Theor Appl Genet (1992) 84: 915–922

THEORETICAL AND APPLIED GENETICS

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Mitochondrial restriction fragment length polymorphisms in wild *Phaseolus vulgaris* L.: insights on the domestication of the common bean

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Received June 17, 1991; Accepted January 18, 1992
Communicated by P. Maliga

Summary. Previous examination of intraspecific mitochondrial DNA (mtDNA) diversity in common bean, *Phaseolus vulgaris*, showed that five restriction fragment length polymorphisms (RFLPs) distinguish the mitochondrial genomes of the two major gene pools of cultivated beans, the Mesoamerican and the Andean. In the study presented here, mtDNA was used to compare the amount of diversity in cultivated beans to that in collections of wild beans to gain an understanding of how and when the mitochondrial genomes of the gene pools became distinct. The mtDNA of six wild bean accessions from Central and South America were digested with nine restriction endonucleases and analyzed by Southern hybridization. A total of twenty RFLPs were detected demonstrating a significantly higher amount of mtDNA variability in wild beans than in cultivated ones. All of the wild beans had the same mtDNA pattern for four out of the five inter-gene pool RFLPs, indicating that the polymorphism arose soon after domestication: two in the gene pool of the cultivated Mesoamerican beans and two in the gene pool of the cultivated Andean beans. The fifth RFLP must have occurred before domestication since the locus was also polymorphic in the wild beans. Wild beans from the south Andes were distinct and less variable than wild accessions of the north Andes and Mesoamerica. The distribution of mtDNA RFLPs among the wild beans supports the concept of two distinct domestication events for *P. vulgaris*.

Key words: Mitochondrial RFLPs – Common bean – Crop domestication – Founder effect – Gene pools

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Introduction

In recent years restriction fragment length polymorphisms (RFLPs) have been used increasingly as molecular markers to construct chromosome maps of crop plants, fingerprint crop cultivars, and mark quantitative trait loci (Beckmann and Soller 1986). In addition, RFLPs are useful in understanding genome evolution, studying plant origins and domestication, and probing genetic diversity.

Organellar DNA RFLPs allow maternal lineages to be followed, since in most plant species, chloroplasts and/or mitochondria are inherited exclusively from the maternal parent (e.g., Palmer et al. 1983). Moreover, plant organelle RFLPs can provide an indication of the cytoplasmic diversity of crops, an important issue if genetic vulnerability, due in part to uniform cytoplasms, is to be avoided. Primarily for this reason, several studies have investigated mitochondrial DNA (mtDNA) variability within various crop species such as soybean (Sisson et al. 1978; Grabau et al. 1989), barley (Holwerda et al. 1986), and oat (Rines et al. 1988). With the exception of annual teosinte (Timothy et al. 1979), maize (Kemble et al. 1983; Weissinger et al. 1983), and carrot (Ichikawa et al. 1989), high degrees of uniformity of plant mitochondrial genomes were revealed in these intraspecific comparisons.

A similar low level of intraspecific mtDNA diversity occurs in the common bean, *Phaseolus vulgaris* L. (Khairallah et al. 1990). Nonetheless, five RFLPs enabled the 23 bean lines studied to be differentiated into the two major gene pools of beans. These are recognized as the small-seeded Mesoamerican and the large-seeded Andean gene pools. In addition to the differences in their seed sizes and centers of origin, the two gene pools are distinguished by differences in growth habits, environ-

