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## ***In Situ* Conservation Strategy for Wild Lima Bean (*Phaseolus lunatus* L.) Populations in the Central Valley of Costa Rica: a Case Study of Short-lived Perennial Plants with a Mixed Mating System**

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### **23.1 Introduction**

A study was conducted in the Central Valley of Costa Rica to support the *in situ* conservation of wild lima bean, *Phaseolus lunatus* L., prone to extinction as a result of growing urbanization and changes in agricultural and land use practices. These populations represent a very important genetic reservoir for the improvement of the various *Phaseolus* bean cultigens, commonly found in many traditional cropping systems not only in Latin America, but also in other tropical regions. *P. lunatus* is also considered a useful plant model due to its reproductive biology. Lima bean is a self-compatible annual or short-lived perennial species with a mixed mating system, e.g. predominantly self-pollinating but with a fair amount of outcrossing, mediated by insects.

In order to achieve the conservation objective, several investigations were conducted in the following areas: (i) ecogeography and metapopulation dynamics; (ii) population demography and phenology; (iii) floral biology and gene flow; (iv) genetic structure of populations using morphological, biochemical and molecular markers; and (v) *in situ* conservation methodologies. A summary of the most relevant information is provided here; further details can be found in Baudoin *et al.* (2004). These investigations were made under the project 'Studies on breeding systems: the case of a short-living perennial, alternatively outbreeder–inbreeder species – *Phaseolus lunatus* – and its consequences for germplasm conservation', which ran in 2–4-year phases from 1992 to 2000 with funding from Belgium's Directorate General for Development Cooperation. It was a collaboration among three partners: International Plant Genetic

Resources Institute (IPGRI)'s Regional Office for the Americas in Cali, Colombia, the Escuela de Biología of the Universidad de Costa Rica (UCR) and the Unité de Phytotechnie tropicale et d'Horticulture of Gembloux Agricultural University (FUSAGx), Belgium.

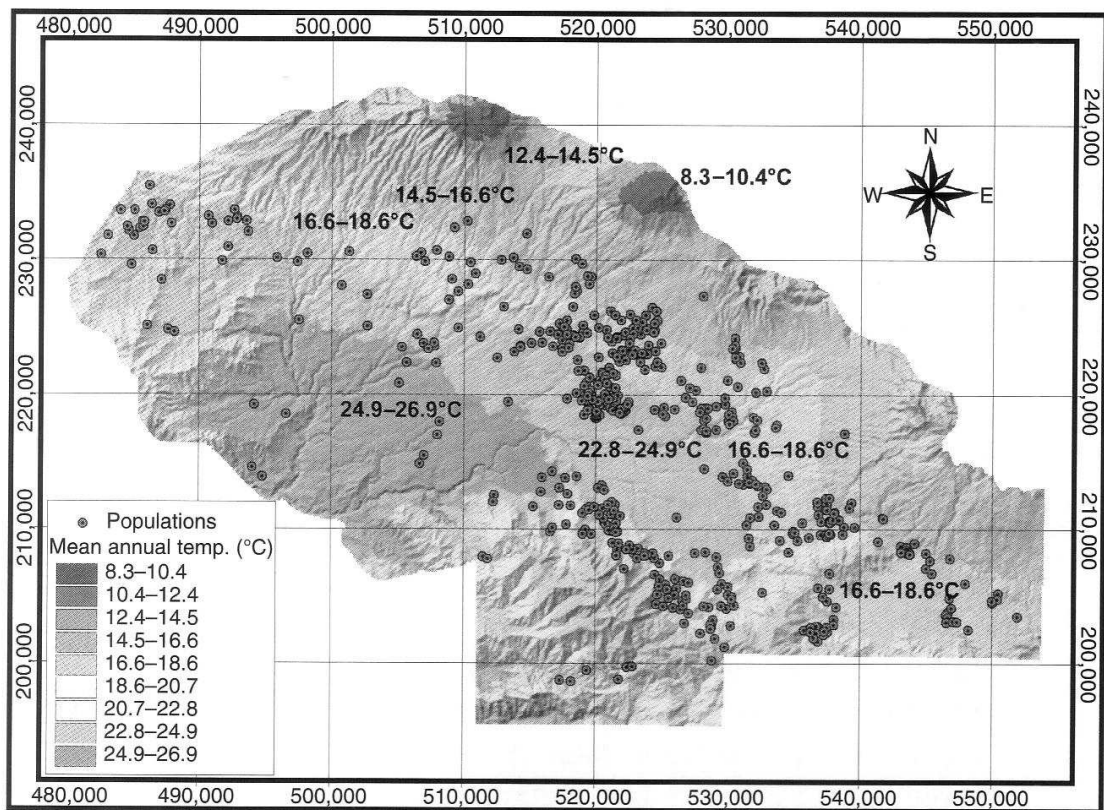
## 23.2 The Study Area and the Target Species

