

Phaseolin-protein Variability in Wild Forms and Landraces of the Common Bean (*Phaseolus vulgaris*): Evidence for Multiple Centers of Domestication¹

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A sample of 106 wild forms and 99 landraces of common bean (Phaseolus vulgaris) from Middle America and the Andean region of South America were screened for variability in phaseolin seed protein using one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE) and two-dimensional isoelectric focusing SDS/PAGE. The Middle American wild forms exhibited phaseolin patterns similar to the 'S' pattern described previously in cultivated forms, as well as a wide variety of additional banding patterns—'M' (Middle America) types—not encountered among common bean cultivars. The Andean wild forms showed only the 'T' phaseolin pattern, also described previously among cultivated forms. Landraces from Middle America showed 'S' or 'S'-like patterns with the exception of 2 lines with 'T' phaseolin. In Andean South America, a majority of landraces had the 'T' phaseolin. Additional types represented in that region were (in decreasing order of frequency) the 'S' and 'C' types (already described among cultivated forms) as well as the 'H' (Huevo de huanchaco) and 'A' (Ayacucho), (new patterns previously undescribed among wild and cultivated beans). In each region—Middle America and Andean South America—the seeds of landraces with 'T' phaseolin were significantly larger than those of landraces with 'S' phaseolin. No significant differences in seed size were observed among landraces with 'T,' 'C,' 'H,' and 'A' phaseolin types of the Andean region. Our data favor 2 primary areas of domestication, one in Middle America leading to small-seeded cultivars with 'S' phaseolin patterns and the other in the Andes giving rise to large-seeded cultivars with 'T' (and possibly 'C,' 'H,' and 'A') phaseolin patterns.

Wild-growing forms of common bean (*Phaseolus vulgaris* L.) have been found in Middle America (Gentry, 1969; Miranda Colín, 1967, 1979) and in the Andes in South America (Burkart and Brücher, 1953; Brücher, 1969; Berglund-Brücher and Brücher, 1976). In Mexico, wild beans have been collected in the west central states at altitudes ranging from 500–1,800 m. In South America, wild beans classified variously as *P. aborigineus* (Burkart, 1943), *P. vulgaris* ssp. *aborigineus* (Burkart and Brücher, 1953), and *P. vulgaris* var. *aborigineus* (Baudet, 1977), have been collected along the eastern slope of the Andes from northern Argentina to western Venezuela at altitudes of 500–2,000 m. The wild beans from Middle America tend to flower later and for a longer time, have longer raceme peduncles, more floral nodes per raceme, larger flower bracteoles, and smaller seeds than those from the Andes (Vanderborght, 1983).

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The existence of 2 geographically and morphologically distinct wild bean populations raises the question of where the domestication of common bean occurred. According to Gentry (1969), common bean was probably domesticated in Mexico from where it was carried to South America. Berglund-Brücher and Brücher (1976) suggested that the common bean might have been domesticated in South America, but did not exclude a separate domestication in Middle America. The archaeological record of the common bean includes findings of similar age in both Middle America (Tehuacán Valley, Mexico: 5,000 B.C.; Kaplan, 1967) and the Andes (Callejón de Huaylas, Peru: 5,680 B.C.; Kaplan et al., 1973). These 2 findings are older than the first documented contacts between Middle and South America (Harlan, 1976). Several authors have suggested the possibility of independent domestications of the common bean in Middle America and the Andes (Harlan, 1971; Heiser, 1965; Evans, 1976). The latter author, in particular, suggested 2 centers of domestication, one in Middle America giving rise to small-seeded cultivars and the other in the Andes leading to large-seeded cultivars.

Analysis of variation in the electrophoretic patterns of seed proteins is a useful method for establishing relationships among plant accessions within a species. Variation in the electrophoretic pattern of phaseolin, the major seed storage protein of the common bean, has been well documented for cultivated forms. One-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE) (Ma and Bliss, 1978) and 2-dimensional isoelectric focusing SDS/PAGE (IEF-SDS/PAGE) (Brown et al., 1981a, 1982) of phaseolin revealed 3 electrophoretic patterns—'S,' 'T,' and 'C'—among cultivars of the common bean. Two-dimensional IEF-SDS/PAGE has revealed that the 'S' and 'T' phenotypes apparently have no polypeptides in common. The 'C' type, on the other hand, shared polypeptides with the 'S' and 'T' types, suggesting that it might represent a rare recombinant. Crossing experiments have shown that the genes coding for the different polypeptides of the phaseolin-banding patterns are tightly linked and inherited as a single Mendelian unit with co-dominant alleles (Brown et al., 1981b). In a survey of 107 bean cultivars, the majority of cultivars screened showed either the 'S' (69%) or 'T' (25%) patterns and only 6% showed the 'C' pattern (Brown et al., 1982).

We report here the phaseolin variability as revealed by one-dimensional SDS-PAGE and 2-dimensional IEF-SDS/PAGE among wild forms and landraces of the common bean in Mexico, Central America, and Andean South America. Our objectives were: 1) to assess the variability of phaseolin among wild forms and landraces of the common bean (A preliminary survey on phaseolin variability in Mexican wild forms of the common bean was carried out by Jeanne Romero-Andreas and published by Bliss and Brown, 1983); 2) to establish the existence of regional distribution patterns, if any, of phaseolin variability; 3) to determine relationships, if any, between wild and cultivated forms in terms of phaseolin variability.

MATERIALS AND METHODS

Plant materials

The 106 wild forms and 99 landraces of *P. vulgaris* originated in Middle and South America and were obtained from: the *Phaseolus* World Collection at the

Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia; the Western Regional Plant Introduction Station, Pullman, WA, USA; the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), El Batán, Mexico; the Seed Savers Exchange, Princeton, MO, USA; Dr. F. A. Bliss, University of Wisconsin, Madison, WI, USA.

Ninety-five of the 106 wild bean accessions came from Mexico, 1 accession from Guatemala, and 10 from South America. Altitudes of collection of the Mexican wild bean (when reported) ranged from 762 m (G12868: Santa María del Oro, Durango) to 2,134 m (G12957: eastern Jalisco) (Table 1). Both the geographic distribution and the altitudinal range of our sample of Mexican wild common beans were similar to those described by Gentry (1969) and Miranda Colín (1967).

In this study, landraces were defined as cultivars with a limited geographic range and adapted to the low input agriculture generally practiced by small farmers in Latin America. Landraces do not result from breeding activities, especially hybridization. To the best of our knowledge, all landrace cultivars included in our sample followed this definition. Forty-six landraces from Middle America (Mexico, Guatemala, El Salvador, Honduras, Nicaragua, and Costa Rica), and 53 from Andean South America (Colombia, Ecuador, Peru, Bolivia, Argentina, and Chile) were included in our study (Table 2).

Preparation of flour samples for electrophoresis

Five seeds of each wild bean accession and 2–5 seeds of each landrace were analyzed by 1-dimensional SDS/PAGE. Initially, a flour sample from the raphe end of each seed was suspended for at least 0.5 h in a 0.5 M NaCl solution at room temperature. The suspension was then centrifuged and the supernatant mixed with an equal volume of cracking buffer (0.625 M Tris-HCl; pH 6.8; 2 mM EDTA; 2% (w/v) SDS; 40% (w/v) sucrose; 1% (v/v) 2-mercaptoethanol; and 0.01% (w/v) bromophenol marker dye) (Brown et al., 1981a). Alternatively, the flour was suspended directly in a mixture containing equal volumes of salt solution and cracking buffer. The mixture was heat-treated at 100°C for 5 min and submitted to electrophoresis. No differences in electrophoresis patterns were observed between the 2 extraction procedures.

