ECOGEOGRAPHIC DISTRIBUTION OF *PHASEOLUS* SPP. (FABACEAE) IN BOLIVIA[1]

ROSANNA FREYRE, RAÚL RÍOS, LORENA GUZMÁN, DANIEL G. DEBOUCK, AND PAUL GEPTS

Freyre, Rosanna, Paul Gepts *(Department of Agronomy and Range Science, University of California, Davis CA 95616-8515),* **Raúl Ríos, Lorena Guzmán** *(Centro de Investigaciones Fitogenéticas de Pairumani, Cochabamba, Bolivia), and* **Daniel G. Debouck** *(CIAT, Apartado Aéreo 6713, Cali, Colombia).* ECOGEOGRAPHIC DISTRIBUTION OF *PHASEOLUS* SPP. (FABACEAE) IN BOLIVIA. *Economic Botany, 50(2):195–215, 1996. Wild* Phaseolus vulgaris *is distributed between northern Mexico and northern Argentina. Analysis of phaseolin and molecular markers (isozymes, Restriction Fragment Length Polymorphisms or RFLPs) indicate that this gene pool consists of two major groups, Mesoamerican and Andean, and a third intermediate group found in northwestern South America. Previous to this study, only four accessions of wild* P. vulgaris *beans from Bolivia had been collected and their genetic relationship with other wild beans from Latin America was not known. Due to the problem of intense erosion in some areas of Bolivia, it was our objective to survey and document* Phaseolus *spp. in this area before their extinction. We conducted a collection expedition in May 1994 in the departments of Cochabamba, Chuquisaca and Tarija. This resulted in collections of four populations of* P. augusti, *two of cultivated* P. lunatus *and two mixtures of cultivated* P. vulgaris. *The first mixture was made of "k'opurus" or beans consumed after toasting, and represented an addition of 17 accessions to the Bolivian collection. The second mixture was made of "porotos" and resulted in the addition of 10 new accessions. Seven germplasm collections of wild* P. vulgaris *were found, which allowed us to increase the number of known populations of wild common bean for Bolivia. Another accession was found as a wild-weed-crop complex. Seven of these wild* P. vulgaris *accessions along with another accession from Bolivia collected previously, and a number of* P. vulgaris *accessions from Mexico (17), Guatemala (3), Colombia (10), Ecuador (6), Peru (17) and Argentina (16) were analyzed with RAPDs. The use of 14 random primers and one SCAR (Sequence Characterized Amplified Region) resulted in 90 bands, of which 83 were polymorphic. This data was used to construct a dendrogram which shows clear separation into three clusters, corresponding to each of the gene pools and an intermediate group. The Bolivian wild* P. vulgaris *beans grouped with the accessions of southern Peru and Argentina into the Andean gene pool. RAPD analysis of genetic diversity correlated well with genetic diversity obtained with other markers. Moreover, the ease of analysis allowed us to obtain a large number of bands which was conducive to greater sensitivity and identification of geographic subgroups and accessions of hybrid origin.*

Distribución Ecogeográfica de *Phaseolus* Sp. (Fabaceae) en Bolivia. Phaseolus vulgaris *silvestre se encuentra distribuído entre el norte de México y el norte de Argentina. Mediante análisis de faseolina y marcadores moleculares (isoenzimas, RFLPs o polimorfismo de largo de fragmentos de restricción). ha sido determinado que este acervo genético consiste de dos grupos principales, Mesoamericano y Andino, y un tercer grupo intermedio hallado en el noroeste de Sudamérica. Previo a este estudio, sólo habian sido colectadas cuatro accesiones de* P. vulgaris *silvestre en Bolivia, y se desconocía su relación genética con otros frejoles silvestres de Latinoamérica. Debido al problema de erosión intensa en ciertas zonas de Bolivia, nuestro objetivo fue estudiar y documentar* Phaseolus *spp. en esta área antes de su extinción. Realizamos una expedición de colección en Mayo de 1994 en los departamentos de Cochabamba, Chuquisaca y Tarija. Como resultado de ésta, coleccionamos cuatro poblaciones de* P. augusti *dos poblaciones de* P. lunatus *cultivado y dos mezclas de* P. vulgaris *cultivado. La primera mezcla consistió de "k'opurus," o frejoles que son consumidos después de tostar, y representaron la adición de 17 accesiones para la colección boliviana. La segunda fue una mezcla de "porotos" que resultaron en la adición de 10 nuevas accesiones. Se encontraron siete colecciones de germoplasma de* P. vulgaris *silvestre, lo cual nos permitió triplicar el número de poblaciones*

[1] Received 7 August 1995; accepted 31 January 1996.

de frejol común silvestre conocidas en Bolivia. Otra entrada fue encontrada como parte de un complejo de silvestre-maleza-cultivo. Siete de estas entradas de P. vulgaris *silvestre así como otra entrada de Bolivia colectada previamente, y varias entradas de* P. vulgaris *silvestre de México (17), Guatemala (3), Colombia (10), Ecuador (6), Perú (17) y Argentina (16) fueron analizadas con el uso de RAPDs (polimorfismo de DNA por amplificación al azar). El uso de 14 cebadores y un SCAR (región amplificada de secuencia caracterizada) dieron como resultado 90 bandas, de las cuales 83 fueron polimórficas. Estos datos fueron utilizados para la construcción de un dendrograma, que muestra clara separación en tres grupos, que corresponden a cada uno de los acervos genéticos y un grupo intermedio. Los frejoles silvestres de Bolivia agruparon con las entradas del sur del Perú y Argentina, dentro del grupo Andino. El análisis de diversidad genética con RAPDs tuvo buena correlación con la diversidad genética obtenida con otros marcadores. Además, la facilidad del análisis permitió obtener un gran número de bandas resultando en mayor sensitividad e identificación de subgrupos geográficos y entradas de origen híbrido.*

Key Words: common bean; *Phaseolus vulgaris*; germplasm exploration; RAPDs.

The cultivated gene pool of common bean (*Phaseolus vulgaris* L.) consists of two major geographic entities, the Mesoamerican and Andean gene pools. Evidence supporting the existence of these two gene pools has been reviewed by Gepts (1993) and includes reproductive isolation between the two groups, differences in morphological and agronomic traits, biochemical markers (phaseolin seed protein, isozymes), and molecular markers (nuclear and mitochondrial DNA). Additional evidence suggests that the divergence between the two groups occurred prior to domestication, which took place in the Americas from a wild ancestral form distributed between northern Mexico and northern Argentina and found as a vine in (semi) dry montane tropical forest (Brücher 1988; Delgado Salinas, Bonet, and Gepts 1988). Analysis of phaseolin (Gepts and Bliss 1986; Gepts et al. 1986; Koenig, Singh, and Gepts 1990), isozymes (Koenig and Gepts 1989), F_1 hybrid weakness (Koinange and Gepts 1992) and RFLP markers (Becerra Velásquez and Gepts 1994) in wild common beans show divergence into two major groups. A third distinct group of wild beans, found in northwestern South America (Ecuador and northern Peru), appears to be intermediate, both at the geographical and molecular levels, between the Mesoamerican and Andean gene pools. This group shows a combination of isozymes from both groups, and a distinct phaseolin type (I) not found in other wild or cultivated bean materials (Debouck et al. 1993; Koenig and Gepts 1989; Koenig, Singh, and Gepts 1990). Sequence analyses of phaseolin, the most abundant storage protein in the seed, suggest that this third group represents the presumed ancestor of *P. vulgaris* (Kami et al. 1995).

Molecular markers have been an important tool to study the pattern of domestication of common bean. However, studies so far have been limited by the nature of the markers utilized. Isozymes are limited in number and polymorphism. Restriction fragment length polymorphism (RFLP) markers have the advantages of potentially unlimited numbers and therefore better genome coverage, and high polymorphism. However, this technology is time-consuming and labor-intensive. Moreover, the study of wild beans is somewhat hindered by the difficulty of obtaining large quantities of good quality DNA (digestible with restriction enzymes) because of their high content of polyphenolics. Random amplified polymorphic DNA (RAPD) markers are based on the amplification of random DNA sequences using arbitrary primers by the polymerase chain reaction (PCR) (Welsh and McClelland 1990; Williams et al. 1990). The advantages of this technique are its simplicity, speed, and requirement for only small amounts of relatively crude genomic DNA (Rafalski, Tingey, and Williams 1991; Waugh and Powell 1992). RAPD markers have been used to study diversity in plant species at different taxonomic levels. Studies include comparisons of genomes within genomes of *Brassica* and *Musa* (Quiros et al. 1991; Howell et al. 1994); of genotypes within amaranth, aspen, *Avena sterilis,* coffee, cruciferous plants, peanut, and wheat (dos Santos et al. 1994; Halward et al. 1991; Hallden et al. 1994; Heun, Murphy, and Phillips 1994; Jain et al. 1994; Liu and Furnier 1993; Orozco-Castillo et al. 1994; Thormann et al. 1994; Transue et al. 1994; Vierling and Nguyen 1992); and of cultivars of celery, rice and rapeseed (Mailer,

Scarth, and Fristensky 1994; Yang and Quiros 1993; Yu and Nguyen 1994). In cultivated common bean, RAPD markers have shown variability between and within the two principal gene pools (Haley et al. 1994; Nienhuis, Tivang, and Skroch 1994).

The presence of wild *P. vulgaris* in Bolivia was first reported by Berglund-Brücher (1967) and Berglund-Brücher and Brücher (1976). These findings were extended by the exploration of Debouck and Rios in 1988 who described four additional populations (Debouck 1988a; Toro, Tohme, and Debouck 1990). Because of the potential value of wild forms for use in tracing evolutionary pathways and contributing genetic diversity to the cultivated gene pool (Gepts 1993), our objective was to extend these collections to additional locations in Bolivia during a germplasm collection expedition undertaken in May 1994. Moreover, we sought to determine, using RAPD markers, the genetic relationships of the recently collected wild *P. vulgaris* beans from Bolivia with their counterparts from elsewhere in the distribution range. Additional objectives were to correlate the RAPD information with geographic origin and previously known biochemical or molecular data, specifically, to document the utility of RAPD markers to distinguish variability between gene pools in wild bean and establish if possible further geographical differentiation below the major gene pool level.

Materials and Methods

Germplasm Exploration

Results of the exploration carried out in 1988 (Debouck 1988a) and the four herbarium voucher specimens deposited in the Herbario Nacional de Bolivia (LPB), Instituto de Botánica Darwinion (SI) and Universidad Nacional Mayor de San Marcos de Lima (USM) served as starting points for this exploration. These records were reported on topographical maps at 1:250,000 purchased from the Instituto Geográfico Militar of Bolivia. Road maps and a physical geography map at 1:1,000,000 published by the Instituto Geográfico Militar in 1993 helped to establish the itinerary. Information gathered included data for 47 descriptors, and the field equipment was as detailed elsewhere (Debouck 1988b).

