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Colombian Common and Lima Beans: Views on their Origin and Evolutionary Significance

ABSTRACT

This article reviews the geographical distribution of wild common and lima beans in the Neotropics, their morphological and ecological attributes, and their biochemical and molecular variation along their ranges. These facts reveal the organization of the genetic diversity into three major gene pools, with one being considered ancestral, and additional subdivisions within the derived ones. The relationships between the ancestral branch and related species are discussed. Colombia appears to be more than a place of contact between gene pools of cultivated materials, but the transit place of the ancestral branches, and a possible place of domestication as well.

Key words: *Phaseolus*, landraces, wild species, geographic distribution, molecular markers, crop evolution, phylogeny

RESUMEN

Título: Los Frijoles Colombianos Lima y Común: Puntos de Vista de su Origen y el Significado de su Evolución

Se presenta una revisión de la distribución geográfica de las formas silvestres del frijol común y del frijol lima en el Neotrópico, de sus características morfológicas y ecológicas, y de la variación bioquímica y molecular a lo largo de esta distribución. Estos hechos muestran que la diversidad genética viene organizada en tres acervos o grupos de genes, uno de ellos siendo ancestral, y con subdivisiones adicionales dentro de los acervos derivados. Se discuten las relaciones filogenéticas entre este ramal ancestral y las especies afines. Más que lugar de contacto entre acervos de materiales cultivados, Colombia aparece como corredor biológico donde transitaron las ramas ancestrales, y como lugar de posible domesticación.

Palabras Claves: *Phaseolus*, especies silvestres, distribución geográfica, marcadores moleculares, evolución de cultivos, filogenia

1. INTRODUCTION

BEANS HAVE LONG been known as part of the major food plants used by the Amerindians (DeCandolle, 1883; Vavilov, 1931, 1939). Perhaps under the influence of their fellow historians who were much impressed by the prestigious pre-Colombian civilizations existing just at the moment of the Conquest, scholars have long focused their attention on Mesoamerica (whose geographic boundaries are here defined as per León, 1992), in particular Mexico, and the Central Andes, in particular Peru. In doing so, other regions as well as the right time perspective might have been overlooked, in order to bring full clarity on events such as crop origin, cultivar diversity, flows of materials, and patterns of domestication. Because of their natural distributions in both Mesoamerica and the Central Andes and beyond (Debouck and Smartt, 1995), the two Neotropical bean (*Phaseolus*, Phaseolinae, Fabaceae) species of worldwide economic importance, *P. vulgaris* L., the common bean, and *P. lunatus* L., the lima bean, offer the possibility to challenge further these early concepts of crop origin and evolution, and would allow us to more fully answer the questions: from which material did these crops arise?, where?, how?, when?, by whom?, all of them of critical importance for the shaping of ge-

netic diversity, and thus in the decision making process of conservation of such a genetic diversity.

When a 'second generation' group of scholars had access to more archaeological information and data from plant explorations carried out mostly prior to World War II, the view that two centers of diversity for beans, most likely independent, was further developed, but was soon in need of additional, novel evidence (Heiser, 1965, 1979). During the 1960s and 1970s, additional material was collected, fortunately enough in a broader perspective than before, and with the advent of molecular genetics and electrophoresis techniques, many more comparisons have become possible, across gene pools or across closely related species, namely where morphological or physiological variation was either inexistent or poorly expressed in phenotypes.

Although we are still perhaps far from a complete understanding of bean evolution over the past thousands of years, it might be tempting for a 'third generation' student of bean evolution to sum up where we are, what we know less, and implications for conservation and use of bean germplasm. I shall consider successively geographic and ecological attributes, genetic variation, and evolutionary histo-

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ries, with main emphasis on wild ancestral forms of these bean species, and I have this time focused this essay deliberately on Colombia. The reasons for doing so should become hopefully obvious to the readership in this essay.

2. Features of wild common bean before Humans

2.1. Distribution of wild common bean: an anomaly in the Andes

The wild common bean is presently distributed in the western mountainous ranges of the Neotropics, from Chihuahua in Mexico to San Luis in Argentina, generally at 1,400-2,200 masl (Gepts and Debouck, 1991; Toro Ch. et al., 1990). It is relatively abundant in western Mexico, particularly along the Eje Volcánico (Nayarit, Jalisco, Michoacán, Guerrero), much less in Central America (Delgado Salinas et al., 1988). In the Andes, its range starts in western Venezuela, and from there extends into Colombia up to Cundinamarca (Debouck et al., 1993). There is a gap in southwestern Colombia (Cauca, Nariño) that needs additional surveys. The range resumes in Chimborazo, Ecuador, and along the Pacific slope of the Andes, extends to Cajamarca, Peru (Debouck et al., 1989a).

