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# WILD COMMON BEAN IN THE CENTRAL VALLEY OF COSTA RICA: ECOLOGICAL DISTRIBUTION AND MOLECULAR CHARACTERIZATION<sup>1</sup>

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## RESUMEN

**Frijol silvestre en el Valle Central de Costa Rica: distribución ecológica y caracterización molecular.** Este trabajo presenta una actualización sobre la distribución de las formas silvestres de frijól común en Costa Rica, su ecología y su caracterización molecular. A la fecha 22 poblaciones fueron encontradas en cuatro cuencas alrededor del Valle Central, generalmente en vegetaciones ruderales (frecuentemente bordes de cafetales), con estatuto de conservación variable (desde protegido a amenazado). Su caracterización molecular indica su pertenencia al acervo genético mesoamericano. Varios marcadores indican una variabilidad aumentada en las formas silvestres y permiten inferir la presencia de un fenómeno de flujo genético e introgresión desde materiales cultivados.

**Palabras claves:** *Phaseolus vulgaris*, genética de poblaciones, biología de la conservación, América Central, flujo de genes.

## ABSTRACT

**Wild common bean in the Central Valley of Costa Rica: ecological distribution and molecular characterization.** This work offers an update on the distribution of wild common bean in Costa Rica, its ecology and molecular characterization. To date 22 populations have been discovered in four watersheds around the Central Valley, usually in man-made habitats (often sides of coffee plantations), under varying conservation status (from protected to threatened). Molecular characterization indicates that the wild common bean belong to the Mesoamerican gene pool. Different markers indicate an increased genetic diversity in some wild populations, and allow to hypothesize the presence of gene flow and introgression from cultivated materials.

**Key words:** *Phaseolus vulgaris*, population genetics, conservation biology, Central America, gene flow.



## INTRODUCTION

Wild relatives of crops have long been known as sources of disease resistance, adaptation to stresses and novel variability. In the case of common bean *Phaseolus vulgaris* L., there are published reports about their interest for bruchid resistance (Cardona *et al.* 1990), photosynthetic efficiency (Lynch *et al.* 1992) and tolerance to salinity (Bayuelo-Jiménez *et al.* 2002), and some progress has been reported in including these traits into elite cultivars (Singh 2001). While the evaluation of wild bean resources surely deserves further attention (Singh 1999), the identification of additional populations in the wild is a prerequisite for any future progress, as their extinction means loss of any enhancement possibility (Freytag & Debouck 2002).

On the other hand, the increased acreage planted with transgenical crops in tropical and subtropical countries (James 2003) invites to document carefully the distribution of genetically compatible wild species as a basic preliminary step for their safe introduction and management (Rissler & Mellon 1996). Common bean is a current target of genetic transformation, and difficulties in achieving effective transformation might be overcome soon (Svetleva *et al.* 2003). Gene flow has been shown to occur in common bean landraces and wild sympatric and conspecific forms in Colombia, Peru, and Mexico (Beebe *et al.* 1997b; Papa & Gepts 2003, respectively). There are some preliminary reports about the presence of gene flow in Costa Rica (Araya *et al.* 2001; González-Torres *et al.* 2003).

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Knowledge about the distribution of wild common bean in native habitats of tropical and subtropical America has progressed substantially (Gepts & Debouck 1991, Toro Ch. *et al.* 1990), since the early records for Argentina (Burkart 1941) and Guatemala (McBryde 1947). Records for Central America outside Mexico are relatively few (Freytag & Debouck 2002). Wild *P. vulgaris* has been reported from Honduras (Beebe *et al.* 1997a), El Salvador (Toro Ch. *et al.* 1990), and Nicaragua (Delgado Salinas 2001). Its presence in Panama is doubtful: Brücher (1988) has claimed it “in the Chiriquí mountains”, but no records are available (Freytag & Debouck 2002; Lackey & D’Arcy 1980).

In Costa Rica, the presence of wild common bean went long unnoticed: it was not reported in the ‘Flora of Costa Rica’ (Standley 1937), nor in the ‘Costa Rican Natural History’ (Boucher 1983). Brücher (1988) claimed to have found it in the “departamento of Santa Cruz”, but there is no department under that name in Costa Rica. The first record for this country seems to be by Debouck *et al.* (1989), from the province of San José. These early findings were confirmed by more records from other provinces (Araya *et al.* 2001). The germplasm accessions resulting from these germplasm explorations have been characterized later on (Tohme *et al.* 1996, Toro Ch. *et al.* 1990), and were shown to be close to Guatemalan accessions in the so-called Mesoamerican genepool (Gepts *et al.* 2000). Their cpDNA haplotype ‘H’ seems however unique, although belonging to a lineage widely distributed in Central America and Colombia and rooted in the Pacific range (Chacón 2001).