Electrophoresis

One-dimensional SDS/PAGE was performed according to the method of Laemmli (1970) modified by Ma and Bliss (1978). Electrophoresis was carried out in 0.75 mm thick, 15% (w/v) polyacrylamide slab gels. Two-dimensional IEF-SDS/PAGE was carried out as described by Brown et al. (1981a) except that 15% polyacrylamide slab gels were used for the SDS dimension.

Seed size

Seed size of the landraces was determined by measuring seed length, height, and width of 5–10 seeds per entry. Seed length represents the longest dimension of the seed, height the distance between the hilum and the side of the seed opposite the hilum, and width the distance between the 2 lateral sides of the seed. Seed

TABLE 1. IDENTIFICATION, ORIGIN, AND PHASEOLIN TYPE OF WILD BEAN ACCESSIONS FROM MIDDLE AMERICA AND ANDEAN SOUTH AMERICA.

Country	State	Identification	Location	Altitude (m)	Phaseolin type ^b	
Mexico	Durango	G10999 ^a	N24.26; W104.10	1,950	'S'*	
		G11025A	N24.30; W104.28	1,930	'M'	
		G11025D	N24.30; W104.28	1,930	'M'	
		G11027	N24.22; W104.28	1,970	'M'*	
		G11027A	N24.22; W104.28	1,970	'M'*	
		G11028	N24.30; W104.35	2,020	'M'	
		G11030A	N24.29; W104.19	2,050	'M'	
		G11034	N23.37; W105.50	1,960	'M'	
		G11125	N24.28; W104.36	2,130	'S'	
		G12868	N25.56; W105.10	762	'M'	
		Nayarit	G12867	N21.05; W104.30	823	'S,' 'M'
		Jalisco	G12891	N20.42; W102.19	1,829	'S,' 'M'
			G12922 to G12935	N20.42; W102.21	1,829	'S'
			G12949	N20.40; W102.23	1,829	'M'
			G12952	N20.40; W102.23	1,829	'M'
			G12953	N20.40; W102.23	1,829	'M'
			G12957	N20.54; W102.22	2,134	'S'
			G9998	Lago Chapala		'M'
			G9998A	Lago Chapala		'M'*
			G12914	N20.32; W103.11	1,524	'S,' 'M'
			G12915	N20.32; W103.11	1,524	'S,' 'M'
			G9989A	Mascota		'S'
			G9989B	Mascota		'S,' 'M'
			G11055	N20.34; W104.46	1,390	'M'*
			G11056	N20.32; W104.46	1,380	'S'
			G12884	N20.32; W104.49	1,524	'S,' 'M'
			G11115	N19.24; W103.24	1,140	'M'
			G12862	N19.57; W103.00	1,280	'S,' 'M'*
			G12862A	N19.57; W103.00	1,280	'M'
			G12862B	N19.57; W103.00	1,280	'M'*
			G12863	N20.47; W104.11	1,402	'S,' 'M'
			G12865	N19.20; W103.15	1,219	'M'*
			G12866	N19.41; W103.29	1,524	'S'
		Colima	G12864	N19.27; W103.37	1,645	'M'
		Guanajuato	G12904	N20.37; W101.43	1,829	'S'
			G12905	N20.37; W101.43	1,829	'M'
			G12910	N20.37; W101.43	1,829	'S'
			G12911A	N20.37; W101.43	1,829	'S,' 'M'
		Michoacán	G10018			'M'
			G10020			'M'
			G11050	N19.41; W101.16	2,040	'M'
			G12861	N19.25; W102.04	1,341	'M'*
			G12869	N19.25; W101.95	1,310	'M'*
			G12888	N20.17; W102.19	1,829	'M'
		Morelos	G10008			'M'*
			G10009			'M'
			G10010			'M'

TABLE 1. CONTINUED.

Country	State	Identification	Location	Altitude (m)	Phaseolin type ^b
		G10012A			'S,' 'M'
		G10013			'M'
		G10014			'M'
		G10015			'M'
		G10016			'M'
		G10017			'M'
		G12872	N18.58; W99.06	1,828	'M'*
		G12872A	N18.58; W99.06	1,828	'M'*
		G12877A	N18.57; W99.13	1,920	'S,' 'M'
		G12877B	N18.57; W99.13	1,920	'M'*
		G13017			'M'
		G13018			'M'
		G13019			'M'*
		G13020			'M'
		G13505			'M'
	México	G12873	N19.35; W99.15	1,981	'M'
		G12873C	N19.35; W99.15	1,981	'M'
	Guerrero	G10000			'S'
		G10001			'M'
		G10002			'M'
		G10003			'M'
		G10004			'M'
		G10005			'M'
		G10006			'M'
		G10007			'M'
		G12870A	N16.37; W98.55	975	'M'*
		G12878	N18.21; W99.46	1,402	'M'*
		G12879	N18.21; W99.59	1,585	'S,' 'M'
		G12881	N18.21; W100.08	1,463	'M'
		G12881A	N18.21; W100.08	1,463	'M'
		G12882	N18.17; W100.09	853	'M'
		G13015			'S'
		G13016			'M'
	Oaxaca	G12875	N17.19; W96.54	1,676	'S,' 'M'
		G13021			'M'
Guatemala		G12851	N14.17; W 90.18	1,829	'S,' 'M'*
Brazil		G6388			'T'
Peru		G7225			'T'*
		G12856A			'T,' 'S'
		G12856B			'T'
		G12859			'T'
Argentina		G996			'T'
		G7469			'T'
		G10021			'T'
		G10024			'T'
		G10025			'T'

^a Number of the *Phaseolus* World Collection, CIAT, Cali, Colombia.

^b 'S' = Sanilac; 'T' = Tendergreen; and 'M' = Middle America banding pattern; lines with an asterisk exhibited 2 or more banding patterns in each category.

TABLE 2. IDENTIFICATION, ORIGIN, PHASEOLIN TYPE, AND SEED SIZES OF LANDRACES FROM MIDDLE AMERICA AND ANDEAN SOUTH AMERICA.

Country of origin	Identification		Phaseolin type ^a	Seed sizes (mm)		
	No.	Name		Length	Height	Width
Mexico	Chih.858	Colores	'S'	11.8	7.4	4.5
	Chis.4A1	Negro tropical	'S'	9.9	6.2	4.3
	Coah.10A	Bayo grande	'S'	12.3	7.7	5.8
	Coah. 10B	Bayo grande	'S'	10.7	6.2	4.3
	DGD78/016A	Flor de Mayo	'S'	11.1	7.0	5.3
	DGD78/017	Pinto Texano	'S'	12.8	8.1	4.8
	DGD78/027	Ejotero	'S'	12.2	6.1	4.6
	DGD78/035G	Bayo	'S'	13.2	9.1	5.7
	DGD78/046B	Pinto	'S'	12.4	8.4	5.9
	DGD78/047A	Sangre de toro	'S'	13.4	9.0	6.0
	DGD78/048	Alubia chico	'S'	8.6	6.0	4.7
	DGD78/066A	Bayo regional	'S'	13.6	9.6	5.9
	DGD78/068	Ojo de liebre	'S'	14.4	8.5	5.1
	DGD78/069A	Bayo	'S'	13.0	8.8	5.9
	Dgo.844	Colores	'S'	10.2	6.8	4.7
	Gro.893	Bayo grande	'S'	—	—	—
	Hgo.4A	Colores	'S'	11.9	7.7	5.0
	Jal.56	Colores	'S'	—	—	—
	Jal.86-2A		'S'	9.4	6.3	5.5
	Jal.132		'S'	9.2	5.2	3.9
	Mex.27-R		'T'	15.2	7.4	6.6
	Mex.80-R		'S'	8.2	6.1	4.1
	Mex.120		'S'	9.0	6.2	4.8
	Mex.235		'S'	12.3	6.8	5.0
	Mor.20-1		'T'	16.6	7.3	6.4
	Mor.32	Colores	'S'	10.5	6.7	3.4
	PI325619	Frijól Machete negro	'S'	—	—	—
	PI325638	Frijól negro	'S'	—	—	—
	Pue.40	Negro arribeño	'S'	9.6	6.2	4.8
	Pue.44A		'S'	15.2	8.3	5.5
	Pue.158C	Colores	'S'	11.0	7.1	3.9
	Tlax.472	Bayo grande	'S'	12.2	7.9	6.8
Ver.6-1	Blancos	'S'	10.7	7.2	6.1	
	Veracruz negro	'S'	9.6	6.5	5.2	
Zac.54-4		'S'	—	—	—	
Guatemala	Blanco137		'S'	10.2	6.7	4.8
	GTA1008		'S'	12.1	7.9	5.2
	GTA1240		'S'	12.1	7.6	5.8
	PI195401		'S'	9.8	6.1	5.2
	PI310740		'S'	11.5	7.2	4.3
El Salvador	PI307824		'S'	9.6	7.1	4.0
Honduras	Zamorano		'S'	10.3	6.3	5.2
Nicaragua		Orgullosa	'S'	9.7	6.8	4.9
	PI310814		'S'	11.3	6.8	4.5
	PI310878		'S'	11.4	6.9	4.8
Costa Rica	51051		'S'	10.3	6.3	4.0
Colombia		Cargamanto	'T'	12.9	9.5	7.2
	Nar.23		'S'	11.0	6.6	4.2
Ecuador	Ecuador299		'S'	11.8	6.5	4.4