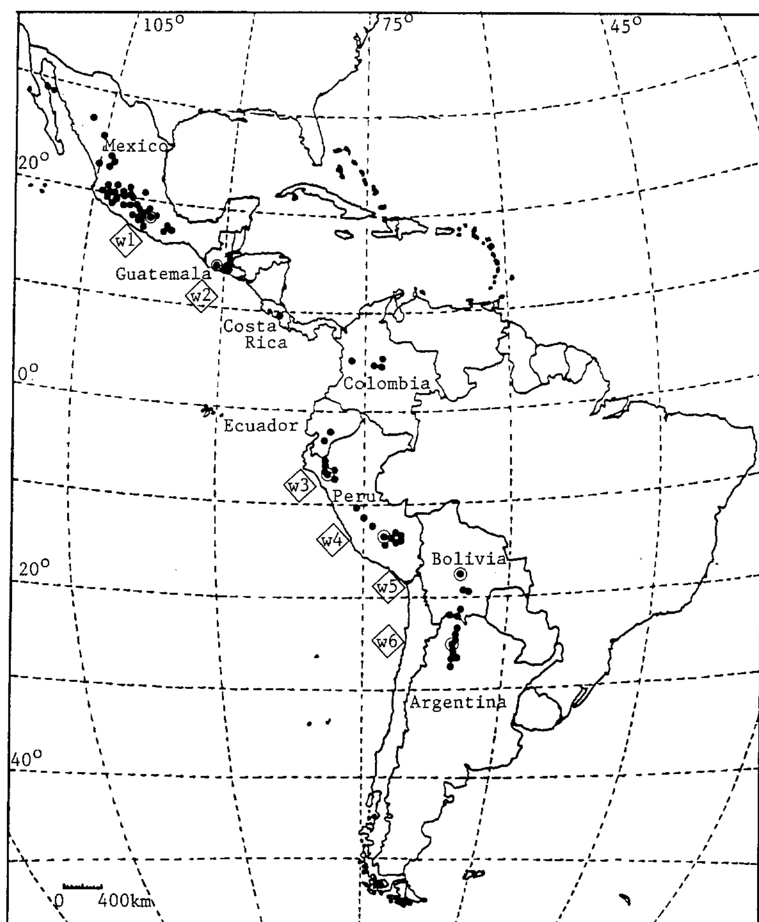

Fig. 1. Known distribution of wild *Phaseolus vulgaris* in North and South America (Toro et al. 1990) and collection site of the six accessions (w1–w6) used in this study

mental adaptation, disease resistance, zymograms, and phaseolin types (reviewed in Gepts 1988b). In order to understand how and when the mitochondrial genomes of the two gene pools became distinct, we decided to examine the mtDNA of extant wild bean populations from both centers of diversity.

As shown in Fig. 1, wild forms of *P. vulgaris* still exist in an almost continuous band extending from the state of Chihuahua, Mexico, in the north to the province of San Luis, Argentina, in South America (Toro et al. 1990). These wild types cross freely with cultivated beans and are classified as botanical varieties of *P. vulgaris* (Baudet 1977; Delgado-Salinas 1985). Morphological and phenological variability exists among the populations in the different geographical areas for such characters as seed size, hypocotyl texture, bracteole size and shape, and days to and duration of flowering (Vanderborght 1983; Debouck and Tohme 1989). In addition, genetic variation has been observed for physiological characters (Lynch et al. 1989) and biochemical attributes (Gepts et al; 1986, Koenig and Gepts 1989; Koenig et al. 1990).

Although the variability appears to be gradual along the range of distribution, the Mexican and the Argentinian forms are the extremes and are quite distinct. Vanderborght (1983) reported an average seed size of 6.2 and 13.9 g/100 seeds for the two forms, respectively. Seed size (Gentry 1969; Evans 1973; Baudet 1977; Gepts et al. 1986) and phaseolin type (Gepts and Bliss 1986; Gepts et al. 1986) were found to vary among wild beans and among domesticated beans in a parallel fashion along the geographic range. This has been interpreted to mean that multiple domestication events have occurred (Gepts 1988a).

The main objectives of the study presented here were to examine mtDNA variability in a sample of wild beans by restriction and hybridization analyses in order to compare the mtDNA diversity of wild and cultivated lines. This investigation was also intended to be a preliminary study to identify new RFLPs that can be used to examine a larger number of wild bean accessions for a better understanding of the origin, evolution, and domestication of the common bean.

Materials and methods

Bean lines

Six wild bean accessions sampled across the range of distribution of wild *Phaseolus vulgaris* were used in this study (Table 1).

Their exact collection locations are indicated in Fig. 1. Seed samples were obtained from Drs. D. Debouck and J. Tohme of the Centro Internacional de Agricultura Tropical (CIAT), Colombia. Two cultivated bean lines from the Michigan State University Malawi collection were used as the representatives of the cultivated Mesoamerican (line 4–11) and Andean (line 4–22) controls (Khairallah et al. 1990). Seeds of the wild bean accessions were scarified prior to germination in vermiculite trays in darkness.

MtDNA RFLP analysis

Seedlings that had been grown in the dark for 10–15 days were used to extract mtDNA. The procedures and conditions for mtDNA isolation, digestion and electrophoresis, Southern blotting, nick translation and hybridization were as described in Khairallah et al. (1990). Nine restriction endonucleases were utilized to digest the mtDNAs: *Asn*I, *Hin*dIII, *Pst*I (Boehringer Mannheim Biochemicals), *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Sal*I (Bethesda Research Lab.) and *Xho*I (New England Biolabs).