The distribution turns again to the eastern slope of the Andes, from Huanuco in Peru through Central Eastern Bolivia and ends up in San Luis in Argentina (Berglund-Brücher and Brücher, 1976; Toro Ch. et al., 1990). It is so far unknown from Chile and likely not present in that country (Debouck, personal observations, 1996). One should note that if the range in Mexico and Guatemala, and in Argentina, is known since the 1940s (Burkart, 1941; McBryde, 1947, respectively), the Pacific range of distribution of wild *P. vulgaris* in South America has been disclosed only recently (Debouck et al., 1993).

2.2. A vine moving from the forest into savannahs

The wild common bean is a viny legume in open, sunny pine-oak forests and oak grasslands in Mexico and Central America with many species of Compositae and Solanaceae (Delgado Salinas et al., 1988; Gentry, 1969), while its South American habitat is somewhat more shady and humid in montane forests with species of *Podocarpus*, *Alnus*, *Celtis* and *Schinus* (Brücher, 1988; Debouck et al., 1993). So, according to a life zone classification (Holdridge et al., 1971), it thrives in *Bosque húmedo montano bajo subtropical* and

Bosque húmedo subtropical templado in Guatemala (de la Cruz S. and Sagastume L., 1983), in *Bosque muy húmedo montano bajo* in Costa Rica (Tosi, 1969), in *Bosque húmedo montano bajo* in Venezuela (Ewel et al., 1976), in *Bosque húmedo premontano* in Colombia (Anonymous, 1988), in *Bosque seco premontano* in Ecuador (Cañadas C. and Estrada A., 1978), in *Bosque seco montano bajo tropical* in Peru (ONERN, 1976), in *Bosque seco montano bajo* in Bolivia (Beck et al., 1993). It grows in *Distrito de las Selvas Montañas* of *Provincia de las Yungas* in Argentina (Cabrera, 1976). In these habitats, where the amount of rainfall ranges 400 -2,000 mm/year but concentrated at the beginning of the growing season, soil nature and fertility vary a lot but with topsoil slightly acidic (pH 6.2-6.7) and rich in organic matter. Given these ecological affinities, the gap in Southwestern Colombia is less understandable - and certainly merits further attention, since according to data published elsewhere (Anonymous, 1988) the *Bosque seco premontano* exists between El Cerrito and Pradera in the Valle del Cauca and in the upper valley of Río Guaitara in the Nariño department.

The almost continuous habitat of wild *P. vulgaris* and its abundance in the Neotropical mid-altitude subhumid mountain forests raises the following question: has the wild common bean seen its habitat altered by human activities such as logging, periodic fires, clearings for shifting agriculture? It seems that perhaps up to half of the habitats where wild *P. vulgaris* thrives in recent decades have been altered, so that it has benefitted from periodic human alterations (Delgado Salinas et al., 1988). But along its range, some populations thrive in true climax vegetations, that are dry variants of Lower montane humid forest or Dry montane forest. So, many populations growing in places so different and distant as the states of Mexico, Nayarit or Jalisco in Mexico, or in Tarija, Bolivia, or in Jujuy, Argentina, truly belong to climax, original forests.

In addition to ecological differences, morphological differences exist along the range of distribution of 8,000 Km, with many intermediary forms between the two geographical extremes: forms with large and obovate leaflets, racemes with many floral insertions and large heart-shaped bracteoles in Mexico (Gentry, 1969), and forms with small rhomboedric leaflets, racemes with few floral insertions and small triangular bracteoles in the Central-Southern Andes (Brücher, 1988). Interest-

ingly enough, a few forms of Southwestern Mexico in Mexico, but also of Costa Rica and Colombia tend to behave as short-living perennials regrowing after the first seed set, with subwoody lower stems and fibrous roots. Some forms of Costa Rica, Colombia and Northern Peru are also particularly late for their first flowering. Not surprisingly, differences are also reflected in physiological parameters, such as the ones controlling photosynthesis (Lynch et al., 1992). According to these results, wild bean populations from Mexico that show adaptation to more open, sunny, savannah-habitats are different from the ones of Guatemala, Peru and Argentina.

