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Allozyme evidence supporting southwestern Europe as a secondary center of genetic diversity for the common bean

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Abstract Genetic diversity within a common bean (*Phaseolus vulgaris* L.) collection, comprising 343 accessions from the Iberian Peninsula, was examined using six allozyme markers. Two major clusters corresponding to the Andean and Mesoamerican gene pools were identified. Both gene pools were characterized by specific alleles, with the former exhibiting $Skdh^{100}$, Me^{100}, $Rbcs^{100\ or\ 98}$ and $Diap\text{-}1^{100}$, and the latter exhibiting $Skdh^{103}$, Me^{100}, $Rbcs^{100}$ and $Diap\text{-}1^{95}$. Some accessions from both clusters, deviating from these allozyme patterns, exhibited $Skdh^{100}$, Me^{100}, $Rbcs^{100}$ and $Diap\text{-}1^{95}$ or $Skdh^{103}$, Me^{100}, $Rbcs^{100}$ and $Diap\text{-}1^{100}$ allozyme profiles and were considered as putative hybrids.The levels of genetic variation has not been eroded since the introduction of the common bean from the American centers of domestication to the Iberian Peninsula. Instead, obvious signs of introgression between the two gene pools were observed, mainly among white-seeded genotypes. The intermediate forms adapted to the Iberian Peninsula could have emerged from initial recombination between Mesoamerican and Andean gene pools. The Iberian common bean germplasm is therefore more complex than previously thought, and contains additional diversity that remains to be explored for genetic and breeding purposes. The Iberian Peninsula could be considered as a secondary center of genetic diversity of the common bean, especially the large white-seeded genotypes.

Keywords *Phaseolus vulgaris* · Genetic diversity · Gene pool · Allozymes · Recombinants

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Introduction

The common bean (*Phaseolus vulgaris* L.) was domesticated in two distinct regions of the New World, one in Mesoamerica (principally Mexico) and another along the eastern slope of the Andes in south America (southern Peru, Bolivia and northwestern Argentina) (Gepts et al. 1986). An additional minor domestication center has been suggested in Colombia, although it is not entirely clear whether this area constitutes a center of domestication or a region of gene flow between wild and domesticated beans (Gepts and Bliss 1986; Beebe et al. 1997). Evidence supporting the divergence of the two major domesticated gene pools came originally from studies of variability in seed size (Evans 1973), phaseolin (the major storage seed protein) (Gepts et al. 1986), morphological (Singh et al. 1991b), isozyme (Koenig and Gepts 1989; Singh et al. 1991a) and DNA markers (Becerra Velásquez and Gepts 1994; Haley et al. 1994). Most cultivars from either the Mesoamerican or the Andean region contain characteristics that are found in wild accessions from the same area, but not in either domesticated or wild accessions from the other gene pool (Koenig and Gepts 1989). In addition to these two major gene pools, recently discovered wild populations constitute a third gene pool located in Ecuador and northern Peru (Debouck et al. 1993; Kami et al. 1995; Tohme et al. 1996). These ancestral populations were not involved in domestication (Debouck et al. 1993) as shown by their phaseolin type, which is absent from the domesticated gene pool.

The divergence between the Andean and Mesoamerican gene pools has implications for bean breeding that have not yet been fully explored. Despite their partial reproductive isolation (Singh and Gutiérrez 1984; Gepts and Bliss 1985; Koinange and Gepts 1992), the two gene pools still belong to the same biological species. Viable and fertile progeny can be obtained, and therefore, genes can be transferred between the two pools, although the transfer of quantitative traits appears to be problematic. Attempts to recombine desirable traits between both

gene pools, such as the large seed size of the Andean gene pool with the yield potential of the Mesoamerican gene pool, have generally failed (Nienhuis and Singh 1986), although there are notable exceptions. The success of Beaver and Kelly (1994), who employed recurrent selection strategies, and of snap bean breeders (Skroch and Nienhuis 1995), suggests that inter-gene pool hybridization could be a valuable resource for bean breeders, although these intermediate forms are attributed to breeding purposes. A first exception is provided by Chilean landraces which showed signs of introgression (Paredes and Gepts 1995) from the Mesoamerican gene pool. This introgression may have taken place between Andean genotypes of race Chile and Mesoamerican genotypes of race Durango and was not due to breeding efforts.

Some limited bean germplasm exchange has taken place in pre-Columbian times between Mesoamerica and south America, but much-more extensive seed movement occurred after the 1500s. Seed exchanges with Europe must have happened since the first visits of Europeans to the Americas. The common bean was introduced into the Iberian Peninsula (Spain and Portugal), mainly from Central America around 1506 (Ortwin-Sauer 1966) and from the southern Andes after 1532, through sailors and traders who brought the nicely coloured, easily transportable seeds with them as a curiosity (Brücher and Brücher 1976; Debouck and Smartt 1995). The principal cultivated bean types in this area are large-seeded white and coloured cultivars of Andean origin and belonging to the white-kidney, canellini, marrow, "Favada", large cranberry, cranberry, red-pinto and "Canela" market classes, and the medium-seeded white and coloured cultivars of Mesoamerican origin and corresponding to the great northern and pinto market classes (Santalla et al. 2001). Most of the Andean cultivars are considered to be closely related and they belong to the race Nueva Granada as described by Singh et al. (1991a), except for the marrow class, which corresponds to the race Chile. The race Nueva Granada is composed principally of growth habit Types I, II and III, whereas the race Chile presents a Type III, and is adapted to moderately wet and cold temperature. Mesoamerican cultivars belong to the race Durango, which is characterized by a Type III growth habit and adaptation to dry highland areas.