The Central Valley is an intermontane valley located in the geographic centre of Costa Rica and enclosing an area of approximately 1500 km<sup>2</sup>, with an altitudinal range between 800 and 1800 m above sea level (masl). The maximum length of the area is about 70 km, running from east to west, and the width is about 30 km, running from north to south. Most soils of the valley can be described as deep, rich in organic material and well drained. Great variation in the microgeographical distribution of rainfall results from the orientation of the mountain ranges and the location of wind passes. Rainfall is seasonal, with a well-defined dry season during December–April. In general, annual rainfall is lower in the eastern valley than in the western valley.

Wild populations of lima bean can be found throughout the Central Valley (Standley, 1937; Rocha *et al.*, 1997, 2002). The populations are usually found in open and disturbed areas with grasses and scattered trees or bushy thickets. They also colonize the coffee plantations from perennial fences (usually *Erythrina* L. and euphorbs) bordering the plots and are found where coffee is grown under shade (traditional coffee plantations), as well as in the waste lands around these plantations. Typically, agricultural activities are less intense in this agroecosystem, and do not rely on heavy use of herbicides for the elimination of weeds (Rocha *et al.*, 1997). However, because of changes in agricultural practices and in land use due to urban development, the populations of lima bean in the Central Valley are fragmented and undergo local extinction and recolonization (Rocha *et al.*, 1997).

## 23.3 Ecogeography and Metapopulation Dynamics

During the course of several surveys in the valley, the geographic location of a total of 565 populations (defined as groups of lima bean individuals isolated at least 500 m from any other) was determined. Studies in subsequent years revealed the appearance of new populations as well as the disappearance of old ones. In order to analyse the physical and ecological attributes of all locations where *P. lunatus* was found, a detailed classification of each site was conducted considering climate, soil, vegetation and topography using a geographical information system (GIS). Lima beans were found in seven different life zones, based on the Holdridge classification (Holdridge, 1966). The species is most abundant in the humid premontane (38% of populations) and the very humid premontane forests (54%). Similarly, the species was observed in 15 different biotic units, these being based on the structure and floristic composition of the plant communities (Gómez, 1986). However, 72% of the populations were



**Fig. 23.1.** Distribution of wild lima bean populations in the Central Valley of Costa Rica in relation to mean annual temperature. Dots indicate lima bean populations.

found in only three of these, i.e. humid, temperate and subtropical areas with a marked dry season lasting 3–6 months (Baudoin *et al.*, 2004). Figure 23.1 indicates the distribution of wild lima bean populations in the Central Valley in relation to mean annual temperature.

The numerous censuses and surveys conducted in the valley clearly demonstrate that wild lima bean populations undergo episodes of local extinction and recolonization in the study area (Rocha *et al.*, 1997, 2002). Therefore, a metapopulation approach would be the most suitable way to study population genetic structure and dynamics. For this purpose, six sampling transects were established along main roads in the valley. In 1994, 103 populations were found along these transects. All populations were visited every 2 weeks from January 1995 to April 2000. During each visit, the phenological status of each population was recorded by counting the number of individual plants that had foliage, flower buds, flowers, immature fruits and/or mature fruits with seeds. In addition, any disturbance experienced by each population was recorded such as fire, weeding (manually or with herbicides) and habitat destruction due to urban development.

This study confirmed that local extinction of lima bean populations is a common event in the Central Valley. Two types of extinction may be recognized:

1. Transient extinction, when all plants at the location disappeared but recolonization occurred during the same year;
2. Effective extinction, when all plants at the location disappeared and no recolonization occurred by the end of the year.

Most locations where extinctions occurred were recolonized during the same year, indicating that the soil seed bank plays a major role in restoring populations. In addition, the number of plants growing at a location appears to be negatively associated with the risk of local extinction. In general, most of the effective extinction events were observed at locations with fewer than ten plants. On the contrary, local extinction was not observed at locations with more than 50 plants. Moreover, once a group of plants became extinct at a given location, the risk of experiencing another extinction after recolonization was also high. In other words, the risk of extinction is not evenly distributed among the locations which were monitored, indicating different intensities of disturbances in the study area.

