

GENE FLOW PROJECT - 2006
CENTRO INTERNACIONAL DE AGRICULTURA TROPICAL
UNIVERSIDAD DE COSTA RICA
BUNDESMINISTERIUM FÜR WIRTSCHAFTLICHE ZUSAMMENARBEIT UND ENTWICKLUNG (GERMANY)



Wild form and landrace of common bean growing close at the experimental site of Quircot (December 2005) (photo: Rodolfo Araya).

TRIP REPORT – COSTA RICA, JANUARY 2006

Daniel G. Debouck¹ and Rodolfo Araya V.²¹Centro Internacional de Agricultura Tropical, and²Universidad de Costa Rica, Estación Fabio Baudrit Moreno**Introduction and Objectives**

This field exploration is part of a series of field studies (Araya et al. 2001; González et al. 2004a) aiming at: (i) identification of populations of wild beans in Costa Rica, (ii) location of places where traditional landraces of common bean are still grown in Costa Rica, above all in the Central Valley of that country, (iii) identification of weedy races possibly resulting from events of gene flow between such landraces and wild forms. Preliminary results seem to indicate that events of gene flow do occur in Central Costa Rica (González Torres et al. 2004b). These preliminary results need confirmation, along four directions: (i) intensity and direction of gene flow (through pollen flow), (ii) presence of gene flow wherever both biological forms are in sympatry and close contact, (iii) persistence of effects of gene flow through time, and (iv) participation of alien species in the gene flow events. Incidentally, if knowledge about the identity and distribution of wild species can be increased, this is an additional objective and net benefit to the biodiversity of that country.

In turn, these objectives are in line with the establishment of the knowledge basis for the safe introduction and management of transgenical beans on the one hand, and for the management of genetic resources on-farm and *in situ* on the other hand, specially if the work by farmers on the ‘wild-weed-crop complexes’ can be evidenced (Beebe et al. 1997). Transgenical common beans with herbicide resistance have been claimed (Agarao et al. 2002), although attempts to reproduce the transformation process have failed (Jacobsen, personal communication, 2004). This situation may mean that transgenical beans may be soon available on the market, and planted for commercial production; accordingly, biosafety procedures if found desirable have to be developed on sound biological background, among others (Rissler & Mellon 1996). One aspect is thus to map on a large scale populations of taxa genetically compatible with the crop; another is to spot the hybrid swarms resulting from gene flow events between the components of the crop gene pool. This may include landraces, wild and weedy forms of the crop (usually considered within the primary gene pool), and wild species partially or totally genetically compatible (Ellstrand et al. 1999; Gepts & Papa 2003).

We were also interested in monitoring the evolution of past gene flow events, and therefore visited again the sites of Aserri and Quircot, in the provinces of San José and Cartago, respectively, where gene flow events were spotted as early as 1987 and 1998, respectively (Debouck et al. 1989; Araya et al. 2001). For the monitoring of gene flow events over time, we sampled materials at the same localities as in previous years; therefore the same collection numbers are used, but with indication of years. It would not make sense (duplicates in the genebank!) to give new collection numbers. If thought pertinent, we did the same for other populations of wild species in order to have a better appraisal of evolution of their demography over time.

During the field work in Quircot in 2004, we found a few plants with characteristics of artificial interspecific hybrids (i.e. showy flowers, pods with few or just one ovule evolving into a seed, shrivelled seeds, ‘cripple’ leaflets) (for further description, see Hucl & Scoles 1985; Smartt 1970). These materials were confirmed as hybrids between *P. vulgaris* and *P. dumosus* Macfad. (González-Torres et al. 2006). So, gene flow may involve another species, but it is not clear to us why it is with *P. dumosus* (an introduced species into Costa Rica, even if it can survive well as feral: Schmit & Debouck 1991), and not the sympatric *P. costaricensis* Freytag & Debouck. The collection of additional material for study in 2006 with help of the different markers established in previous works (González Torres et al. 2004b, 2006) was thus appropriate.

Schedule and journeys

January 16: flight Cali – San José.

