

Population Structure and Evolutionary Dynamics of Wild–Weedy–Domesticated Complexes of Common Bean in a Mesoamerican Region

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ABSTRACT

The diversity, structure, genetic flow, and evolutive relationships within and among three wild–weedy–domesticated complexes and a wild population isolated from crops of common bean (*Phaseolus vulgaris* L.) were analyzed under traditional agriculture, within a region of the Mesoamerican center of domestication. Their diversity was compared with the diversity of a local commercial variety and a breeding line. A morphological marker and 37 polymorphic ISSR loci were used. Total genetic diversity within the wild, weedy, and domesticated populations across the complexes was very similar (0.24, 0.22, and 0.26, respectively). Weedy populations within each complex were more closely related to the domesticated than to the wild populations, suggesting they originated from introgression of wild-type alleles into the domesticated populations or predominant gene flow from domesticated to wild populations. The wild population in closest proximity to the crop within its complex was more similar to the domesticated and weedy populations of its complex than to the rest of the wild populations, suggesting displacement of the wild genetic diversity by gene flow from the domesticated population within its complex. The high values of differentiation among wild, weedy, and domesticated populations within each complex suggest high autogamy or genetic drift. However, the values of gene flow among populations within the complexes were close to one, theoretically sufficient to counteract genetic drift and/or autogamy. We therefore assume that human selection is the most important evolutionary mechanism for maintaining the high wild-domesticated differentiation by negative farmer selection of cultivated plants with morphological characters that suggest introgression. Farmers may influence the magnitude and characteristics of gene flow among populations within each complex by the management of the distance between the crops and the wild populations, the diversity within the landraces sown, and the tolerance and harvesting of weedy populations. The high geographic differentiation of the wild populations, together with the local differences in human selection practices and agronomic management, could have generated multiple evolutionary lineages after domestication. Domesticated populations within complexes were between two and four times more diverse than the local commercial variety and four and nine times more diverse than the breeding line. New conservation and breeding strategies are suggested to maintain and use the gene pools from these complexes.

IN MEXICO, the common bean is the main source of protein for the human population. Its present yield is calculated at around 650 kg/ha, while the potential

yield is estimated between 4000 and 5000 kg/ha (Gepts, 1993). Low yields can be attributed in part to the poor knowledge and limited use of the genetic diversity of wild populations of this species (Gepts and Debouck, 1991; Gepts et al., 1999). Several studies have suggested that wild bean populations present greater genetic variability than domesticated populations, which, because of the founder effect during the domestication process, excluded valuable genetic variability for adaptive and productive characteristics (Romero and Bliss, 1985; Romero et al., 1986; Debouck and Tohme, 1989; Koenig et al., 1990; Acosta-Gallegos et al., 1998; Gepts et al., 1999). During domestication, humans selected a set of morphological and physiological characters related to the so-called domestication syndrome, including seed dispersal (pod suture fibers, pod wall fibers), growth habit (determinacy, twining, number of nodes on the main stem, number of pods, internode length), pod length and seed weight, number of days to flowering, photoperiod sensitivity, harvest index, and seed pigmentation (Gepts and Debouck 1991; Koinange et al., 1996).

Genetic compatibility between wild and domesticated populations leads to wild–weedy–domesticated hybrid complexes in sites with sympatric distribution by introgression of genes from wild populations to domesticated ones or vice versa. Weedy populations are defined here as wild populations growing in crop fields that were not planted by farmers. Because they may be the result of this introgression, they usually show morphological traits reminiscent of one or the other parent, such as larger seeds than the wild parent or seed color or color patterns similar to those observed in wild beans. These hybrid complexes constitute a valuable source of genes to the farmer or to the plant breeder (Debouck and Smartt, 1995; Beebe et al., 1997); however, at the same time they also represent a risk for the massive introduction of genes from the domesticated to wild populations (Gepts et al., 1999; Papa and Gepts, 2003).

Comparisons of wild and domesticated populations in their whole area of distribution indicate two main gene pools in which domestication took place (Becerra-Velásquez and Gepts, 1994; Debouck and Smartt, 1995; Gepts and Debouck, 1991; Koenig and Gepts, 1989; Tohme et al., 1996). One center of domestication is located in Mesoamerica and the other in the Andes. Studies on gene dynamics of wild–weedy–domesticated complexes within the Mesoamerican area of domestication are important, since four of the five domesticated species and 45 of the 50 species of the genus *Phaseolus* grow in this area, and their natural reproductive rela-

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Published in Crop Sci. 45:1073–1083 (2005).

doi:10.2135/cropsci2004.0340

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Abbreviations: ISSR, inter simple sequence repeat; QTLs, quantitative trait loci.

tionships are mostly unknown at present. Genetic bridges may exist between these species, as in the case reported by Escalante et al. (1994) regarding gene flow between wild populations of *P. vulgaris* and *P. coccineus* L.

Recent studies on wild populations of Mesoamerica, in areas where traditional agriculture continues, indicate that opportunities for wild-domesticated hybridization are common in spite of a predominantly autogamous reproductive system. They suggest that gene flow is asymmetric, at least three times greater from the domesticated to the wild, compared with the opposite direction (Papa and Gepts, 2003; Payró de la Cruz et al., 2005). Thus, a displacement of genetic diversity in wild populations because of gene flow from the domesticated populations can take place (Papa and Gepts, 2003). Farmers in traditional farming systems can have an influence on the magnitude and characteristics of gene flow by distancing the crop from the wild populations and by the management of diversity in domesticated landraces (Payró de la Cruz et al., 2005).