Metapopulation dynamics was studied by determining the transition probabilities among five possible population stages: (i) populations that remain vegetative during the year; (ii) populations that flowered during the year; (iii) populations that produced seeds during the year; (iv) populations that became extinct; and (v) populations that recolonized a site. Lefkovitch matrices (Lefkovitch, 1965) were used to describe the dynamics of these populations. The populations differed in their capacity to flower and to produce seeds every year and to maintain a seed reservoir in the soil (Rocha *et al.*, 2002). Populations that produce seeds in a given year have a high probability of producing seeds in future years, while those that become extinct also have a high probability of staying extinct. The UNIFIED LIFE MODEL software program (Legendre and Clobert, 1995) was applied to determine demographic parameters, in particular metapopulation growth rate ( $\lambda$ , the rate at which the number of populations in the metapopulation increases or decreases). Details are given in Rocha *et al.* (2002). In spite of the frequent extinctions recorded in this study, the metapopulation growth rate was rather close to 1 ( $\lambda = 0.990$ ), indicating that the lima bean is not under a clear risk of overall extinction in the Central Valley.

## 23.4 Demography

Very few data are available about the demographic behaviour of tropical food legumes like *Phaseolus* (Baudoin *et al.*, 2004). However, demography is widely considered to be a key to the formulation of *in situ* conservation strategies (Oostermeijer *et al.*, 1996; Ehrlén and Van Groenendael, 1998). Matrix models have been used to determine which stages of the life cycle of a plant are most vulnerable (Charron and Gagnon, 1991; Damman and Cain, 1998) and which life stage transitions most strongly affect population growth (Caswell, 1989), hence improving our understanding of how plant populations respond to changes in the environment, including the impact of human activities.

### 23.4.1 Field observations

Demography of five wild Lima bean populations in the Central Valley of Costa Rica was analysed for 3 years. These populations were located in highly disturbed



sites along trails, at the border of coffee plantations or in more natural sites such as at the edge of secondary regeneration forests. The following parameters were monitored in each population: (i) seed germination and longevity in the soil seed bank; (ii) survival and growth of seedlings and adult plants; and (iii) reproductive output of individual plants.

Matrix population models were used to compare the responses of the species to different environments and to identify the most critical life stages in each population.

The life cycle of lima bean was described on the basis of monthly field observations at each study site. In all, 49 quadrats (1 m<sup>2</sup> each) were sampled; all adult individuals were present and all seedlings appearing during the course of the study were labelled and identified by a code number. Developmental stage (vegetative or lignified stem), stem diameter (at 2 cm height), fecundity of each individual (number of seeds produced per year) and mortality rate in each quadrat were documented. Germination tests were conducted with painted seeds placed in plastic plates in the vicinity of each quadrat (Degreef *et al.*, 1997; Degreef, 1998).

#### 23.4.2 Seed bank dynamics

Germination rates ranged from 70% to 86% after 1 year in the soil and from 89% to 94% after 2 years, for the five populations. Seed coat dormancy is likely to be the major factor responsible for this delay in germination. This dormancy is induced by drought stress, which may occur on the soil surface just after seed dispersal (Degreef *et al.*, 2002). Hypothesizing that the annual germination rate is similar from year to year, it was estimated that 96–99% of seeds will germinate within 3 years after dispersal. The design of the model was simplified by assuming that all seeds were germinated within this period.

#### 23.4.3 Matrix demographic model

A life cycle graph was prepared for the species, in which each node is associated with a specific stage in the life of each individual. Due to the presence of a soil seed bank, seed classes were identified according to age. Juvenile and adult plants were grouped according to their developmental stage and stem diameter. Results are given in Degreef (1998).

A generalized projection model, using the UNIFIED LIFE MODELS software (Legendre and Clobert, 1995), was developed to describe the demography of lima bean populations. This allowed the determination of the asymptotic growth rate of each population when it reaches its stable structure; this rate can be used as a measure of fitness for the population in its particular environment, indicating either a decrease or an increase in the number of individuals, according to the location in threatened or more isolated areas.

Rocha *et al.* (1997) reported that wild lima bean populations in the Central Valley experience frequent extinctions and population fragmentation. These phenomena were recognized as resulting mainly from human perturbations,

but some populations not subject to weeding and other disturbances also showed oscillation in the number of their individuals in the course of time. Therefore, the critical status of some populations, indicated by low values of their asymptotic growth rate, probably results from perturbations occurring at a very early developmental stage and still having an impact on their dynamics. The status of each population, whether declining or recolonizing a site, has to be taken into account when designing a conservation plan and making management recommendations.