January 17: Cartago: Quircot, Poroses. Collections in open vegetations (# 3125, 3126, 3142, 3149), farmers' fields (# 3152, 3153, 3154, 3194, 3195), and field borders (# 3126). As indicated elsewhere (Araya et al. 2001; González Torres et al. 2004a), Quircot has been our major site for field observations since 1998. Plots are planted by farmers associated through a cooperative, in a modified slash-and-burn practice, with less fallow between plantings. Plots are planted with maize, beans (both grain and snap bean varieties of common bean, and *P. dumosus* Macfad. 'cuba'), and vegetables (cabbage, pea, squash, arracacha).

January 18: San José: Aserrí, Alajuelita. Collections in open vegetations (# 2111, 2112, 2116, 3131, 3146, 3176, 3177) and old fallows (# 2111, 2112).

January 19: San José: Santa Eduvigis, Santa María de Dota. Collections in open vegetations (# 3168, 3170, 3172, 3173, 3182). The Santa María area was visited again in order to better evaluate the presence of wild common bean in that watershed, since only one plant was found in 2003.

January 20: San José: Vuelta de Jorco (# 3190), Palmichal (# 3191), Pabellón above Santa Ana (# 3192, 3193).

January 21: preparation of samples and report.

January 22: flight San José – Cali.

Results and Discussion

General

A list of materials found can be seen in Table 1.

Table 1 – List of populations and materials found, species, sites, watershed, and coordinates.

| Collectors' Number | Date | Species | Province, district, closest site | Watershed | Longitude | Latitude | Altitude (masl) |
|--------------------|------|------------------|---|--------------|-----------|----------|-----------------|
| 3125 | 17/1 | <i>xantho</i> | Cartago, San Nicolás, Quircot | Reventazón | 83°56'W | 9°54'N | 1540 |
| 3126 | 17/1 | <i>vulg silv</i> | Cartago, San Nicolás, Quircot | Reventazón | 83°56'W | 9°54'N | 1540 |
| 3127 | 17/1 | <i>costar</i> | Cartago, San Nicolás, Quircot | Reventazón | 83°56'W | 9°54'N | 1540 |
| 3149 | 17/1 | hybrid? | Cartago, San Nicolás, Quircot | Reventazón | 83°56'W | 9°54'N | 1540 |
| 3165 | 17/1 | <i>leptos</i> | Cartago, San Nicolás, Quircot | Reventazón | 83°56'W | 9°54'N | 1540 |
| ? | 17/1 | <i>vulg cult</i> | Cartago, San Nicolás, Quircot | Reventazón | 83°56'W | 9°54'N | 1540 |
| 3142 | 17/1 | <i>costar</i> | Cartago, San Nicolás, 5km NNW Quircot | Reventazón | 83°55'W | 9°55'N | 1850 |
| 2111 | 18/1 | <i>vulg silv</i> | San José, Aserrí, Piedra de Aserrí | Virilla sur | 84°07'W | 9°52'N | 1540 |
| 2112 | 18/1 | <i>xantho</i> | San José, Aserrí, Piedra de Aserrí | Virilla sur | 84°07'W | 9°52'N | 1540 |
| 2116 | 18/1 | <i>costar</i> | San José, Aserrí, Piedra de Aserrí | Virilla sur | 84°07'W | 9°52'N | 1540 |
| 3146 | 18/1 | <i>leptos</i> | San José, Aserrí, Piedra de Aserrí | Virilla sur | 84°07'W | 9°52'N | 1550 |
| 3131 | 18/1 | <i>vulg silv</i> | San José, Desamparados, Jericó | Candelaria n | 84°03'W | 9°49'N | 1540 |
| 3176 | 18/1 | <i>xantho</i> | Sn José, 1.5km SW Llano Alajuelita | Virilla sur | 84°07'W | 9°52'N | 1400 |
| 3177 | 18/1 | <i>lun silv</i> | Sn José, 2.4km SW Llano Alajuelita | Virilla sur | 84°07'W | 9°52'N | 1470 |
| 3172 | 19/1 | <i>hygroph</i> | Sn José, Sn Isidro, Sta Eduvigis | Savegre | 83°45'W | 9°30'N | 1580 |
| 3173 | 19/1 | <i>hygroph</i> | Sn José, Sn Isidro, Las Nubes | Savegre | 83°46'W | 9°28'N | 1550 |
| 3168 | 19/1 | <i>vulg silv</i> | Sn José, Sta. María Dota, 1km W Sta Mar | Pirris | 83°59'W | 9°39'N | 1860 |
| 3170 | 19/1 | <i>tuerck</i> | San José, Sta María Dota, Los Angeles | Pirris | 83°58'W | 9°38'N | 1500 |
| 3182 | 19/1 | <i>talaman</i> | Sn José, Sta. María Dota, 1km W Sta Mar | Pirris | 83°59'W | 9°39'N | 1500 |
| 3190 | 20/1 | <i>vulg silv</i> | San José, San Gabriel, Vuelta de Jorco | Candelaria n | 84°08'W | 9°48'N | 1480 |
| 3191 | 20/1 | <i>lun silv</i> | San José, Sn Ignacio, 4km NE Palmichal | Candelaria n | 84°11'W | 9°52'N | 1390 |
| 3192 | 20/1 | <i>lun silv</i> | Sn José, Sta Ana, 1km N Pabellón | Virilla sur | 84°12'W | 9°55'N | 1280 |
| 3193 | 20/1 | <i>costar</i> | Sn José, Sta Ana, 0.5km S Pabellón | Virilla sur | 84°12'W | 9°54'N | 1590 |
| 3194 | 17/1 | hybrid? | Cartago, San Nicolás, Quircot | Reventazón | 83°56'W | 9°54'N | 1540 |
| 3195 | 17/1 | <i>dumos ?</i> | Cartago, San Nicolás, Quircot | Reventazón | 83°56'W | 9°54'N | 1540 |