Molecular Studies

Plant Materials

Six of the seven accessions of wild *P. vulgaris* collected by the authors in 1994 in Bolivia were used in the molecular study. These accessions had been collected in the departments of Cochabamba, Chuquisaca and Tarija (southern Bolivia) (Fig. 1). One of these accessions had seeds of two different colors which were analyzed separately. Additionally, one accession collected in a previous expedition to Bolivia (Toro, Tohme, and Debouck 1990) was also included. Accessions from other countries were obtained from the *Phaseolus* World Collection at the Centro Internacional de Agricultura Tropical (Cali, Colombia) or were collected by the authors in previous expeditions. The identification and collection site of these accessions is shown in Table 1. For identification in this study, accessions were ordered by country and latitude going from north to south, and then labelled with the country name followed by a number in ascending order. The total number of accessions utilized was seventy-eight. Seventeen accessions were from Mexico (six states), three from Guatemala (one department), ten from Colombia (one department), six from Ecuador (three provinces), seventeen from Peru (six departments), nine from Bolivia (two departments) and sixteen from Argentina (three provinces). Based on the high levels of uniformity observed within accessions in previous analyses, only one seed per accession was used for the analyses. Two accessions had seeds of different color, which were analyzed separately: CLB2 and CLB3 corresponded to brown and black seed of accession Leroi Col-47, and BOL8 and BOL9 corresponded to cream and brown seed of accession DGD3024.

Seed Protein Gel Electrophoresis

Phaseolin seed protein type for all accessions except the ones from Bolivia, have previously been described (Debouck et al. 1993; Koenig, Singh, and Gepts 1990; Toro, Tohme, and Debouck 1990). This analysis was confirmed on all accessions by one-dimensional SDS/PAGE using a flour sample from one seed from each accession following the method described by Koenig, Singh, and Gepts (1990). The flour sample was obtained from the raphe end of the seed without destroying the embryo.

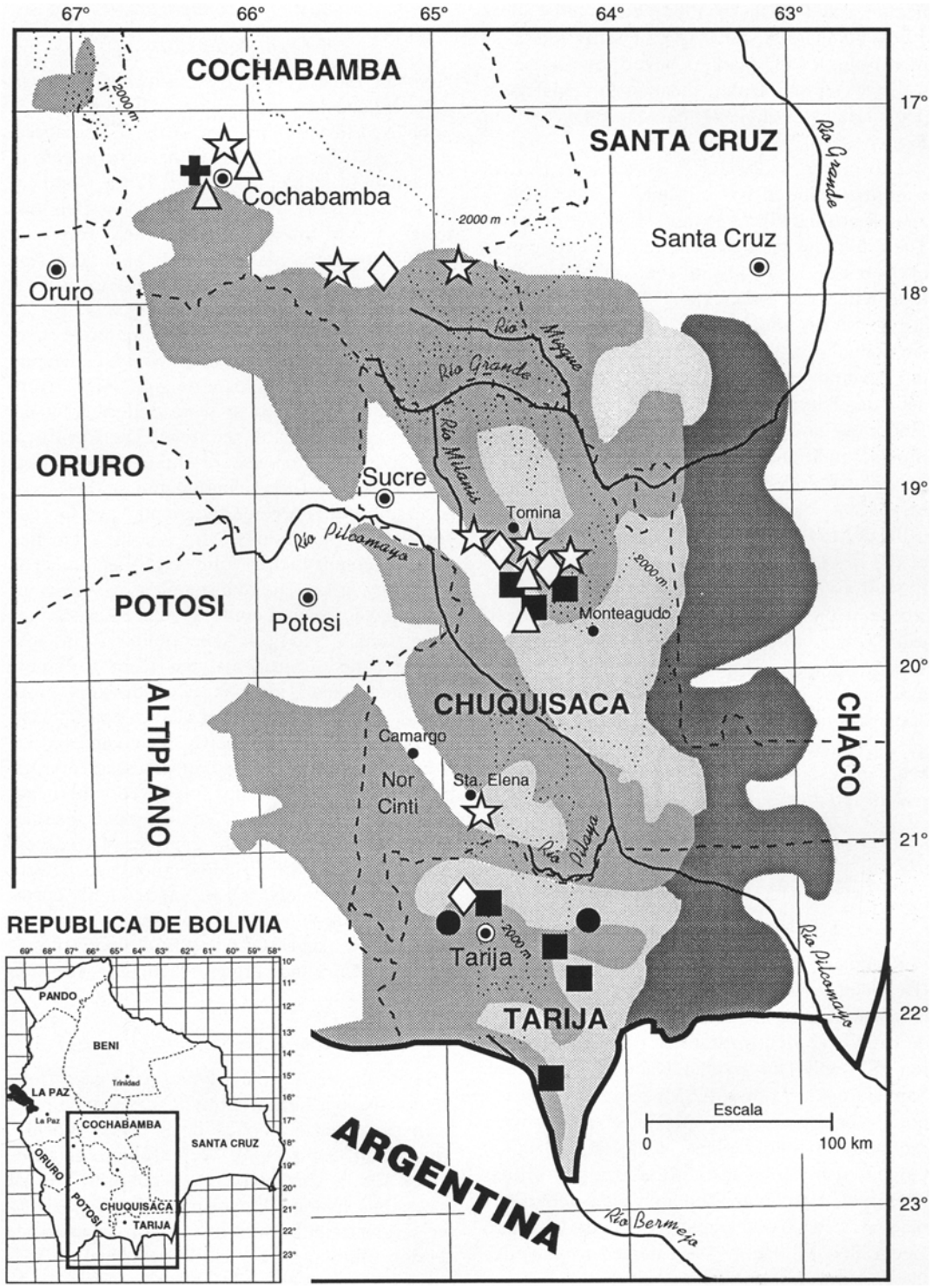

Fig. 1. Geographic distribution of *Phaseolus* spp. accessions collected in Bolivia. Open circles with dots refer to department capitals. Shaded areas correspond to vegetation types (adapted from Beck, Killeen, and

DNA Extraction

The seed used for seed protein analysis for each accession was germinated. A newly expanded young leaf was used for DNA extraction following a "mini-prep" extraction method as described by Afanador, Haley, and Kelly (1993). The concentration of DNA was quantified using a fluorometer (TK100, Hoefer Scientific, San Francisco) and then diluted to a concentration of 4 ng/μl.

Random Amplified Polymorphic DNA (RAPD) Markers

For this part of the study, accessions were studied in the order specified by their UC Davis identification, which is a consecutive introduction number and is independent of geographic and latitudinal origin. Hence, accessions of widely different origins were analyzed in the same batch thus avoiding potential confounding effects arising from uncontrollable variation in experimental conditions. A set of 14 random primers (Operon Technologies, Alameda, CA), which had produced RAPD bands recently mapped on the *P. vulgaris* genetic map (Nodari et al. 1993; R. Freyre and P. Gepts, unpubl. results) were selected. These primers were: AD19, D12, D20, E4, E9, E10, F1, F7, F10, G5, G19, I19, J17 and S13. PCR reactions used were: 1X buffer (50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100, pH 9.0), 2 mM $MgCl_2$, 100 μM dNTPs, 0.4 μM primer, 20 ng DNA and 1 U *Taq* polymerase in a 25 μl volume. The thermocycler (96-well Twinblock, Ericomp) was programmed for 1 cycle of 2 min at 94°C, 3 cycles of 1 min at 94°C, 1 min at 35°C and 2 min at 72°C, 32 cycles of 10 sec at 94°C, 30 sec at 35°C, and 1 min at 72°C, followed by 5 min extension at 72°C. Additionally, a SCAR constructed for a band produced by primer J1 (J1d) and located on the *P. vulgaris* genetic map (Adam-Blondon et al. 1994), was also used. Sequences for this SCAR are 5'CCCGGCATAATTATTCTTCTTTTCT3' and 5'CCCGGCATAAAGAACCAAAGATGA3'. In this case, cycling conditions used were 1 cycle of 2 min at 94°C, 40 cycles of 1 min at 94°C, 1 min at 60°C and 2 min at 72°C, followed by 5 min at 72°C.

Amplification products were separated on 1.5% agarose gels in 1X TAE buffer (0.04 M Tris-acetate, 1 mM EDTA). Lambda DNA digested with *Hind*III and *Eco*RI was used as size marker. Gels were stained with ethidium bromide and photographed under UV light. All clearly distinct bands per primer were labelled using the primer number and a letter (a, b, c . . . n) according to decreasing molecular weights. Each accession was scored for the presence or absence of a band coded as 1 and 0, respectively.

Cluster Analysis and Estimation of Genetic Diversity

RAPD data was analyzed with the program NTSYS-pc version 1.8 (Exeter Software, Setauket, NY). The original data was converted into a similarity matrix using Jaccard's similarity coefficient (Jaccard 1908). Cluster analysis was then performed based on the unweighted pair-group method using arithmetic averages (UPGMA, Sneath and Sokal 1973) and the resulting clustering was expressed as a dendrogram. Genetic diversity (RAPD marker variance) was estimated following Nienhuis and co-workers (1994). Within each major cluster, the sum over all 90 RAPD bands was scored for the variance of a binomial, $(n*p*q)/(n - 1)$, where n = the number of genotypes in each group, and p and q refer to the frequency of the presence (1) or absence (0) of a band, respectively.

Results

Description of the Study Area in Bolivia

The area of study could be defined as a rectangle covering the interandean valleys and

←

García E. 1993): lightly shaded area: 'Bosque Tucumano-Boliviano'; medium shaded area: 'Valles Secos Interandinos'; and heavily shaded area: 'Bosque Serrano Chaqueño.' Symbols refer to *Phaseolus* collections as follows: 1) wild *P. vulgaris*: closed circles: herbarium voucher specimens prior to 1988; open rhombuses: germplasm collections of 1988; closed squares: germplasm collections of 1994; 2) wild *P. augusti*: solid cross: herbarium voucher specimen prior to 1988; open stars: germplasm collections of 1988; open triangles: germplasm collections of 1994. One voucher specimen (*S. G. Beck 8659*, at LPB) of *P. augusti* from Sorata, Dept. of La Paz, is not represented.

TABLE 1. IDENTIFICATION, LOCATION OF ORIGIN, AND PHASEOLIN TYPE OF WILD *PHASEOLUS VULGARIS* ACCESSIONS.