There is evidence that the domestication of *P. vulgaris* in Mesoamerica originated in the central western sector of Mexico, where the modern states of Jalisco, Michoacán, and Guanajuato meet (Gepts and Debouck, 1991). Primitive cultivars could have spread from this area to other regions where they crossed with local wild populations, giving rise to present day landraces (Gentry, 1969; Beebe et al., 2000). This area of possible domestication of common bean corresponds to the early frontier of Mesoamerica with Aridoamerica, in the southern portion of an old lakes system formed by the river Lerma. Some of the lakes were drained by the Europeans during the first hydraulic works of drainage and irrigation performed in the Americas around 1548, allowing the establishment of one of the most important regions of intensive agriculture in Mexico, known as El Bajío. Alongside this intensive agricultural area, on the slopes of the surrounding mountains, traditional agricultural systems are still practiced today in extremely stony soils of volcanic origin, without the possibility of irrigation or mechanization.

In this area, we find a climatic transition from sub-humid to semiarid and vegetation varying from tropical deciduous forest to a forest of mesquite, *Prosopis laevigata* (Humb. et Bonpl.) Johnst. The average annual rainfall is around 700 mm, with a high coefficient of variation between years, close to 25%, with a high variation in the initiation and establishment of the rainy season, and with an intraseasonal dry period that is variable in intensity, amplitude, and date of appearance. Traditional agriculture, therefore, is performed with a high risk factor. As a consequence, the production rationale of the farmer focuses on securing the harvest using plant genetic resources with a high diversity of response (Zizumbo-Villarreal et al., 1988). Within this area, evidence has been found of agricultural villages dating back to the Preclassic period (800–100 BCE) (Branniff, 1975; Oliveros, 1975). At present in this area, there is a high incidence of seasonal migration of farmers to the USA, where some of them work in the cultivation of bean.

Occasionally, these farmers incorporate varieties from the USA in their crops (Zizumbo-Villarreal, 1985).

According to Zizumbo-Villarreal (1985), three main types of agriculture are practiced in this area, each one in a different agro-ecological condition.

1. Mechanized and irrigated intensive farming, performed in flat terrains with few stones in the soil. Beans are sown in monocropping in which genetically improved commercial varieties have been introduced and tested by INIFAP and private breeding companies.
2. Dry land farming with animal traction, performed on stony ridges. Local landraces of bean are cultivated in association with maize and squash. In some cases, weedy populations of bean grow on this land.
3. Dry land farming with human energy, performed in extremely stony terrain. Local landraces of beans are also cultivated, associated with maize and squash. Weedy populations of bean generally also grow on this land. Adjacent to the dry land farming areas, wild populations are found in sites with disturbed natural vegetation and high light incidence, close to seasonal streams in small ravines with extremely rocky soil, on bushes and trees of the mesquite forest and tropical deciduous forest. It is common to find goats grazing in these areas, subjecting the wild bean populations to constant pressure from these animals.

The aims of this work were (i) to analyze the morphological variation, diversity, structure, genetic flow and evolutionary relationships within and among three wild-weedy-domesticated complexes of *P. vulgaris* growing under traditional agricultural systems in the El Bajío region, Mexico, in addition to one wild population isolated from crops in the same region; (ii) to compare their levels of diversity with the diversity of a local commercial variety and a breeding line, both cultivated with modern technology; and (iii) to analyze the role played by farmers in the present evolutionary dynamics through selection and agronomic management.

MATERIALS AND METHODS

Selection of Sites and Complexes and Seed Collection

Sites studied were a subset of those included in a previous study (Payró de la Cruz et al., 2005) with regard to the spatial distribution of genetic diversity in wild populations of *P. vulgaris* in the old Mesoamerican frontier in the states of Michoacán and Guanajuato in the southern part of El Bajío region (Fig. 1, Table 1). Three wild-weedy-domesticated complexes were selected, each one growing on the slopes of the mountains of a different valley, under traditional dry land farming with human energy conditions. Field plots were managed by farmers over 60 yr old with long agricultural experience. We collected seeds from an average of 29 plants from each population with the participation of the farmers. Care was taken to sample the whole area occupied by populations and to collect samples from plants that were more or less equidistant. Domesticated populations were constituted by those plants recognized as