N.B.: collection numbers in bold mean that herbarium vouchers were made and deposited initially at CR, INB and USJ (label in annex).

Per species

Phaseolus costaricensis Freytag & Debouck

We found this species but with populations of smaller size in Quircot (# 3127) and close to Poroses (# 3142). A few plants in the Quircot population display speckled black seed, while the normal phenotype is speckled brown grey. A new population (# 3193) of this species was found south of Pabellón, county of Santa Ana; it seems to be the one to the extreme north-west in the mountainous range “Cerros de Escazú”. It was found reduced to a few scattered plants while gaining altitude in this sector in search of wild common bean. The latter species was not found, meaning that the population of Matinilla (# 3137) is the one to the extreme west in that mountainous range. With this addition (see label in annex), there are now twenty-one populations of *P. costaricensis* sampled. The site south of Pabellón has been much modified, with grassland replacing the original climax humid mountain forest. In this ecological zone, it is likely that common bean would be heavily affected by anthracnose, a fungal disease for which it might be useful to screen the *P. costaricensis* germplasm.

Phaseolus x hybrid

At Quircot we found again (# 3149) in the open vegetation (named sector 6, to the east of the Quircot site) a material that could well be a natural interspecific hybrid, and in the middle of the cultivated field (named sector 3, see Figure 1) another possible hybrid (# 3194). It is not sure that this material has been planted on



Figure 1. The experimental site of Quircot. To the right is the streamlet Quebrada Norberta, and sector 6 (outside the actual picture). The steep slope in the center has been planted in 2005-2006 with a maize- bean (‘cuba’, *P. dumosus*) association.

purpose by the farmer Mr. Tali, even if it was growing on a maize stem. Both materials showed pods with reduced fertility: on average only one ovule (out of 4-5) developed into full mature seed, although they both showed vigorous vine development. One should note that # 3149 was found at about the same place in January 2003 and January 2006, being the same plant or its progeny. As noted by Freytag & Debouck (2002), perennialism is a trait of the Phaseoli. While wild *P. vulgaris* behaves as an annual herb in Costa Rica, other sympatric species such as *P. costaricensis* and *P. dumosus* behave as short-lived perennial vines. If proven as interspecific hybrids (González-Torres et al. 2006), the materials # 3149 and 3194 could be used as to transfer (or better, say, to recover) perennialism to cultivated common bean, or as bridge in breeding programs (Singh 2001).

A special collection might be # 3195, and could tentatively be reported as *dumosus* hybrid (see below), possibly with other forms of the year-bean, or with *P. costaricensis*. The latter is sympatric in the valley of the streamlet Quebrada Norberta. Its red seeds, sometimes with bayo streaks, is quite unusual in *P. dumosus* (Schmit & Debouck 1991). Because it has been found since 2003, it seems to be positively selected for by the local farmers; it might also be a true stable variant of *P. dumosus*, though initially coming from open pollination.