Collector number	Other number	Country and number[1]	Province	County	Latitude	Longitude	Alt.[2]	Phas[3]
GN84154	L625	MEX1	Chihuahua	Yepachic	28.20N	108.30W	1530	S
Gentry 22043		MEX2	Durango	Durango	24.10N	104.41W	1829	Sd
Leroi Mex-2	X663	MEX3	Durango	El Saltito	23.40N	105.00W		S
M7278-D		MEX4	Jalisco	Arandas	20.42N	102.21W	1829	S
M7408-P		MEX5	Jalisco	Arandas	20.40N	102.23W	1829	M
DGD489		MEX6	Jalisco	Mascota	20.34N	104.46W	1390	M
M7016		MEX7	Jalisco	Mascota	20.32N	104.49W	1524	S,M
Gentry 22202		MEX8	Jalisco	Cd. Guzmán	19.41N	103.29W	1524	S
Gentry 22199	L13	MEX9	Jalisco	Tecalitlán	19.20N	103.15W	1219	M
M7240-B-1		MEX10	Guanajuato	Pénjamo	20.37N	101.43W	1829	M
DGD439		MEX11	Michoacán	Morelia	19.41N	101.16W	2040	M
Gentry 22492		MEX12	Morelos	Huitzilac	19.00N	99.15W	1981	M
Gentry 22404		MEX13	Morelos	Tepoztlán	18.58N	99.06W	1828	M
Gentry 22530		MEX14	Morelos	Tepoztlán	18.57N	99.13W	1920	M
Morelos 635		MEX15	Morelos	Jiutepec	18.53N	99.09W	1430	M
Morelos 654		MEX16	Morelos	Jiutepec	18.53N	99.09W	1430	M
DGD1619		GTA1	Sacatepéquez	San Miguel Dueñas	14.33N	90.50W	1820	S
DGD1610		GTA2	Sacatepéquez	Santa María de Jesús	14.27N	90.42W	1550	S
DGD1611		GTA3	Sacatepéquez	Alotenango	14.27N	90.49W	1280	M
	X636	CLB1	Cundinamarca	Macheta	5.05N	73.32W	1900	B
Leroi Col-47	X654	CLB2	Cundinamarca	Manta	5.01N	73.33W	1900	CH
Leroi Col-47	X654	CLB3	Cundinamarca	Manta	5.01N	73.33W	1900	CH
Leroi Col-24	NI928	CLB4	Cundinamarca	Manta	5.01N	73.33W	1600	CH
Leroi Col-28	NI937	CLB5	Cundinamarca	Tena	4.40N	74.24W	1560	CH
Leroi Col-22	X643	CLB6	Cundinamarca	Choachi	4.32N	73.55W	1800	CH
Leroi Col-23	NI926	CLB7	Cundinamarca	Choachi	4.32N	73.55W	1800	S **CH**
Leroi Col-13	X634	CLB8	Cundinamarca	Ubaque	4.29N	73.56W	1750	CH **S**
Leroi Col-14	NI922	CLB9	Cundinamarca	Ubaque	4.29N	73.56W	1900	CH
DGD2889		ECD1	Chimborazo	Pallatanga	1.58S	78.57W	1610	I
DGD2769		ECD2	Chimborazo	Huigra	2.16S	78.58W	1710	I
DGD2762		ECD3	Azuay	Girón	3.11S	79.10W	1990	I
DGD2763		ECD4	Azuay	Girón	3.12S	79.11W	1930	I, T **I**
DGD2765		ECD5	Azuay	Girón	3.12S	79.11W	1600	I
DGD2881		ECD6	Loja	Macará	4.19S	79.56W	960	I
DGD2788		PER1	Piura	Huancabamba	5.24S	79.37W	920	I
DGD2854		PER2	Piura	Huancabamba	5.56S	79.33W	1100	I
DGD2855		PER3	Cajamarca	Chota	6.21S	79.24W	930	I
DGD2858		PER4	Cajamarca	Chota	6.21S	79.24W	1250	I
DGD1962		PER5	Cajamarca	San Miguel	7.07S	78.47W	1790	I
DGD1956		PER6	Cajamarca	San Pablo	7.11S	78.50W	2020	I
PV-1		PER7	Huánuco	San Rafael	10.20S	76.11W		T,S **T**
DGD2147		PER8	Junín	Tarma	11.12S	75.29W	1770	T
DGD2152		PER9	Junín	Huancayo	12.01S	74.53W	2650	C
DGD2581		PER10	Cuzco	Anta	13.30S	72.29W	2080	C,H **H**
DGD2594		PER11	Cuzco	Paruro	13.49S	71.51W	2780	H

TABLE 1. CONTINUED.

Collector number	Other number	Country and number[1]	Province	County	Latitude	Longitude	Alt.[2]	Phas[3]
DGD2600		PER12	Cuzco	Anta	13.30S	72.39W	2370	T
DGD2586		PER13	Cuzco	Anta	13.32S	72.31W	2300	T
DGD2295		PER14	Apurímac	Andahuaylas	13.37S	73.12W	2440	C
Apurimac 76		PER15	Apurímac	Abancay	13.40S	72.53W	2300	T
								T,S
DGD2157		PER16	Apurímac	Abancay	13.51S	72.58W	2050	H,C
								C
DGD2156		PER17	Apurímac	Aymaraes	14.00S	73.10W	2560	C
DGD3007		BOL1	Chuquisaca	Tomina	19.17S	64.21W	2080	**T**
DGD3012		BOL2	Chuquisaca	Tomina	19.32S	64.27W	1960	**T***
DGD3025		BOL3	Tarija	Mendez	21.18S	64.51W	2180	**T**
DGD3021		BOL4	Tarija	O'Connor	21.25S	64.16W	1670	**T**
DGD3020		BOL5	Tarija	O'Connor	21.30S	64.09W	1270	**C**
DGD3023		BOL6	Tarija	Cercado	21.32S	64.45W	1890	**C**
DGD2501		BOL7	Tarija	Méndez	21.32S	64.52W	2100	**T**
DGD3024A		BOL8	Tarija	Arce	22.14S	64.36W	1130	**?**
DGD3024B		BOL9	Tarija	Arce	22.14S	64.36W	1130	**?**
DGD1716		ARG1	Salta	Santa Victoria	22.15S	65.00W	2600	T
DGD1713		ARG2	Jujuy	Tumbaya	23.55S	65.21W	1880	T
DGD1711		ARG3	Jujuy	Tumbaya	24.03S	65.27W	1850	J
DGD1712		ARG4	Jujuy	Capital	24.04S	65.22W	1670	T
DGD621		ARG5	Jujuy	Capital	24.07S	65.25W	1480	T,C
								C
DGD623		ARG6	Jujuy	Capital	24.10S	65.36W	1850	T
DGD624		ARG7	Jujuy	Capital	24.15S	65.17W	1200	T
DGD630		ARG8	Salta	Caldera	24.38S	65.29W	1640	H
DGD628		ARG9	Salta	Chicoana	25.07S	65.37W	1340	T
DGD639		ARG10	Tucumán	Trancas	26.13S	65.35W	1520	C,T
								C
DGD637		ARG11	Tucumán	Trancas	26.26S	65.31W	1350	J
DGD649		ARG12	Tucumán	Tafi	26.56S	65.42W	1900	T
DGD643		ARG13	Tucumán	Chicligasta	27.19S	65.55W	1400	J
DGD644		ARG14	Tucumán	Chicligasta	27.20S	65.57W	1650	C
L326	NI029	ARG15						T
	NI190	ARG16						T

[1] MEX: Mexico; GTM: Guatemala; COL: Colombia; ECD: Ecuador; PER: Peru; BOL: Bolivia; ARG: Argentina.

[2] Alt. corresponds to altitude in meters above sea level.

[3] Phas. corresponds to phaseolin seed protein type. Phaseolin type for accessions determined for the first time in this study, or for those different from specified in Toro et al. (1990) are denoted in bold type.

foothills of south-central Bolivia (Fig. 1), 17°19′S (latitude of Cochabamba) to 22°14′S (latitude of the site in the valley of Río Bermejo), 66°19′W (longitude of the site close to Quillacollo, in the Cochabamba valley) to 63°48′W (longitude of the site east of Monteagudo, entering the Bolivian Chaco). Westward lies the Altiplano, a flat highland topping at 3800 masl with salty lands (e.g., Salar de Uyuni) and lakes (e.g., Lake Poopo). Eastward lies the Chaco at 400 masl with dense dry thickets. The study area includes the drainages of the Río Grande, Río Pilcomayo, and Río Bermejo. It is a topographically accidented country with mountainous ranges oriented N–S from the border with Argentina to Sucre, and NW–SE from Sucre to Cochabamba, and thus with very few roads crossing the mountain ranges in W–E direction. Vegetation types are of three sorts: "Valles Secos Interandinos" or dry forests and shrubs in the range of 500–3000 masl, "Bosque Tucumano-Boliviano" or subhumid montane forests from 800–3000 masl (extending from Valle Grande, Santa Cruz, down to Tucumán,

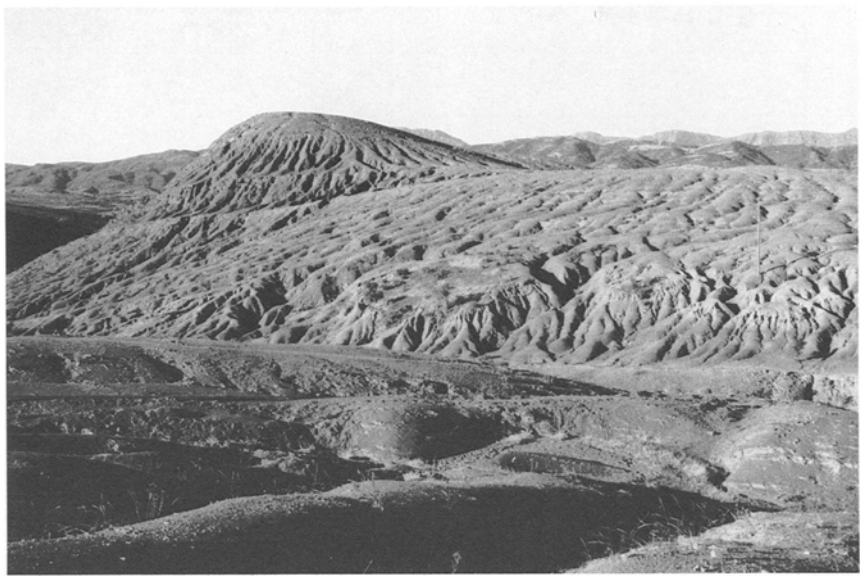

Fig. 2. Magnitude of erosion encountered in Bolivia near the town of Padilla, Dept. of Chuquisaca (2060 m).

Argentina), and "Bosque Serrano Chaqueño" or subtropical dry forest typically colonizing the Andean foothills from 500–1500 masl from the Argentinean border up to Santa Cruz (Beck, Killeen, and García E. 1993). These three zones would correspond to climates BSwh, Cwa and BSwh' according to Montes de Oca (1989), following the Köppen classification, or approximately steppe with dry warm winter, mesothermic with dry cool winter, and steppe with dry very warm winter, respectively. Rainfall varies from 250 to 1100 mm/year with dry months from May to September (driest months: June, July) (Johnson 1976; Montes de Oca 1989). In the study area, climate could be classified as temperate, with isotherms between 12–18°C and a few days of frost in the period June–July (increasing with altitude and as one goes westward to the Altiplano). The geology is the most complex in this region with many folds of Ordovician, Devonian, Carboniferous and Triassic age in the interandean valleys, and Tertiary and Quaternary sediments towards the Chaco (Montes de Oca 1989). Erosion is intense, and accelerated by overgrazing and deforestation, which makes it important to survey and document all attributes of this rich flora before its extinction (Fig. 2).

Geographical and Ecological Distribution of *Phaseolus* spp.

Wild P. augusti *Harms*

Berglund-Brücher (1967) and Berglund-Brücher and Brücher (1976) mentioned the existence of populations of *P. vulgaris* in three sites—Quillacollo, Liriuni and Taquiña—at the edges of the valley in which the city of Cochabamba is located. The expansion of the city, which already occupies most of the valley (and is now expanding east towards Sacaba), made a visit to these places even more urgent. All three places are located in the foothills of the Cordillera de Cochabamba (just north of the city) and the Cordillera del Tunari (west of Cochabamba). We also sought to locate the actual site of the type of *P. bolivianus* Piper ["... collected at Cochabamba, Bolivia, March 14, 1920, by E.W.D. and Mary M. Holway (no. 411)" according to Piper (1926)] for further taxonomical studies.