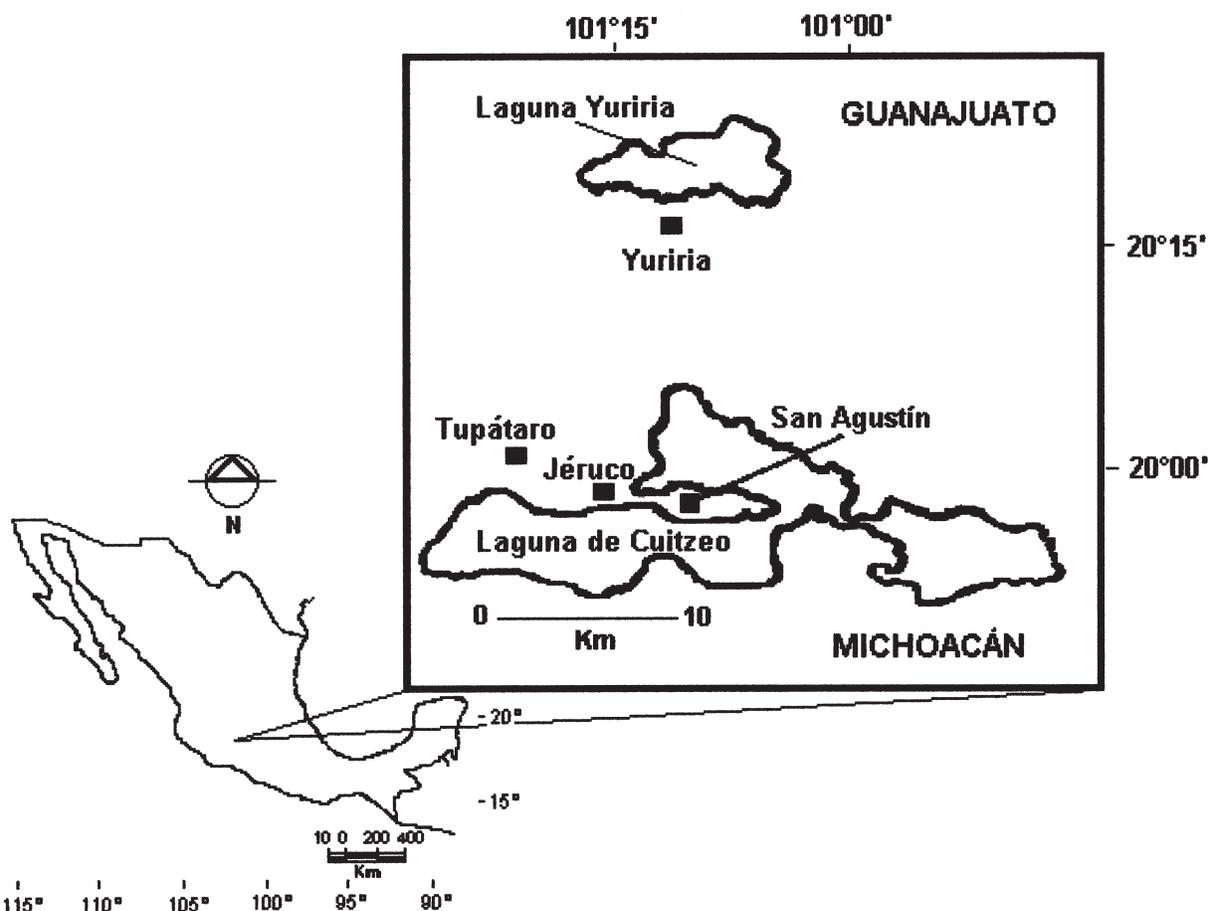


Fig. 1. Location of the three wild-weedy-domesticated complexes and the wild isolated population of *Phaseolus vulgaris* L. studied.

planted and cultivated by them in their crop fields, weedy populations by those plants growing in the crop field but out of the furrows, in an irregular pattern because they were not planted by the farmers, and wild populations by those plants growing outside the crop fields. Wild populations were growing in abrupt and rocky areas. The populations growing closest to the field crops were chosen. One wild population that was growing in a different valley and was isolated from the crops was also included in the study. Furthermore, a local variety named Flor de Junio and a variety released by the governmental breeding institution INIFAP (Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias) named Anita were added as well. These two varieties are cultivated in the same region but under modern agricultural management, in plots located in valleys with irrigation. Their seeds were bought in the local market for the analyses. The study was performed during the summer-autumn agricultural cycle of 1998.

Morphological Variation in Seed

Seed mass (expressed in grams per 100 seeds) was used as an indicator of morphological variation because of the marked

difference in this character between wild and domesticated beans. Seed mass has a high heritability (Motto et al., 1978), four QTLs explain 57% of its variation between wild and domesticated beans (Koinange et al., 1996), and it is also easy to measure in the field and the laboratory. In each wild population, an average of 29 plants were evaluated; in the weedy and domesticated populations, an average of 16 and 28, respectively, were evaluated. The mean value (M) and the coefficients of variation (CV) per population were estimated by the Statistical Analysis System software release 6.03 (SAS 1992). The same software was used to performed a one-way analysis of variance (ANOVA) and a Bonferroni means separation test for multiple comparisons, to estimate the differences in seed mass among wild, weedy, and domesticated populations across the three complexes and the isolated wild population.

Diversity, Genetic Structure, Gene Flow, and Evolutionary Relationships

Diversity and genetic structure were evaluated by polymorphism for ISSR markers, which is a dominant molecular

Table 1. Geographic characteristics of the three wild-weedy-domesticated complexes and the isolated wild population of *Phaseolus vulgaris* L.

Complex or population name	Municipality	State	Latitude	Longitude	Altitude (m)	Distance (m)†
Jéruco	Cuitzeo	Michoacán	19°57'	101°10'	1850	10
Tupátaro	Tupátaro	Michoacán	20°01'	101°51'	2100	25
Yuriria	Yuriria	Guanajuato	20°11'	101°08'	2000	60
San Agustín	Cuitzeo	Michoacán	19°58'	101°04'	1900	110

† Between wild population and nearest cultivated bean field.

marker. An average of 20 individuals was used per population, chosen at random among the individuals that were evaluated morphologically. Domesticated individuals were analyzed without identification of the landrace to which they belonged. The genomic DNA was obtained from young leaves by the CTAB method (González et al., 2005) and three ISSR primers [(GACA)₃ RG, (GACAC)₂, and (GA)₈ RG] were used that had been reported to be highly polymorphic (González et al., 1988). Each 20- μ L amplification reaction consisted of 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% (v/v) Triton X-100, 2 mM MgCl₂, 200 μ M each dNTPs, 1 μ M of primer, 1 unit of Taq polymerase (Promega, Madison, WI) and 50 ng of template DNA. Amplification was performed in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) following the conditions established by González et al. (2005). The fragments of DNA generated were separated by electrophoresis (Hoefer SQ3 sequencer, Amersham Pharmacia Biotech, San Francisco, CA) in 320- \times 380- \times 0.4-mm gels containing 5% (w/v) of nondenaturing 29:1 acrylamide-bisacrylamide (González et al., 2005). Visualization of the fragments was performed 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 and analyzed as diploid data for dominant markers by standard POPGENE 1.31 (Yeh et al., 1999) procedures for summary multilocus genetic statistics: percentage polymorphic loci (%) in the populations within complexes, in the populations of the same type across the complexes, and in the whole sample, Nei's (1973) genetic diversity (h) and Shannon's information index (I) (Lewontin, 1972) for populations within complexes, genetic structure for populations within complexes and for populations of the same type across complexes [total diversity (H_t), intrapopulation diversity (H_s), population differentiation (G_{st}) (Nei, 1987)], and homogeneity tests of gene frequencies across populations within each complex (Sokal and Rohlf, 1995). Since some studies have demonstrated high levels of autogamy in the common bean and others have demonstrated allogamy when pollinators are abundant (Brunner and Beaver, 1989; Triana et al., 1993; Ibarra-Pérez et al., 1997), the statistics just mentioned were calculated twice: assuming Hardy-Weinberg equilibrium (H-W) ($F_{is} = 0$) and assuming autogamy ($F_{is} = 0.95$) to decide which assumption should be made under our study conditions. As most estimators were different under both assumptions, the values presented here were those obtained under the assumption of autogamy (H-W disequilibrium). Gene flow (Nm) was estimated indirectly by calculating the number of migrants from G_{st} (McDermott and McDonald, 1993), since this estimator considers the combined effects of flow of genes (by means of pollen or seeds) and its selection in a large number of populations over a wide temporal scale (Slaktin and Barton, 1989). It was estimated among populations within each complex and among populations of the same type from different complexes, considering that even though complexes were in different valleys, seed movement by humans may have occurred. Results were the same when analyzed as haploid data, assuming total autogamy, as Papa and Gepts (2003) assumed in a study of this same species.