Phaseolus hygrophilus Debouck

The two populations known to date of this species were visited again in 2006, basically in order to assess their status and phenology. While population # 3172 was found stable with more than twenty individuals (many flowering and setting seeds), population # 3173 was found reduced to just four plants. The forested sites should be included into some protection schemes, with less expensive weeding. Soil protection would invite to maintain a forest cover anyway. Overall morphology and pod attributes of this taxon would indicate its belonging to the *Brevilegumeni* section (Freytag & Debouck 2002), so increasing it to four taxa; if validated, there would be no interference with transgenical beans. Also because beans would not be able to survive in those rainy habitats; local informants reported about a 'dry' season of only two months per year (also marginal for the survival of the introduced year-bean *P. dumosus*; see Schmit & Debouck 1991).

Phaseolus leptostachyus Benth

Although our visit was late for this early taxon, we still found flowering stems with mature seed, usually infested by weevils. We found it (# 3165) at Quircot on the edges of grasslands invaded by *Hyparrhenia rufa* (indicating frequent fires), and among rocks in Aserrí (# 3146). Its belonging to the section *Falcati* (Freytag & Debouck 2002) with $2n=2x=20$ (Mercado Ruaro & Delgado Salinas 1996) makes it impossible to cross with common bean under natural conditions. This would be another species out of concern for biosafety.

Phaseolus lunatus L.

This is a wild species quite common in the Central Valley, though some populations are endangered by other land uses (e.g. urbanization, for # 3177; pasture land for cattle grazing, for 3191). For the record we added two novel populations on the southern slope of Cerros de Escazú (# 3191, 3192; see a label in annex). It has toxic seeds (Baudoin et al. 1991), and thus less prone to pests attacks. It is the type species of the *Paniculati*, and quite distant from the common bean (see a review by Debouck 1999), and thus not a concern in biosafety for the latter.

Phaseolus talamancensis Debouck & Torres

The population # 3182 was visited once more while searching for wild *P. vulgaris* (# 3168). It was already at the full seed dispersal phase. One should note that it is the first population of this species (there are only four known to date) reported outside the range of the Cordillera de Talamanca (an indication that the senior author walked outside his shoes when naming it ?!). But this opens the possibility if searches are carried out early enough to find it out in other mountainous ranges of Costa Rica. A preliminary report (Gaitán et al. 2000) indicates its closeness with *P. tuerckheimii* in the *Brevilegumeni* section, thus likely far away from the *Phaseoli*.

Phaseolus tuerckheimii Donnell-Smith

The population # 3170 (a beautiful picture of it was on display for the PITTA Frijol meeting of 2005) was visited when searching for more wild common bean in the county of Dota. It is currently threatened by the extension of coffee plantations. It belongs to the *Brevilegumeni* (Freytag & Debouck 2002), together with *P. oligospermus* Piper, *P. campanulatus* Freytag & Debouck, and possibly *P. hygrophilus* Debouck. Preliminary results with ITS sequencing (Gaitán et al. 2000) would indicate that it is quite remote from the *Phaseoli*, and thus out of concern in biosafety issues for common bean.

Phaseolus vulgaris L.

As indicated above, searches for new populations of wild common bean proved unsuccessful (with the exception of # 3168, see below). The total of populations for Costa Rica to date would thus be 22 (González Torres et al. 2004), and their precise location now fully documented (with samples in both genebanks and herbaria) is important for biosafety. Important variations in demography from year to year have been noticed. This is exemplified by # 3168 (Sta María de Dota): in December 2003, only one single plant was found E of Santa María de Dota. In January 2006, the progeny of that plant was not found, but a large population W of Santa María (not noticed in 2003!). The population # 2111 was found abundant at the cross site of Piedra de Aserri in 1987 and again in 2002, and absent in 2006.

Some variation was displayed in seed of the wild form (# 3126) at Quircot, with black seeded variants. Similarly, variants with large pods and purplish pods (at full physiological maturity) were found. Escaped forms found in 2003 all as pole types, i.e. black seeded (# 3154), brownish stripped ('Higuerilla', # 3152) and a solid yellow (# 3153) were again found in 2006 in the cultivated plots and borders, indicating that if certain ecological conditions are fulfilled (overall ecology fitting for wild forms, vegetation growing in waste ground, and absence of herbicides) some escaped forms can survive without human planting and harvesting.