Southern blots were consecutively hybridized to five cosmid clones (C2, C3, C4, C6, C8) and strip-washed after each hybridization according to Gatti et al. (1984). The clones were provided by Dr. C. D. Chase (University of Florida, Gainesville) and contained random inserts from the bean mitochondrial genome ranging in size from 29 to 38 kb.

Results

Mitochondrial DNA from six wild *P. vulgaris* accessions and two cultivated lines representing the Mesoamerican and the Andean types were digested with nine restriction endonucleases, and the Southern blots were hybridized to five cosmid clones. Seven of the endonucleases were used previously to probe mtDNA diversity among cultivated bean lines, but only two of these, *Eco*RI and *Dra*I, revealed RFLPs (Khairallah et al. 1990). These two enzymes differ from the other five in that they contain a minimum of four adjacent AT pairs in their recognition sequences – GAATTC and TTTAAA, respectively. On the basis of this observation, two other enzymes having this trait, *Eco*RV (GATATC) and *Asn*I (ATTAAT), were included in this analysis of wild bean mtDNA diversity.

The restriction fragment profiles for all of the enzymes were complex and relatively uniform across the eight lines tested, as expected for an intraspecific comparison. However, a few more polymorphic bands were observed in this analysis than with the mtDNA isolated from cultivated material (Khairallah et al. 1990). Differences in ethidium bromide-stained gels were observed repeatedly in the case of *Dra*I (Fig. 2a). With the other enzymes, RFLPs on stained gels were not as clear, and thus are not considered in this analysis. Hybridization experiments provided more conclusive results, and the RFLPs thus detected are listed in Table 2. Six enzymes out of nine revealed 20 polymorphisms, although *Dra*I, *Eco*RI, *Eco*RV, and *Asn*I produced most of the variation.

Figure 2 shows the six RFLPs detected with *Dra*I. The cultivated Mesoamerican line showed an extra 12.7-

Table 1. Origin and some characteristics of the wild bean accessions used in this study

Line	CIAT number	Collection number	Country, State	Seed size (g/100 seeds)	Phaseolin[a]
w1	G12878	Gentry 22535	Mexico, Guerrero	8.5	M1
w2	G19906	DGD 1610	Guatemala, Sacatepequez	5.7	S, Sd
w3	G21245	DGD 1962	Peru, Cajamarca	8.8	I
w4	G23455	DGD 2581	Peru, Cuzco	9.0	C, H
w5	G23442	DGD 2484	Bolivia, Cochabamba	8.7	To
w6	G19892	DGD 629	Argentina, Salta	7.0	T

[a] Phaseolin types were determined at the Centro Internacional de Agricultura Tropical (CIAT). Types S, T, C were first described in Brown et al. (1981); M1 in Romero-Andreas and Bliss (1985); H in Gepts et al. (1986); I and Sd in Koenig et al. (1990) and To in Vargas et al. (1990)

Table 2. RFLPs detected in wild and cultivated bean mtDNA

RFLP number	Enzyme	Revealed by	Definition of pattern # 1[b]	Definition of pattern # 2[b]	Number of polymorphic fragments
1	*Dra*I	Gel	12.7 kb	no 12.7 kb	1
2	*Dra*I	Gel	no 9.0 kb	9.0 kb	1
3	*Dra*I	Gel	no 4.9 kb	4.9 kb	1
4	*Dra*I	Gel	no 3.7 kb	3.7 kb	1
5	*Dra*I	C3/C6[a]	3.45 kb	3.37 kb	2
6	*Dra*I	C8[a]	no 6.2 kb	6.2 kb	1
7	*Eco*RI	C2	18.6 + 4.3 kb	22.3 kb	3
8	*Eco*RI	C2	1.6 kb	1.2 + 0.7 kb	3
9	*Eco*RI	C4	6.2 kb	11.2 kb	2
10	*Eco*RV	C3	18.3 + 12.7 kb	no 18.3, 12.7 kb	2
11	*Eco*RV	C3	no 6.6 kb	6.6 kb	1
12	*Eco*RV	C8/C4	7.2 + 0.7 kb[c]	6.7 + 1.1 kb[c]	4
13	*Asn*I	C2	4.9 kb	11.8 kb	2
14	*Asn*I	C3	24.9 + 17.3 kb	21.4 kb	3
15	*Asn*I	C3/C6	3.4 kb	6.4 kb	2
16	*Asn*I	C6	10.4 + 4.5 kb	no 10.4, 4.5 kb	2
17	*Asn*I	C8/C4	10.7 + 1.5 kb	12.7 kb	3
18	*Asn*I	C8	17.3 kb	23.6 kb	2
19	*Hin*dIII	C4	2.3 + 1.5 kb	3.8 kb	3
20	*Xho*I	C6	8.8 kb	no 8.8 kb	1

