Additional evidence on gene flow events in *Phaseolus vulgaris* in Costa Rica

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We present here evidence on gene flow between wild and cultivated forms of common bean in Costa Rica in addition to our previous work (González-Torres et al. 2003).

Seeds were collected from natural populations in the Central Valley of Costa Rica (Figure 1) as previously reported (González-Torres et al. 2003). We focus on 226 'weedy' or 'intermediate' materials initially selected on morpho-agronomic characteristics, which phenotype is inherited from possible hybridization between wild and cultivated materials. A similar procedure has been used by Papa & Gepts (2003). The analyses were conducted on: 1) morpho-agronomic evaluation; 2) biochemical analysis of phaseolin by SDS-PAGE (Gepts et al. 1986), and isozymes: diaphorase (DIA) and peroxidase (PRX) according to Ramírez et al. (1987), and 3) molecular marker analysis: eight microsatellite primers reported by Gaitán-Solis et al. (2002), and cpDNA polymorphisms by PCR- RFLPs following the protocol of Chacón-Sánchez (2001).

The wild populations showed mainly two phaseolin patterns, S-4 and S (Table: morphological, biochemical and molecular markers used and No. individuals analyzed for each parameter). In cultivated materials, the phaseolins T, Sb and S-4 were also observed although in low frequency.

Biological status	Seed average weight (g)	Phaseolin Type	Isozymes		Microsatellites		cpDNA haplotypes
			Pattern[1]	Allele[2]	Primer	Allele	
Wild	6 **N=443**	"S-4" *"S"* **N=402**	DIA –1 **N=227**	PRX 100 **N=204**	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 **N=134**	160 ***89*** 162 110 163 146 137 122	G, H **N=97**
Weedy	13 **N=226**	"C" "CH" "H" *"S"* "X-7"[3] "S-4" **N=191**	DIA-1 DIA-2 DIA-4 **N=170**	PRX 100 PRX 98 **N=170**	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 **N=142**	160, 177 ***89*** 162, 183 110, 106 163, 189 146, 150 137, 174 122, 135	G, H J, K, L **N=100**
Cultivated	23 **N=188**	*"S"* "X-7" "CH" **N=186**	DIA –2 DIA –4 **N=150**	PRX 98 **N=150**	BM140 BM172 BM175 BM183 BM187 BM188 BM189 BM205 **N=35**	177 ***89*** 183 106 189 150 174 135	J, K, L **N=33**

[1] According to Sprecher (1988); [2]According to Koenig & Gepts (1989);
[3] Phaseolin pattern to be checked.

The figure 2 is a representation of markers used on a selection of individuals; bar height shows the weight (g) of 100 seeds. The shortest bar represents mainly wild characteristics and the longest bar is a description of cultivated materials. The other bars show exchange among individuals of the following markers: shared SSR alleles, change in cpDNA haplotypes, seed weight, isozymes and phaseolin patterns.

In individuals 1 and 2, all the evaluated parameters are "wild" and they have a hybrid SSR locus, which suggests a recent crossing of wild material with pollen of cultivated material. Seed size of individual 3 could be a phenotypic consequence of more than one past event of gene flow from cultivated material into the wild form, because all evaluated parameters are "wild" including hypocotyl color (purple), purple flower, 85 days to flowering and growth habit IV. Besides, its F2 displays a weight of 10.3 g, which suggests that it has kept "wild" characteristics and acquired a "cultivated" seed size. Individual 8 has hybrid isozymes, "wild" microsatellites and phaseolin, but it has a "cultivated" chloroplast haplotype.