TABLE 2. CONTINUED.

Country of origin	Identification		Phaseolin type ^a	Seed sizes (mm)		
	No.	Name		Length	Height	Width
	PI152311	Blanca torta	'T'	12.3	7.9	4.8
	PI152313	Burrito	'C'	11.2	8.0	5.9
	PI152322	Matahambre	'S'	9.0	6.4	5.2
	PI152324	Misturiado roasado	'T'	12.4	8.9	7.0
	PI152326	Negro redondo	'S'	9.4	6.6	4.8
	PI299019	Amarillo	'T'	13.3	7.2	5.1
	PI299020	Bajo	'T'	14.3	9.2	6.2
	PI299021	Blanco de bajo	'S'	9.2	6.3	5.4
	PI299022	Rosado	'T'	16.7	8.9	6.9
Peru	BN1280		'S'	12.4	7.6	5.7
	BN1281		'T'	13.6	7.1	5.6
	BN1282		'T'	16.1	8.2	5.6
	BN1283		'T'	10.9	6.9	5.5
	BN1284		'T'	13.9	6.8	5.5
	PI153714	Huasca Huallaga Colorado	'T'	16.7	8.9	6.0
	PI290992	Sangre do toro	'T'	14.1	8.6	5.7
	PI290993	Algarrobo	'T'	17.5	9.7	7.5
	PI290995	Canario	'T'	11.8	7.0	5.3
	PI290996	Panamito	'S'	7.4	5.6	4.2
	PI290998	Frijól negro	'C'	12.6	7.4	5.6
	PI306149	Caraota	'S'	9.1	5.8	4.0
	PI415951	Poroto negro	'T'	13.3	8.5	6.3
	PI415953	Poroto blanco chico	'S'	7.0	5.6	4.8
	PI415954	Poroto blanco grande	'T'	9.8	7.4	6.4
	PG0120	Ñuña Conejita	'H'	11.4	9.3	8.1
	PG0133	Ñuña Maní roja	'C'	12.3	9.2	7.7
	PG0139	Ñuña Huevo de huanchaco	'H'	10.5	9.5	8.7
		Ayacucho	'A'	9.8	8.1	6.2
Bolivia	PI152206A		'T'	14.0	8.7	6.3
	PI152206B		'T'	11.3	8.9	7.3
	PI152208		'C'	15.9	10.6	7.4
	PI152215		'T'	12.0	8.7	6.3
	PI156669A	Bayo chico	'S'	9.7	6.6	4.8
	PI156669B		'T'	13.1	5.9	6.2
Chile	BN487	Bayo horticulture	'C'	14.2	6.7	6.4
	PI151014	Amarillos	'T'	13.1	6.5	4.9
	PI151015	Azulillo	'C'	13.6	8.8	5.8
	PI151017	Bayo palo	'S'	12.3	8.4	5.4
	PI151020	Burrito	'C'	10.6	7.3	6.5
	PI151023	Coscorrones	'H'	13.2	9.1	7.3
	PI281984	Almidón	'C'	11.9	8.7	6.6
	PI281990	Señorita	'C'	10.9	8.4	6.7
	PI281996	Pajaritos	'C'	13.9	10.4	7.5
	PI281998	Valsecía	'T'	14.1	9.7	7.8
	PI282000	Tórtola Los Angeles	'C'	12.4	7.9	6.5
	PI282016	Algarrobeño	'T'	16.9	8.6	7.6
Argentina	BN250	Pampa	'H'	16.6	10.3	7.0
	BN252	Bahía blanca	'T'	11.4	8.2	7.2
	BN253	Gaucho	'T'	12.9	6.6	5.1
	BN386	Tongue of fire	'T'	14.0	9.4	7.5

^a 'S' = Sanilac; 'T' = Tendergreen; 'C' = Contender; 'A' = Ayacocho; 'H' = Huevo de huanchaco phaseolin-banding pattern.

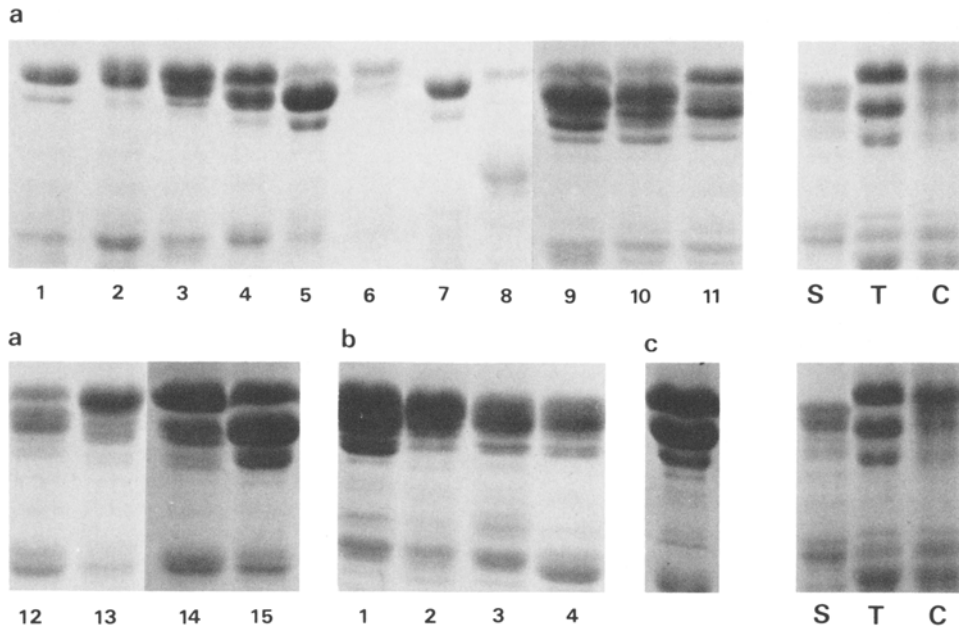


Fig. 1. One-dimensional SDS/PAGE of phaseolin of wild beans from Middle America (a-b) and Andean South America (c). a: 'M' phaseolin: 1) G11025A; 2) G12868; 3) G12861; 4) G9998; 5) G12864; 6) G12872A; 7) G11115; 8) G12891; 9) G12882; 10) G12867; 11) G12869; 12) G11050; 13) G12877B; 14) G11034; 15) G11005. b: 'S' phaseolin: 1) G11056; 2) G12863; 3) G12884; 4) G12914. c: 'T' phaseolin: G7469. S, T, and C: reference phaseolins of cultivars Sanilac, Tendergreen, and Contender, respectively.

sizes for the individual accessions are presented in Table 2. Within each region (Middle America and Andean South America), the statistical significance of the differences in seed size observed between classes of cultivars showing different phaseolin-banding patterns was assessed using Duncan's multiple range test (Steel and Torrie, 1980).