[a] Also detected in the stained gel

[b] The RFLP pattern numbers were assigned so that the cultivated Mesoamerican control always displayed pattern # 1

[c] Only the 7.2- and 6.7-kb fragments hybridized to C8, whereas all four fragments hybridized to C4

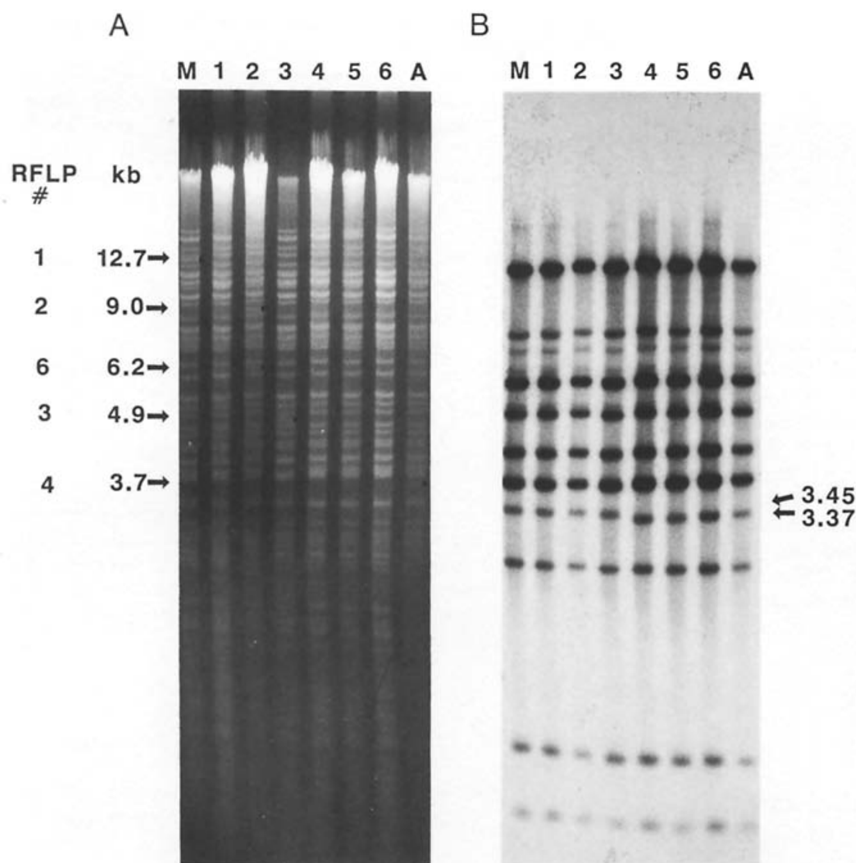

Fig. 2 A, B. *Dra*I mtDNA digestion patterns of six wild and two cultivated bean accessions. *M, A* Mesoamerican and Andean controls, respectively. *Numbers 1–6 above* the gel lanes indicate the wild bean accessions as in Table 1. **A** Ethidium bromide-stained gel. *Arrows* indicate polymorphic fragments; RFLP numbers and sizes are given at the *left* of the gel. **B** Southern blot hybridized with clone C3 showing RFLP #5; sizes are indicated at *right*

kb fragment (RFLP #1), while line w2 showed two extra fragments, a 9.0-kb band (RFLP #2) and a 6.2-kb band that was also seen with clone C8 (RFLP #6). Line w3 and the cultivated Andean line had, respectively, a unique band of 4.9-kb (RFLP #3) and 3.7-kb (RFLP #4). RFLP 5 was detected with probes C3 and C6 and is most probably due to a small length mutation on the order of 80 basepairs since the cultivated Mesoamerican line and lines w1, w2, and w3 had a 3.45-kb band while the cultivated Andean line and lines w4, w5, and w6 had a 3.37-kb fragment.

Figure 3 shows the two RFLPs seen in the Southern blot of the *Eco*RI digest hybridized to clone C2. In both cases, the three polymorphic band may result from a basepair mutation in an *Eco*RI recognition site. As indicated in this figure for both RFLP #7 and 8, the total of the two fragments present in pattern #1 is approximately equal to the size of the third fragment present in pattern #2. While RFLP #7 differentiates the cultivated Andean line from all of the others, RFLP #8 differentiates line w3 from all of the others.