The objective of this note is to provide an update about the distribution of wild common bean in Costa Rica, its ecology and conservation status, and characterization through molecular markers.

## MATERIALS & METHODS

For the disclosure of wild populations, we applied a technique of ecogeographic surveying described elsewhere (Debouck 1988), taking into account the results of previous works (Debouck *et al.* 1989; Araya Villalobos *et al.* 2001). For the molecular characterization, we used seeds and seedlings of 443 individuals coming from six populations collected in 1987, 1998 and 2003: Chagüite (102), Zarcero (48), Aserrí (84), Tarbaca (18), Jérico (64), and Quircot (119). Seed storage protein phaseolin and two enzymes (diaphorase and peroxydase) were analyzed following

the protocol by Lareo *et al.* (1993) and Ramírez *et al.* (1987), respectively. The interpretation of the diaphorase pattern was done along Sprecher (1988), while we followed the procedure by Koenig & Gepts (1989) for peroxydase. Nine *loci* of microsatellites were studied along the protocol by Gaitán-Solis *et al.* (2002). Polymorphism of non-coding regions of chloroplast (cp) DNA was studied along a procedure of RFLPs-PCR developed by Chacón (2001).

## RESULTS

### Distribution, ecology and current conservation status

To date twenty-two populations of wild common bean are known for Costa Rica, and distributed in four watersheds in the central part of that country (Table 1; Figure 1): Virilla, Candelaria, Pirrís and Reventazón. For our purpose, we found useful to split the Candelaria and Pirrís watersheds, while both contribute to the Parrita watershed (Gómez Pignataro 1986). Ten populations have been found in the Central Valley or watershed of Río Virilla (ending into Río Grande de Tárcoles): seven on the southern slope (#2097 Tarbaca, 2111 Aserrí, 3136 San Miguel Desamparados, 3137 Bebedero, 3140 Parque Iztarú, 3143 Hda. Tres Ríos, and 3178 Guatuso), and three on the northern slope (#3106 Chagüite, 3132 Zarcero, and 3133 Sabana Redonda). Ten populations have been found in the upper valley of Río Grande de Candelaria: six on the northern slope (# 3131 Jérico, 3134 Tranquerillas, 3135 Chirogres, 3147 El Tigre, 3148 Manzano, and 3190 Vuelta de Jorco), and four on the southern slope (# 3184 Río Tarrazú, 3186 Bajo Los Angeles, and 3188 and 3189 in the surroundings of San Andrés).

The mountainous range that separates these two watersheds – Cerros de Cedral o de Escazú – has thus the largest number of populations: thirteen (7+6). One population has been found in the upper valley of Río Reventazón (# 3126 Quircot), which is the only one so far on the Atlantic slope of the continental divide (Fig. 1). One population has been found in the upper valley of Río Pirrís (# 3168 Copey), the southernmost population to the southeast of the country. Our attempts to find wild common bean in other parts of Costa Rica, namely the upper Río Savegre, Río División and Río Chirripó Pacífico have failed so far. There might still be one population in the watershed of Río Pirrís (the slope north of San Marcos de Tarrazú, but heavily cleared for coffee plantations), and one on the slope of Fila Bustamante. All vegetation types where it thrives, once