RESULTS

One-dimensional electrophoretic analysis of phaseolin variability in wild beans

Wild bean accessions from Middle America (95 from Mexico and 1 from Guatemala) exhibited a high diversity of phaseolin electrophoretic patterns, as seen by 1-dimensional SDS-PAGE (Fig. 1). Some accessions showed patterns analogous to the 'S' patterns described previously in cultivated forms (Fig. 1b; Ma, 1977; Brown et al., 1981a). The majority of the Middle American wild beans, however, showed phaseolin patterns not encountered among cultivated forms. A sample of these patterns, of which that in lane 1 appeared to be quite common, is shown in Fig. 1a. We propose to call this mixed group of electrophoretic patterns the 'M' (Middle America) types. Approximately one-third of the accessions were heterogeneous and exhibited 2 or more phaseolin patterns (Table 1). Several accessions, such as G12877A, G12911A, and G12914, which showed both 'S' and 'M' patterns, exhibited one or more morphological traits typical of cultivated

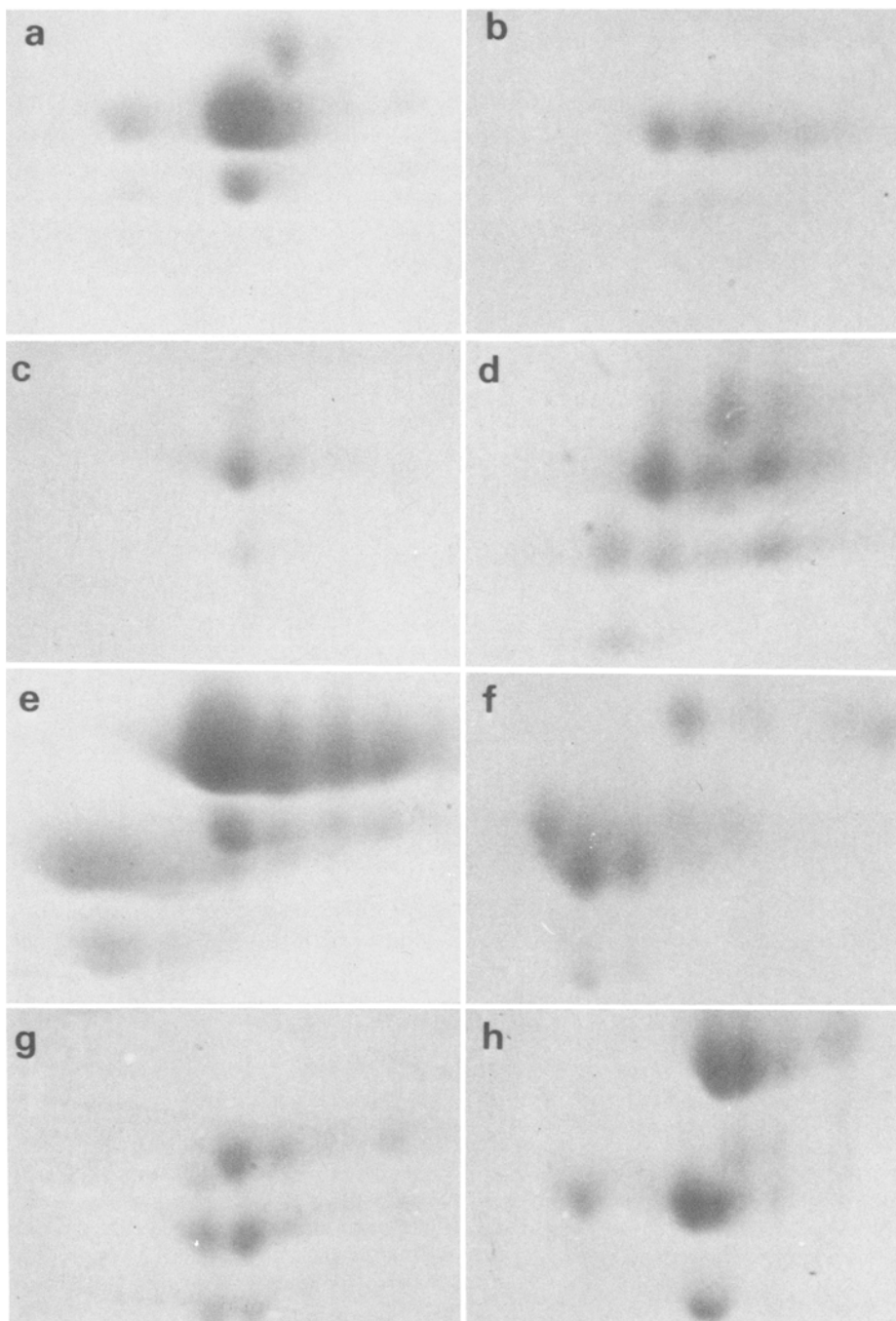


Fig. 2. Two-dimensional IEF-SDS/PAGE of phaseolins from selected wild bean lines from Middle America (a-g) and the Andes (h). a-f: 'M' phaseolin: a) G12864; b) G12949; c) G12891; d) G11034; e) G11050; f) G12877B; g) 'S' phaseolin: G12934; h) 'T' phaseolin: G6388.

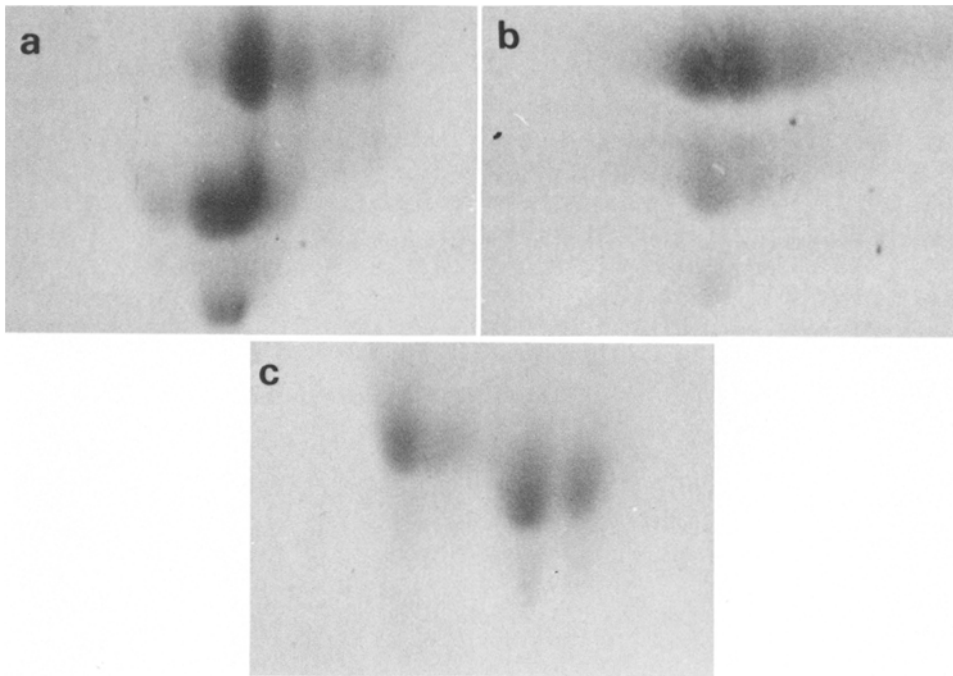


Fig. 3. Two-dimensional IEF-SDS/PAGE of phaseolin mixtures from selected wild bean lines: a) G6388 (‘T’ phaseolin) + Tendergreen (‘T’); b) G11030A (‘M’) + Tendergreen (‘T’); c) G11115 (‘M’) + *P. coccineus* subsp. *coccineus* cv. Painted Lady: the two polypeptides to the right are from *P. coccineus* subsp. *coccineus*.

forms, such as determinate branch termination and large seed size (Vanderborght, 1982).