As summarized in Table 2, the hybridization of the *Eco*RV blot to C8 revealed RFLP #12, which differentiates line w1 (6.7-kb) from all of the other lines (7.2-kb). Clone C4 hybridizes to the same two polymorphic bands and two others, 1.1-kb in w1 and 0.7-kb in all other lines (blots not shown). These four polymorphic fragments defining RFLP #12 could be due to an inversion because 6.7 + 1.1-kb is approximately equal to 7.2 + 0.7-kb.

The six RFLPs detected in the *Asn*I digest are shown in Fig. 4. Hybridization to clone C2 shows the absence of an 11.8-kb band and the presence of a 4.9-kb band in the cultivated Mesoamerican and w2 lines (RFLP #13, Fig. 4A). RFLP #14 (clone C3) differentiates line w3 from all of the others by the absence of two bands (24.9 and 17.3-kb) and the presence of a 21.4-kb fragment (Fig. 4B). On the other hand, RFLP #15, seen with both clones C3 and C6, separates line w1 from all the others by the presence of a 6.4-kb band instead of a 3.4-kb fragment (Fig. 4B, C). RFLPs #16, 17, and 18 again distinguish line w3 from all other lines. For RFLP #16, which is detected with clone C6 (Fig. 4C), w3 is missing two fragments (10.4 and 4.5-kb). For RFLP #17, which is seen with clone C8 (Fig. 4D), w3 lacks a 10.7- and a 1.5-kb fragment but has a 12.7-kb band, which indicates a basepair mutation event. Finally, for RFLP #18 (C8), the w3 line shows a 23.6-kb band and lacks a 17.3-kb one (Fig. 4D).

Although our interpretation of some RFLPs as restriction mutations or inversions has already been men-

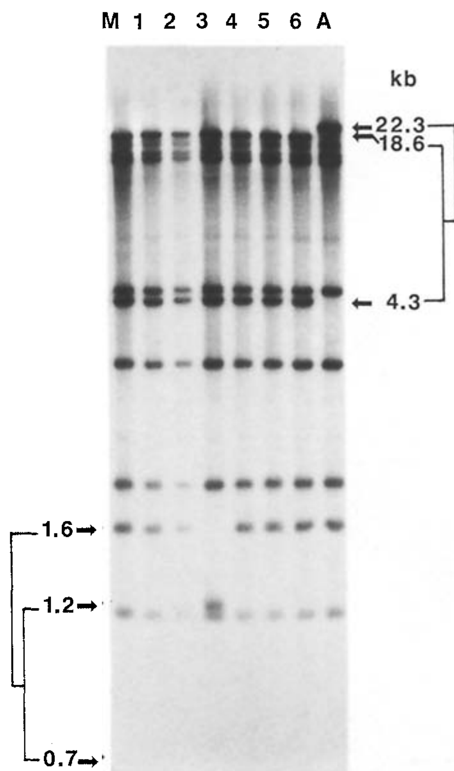

Fig. 3. Hybridization of *Eco*RI-digested mtDNAs from cultivated and wild beans to clone C2. *M*, *A* numbers 1–6 as in Fig. 2. *Arrows* on the *right* show RFLP #7 and on the *left* RFLP #8 (Table 2)

Table 3. Organization of the 20 mtDNA RFLPs detected in the cultivated and wild bean lines

RFLP number[a]	Enzyme	Gel or probe	Bean lines[b]							
			cM	w1	w2	w3	w4	w5	w6	cA
1	*Dra*I	Gel	1	2	2	2	2	2	2	2
9	*Eco*RI	C4	1	2	2	2	2	2	2	2
4	*Dra*I	Gel	1	1	1	1	1	1	1	2
7	*Eco*RI	C2	1	1	1	1	1	1	1	2
5	*Dra*I	C3/C6	1	1	1	1	2	2	2	2
13	*Asn*I	C2	1	2	1	2	2	2	2	2
10	*Eco*RV	C3	1	2	1	1	1	1	2	1
2	*Dra*I	Gel	1	1	2	1	1	1	1	1
3	*Dra*I	Gel	1	1	1	2	1	1	1	1
6	*Dra*I	C8	1	1	2	1	1	1	1	1
8	*Eco*RI	C2	1	1	1	2	1	1	1	1
11	*Eco*RV	C3	1	2	1	1	1	1	1	1
12	*Eco*RV	C8/C4	1	2	1	1	1	1	1	1
14	*Asn*I	C3	1	1	1	2	1	1	1	1
15	*Asn*I	C3/C6	1	2	1	1	1	1	1	1
16	*Asn*I	C6	1	1	1	2	1	1	1	1
17	*Asn*I	C8/C4	1	1	1	2	1	1	1	1
18	*Asn*I	C8	1	1	1	2	1	1	1	1
19	*Hin*dIII	C4	1	1	1	1	1	1	2	1
20	*Xho*I	C6	1	1	1	2	1	1	1	1