These visits lead to the discovery of two populations of wild *P. augusti* in the valley of Cochabamba (marked as open triangles on Fig. 1): DGD3000 (north of Pairumani experimental station, north-west of Quillacollo) and DGD3001 (next to the Taquiña brewery). In a germplasm exploration in 1988, we had already identified

another population of wild *P. augusti* (DGD2506) on the road to Liriuni (marked as a star on Fig. 1). A search for additional populations on the road to Sacaba, Villa Copacabana, Santibañez, Parotani, Sipe Sipe and Chacapaya, was unsuccessful. The three populations can be considered as endangered and potentially extinct within the next 20–25 years if no protective measures are taken, because of overgrazing and expanding urbanization. Population DGD3001 is benefiting indirectly from restricted access imposed by the brewery to protect its water sources. Unfortunately, geographical data about *P. bolivianus* (presumably either DGD3000 or DGD3001) are not accurate enough to permit monitoring of its geographic distribution over a longer period of time.

In addition, two other *P. augusti* populations (DGD3006, DGD3010) were discovered in Chuquisaca, resulting in eleven populations sampled throughout the country, when adding the seven populations sampled in 1988 (Debouck 1988a) (Fig. 1). Their distribution corresponds to the montane moist and subhumid forests in the subtropical and temperate zones [included into the "Valles Secos Interandinos" according to Beck, Killeen, and García E. (1993)], between 1900 and 2900 masl [Debouck (1988), and present results]. For all of them, enough seed germplasm was collected, since it can be considered as threatened because of overgrazing. While DGD3006 might be seen as an extension of population DGD2496 but further SE in the Sillani canyon, population DGD3010 was found in the upper valley of Río Milanis, which had not been explored so far. Both were found in heavily disturbed forests of *Podocarpus* with *Schinus* and *Caesalpinia.* Population DGD2496 was re-visited, and as a possible result of severe drought this year, only two vegetative stems were found. Plants were damaged by lace-bug (*Gargaphia* sp., Hemiptera) in DGD3000 and 3006. Collection DGD3000 was known as "algo chui" or dog bean, and was used as a toy by children.

Cultivated P. lunatus *L.*

Two collections of this taxon were made (DGD3017, 3046). Both belong to the large-seeded type of lima bean or Big Lima (Baudet 1977). Seeds of the material DGD3017 had a white background and a large dark yellow tan spot from the micropyle side of the seed; they were found in a dooryard garden in eastern Tomina, Dept. of Chuquisaca, at an unexpectedly low altitude for such a cultigroup (1200 m). Its name "poroto" may well indicate an origin outside this area; this might be reinforced by the fact that farmers there were recently arrived settlers coming from the highlands. It was new to the Bolivian collection, however (seed pattern different from DGD2499 of 1988), and was therefore collected. Seeds of the other material, DGD3046, had a white background and large black spots irradiating from the hilum. This color pattern was quite similar to that of DGD2500 of 1988, but the color was black instead of wine red. It was found in Santa Elena, east of Camargo, Chuquisaca, at 2160 m and called "chorca" or "pallar" as other types discovered in the Valle Cinteño in 1988 (DGD2488, 2494, 2499, 2505) (Debouck 1988a). Seeds were used in the immature, green stage in soups, but much less as dry bean because of reportedly heavy damage by weevils. They were also used as toys by children. As mentioned by Debouck (1988a), we believe that this crop had been introduced in Bolivia in pre-Columbian times from Ecuador and northern Peru where it was domesticated (Gutiérrez Salgado, Gepts, and Debouck 1995). As for many traditional landraces of Andean crops, this kind of germplasm is suffering from neglect and will sooner or later disappear. Ex situ conservation appears to be a safe back-up measure for the time being.

Cultivated P. vulgaris *L.*

Two mixtures were collected: one in Sillani, Tomina, Dept. of Chuquisaca, at 2140 m and another one in Santa Elena, Nor Cinti, Dept. of Chuquisaca, at 2160 m. The first mixture was made of "k'opurus" or beans consumed after toasting (Tohme et al. 1995). It included the 27 following morphotypes: CF-36, CF-41, CF-54, CF-97, CF-110 (of the collection at Pairumani), DGD2517, 2520, 2523, 2535, 2567 (of the 1988 exploration), DGD3029 to 3045 (new and not present in the Pairumani collection nor collected in 1988). This represented an addition of 17 accessions to the Bolivian collection of *k'opurus.* These morphotypes were obtained from a farmer who used to plant them together with "j'anka sara," a tall maize variety (2.5 m high) also for local consumption as toasted grain (note: *sara* is the Quechuan name for maize, and *j'anka* would refer to the toasting). It might correspond to the "Checchi" race of maize, used almost exclu-

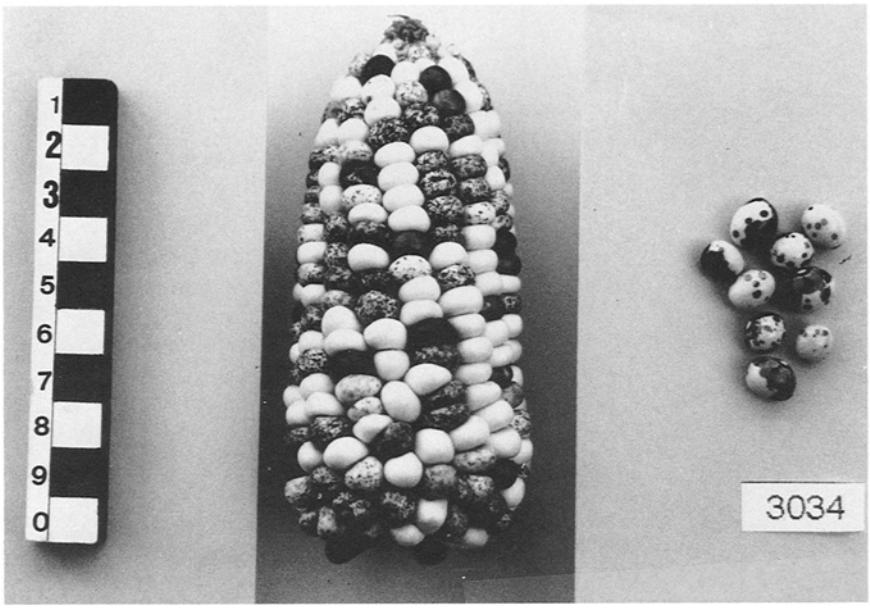

Fig. 3. Comparison of seed types in pop (Checchi) corn and popping beans (K'opuru); scale in cm.

sively for parched corn, although plant height does not correspond to the original description (Ramírez E. et al. 1960). This farmer also had Maíz Blanco, all planted in November and intermixed with *Cucurbita ficifolia* Bouché ("lacayote"), *Cucurbita maxima* Duch. ex Lam. ("angula"), *Lagenaria siceraria* (Mol.) Standl. ("calabaza"), and *Amaranthus caudatus* L. ("coimi" or "millmi," also consumed after toasting). This collection might be the extreme south of the range of toasted beans also called *nuñas* or *reventones* in other parts. According to G. Avila (pers. comm., 1994), Quechuan Indians look for similarities in speckled patterns between both crops, using the Checchi with its dotted aleurone as standard for comparison and as criterion for good toasting potential (Fig. 3). According to Ramírez E. and co-workers (1960), *checchi* name refers to a grey colour on a white background.

The second mixture was made of "porotos," dry beans planted in association with maize. It included 18 morphotypes: CF-16, CF-33, CF-49, CF-57, FI-83, FI-109, FI-121 (of the Pairumani collection), and DGD3047 to 3056 (apparently new and not present in the Pairumani collection nor collected in 1988, resulting in 10 new accessions for the Bolivian national collection). Collections DGD3050 and 3055, because of their almost spherical shape, could well be "chui," generally used as toys (Cárdenas 1989). Given social changes affecting rural populations (younger generations leaving the countryside), cultivation of this type is likely to be discontinued. If some beans might indeed have been domesticated for play (Debouck 1989), they might provide for an interesting study of genetic variability based on phenotypic traits and biochemical/molecular markers.

Wild P. vulgaris *L.*

This trip allowed us to increase the number of known populations of wild common bean for Bolivia by seven germplasm collections (DGD-3007, 3011, 3012, 3020, 3021, 3024, 3025) (marked as solid squares on Fig. 1). Collection DGD3023 will be discussed below under weedy forms. Although the material was already at mature, dry stage in some places, we were still able to make herbarium specimens for most of them (DGD3007, 3011, 3012, 3020, 3024 and 3025). Amounts of seeds were normal to abundant (more than 1000 original seeds).

The twelve populations originating in Bolivia (Fig. 1) provide a better picture of the distribution of wild *P. vulgaris* in that country. First, the range of distribution of wild *P. vulgaris* in Bolivia is restricted to interandean valleys south of

parallel 17°30′S, between 65°00′W and 64°00′W, and limited southward to the Río Bermejo valley. That rectangle encompasses the valleys of Río Mizque, Río Grande, Río Pilcomayo, Río Bermejo and tributaries (northernmost accession: DGD2484, 17°47′S; southernmost accession: DGD3024, 22°14′S; easternmost accession: DGD3020, 64°09′W; westernmost accession: DGD2484, 65°10′W). Valleys are generally oriented from north to south, centrally located along an east–west gradient, and organized in a succession of increasing altitude towards the Altiplano to the west. Along that succession, if humidity is too high, wild bean populations will be found on the western slope of one *cordillera* (as in DGD3020, just north of the village of Entre Rios); if humidity is too low, they will be found on the eastern slope facing humid winds blowing westward from the East (as in DGD2501, west of the city of Tarija).

Second, populations of wild *P. vulgaris* display morphological variation along a north to south gradient. The northernmost population, DGD2484 (found in 1988 south of the place called Totora, in the Department of Cochabamba; collections of 1988 are marked as open rhombuses on Fig. 1), resembles the Peruvian morphotypes of Cuzco and Apurimac with larger racemes with 2–4 primary bracts, while the southernmost population DGD3024 (discovered this year south of the site called La Mamora, in the Río Bermejo valley, Department of Tarija) is close to the Argentinean morphotypes of Jujuy, Salta and Tucumán with rhombohedric leaflets and short racemes with 1–2 primary bracts, as described by Brücher (1988).

Third, from the present distribution, wild common bean appears to be a floristic element of both the Tucumano-Bolivian forest and the humid variants of the dry forest of "Valles Secos Interandinos" described by Beck, Killeen, and Garcia E. (1993). Population DGD3024 was found in a humid temperate forest in the upper Bermejo valley, rich in Bambusoideae, Bignoniaceae (*Tecoma*), Convolvulaceae (*Ipomoea*), Juglandaceae (*Juglans boliviana*), Lauraceae (*Ocotea*), Melastomataceae, Meliaceae (*Cedrela*) and Myrtaceae. There were no indications of any disturbance suggesting that common bean is wild in Bolivia, and not weedy (i.e., escape from cultivation or a result from a wild × cultivated hybridization) as once claimed (Gentry 1969). It also enters into the *Podocarpus* forest, now disturbed in most places (populations DGD3011, 3021 of 1994, DGD2501 of 1988 re-visited this year). This forest is a moist temperate forest with a winter dry period of approximately four months (Unzueta, 1975). It is possible that in places where forests have been cleared to give place to secondary thickets with *Acacia, Physalis, Schinus, Solanum, Verbesina,* the range of wild common bean has been extended, provided that cattle have not been allowed to graze (as for population DGD3025; Fig. 4). In this case, grazing is delayed until corn is harvested, allowing a growing period of 6–8 months, a duration long enough for the wild bean to set seeds and escape destruction. Giving the presence of goats ranging freely in most southern Chuquisaca and northern Tarija, we considered that many wild bean populations are not presently secure in situ, and we therefore collected germplasm.