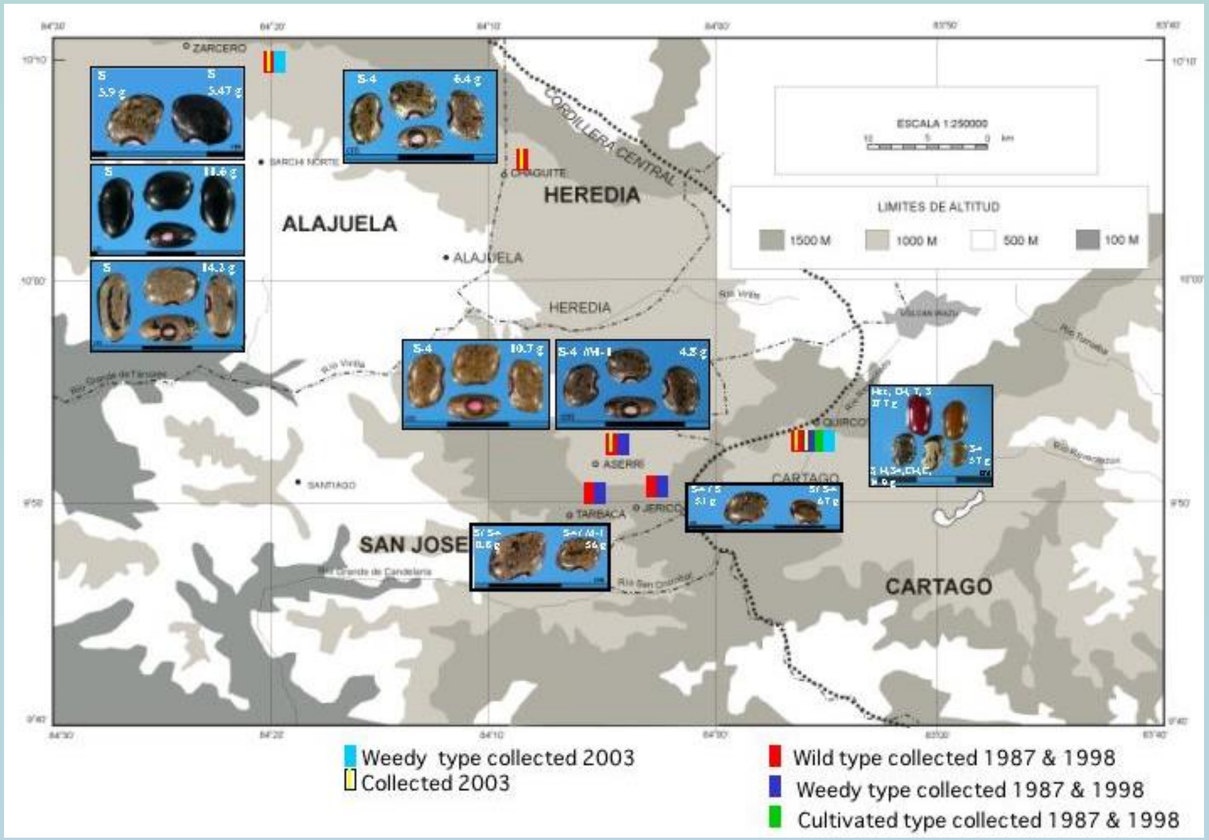

Figure 1. Geographical distribution of wild, weedy and cultivated materials from Central Valley of Costa Rica analyzed in this study. Seed weight and the most frequently found phaseolin of each population are shown.