[a] The RFLP numbers as defined in Table 2
[b] cM, cA = cultivated Mesoamerican and Andean beans, respectively; w1–w6 as in Table 1; numbers indicate the occurrence of pattern #1 or #2 for each probe × enzyme combination as defined in Table 2

tioned, the nature of the mutations giving rise to some RFLPs was ambiguous and difficult to decipher. The absence of large fragments in some lines (e.g., RFLPs #6, 10, 11, 20) or a larger difference in fragment sizes in the two patterns (e.g., RFLPs #9, 13, 14, 15, 18) are not likely to be due to large length mutations, since these differences should have then been detected with other enzymes using the same probe. If, as in other plant species, the bean mitochondrial genome has a set of small repeat elements, the deletion of one of those may prevent hybridization of a fragment to a clone that has sequences similar to the repeat rather than to the fragment itself. Alternatively, these RFLPs may be due to basepair mutations or inversions, which are not readily observed because the probe(s) may not extend into the other polymorphic fragments.

The scores (pattern #1 or #2) of the eight bean lines with respect to the polymorphic enzyme × probe combinations are organized in Table 3 to show the patterns of variation. The first six RFLPs (#1, 9, 4, 7, 5, and 13) differentiated the gene pools of the Mesoamerican and Andean cultivated beans. The other 14 RFLPs distinguished one or two of the wild bean lines from all of the others. This is a good indication of the higher variability of mtDNA in the wild material.

Accession w3 was remarkably distinct from all of the others, as exhibited by seven unique polymorphisms: RFLPs #3, 8, 14, 16, 17, 18, and 20. It is followed by the Mexican accession w1, with three unique polymorphisms (RFLPs #11, 12 and 15), then by w2 from Guatemala with two unique RFLPs (#2, 6), and finally by w6 from Argentina with a single unique RFLP, #19. Accessions w4 and w5 were the most uniform.

Discussion

Timing and direction of the mtDNA mutations that distinguish the two gene pools of cultivated beans

Examination of 23 cultivated bean lines for intraspecific mtDNA variability has revealed very uniform restriction patterns (Khairallah et al. 1990). However, five RFLPs were identified that divided the bean lines into two groups corresponding to the Mesoamerican and Andean gene pools of *P. vulgaris.* By examining the mtDNA of extant wild bean populations from across the centers of bean diversity in this study, we sought to understand how and when the cytoplasms of the two gene pools diverged. The first five RFLPs in Table 3 are the ones that differentiated the mtDNA of the cultivated bean gene pools. In