2.3. An impressive variation at biochemical and molecular levels

The variation of wild *P. vulgaris* revealed by ecological, morphological and physiological characteristics is also reflected at the biochemical (seed proteins, allozymes) and molecular (RFLPs, AFLPs) levels. An important and easy-to-detect polymorphism has been observed in the main storage protein, phaseolin, with more than twenty electromorphs found in Mesoamerica, slightly less in the Central and Southern Andes (Toro Ch. et al., 1990). In Mesoamerica, wild beans show 'S' and several 'M' types (Gepts et al., 1986; Koenig et al., 1990; Toro Ch. et al., 1990), while in the Central and Southern Andes of Peru (Junín, Apurímac, Cuzco), Bolivia (Cochabamba, Chuquisaca, Tarija) and Argentina (Jujuy, Salta, Tucumán), they show 'T', 'C', 'H', 'A' and 'J' types (Gepts et al., 1986; Koenig et al., 1990; Tohme et al., 1989; Toro Ch. et al., 1990). So, given the attributes of phaseolin as an evolutionary marker (Gepts and Bliss, 1985b), two gene pools were recognized. More subtle differences were revealed afterwards using RFLPs on mtDNA (Khairallah et al., 1992), namely that Guatemalan wild forms might be different from the ones in Mexico, and that the forms of Bolivia and Argentina would be much alike.

Colombian wild beans display two interesting features. First, they display some phaseolin types such as 'S', 'CH' (Gepts and Bliss, 1986), that are present elsewhere in Mesoamerica (Toro Ch. et al., 1990). Second, they also display phaseolin types such as 'L' that are apparently unique to Colombian materials (Chacón S. et al., 1996). Recent work using AFLPs markers has shown that for those wild beans of Colombia sharing 'Mesoamerican' phaseolins differences at the DNA level for non-phaseolin coding genes are important

(Chacón S. et al., 1996, Tohme et al., 1996), indicating that these Colombian wild forms are already distinct from their Central American counterparts.

In contrast, Ecuadorian and northern Peruvian (Piura, Cajamarca) wild forms display little variation in phaseolin, since a single 'T' type has been described for the different populations analyzed so far (Debouck et al., 1993; Koenig et al., 1990; Neema et al., 1994). Diversity analysis using allozymes confirms their unique position in comparison to the two major gene pools (Koenig and Gepts, 1989), but it was then not possible to conclude whether that uniqueness resulted from hybridizations between the two major gene pools because of contact through millenia or was due to any other factor. Later, numerous and unique polymorphisms have been revealed using RFLPs on mtDNA in these materials in comparison to those of other regions (Khairallah et al., 1992), giving support to the hypothesis that factors different from simple contact and resulting hybridization cause originality of that range of wild materials.

The genetic variation in wild common bean is thus strongly structured along space gradients, at the regional level first (with an Mesoamerican and an Andean gene pool, and even perhaps a North Andean gene pool), at the subregional level next (with differences within each gene pool). The hypothesis that wild bean populations growing in mountainous ranges every time separated by geological fractures are different (Bannerot and Debouck, 1992) has gained some support. As analysis progresses and more performant markers are becoming available, it is likely that differences shall be revealed eventually at the level of each population.

2.4. Can we trust the biochemical and molecular variation and how did it arise?

Given the level of outcrossing in wild common bean (Triana et al., 1993; Vanderborcht, 1983), one wonders whether such a biochemical and molecular variation is not the result of introgressive hybridization with cultivated bean varieties or even other *Phaseolus* species (the work by Wall and Wall, 1975, shows that this possibility is plausible). The molecular complexity of many markers, the uniqueness of many electromorphs and their absence in any cultivated common bean variety so far gives some insurance that the polymorphisms observed are the true reflect of genetic variation accumulated over time in

the wild, that is the accumulation of small mutations, translocations and inversions in the DNA molecules (that can now be revealed directly) and so in the genes coding for certain proteins or enzymes. There are however cases where undoubtedly introgression has produced "mismatches" in the expected patterns, and it is likely that with circulation of cultivated varieties around this phenomenon may increase in the future. So, 'S' phaseolin was observed in wild and weedy forms present in Apurimac, Peru (Gepts et al., 1986), likely coming from the human-introduced 'Panamito' well distributed this century in Coastal and Central Peru (Voyses, 1983). In spite of the presence of reproductive barriers due to a long evolution in isolation (Gepts and Bliss, 1985a; Gutiérrez and Singh, 1985), some introgressive hybridization between gene pools is possible (for instance in the Chilean material: Paredes and Gepts, 1995), because the common bean is not yet a closed, compartmentalized biological system.