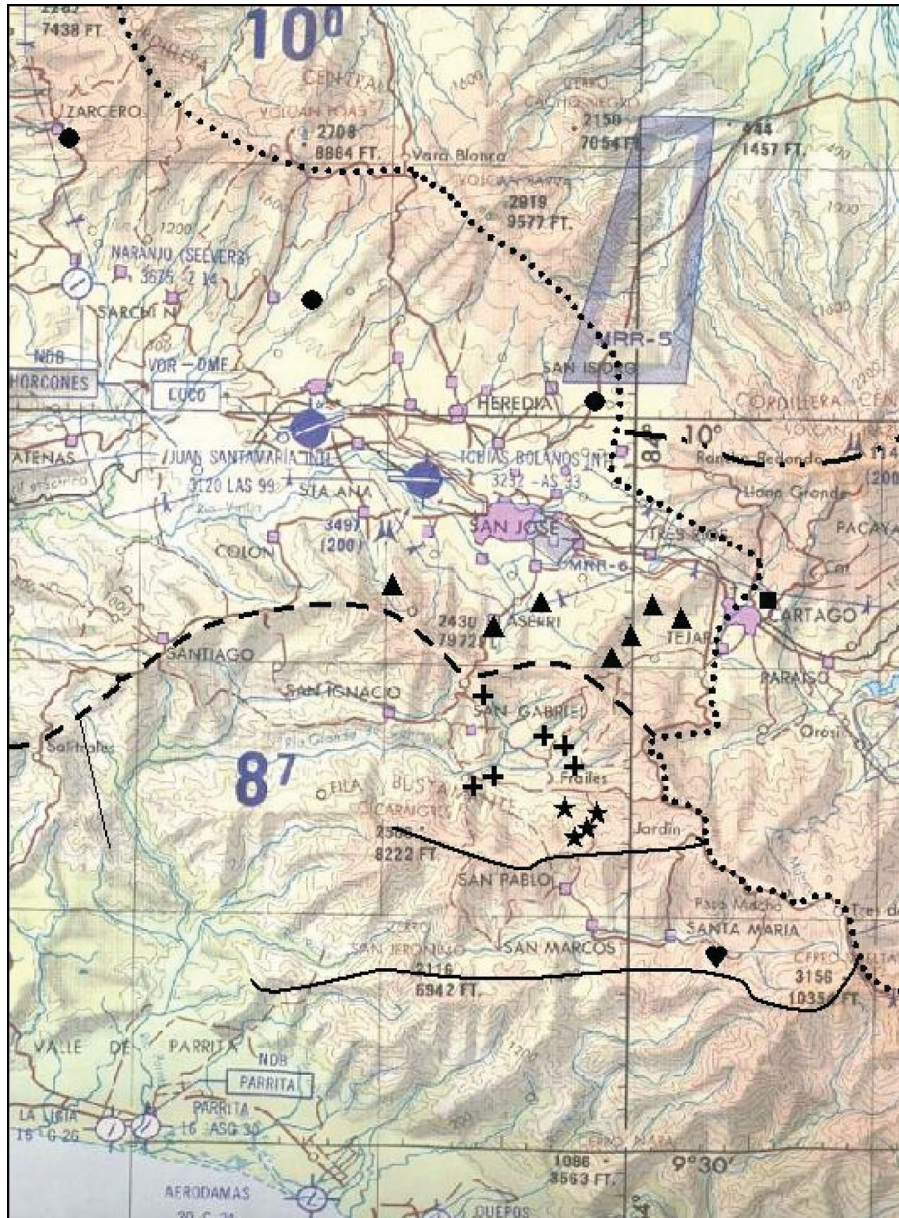
**Table 1** – List of populations of wild common bean found, sites, watershed, coordinates and year. Costa Rica.

Collector's Number	Province, district, closest site	Watershed	Longitude	Latitude	Altitude (masl)	Year found
1. 2097	San José, Tarbaca	Virilla sur	84°07'W	9°49'N	1750	1987
2. 2111	San José, Aserrí	Virilla sur	84°07'W	9°52'N	1560	1987
3. 3106	Alajuela, Carrizal, Chagüite	Virilla norte	84°10'W	10°06'N	1510	1998
4. 3126	Cartago, San Nicolás, Quircot	Reventazón	83°56'W	9°54'N	1540	1998
5. 3131	San José, Desamparados, Jericó	Candelaria n	84°03'W	9°49'N	1540	1998
6. 3132	Alajuela, Alfaro Ruiz, Zarcero	Virilla norte	84°23'W	10°10'N	1610	2002
7. 3133	Alajuela, Poas, Sabana Redonda	Virilla norte	84°14'W	10°07'N	1380	2002
8. 3134	San José, Aserrí, Tranquerillas	Candelaria n	84°07'W	9°48'N	1500	2002
9. 3135	San José, Aserrí, Chirogres	Candelaria n	84°06'W	9°48'N	1480	2002
10. 3136	San José, Desamparados, San Miguel	Virilla sur	84°04'W	9°51'N	1370	2002
11. 3137	San José, Escazú, Bebedero	Virilla sur	84°10'W	9°54'N	1600	2002
12. 3140	Cartago, La Unión, Pque Iztarú	Virilla sur	83°58'W	9°54'N	1750	2002
13. 3143	Cartago, La Unión, Hda Tres Ríos	Virilla sur	83°59'W	9°54'N	1500	2002
14. 3147	San José, Aserrí, El Tigre	Candelaria n	84°06'W	9°49'N	1450	2003
15. 3148	San José, Desamparados, Manzano	Candelaria n	84°05'W	9°49'N	1370	2003
16. 3168	San José, Sta. María de Dota, Copey	Pirris	83°57'W	9°39'N	1600	2003
17. 3178	San José, Desamparados, Guatuso	Virilla sur	84°02'W	9°51'N	1380	2003
18. 3184	San José, San Pablo, Río Tarrazú	Candelaria s	84°01'W	9°44'N	1450	2003
19. 3186	San José, San Gabriel, Bajo Los Angeles	Candelaria s	84°05'W	9°44'N	1200	2004
20. 3188	San José, San Gabriel, Sn Andrés León Cor.	Candelaria s	84°05'W	9°43'N	1250	2004
21. 3189	San José, San Gabriel, Sn Andrés León Cor.	Candelaria s	84°05'W	9°43'N	1300	2004
22. 3190	San José, San Gabriel, Vuelta de Jorco	Candelaria n	84°08'W	9°43'N	1480	2004