In contrast to the diversity in phaseolin patterns exhibited by the Middle American wild beans, the 10 Andean wild bean lines—with the exception of accession G12856A—showed only ‘T’ patterns (Fig. 1c, Table 1). Accession G12856A showed both ‘T’ and ‘S’ phenotypes.

Two-dimension electrophoretic analysis of phaseolin in wild bean accessions

Single seeds of selected wild bean accessions from Middle America and Andean South America were analyzed by 2-dimensional IEF-SDS/PAGE. For each of the wild accessions tested, the phaseolin proteins migrated to the same specific area of the gel as reported for phaseolin from common bean cultivars (Brown et al., 1980), indicating that the phaseolins of both wild and cultivated beans had similar molecular weights and isoelectric points.

As expected from the variability observed with 1-dimensional electrophoresis, accessions with ‘M’ phaseolin types showed several 2-dimensional phaseolin patterns (Fig. 2a–f). One pattern was very simple, having only one major and one minor protein subunit (Fig. 2c). Middle American accessions having ‘S’-like phaseolins had 2-dimensional phaseolin patterns (one is shown in Fig. 2g) similar to that reported for Sanilac (Brown et al., 1981a). Two-dimensional IEF-SDS/PAGE confirmed the absence of any ‘T’ or ‘C’ phaseolins among the Middle American

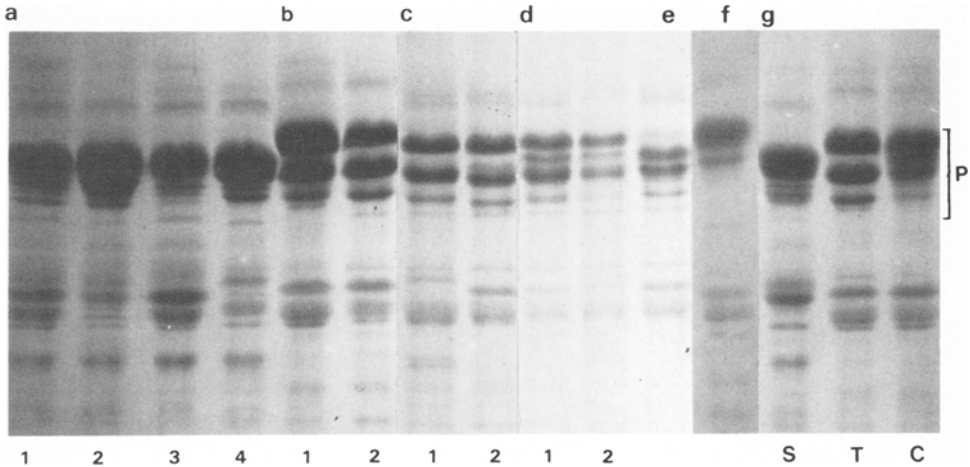


Fig. 4. One-dimensional SDS/PAGE of phaseolin (P) of landraces from Middle America (a-b) and Andean South America (c). a: 'S' phaseolin: 1) DGD78/047A (Mexico); 2) Ver.6-1 (Mexico); 3) DGD78/027 (Mexico); 4) Orguloso (Nicaragua). b: 'T' phaseolin: 1) Mor.20-1 (Mexico); 2) Mex.27-R (Mexico). c: 'T' phaseolin: 1) PI153714 (Peru); 2) PI152206 (Bolivia). d: 'C' phaseolin: 1) PI 151015 (Chile); 2) PI 281984 (Chile). e: 'H' phaseolin: PI 151023 (Chile). f: 'A' phaseolin: 'Ayacucho.' g: S, T and C: references phaseolins of cultivars Sanilac, Tendergreen, and Contender, respectively.

wild lines tested. In particular, some of these wild lines whose one-dimensional phaseolin pattern bore some resemblance to the 'T' phaseolin pattern (Fig. 1a, lanes 14 and 15), were shown to have a 2-dimensional phaseolin pattern quite different from the 'T' type (Fig. 2e, or f). The Andean wild lines on the other hand had a 2-dimensional pattern very similar to the 'T' type (Fig. 2h). Analysis of a mixture of an Andean 'T'-type phaseolin and Tendergreen phaseolin showed that most of the polypeptides of each pattern co-migrated in the mixture (Fig. 3a). One of the 'M' phaseolin types (Fig. 1a, lane 7), which appeared to have a 1-dimensional phaseolin pattern similar to that of some *P. coccineus* subsp. *coccineus* cultivars, is shown in a mixture with *P. coccineus* subsp. *coccineus* cv. Painted Lady phaseolin (Fig. 3c). The phaseolins from these 2 sources had different isoelectric points and slightly different molecular weights (the 2 polypeptides to the right are from *P. coccineus* subsp. *coccineus*).

One-dimensional electrophoretic analysis of phaseolin variability in landraces

When analyzed by 1-dimensional SDS/PAGE, the landraces showed the 3 phaseolin patterns ('S,' Fig. 4a; 'T,' Fig. 4b and c; 'C,' Fig. 4d) previously identified among cultivated forms of the common bean (Ma, 1977; Brown et al. 1981a), and 2 new banding patterns that had not been identified previously among wild or cultivated forms (Fig. 4e,f). We propose to name these the 'H' and 'A' type phaseolins after the Peruvian landraces, 'Huevo de huanchaco' and 'Ayacucho,' respectively, in which they were first observed. Contrary to the wild bean accessions, all the landraces were homogeneous, each showing only a single pattern. However, the frequency of the 5 patterns varied according to the region of origin (Table 3). In Middle America, the 'S' type predominated; of 46 landraces screened, 44 had an 'S' pattern, and 2 had a 'T' pattern. No 'A,' 'C' or 'H' types were found.

TABLE 3. GEOGRAPHIC FREQUENCY DISTRIBUTION OF PHASEOLIN TYPES AMONG LANDRACES FROM MIDDLE AMERICA AND ANDEAN SOUTH AMERICA.

Country of origin	Phaseolin type					Total number of lines
	'S'	'T'	'C'	'H'	'A'	
1. Middle America						
Mexico	33	2	0	0	0	35
Guatemala	5	0	0	0	0	5
El Salvador	1	0	0	0	0	1
Honduras	1	0	0	0	0	1
Nicaragua	3	0	0	0	0	3
Costa Rica	<u>1</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>1</u>
Totals	44 (96%) ^a	2 (4%)	0 (0%)	0 (0%)	0 (0%)	46
2. Andean South America						
Colombia	1	1	0	0	0	2
Ecuador	4	5	1	0	0	10
Peru	4	10	2	2	1	19
Bolivia	1	4	1	0	0	6
Chile	1	3	7	1	0	12
Argentina	<u>0</u>	<u>3</u>	<u>0</u>	<u>1</u>	<u>0</u>	<u>4</u>
Totals	11 (21%)	26 (49%)	11 (21%)	4 (8%)	1 (2%)	53

^a % of total number of lines for the region.