During this trip, we could not enter the upper Pilcomayo region (e.g., we could not reach Azurduy from Sopachuy), because of a lack of roads and bridges. This region would constitute the only noteworthy missing spot for a general survey of the distribution of *Phaseolus* spp. in Bolivia. Another place of potential interest might be the valley of Río Pilaya (in the surroundings of Pampa Grande, on the border of the Departments of Chuquisaca and Tarija), which is not accessible by road yet.

Symptoms similar to those of anthracnose (caused by *Colletotrichum lindemuthianum*) were observed in several populations (DGD3007, 3011, 3012, 3020, 3024, 3025), with the highest severity observed in DGD3024. Pod damage caused by birds was observed in populations DGD3011, 3012, 3024 and 3025, as already seen in other parts of the range (Debouck et al., 1993). Wild beans were known as "monte chui" (DGD3007), "porotito" (DGD3011, 3025), "campo purutu" (DGD3012), and "poroto del zorro" (DGD3021, 3024). They were locally eaten by rural inhabitants in southern Chuquisaca (DGD3011).

The Wild-Weed-Crop Complexes

Observations made in 1988 on naturally occurring hybridizations between wild and cultivated beans (Debouck 1988a), were confirmed and extended during this trip. In close proximity (within a 100 m radius) of a wild *P. vulgaris* accession (DGD2501, Fig. 5a), a weedy accession, DGD2502 (Rincón de la Victoria, north-

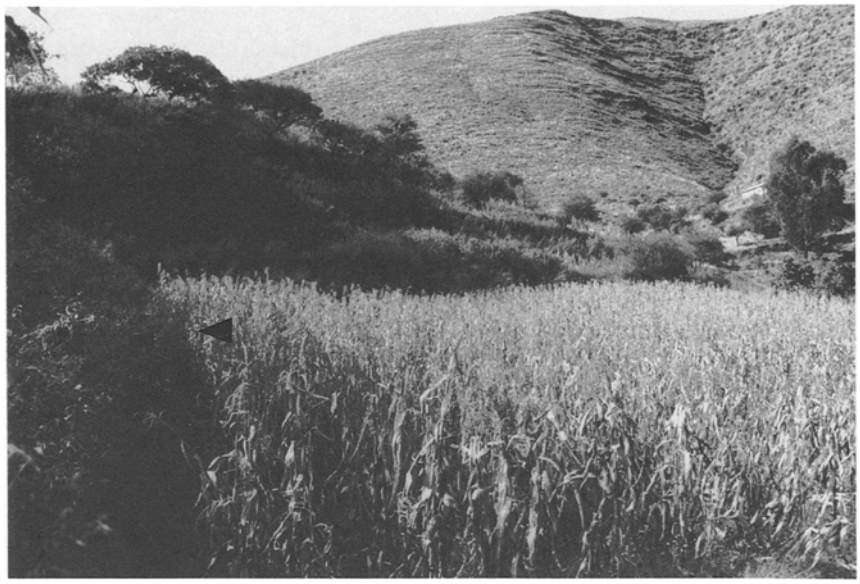

Fig. 4. Habitat of wild *P. vulgaris* accession DGD3025, close to Capilla de Trancas, Dept. of Tarija (2180 m). Secondary vegetation is left only around maize fields where wild common beans (arrow) still survive.

west of the city of Tarija), with a viny growth habit and seeds of cultivated size but of agouti color (Fig. 5b), was found again in the same thickets intermixed with remaining spots of *Podocarpus,* as an indication that weedy regressive forms can maintain themselves in some environments for a period of at least a few years. To the authors' knowledge, this is relatively rare in *P. vulgaris,* but appears to be possible in appropriate ecological conditions. An additional case of introgression was observed in the suburbs southwest of the city of Tarija, in a refuse heap at Tabladita on the road to San Jacinto. A wild bean (DGD3023) growing on *Opuntia* and *Schinus,* was observed mixed with a Bayo (light cream colored seed) cultivated type with at least four intermediate, black-stripped seed types with grey-cream background (Fig. 5c). Introgression was also observed in DGD3025, found on borders of maize fields near Trancas, approximately 23 km NNW of the city of Tarija, with bayo and brownish black-spotted seed types larger than the sympatric wild seed type.

Molecular Analyses of Wild *P. vulgaris*

The relationships of the Bolivian wild *P. vulgaris* accessions with other wild *P. vulgaris* accessions were investigated with biochemical (phaseolin seed protein) and molecular markers (RAPDs).

Phaseolin Seed Protein

The phaseolin type for each accession in the study is shown in Table 1. In previous studies of phaseolin type, four or more seeds per accession were utilized. If the accession consisted of heterogenous seed, it resulted in the identification of more than one phaseolin type. For those cases, the phaseolin type identified in this study is indicated. In only two cases was there discrepancy between these results and previous publications: CLB7 (Leroi Col-23) and CLB10 (Leroi Col-13) were found to have CH and S types, respectively, in this study but had previously been identified as S and CH types. PER15 (Apurimac 76) had been previously described as a T type, but when more than one seed was used in this study, it was found to be heterogeneous and consisting of both S and T types.

Most accessions from Bolivia were identified as T or C types. However, there were three instances where the phaseolin type could not be identified clearly. BOL2 (DGD3012) had a phaseolin pattern that resembled a T type but seemed to have an additional band smaller than the usual lowest molecular weight band of 45kD. BOL8 and BOL9 (which are different colored seeds

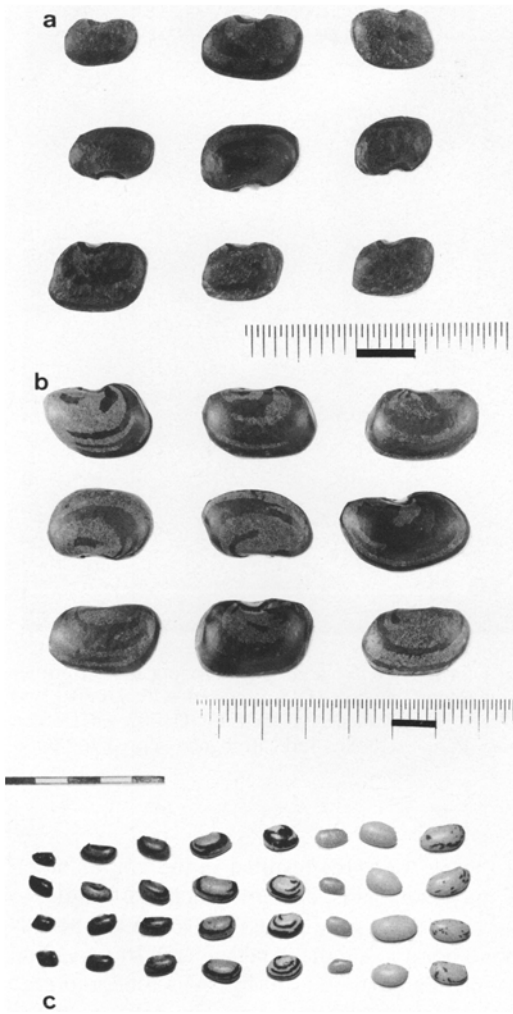

Fig. 5. Seeds from wild × cultivated *Phaseolus vulgaris* hybrids. a: DGD2501: wild (solid bar: 5 mm); b: DGD2502: feral (solid bar: 5 mm); c: DGD3023 (scale in cm).

from accession DGD3024) both showed the 45 kD and 48–49 kD bands, thus resembling accessions with I type phaseolin, but seemed to have an additional band of approximately 50–51 kD.

RAPD Analysis and Construction of the Dendrogram

A total of 90 distinct bands were scored, of which 83 were polymorphic (92%). An example of bands scored for Operon primer E10 is shown in Fig. 6. The number of polymorphic bands scored per primer ranged from one to fifteen, with an average of 5.4. The size of the bands scored ranged from 180 to 2100 bp, with an average size of 980 bp.

A UPGMA cluster analysis based on Jaccard's similarity coefficient clearly separated the wild *P. vulgaris* accessions into two clusters, which correspond to the Mesoamerican (Mexico, Guatemala, Colombia) and Andean (southern Peru, Bolivia, Argentina) gene pools. The similarity coefficient between these two groups was 0.34. A third distinct cluster, termed Intermediate, corresponded to accessions from Ecuador and northern Peru. This cluster was slightly more closely related to the Andean group, with a similarity coefficient of 0.35. There were three outliers to this clustering: PER15 (Apurimac 76) which grouped with the Mesoamerican gene pool, ECD4 (DGD2763) grouping with the Andean gene pool, and PER7 (PV-1), within the Andean gene pool but clearly separated from all other accessions and more similar to ECD4 and the Intermediate cluster (Fig. 7).

The three outliers to this clustering are interesting cases. PER15 showed two bands which are unique and monomorphic (i.e., present in all accessions) in the Mesoamerican group, one band which is unique and monomorphic in the Andean group, and another band which is only present in some of the Andean accessions. Furthermore, as already mentioned, in this study PER15 was found to be a heterogeneous accession consisting of seeds with both S and T type phaseolin. ECD4 was distinct from the Intermediate cluster by the presence of six bands and the absence of two bands which are monomorphic in all other accessions in this group. It was also distinct from the Andean cluster by showing 2 bands absent in all other Andean accessions and lacking four bands monomorphic to this group. PER7 was distinct from the Andean group in showing one band which is monomorphic and unique to the Mesoamerican group, one band which is present only in some Mesoamerican and some Intermediate accessions, and lacking one band which is unique and monomorphic in the Andean group. This accession also showed one unique band, absent in all other accessions. These three accessions were excluded from further genetic diversity analyses.