Elasticity is another parameter given by the model, which is of particular interest to determine which life cycle phase of the individuals is the most critical for the survival of the population, to quantify the contribution of each vital rate to population growth and to evaluate the effects of environmental perturbations on population dynamics (De Kroon *et al.*, 1986; Caswell, 1989; Mesterton-Gibbons, 1993; Degreef, 1998; Menges and Dolan, 1998). Analysis of elasticity values shows the importance of the direct transition from a seed present in the soil to a ligneous, potentially fertile individual, on population growth rate. This fate is only possible if seeds germinate soon after dispersal. Consequently, rapid germination is a key factor in the population dynamics of wild lima beans in the Central Valley of Costa Rica.

The elasticity analysis also reveals the relevance of the soil seed bank in the dynamics of populations. The growth rate of populations that are decreasing in size is particularly influenced by the arrival of new seeds into the soil seed bank. On the contrary, in increasing populations, elasticity for this particular transition is low.

Furthermore, the analysis shows the importance of the growth of ligneous individuals, mainly favoured by adequate air and soil moisture; and, finally, the survival of well-established ligneous and fertile individuals appears to be critical for the growth rate in the two populations located in the most natural sites.

It is generally recognized that the life history component which most strongly affects population growth depends on the habitat where the population grows (Bierzychudek, 1982; Silvertown *et al.*, 1996; Damman and Cain, 1998). Higher elasticities for growth and fecundity are typical of open habitats while higher elasticities for survival are characteristic of closed habitats (Menges and Dolan, 1998). In particular, for wild lima beans, this pattern is best shown by the extent and the frequency of disturbances to which populations are exposed. Higher growth and fecundity elasticities were obtained in populations experiencing perturbations and lower environmental stability. In contrast, populations in more stable habitats had higher survival elasticities. These results are essential for the implementation of an *in situ* conservation programme in the region.

## 23.5 Genetic Diversity

A major component of this project was to evaluate the genetic diversity of the wild lima bean populations found in the Central Valley. This diversity was studied at both the intra- and inter-population level, with the aim to analyse factors

responsible for the genetic structure and the microgeographical patterns of the *P. lunatus* gene pool in the region.

Despite the relatively small size of the study area and the small sample size taken from each population (five seeds from a bulked sample), significant phaseolin variation was found, mainly among the wild populations analysed, all belonging to the meso-american gene pool of *P. lunatus* (Vargas *et al.*, 2000, 2001).

Assessment of genetic variability was made in 330 populations and 1–60 individuals per population using both enzyme and microsatellite markers (Maquet *et al.*, 1996; Zoro Bi, 1999; Ouédraogo, 2003; Zoro Bi *et al.*, 2005). Table 23.1 presents results for 28 populations evenly selected throughout the valley. Microsatellite markers showed more allelic and genetic diversity than enzyme markers. However, the two markers revealed a relatively low percentage of polymorphic loci at the intra-population level (probably due to the belonging of the populations to the single Meso-american gene pool), few alleles per locus and a lack of heterozygotes, attributed mainly to self-fertilization. The coefficient of gene differentiation ( $G_{ST}$ ), reflecting the contribution of among population genetic diversity ( $D_{ST}$ ) to total genetic diversity ( $H_T$ ), is relatively high and similar for the two markers. Each wild lima bean population may therefore constitute a valid *in situ* conservation unit. In another study, based on 96 well-scattered wild populations, significant heterogeneity of allele frequencies was found in all enzyme markers (Table 23.2). The Wright consanguinity coefficient ( $F_{IT}$ ) showed deviation of populations from Hardy–Weinberg equilibrium, due to genetic differentiation among populations ( $F_{ST}$ ) and non-random mating within populations ( $F_{IS}$ ).

**Table 23.1.** Genetic diversity and structure of 28 wild lima bean populations in the Central Valley of Costa Rica with the use of two markers.

Marker	Intrapopulation polymorphism indices (means $\pm$ SE)			
	$P^a$	$A^b$	$H_0^c$	$H_e^d$
Enzymes	20.57 $\pm$ 5.07	1.227 $\pm$ 0.051	0.020 $\pm$ 0.021	0.080 $\pm$ 0.024
Microsatellites	48.89 $\pm$ 21.47	1.644 $\pm$ 0.384	0.012 $\pm$ 0.009	0.143 $\pm$ 0.058
	Nei's genotypic diversity indices (means)			
	$H_T^e$	$H_S^f$	$D_{ST}^g$	$G_{ST}^h$
Enzymes	0.120	0.083	0.036	0.303
Microsatellites	0.220	0.153	0.067	0.303

<sup>a</sup>Percent of polymorphic loci.