Previous studies have indicated that most of the Iberian cultivars may have been introduced from Chile due to a high frequency of the "C" phaseolin pattern (Gepts and Bliss 1988; Gil and Ron 1992) although other studies (Escribano et al. 1998) indicated a high frequency of the "T" phaseolin pattern, which was also observed in western Europe (Gepts and Bliss 1988). Subsequently, the Iberian peninsula landraces could have been introduced in other parts of Europe such as Greece, Cyprus and Italy, as indicated by the high proportions of "T" and "C" types in these areas (Lioi 1989; Limongelli et al. 1996). These studies have provided evidence for the existence in the Iberian Peninsula of the two major gene pools, Andean and Mesoamerican. The variation in bean-growing environments, cropping systems and consumer preferences for seed types in this area might have played a significant role in the common bean crop diversity and could give rise to the preservation of a large variation in the domesticated common bean's characteristics. Hence, bean populations cultivated in the Iberian Peninsula might find their roots back in the Americas with isozyme markers being a very efficient tool to study crop evolution (Doebley 1990) and the genetics of populations (May 1992). Additional information and understanding about the genetic diversity and the organization of the common bean crop in the Iberian Peninsula is required for its efficient management and use in breeding. The objectives of the present study were to conduct an extensive survey of the common bean germplasm for allozyme variation, to calculate frequencies of Andean and Mesoamerican accessions, and to examine the evidence for new genetic diversity in the common bean crop in southwestern Europe.

Materials and methods

Plant materials

From the Misión Biológica de Galicia-CSIC germplasm collection, 316 common bean landraces were studied, which had been collected in the main dry bean production regions of the Iberian Peninsula (see Fig. 1A). In this study, landraces were defined as cultivars locally adapted to the low input agriculture generally practised by small farmer holders in Spain and Portugal. These landraces were classified according to Voysest (2000) and Santalla et al. (2001) into eight different dry bean market classes of seed colour groups (Table 1). This classification by market class is important to highlight breeding efforts in some particular bean types. Twenty seven commercial cultivars representing all the different dry bean market classes were also included.

Isozyme assays

For each accession, at least 12 seeds were sown and plant and root tissues, collected at the first leaf stage (approximately 20 days after sowing), were analysed. A crude tissue homogenate was produced by grinding the leaf or root apex tissue (depending on the enzymes assayed) in a potassium phosphate grinding buffer 0.1 M pH 7.0 containing 20% sucrose (w/v), 5% PVP-40, 0.5% Triton X-100 and 14 mM of 2-mercaptoethanol. The homogenate, absorbed onto paper wicks, was loaded on a 12% starch gel and subjected to electrophoresis in a lithium borate/tris citrate discontinuous system. Wicks from 24 samples along with one check were inserted into a vertical slice 4 cm from the base of the gel. Electrophoresis was carried out at 25 mA for 20 min to load the proteins into the gel. The wicks were then removed and electrophoresis resumed at 30 mA. After the Borate front migrated 8.0 to 9.0 cm, both anodal and cathodal sections of a gel slice 1.5 mm thick were placed in a tray along with the enzyme assayed. The gels were incubated in the dark at 37 °C or at room temperature depending on the enzyme assayed, and scored after 2 h. Following preliminary assays with 12 enzyme systems to determine the plant tissue with maximum enzyme expression and the polymorphism observed in the accessions, six enzyme systems were assayed: malic enzyme (*Me*; E.C.1.1.1.40), shikimate dehydrogenase (*Skdh*, E.C.1.1.1.25), ribulose bisphosphate carboxylase (*Rbcs*, E.C.4.1.1.39), peroxidase (*Prx*, E.C. 1.11.1.7), malate dehydrogenase (*Mdh*, E.C.1.1.1.37) and diaphorase (*Diap*, E.C.1.6.99). The *Mdh* and *Diap* enzyme systems each had two independent loci. Loci were labelled se-

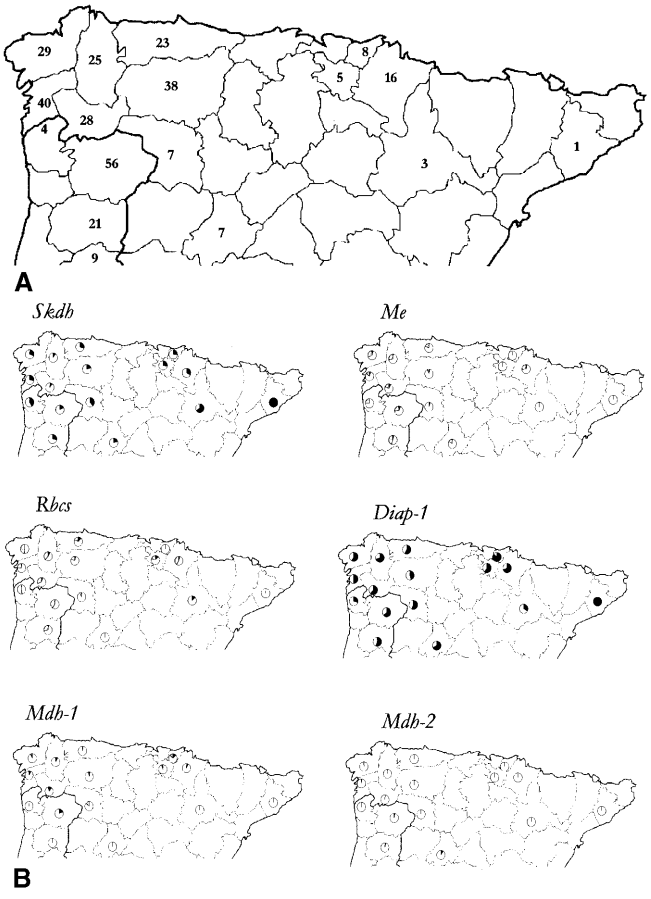

Fig. 1 **A** Iberian Peninsula regions from which common bean germplasm was sampled. The number of accessions studied per region is indicated. **B** Distribution of allozymes into major dry bean regions from the Iberian Peninsula. Skdh: solid Skdh^{103}, open Skdh^{100}; Me: solid Me^{102}, open Me^{100}, shaded Me^{98}; Rbcs: solid Rbcs^{102}, open Rbcs^{100}, shaded Rbcs^{98}; Diap-1: solid Diap-1^{100}, open Diap-1^{95}; Mdh-1: solid Mdh-1^{103}, open Mdh-1^{100}, shaded Mdh-1^{98}; Mdh-2: solid Mdh-2^{102}, open Mdh-2^{100}