A dendrogram based on Nei's genetic distance (1972) using UPGMA (Unweighted Pair Group Method with Arithmetic Means). Swofford and Olsen (1990) was computed by the TFPGA program (Tools for Population Genetic Analyses; Miller, 1997) using the bootstrapping option for 1000 permutations (Felsenstein, 1985), which reports the proportion of permuted data sets that result in the formation of the node seen in the original data set.

RESULTS

Selection and Traditional Management of the Wild-Weedy-Domesticated Complexes

On the basis of a previous study of the traditional agricultural systems in the area (Zizumbo-Villarreal, 1985) and on the participatory observations performed throughout the study year, we were able to describe the richness of local domesticated landraces, their agricultural management, harvesting methods, and the criteria for selection and consumption of seeds by farmers and their families. The three farmers studied, Pedro Melo (Yuriria, Guanajuato), Nicolás Rico (Jéruco, Michoacán), and Ignacio Fernández (Tupátaro, Michoacán), were capable of distinguishing the weedy plants during hand weeding. They protected these plants, rather than eliminating them. As all three of them had consumed their seeds in the past and because of their sweet taste in the immature stage, they decided it was convenient to tolerate these populations, arguing that they are more resistant than the domesticated populations to droughts, excessively wet periods, and the attack of fungi. These were qualities, which, in the case of adverse conditions, would allow them to harvest these populations, whereas the domesticated ones might succumb. Two of the producers decided to harvest and consume them at a later date. Harvesting was performed before the harvesting of domesticated beans and maize (*Zea mays* L.), before natural pod dehiscence. The harvest is not particularly exhaustive; therefore, some weedy plants escape and are able to produce seed and disperse naturally. The fresh pods were taken to barns and placed in a corner; care was taken not to mix them with the domesticated beans (Fig. 2a). When the pods were dry, they opened naturally and the farmers then selected the seeds for consumption on the basis of their color. Light colors (such as white and cream) were favored, while the dark or black seeds were eliminated (Fig. 2b). Black seeds, both in weedy and domesticated populations, are selected against because they are considered of bad quality, in contrast with the more lightly colored seeds.

The three farmers cultivated simultaneously several local landraces in their plots planting their seeds in mixture. Farmer Pedro Melo planted eight landraces (Apetito, Cacahuatate, Café, Canelo, Flor de Mayo, Higüerillo, Morado, and Vaca). Farmer Ignacio Fernández planted eleven (Apetito, Bayo, Cacahuatate, Canelo, Flor de Mayo, Guindo, Higüerillo, Morado, Ojo de Cabra, Palacio, and Vaca), whereas farmer Nicolás Rico only five (Cacahuatate, Flor de Mayo, Higüerillo, Morado, and Vaca). The pods were harvested when they were almost dry and were transported to the barns to complete the drying. Cultivated plants pods were kept separate from the weedy ones that had been harvested previously to avoid mixing. When the cultivated seeds were extracted from the pods, they were stored in sacks with all the landraces mixed together. When the seeds were required for subsistence or sale in the local market, they were then selected and separated according to landrace because seed mixtures are penalized in the local market. For local commercialization, the selection was very strict,



Fig. 2. (a) Weedy and domesticated populations harvested and stored in home, (b) selection of weedy seeds in home.

especially in the case of the Cacahuate and Morado landraces, which are the most preferred and reach a higher commercial value. The farmers' wives also separate the seeds because they prefer to eat first the landraces, such as *Apetito*, *Cacahuate*, *Flor de Mayo*, *Higüerillo*, and *Morado*, which cook more quickly and homogeneously and have good flavor in their opinion.

In years when the production of domesticated populations is low, the seeds from weedy plants are harvested and consumed, which was the case in the year this study was performed. The farmers' wives mentioned that weedy seeds cook very slowly and heterogeneously;

however, they are appreciated because they help to complement the food requirements for the family. The wives also mentioned that the consumption of these seeds is considered socially demeaning. The farmers store part of the harvest for planting the following year. The infected or damaged seeds are eliminated before planting, and the landraces mixed together are once again cultivated.

Morphological Variation of the Seed

Significant differences were found in seed mass among wild bean populations (6 g/100 seeds), weedy bean pop-

Table 2. Complex or population name, number of plants (*n*), mean seed mass (g/100 seeds) (*M*), coefficient of variation of seed mass (*CV*), and level of significance of differences among mean seed mass in three wild-weedy-domesticated complexes and a wild population of *Phaseolus vulgaris* L.