<sup>b</sup>Mean number of alleles per locus.

<sup>c</sup>Observed heterozygosity.

<sup>d</sup>Expected heterozygosity.

<sup>e</sup>Total genic diversity.

<sup>f</sup>Intra-population gene diversity.

<sup>g</sup>Inter-population gene diversity.

<sup>h</sup>Genic differentiation coefficient.

**Table 23.2.** Heterogeneity of allele frequencies and  $F$ -statistics estimated for 96 wild lima bean populations of the Central Valley of Costa Rica with the use of enzyme loci.

Locus	G-test (df)	$F$ -statistics $\pm$ standard deviation		
		$F_{IT}^a$	$F_{IS}^b$	$F_{ST}^c$
Adh-2	529.24 (52)***	0.873 $\pm$ 0.026	0.778 $\pm$ 0.079	0.433 $\pm$ 0.079***
Dia-1	145.00 (16)***	0.921 $\pm$ 0.061	0.874 $\pm$ 0.111	0.376 $\pm$ 0.136*
Gpi-1	26.61 (10)**	1.000 $\pm$ 0.000	1.000 $\pm$ 0.000	1.000 $\pm$ 0.007***
Mdh-2	402.16 (62)***	0.823 $\pm$ 0.035	0.747 $\pm$ 0.038	0.299 $\pm$ 0.077***
Pgm-2	874.11 (48)***	0.917 $\pm$ 0.035	0.777 $\pm$ 0.065	0.625 $\pm$ 0.122***
Mean		0.882 $\pm$ 0.026	0.761 $\pm$ 0.012	0.504 $\pm$ 0.094***

<sup>a</sup>Coefficient of consanguinity.

<sup>b</sup>Coefficient of intra-population consanguinity.

<sup>c</sup>Genetic differentiation between populations.

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; the comparisons being based on G-tests for allelic frequencies among populations and Student  $t$ -tests for  $F_S$ .

Despite small population sizes, common alleles at several loci, very few heterozygous individuals and low pollen and seed dispersal (see Section 6), significant polymorphism within populations was observed. This result, apparently contradictory with the low percentage of polymorphic loci at the intra-population level, could be due partly to the existence of gene flow over long distances, and partly to non-random mating within populations. Significant correlation was observed between population size and genetic variability. The loss of genetic diversity in small-sized populations could be attributed to inbreeding and bottleneck effects in some populations.

Through phaseolin characterization, populations at different sites of the valley were arranged in groups that were also congruent in terms of geographical proximity and phenology (Vargas *et al.*, 2001). This could be explained, at least in part, by climatic factors. Using isozyme markers, a similar study on 96 populations showed a high heterogeneous allelic distribution through all the polymorphic loci (Zoro Bi, 1999). Alleles were present in either very few or numerous populations and had an irregular geographic distribution. This non-random spatial distribution of alleles might result from limited gene flow between populations, and/or high localized selection pressure caused by biotic or abiotic stresses.

Genetic variation among populations and its geographical pattern are affected by several factors, including population dynamics and environmental conditions of the valley (Baudoin *et al.*, 2004). For example, wild populations might undergo repeated bottlenecks, as weeding and other agricultural practices only allow a few plants to survive and reproduce. These processes lead to significant reductions in effective population size, and to high levels of inbreeding, favouring the decrease of heterozygotes in the populations. The recurrent reduction in population size will also favour genetic differentiation among populations. The discontinuity of the habitats where wild lima beans are most likely to be found in the valley also promotes genetic differentiation among populations.



Such fragmentation is mainly the result of replacement of traditional coffee plantations by modern, intensive plantations and accelerated urban development. Differences in abiotic (climate and soil) and biotic factors also affect levels and patterns of genetic variation in the valley.

## 23.6 Gene Flow

Field and laboratory investigations have revealed a considerable amount of information relevant to gene flow in wild lima beans in the valley, such as the high frequency of small population sizes (66% of populations with fewer than 30 individuals), the low allogamy rate ( $t \leq 10\%$ ), the presence of major alleles at several loci, the low frequency of heterozygous individuals and the high intra-population polymorphism (indicated by significant  $G_{ST}$  values for both isozyme and microsatellite markers).