quentially, with those migrating closest to the anodal end being designated as number 1 (Koenig and Gepts 1989). The most common allele was designated as 100 and all other alleles were measured in millimetres from the standard. In each gel, the cultivar ICA-Pijao was included as a standard. ICA-Pijao has the following genotype at the polymorphic enzyme loci: *Rbcs^{100}*, *Skdh^{103}*, *Prx^{98}*, *Me^{100}*, *Mdh-1^{100}*, *Mdh-2^{100}*, *Diap-1^{95}* and *Diap-2^{105}*.

Five seeds per accession were used for polyacrilamide gel-electrophoresis analysis of phaseolin seed-storage proteins following the procedures of Brown et al. (1981) and electrophoresed by one-dimensional SDS/PAGE according to the method of Laemmli (1970) as modified by Ma and Bliss (1978). The phaseolin phenotypes of the accessions were scored by comparing the patterns to those of reference genotypes (Boyaca 22-"B" phaseolin pattern, Sanilac-"S" phaseolin pattern, Contender-"C" phaseolin pattern, Tendergreen-"T" phaseolin pattern and Huevo de Huanchaco-"H" phaseolin pattern).

Table 1 Iberian cultivated common bean accessions used in this study

Colour group	Seed size[a]	Market class	Number of accessions[b]
1. White	Small	Small white	7
		Navy	8
	Medium	Great northern	29
		Marrow	23
		Hook	2
	Large	Large great northern	12
		Canellini	51
		White kidney	15
		"Favada"	16
1. White (bi-coloured)	Large	"Hen Eye"	1
		Rounded caparrón	3
		Red caparrón	2
		Kidney caparrón	1
		"Favada Pinto"	2
2. Cream	Small	"Carioca"	1
		Mulatinho	3
	Medium	Pinto	4
		"Sargaçco"	3
	Large	Dark garbanzo	3
		Mottled canellini	3
		"Ojo de Cabra"	13
		"Viscado"	2
		"Bayo Gordo"	19
		Cranberry	14
		"Canela"	23
		Large cranberry	18
3. Yellow	Medium	Small yellow	2
		"Garbancillo"	2
	Large	"Canario Bola"	4
		Azufrado	4
4. Brown	Small	Chumbinho	1
		Brown marrow	5
	Large	Brown garbanzo	1
		Brown mottled	3
		Manteca	2
5. Pink	Large	Rosada	12
		Light red kidney	1
6. Red	Small	Small red	1
		"Guernikesa"	6
	Medium	"Sangretoro"	2
		Dark red kidney	8
	Large	Red pinto	7
		Large red mottled	1
7. Purple	Medium	Morado	1
		Purple caparrón	2
8. Black	Small-opaque	Black turtle	3
	Medium-brilliant	Negro brillante	14
		Black canellini	1

[a] Seed size: small (<25 g/100 seeds), medium (25–40 g/100 seeds) and large (>40 g/100 seeds)
[b] Some accessions are a mixture of seed types

Statistical analysis

Estimation of the frequency of allozymes was limited to those accessions that showed consistent and scorable polymorphism. Allele frequencies were estimated after grouping bean accessions according to their electrophoretic seed-protein pattern (Mesoamerican and Andean groups), geographical origin and economical status (landraces and commercial cultivars). Nei's (1973) genetic diversity statistics were used to measure the total genetic diversity (Ht) of the allozyme data as well as the intra-population differences (Hs) for each polymorphic locus. Gene diversity due to variation among accessions (Dst) was related to the total diversity to

Table 2 Distribution of allozyme variants in cultivated common bean from the Iberian Peninsula according to the phaseolin pattern

Origin[a]	n[b]	*Skdh*		*Me*			*Rbcs*			*Diap-1*		*Mdh-1*			*Mdh-2*	
		103	100	102	100	98	102	100	98	100	95	103	100	98	102	100
M	76	0.64	0.36	0.14	0.82	0.04	0.03	0.89	0.08	0.13	0.87	0.06	0.90	0.04	0.02	0.98
A	285	0.12	0.88	0.02	0.73	0.25	0.04	0.59	0.38	0.71	0.29	0.08	0.91	0.02	0.01	0.99

[a] M, Mesoamerican phaseolin pattern ("S" and "B" types); A, Andean phaseolin pattern ("C", "T" and "H" types)
[b] Total number of accessions analyzed

determine the proportion residing among accessions (Gst). A dendrogram based on Nei's (1973) genetic distance was constructed according to the unweighted paired group method or UPGMA (Sneath and Sokal 1973). Allozyme frequencies were also analysed by principal component analysis. The multivariate distance approach permits one to identify identical genotypes which can give rise to similar phenotypes and to identify divergent genotypes which can also give rise to similar phenotypes.