In the Andean countries of South America, 49% of the landraces screened had a 'T' phaseolin pattern, 21% a 'C' pattern, 21% an 'S' pattern, 8% an 'H' pattern, and 2% an 'A' pattern.

Two-dimensional electrophoretic analysis of phaseolins in landraces

Seed proteins of selected landraces from Middle America and Andean South America were analyzed for variation in phaseolin pattern using 2-dimensional IEF-SDS/PAGE. Middle American landraces with 'S' phaseolin types (one is shown in Fig. 5a) showed some variability, but closely resembled the pattern reported for 'Sanilac' (Brown et al., 1981a). The two Middle American landraces with 'T' phaseolin types had the same 2-dimensional phaseolin pattern (gels not shown) as reported previously for 'Tendergreen' (Brown et al., 1981a). Among the Andean landraces, those with the 'C' phaseolin type had a 2-dimensional phaseolin pattern (Fig. 5c) identical to that of 'Contender' (Brown et al., 1981a). The 'H' and 'A' phaseolin types had unique 2-dimensional patterns, which had not been identified previously (Fig. 5d,e). The 2-dimensional pattern of the 'T' phaseolin type found in Andean landraces (Fig. 5b) was identical to that of the wild Andean accessions and closely resembled the 'Tendergreen' pattern (Brown et al., 1981a).

Relationship between seed size and shape and phaseolin-banding pattern in landraces

In both regions, Middle America and the Andes, significant differences in seed sizes were observed among cultivars with different phaseolin types, except for seed height of Middle American 'T' and 'S' phaseolin cultivars (Table 4). 'T,' 'H,' 'C,' and 'A' cultivars tended to have larger seed dimensions than 'S' phaseolin cultivars, with the exception of the cultivar with an 'A' phaseolin where seed length was similar to the seed length of Andean 'S' phaseolin cultivars (Table 5).

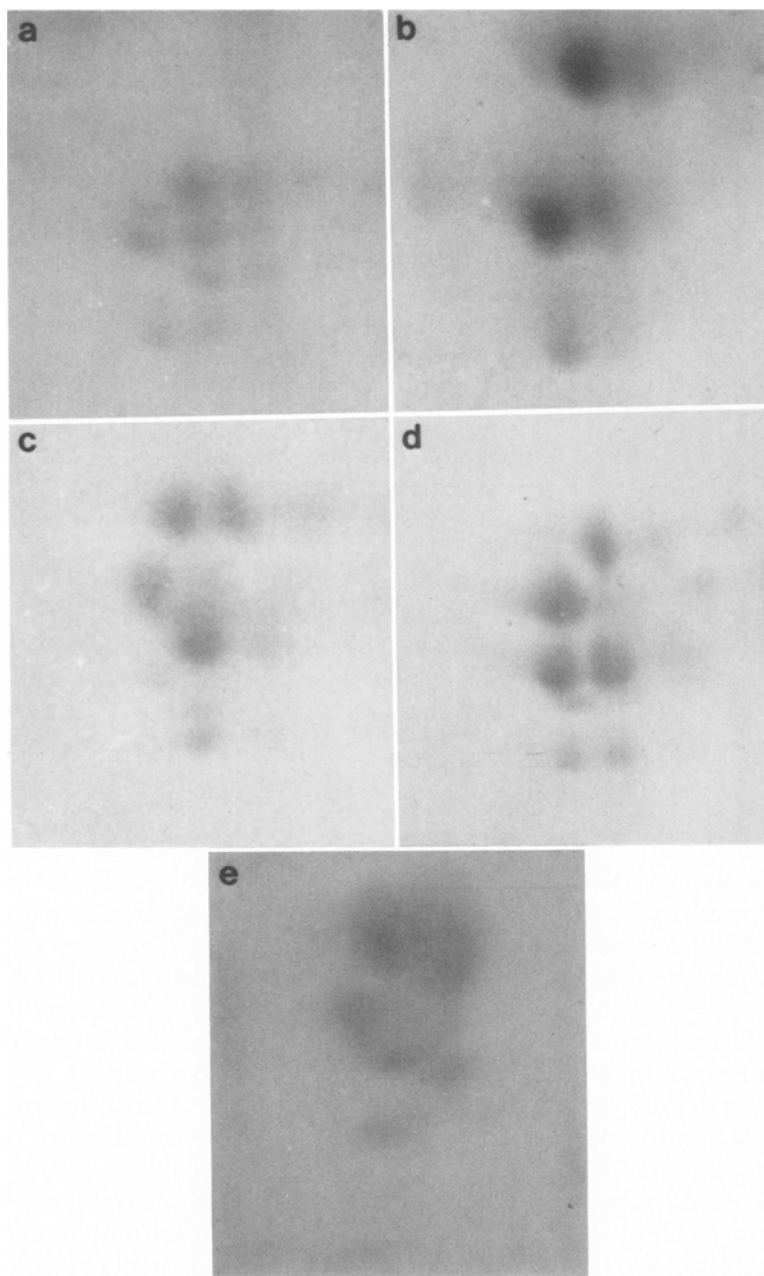


Fig. 5. Two-dimensional IEF-SDS/PAGE of phaseolin from Mexican and Andean landraces: a: 'S' phaseolin: DGD78/027 (Mexico); b: 'T' phaseolin: Cargamanto (Colombia) c: 'C' phaseolin: BN 487 (Chile); d: 'H' phaseolin: PG0139 (Peru); e: 'A' phaseolin: 'Ayacucho' (Peru).

DISCUSSION

Four findings emerge from our results: First, the wild forms of the common bean show much greater diversity in phaseolin patterns than the cultivated forms. While the latter have consistently shown only the 'S,' 'T,' 'C,' 'H,' and 'A' banding patterns (Ma, 1977; Brown et al., 1981a, 1982; our present results on landraces),

TABLE 4. ANALYSIS OF VARIANCE OF SEED SIZES OF CULTIVARS WITH DIFFERENT PHASEOLIN TYPES.

Area	df ^a	F value ^b		
		Length	Height	Width
Middle America	1,39	13.00***	0.03 ns	8.88**
Andes	4,48	7.81***	8.15***	12.76***

^a df: degrees of freedom of the numerator and denominator, respectively.

^b ***, **, *: significant differences at the $P = 0.001$, $P = 0.01$; and $P = 0.05$ levels, respectively; ns: absence of significant differences at the $P = 0.05$ level.

the wild beans exhibited a wide variety of phaseolin patterns, in addition to those observed in the cultivated forms of the species. Approximately one-third of the wild bean accessions were heterogeneous for phaseolin patterns, in contrast to the homogeneity among landraces. This heterogeneity may be attributed to higher levels of cross-pollination among wild beans (Vanderborcht, 1982). For some wild bean accessions, these outcrosses may have involved cultivated types as inferred by their large seed size, their determinate growth habit, and the presence of cultivated phaseolin electrophoretic patterns.

Second, the great variability of phaseolin-banding patterns is found exclusively among wild beans from Middle America. This contrasts with the Andean wild beans of our sample, which have shown only the 'T' type pattern. Accession G12856A, which exhibited both 'T' and 'S' phaseolins, has been characterized by Vanderborcht (1982) as a weedy type and may therefore have resulted from an out-cross between a wild bean with a 'T' phaseolin and a cultivated type with an 'S' phaseolin. However, our sample of Andean wild beans was much smaller than the Middle American sample. A better assessment of the degree of diversity must be postponed until more Andean wild beans become available.

Third, the phaseolin patterns of the landraces display a geographic pattern. The 'S' category predominates in Middle America and the 'T' category prevails in Andean South America. The 'C,' 'H,' and 'A' types were found only among Andean landraces.