2.5. Two or three major gene pools?

Prior to the discovery of the wild beans of the Pacific Andean range (Debouck et al., 1989a), it seemed logical to organize the diversity of wild *P. vulgaris* into two gene pools, one centered in Mesoamerica, and another one in the Central and Southern Andes (Gepts et al., 1986; Koenig and Gepts, 1989). If subdivisions and regional differences could be recognized within each major gene pool (for instance, between Mexico west of the Isthmus of Tehuantepec and the entity Chiapas-Guatemala: Bannerot and Debouck, 1992; Chacón S. et al., 1996) - and likely will be revealed further (Tohme et al., 1996), the presence of two major gene pools is today little questioned (Becerra Velásquez and Gepts, 1994; Gepts, 1993; Hamann et al., 1995; Zink et al., 1994). Not surprisingly, the organization of diversity in the host plant was also paralleled with that of some fully specific pathogens - evidenced on cultivated forms - such as angular leaf spot (Guzman et al., 1994) and anthracnose (Pastor-Corrales et al., 1995), where two gene pools can also be recognized.

The existence of two major gene pools - at the level of wild forms - unavoidably raises the question of their genesis. It seems first that *P. vulgaris* as a species did not arise by morphological convergence. In other words, the possibility that two wild legumes completely different from an evolutionary perspective gave rise to two morphologically and genetically so close

entities is quite remote. Data accumulated on cpDNA (Llaca et al., 1994; Schmit et al., 1993) among various materials of *P. vulgaris* show a very low level of polymorphism, compatible with that observed in other species of Spermatophyta (Soltis et al., 1992). Two genesis scenarios were thus possible: either i) one gene pool derives from the other, or ii) the two gene pools arise from a third one, that could be seen as the ancestral pool. Since most *Phaseolus* species are today distributed in Mexico and Central America (Debouck, 1991; Delgado Salinas, 1985), it looked plausible that *P. vulgaris* originated first in Mesoamerica and that the Andean gene pool arose as a branch deriving from the Mesoamerican pool. The alternate scenario - that the wild common bean arose first in South America and then migrated towards Mesoamerica - was a more remote possibility, although once considered by some (Burkart and Brücher, 1953). Both scenarios were however challenged by the molecular complexity of the phaseolin marker (Gepts, 1988), and the fact that the dominant Mesoamerican phaseolin 'S' was slightly more complex than the dominant Andean type 'T' (Bliss and Brown, 1983). In other words, these phaseolins could not derive straight from each other. In addition, RFLP polymorphisms on mtDNA between Mesoamerica and the Central-Southern Andes (Khairallah et al., 1992) were important enough as to raise doubts about a simple migration scenario. There were however two cases reported of a direct similarity between Argentinean and Mexican wild bean germplasm: i) the collection DGD-621 of Jujuy displays the Mexican allele of diaphorase (Koenig and Gepts, 1989), and ii) DGD-629 of Salta displays a RFLP polymorphism present in a wild Mexican form of Guerrero (Khairallah et al., 1992).

2.6. In search of the ancestral branch

In contrast with the situation prevailing in Mesoamerica and in the Central-Southern Andes, we have seen that the wild beans of Ecuador and northern Peru show no variation in phaseolin type but a single 'T' type. This phaseolin type is outstanding because of two characteristics. First, it lacks the 52kD high molecular weight subunit of phaseolin (Koenig et al., 1990). In a comparative study of nucleotide sequences responsible for the synthesis of 'I', 'S' and 'T' phaseolins, (Kami et al., 1995) showed that 'I' phaseolin genes lack a 27-base pair tandem direct repeat, whereas the 'S' and 'T' phaseolin genes do

have it. This study together with results of phaseolin gene sequencing (Kami and Gepts, 1994) indicates that gene sequences responsible for the synthesis of phaseolin have gained complexity by endoreplication, instead of losing sequences (the alternate scenario, less plausible because random deletions would have prevented or hindered phaseolin formation - apparently a selective advantage in this species). These results have been confirmed elsewhere (Schumann and Nagl, 1995). The nucleotide sequences responsible for 'I' phaseolin can thus be considered as primitive or ancestral in comparison to other ones in common bean. Second, the two-dimension structure of 'I' phaseolin is close to the one of a globulin present in wild *P. polyanthus* Greenman (Schmit and Debouck, 1991). CpDNA analysis has shown a close similarity between wild *P. vulgaris* of Ecuador (with 'I' phaseolin) and wild *P. polyanthus* of Central Guatemala as they would belong to the same lineage (Schmit et al., 1993). One should note that another species, *P. costaricensis* Freytag & Debouck, belonging to the same lineage (Schmit et al., 1993) and possibly involved in the parentage of the syngameon including *P. vulgaris*, *P. polyanthus* and *P. coccineus* L., exists in mountainous ranges of eastern Costa Rica and western Panamá (Freytag and Debouck, 1996); phaseolin genes of this species have not been sequenced so far.