Complex or population name	Wild			Weedy			Domesticated		
	<i>n</i>	<i>M</i>	<i>CV</i>	<i>n</i>	<i>M</i>	<i>CV</i>	<i>n</i>	<i>M</i>	<i>CV</i>
Jéruco	24	7	50	26	21	32	27	42	11
Tupátaro	33	5	24	7	20	28	9	38	18
Yuriria	24	6	27	14	19	21	49	37	11
San Agustín	33	4	12	—	—	—	—	—	—
Mean	29	6	28	16	20	27	28	39	13
Significance*		A			B			C	

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

ulations (20 g/100 seeds), and domesticated bean populations (39 g/100 seeds) (Table 2). Similar results were presented by Delgado-Salinas et al. (1988) and Tohme et al. (1996).

Diversity, Genetic Structure, Gene Flow, and Evolutionary Relationships

For the genetic analysis, we studied 37 bands (putative loci), obtained with three ISSR primers, seven with primer (GACA)₃ RG, 21 with primer (GACAC)₂, and nine with primer (GA)₈ RG (Fig. 3, Table 3). The frequency of polymorphic loci ranged from 35 to 84% among the populations within each complex (Table 4) and was 87, 84, and 95% in the wild, weedy, and domesticated populations across the complexes, respectively (Table 5). Over the three complexes and the wild isolated population, the total polymorphism was 100%.

The values of total genetic diversity within the wild, weedy and domesticated populations across the complexes were very similar (0.24, 0.22, and 0.26, respectively) (Table 5). However, within the complexes, the wild population showed, in general, lower values than the domesticated population of its complex (Table 4).

This is in agreement with the high geographic differentiation ($G_{st} = 0.4$) among wild populations across complexes and the low geographic differentiation among cultivated populations across complexes ($G_{st} = 0.26$) (Table 5). Within the Jéruco complex, all three populations showed similar values of diversity (Table 4).

The homogeneity test indicated a high percentage of alleles with different frequencies among the populations of each complex ($P < 0.05$): Tupátaro 62%, Yuriria 57%, and Jéruco 54%, which suggests high autogamy in these populations. The values of H_s and H_t were positive and different from zero within the complexes, also indicating effects of autogamy (Table 6). These results are reflected in the high values obtained for the average genetic structure of the three complexes ($G_{st} = 0.31$), for which the Tupátaro and Jéruco complexes showed the greatest genetic structure (Table 6). Thus, between 26 and 34% of the diversity observed is explained by the genetic differences among the populations within each complex.

Gene flow among wild and weedy populations from different complexes was low ($Nm = 0.77$ and 0.79 , respectively), whereas among the domesticated popula-

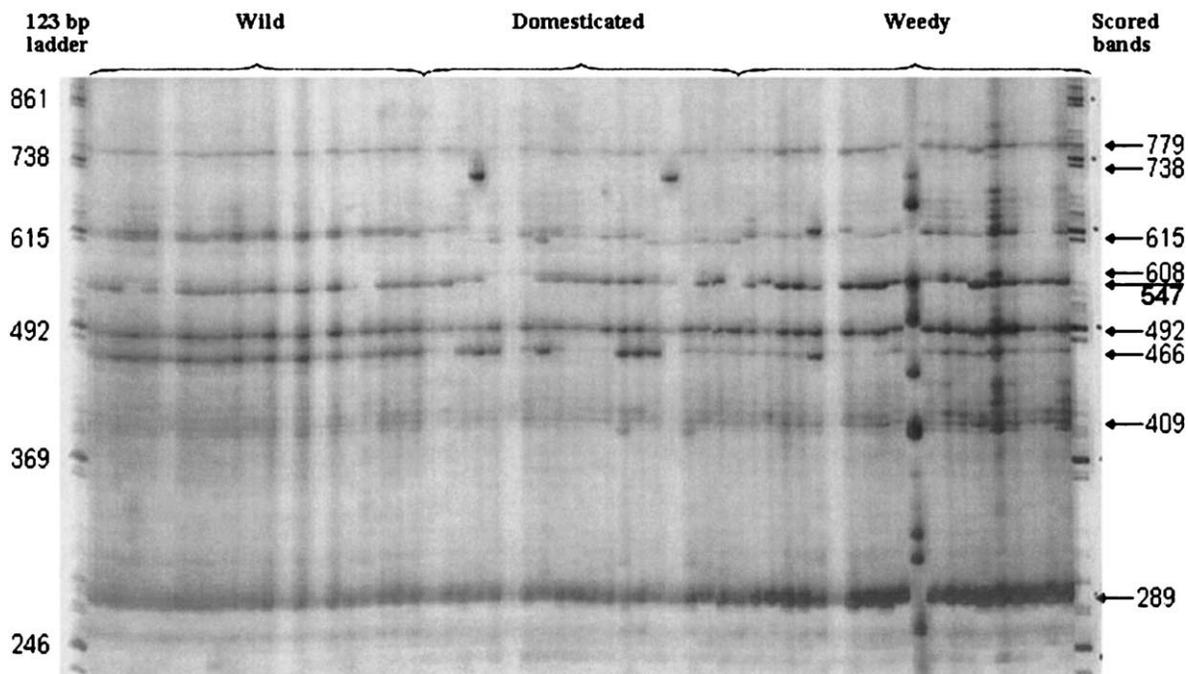


Fig. 3. Samples of ISSR profiles for the (GA)₈ RG primer of the Yuriria complex. Arrows on the right indicate bands scored with size in bp.

Table 3. Primers used in intersimple sequence repeat (ISSR) analyses of four wild-weedy-domesticated complexes of *Phaseolus vulgaris* L. and size of the bands they produced. $R = A, G$.

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

tions it was higher than one ($Nm = 1.4$; Table 5). Among the populations within each complex, gene flow was close to one in all cases (Table 6), which may be sufficient to counteract the effects of genetic drift and autogamy under natural conditions (Hedrick, 2000).

UPGMA analysis indicated a small genetic distance between the weedy and domesticated populations from each complex, grouping them together with a support between 0.73 and 0.99 for 1000 permutations (Fig. 4). Wild populations of Tupátaro and Yuriria grouped together with the wild population of San Agustín, which was isolated from bean crops, with a support of 0.54, whereas the wild population from the Jéruco complex, the one closest to the crop field, clustered with the domesticated and weedy population from the complex, although with a weak support of 0.32 (Fig. 4).