reported on maps of life zones (Bolaños M. and Watson C. 1993, Gómez Pignataro 1986, Herrera and Gómez Pignataro 1993, Tosi 1969), have been visited.

Wild common bean is usually found in subhumid montane forests (bmh-P, bh-MB and bmh-MB: Araya Villalobos *et al.* 2001) at intermediate altitudes (Table 1), now largely cleared for coffee plantations and replaced by urban areas. These are seasonal forests with a marked dry season (Matamoros 1996). In this habitat, the end of the rainy season coincides with the flowering period, and mists are not frequent; bean plants thus escape pressures from diseases such as anthracnose and root rots, as well as drought stresses, and seed dispersal will occur during the dry season (3-4 months; late December to April) (Araya Villalobos *et al.* 2001). Germination of wild bean will occur from July onwards, with flowering in September. Carpenter bees, bumble bees and honey bees have been seen as active in the pollination at Quircot, Jericó, and Sabana Redonda, as indicated by the field notes.

While certain wild bean populations seem stable (e.g. Aserrí, Bebedero, San Miguel), others are endangered. There are important variations in demography of wild bean populations (as observed for # 2097, 3148), likely due to important climatic variations (rainfall) from one year to another, so a critical assessment of the conservation status is not easy. The site of Zarcero has been converted into a quarry, and the population # 3132 seems gone. Urbanization with the conversion of original land into housing compounds is a threat to population # 3178 in 2003-2006. In Quircot the use of atrazin in maize has virtually eliminated the wild bean # 3126 from certain plots. In Chirogres and Copey, weeding has seriously reduced the wild bean to just a very few plants. Coffee plantations and use of herbicides therein have eliminated the population # 3131. Paradoxically, the including of a population (# 3140) into a protected area, viz. Parque Iztarú, may not guarantee high numbers of plants, as the closing of the forest canopy restricts light and increases disease pressures.



**Figure 1.** Distribution of wild common bean in the Central Valley of Costa Rica (base map: IGN-DGAC, 1991). Solid square: Reventazón, one population; solid heart: Pirrís, one population; closed circles: Virilla north, 3 populations; solid triangles: Virilla south, 7 populations; crosses: Candelaria north, 6 populations; and closed stars: Candelaria south, 4 populations. Dotted line represents the continental divide, while the other lines limit the different watersheds (see text).

### Molecular characterization

Dominant phaseolin types for the six populations were 'Simple-4' (83.4 %) and 'S' (10.43 %) (Table 2). 'M1' was observed in the populations of Aserri and Tarbaca, while 'S' phaseolin was noted in the populations of Jérico and Quircot.

In the six populations analyzed for diaphorase, the Dia<sup>100</sup> pattern was found in 94% of the cases, while the Dia<sup>95</sup> pattern was found in 14 individuals of Chagüite, Jérico and Quircot. For peroxydase (PRX) individuals displayed mainly the Prx<sup>100</sup> allele; however 35 individuals out of 197 showed the Prx<sup>98</sup> allele in the populations of Quircot, Zarcero, Jérico and Chagüite (one case).