From the above, it seems that the wild beans with 'I' phaseolin represent an ancestral branch of the species *P. vulgaris* prior to its separation into two major gene pools. One could thus assume the existence of a nuclear material that was growing in one area from which two major branches progressively separated, specialized and migrated, likely hundreds of thousands of years ago. What can we infer about that area? The present geographic location of these beans with 'I' phaseolin is somewhat puzzling, since it falls outside the regular range of wild beans on the eastern slope of the Andes in South America. It is not sure that they have always been in that part, from Chimborazo to southwestern Cajamarca, which might have functioned as refugia at the moment of migrations of flora during the late Pliocene - early Pleistocene (Haffer, 1987; van der Hammen, 1992). It is worth mentioning here that another phaseolin type called 'Mu' which displays a simple structure in SDS-PAGE electrophoresis exists in Cundinamarca, Colombia (Gutiérrez et al., 1994). So, a few

'simple' phaseolin types would exist in the Northern Andes among more complex ones, with the possibility that the latter would have derived from the former by duplication of nucleotide sequences (Kami and Gepts, 1994). On the other hand, as we have seen, all presently known species belonging to the same cpDNA lineage as *P. vulgaris*, exist today in Central America. With presently available evidence, one could say that the common bean evolved as *P. vulgaris* in an area located between Guatemala and Northern Peru. Because speciation is an evolutionary process, it does not automatically take place in a single locality or geographic spot. The hypothesis that the species *P. vulgaris* did not have a static distribution at the moment of its formation, but rather moved back and forth in an area between what is today Guatemala and Northern Peru, can be supported by the presence of common phaseolin types such as 'CH' in Central America and Colombia (Toro Ch. et al., 1990), or the fact that on the basis of allozyme evidence the Colombian wild forms are close to the Central American ones (Koenig and Gepts, 1989). While nucleotide sequences responsible for phaseolin synthesis were conserved, other sequences continued to evolve, resulting in base pair changes and substitutions as revealed by AFLPs analysis (Chacón S. et al., 1996). Finally, migration towards higher latitudes close to the Tropics parallels and slightly beyond would be a more recent phenomenon.

2.7. A higher diversity in Mesoamerica?

A slightly higher diversity has been long observed in wild beans of Mexico and Central America in comparison to the Central-Southern Andes, on the basis of seed protein markers (Gepts et al., 1986; Ishimoto et al., 1995; Toro Ch. et al., 1990), or RFLP markers on different DNAs (Becerra Velásquez and Gepts, 1994; Khairallah et al., 1992; Sonnante et al., 1994), and has been long claimed as evidence supporting a single Central American (or Mexican) origin for the *P. vulgaris* species. Two different features could have acted together and explain that higher diversity in Mesoamerican wild beans: first, under relatively less changing though favourable ecological conditions, the wild beans colonize in Central America and Mexico an expanded niche along a longitude gradient that is not so wide in the Andes (Debouck, 1986). So, the longitude gradient in Central America and Mexico would have been of the magnitude of

84°07'W-108°30'W, while of 64°19'W-79°24'W in the Andes (Toro Ch. et al., 1990). Second, *P. vulgaris* as it came into being did not migrate alone further into Mesoamerica, but with companion species such as *P. costaricensis* and *P. polyanthus*, that would eventually share the same cpDNA polymorphisms (Schmit et al., 1993), or even *P. coccineus* with which it would share some mtDNA sequences (Hervieu et al., 1994), while for still unknown reasons it did migrate alone into the Andes. Worth mentioning here is the presence of certain peptides in seed storage proteins of common bean that could come from *P. coccineus* (Lioi and Hammer, 1989), a species that in its very early evolution was close to *P. vulgaris* (Hervieu et al., 1994), indicating that some gene exchange through natural outcrossing indeed took place. It is important to note that *P. coccineus* and *P. polyanthus* were probably introduced by Man into the Andes, yet as very primitive materials, thousands of years after the Andean branch of *P. vulgaris* separated from the bulk of migrating *P. vulgaris* and started colonizing the Andes (and already in complete isolation from the Chimborazo-Cajamarca range).