The origin of this significant intra-population diversity is in part related to the importance of short- and long-distance gene flow. This can be estimated at the within- and between-populations levels, using direct (field measures) and indirect methods.

### 23.6.1 Direct methods of gene flow estimation

According to Crawford (1984) and Gliddon *et al.* (1987), estimation of gene flow is based upon the number of individuals in a local random breeding unit, i.e. a 'neighbourhood', defined more precisely as the genetic neighbourhood area (NA) and the effective neighbourhood size (Nb). These two parameters are determined from two equations: (1)  $NA = 4\pi(1/2t \times \sigma_n^2 + \sigma_s^2 + \sigma_v^2)$ ; and (2)  $Nb = NAd(1 + t)/2$ , where  $\sigma_n^2$ ,  $\sigma_s^2$  and  $\sigma_v^2$  are dispersal variances, respectively for pollen, seed and flower (or vegetative growth);  $t$  is the outcrossing rate and  $d$  is adult plant density.

Gene flow was measured by this method in three selected populations of the valley (Hardy *et al.*, 1997; Baudoin *et al.*, 1998). Pollen grains and seeds were labelled *in vivo* using stains and fluorescent dye. Pollinators were identified by observing each population at different dates during their flowering period and estimating the lima bean pollen load on the insect body.

In the valley, lima bean blooms during the dry season, from about mid-November to mid-February. Mean pollen/ovule ratio is about 863 (Hardy *et al.*, 1997). According to Cruden (1977), this ratio is typical of species with a mating system qualified as facultative allogamy. In the target area, the major pollinator is *Apis mellifera* L. In all studied populations, most pollen transfers occurred across distances of less than 1 m, confirming that common bees disperse pollen mostly over short distances. The frequency of pollen transfers dropped quickly around 1 m, although transfer could reach a maximum value of 5.5 m. The corresponding dispersal variance for pollen was  $\sigma_p^2 = 1.7 \text{ m}^2$ . Measures of flower dispersal showed great variability in the vegetative growth of wild lima bean individuals. Distances separating each inflorescence from its

respective plant base ranged between 0.37 and 6.5 m, according to the presence of surrounding vegetation that constituted a support allowing the plant to climb. In the tested populations, the mean flower dispersal variance was  $\sigma_v^2 = 2.7 \text{ m}^2$ . Seeds of wild lima beans are too heavy to be carried by air. The most important contribution to seed dispersal occurs when dehiscent pods open and project their seeds on the ground. In the studied populations, the maximum distance of seed dispersal was 5.5 m from the pod to the site on the ground. The mean seed dispersal variance was  $\sigma_s^2 = 1.68 \text{ m}^2$ .

Using isozymes, Maquet *et al.* (1996) and Hardy *et al.* (1997) calculated a mean outcrossing rate and a mean adult plant density of, respectively,  $t = 0.1$  and  $d = 0.235 \text{ plants/m}^2$ . From the two equations (1) and (2), the neighbourhood area (NA) and the effective neighbourhood size (Nb) were, respectively,  $56 \text{ m}^2$  and 7.23 individuals. According to Wright (1946), the low value for Nb (<20 individuals) corresponds to a high probability of random local genetic differentiation within the population. As the populations in the region spread over areas ranging from about  $100 \text{ m}^2$  to more than  $1000 \text{ m}^2$ , they could contain several to many neighbourhood areas. Therefore, allelic distribution in a single population is expected to be highly structured and there is a need to sample systematically at many sites of the population for collecting the whole genetic diversity.

### 23.6.2 Indirect methods of gene flow estimation

A conventional approach to quantify gene flow is to transform measures of population structure into indirect estimates of the average number of migrants exchanged per generation: this can be done by using the island model (Wright, 1951) or the isolation-by-distance model or Slatkin's private alleles model, in which the rate of gene flow is expected to decline monotonically with increasing geographic distance between continuous populations (Slatkin, 1985, 1993). Both methods were applied using enzyme and microsatellite markers and analysing polymorphic loci in populations located at various distances from each other.

Using isozymes, Wright's method produced a  $G_{ST}$  value of 0.575, corresponding to a number of migrants per generation (Nm) of 0.18, suggesting restricted gene flow among subpopulations ( $Nm < 1$ ). By the method of Slatkin using rare alleles, Nm was estimated at 0.08.

Using microsatellites, the fixation index ( $F_{ST}$ ) was 0.346, and the average inbreeding coefficient within populations was high ( $F_{IS} = 0.916$ ). The number of migrants per population and per generation from Wright's method was 0.47. By the method of Slatkin using private alleles, Nm was estimated at 0.06.