Fourth, there seems to be an association between seed size and phaseolin pattern among the landraces. Landraces with banding patterns belonging to the 'T,' 'C,' 'H,' or 'A' categories generally have larger seeds compared to landraces with an 'S' type pattern. No significant seed size differences were observed among landraces with 'T,' 'C,' 'H,' and 'A' phaseolin patterns.

These 4 findings elicit several questions. The first question relates to the limited variation in phaseolin patterns among cultivated forms of the common bean compared to wild forms, especially in Middle America. This contrasts markedly with the large amount of variability observed for the main storage proteins in seeds of cultivated barley (Doll and Brown, 1979; Shewry et al., 1978), maize (Gianazza et al., 1976), wheat (Autran and Bourdet, 1975), peas (Casey, 1979), and soybeans (Mori et al., 1981).

Why are the 'M' types of phaseolin not represented among cultivars? Several hypotheses can be suggested for this absence. Efficient protein deposition leading to higher-yielding genotypes with a higher seed protein content might be associated with the phaseolin types found in the cultivated forms. Cultivars with these

TABLE 5. MEAN SEED DIMENSIONS OF MIDDLE AMERICAN AND ANDEAN LANDRACES WITH DIFFERENT PHASEOLIN TYPES.

Area	Phaseolin type	n	Seed sizes (mm)*		
			Length	Height	Width
Middle America	'T'	2	15.9a	7.4a	6.5a
	'S'	44	11.4b	7.2a	5.1b
Andes	'T'	26	13.6a	8.2ab	6.3b
	'H'	4	12.9ab	9.5a	7.8a
	'C'	11	12.7ab	8.5a	6.6ab
	'S'	11	9.8b	6.5b	4.8c
	'A'	1	9.8b	8.1ab	6.2b

* For each area within columns, values followed by the same letter are not significantly different at the $P = 0.05$ level, according to Duncan's multiple range test (Steel and Torrie, 1980).

phaseolin types would then have been selected by the farmers and gradually would have replaced cultivars with 'M' phaseolin types. Along a similar line, the biological value of the 'S,' 'T,' 'C,' 'H,' and 'A' phaseolin types might be higher than the 'M' phaseolin types, and selection would have favored the cultivars with the biologically more valuable types.

One might also hypothesize a limited number of domestication events involving only genotypes with the 'S,' 'T,' 'C,' 'H,' or 'A' phaseolins. Alternatively, the 'M' types might have arisen after the domestication of the common bean. It seems unlikely, however, that the variability we have observed among 'M' phaseolin types in Middle America would have been generated in the time span of approximately 7,000 yr—age of the earliest archaeological findings (Kaplan, 1965; Kaplan et al., 1973). Lastly, a genetic linkage or an association might have existed between the alleles coding for the 'S,' 'T,' 'C,' 'H,' or 'A' phaseolin types, and a gene or a group of genes determining an essential aspect of the cultivated phenotype, such as the delayed or reduced dehiscence of pods.

The second question relates to the region of domestication of the common bean. Our observations on phaseolin variability in wild forms and landraces suggest 2 hypotheses: either the common bean was domesticated in one of the 2 regions (Middle America or the Andes) and cultivated forms subsequently spread to the other regions, or domestication occurred independently in Middle America and in the Andes.

Under the first hypothesis, domestication in either of the 2 regions (Middle America or the Andes) resulted in small-seeded cultivars with an 'S' phaseolin type and large-seeded cultivars with a 'T,' 'C,' 'H,' or 'A' phaseolin type. Subsequently, the different cultivar types spread to the other region (the Andes or Middle America, respectively). Exchanges between Middle America and the Andes around 2,000–1,000 B.C. have been documented by archeologists. Both Middle America and the Andes have been recognized as domestication centers (Harlan, 1971), and Andean South America is now considered to be a secondary diversification center for maize (Goodman and Bird, 1977). Mackie (1943) has suggested that the lima bean (*Phaseolus lunatus* L.) was domesticated in Middle America from where it spread to Andean South America (Inca Branch). Huauzontle (*Chenopodium nuttaliae*) may actually represent an early migrant quinoa population introduced from the Andes into Mexico (Simmonds, 1976).

Arguing against this hypothesis is the absence of any 'T,' 'C,' 'H,' or 'A' phaseolin types among the Middle American wild beans, or of any 'S' phaseolin-banding pattern among the Andean wild beans (with the exception of G12856A, which may have resulted from a cross with an 'S' phaseolin cultivar). It cannot be excluded, however, that additional samples may reveal the presence of these phaseolin patterns in either the Andes or Middle America.

Under the second hypothesis, domestication in Middle America would have resulted in small-seeded cultivars with 'S' phaseolin patterns, and an independent domestication involving the Andean wild beans would have led to large-seeded cultivars with a 'T' phaseolin type. It is unlikely that the wild beans in either region are actually escapes from cultivation. Cultivated beans have reduced fitness in a wild state mainly because of the lack of seed dormancy, the presence of nonshattering pods, and the nonmimetic seed color. This reduced fitness would make naturalization of cultivars highly unlikely in the absence of wild-growing beans. In addition, the Andean wild beans occur only on the eastern slope of the Andes in a well-defined ecological zone (Berglund-Brücher and Brücher, 1976), whereas escapes from cultivation would be expected to occur more widely in the bean-growing areas of the Andes. The most ancient archaeological findings consist of cultivated forms and have been dated to about 5,000 B.C., which is well before the first documented exchanges between Middle America and the Andes (Kaplan, 1967; Kaplan et al., 1973).

Our results show a broad correspondence between the phaseolin patterns of the wild forms and the landraces within both Middle America and the Andes. The presence of the 'S' phaseolin type in Middle American wild beans and the 'T' phaseolin type among Andean wild beans is reflected in the predominance of landraces with an 'S' phaseolin type in Mexico and Central America and with a 'T' type in the Andes. The 2 Mexican cultivars with a 'T' phaseolin banding pattern (Mex. 27-R and Mor. 20-1) have significantly larger seeds than the Mexican 'S'-type landraces and might represent introductions from the Andes. The 'S' phaseolin-type landraces from the Andes are all small-seeded and might represent introductions from outside the Andean region. All the white small-seeded cultivars in our sample—PI 152322 and PI 299021 from Ecuador, PI 290996 and PI 415953 from Peru—showed an 'S' phaseolin type. One of them, PI 290996, is named Panamito, which might reflect its Central American origin. Likewise, the 2 black-seeded cultivars studied (PI 152326 from Ecuador and PI 306149 from Peru), and the two Bayo types (PI 156669A from Bolivia and PI 151017 from Chile) also showed an 'S' phaseolin pattern and might have been introduced from Venezuela and Mexico, respectively, where these seed types are prevalent. Overall, the data from our study favor 2 primary independent centers of domestication for the common bean. In Middle America, domestication would have given rise to small-seeded cultivars with an 'S' phaseolin-banding pattern and, in the Andes, domestication would have led to large-seeded cultivars with a 'T' phaseolin type.

Independent domestication events for the common bean have been suggested previously by Evans (1976) on the basis of seed size, with small-seeded cultivars having been domesticated in Middle America and large-seeded cultivars in the Andes. As pointed out by Heiser (1965), plant domestication has followed parallel paths in Middle America and in South America. Several genera contain species that were domesticated independently in these 2 regions, e.g., *Amaranthus*, *Cap-*

sicum, *Cucurbita*, *Gossypium*, and *Pachyrrhizus*. Independent domestications have also been suggested for cassava (*Manihot esculenta*) and sweet potato (*Ipomoea batatas*).