**Table 2.** Results for the different descriptors and markers; N= total number of individuals analyzed.

Average of seed weight (g)	Phaseolin type	Isozymes		Chloroplast haplotype
		Dia <sup>1</sup> Pattern	Prx <sup>2</sup> allele	
6 (2.5-7)	'Simple-4' (327)	Dia <sup>100</sup> (202)	Prx <sup>100</sup> (162)	G ó H (104)
	'S' (41)	Dia <sup>90</sup> (14)	Prx <sup>98</sup> (35)	L (66)
	'M1' (23)	Dia <sup>95</sup> (7)		J (22)
	'Simple-3' (1)	Dia <sup>98</sup> (6)		
N=443	N=392	N=229	N=197	N=192

<sup>1</sup>Sprecher (1988); <sup>2</sup> Koenig & Gepts (1989).

The haplotype G or H (we are currently sequencing the *ndhA* non-coding intron fragment in order to distinguish between them) was found in 54% of the cases (Table 3). Haplotype L was found at lower frequency, in 43, 17, 4 and 2 individuals in Quircot, Zarcero, Aserrí and Chagüite, respectively. Haplotype J was found in 12, 1 and 9 individuals in Quircot, Zarcero and Chagüite, respectively.

Generally speaking, two or three SSR alleles used to be dominant at each locus analyzed, while the other alleles were at low frequencies. Table 4 indicates that certain alleles might be present only in one location, while others seem widespread (for instance BM140-164 seems both dominant and widespread). Further, certain alleles present in one year do not appear in a subsequent sampling, while others appear for the

first time. For instance, for the population at Aserrí, thirteen alleles seem to be lost between the samplings of 1987 and 2002, while two are found for the first time. The loss over years seems more severe at Jérico, while diversity seems on the rise at Quircot.

## DISCUSSION

These results elicit the following points for discussion. First, as compared to previous works (Debouck *et al.* 1989, Araya Villalobos *et al.* 2001), we have found over the past few years sixteen additional populations of wild common bean. Targeted collecting, namely when the collecting team knows more about the ecology of the target species, can thus result in the

**Table 3.** Alleles found for the different microsatellites. N is the total number of individuals analyzed for each primer, while numbers in brackets refer to the number of individuals for each allele.

<i>Loci of microsatellites evaluated in the six wild populations</i>								
BM140	BM172	BM175	BM183	BM187	BM188	BM189	BM205	GATS91
160 (60)	80 (103)	160 (65)	110 (80)	165 (61)	147 (93)	138 (86)	122 (44)	224 (38)
164 (42)	72 (5)	164 (30)	100 (6)	160 (17)	150 (6)	160 (5)	136 (27)	216 (29)
139 (2)	90 (3)	153 (3)	106 (3)	167 (8)	178 (1)	148 (2)	140 (16)	212 (16)
166 (2)	104 (2)	167 (2)	112 (1)	177 (7)	160 (1)	131 (1)	132 (1)	230 (3)
168 (2)	83 (1)			174 (3)				250 (2)
178 (1)	78 (1)			163 (3)				243 (2)
180 (1)	75 (1)			189 (1)				253 (1)
154 (1)								220 (1)
								200 (1)
N=111	N=116	N=100	N=90	N=100	N=101	N=94	N=88	N=93

**Table 4.** Alleles found for the different microsatellites at three sites (Aserrí, Jérico and Quircot) and different years. N is the total number of individuals analyzed for each primer, while numbers in brackets refer to the number of individuals for each allele. Figures in bold face refer to alleles not found in later periods (2002 or 2003), while figures in bold italics refer to new alleles found in the respective populations.

Locus	Aserrí		Jérico		Quircot	
	1987	2002	1998	2003	1998	2003
BM140	139,160,164	164	160,164, <b>166,168,178</b>	160,164, <b>180</b>	160,164,178	<b>154</b> ,160,164, <b>166,168</b> ,178
BM172	<b>75,78</b> ,80	80, <b>90</b>	80, <b>104</b>	<b>72,80,90</b>	78,80	<b>75,78,80,83</b>
BM175	<b>153,160,164</b>	160	160,164	160,164	153,160	153,160, <b>164,167</b>
BM183	100,110	100,110	<b>100,106</b> ,110, <b>112</b>	110	106, 110, 112	<b>100</b> ,106,110,112
BM187	<b>165</b> ,167	167	<b>160,163,165,177</b>	163,167	163,165,167,174	163,165,167,174, 177, <b>189</b>
BM188	147, <b>150</b>	147	147,150, <b>178</b>	147,150	147,150	<b>142</b> ,147,150
BM189	138	138	<b>131</b> ,138,148, <b>160</b>	138,148	138,145,148	<b>131</b> ,138,145,148,160
BM205	<b>122,140</b>	<b>136</b>	122,136,140	122,136,140	122,132,136,140	122,132,136,140
GATS91	<b>212,220</b> 224, <b>230</b>	224	212,216,220,224, <b>230,253</b>	220,224	212,216,224,237, 243,250	<b>200</b> ,212,216, <b>220</b> ,224,237, 243, 250