The two models of indirect gene flow estimation used in this study are useful for understanding the evolution of genetic structure in plant species with metapopulation dynamics. Results indicated low to moderate levels of gene flow (0.06–0.47) for the wild populations of the valley, although heterogeneity in the number of individuals per population could cause underestimation (Slatkin, 1985). On the basis of enzyme and microsatellite markers, very high divergence occurs among populations. This is probably due to restricted gene

flow, with genetic drift, therefore playing a major role in the genetic structure of lima bean populations in the study area.

Gene flow was not related to geographical distances at larger scales (Baudoin *et al.*, 2004). This could be due to heterogeneity among the gene flow values when all populations are considered or, in the isolation-by-distance model, to the assumption that populations must be in equilibrium between migration and genetic drift.

## **23.7 In Situ Conservation of Wild *P. lunatus* in the Central Valley**

On the basis of the data of genetic diversity, gene flow, demography and population dynamics, an *in situ* conservation strategy was developed for the Central Valley. Two complementary options were investigated. One was to identify and protect specific existing populations. The other was to establish new, synthetic populations in protected areas.

### **23.7.1 In Situ conservation areas for existing populations**

Life zones and soil type maps were used to identify all possible environmental combinations in which lima beans occur. Two combinations (bh-P and bmh-P life zones on inceptisol) are particularly important, including more than 75% of the total number of wild lima bean populations recorded in the valley. Only sites likely to maintain their integrity were considered. In view of the two main agents of genetic erosion in the valley, i.e. urban expansion and intensifying agricultural practices, and the small size of many lima bean populations, sites prone to human pressure (such as modern coffee plantations, small-scale farms and hedges near roads) were discarded. Some isolated sites, remote from cultivated land or human settlements, mainly located along water streams and deep slopes, and legally protected from cutting and weeding, were identified as potential conservation areas. In addition, with the purpose to preserve the available genetic diversity of wild lima beans, priority was also given to sites where populations are characterized by the presence of localized and private alleles (Zoro Bi, 1999).

In the end, the survey of the Central Valley identified 30 conservation sites, representing 24 combinations of life zone and soil type and distributed at elevations from 340 to 1980 m asl. Most of these sites are located at the margins of secondary forests or near a river or stream. The micro-environment was very often characterized by a diverse overstorey, producing a layered vertical structure and allowing light penetration and support for the climbing individuals.

The populations maintained *in situ* are large, covering an area of 1000 m<sup>2</sup> or more and containing at least 100 individuals. Their field management should follow specific recommendations derived from the results of the demographic studies, and be adapted to the disturbance level of their environment. In perturbed sites, such as in the vicinity of coffee plantations or along trails, conservation must favour the growth of young lignified individuals, which closely

depends upon the humidity of the environment. The suggested management is to maintain a mulch on the soil surface at the end of the rainy season or to install a vegetation cover favouring both high air and soil moisture. In addition, recruitment of new individuals from seeds can be promoted by weeding the soil surface just after seed dispersal at the end of the dry season. In contrast, in the more natural, undisturbed sites, mainly located at the border of secondary forests, it is important to favour the survival of large lignified individuals. Selective clearings should be carried out in these sites to maintain potential reserves of lignified adult plants.

### 23.7.2 Establishment of *in situ* synthetic populations

Interesting populations of smaller size and at risk of anthropogenic disturbance can only be preserved *in situ* in the long term by moving them to protected areas in the previously defined 24 environmental combinations. For this purpose, we developed a process involving synthetic populations. A synthetic population is defined as a group of individuals derived from seeds collected from original sites of wild lima bean populations, then sown in a protected site selected to allow optimal plant development and gene flow within and among populations. The protected sites are located in the same life zones as the original wild lima bean populations, or at least in similar ecological niches. The first synthetic populations were established in June 1998 in protected microsites covering four life zones of the Central Valley.

At each of these sites, synthetic populations were sown with seeds collected from nearby populations found in natural areas. The microconservation plots containing the synthetic populations were designed to fulfil several requirements, with regard to gene flow, plant dispersal, population size and fragmentation, extinction and recolonization processes. Such requirements were identified on the basis of our investigations in the valley and also from the studies made by Given (1993), Maxted *et al.* (1997), Tiebout and Anderson (1997) and Yonezawa (2000). Taking into account the patchy distribution of wild lima beans in the Central Valley, the usually small population size, the twining vegetative growth and the possibility of gene exchange between nearby populations through pollen and seed dispersal, two types of conservation microreserves were designed and established (Meurrens *et al.*, 2001):

- Circular design, consisting of circular cleared patches, linked by corridors. In each patch, seeds from nearby populations were sown at the base of an existing tree or shrub, to provide a support for the vines. The cleared corridors are meant to facilitate gene flow between patches through pollen movement or development of very long twining branches, bearing racemes at leaf nodes.
- Linear conservation design, consisting of several groups of nearby population seeds sown every metre along thickets.