Without taking into consideration the three outliers, each cluster or gene pool can be characterized by specific RAPD bands. The Meso-

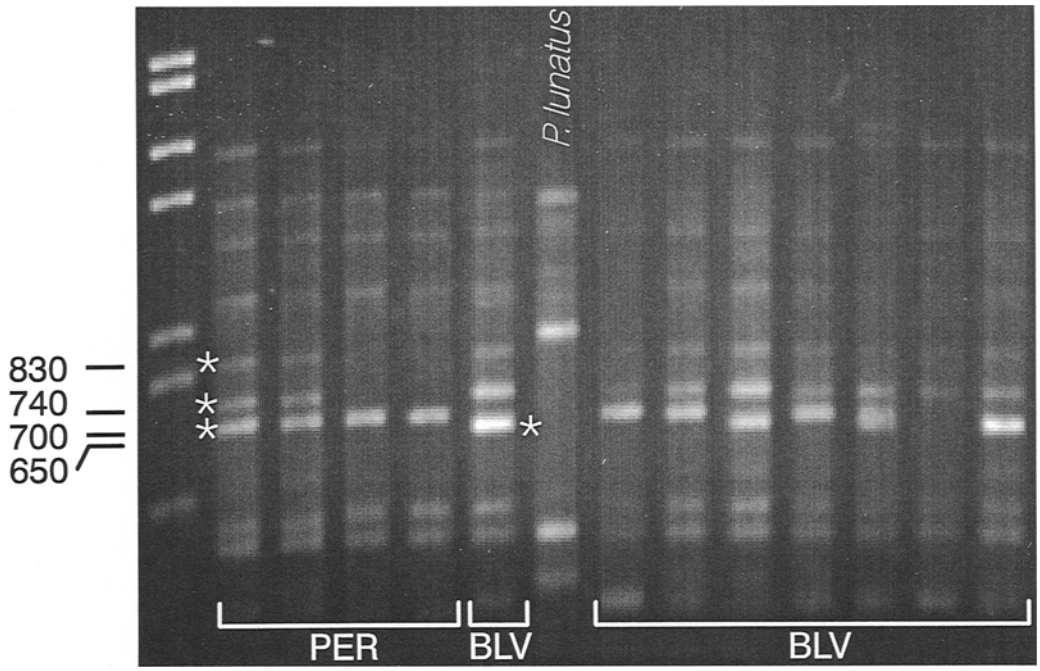

Fig. 6. Analysis of genetic diversity among a sample of wild *Phaseolus vulgaris* lines using RAPD primer Operon E04. Lanes are from left to right: Molecular size markers (lambda DNA digested with *Hin*dIII and *Eco*RI), DGD2594, DGD2600, DGD2584, DGD2858, DGD2501, *P. lunatus,* DGD2504, DGD3007, DGD3012, DGD3021, DGD3023, and DGD3024B. BOL: Bolivia, PER: Peru. Asterisks indicate bands scored for polymorphisms.

american group had 20 unique bands (i.e., found only in the Mesoamerican group), none of which, however, was monomorphic across all accessions. The Intermediate group had six unique bands, two of which were monomorphic. This group was also distinct by the absence of two bands present and monomorphic in both other groups. The Andean group had five unique bands, one of which was monomorphic. The use of the SCAR (J1d) resulted in the amplification of only two bands, each of which was unique to the two major gene pools: J1dA (1590 bp) was monomorphic in the Andean and Intermediate groups, and J1dC (1200 bp) was monomorphic in the Mesoamerican group. The only exception was accession PER17 (DGD2156) with band J1dB (1500 bp). The Intermediate group showed 14 monomorphic bands: two of these were unique, eight were present in both other groups, three were present in some Mesoamerican accessions, and one was monomorphic in the Andean group.

TABLE 2. NUMBER OF MONOMORPHIC AND POLYMORPHIC RAPD BANDS SCORED FOR EACH CLUSTER OF WILD BEANS.

	Mesoamerican	Intermediate	Andean
No. of monomorphic bands			
present	12 (13.3)*	21 (23.3)	16 (17.8)
absent	19 (21.1)	47 (52.2)	37 (41.1)
No. of polymorphic bands	59 (65.6)	22 (24.4)	37 (41.1)

* Numbers in parentheses indicate percentage.

The number of monomorphic bands (either present or absent) and polymorphic bands for each gene pool is shown in Table 2. The Mesoamerican gene pool showed the most polymorphic bands (65.6%) compared to 41.1% for the Andean group and 24.4% for the Intermediate group. This diversity was also evident in the dendrogram, where the similarity coefficient for all the accessions in the Mesoamerican group was 0.47 compared with similarity coefficients

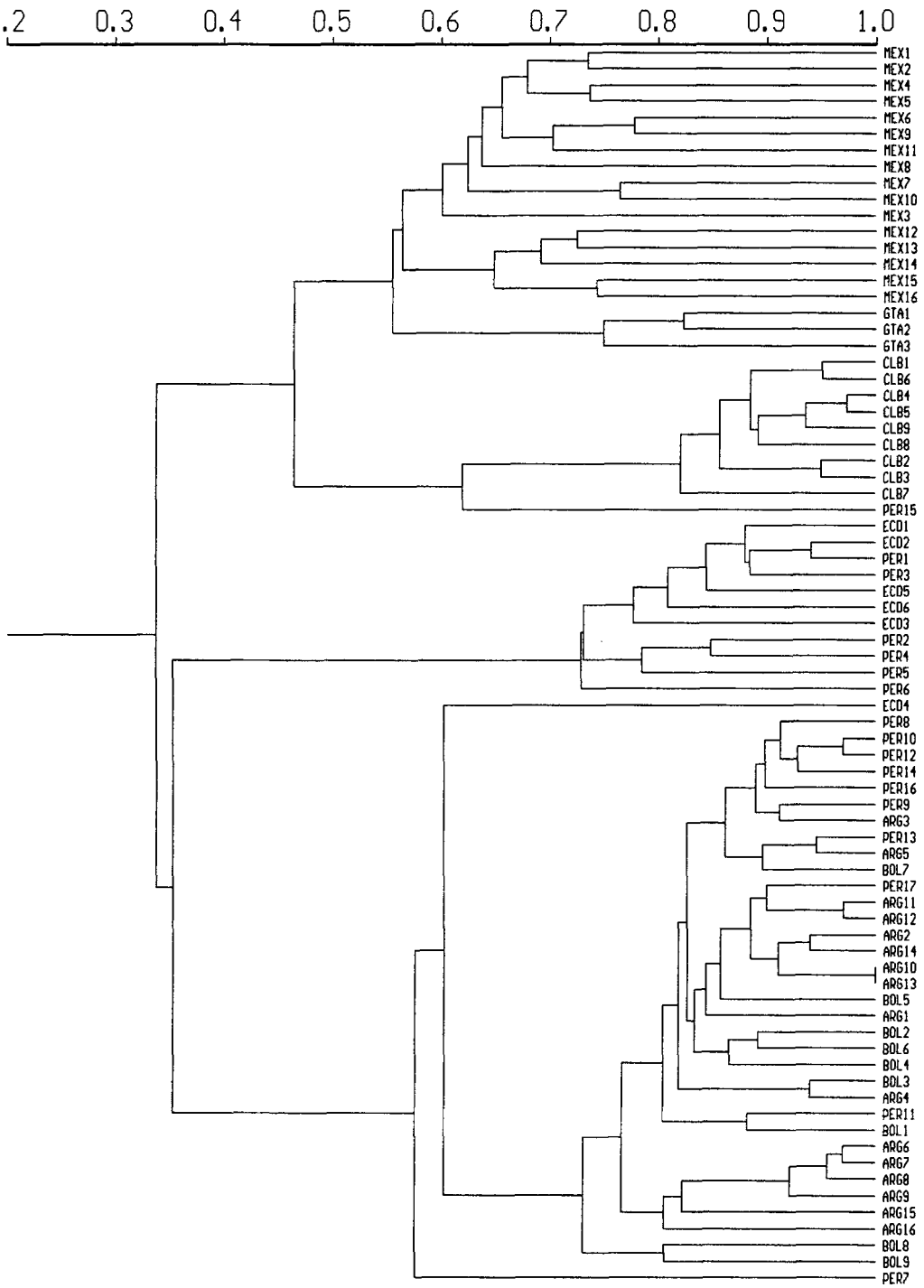

Fig. 7. UPGMA dendrogram based on Jaccard's similarity coefficient of RAPD diversity among wild *Phaseolus vulgaris*. ARG: Argentina, BOL: Bolivia, CLB: Colombia, EDU: Ecuador, GTA: Guatemala, MEX: Mexico, PER: Peru.

TABLE 3. LIST OF PLACES WITHOUT WILD *PHASEOLUS* SPP.

Department	Coordinates and altitude	Possible reason(s)
Cochabamba	66°15'W 17°35'S 2400	Naturally too dry
Cochabamba	66°22'W 17°26'S 2600	Overgrazed
Cochabamba	65°22'W 17°53'S 2000	Overgrazed, crops
Cochabamba	65°14'W 18°06'S 2150	Overgrazed
Cochabamba	64°56'W 18°10'S 2140	Overgrazed (goats)
Cochabamba	65°15'W 18°18'S 2280	*Vigna* sp.
Chuquisaca	64°58'W 18°53'S 2400	Too dry this year?
Chuquisaca	64°18'W 19°11'S 2060	Overgrazed
Chuquisaca	64°29'W 19°20'S 2250	Completely eroded
Chuquisaca	64°11'W 19°27'S 1900	*Vigna lasiocarpa* (?)
Chuquisaca	64°45'W 20°35'S 2160	Too far east?
Tarija	64°50'W 21°35'S 2250	Overgrazed, fire
Tarija	64°50'W 21°27'S 2250	Overgrazed

of 0.73 for both other groups (without considering the outlier genotypes). The Mesoamerican cluster was further subdivided into two subclusters: one consisting of Mexican and Guatemalan accessions, with similarity coefficient 0.55, and the other with Colombian accessions and similarity coefficient 0.82. Mexican accessions could be further subdivided in a cluster of accessions from the northern and western part of the country (MEX1 to MEX11: states of Chihuahua, Durango, Guanajuato, Jalisco, and Michoacán) and a cluster of accessions from the state of Morelos (MEX12 to MEX16). No geographic subdivisions were readily apparent in the Andean or Intermediate groups. Another estimate of genetic diversity for each group is provided by the RAPD marker variance (Nienhuis, Tivang, and Skroch 1994). This value was estimated as 9.86 for the Mesoamerican group, 4.11 for the Intermediate, and 3.62 for the Andean group.

DISCUSSION

No signs of the presence of wild *P. vulgaris* were observed around Cochabamba. Thus, it is doubtful that wild *P. vulgaris* was present in the places reported by the Brüchers, who did not leave any seed samples nor herbarium specimens. In the authors' view, wild *P. vulgaris* may have been confused with wild *P. augusti*. The altitude of the Cochabamba valley (2500 m) is slightly too high for wild *P. vulgaris* at this latitude. If wild *P. vulgaris* had ever been present in the valley, it could have been distributed in more humid places (river beds of Río Rocha, Estancia Alba, Rancho Champa), all of which are presently occupied by the city and its suburbs.

Several locations were searched in vain for the presence of wild *Phaseolus* spp. (Table 3). The absence of wild populations might be due to a lack of suitable habitat, inappropriate growing conditions (drought!), overgrazing, and vicariance with other wild legumes such as *Vigna*. An abnormally dry year led to lack of germination of entire populations as suggested by the absence in 1994 of accession DGD2497 discovered in the 1988 exploration. The seed dormancy mechanism, conditioned by impermeability of the seedcoat to water, may have played an important role in this adaptation to insufficient rainfall. This observation also suggests that surveys of biological diversity need to be conducted over several years to obtain a more accurate image of species distribution.

Pending confirmation with additional morphological and molecular studies, it seems that crosses occur where cultivated and wild common bean populations are in close contact (a radius of 100 m or less) in Bolivia, as has been observed in some other parts of the range of wild *P. vulgaris* in Latin America (Acosta Gallegos, Gepts, and Debouck 1994; Debouck et al. 1989) but not in others (Debouck et al. 1993). The case of Tabladita (DGD3023) is most interesting. We looked for typical, pure wild bean stands but could not find any, which in retrospect was to be expected given the high level of habitat disturbance. The original wild bean at this site may have been genetically absorbed into the weedy complex through repeated hy-

bridizations and may have therefore disappeared as a purely wild material. The survival of the weedy accession DGD2502 for at least five years is unusual but raises the issue as to which genetic or environmental factors are most important in this survival. Further research in this respect may be important in the light of future releases of transgenic beans in their centers of domestication.