The farmer community of Quircot continues to change crops and varieties (when they continue to practice agriculture), with increased interest towards cash crops (cabbage, onions, parsley, camomile, sweet pea, snap bean, year-bean), while maintaining some crops for consumption on-farm (maize, beans, arracacha, squash, cassava, cocoyam). The old system of the maize-bean crop association called 'tapado' with a long fallow period seems almost gone, but interestingly because of good prices in Cartago year-bean 'cuba' (*Phaseolus dumosus* Macfady.) has been planted repeatedly over the past years in Quircot. Apart from the frequent yellow seeded type (see Schmit & Debouck 1991), a red *P. dumosus* (# 3195) is also planted (see above); until more analysis is performed on that group it is not sure where that unusual variability is coming from (crosses within *P. dumosus* itself or with *P. costaricensis*). On the other hand, the farmer community of Quircot continues to "experiment" with new introductions of bean germplasm: they are keen on introducing 'chilenos' or Andean types of the 'Nueva Granada' race (Singh et al. 1991) or red speckled Calima group (Voysset 2000).

Phaseolus xanthotrichus Piper

Plants of this species at the moment of our visit were all in stage of mature pods and almost completely defoliated. Its tuberous root makes it a bit tolerant to frequent fires of secondary bushes, and thus it is still frequent although often unnoticed because of its earliness, but urbanization means the extinction of local populations (e.g. # 3176). The special flower structure that resulted in the definition of a special section *Xanthotricha* (Delgado Salinas 1985; Freytag & Debouck 2002) makes the possibility of widecrossing with common bean quite remote, and thus leaves this species outside the biosafety considerations for common bean.

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Trabajo de Recolección de Germoplasma de *Phaseolus*

Misión colaborativa entre el Centro Internacional de Agricultura Tropical (Cali, Colombia) y ~~la~~ Estación Experimental Fabio Baudrit de la Universidad de Costa Rica, con el apoyo de Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung de Alemania.

HERBARIO

Nombre científico: Phaseolus lunatus L. forma silvestre
 Determinavit: D.G. Debouck Fecha: 20/01/2006
 Nombre vulgar: desconocido
 País/Estado/Municipio/Localidad: COSTA RICA, SAN JOSE, Santa Ana, 1 km al N de Pabellón.
 Longitud: 84 ° 12 ' W Latitud: 9 ° 55 ' N Altitud: 1280 m
 Fecha de Recolección: 20 de Enero de 2006.

Observaciones: en bosque tropical de transición muy modificado convirtiéndose en matorral con Compuestas, Gramíneas, Senna, Rubus, Vigna. Soleado, abierto. Suelo café orgánico derivado de esquistos. Escaso. En floración (flor rosado lila), vainas verdes. Tallos volubles 2-4 m de alto.

Colectores: D.G. Debouck, Walter Barrantes & Nestor Chaves
 Nº: 3192 Se colectaron semillas bajo el Nº: _____

Trabajo de Recolección de Germoplasma de *Phaseolus*

Misión colaborativa entre el Centro Internacional de Agricultura Tropical (Cali, Colombia) y ~~la~~ Estación Experimental Fabio Baudrit Moreno de la Universidad de Costa Rica, con el apoyo de Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung de Alemania.

HERBARIO

Nombre científico: Phaseolus costaricensis Freytag & Debouck
 Determinavit: D.G. Debouck Fecha: 20/01/2006
 Nombre vulgar: desconocido
 País/Estado/Municipio/Localidad: COSTA RICA, SAN JOSE, Santa Ana, 0,5 km al S de Pabellón.
 Longitud: 84 ° 12 ' W Latitud: 9 ° 54 ' N Altitud: 1590 m
 Fecha de Recolección: 20 de Enero de 2006.

Observaciones: en bosque de Ciprés muy alterado convirtiéndose en potreros. Helechos, Compuestas, Gramíneas, Orquideas terrestres. Mi soleado abierto. Suelo amarillo beige detrítico derivado de esquistos, orgánico fresco, lado de cañada. Grupo pequeño, en floración (flor rosado fuschia) - vainas verdes. Plantas trepadoras 2-3 m alto. Encontrado a 1 km de la cima que está en 1750 m.

Colectores: D.G. Debouck, Walter Barrantes & Nestor Chaves
 Nº: 3193 Se colectaron semillas bajo el Nº: _____