Comparison of Genetic Diversity of Local Landraces, a Local Commercial Variety, and a Breeding Line

In the local variety Flor de Junio, sold in the market of Yuriria, low genetic diversity ($h = 0.06$, $I = 0.10$) was found. The breeding line Anita, cultivated in monoculture, showed an even lower diversity ($h = 0.03$, $I = 0.07$), in comparison with that found in the domesticated populations of the three complexes ($h = 0.13$, $I = 0.19$ to $h = 0.26$, $I = 0.40$) (Table 4).

DISCUSSION

Diversity, Structure, Gene Flow, and Evolutionary Relationships

Results from this work indicate that in the area studied, under present day traditional agricultural practices, the diversity of the domesticated populations within the complexes is the same as or higher than that of the wild

Table 5. Population type, number of plants (n), percentage of polymorphic loci, total diversity (Ht), interpopulation diversity (Gst) and gene flow (Nm), assuming predominant selfing ($Fis = 0.95$).

Population	n	Percentage of polymorphic loci	Ht	Gst	Nm
Wild	85	87	0.24	0.40	0.77
Weedy	62	84	0.22	0.39	0.79
Domesticated	64	95	0.26	0.26	1.40

populations of the same complex; thus, no reduction in the genetic diversity of the domesticated pool is observed in relation to the wild gene pool of the same complex. This result suggests that the farmers, by interchanging seeds among complexes and by cultivating a large number of local landraces in the same plot, can maintain a high diversity and increase it through spontaneous crossing among landraces.

Diversity of domesticated populations within the complexes is positively related to the number of landraces in the mixture sown. Zizumbo-Villarreal (1985), in a study of the traditional agriculture in one of the locations included in the present study (Yuriria), found that this number is the result of a balance struck by the farmer between his need to confront the variability and adversity of the agroecological conditions under which he cultivates and his need to obtain cash through the sale of bean seeds in the local markets. Planting a high number of landraces with different ecological requirements permits him to have some harvest regardless of the annual growing conditions. At the same time, he needs to reduce this number and plant only those landraces that fetch high market prices. This is what we found in the three locations studied: Tupátaro (farmer Ignacio Fernández with 11 landraces) is the town with less dependence on the markets and Jéruco (farmer Nicolás Rico with 5 landraces) with the greatest.

Although the presence of Cacahuete landraces, which may be of Andean origin, could inflate the genetic diversity of domesticated types, this is unlikely to have a major effect since Cacahuete was but one of several components grown by the three farmers: Melo: 1/8; Fernández (1/11); and Rico (1/5). Furthermore, one of the goals of this research was to investigate the possible role of farmers in the maintenance of genetic diversity, regardless of the evolutionary origin of the landraces, which is actually unknown to the farmers.

Table 4. Complex, population or variety name, number of plants (n), percentage of polymorphic loci, Nei's gene diversity (h) (Nei, 1973), Shannon's information index (I) (Lewontin, 1972) of populations studied, assuming predominant selfing ($Fis = 0.95$).

Complex, population, or variety name	n	Population type	Percentage of polymorphic loci	h	I
Jéruco	20	wild	59	0.13	0.22
	22	weedy	43	0.13	0.20
	21	domesticated	35	0.13	0.19
Tupátaro	22	wild	65	0.18	0.28
	20	weedy	54	0.13	0.22
	20	domesticated	84	0.26	0.40
Yuriria	22	wild	57	0.14	0.22
	22	weedy	57	0.16	0.25
	21	domesticated	68	0.20	0.31
San Agustín	21	wild	35	0.14	0.20
Flor de Junio	20	local commercial variety	22	0.06	0.10
Anita	20	breeding line	14	0.03	0.07

Table 6. Genetic structure in three wild-weedy-domesticated complexes of *Phaseolus vulgaris* L. Total diversity (*Ht*), intrapopulation diversity (*Hs*), interpopulation diversity (*Gst*) and gene flow (*Nm*), assuming predominant selfing (*Fis* = 0.95).

Complex name	<i>n</i>	<i>Ht</i>	<i>Hs</i>	<i>Gst</i>	<i>Nm</i>
Jéruco	63	0.19	0.13	0.33	1.04
Tupátaro	62	0.29	0.19	0.34	0.98
Yuriria	63	0.23	0.17	0.26	1.39

The lower geographic differentiation among the domesticated populations across the complexes ($Gst = 0.26$) compared with the value for the wild ones ($Gst = 0.40$), and the higher value of gene flow among domesticated populations across the complexes ($Nm = 1.4$) compared with the value for the wild populations ($Nm = 0.77$) support our hypothesis that landraces are actively exchanged by farmers. Papa and Gepts (2003) also observed lower levels of spatial autocorrelation in domesticated populations compared to wild populations in Mexico, consistent with a lower level of geographic differentiation in the former compared with the latter. Furthermore, by protecting the weedy populations within the cultivated plots, the farmers further encourage the formation of hybrids and backcrosses. In contrast, the genetic diversity of the local commercial variety cultivated under modern agricultural technology was between two and four times lower than that of the domesticated populations cultivated under traditional agriculture. This is even more noticeable in the breeding line cultivated in monoculture, whose diversity was between four and nine times lower. This has considerable importance for the design of germplasm collecting strategies for ex situ

conservation programs, which must focus on collecting in the traditional agroecosystems instead of in local markets, to achieve representativeness of the diversity of domesticated populations.

The high level of autogamy suggested by the high percentage of alleles with different frequency among populations within the complexes, in spite of gene flow values close to one, could be a result of a strong negative farmer selection of cultivated plants with morphological characters suggesting introgression. Similar gene flow values were observed between wild and domesticated populations in Chiapas, and evidence that the selection of alleles of the domestication syndrome is the evolutionary mechanism that maintains the identity of wild and domesticated populations in sympatric situations (R. Papa and P. Gepts, unpublished data).