Results and discussion

The use of allozymes combined with phaseolin-seed protein patterns provided a clear picture of genetic diversity in the cultivated common bean in the Iberian Peninsula. The total genetic diversity (Ht) was 0.317 for the entire array of accessions included in this study, which was higher than that found in the wild common bean but was similar to that of other *Phaseolus* species (Koenig and Gepts 1989; Maquet et al. 1997). However, the total genetic diversity could be overestimated because only isozymes that had been shown to be polymorphic were included. There was little within-accession diversity (Hs = 0.052) but between-accession genetic diversity (Dst = 0.265) was moderate. Koenig and Gepts (1989) also reported low intrapopulation genetic diversity (Hs = 0.006) in the common bean. Loveless and Hamrick (1984) indicated that annual selfing species have few alleles per locus and more skewed allele frequencies, a pattern that is indicative of reduced gene flow. Hartl and Clark (1989) showed that the drift in a selfing breeding system is also more pronounced in small populations, a situation commonly found in the Iberian common bean landraces, which are mainly cultivated in small gardens as subsistence agriculture (Ron et al. 1997).The coefficient of gene differentiation among populations (Gst) was 0.780, which measures the proportion of variation among populations relative to the total species diversity, and was higher than that observed in lima bean (Maquet et al. 1997).

An analysis of genetic diversity was performed among groups of accessions based on the phaseolin seed-protein pattern (Mesoamerican phaseolin pattern = "S"and "B" types, and Andean phaseolin pattern = "T", "C" and "H" types) and commercial status (landraces and commercial cultivars). The total genetic diversity of the Andean landraces (Ht = 0.288) was similar to that of Mesoamerican Landraces (Ht = 0.253) and Andean commercial cultivars (Ht = 0.255) but higher than that of Mesoamerican commercial cultivars (Ht = 0.177). The reduced genetic diversity found among recent Mesoamerican commercial cultivars could be attributed to consumer preferences and have serious consequences for bean breeding. The establishment of new market class cultivars to satisfy consumer preferences (e.g. seed colour and size) could increase genetic drift and inbreeding in Mesoamerican germplasm. A similar reduction in genetic diversity within USA cultivars was observed by McClean et al. (1993) and Sonnante et al. (1994). Furthermore, the genetic diversity was higher in the north and northwest of the Iberian Peninsula than in the other dry bean areas (Fig. 1B), although it could be because the number of accessions from those regions was higher. *Me*^{102}, *Rbcs*^{102} and *Mdh-1*^{103} alleles were mainly found in accessions from the northwest and north of the Iberian Peninsula, and the *Mdh-2*^{102} allele was only found in three Iberian regions. Other allozymes showed a less-pronounced geographical pattern.

The Andean and Mesoamerican accessions from the Iberian Peninsula exhibited contrasting alleles at the *Skdh*, *Diap-1*, *Me* and *Rbcs* loci (Table 2). This supported the earlier reports by Sprecher (1988) and Singh et al. (1991a) in the cultivated common bean from Malawi, and from Mexico to Argentina and Chile, respectively. Approximately, 90% and 70% of the Andean accessions showed the *Skdh*^{100} and *Diap-1*^{100} alleles, respectively, and 60% and 90% of the Mesoamerican accessions exhibited the *Skdh*^{103} and *Diap-1*^{95} alleles, respectively. The *Me*^{102} allele was mainly found in Mesoamerican germplasm while the *Me*^{98} and *Rbcs* ^{98} alleles were detected in Andean germplasm, although these alleles were observed in a low frequency. Two isozyme loci (*Prx* and *Diap-2*) did not show any polymorphism among the accessions examined. The *Diap-2*^{105} allele was not found in the genetic material studied, which may be attributed to an insufficient sampling of Mesoamerican accessions because this allele had only been described in the cultivated common bean from race Mesoamerica (Singh et al. 1991b) and in the wild common bean from Ecuador (Debouck et al. 1993).

A total of 200 accessions, each with its distinctive allele combination at the six isozyme loci, was identified among the 343 cultivated accessions studied. Because several identical allelic combinations were observed in more than one accession, only one accession per allelic combination was included in the cluster analysis. The cluster analysis based on Nei's (1973) genetic distance