3. Common bean domestication in a Colombian perspective

We don't know when the common bean has been domesticated, neither the exact place, nor the identity of its domesticators, nor the reasons behind it. Some evidence has been however accumulated in recent years which shows that:

i. Bean domestication is likely ancient, even if a novel dating method (Kaplan, 1994) has recently pleaded for a revision of early assumptions (see Kaplan and Kaplan, 1988; McClung de Tapia, 1992; McNeish, 1992; Pearsall, 1992, for reviews).

ii. Bean domestication surely happened in different locations of the range of the wild common bean, independently in Mesoamerica and in the Andes (Gepts, 1993; Gepts and Debouck, 1991; Gepts et al., 1986). Further, the existence of races in common bean (Becerra Velásquez and Gepts, 1994; Singh et al., 1991a) would suggest the possibility of at least two domestication events in Mesoamerica, and two in the Central-Southern Andes.

iii. Bean domestication might have taken place at different moments in time, perhaps repeatedly in some parts, while unique in time in others. This means that certain groups of beans might be more ancient than others. For Mesoamerica ex-

tending to the Southwestern USA, for which more data are available, a south-to-north gradient shows clearly that beans were used much earlier in South-Central Mexico in comparison to other places (Carter, 1945; Kaplan, 1956, 1967, 1985). Other data (Kaplan and McNeish, 1960) as well as the unfinite nature of the archaeological argument might however invite us to consider this point carefully.

iv. Bean domestication might have taken place for other reasons than consumption as a boiled food item. Purposes diverse enough as consumption as snap bean (Debouck, 1989) or as toasted beans (Tohme et al., 1995; Toro Ch. and Debouck, 1995) or as play (Debouck, 1989) have been proposed. Given the fact that beans have appeared as fully domesticated in several pre-ceramic contexts especially in the Andes (Engel, 1987; Lynch et al., 1985; McNeish, 1977), it is not sure that consumption as a boiled food item was the prime reason for domesticating beans. Such a way of cooking beans might have come later, once boiling was well established, perhaps because of other food plants.

Data from different disciplines are critically lacking in the case of Colombia. Archaeological data are few (Kaplan and Smith, 1985), and not conclusive, but from historical records (Patiño, 1964), we know that common beans are ancient in that country. For reasons explained above, it seems that at least a few wild common beans present in North-Eastern Colombia are not weedy escapes, but true wild forms. The comparison of Colombian landraces with sympatric wild forms with the use of the phaseolin marker has shown the massive introduction, likely in pre-Columbian times, of cultivars from Central America and from the Central-Southern Andes, with the exception of a few landraces apparently domesticated in Boyacá-Cundinamarca (Gepts and Bliss, 1986). These findings were confirmed with the screening of more landraces with the use of AFLPs (Chacón S. et al., 1996). Such analyses show further that some Colombian landraces with 'S' or 'CH' phaseolin of suspected Central American origin group together with Colombian wild forms (Chacón S. et al., 1996), indicating either their true origin in Colombia or a reticulate origin with extensive wide-crossing. The presence of 'L' phaseolin in both wild and cultivated forms of Colombia is an additional indication of a separate domestication event (Chacón S. et al., 1996).

The reasons for such an in masse introduction of Central American and Andean bean cultivars in Colombia are still unclear, and perhaps are related to the peopling of Colombia. In this regard, one should note that most of the materials of Central American origin concentrate in the North and in the Cauca and Magdalena valleys below 1,200 masl, while those of Central-Southern Andean origin are grown in the South and generally above 1,400 masl (Debouck et al., 1993; Gepts and Bliss, 1986). Materials with 'B' or 'CH' or 'L' phaseolin do not seem to cumulate negative characters that would have been culled out during selection. At this stage, given the lack of data, these materials might equally represent an old 'center' of domestication, later on dominated by bean introductions from other parts, or a recent one. Not surprisingly, the race 'Nueva Granada' defined elsewhere (Singh et al., 1991b; Singh et al., 1991c) still lacks clear-cut boundaries, as it would be made of several genetical bean entities with different evolutionary histories. Since sampling of truly traditional cultivars is still fragmentary, and might thus introduce a dangerous bias, these results are very preliminary, and more material of Colombia, wild and cultivated, needs to be studied, also paying particular attention to materials from Ecuador and Venezuela (from where little has been thoroughly studied, particularly for cultivated materials).