The interesting scenario whereby beans and maize were selected jointly for popping ability and concomitantly for speckled and spotted seeds faces some difficulties. Corn was domesticated in Mesoamerica and was introduced into the Andes only at a later stage, at a time when common bean had already been domesticated there (Pearsall 1992). The four primitive varieties of pop corn from Mexico do not present the dotted aleurone (Wellhausen, Roberts, and Hernández X. 1952). Several varieties of common bean with good potential for toasting have uniform color patterns or very large color dots not present in any traditional maize landrace (Tohme et al. 1995). One could, however, propose the converse hypothesis, that is, some maize types have been selected specifically for toasting on the basis of characteristics already present in the common bean types. Tohme and co-workers (1995) suggested that common bean may have been selected first for toasting in the Andean region. The habit of toasting grains, by then already applied to *k'opurus* and *coimi,* could have been extended to the other grain crop—maize—once introduced from Mesoamerica in preceramic times.

Previous studies of wild common bean at the molecular level identified divergence into the two major gene pools, and an intermediate, presumed ancestral group. However, these studies were limited by the type of markers used. Studies of diversity in wild bean were based on isozyme systems, of which eight were polymorphic, resulting in nine loci (Debouck et al. 1993; Koenig and Gepts 1989). Mitochondrial RFLP studies were performed in six wild bean accessions using nine restriction enzymes and five probes, resulting in 20 polymorphisms scored (Khairallah, Sears, and Adams 1992). Nuclear RFLP studies were performed in 22 wild bean accessions, using 12 probes (Becerra Velásquez and Gepts 1994). A hindrance to the RFLP technique is in obtaining high quantities of quality DNA of wild beans suitable for digestion with restriction enzymes. This problem is not encountered with cultivated beans, which is probably due to the presence of a lesser amount of polyphenolics or other secondary metabolites. Higher quality DNA can be obtained using cesium chloride purifications, or following a method for isolation of large amounts of mostly nuclear and very pure DNA as described by Llaca (1994). Both of these alternatives have the disadvantage of increased cost and time involved. For RAPD amplification, the miniprep DNA extraction proved adequate, and the simplicity and ease of the method allowed us to obtain DNA for up to 40 accessions in a day.

In this study, 76 wild bean accessions were surveyed with 15 primers, resulting in 90 RAPD markers. The level of polymorphism (92%) was very high and similar to the value of RAPD polymorphism found between gene pools in cultivated beans (83%) (Haley et al. 1994). Both values are consistent with estimates of nuclear RFLP-based variation of 80% to 90% polymorphism between gene pools (Chase, Ortega, and Vallejos 1991; Nodari et al. 1992). There are certain problems associated with RAPD markers which may result in errors in scoring data. The complete sequence of the amplification products is not known. A primer might produce identically sized products in two genotypes, but possible divergence within the internal loci cannot be detected. A second limitation is that very small differences in product size may be impossible to detect. The magnitude of both problems can be reduced by using a larger number of primers and, hence, scoring more bands. For data in cultivated bean, Nienhuis and co-workers (1994) indicated that sampling 80 bands resulted in coefficients of variation as low as 20% for estimating the genetic relationship between the average individual genotypes.

RAPD data was effective in clustering the wild accessions into the two major gene pools and the intermediate group, which is consistent with their geographical origin. The Bolivian wild *P. vulgaris* grouped with the accessions of southern Peru and Argentina in the Andean gene pool. Moreover, the RAPD technique was sensitive enough to detect three outliers to this grouping (ECD4, PER7 and PER15), which are explained probably by their hybrid origin. Accession PER15 (Apurimac 76), grouping within the Mesoamerican cluster, showed bands that were unique and monomorphic to each of the

two major gene pools. It also proved to be a heterogeneous accession consisting of seeds with both S (Mesoamerican) and T (Andean) type phaseolin. This accession was collected in Apurímac (southern Peru), and might have been subjected to introgression from cultivated beans of Mesoamerican origin (e.g., "panamito," Voysest 1983), introduced and grown in the country. Visiting that department in 1987, one of us (DGD) found "panamito" growing close to wild common beans in the Abancay valley. Accession PER7 (PV-1) is distinct from all other accessions in the Andean group by showing one band which is present only in some Mesoamerican and some Intermediate accessions, and lacking one band which is unique and monomorphic in the Andean group. This is the only accession collected in Huánuco (central Peru) included in the study, and its geographic origin is also consistent with a clustering position between the Andean and Intermediate groups. However, the presence of SCAR band J1dC, of Mesoamerican origin, and a unique band, absent in all other accessions, could suggest introgression from cultivated Mesoamerican material as in the previous case. Interestingly, a study of Mesoamerican and Andean beans based on isozyme loci, also grouped both PER7 and PER15 within the Mesoamerican gene pool (Koenig and Gepts 1989). Finally, ECD4 (DGD2763) is distinct from the intermediate cluster and more similar to the Andean cluster. This is consistent with the fact that this accession was previously reported as heterogeneous with both I and T type phaseolins, and was the only accession in this group showing an Andean allele at the malic enzyme locus (Debouck et al. 1993). Accession ARG5 (DGD621) which was the only Andean accession included in the Mesoamerican group based on isozyme data (Koenig and Gepts 1989) is grouped in this study within the Andean cluster.

RAPD data variability was greater within the Mesoamerican cluster, as evidenced by the number of polymorphic loci (65.6%), total marker variance (9.86), and number of unique bands (21). This result is similar to that found with phaseolin (Gepts and Bliss 1986) and isozymes (Koenig and Gepts 1989). Even though ecological conditions where wild beans are found are similar throughout America (Debouck et al. 1993), the greater area of the Mesoamerican region particularly its spread in longitude could partly account for this higher variability. The range between collection sites for accessions in this region was 23.91° in latitude and 34.74° in longitude, respectively. The Mesoamerican cluster can be subdivided into two subclusters with similarity coefficient of 0.47, which correspond to Central American (Mexico and Guatemala) and South American (Colombia) geographical regions. Accessions from Colombia had the highest similarity coefficient (0.82), consistent with the fact that they were all collected in the same department (Cundinamarca). Accessions from Mexico and Central America have the highest diversity, with a similarity coefficient of 0.55. These accessions have been further subclustered into three groups which correspond to northern and southern Mexico, and Guatemala. Interestingly, wild bean populations from Mexico and Guatemala were different for traits such as photosynthetic rates and instantaneous photosynthetic nitrogen-use efficiency (Lynch et al. 1992). The northern Mexican group includes accessions of the states of Chihuahua, Durango, Jalisco, Guanajuato, and Michoacán, whereas the southern group includes accessions from the state of Morelos. Additional regions of Mexico need to be sampled including the states of Oaxaca and Chiapas. Furthermore, additional accessions from Central America (e.g., Honduras, Costa Rica, and possibly Panama) also need to be analyzed to obtain a more complete picture of genetic variation in the Mesoamerican gene pool of wild *P. vulgaris*.

Within the Intermediate group, the similarity coefficient was 0.73. This group had the least number of polymorphic loci (24.4%), and the total marker variance was 4.11. The ecological niche for wild beans in Ecuador and northern Peru is fairly narrow compared to its niche in other regions (Debouck et al. 1993). The distribution of wild beans in this region is within a range of latitude of 5.53°, and longitude of 1.06°. Within the Andean group, the similarity coefficient was also 0.73. Even though this group had a higher number of polymorphic bands (41.1%), marker variance is the lowest of all three groups (3.62). The range of distribution for accessions collected in this area is 16.08° in latitude and 11.2° in longitude. The accessions in this group do not appear to cluster by region (country), and there is no apparent grouping due to similar latitude, longitude, or altitude of collection. It is interesting to note that BOL8 and BOL9 (both corresponding to DGD3024), which appeared to

show a phaseolin variant, are the most dissimilar to the rest of the group.

This study confirmed a separate cluster for the Intermediate group of Ecuador and northern Peru. From phaseolin and isozyme studies in this group, two explanations were put forward to account for the biochemically and geographically intermediate nature of this group (Debouck et al. 1993). First, they might constitute hybrids between the Mesoamerican and Andean gene pools. An initial cross may have led to recombinant material adapted to the particular conditions prevailing on the western slope of the Andes in this region. Second, these populations represent relics of an ancestral *P. vulgaris* type from which the two major branches gradually diverged and were dispersed. Molecular marker data obtained so far appears to support the second hypothesis. Studies of the primary structure of phaseolin genes indicate that the I type phaseolin genes lack three tandem repeats present in other phaseolin types (e.g., S or T), suggesting that they represent an ancestral phaseolin type in *P. vulgaris* (Kami 1993; Kami et al., 1995; Llaca 1994).

In conclusion, our explorations have allowed us to expand our knowledge about the broad geographic and ecological distribution of *Phaseolus* spp. in Bolivia. RAPD analysis of genetic diversity correlated well with genetic diversity obtained with other markers such as isozymes and seed proteins and was instrumental in identifying geographic subgroups within the Mesoamerican gene pool. The recently collected wild *P. vulgaris* from Bolivia were closely related with their southern Peruvian and Argentinian counterparts. Additional explorations are necessary in Central America and southern Mexico, and perhaps also in the Alto Pilcomayo region of Bolivia (Fig. 1).

ACKNOWLEDGMENTS

The exploration and lab analyses were funded by a grant of the USDA/OICD. We thank Dr. Gonzalo Avila for his support of the germplasm exploration. The help of Alcira Arias for the map is fully acknowledged.

LITERATURE CITED

Acosta Gallegos, J. A., P. Gepts, and D. G. Debouck. 1994. Observations on wild and weedy forms of common bean in Oaxaca, Mexico. Annual Report of the Bean Improvement Cooperative 37: 137–138.

Adam-Blondon, A. F., M. Sévignac, H. Bannerot, and M. Dron. 1994. SCAR, RAPD and RFLP markers linked to a dominant gene (*Are*) conferring resistance to anthracnose in common bean. Theoretical and Applied Genetics 88:865–870.

Afanador, L. K., S. D. Haley, and J. D. Kelly. 1993. Adoption of a "mini-prep" DNA extraction method for RAPD marker analysis in common bean (*Phaseolus vulgaris* L.). Annual Report of the Bean Improvement Cooperative 36:10–11.

Baudet, J. C. 1977. The taxonomic status of the cultivated types of lima bean (*Phaseolus lunatus* L.). Tropical Grain Legume Bulletin, International Institute for Tropical Agriculture, Ibadan, Nigeria 7: 29–30.

Beck, S. G., T. J. Killeen, and E. García E. 1993. Vegetación de Bolivia. Pages 6–24 *in* T. J. Killeen, E. García E., and S. G. Beck, eds., Guía de arboles de Bolivia. Quipus S.R.L., La Paz, Bolivia.

Becerra Velásquez, V. L., and P. Gepts. 1994. RFLP diversity in common bean (*Phaseolus vulgaris* L.). Genome 37:256–263.

Berglund-Brücher, O. 1967. Wildbohnen-Funde in Südamerika. Naturwissenschaft 54:466–468.

———, **and H. Brücher.** 1976. The south American wild bean (*Phaseolus aborigineus* Burk.) as ancestor of the common bean. Economic Botany 30: 257–272.