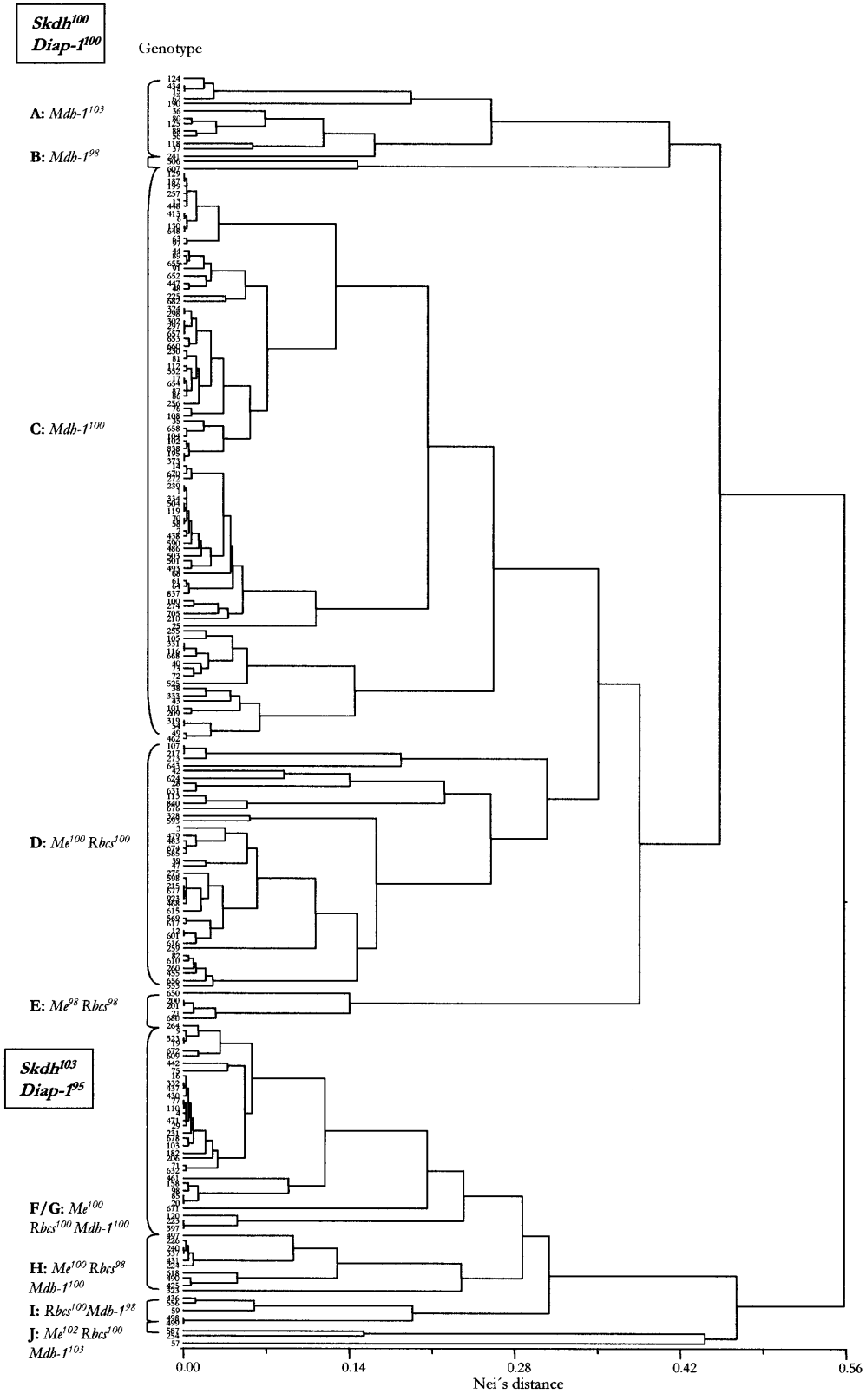

Fig. 2 Dendrogram of allozyme diversity in the cultivated common bean from the Iberian Peninsula. To the left of the dendrogram is the number of the cultivated accession. Letters *A to J* indicate clusters of accessions sharing a common allozyme profile (as indicated to the right of the cluster letter)

(Fig. 2) revealed the existence of two major groups of accessions. Such a cluster can be interpreted as the result of two independent domestications, as was found in previous allozyme studies by Koenig and Gepts (1989) and Singh et al. (1991a), and has provided evidence that the genetic diversity in common bean is organized into two major groups, Mesoamerican and Andean forms. Most of the landraces of the upper group originated in the An-

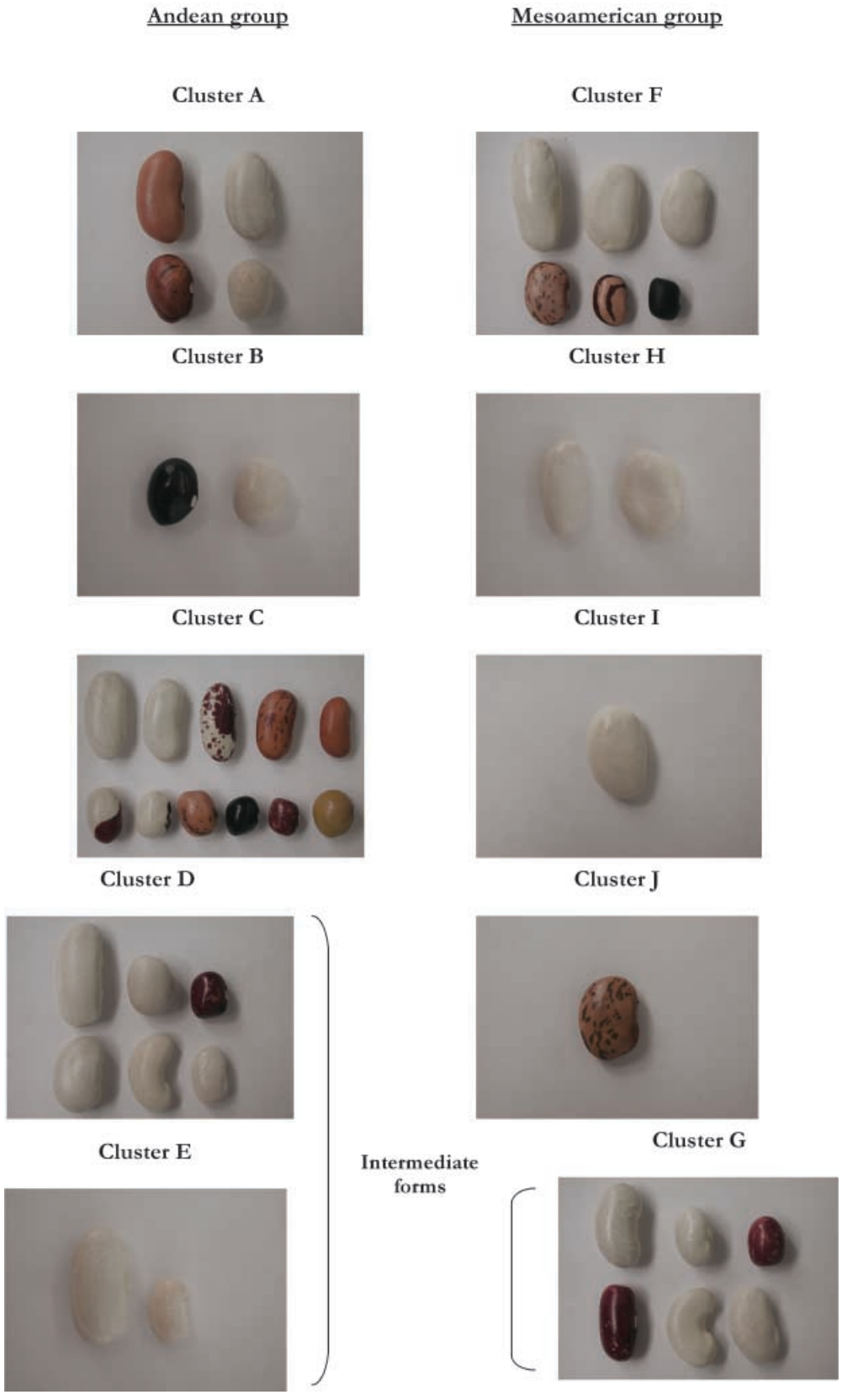

Fig. 3 Representative bean market classes included in each cluster

Table 3 Clusters of cultivated common bean from the Iberian Peninsula based on a characteristic common allozyme

