*Fis* = 0.95)
San Agustín	21	35.1	1.35	1.35	1.25	1.25	0.14	0.13	0.21	0.20
Yuriria	22	56.8	1.58	1.57	1.38	1.21	0.21	0.14	0.31	0.22
Tupátaro	22	64.9	1.65	1.64	1.31	1.29	0.19	0.18	0.29	0.28
Jeruco	20	59.5	1.6	1.59	1.2	1.19	0.13	0.13	0.22	0.22
Cepio	21	56.8	1.55	1.56	1.34	1.33	0.20	0.20	0.30	0.29

Number of plants (*n*) Percentage of polymorphic loci (%), observed number of alleles (na), effective number of alleles (ne); Nei's gene diversity (*h*), Shannon's information index (*I*). Assuming Hardy–Weinberg equilibrium (*Fis* = 0) or assuming mostly selfing (*Fis* = 0.95).

Table 5. Geographic (km; above diagonal) and genetic (below diagonal) distance between five wild populations of *P. vulgaris* L. studied.

Population	Jeruco	Tupatáro	Yuriria	San Agustín	Cepio
Jeruco	****	2	4.7	1.6	2.3
Tupátaro	0.14	****	4.1	3.2	1.3
Yuriria	0.19	0.16	****	4.5	2.9
San Agustín	0.2	0.13	0.1	****	3.7
Cepio	0.23	0.28	0.21	0.3	****

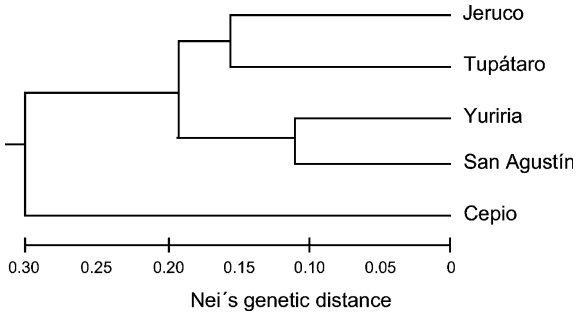

Figure 4. UPGMA dendrogram based on Nei's distances derived from 37 ISSR bands among five wild bean populations studied.

(Table 4). The difference between the number of observed and effective alleles was low. There were no differences between populations regarding the number of effective alleles, but there were differences in relation to Nei's diversity index (*h*) and Shannon's information index (*I*). The SA, YU and JE populations were the least diverse (*h* = 0.13–0.14) (*I* = 0.20–0.22), while the CE and TU populations were the most diverse (*h* = 0.18–0.20 and *I* = 0.28–0.29).

The population differentiation estimates, *Ht*, *Hs*, *Gst* and *Nm*, were similar under *Fis* = 0 and 0.95 assumptions. The total diversity (*Ht*) and the diversity within populations were high under both assumptions: *Ht* = 0.28–0.29, and *Hs* = 0.16–0.18. Diversity between populations was high, between *Gst* = 0.40 and 0.45, indicating that between 40% and 45% of the variation is explained by differences among the sub-populations. Gene flow between populations was low, between *Nm* = 0.6 and 0.7.

The UPGMA clustering, using the genetic distances (Table 5; Figure 4) did in fact indicate a high differentiation between populations. Three groups of populations were identified: the CE population, which was the most differentiated from the rest of the populations, a group comprising JE and TU

populations, and finally YU and SA populations. Thus, the greatest genetic distance was observed between CE population, the most diverse, and SA population, the least diverse ($D = 0.3$).

We found a significant highly negative correlation between the percentage of polymorphic loci and the distance of the populations from the nearest crop field (Figure 2, $r = -0.88$; $P = 0.046$), suggesting gene flow from the domesticated to the wild populations. We did not find correlation between the distance from crop field and Nei's diversity (Figure 2, $r = -0.53$; $P = -0.67$) and Shannon's index (Figure 2, $r = -0.67$; $P = 0.32$). The SA population, which was isolated from the domesticated plants, showed the lowest values in the estimates of diversity. We did not find correlation between the genetic and geographic distance ($r = -0.37$; $P = 0.32$).

Discussion

The populations of wild beans in the area under study grow in places where agricultural development is not possible, due to the high concentration of stones in the soil, rugged topography or the distance from urban centers and lack of roads. The populations survive on trees, thorny shrubs or cacti, where they can escape grazing by sheep, horses or goats. This would indicate the possibility that the size of these populations has been decreasing as a result of the expansion of agriculture, cattle raising and deforestation in the region. Another factor which may have had a negative influence is the introduction of agricultural implements such as the plough with animal traction 500 years ago and the introduction of agricultural machinery in the last 50 years. Traditional farming knowledge of the wild populations is poor and consumption by humans is occasional. No agricultural practices have been observed that protect or favor these plants. It is safe to say, therefore, that mankind has had a negative effect on the distribution and abundance of these populations.

The values of polymorphism and genetic diversity in the five populations studied were greater than those reported by Koenig and Gepts (1989). They observed values of $Ht = 0.13$ and $Hs = 0.006$, when they used 9 isoenzymatic loci and included 83 accessions of wild bean covering a wide geographic range in Mesoamerica and the Andes. These differences can be explained by the greater sensitivity of the molecular markers in the detection of polymorphism, as was pointed out by Becerra-Velásquez and Gepts (1994) and Tohme et al. (1996). The percentage of polymorphic loci found (89%) was also higher than that reported by Becerra-Velásquez and Gepts (1994) (76%), when they used RFLP markers in a group of 85 accessions of wild and domesticated populations from both centers of domestication, but the total genetic diversity in our study was lower ($Ht = 0.28$) in comparison with their report ($Ht = 0.38$). This difference could be attributed to the fact that we only included five wild populations from the Mesoamerican center and did not include domesticated populations.