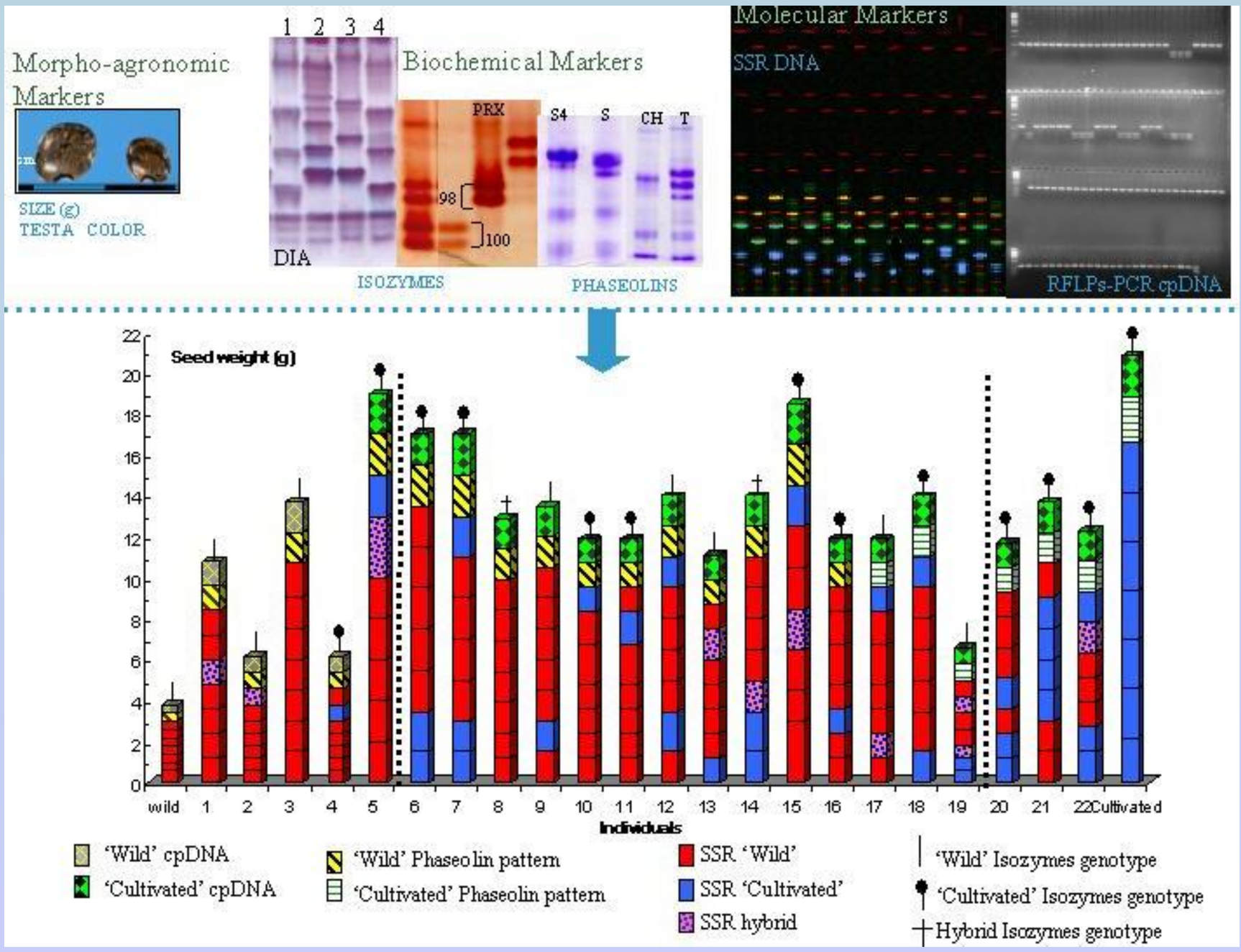

Figure 2. Some cases of gene flow found in this study

Individual 9 has the same characteristics as individual 8 but it has "wild" isozymes. These materials may represent cases of repeated gene flow of cultivated materials crossed with wild forms. Individual 14 is hybrid (PRX enzyme and one SSR locus), meaning that it comes from recent flow of "wild" pollen into a cultivated form. The evaluation of 22 cases from Costa Rica indicates that all materials are indeed product of a hybridization showing that the methodology implemented in the selection of the intermediate materials was the appropriate one. Papa & Gepts (2003) found in intermediate materials of Mexico that the contribution of cultivated parental population was significantly higher than the wild parental one. So far, for these materials of Costa Rica, we have found a more important gene flow from wild material into the cultivated type.

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