The UPGMA dendrogram indicates that, in the Jéruco complex, where the geographic distance between the wild and the domesticated population is the smallest (10 m), the wild population grouped with the domesticated and the weedy populations, also differentiating it from the other wild populations of the region. This observation suggests that gene flow from domesticated to wild populations has decreased the genetic distance between wild and domesticated types at Jéruco, presumably by displacement of the wild genetic diversity in wild populations, as indicated by Papa and Gepts (2003). These authors found that the gene flow is asymmetric, three to four times greater from domesticated to wild, which could be explained by the greater size of the domesticated populations and a greater adaptation of the hybrids to the natural environment promoted by

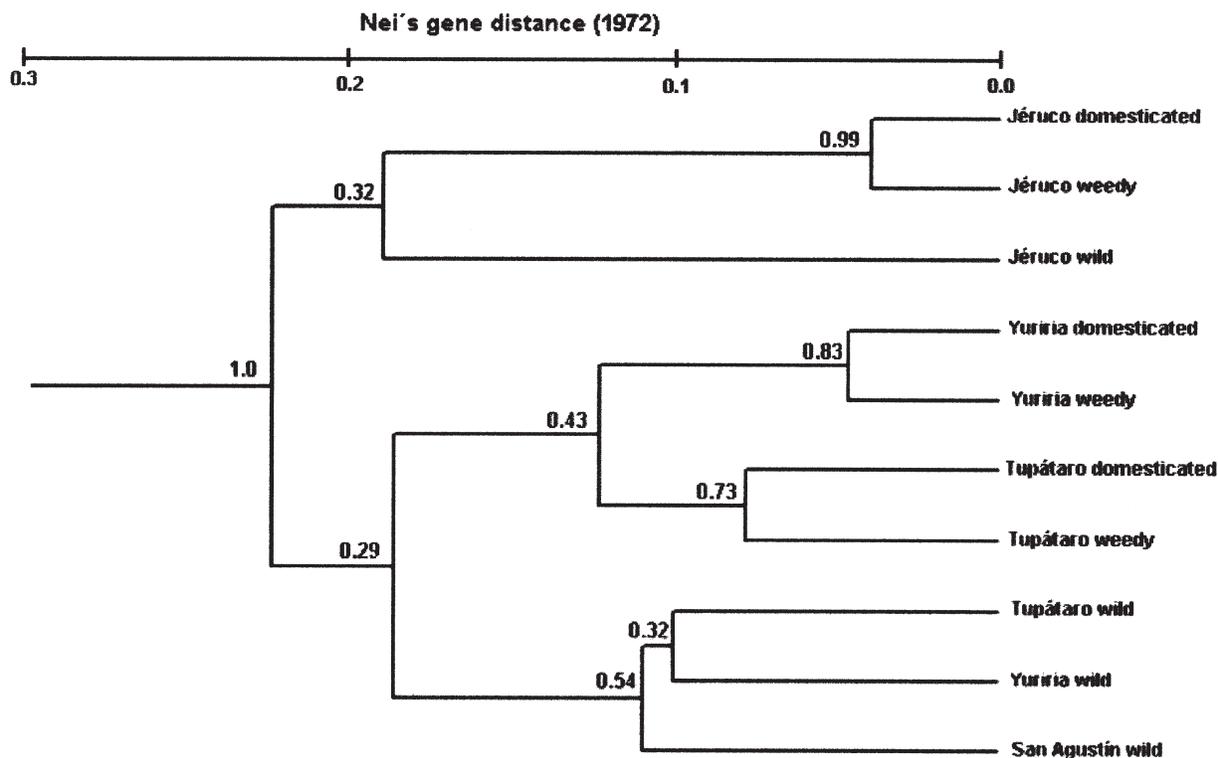


Fig. 4. UPGMA dendrogram based on Nei's gene distance (1972) derived from 37 ISSR bands among the populations of three wild-weedy-domesticated bean complexes and a wild population isolated from crops. Values at each node are the bootstrapping results for 1000 permutations.

the dominance of the wild phenotype. Payró de la Cruz et al. (2005) reported a negative correlation between the wild–domesticated distance and the genetic diversity in wild populations, with the exception of the wild population in the Jéruco complex. They argued that the genetic diversity of the nearest domesticated population was probably low. The results presented in this study further support this assumption. The genetic diversity of the nearest domesticated population was low and similar to that of the wild population ($h = 0.13$).

The high genetic differentiation of populations within the complexes is explained mainly by the genetic distance from the wild to the domesticated population, the farmer selection of seeds generation after generation, and the effects of natural selection in two contrasting environments. The smaller weedy–domesticated genetic distance can be explained by the selection performed by the farmer for domesticated traits in both types of populations, by similar selection pressures in the domesticated plot, and predominant gene flow from domesticated to wild-types.

The wild populations of Yuriria and Tupátaro, separated from the domesticated population by 25 to 60 m, showed high genetic similarity with the wild population of San Agustín, isolated from bean crops, indicating that the gene flow from the domesticated population to the wild has not been sufficiently high to result in a grouping with their respective domesticated populations. The data mentioned above indicate, therefore, that the geographic distance between domesticated and wild has a strong influence on the level of genetic infiltration and is a factor of great importance for in situ conservation.

Origin of Weedy Populations

The analysis of variance of seed mass (Table 2) suggests that the weedy populations are a product of genetic infiltration between wild and domesticated populations, since they present an intermediate seed mass between wild and domesticated and a high coefficient of variation in this character. These results concur with those suggested by Delgado-Salinas et al. (1988) on the basis of the purple coloring of the flowers and pod dehiscence in weedy types, characters which are found in wild populations. The short genetic distance obtained between domesticated and weedy populations suggests, in effect, that they were indeed derived from hybridization between wild and domesticated populations, as indicated by Papa and Gepts (2003). The hybrid character of the weedy types was demonstrated by Beebe et al. (1997) on the basis of the segregation of wild and domesticated characters in weedy types. The cases in which this segregation did not occur were explained by Beebe et al. (1997) as resulting from character fixation due to a historic selective process by humans.