The maximum differentiation distance between the wild populations studied was 0.30, similar to the value of 0.31 reported by Tohme et al. (1996), using AFLP polymorphisms in 975 accessions of gene pools of wild beans from both centers of domestication. The value obtained is within the range of 0.3–0.4 reported for the maximum distance observed in studies that utilized RFLPs, RAPDs or AFLPs markers to characterize genetic diversity of wild and weedy accessions, landraces and breeding lines (Beebe et al. 1995; Tohme et al. 1996; Skroch et al. 1998). Diversity within the populations was high and no differences were found between the number of effective and observed alleles, suggesting high levels of homozygosity and limited gene flow between populations, consistent with the predominantly selfing system of reproduction in common bean. Between 40% and 45% of the diversity found was explained by the differences among the populations, indicating high local differentiation and confirming the limited genetic flow between populations as shown the *Nm* values around 0.6.

The topology of the dendrogram suggests geographical isolation. This isolation could be due to the presence of hills or mountains as geographical barriers between the valleys of the area. This could be the case in the CE population, which is geographically close but genetically distant from the TU and YU populations. This phenomenon was not observed in the SA and YU populations. In spite of being geographically distant and

separated by Lake Cuitzeo, they were genetically close and were grouped in the same clade. These two populations were the most distant from cultivated plots and showed little influence of hybridization with domesticated plants (low morphological and genetic variability), suggesting that isolation from domesticated populations has allowed them to remain similar to each other and distinct from the other wild populations. This result suggests the existence of another factor favoring genetic differentiation among wild populations, which could be gene flow from domesticated populations. The high values in the coefficients of variation and the greater number of patterns and colors of the seeds in some populations studied suggest a high level of morphological genetic diversity. The high negative correlation between the coefficient of variation of average seed size with the distance to the nearest crop field suggests the existence of gene flow from the domesticated to the wild populations, as has been suggested by different authors (Delgado-Salinas et al. 1988; Debouck and Smart 1995; Beebe et al. 1997; Gepts et al. 1999). The highly negative correlation found between the percentage of polymorphic loci and the distance to the nearest crop field supports this hypothesis. Although the correlation coefficients between Nei's diversity and Shannon's information indices and the distance to the nearest cultivated field were not statistically significant, they were highly negative as well and consistent with correlations observed for seed size coefficient of variation and the number of polymorphic loci. Furthermore, Papa and Gepts (2003) have shown that gene flow between wild and domesticated beans is three-fold more important in the domesticated to wild than in the opposite direction. Overall, the spatial approach to studying gene flow appears to be quite useful to identify the presence of gene flow, even in predominantly selfing species such as common bean.

Given the previously mentioned circumstances, the farmer, with his or her agricultural activity, could be affecting the magnitude and the characteristics of gene flow between domesticated and wild populations, through the distance of the domesticated plots from wild populations and the genetic diversity included in cultivated fields, thereby influencing the process of differentiation among wild populations. An example illustrating this fact can be observed in the JE population, which is located close to domesticated populations and showed morphological effects of genetic infiltration (large seeds with a high coefficient of variation). This population did not group with the SA population, the closest geographically speaking, but with the TU population, another population close to domesticated plants. The JE population, however, showed low levels of genetic diversity, suggesting also that the diversity of the bean grown in the nearest domesticated plot was low.

Our study suggests that programs of *ex situ* conservation, collection strategies should be implemented that include a large number of populations both isolated from domesticated plants and in proximity to them, in order to represent the genetic diversity of the wild gene pool. Information as to the proximity of cultivated fields should be added to the passport date of individual wild accessions. As for conservation *in situ*, there is a need to establish strategies for maintaining wild populations isolated from domesticated plants in order to minimize gene flow. This would be difficult to achieve with strips of natural vegetation functioning as barriers, as this is precisely where wild populations grow. The evidence obtained in this study regarding gene flow from domesticated to wild populations suggests that the possible introduction to the Mesoamerican area of bean varieties from the Andean domestication center with the intention of increasing production, could affect the local wild populations through gene flow, as was suggested by Tohme et al. (1996). The introduction of transgenic plants could lead to the incorporation of transgenes into wild populations conferring them with greater adaptive capacity, thus allowing them to thrive under natural conditions, conditions such as the edges of cultivated areas or within the cultivated plots, and complicating both *in situ* conservation and the control of weeds as has been pointed out by Gepts et al. (1999). Additional studies are necessary that would permit us to better estimate potential risks associated with the introduction of transgenic plants. These studies include observations on the diversity, abundance and distribution of populations of pollinating insects of *Phaseolus*, estimates of the rates of cross-breeding in wild populations under different conditions associated with the presence or absence of pollinators, a better definition of the role of the floral characteristics of

wild and domesticated populations, the evolutionary dynamics of wild-weed–domesticated complexes in traditional agricultural systems and the surrounding vegetation, and the role and impact of farmers on these evolutionary processes. This analysis must consider the production rationale of the producers themselves, which involves technological and organizational aspects as well as forms of selection, utilization and consumption.

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