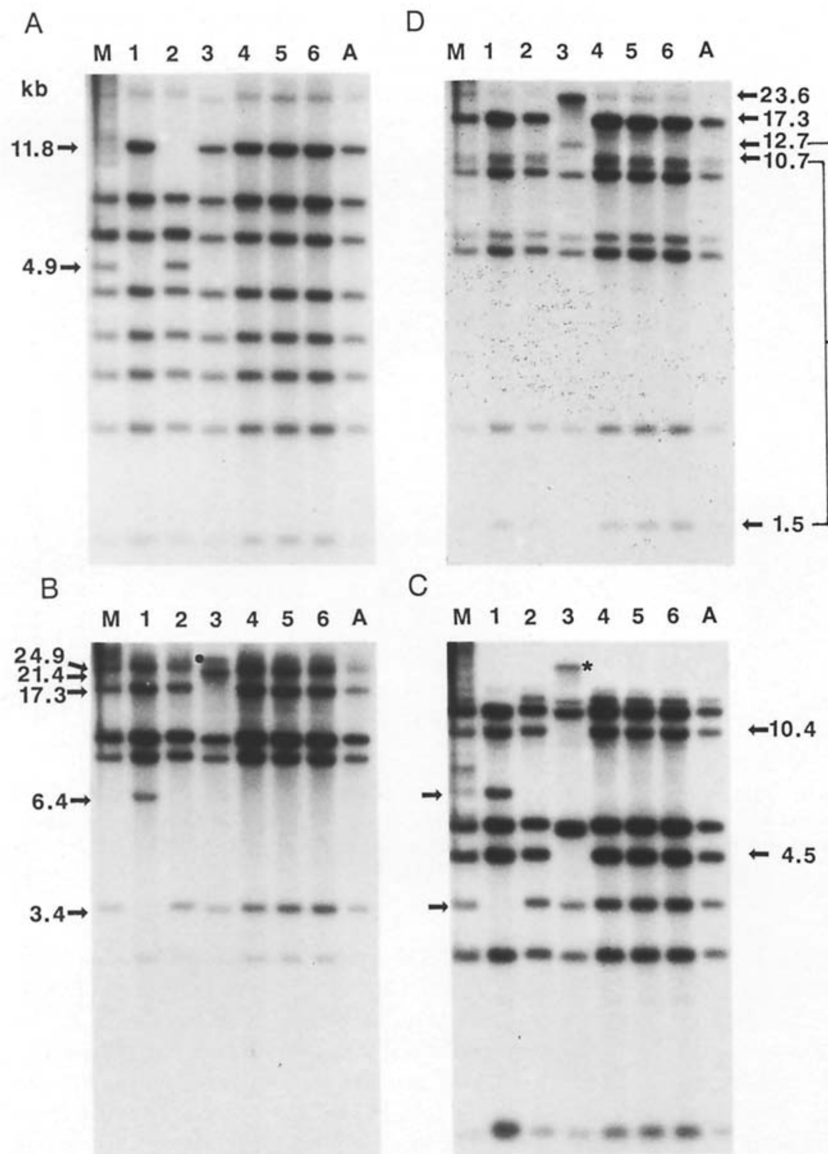

Fig. 4A–D. Hybridization of *Asn*I-digested mtDNAs from cultivated and wild beans to clones C2, C3, C6 and C8. *M*, *A* numbers 1–6 as in Fig. 2. **A** Probe C2; RFLP #13. **B** Probe C3; the *upper three arrows* point to RFLP #14, the *lower two* to RFLP #15 (also seen with clone C6). The *dot* shows a probable partially digested band in line 3. **C** Probe C6; *arrows* on the *left* show RFLP #15 and on the *right* RFLP #16. The * shows a fragment remaining from the previous hybridization with clone C8. **D** Probe C8; the *upper two arrows* point to RFLP #18 and the *lower three* to RFLP #17 (shared with clone C4)

RFLPs #1 and 9, all six wild beans are similar to the cultivated Andean beans, while in RFLPs #4 and 7 they are similar to the cultivated Mesoamerican beans, demonstrating that the mutations occurred in the Mesoamerican gene pool giving rise to the first two RFLPs and in the Andean gene pool resulting in the other two RFLPs. Although these results indicate that the mutations occurred after domestication, they must have happened early enough such that all the representatives of cultivated beans of an individual gene pool that were examined were the same. This also argues against multiple domestication events along the range of the wild beans.

As to the fifth polymorphism (Table 3, RFLP #5), it obviously occurred before domestication since the wild beans themselves exhibit both patterns. Surprisingly, however, accession w3 from northern Peru showed the same pattern (#1) as the Mesoamerican wild (w1, w2) and cultivated beans rather than pattern 2, that was seen in the other Andean wild (w4, 5, 6) and cultivated beans. Consistent with this observation, Koenig and Gepts (1989) found that allozyme allelic frequencies in accessions from Colombia and northern Peru are more similar to those of Mexican accessions than to those of populations from southern Peru and Argentina. On the basis of a survey of phaseolin types in wild and cultivated beans, Colombia was initially suggested to be a minor domestication center of the common bean and a geographical meeting place for the Mesoamerican and Andean cultivated forms (Gepts and Bliss 1986). Koenig and Gepts (1989) later defined the Colombia-Peru region as a geographical transition zone. Analysis of a larger number of

wild bean accessions from that region for mtDNA variability may help clarify the validity and/or significance of that "transition zone". On the other hand, Debouck (1986) recognized three centers of diversification of the genus *Phaseolus* in general and of *P. vulgaris* in particular: Mesoamerica, the north Andes (western Venezuela to northern Peru), and the south Andes.