The Role of the Farmer in the Evolutionary Dynamics

The protection of wild populations in the plots by traditional farmers can lead to hybridization of wild and domesticated populations, thereby generating weedy

plants. In the same way, this protection favors backcrossing of weedy with domesticated plants and subsequently the establishment of segregants with high morphological similarity to the domesticated. These segregants are harvested and eventually returned to the plots in the next agricultural cycle. As the harvesting of weedy types is not particularly exhaustive, some of these escape and disperse naturally, appearing in the same way in the following agricultural cycle. The harvesting and storage of weedy plants in close proximity to seeds from domesticated plants in the barns, may also allow the weedy seeds to return eventually to the plot and to be cultivated inadvertently. In this way, the farmer himself has facilitated the existence of genetic complexes. The management of geographic distance between wild and domesticated populations, the selection and management of domesticated diversity, and farmer tolerance and harvesting of weedy populations, are traditional agricultural practices that influence the magnitude and characteristics of gene flow in wild–weedy–domesticated complexes, and which have made recurrent evolutionary processes possible after the initial domestication in the Mesoamerican area.

Under these conditions, how could the Mesoamerican farmer isolate the desired characters involved in the domestication syndrome, with the presence of both wild and domesticated populations within outcrossing distance? Probably, the mechanisms involved in the asymmetry of wild–domesticated gene flow permitted this. Harvesting before pod dehiscence to avoid the loss of seed and selection of large, white or light-colored seeds, could have had an influence on the process of reproductive isolation. Selection of precocious plants with early and short periods of pollen receptivity and seed production as well as attractiveness to insects (white flowers) may have limited gene flow from wild to domesticated types.

Conservation In Situ

Bean breeding and germplasm conservation programs require new approaches to achieve an increase in productivity and adaptation of the domesticated gene pool through the use of genes from wild and weedy populations (Singh et al., 1995; Beebe et al., 1997; Mauro Herrera, 2003). The in situ conservation of the wild–weedy–domesticated complexes within the traditional agricultural systems, where the farmers are producing advanced generations of wild–domesticated hybrids, could be an important approach to achieve this goal. The importance of weedy seeds for the survival of traditional farmers is a factor that has permitted and facilitated their presence in the agricultural system and the continuity of genetic relationships between the populations that form the wild–weedy–domesticated complexes. Our results also encourage further emphasis on both ex situ and in situ collections of common bean and other crops. For ex situ collections, the current *Phaseolus* world collection at CIAT (Colombia) contains some 25 000 domesticated accessions of *P. vulgaris* but only some 1200 accession of wild *P. vulgaris*. Wild popula-

tions that have been the object of gene flow studies conducted recently on common bean, including this one, are not well represented in ex situ collections. Thus, more intensive exploration and collection of wild populations are warranted. For in situ conservation, our results suggest that increased efforts should be placed on the conservation of wild populations in more remote areas. Diversity in wild populations is very localized so its conservation is difficult and should include farmers' participation under a strategy that evaluates economically their agricultural products from an economic standpoint, to decrease economic pressures to extend the cultivated areas, eliminating wild populations or planting domesticated beans too close to them.

One important survival strategy of the traditional farmers in the study area has been the incorporation of exogenous varieties to their crops as a test; the idea being to test both their adaptive capacity to the traditional agricultural systems and consumer preferences. These varieties have come from places as far away as the Central Valley of California, brought back by temporary migratory workers (Zizumbo-Villarreal, 1985). Both the introduction of new varieties and the maintenance of hybrid forms and their segregants could be favored by the farmers, thereby complicating the conservation of the genetic resources of wild populations in situ.

Under the technological and socioeconomic conditions in which traditional agriculture is performed, should new genes be introduced, the deployment of new cultivars, whether transgenic or not, could signify the introduction of these new genes into local landraces and wild populations, given the intense interchange of seeds among peasant farmers and the practice of cultivating various types of seeds, even in spite of limited genetic flow. *Phaseolus vulgaris* has been already transformed (Russell et al. 1993; Aragão et al., 1996, 1998, 1999, 2002) and the first field trials of transgenic beans have been approved in Brazil. Thus, it is just a matter of time before such beans might be introduced to other countries. Availability of the data presented here suggests that although common bean is a selfing species, genes from domesticated beans (including transgenes) will be introduced in other populations, whether wild or domesticated. Whereas wild populations that are more distantly located from domesticated ones are less affected by gene flow, they tend to be less frequent. Thus, wild populations closer to cultivated fields, which are more abundant, will suffer the brunt of the impact of gene flow. On the other hand, gene flow estimated by *N_m* considers the combined effects of flow of genes (by means of pollen or seeds) and its selection in a large number of populations over a wide temporal scale. This study shows low influence of domesticated populations on wild ones but this result could be influenced by a low adaptive value of the domesticated alleles. Other genes that could be introduced (including transgenes) may have a higher adaptive value and eventually persist in the wild populations.

ACKNOWLEDGMENTS

We thank UC-MEXUS Program (University of California-CONACYT-México) for a posdoctoral scholarship at the Uni-

versity of California-Davis for the first author, and the Bean Network of the SINAREFI (SAGARPA-México) for financial support; to Julián Coello for his technical help in the laboratory; to Francisco Campos and Pedro Melo (Yuriria, Guanajuato), Nicolás Rico and Roberto Zizumbo (Jéruco, Michoacán), and Ignacio Fernández (Tupátaro, Michoacán) for their support in the field and for the information contributed; to Consuelo Zizumbo for her hospitality in Jéruco. Thanks to the three anonymous reviewers for their valuable suggestions.

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