Clusters	Number of accessions	Characteristic allozyme	Phaseolin pattern[a]	Representative	
				Market classes	Accessions
	Andean group (*Skdh*^{100} *Diap-1*^{100})				
A	17	*Mdh-1*^{103}	T, H	"Canela", white kidney, marrow, "Ojo de Cabra"	124, 53, 241, 434
B	2	*Mdh-1*^{98}	T, C	Marrow, negro brillante	506, 607
C	174	*Mdh-1*^{100}	T, C, H	Canellini, "Favada Pinto", "Favada", "Canela", large cranberry, cranberry, red caparrón, "Hen Eye", negro brillante, bayo gordo, "Guernikesa"	838, 413, Collacia, 373, 648, 298, 404, 17, 502, 837, 486
	Intermediate forms (*Skdh*^{100} *Diap-1*^{95})				
D	62	*Me*^{100} *Rbcs*^{100}	T, C, H, S	"Favada", "Guernikesa", marrow, hook, great northern, small white	917, 631, 589, 593, 610, 676
E	7	*Me*^{98} *Rbcs*^{98}	T, C, B	Canellini, small white	201, 551
	Mesoamerican group (*Skdh*^{103} *Diap-1*^{95})				
F	55	*Me*^{100} *Rbcs*^{100} *Mdh-1*^{100}	S, T, H	Black turtle, "Carioca", "Favada", great northern, large great northern, pinto	397, 4, Andecha, 609, 55, 75
H	5	*Me*^{100} Rbcs^{98} *Mdh-1*^{100}	S, T	Canellini, large great northern	499, 59
I	2	*Rbcs*^{100} *Mdh-1*^{98}	S	Great northern	587
J	1	*Rbcs*^{100} *Me*^{102} *Mdh-1*^{103}	S	Pinto	57
	Intermediate forms (*Skdh*^{103} *Diap-1*^{100})				
G	18	*Me*^{100} *Rbcs*^{100} *Mdh-1*^{100}	S, T, C	Canellini, white kidney, "Guernikesa", red pinto, hook, large great northern	555, 240, 490, 337, 623, 224

[a] M, Mesoamerican phaseolin pattern ("S" and "B" types); A, Andean phaseolin pattern ("C", "T" and "H" types)

des, having an Andean seed-protein pattern and *Skdh*^{100} and *Diap-1*^{100} alleles. However, the landraces of the lower group originated in Mesoamerica with a Mesoamerican seed-protein pattern and *Skdh*^{103} and *Diap-1*^{95} alleles. The Andean and Mesoamerican groups could be classified into clusters of accessions which shared a common allozyme profile. Table 3 and Fig. 3 provide the summary of five cluster groups in the Andean and Mesoamerican cultivars, each with their characteristic allozyme profile and representative cultivars.

Accessions in clusters A, B and C displayed a true Andean allozyme and seed protein pattern, except for two accessions which had a Mesoamerican seed protein pattern ("S" type) and belonged to the great northern and small yellow market classes. The predominant allozyme allele in accessions from cluster A was *Mdh-1*^{103} combined with the "T" and "H" types as phaseolin patterns. Most of these accessions had large and often round or oval, although can also be elongated, seeds. These morphologically and allozyme characteristics were detected by Singh et al. (1991a) in germplasm from the race Peru. Accessions included in cluster B exhibited the characteristic allozyme allele *Mdh-1*^{98} and the phaseolin patterns were "T" and "C" types. Morphologically, these accessions largely resemble germplasm from the race Chile, with round or oval seeds (Singh et al. 1991b). The most-common allozyme allele in germplasm from cluster C was *Mdh-1*^{100} and the phaseolin patterns were "T", "C" and "H" types. This characteristic allozyme allele was found in accessions from the races Nueva Granada and Chile, and morphologically accessions from this cluster group are representative of these Andean genotypes.

Accessions in clusters D and E presented an Andean allele *Skdh*^{100} and a Mesoamerican allele *Diap-1* ^{95}, and they could be considered as intermediate forms between both gene pools. Some accessions had a Mesoamerican seed-protein pattern ("S" and "B" types) and they mainly belong to the small-white, great-northern, large-great-northern and hook market classes. Other accessions showed an Andean seed-protein pattern ("T", "C" and "H" types) and largely resembled the "Favada", canellini, marrow and "Guernikesa" market classes. The two clusters exhibited a different allozyme profile at the *Me* and *Rbcs* loci. Cluster D showed the *Me*^{100} and *Rbcs*^{100} alleles whereas cluster E had *Me*^{98} and *Rbcs*^{98} alleles. The red-coloured accessions from the market class "Guernikesa" may have inherited their banding patterns from red-pinto accessions because this stripping pattern is confined to those large-seed beans, and the small seed size from small white accessions. One "Guernikesa" accession probably resulted from segregation of a heterozygous plant in some earlier generation because 25% of its individuals had hybrid seed-protein pattern. The intercrossing between small-seeded-white and large-seeded red-stripped beans must have occurred in an earlier generation. Thus, most of the "Guernikesa" accessions are an advanced-generation true-breeding recombinants, except for one accession that is still segregating or is a