4. Features about wild lima bean before Humans

4.1. Two families of wild lima beans

Wild lima beans with small triangular, parchment-like, almost glabrous leaflets, small seeds, small flowers with greenish and hairy standard and purplish wings, are widespread in the Neotropics, generally below 1,600 masl to sea level (Debouck and Smartt, 1995). The range of distribution of this form extends from Sinaloa in Mexico to Panamá, and from Tamaulipas in Mexico through the Caribbean up to coastal Colombia; elsewhere in South America, it is known from Bahia, Brazil, and eastern Peru, and extends up to Salta in Argentina (Gutiérrez Salgado et al., 1995).

In contrast, a group of wild lima bean with larger somewhat hairy leaflets, slightly larger seeds, deep purple standard and wings, has been disclosed in inter Andean valleys on the western slope of the Andes, from Imbabura in Ecuador to Southwestern Cajamarca in Peru (Debouck et al., 1987). The presence of this form

in Colombia is possible (Toro Ch. et al., 1993), but requires additional surveys. It occupies niches at slightly higher altitudes and more xeric habitats in comparison to the tropical 'lowland' wild lima bean.

The presence of a hydrocyanic acid precursor in mature dry seeds especially high in wild forms (Baudoin et al., 1991) leaves little possibility for their active transportation by humans or animals. Seeds of wild forms, either grey mottled or solid black, as in most *Phaseolus* species, are unobtrusive and likely not noticed by rodents or birds. These features would allow to consider the present range of distribution as resulting rather from natural mechanisms of seed dispersal with brutal pod dehiscence, hard seed coat, etc, than from the intervention of human factors.

4.2. Two gene pools, probably ancient

The wild lima bean distributed in the lowland Neotropics displays, on the basis of results available so far in polymorphism of seed lectins and arcelins, a distinct and more important diversity in comparison to the Andean wild lima bean (Debouck et al., 1989b; Gutiérrez Salgado et al., 1995; Maquet et al., 1990). These results would allow to conclude to the existence of two gene pools in wild lima bean. The existence of such gene pools in the wild was further supported by allozyme analysis (Maquet et al., 1994) and RFLP analysis of rRNA genes (Jacob et al., 1995). Not surprisingly, given the extent of its range over 7,000 Km, the lowland Neotropical wild lima bean shows a wider genetic variation, particularly in Mesoamerica (Gutiérrez Salgado et al., 1995; Maquet et al., 1994), in spite of insufficient sampling.

4.3. Very early evolution

Differences at the morphological level between the two wild forms of lima bean are stronger in comparison to those separating gene pools in wild common bean if we assume equal speeds of differentiation. However we presently do not have evidence that these two families of wild lima bean would represent two different evolutionary pathways in American Phaseolinae which by morphological convergence would constitute an artificial legume species. Rather, present evidence could be interpreted as if separation of the two families of wild lima bean took place earlier in comparison to what occurred in common bean. From a nuclear stock, two branches separated, one colonizing the Neotropical savannahs (of both Central America and South America), while the

other was restricted to the montane dry forests of the Northwestern Andes. Present evidence although scarce would locate this nuclear stock in an area extending from Costa Rica to Ecuador. Indeed wild materials showing both sets of alleles have been disclosed in that area (on allozyme evidence: Maquet et al., 1994), but it is difficult at this stage to assert that they do represent the nuclear stock (that would have evolved since anyway) or are instead the result of wide hybridizations, although Andean Big Lima beans are unknown in archaeological times north of Panamá (Gutiérrez Salgado et al., 1995; Kaplan and Kaplan, 1988). It seems that: i) a true nuclear stock has not been formally identified in wild lima beans, and ii) in contrast to what happened with the Andean branch of *P. vulgaris*, *P. lunatus* has migrated or was in the Andes with companion species such as *P. augusti* Harms, *P. pachyrrhizoides* Harms (if these two are different!), and *P. mollis* Hooker whose range restricts to the Galapagos Islands (Wiggins and Porter, 1971). The little difference between *P. augusti* and *P. pachyrrhizoides* might indicate that speciation process still goes on. Finally, one has to note the extended possibilities for widecrossing with lima bean, with species such as *P. jaliscanus* Piper, *P. maculatus* Scheele, *P. ritensis* Jones, *P. salicifolius* Piper (Katanga and Baudoin, 1990), all today distributed in Mexico and the Southwestern USA (Debouck, 1991). Such possibilities let us to consider genetic affinities between these species and the nuclear stock leading to *P. lunatus*.