disclosure of additional populations. From the perspectives of monitoring genetic erosion of natural vegetations and advising on the safe introduction and management of transgenical crops, the question is obviously whether more populations are still to be discovered in Costa Rica. The vegetation types where the wild bean grows, once reported back on maps of life zones (Bolaños & Watson 1986; Gómez Pignataro 1986; Herrera & Gómez Pignataro 1993; Tosi 1969) and considered as with high potential, have all been visited. As indicated above, two areas might still be of promise, while the others not. Perhaps the further development of a current GIS tool (Jones *et al.* 1997) might help to be certain about the location of new populations. Many of the existing GIS tools predict about the presence of wild species on the basis of past records (germplasm accessions, herbarium vouchers), while plant explorers discover new species or populations on little background.

Second, the populations of wild common bean display different levels of polymorphisms. The phenotypic biochemical markers, phaseolin and isozymes, clearly indicate their belonging to the Mesoamerican genepool (Claros *et al.* 1994; Koenig & Gepts 1989; Toro Ch. *et al.* 1990), as similar electromorphs have been observed in wild beans of Mexico and Guatemala (Gepts 1988). It remains to be checked in future analysis whether the 'S' phaseolin is concentrated only in Quircot and Jérico where cultivated common bean has been observed in the past. Along this hypothesis, 'S' phaseolin would not be part of the original variability of wild beans in Costa Rica, but has become so because of gene flow events over the

past centuries. The dominant cp DNA haplotype would confirm the findings of Chacón (2001), with one haplotype being present in the populations of wild common bean in Costa Rica – a result to be expected from a slow evolving molecule (Avice 1994; Dowling *et al.* 1996). The large numbers of individuals with haplotypes L and J typically found in wild forms of Colombia, western Mexico and Guatemala (Chacón 2001), respectively, is puzzling, and might be indicative of novel variation in Costa Rica. It might also indicate gene flow events through cytoplasm capture from introduced landraces from these countries east and west of Costa Rica. Along this scenario the wild forms have crossed once with the cultivated materials and through repeated crossing have returned fully to the wild phenotype but keeping the 'cultivated' cytoplasm. Many individuals with 'unexpected' cpDNA were found in Quircot, Zarcero and Aserrí where gene flow has been observed in the past (Debouck *et al.* 1989; Araya *et al.* 2001).

Third, one would like to test whether the genetic diversity is organized along the watersheds. Abe (2000) has shown that diversity in wild soybean is highly structured along watersheds in northern Honshu, Japan, namely along the continental divide. Unfortunately, we have only one wild population east of the continental divide (Quircot), and it might be affected by gene flow and the presence of cultivated beans, since it was found on field borders (Araya *et al.* 2001). Many alleles are rare (displayed by single individuals) for example BM187-160, BM188-178 and GATS91200, and the question whether they can characterize individual populations is worth investigating. On the basis of the

microsats data, it seems that variability is gained or lost in the different populations over time (Table 4). These changes point out first to the sampling; the strategy (Debouck 1988) was to collect large numbers of seed from original populations, but collectors were limited to amounts available at time of visit. The continuing presence of the populations year after year is an indication that the collectors have not harvested excessive amounts of seed. More original seed needs to be analyzed in order to confirm the progressive loss of alleles at Aserrí, and the apparent gain of diversity at Quircot. Bean planting seems abandoned at Aserrí since our first visit in 1987, or limited to few plants in the coffee plantations, while in Quircot it went on though with important variations mainly due to price fluctuations. It is tempting to think about gene flow, with decreasing frequency in Aserrí due to the lack of continuing contact between the wild relative and the crop, and increasing frequency in Quircot because the collectors there were specifically looking for intermediate weedy forms (González-Torres *et al.* 2003). In the former case fifteen years after the gene flow event most alleles (of neutral selective value) seem to be lost. These aspects deserve further study given their consequences for the long-term survival of the populations (Avisé 1994; Hamrick & Godt 1996).

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