Brücher, H. 1988. The wild ancestor of *Phaseolus vulgaris* in South America. Pages 185–214 *in* P. Gepts, ed., Genetic resources of *Phaseolus* beans. Kluwer Academic Publishers, Dordrecht, Holland.

Cárdenas, M. 1989. Manual de plantas económicas de Bolivia—2da edición. Editorial Los Amigos del Libro, La Paz, Bolivia.

Chase, C. D., V. M. Ortega, and C. E. Vallejos. 1991. DNA restriction fragment length polymorphisms correlate with isozyme diversity in *Phaseolus vulgaris* L. Theoretical and Applied Genetics 81:806–811.

Debouck, D. G. 1988a. Recolección de germoplasma de *Phaseolus* en Bolivia. Centro Internacional de Agricultura Tropical, Cali, Colombia, Mimeographed.

———. 1988b. *Phaseolus* germplasm exploration. Pages 3–29 *in* P. Gepts, ed., Genetic resources of *Phaseolus* beans. Kluwer Academic Publishers, Dordrecht, Holland.

———. 1989. Early beans (*Phaseolus vulgaris* L. and *P. lunatus* L.) domesticated for their aesthetic value? Annual Report of the Bean Improvement Cooperative 32:62–63.

———, **M. Gamarra Flores, V. Ortiz Arriola, and J. Tohme.** 1989. Presence of a wild-weed-crop complex in *Phaseolus vulgaris* L. in Peru? Annual Report of the Bean Improvement Cooperative 32: 64–65.

———, **O. Toro, O. M. Paredes, W. C. Johnson, and P. Gepts.** 1993. Genetic diversity and ecological distribution of *Phaseolus vulgaris* (Faba-

ceae) in northwestern South America. Economic Botany 47:408–423.

Delgado Salinas, A., A. Bonet, and P. Gepts. 1988. The wild relative of *Phaseolus vulgaris* in Middle America. Pages 163–184 *in* P. Gepts, ed., Genetic resources of *Phaseolus* beans. Kluwer Academic Publishers, Dordrecht, Holland.

dos Santos, J. B., J. Nienhuis, P. Skroch, J. Tivang, and M. K. Slocum. 1994. Comparison of RAPD and RFLP genetic markers in determining genetic similarity among *Brassica oleracea* L. genotypes. Theoretical and Applied Genetics 87:909–915.

Gentry, H. S. 1969. Origin of the common bean, *Phaseolus vulgaris*. Economic Botany 23:55–69.

Gepts, P. 1993. The use of molecular and biochemical markers in crop evolution studies. Pages 51–94 *in* M. K. Hecht, ed., Evolutionary biology. Volume 27. Plenum Press, New York.

———, and F. A. Bliss. 1986. Phaseolin variability among wild and cultivated common beans (*Phaseolus vulgaris*) from Colombia. Economic Botany 40:469–478.

———, T. C. Osborn, K. Raska, and F. A. Bliss. 1986. Phaseolin protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris*): evidence for multiple centers of domestication. Economic Botany 40:451–468.

Gutiérrez Salgado, A., P. Gepts, and D. G. Debouck. 1995. Evidence for two gene pools of the lima bean, *Phaseolus lunatus*, in the Americas. Genetic Resources & Crop Evolution 42: in press.

Haley, S. D., P. N. Miklas, L. Afanador, and J. D. Kelly. 1994. Random amplified polymorphic DNA (RAPD) marker variability between and within gene pools of common bean. Journal of the American Society for Horticultural Science 119: 122–125.

Hallden, C., N. O. Nilsson, I. N. Rading, and T. Sall. 1994. Evaluation of RFLP and RAPD markers in a comparison of *Brassica napus* breeding lines. Theoretical and Applied Genetics 88:123–128.

Halward, T., T. T. Stalker, E. LaRue, and G. Kochert. 1992. Use of single-primer DNA amplification in genetic studies of peanut (*Arachis hypogaea* L.). Plant Molecular Biology 18:315–325.

Heun, M., J. P. Murphy, and T. D. Phillips. 1994. A comparison of RAPD and isozyme analysis for determining the genetic relationships among *Avena sterilis* L. accessions. Theoretical and Applied Genetics 87:689–696.

Howell, E. C., H. J. Newbury, R. L. Swennen, I. A. Withers, and B. V. Ford-Lloyd. 1994. The use of RAPD for identifying and classifying Musa germplasm. Genome 37:328–332.

Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. Bulletin Société Vaudoise Sciences Naturelles 44:223–270.

Jain, A., S. Bhatia, S. S. Banga, S. Prakash, and M. Lakshmikumaran. 1994. Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica juncea*) and its relationship to heterosis. Theoretical and Applied Genetics 88:116–122.

Johnson, A. M. 1976. The climate of Peru, Bolivia and Ecuador. Pages 147–218 *in* W. Schwerdtfeger, ed., Climates of Central and South America. Elsevier Scientific Publishing Co., Amsterdam, The Netherlands.

Kami, J. A. 1993. Molecular evolution of phaseolin. Ph.D. Dissertation. University of California, Davis.

Kami, J., V. L. Becerra Velásquez, D. G. Debouck, and P. Gepts. 1995. Identification of the presumed ancestor of *Phaseolus vulgaris*. Proceedings of the National Academy of Sciences (USA) 92: 1101–1104.

Khairallah, M. M., B. B. Sears, and M. W. Adams. 1992. Mitochondrial restriction length polymorphisms in wild Phaseolus vulgaris L.: insights on the domestication of the common bean. Theoretical and Applied Genetics 84:915–922.

Koenig, R. L., and P. Gepts. 1989. Allozyme diversity in *Phaseolus vulgaris*: further evidence for two major centers of genetic diversity. Theoretical and Applied Genetics 78:809–817.

———, S. P. Singh, and P. Gepts. 1990. Novel phaseolin types in wild and cultivated common bean (*Phaseolus vulgaris*, Fabaceae). Economic Botany 44:50–60.

Koinange, E. M. K., and P. Gepts. 1992. Hybrid weakness in wild *Phaseolus vulgaris* L. Journal of Heredity 83:135–139.

Llaca, V. 1994. Aspects of genome evolution in the *Phaseolus vulgaris* complex. Ph.D. Dissertation. University of California, Davis.

Liu, Z., and G. R. Furnier. 1993. Comparison of allozyme, RFLP, and RAPD markers for revealing genetic variation within and between trembling aspen and bigtoothaspen. Theoretical and Applied Genetics 87:97–105.

Lynch, J., A. González, J. M. Tohme, and J. A. García. 1992. Variation in characters related to leaf photosynthesis in wild bean populations. Crop Science 32:633–640.

Mailer, R. D., R. Scarth, and B. Fristensky. 1994. Discrimination among cultivars of rapeseed (*Brassica napus* L.) using DNA polymorphisms amplified from arbitrary primers. Theoretical and Applied Genetics 87:697–704.

Montes de Oca, I. 1989. Geografía y recursos naturales de Bolivia. Editorial Educational, Ministerio de Educación y Cultura, La Paz, Bolivia.

Nienhuis, J., J. Tivang, and P. Skroch. 1994. Analysis of genetic relationships among genotypes based on molecular data. *In* Proc. Symp. Anal. Mol. Marker Data Aug. 5-6, 1994, Corvallis, OR, Joint Plant Breed. Symp. Series, Amer. Soc. Hort.

Sci., Alexandria, VA, and Amer. Soc. Agron., Madison, WI: pp. 8–14.

Nodari, R. O., E. M. K. Koinange, J. D. Kelly, and P. Gepts. 1992. Towards an integrated linkage map of common bean. 1. Development of genomic DNA probes and levels of restriction fragment length polymorphism. Theoretical and Applied Genetics 84:186–192.

———, S. M. Tsai, R. L. Gilbertson, and P. Gepts. 1993. Towards an integrated linkage map of common bean. 2. Development of an RFLP-based linkage map. Theoretical and Applied Genetics 85: 513–520.

Orozco-Castillo, C., K. J. Chalmers, R. Waugh, and W. Powell. 1994. Detection of genetic diversity and selective gene introgression in coffee using RAPD markers. Theoretical and Applied Genetics 87:934–940.

Pearsall, D. M. 1992. The origins of plant cultivation in South America. Pages 173–205 *in* C. W. Cowan and P. J. Watson, eds., The origins of agriculture—an international perspective. Smithsonian Institution Press, Washington, D.C.

Piper, C. V. 1926. Studies in American Phaseolinae. Contributions to the US National Herbarium 22: 663–701.

Quiros, C. F., J. Hu, P. This, A. M. Chevre, and M. Delseny. 1991. Development and chromosomal localization of genome-specific markers by the polymerase chain reaction. Theoretical and Applied Genetics 82:627–632.

Rafalski, J. A., S. V. Tingey, and J. F. K. Williams. 1991. RAPD markers—a new technology for genetic mapping and plant breeding. Agbiotech News and Info. 3:645–648.

Ramírez E., R., D. H. Timothy, E. Díaz B., and U. J. Grant. 1960. Races of maize in Bolivia. National Academy of Sciences, National Research Council, Publication 747, Washington, D.C.

Sneath, P. H. A., and R. R. Sokal. 1973. Numerical taxonomy. W. H. Freeman, San Francisco, California.

Thormaun, C. E., M. E. Ferreira, L. E. A. Camargo, J. G. Tivang, and T. C. Osborn. 1994. Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. Theoretical and Applied Genetics 88:973–980.

Tohme, J., O. Toro, J. Vargas, and D. G. Debouck. 1995. Variability studies in Andean *nuña* common beans (*Phaseolus vulgaris,* Fabaceae). Economic Botany 49:78–95.

Toro, O., J. Tohme, and D. G. Debouck. 1990. Wild bean (*Phaseolus vulgaris* L.): description and distribution. Centro Internacional de Agricultura Tropical, Cali, Colombia.

Transue, D. K., D. J. Fairbanks, L. R. Robison, and W. R. Andersen. 1994. Species identification by RAPD analysis of grain amaranth genetic resources. Crop Science 34:1385–1389.

Unzueta, O. 1975. Mapa ecológico de Bolivia. Memoria explicativa. Ministerio de Asuntos Campesinos y Agropecuarios. División de Riegos e Ingeniería, La Paz, Bolivia.

Vierling, R. A., and H. T. Nguyen. 1992. Use of RAPD markers to determine the genetic diversity of diploid wheat genotypes. Theoretical and Applied Genetics 84:835–838.

Voysest, O. 1983. Variedades de frijol en América Latina y su origen. Centro Internacional de Agricultura Tropical, Cali, Colombia. 87 pp.

Waugh, R., and W. Powell. 1992. Using RAPD markers for crop improvement. Trends in Biotechnology 10:186–191.

Wellhausen, E. J., L. M. Roberts, and E. Hernández Xolocotzi. 1952. Races of maize in Mexico. Their origin, characteristics and distribution. Bussey Institution, Harvard University, Harvard, Massachusetts, USA.

Welsh, J., and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18:7213–7218.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531–6535.

Yang, X., and C. Quiros. 1993. Identification and classification of celery cultivars with RAPD markers. Theoretical and Applied Genetics 86:205–212.

Yu, L. X., and H. T. Nguyen. 1994. Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). Theoretical and Applied Genetics 87:668–672.