5. Gene pools in cultivated lima bean

Probably as the result of independent domestications in the two different families of wild lima bean, there are two gene pools in cultivated lima beans, as evidenced on seed proteins (Debouck et al., 1989b; Gutiérrez Salgado et al., 1995; Maquet et al., 1990), allozymes (Maquet et al., 1994), RAPDs markers on total genomic DNA (Nienhuis et al., 1995) and RFLPs of rRNA genes (Jacob et al., 1995). We lack evidence as to ascertain that there were more than one domestication event within each family of wild forms, but such possibility might exist at least in the Andes (Gutiérrez Salgado et al., 1995). This needs additional support since the presence of high cyanogenic glucoside in the wild would rather be a limiting factor for independent multiple domestications.

The two gene pools of cultivated materials are present in Colombia, with a

particular distribution according to altitude, the small-seeded materials being majorily distributed on the Atlantic coast of Colombia and in inter-Andean valleys below 1,200 masl, and the large-seeded cultivars being distributed in the South and in the *cordilleras* above 1,600 masl (Gutiérrez Salgado et al., 1995). As an example that the two gene pools still belong to the same biological species, and therefore do not merit a special Latin nomenclature (Debouck, 1991), a few materials with hybrid pattern exist in Colombia as a result of past hybridizations (Gutiérrez Salgado et al., 1995). The question of the specific origin for the Colombian cultivars is still quite open, since wild materials of both gene pools may exist in that country, opening the possibility of separate domestications, although separate introductions might be a more likely scenario for historic reasons (Patiño, 1964). More analysis is required, in relation to the germplasm of Panamá, Venezuela and Ecuador, but first of all a better sampling of the wild forms in Colombia.

6. Colombia: at the crossroads for sure

Colombia is a place of particular relevance in the study of the two most important pulses of American origin, since it might have been the scene (or very close to it) of: i) the processes leading to the formation of both species as such from a bulk of companion species, ii) the separation of their original stocks into Mesoamerican and Andean branches (all wild) as further steps of the speciation process, and iii) separate domestication events or selection of recombinants in cultivars domesticated elsewhere. Evidence is coming that Colombia is more than a simple zone of contact, because of its geographical position, between the major gene pools for these two pulses.

There is some parallelism between the situations prevailing in the wild common bean and the wild lima bean, although much evidence is still lacking, particularly for the latter. But there are also major differences in such a parallelism, namely in the timing of the events, that maintains these two taxa well apart in the evolution of the genus (Debouck, 1991; Maréchal et al., 1978). Both species were formed by separation from a bulk of companion species, which today represent the secondary and tertiary gene pools (Debouck, 1991) and possibilities for promising widecrossing. These companion species migrated to Central America and Mexico in the case of common bean, and to the

Andes in the case of lima bean. There are indications although preliminary that these events took place in Colombia or close to it. Both *P. vulgaris* and *P. lunatus* expanded as wild forms in the other region, in contrast to most *Phaseolus* species, in part because of man-made alterations in their habitats, that in turn they were able to exploit (as a preamble to their domestication: Hawkes, 1969; Heiser, 1969), while most *Phaseolus* species were not. The Northwestern Andes still harbour the two branches for both species or better say their descendants, so that it would be possible to understand the early formation of the gene pools in the wild.

The parallelism finally extends to the domestication histories, where different wild forms growing in different ecological zones were domesticated separately resulting in vicariance (Debouck, 1992). One example is found in the range extending from Chimborazo to Cajamarca where the large-seeded lima bean was probably domesticated, while the wild common bean with 'I' phaseolin growing in a complementary habitat in the same range was not. The explanation may lie in the non-necessity for early farmers to domesticate two wild legumes (that were not necessarily sympatric) at the same time for similar purposes. On the other hand, domestication processes of common and lima beans and within each cultigen although independent were conducted much along the same principles (e.g. soon growing beans with maize and squashes, or cooking beans with ceramics), leading to the same evolutionary changes (detailed by Smartt, 1988) and consequences (e.g. an important reduction of diversity upon domestication). Yet, markers have revealed different amounts of diversity among groups within each cultigen (for *P. vulgaris*: Becerra Velásquez and Gepts, 1994; Debouck and Tohme, 1989, and for *P. lunatus*: Gutiérrez Salgado et al., 1995; Nienhuis et al., 1995), indicating that on marker base these groups are not at the same evolutionary stage, and so opening the way to better use the original potential of these American Phaseolinae.

All Neotropical countries where these wild beans are present do all harbour genetic resources, some of which have been critical in the past evolution of these species, as wild forms or as cultigens. As perhaps all these Neotropical countries are interested in enhancing their agrobiodiversity, a priority is to collect, document and study further these resources before their extinction.

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