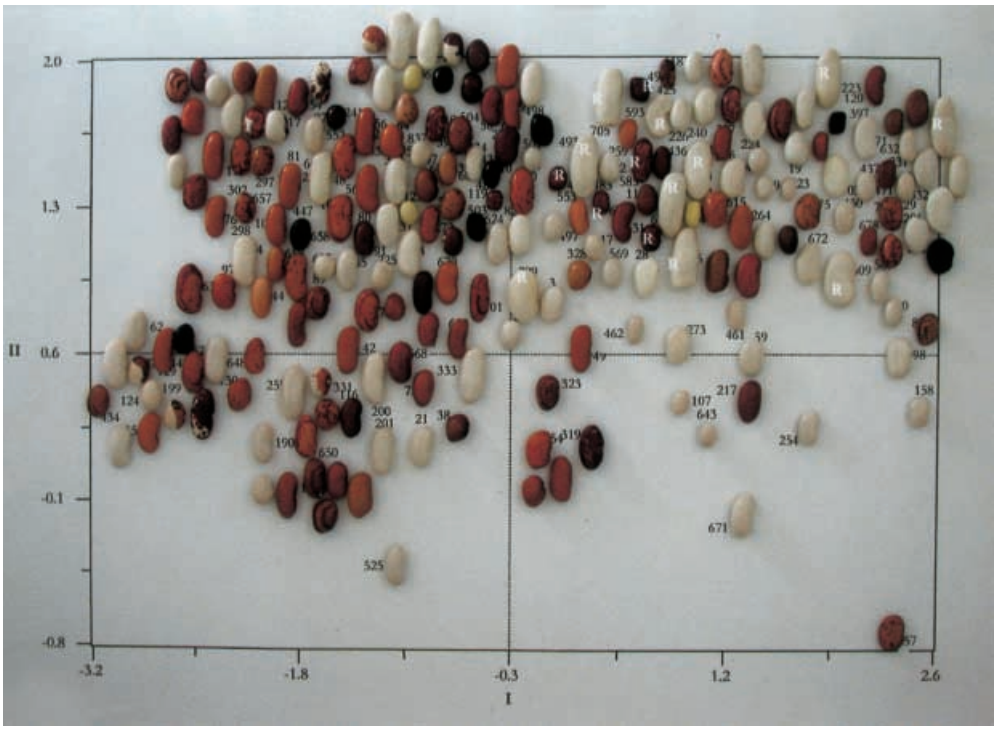

Fig. 4 Principal component plot of isozyme diversity in the Iberian accessions. Letter R indicates intermediate forms

mixture. This hybridization could be explained because Iberian landraces are cultivated in small gardens in proximity. This close proximity of the cultivation of landraces in the Iberian Peninsula and the exchange of seeds among neighbouring farms may have resulted in occasional outcrossing and gene flow. Outcrossing rates are usually below 10% (Park et al. 1996) although higher rates have been reported (Wells et al. 1988; Ibarra-Pérez et al. 1997). These outcrossing rates have been found outside the distribution range of wild beans in Latin America. Therefore, insect pollinators could have been attracted by comparatively larger flowers of the cultivated bean in the Iberian Peninsula and facilitated gene flow between both gene pools.

Clusters F, H, I and J included accessions with a true Mesoamerican allozyme (*Skdh*^{103} and *Diap-1*^{95} alleles) and seed-protein pattern ("S" type), although some accessions from cluster groups F and H had an Andean seed-protein pattern ("T" and "H" types). Clusters F and H, comprising 55 and 5 accessions, respectively, are characterized by the same allozyme profile at *Me* and *Mdh-1* (allele 100), combined with the *Rbcs*^{100} and *Rbcs*^{98} alleles, respectively. Andean accessions belonging to clusters F and H are mostly representative of the market classes "Favada" and canellini, and accessions in cluster H exhibited the *Rbcs*^{98} allele which was previously found in Andean germplasm (Singh et al. 1991a). The Mesoamerican accessions largely resemble the germplasm from races Mesoamerica and Durango (Singh et al. 1991b). Alleles *Rbcs* ^{100} and *Mdh-1*^{98} were found in accessions from cluster I, while cluster J exhibited the *Rbcs*^{100}, *Me*^{102} and *Mdh-1*^{103} alleles. These alleles, combined with the seed type, are representative of the Mesoamerican genotypes from race Durango (Koenig and Gepts 1989; Singh et al. 1991b; Debouck et al. 1993).

Accessions in cluster G displayed an intermediate position between Mesoamerican and Andean gene pools. At two isozyme loci, these accessions showed an allele characteristic of the Mesoamerican gene pool (*Skdh*^{103}), whereas at the other loci an Andean allele was displayed (*Diap-1*^{100}). This allozyme profile was also found in wild common bean in a geographical area (Colombia-Peru) which was considered as transition between the Mesoamerican and Andean gene pools (Koenig and Gepts 1989; Debouck et al. 1993). This cluster included accessions with a Mesoamerican ("S" type) and Andean ("T" and "C" types) seed-protein pattern, and resemble white kidney, canellini, "Guernikesa", red pinto, hook and large great-northern class cultivars.

A principal component analysis (Fig. 4) of the allozyme gene frequencies was carried out among the cultivated common bean from the Iberian Peninsula. Variation along the first three principal components accounted for 25%, 15% and 13% of the total variation, respectively. Discrimination along the first principal component was accounted for by variation at the *Skdh* and *Diap-1* loci, and along the second principal component by variation at the *Me* locus. The differences among accessions by the first principal component identified the Mesoamerican and Andean germplasm. Mesoamerican accessions were plotted on the right side of the corresponding graph (*Skdh*^{103} and *Diap-1*^{95} alleles), while Andean accessions were plotted on the left side of the graph (*Skdh*^{100} and *Diap-1*^{100} alleles). The graph also indicated a close relationships between Andean accessions which largely resembled "Favada", canellini, red-pinto and

"Guernikesa" class cultivars with Mesoamerican accessions mainly from the great-northern, large great-northern, hook and small-white market classes. There is evidence, then, of gene exchange between the two groups. Thus, the genetic incompatibility between large-seeded and small-seeded germplasm does not appear to have been complete in these Iberian accessions.