For the two conservation models, the minimum area of the patches varied from 56 to 150m<sup>2</sup> according to the neighbourhood area calculated in our investigations.



The number of seeds to be introduced in each patch was determined with the aim of reaching a mean density of 0.35 adult plants/m<sup>2</sup>, a situation frequently observed in the valley (Degreef, 1998). The seeds sown in each patch should represent the original genetic variability of the populations in its original site, following the sampling recommendations of Zoro Bi *et al.* (1998) regarding number of plants per population and number of seeds per plant.

Considering the results of the demographic studies, the vegetation in each patch was slightly disturbed during the development stage of introduced populations in order to obtain optimal germination and rapid growth of the plantlets and to avoid uncontrolled dispersal.

The effectiveness of this methodology was assessed by comparing the demography of the synthetic populations with that of natural populations located in the same environments. Similar field observations and measurements were taken with the two types of populations. Results of the comparison are presented in details by Meurrens *et al.* (2001), showing a positive impact for at least three important demographic parameters: germination percentage of seeds sown, lignification and death rates.

The increase in germination percentage in synthetic populations can be explained by the management method used to break seed dormancy, i.e. successive weeding after sowing. This practice moves the seeds to the soil surface, where they are exposed to high temperature, a factor favouring the breaking of seed coat dormancy (Degreef *et al.*, 2002). When establishing synthetic populations, the first step is to allow as many seeds as possible to germinate and to produce seedlings. When a sufficient number of adult plants are reached to ensure progenies, it is important to let the populations constitute their own soil seed bank. If sufficiently large, this seed reserve will buffer the poor seed production that could occur some years and enhance the demographic stability of the population. After successful germination, the possible fates of a seedling within the first year are either to die or to reach the juvenile or the lignified stage. Reducing the mortality of seedlings is an important challenge for *in situ* conservation of wild lima beans in the Central Valley, as most individuals die at this stage in natural populations (Degreef, 1998). In synthetic populations, death rates can be decreased by appropriate management practices, in particular using mulch to maintain soil moisture during the dry season.

## 23.8 Conclusions and Recommendations

The project has developed a uniquely detailed and comprehensive data set of ecogeographic, genetic and demographic information on a wild crop relative. It has then used this data set to develop an *in situ* conservation strategy for *P. lunatus* in the study region based on the selection of key natural sites, the establishment of complementary synthetic populations and targeted management interventions.

In order to build on these scientific achievements, it is important to improve the strategy of conservation and the methodology used to reach it. Some further research activities could be highlighted.

- Monitoring (through genetic characterization as well as phenological and demographic observations) and refinement of the management strategy should be carried out in the two types of populations (existing and synthetic) to ensure long-term conservation. In particular, plant growth and development, extent of gene flow among patches within microconservation sites and appearance of novel multilocus genotypes through genetic hybridization should be analysed.
- GIS tools should be used to examine in more depth any relationship between the distribution of genetic variation in the Central Valley and eco-geographic factors. For the conservation objective, it is essential to identify those populations that best represent ecological and genetic diversity. Data from various genetic markers (biochemical and molecular) could be tested against microscale passport data to highlight the combinations of factors that best orient the choice of individuals or populations for inclusion in conservation programmes. Such data will also be relevant for the determination of the minimum sample size required for maintaining a given level of allelic diversity.
- A 'carrying capacity' component should be added to the demographic model which has been developed, in order to determine the effects of plant density on mortality and growth rates of lima bean individuals. A study should be carried out to evaluate the impact of the 'extinction–recolonization' process on the genetic structure of populations. As the soil seed bank plays a significant role in population survival, it should be interesting to compare the genetic diversity between populations established from the soil seed bank after a local extinction and populations established with previously collected seeds from the same original populations.

As gene flow is a key element in determining the genetic structure of the wild populations, it is essential to follow-up their study in selected regions of the Central Valley by using microsatellite markers. A specific objective will be to measure the impact of gene flow on a very large scale through the sampling of individuals located at large distances from a central population.

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