It is most likely that there is a relationship between the Andean and Middle American phaseolin patterns. A Middle American accession that was heterozygous for 'S'-like and 'M' phaseolin types has a pattern identical to that of Contender. Polypeptides of a common 'M' phaseolin type co-migrated with polypeptides in the 'Tendergreen' pattern (Fig. 3b). These findings reflect the fact that Andean and Middle American wild beans had a common progenitor.

The events leading to the appearance of the cultivated types with 'C,' 'H,' and 'A' phaseolin types cannot be ascertained from our data. They could have been domesticated in Middle America from where they would have spread to the Andes. However, no 'C,' 'H,' or 'A' phaseolin types were found among wild or landrace accessions from Middle America. Alternatively, they could have appeared in the Andes either before or after domestication. Although the 'C,' 'H,' and 'A' phaseolin types were not observed in wild Andean accessions, the number of Andean accessions available for study was small. Further collection of wild Andean accessions may uncover 'C,' 'H,' and 'A' phaseolin types within this group. Brown et al. (1981a) have suggested that the 'C' phaseolin genotype might have appeared through a translocation or an unequal crossing-over in a hybrid between 2 lines having 'T' and 'S' patterns. This event might have occurred after the introduction of cultivars with 'S' phaseolin types into the Andean region.

Our study demonstrates the usefulness of electrophoretic techniques applied to seed proteins for the study of domestication of the common bean. It emphasizes the importance of wild beans and landraces and stresses the need for additional germplasm collections especially of wild beans in the Andes.

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LITERATURE CITED

- Autran, J. C., and A. Bourdet. 1975. L'identification des variétés de blé: établissement d'un tableau général de détermination fondé sur le diagramme électrophorétique des gliadines du grain. *Ann. Amélior. Pl.* 25: 277-301.
- Baudet, J. C. 1977. Origine et classification des espèces cultivées du genre *Phaseolus*. *Bull. Soc. Roy. Bot. Belgique* 110: 65-76.
- Berglund-Brücher, O., and H. Brücher. 1976. The South American wild bean (*Phaseolus aborigineus* Burk.) as ancestor of the common bean. *Econ. Bot.* 30: 257-272.
- Bliss, F. A., and J. W. S. Brown. 1983. Breeding common bean for improved quantity and quality of seed protein. In J. Janick, ed, *Plant Breeding Reviews*, Vol. 1, p. 59-102. AVI, Westport, CT.
- Brown, J. W. S., F. A. Bliss, and T. C. Hall. 1980. Microheterogeneity of globulin-1 storage protein of French bean with isoelectrofocusing. *Pl. Physiol.* 59: 838-840.

- , Y. Ma, F. A. Bliss, and T. C. Hall. 1981a. Genetic variation in the subunits of globulin-1 storage protein of French bean. *Theor. Appl. Genet.* 59: 83–88.
- , F. A. Bliss, and T. C. Hall. 1981b. Linkage relationships between genes controlling seed proteins in French beans. *Theor. Appl. Genet.* 60: 251–259.
- , J. R. McFerson, F. A. Bliss, and T. C. Hall. 1982. Genetic divergence among commercial classes of *Phaseolus vulgaris* in relation to phaseolin pattern. *HortScience* 17: 752–754.
- Brücher, H. 1969. Die Evolution der Gartenbohne *Phaseolus vulgaris* L. aus der südamerikanischen Wildbohne *Ph. aborigineus* Burk. *Angew. Bot.* 42: 119–128.
- Burkart, A. 1943. Las Leguminosas Argentinas Silvestres y Cultivadas. Acme, Buenos Aires, Argentina.
- and H. Brücher. 1953. *Phaseolus aborigineus* Burkart, die mutmassliche andine Stammform der Kulturbohne. *Züchter* 23: 65–72.
- Casey, R. 1979. Genetic variability in the structure of the α -subunits of legumins from *Pisum*—a two-dimensional gel electrophoresis study. *Heredity* 43: 265–272.
- Doll, H., and A. H. D. Brown. 1979. Hordein variation in wild barley. *Canad. J. Genet. Cytol.* 21: 391–404.
- Evans, A. M. 1976. Beans. In N. W. Simmonds, ed, *Evolution of Crop Plants*, p. 168–172. Longman, London, England.
- Gentry, H. S. 1969. Origin of the common bean, *Phaseolus vulgaris*. *Econ. Bot.* 23: 55–69.
- Gianazza, E., P. G. Righetti, F. Pioli, E. Galante, and C. Soave. 1976. Size and charge heterogeneity of zein in normal and opaque-2 endosperms. *Maydica* 21: 1–17.
- Goodman, M. M., and R. M. Bird. 1977. The races of maize. IV. Tentative grouping of 219 Latin American races. *Econ. Bot.* 31: 204–221.
- Harlan, J. R. 1971. Agricultural origins: Centers and noncenters. *Science* 174: 468–474.
- . 1976. *Crops and Man*. American Society of Agronomy, Madison, WI.
- Heiser, C. B. 1965. Cultivated plants and cultural diffusion in nuclear America. *Amer. Anthropol.* 67: 930–949.
- Kaplan, L. 1965. Archaeology and domestication in American *Phaseolus* (beans). *Econ. Bot.* 19: 358–368.
- . 1967. Archaeological *Phaseolus* from Tehuacan. In D. E. Beyers, ed, *The Prehistory of the Tehuacán Valley*. Vol. 1, Environment and subsistence, p. 201–211. Univ. Texas Press, Austin, TX.
- , T. F. Lynch, and C. E. Smith. 1973. Early cultivated beans (*Phaseolus vulgaris*) from an intermontane Peruvian valley. *Science* 179: 76–77.
- Laemmli, U. K. 1970. Cleavage of structure proteins during assembly of the head of bacteriophage T4. *Nature* 22: 680–685.
- Ma, Y. 1977. Improvement of Nutritive Value of Dry Bean Seeds (*Phaseolus vulgaris*). Ph.D. Thesis, Univ. Wisconsin–Madison, Madison, WI.
- , and F. A. Bliss. 1978. Seed proteins of common bean. *Crop Sci.* 18: 431–437.
- Mackie, W. W. 1943. Origin, dispersal and variability of the Lima bean, *Phaseolus lunatus*. *Hilgardia* 15: 1–24.
- Miranda Colín, S. 1967. Origen de *Phaseolus vulgaris* L. (frijól común). *Agrociencia* 1: 99–109.
- . 1979. Evolución de *Phaseolus vulgaris* y *P. coccineus*. In E. M. Engleman, ed, *Contribuciones al Conocimiento del Frijól (Phaseolus) en Mexico*, p. 83–99. IEICA, Chapingo, Mexico.
- Mori, T., S. Utsumi, H. Inaba, K. Kitamura, and K. Harada. 1981. Differences in subunit composition of glycinin among soybean cultivars. *J. Agric. Food Chem.* 29: 20–23.
- Shewry, P. R., H. M. Pratt, and B. J. Mifflin. 1978. Varietal identification of single seeds of barley by analysis of hordein polypeptides. *J. Sci. Food Agric.* 29: 587–596.
- Simmonds, N. W. 1976. Quinoa and relatives. In N. W. Simmonds, ed, *Evolution of Crop Plants*, p. 29–30. Longman, London, England.
- Steel, R. G. D., and J. H. Torrie. 1980. *Principles and Procedures of Statistics*. McGraw-Hill, New York.
- Vanderborcht, T. 1982. Seed increase and evaluation of the wild *Phaseolus vulgaris* L. germplasm. Mimeographed, Centro Int. Agric. Trop., Cali, Colombia.
- . 1983. Evaluation of *Phaseolus vulgaris* wild types and weedy forms. *Pl. Genet. Res. Newslett.* 54: 18–24.