A sixth polymorphism (RFLP #13), detected with *Asn*I and clone C2, differentiated the cultivated Mesoamerican and Andean beans used in this study and was also obvious in the wild beans, indicating that the mutation happened before domestication. In this case, however, only line w2 from Guatemala showed the same pattern as the Mesoamerican control. Moreover, the distribution of the polymorphism within domesticated beans is unknown, since this enzyme was not utilized in the previous study of bean mtDNA diversity (Khairallah et al. 1990).

More mtDNA variability in the wild

It is obvious from Table 3 that the wild beans contain quite a few more mtDNA polymorphisms than do the cultivated forms. A similar observation has been made for chloroplast DNA (cpDNA) in barley (Clegg et al. 1984; Neale et al. 1988), although a study of both cpDNA and mtDNA diversity in wild and cultivated barley produced contradictory results (Holwerda et al. 1986). In *P. vulgaris*, wider genetic diversity in wild populations, illustrated by phaseolin studies (Gepts and Bliss 1986; Gepts et al. 1986), led Debouck and Tohme (1989) to postulate a founder effect of domestication. The reduction in genetic diversity in the crop has important implications to bean breeders who are in continuous search for useful genes to incorporate into their improved cultivars.

Our results confirm previous studies of phaseolin (Gepts et al. 1986; Koenig et al. 1990) and allozyme (Koenig and Gepts 1989) diversity in showing that wild beans accessions from Mesoamerica and Colombia contain higher genetic variability than those from the southern Andes. However, it is still unclear if this indicates a Mesoamerican origin of wild *P. vulgaris* or whether the higher variation in Mesoamerica is due to conditions eliciting higher levels of genetic diversity.

The significantly higher mtDNA variability of accession w3 is quite peculiar. Koenig and Gepts (1989) have reported allozyme data showing that this same accession (DGD 1962) from northern Peru is genetically distinct from 82 other wild bean accessions that clustered into two major groups. Until more accessions from that region are examined, it will not be clear whether this higher variability is characteristic of the area in general or whether it is unique to that particular accession.

The similarity of mtDNA from the cultivated Andean beans to mtDNA of the south Andean wild accessions, especially w4 and w5, is remarkable. These data suggest that the south Peru-Bolivia area may be the initial domestication site of the Andean beans. The results also favor at most a subspecific classification of the Andean wild bean, as suggested by Burkhart and Brücher (1953), *P. vulgaris* ssp. *aborigineus* Burk., or Baudet (1977), *P. vulgaris* var 'aborigineus' (Burk.) Baudet, in contrast to placing it as a separate species, *P. aborigineus* Burk., as was initially done (Burkhart 1952) and as Brücher (1988) currently favors.

On the other hand, the mtDNA patterns of the Mesoamerican cultivated beans are most similar to those of the wild bean accession from Guatemala (Table 3), which, in a similar fashion, may point to a specific area of bean domestication in Mesoamerica. A primary center of *P. vulgaris* origin in the western Mexico-Guatemala area has been suggested (Miranda-Colín 1967), although Gentry (1969) proposed multiple origins of cultivated beans in Mesoamerica on the basis of botanical, genetic, and archaeological evidence. Because several RFLPs distinguish the cultivated Mesoamerican beans from the wild accessions, analysis of more plant samples, both wild and cultivated, is essential before similar conclusions about a domestication center can be made.

Finally, it was surprising to see that the mtDNA of the bean accessions from the extremes of the range of distribution of wild *P. vulgaris*, w1 and w6, shared one polymorphism that differentiated them from all other wild beans (Table 3, RFLP #10). It is not clear, though, whether this RFLP arose twice independently or if it is due to common descent.

In summary, this study has used cytoplasmic variability to document a founder effect on bean domestication. It has also allowed the identification of four restriction endonucleases that can detect significantly more mtDNA RFLPs in wild bean germ plasm than other enzymes. These enzymes should be useful for a more extensive survey of mtDNA polymorphisms in wild populations, especially when seed amounts are limiting. Many questions concerning bean origin and domestication have been raised. Because the bean mitochondrial genome is evolutionarily conserved and yet shows a fair amount of polymorphism, it provides an ideal system for addressing these questions. A survey of mtDNA RFLPs from 26 accessions from across the range of distribution of wild *P. vulgaris* is presently underway.

Acknowledgements. The authors thank D. G. Debouck and J. M. Tohme for supplying the seeds of the wild beans, C. D. Chase for providing the cosmid clones, and J. F. Hancock, J. D. Kelly, and J. M. Tohme for reviewing the manuscript. M. M. K. was supported by the Bean/Cowpea Collaborative Research Support Program, Malawi project. Funding from the Michigan State University Agricultural Research Station is acknowledged.

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