The use of allozymes complements the data obtained from seed-protein electrophoresis (Escribano et al. 1998) providing a clear picture of the organisation of genetic diversity in the cultivated common bean in the Iberian Peninsula. The total genetic diversity is evenly distributed among the Mesoamerican and Andean gene pools, and occurs mainly among accessions. Therefore, more accessions should be preserved to ensure the retention of allelic and genotypic diversity for both gene pools. This result indicates that the levels of genetic variation in the common bean has not been eroded since the introduction of the common bean from the American centers of domestication to the Iberian Peninsula. However, small-seeded Mesoamerican commercial cultivars from the Iberian Peninsula showed a reduction in genetic diversity, which could be attributed to a reduced demand for these market classes in the region in particular and the Iberian Peninsula at large. Moreover, in recent years breeding activities have focussed on only a few highly priced large-seeded market classes which could have contributed to a reduction in genetic diversity within the small-seeded less-desirable cultivars. Hence, an important goal of the Spanish bean breeding programs should be to broaden the genetic diversity of each specific commercial market class.

An important feature of allozyme diversity among cultivated accessions from the Iberian Peninsula is the existence of a high number of large-seeded white accessions of predominantly Andean phenotypes (e.g. "Favada", canellini and marrow) and Mesoamerican phenotypes (e.g. great northern, large great northern and hook) which have a combination of Mesoamerican and Andean alleles. As discussed above, these could constitute intermediate forms or recombinants between the Mesoamerican and Andean gene pools. Paredes and Gepts (1995) also showed signs of introgression from the Mesoamerican gene pool in Chilean landraces based on seed-protein and allozyme data. However, Johns et al. (1997) – based on a different set of materials – found that Chilean landraces could be classified into the Andean and Mesoamerican gene pools, based on RAPD data. Many of the Iberian recombinants had morphological traits that did not correspond with the characterization of Singh et al. (1991b). Previous studies (Escribano 1992; Casquero 1997; Rodiño 2001) indicated that Iberian Andean landraces generally have larger seeds than Iberian Mesoamerican landraces, but there is a considerable overlap between the two groups. In addition, growth habit showed a similar distribution: many Andean landraces from the race Nueva Granada ("Favada" and canellini class cultivars) and Mesoamerican landraces from the race Durango (large-great-northern and hook-class cultivars) had climbing type IV. In contrast, the *fin* allele, responsible for determinate bush growth habit, occurs at higher frequency in the Andean cultivated gene pool (race Nueva Granada) compared with the Mesoamerican gene pool (race Mesoamerica) (Singh et al. 1991b). The common bean is still grown in association with maize in Iberian regions close to the Atlantic coast (Santalla et al. 1994), where the widest genetic diversity was observed. This could have favoured the evolution of these recombinant indeterminate types. Occasional outcrossing in home gardens and selection for larger seeds may also account for the evolution of these intermediate materials and the additional variation observed. Thus, the large-seeded landraces belonging to the "Favada" (98 g/100 seeds), large-great-northern (58 g/100 seeds) and hook (45 g/100 seeds) market classes (Santalla et al. 2001), which are exclusively cultivated in the Iberian Peninsula, may be of interest to breeders who have had trouble recombining the two major gene pools. These could serve as a bridging germplasm to transfer genetic diversity between the Andean and Mesoamerican gene pools if that could not be achieved by direct crosses. Productivity potential and the breeding value of these recombinant genotypes has yet to be determined.

Iberian landraces suggest interesting questions about the nature of the variation described, as well as the evolutionary forces affecting the current European common bean germplasm. Mesoamerican germplasm was introduced into the Iberian Peninsula after around 1500, mainly from Mexico, due to extensive native commerce between Central Mexico and islands from the Caribbean. These beans were only red and white in colour. They were known as "frijoles", which came into general use for all kinds of common beans in Spain and Portugal due to the similarity to "faxones" and "fexoes", Spanish names for the common bean (Ortwin-Sauer 1966; Gepts and Debouck 1991). This reduced genetic variation, represented by a small population size (population bottlenecks), and further establishment of new populations from only a few individuals (founding events) based on farmer's preferences, could have increased genetic drift. However, later germplasm introductions from the southern Andes after around 1532, principally from Peru, which were mentioned as "purutus" and were large and rather round, could have broaden the genetic diversity (Brücher and Brücher 1976). Occasional outcrossing, adaptation to particular environmental conditions (for temperature, moisture, photoperiod, soil fertility, diseases and insects), cropping systems and strong selection for consumer preferences for seed types, might have played a significant role in the evolution of the new variation in the common bean in the Iberian Peninsula. Thus, new germplasm (e.g. "Favada", hook and large-great-northern class cultivars) that could be considered as "Iberian forms" could have probably emerged from initial recombination events between the Mesoamerican and Andean gene pools that are better adapted to the conditions prevailing on the Iberian Peninsula. The new Iberian forms

could have subsequently been disseminated to other parts of Europe, thus contributing to much-wider variation observed in European germplasm (Lioi 1989; Gil and Ron 1992; Limongelli et al. 1996; Zeven 1997; Escribano et al. 1998). Hence, the Iberian Peninsula, mainly the north and northwest regions,could be considered as a secondary center of genetic diversity for the common bean, especially the large white-seeded market-class cultivars.

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