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Case studies on breeding systems and its consequences for germplasm conservation

1. Isoenzyme diversity in wild Lima bean populations in central Costa Rica

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Summary

This study gives the results of allozyme diversity within and among 20 wild Lima bean populations uniformly distributed throughout the Central Valley of Costa Rica. The electrophoretic analysis of seven enzyme systems show five monomorphic loci and a relatively low level of polymorphism for the other loci. This moderate level of polymorphism is unexpected for a species for which a fair amount of allogamy rates has been reported, at least among the cultivated forms. The genetic parameters (mainly percentage of polymorphic loci, mean genetic diversity, percentage of heterozygotes and fixation index) indicate a tendency for a predominantly selfing breeding system in the wild Lima beans although some values range between selfing and mixed-animal breeding systems. Very low within-population diversity is observed while a good diversity is found among populations. Results also show a departure from Hardy-Weinberg equilibrium on most analyzed populations in the target site This might be due to populations divided into subpopulations among which no natural crosses occur randomly, to weeding practices or to overlap of generations within some populations. All the results obtained in this work are discussed in view of further studies for the planning of *in situ* conservation in a quickly evolving tropical environment.

Introduction

Conservation of genetic resources

The conservation of genetic resources has attracted growing scientific interest in the past twenty years. During this period, some topics have generated various levels of controversy among the scientists involved. For example, there has been some debate concerning the most appropriate method for conservation (*in situ* vs *ex situ*), the optimal sampling strategy in the field, and the types of genetic variants to be conserved (Altieri & Merrick, 1987; Astley, 1992; Benz, 1988; Brush, 1991; Cohen et al., 1991; Crossa et al., 1993; Frankel & Soulé, 1981; Marshall, 1990).

For plant genetic resources, a special attention is now given to the wild ancestral populations of the cultigens. This interest is due to their poor representation in germplasm collections, their high value as large stores of genetic variation, and their potential value as a source of novel variants for plant breeding (Benz, 1988; Bianchi-Hall et al., 1993; Brown, 1978; Debouck & Tohme, 1989; Delgado Salinas et al., 1988; Frankel, 1974; Harlan, 1976; Marshall, 1990). However, wild materials are not easy to maintain in gene banks. The limited information available on the ecogeographic distribution of these populations and the difficult task of assembling genetic stocks sufficiently representative of the total genetic variability displayed by wild populations are among the major reasons for this difficulty. Furthermore, seed dispersal and variation in both seed ripening and dormancy

among individual plants and populations are also additional constraints for an adequate *ex situ* storage of wild populations (Debouck et al., 1993; Ehrman & Cocks, 1990; Marshall, 1990).

In order to circumvent such obstacles, *in situ* conservation—e.g. in the environment in which wild populations evolve—is regarded as a dynamic conservation system which facilitates the continuing evolution of the crop gene pool. It maintains the genetic integrity and the potential of adaptation of each population and complements also *ex situ* conservation avoiding the inadequate field sampling of ecotypes for storage in gene banks (Altieri & Merrick, 1987; Astley, 1992; Brush, 1991; Ingram & Williams, 1984; National Research Council, 1992). A sound programme of *in situ* genetic conservation is aimed at ensuring the preservation of representative populations throughout their natural geographic range (Marshall, 1990).

To achieve this objective, a prerequisite is to define an appropriate measure of genetic variation and to determine that variation which is potentially more useful and should have priority for *in situ* or *ex situ* conservation (Hedrick & Miller, 1992; Kresovich & McFerson, 1992; Lefort-Buson et al., 1988; Marshall & Brown, 1975). A good measure of genetic variation is the number of alleles per locus for each population, using qualitative marker loci such as isozymes or DNA hypervariable segments (Brown & Allard, 1970; Brown, 1990; Clegg, 1980; Hamrick & Godt, 1990; Hoelzel, 1992; Nakamura et al., 1987). Once the alleles are identified and their frequencies measured, the conservationist should preserve as many variants as possible at each locus, without weighing a priori a group of genes for its usefulness (Breese, 1989; Chambers, 1983; Marshall & Brown, 1975). The purpose will be to conserve locally common and rare alleles over a maximum number of sites, bearing in mind that each variant occurring in any population may assume special significance under unforeseen conditions and consequently respond to future and unpredictable needs of plant breeding (Singh & Williams, 1984).

Components of an in situ *conservation programme*

In situ conservation planning will necessarily integrate several operations centred on the plant populations in the designated habitats. These involve mainly eco-geographical surveys, regional environmental analysis, formulation of practical conservation objectives, delimitation of sites, choice of population number and size and on-going monitoring of conserved populations (Ingram & Williams, 1984). In coordinating these various operations, a central and essential preliminary will be to link closely the conservation of both genetic resources and nature reserves where the wild populations are scattered, through the establishment of genetic resources. In particular, genetic reserves should be designed to contain sufficiently large and diverse populations and habitat units so as to sustain the levels of allelic variability identified in the target regions (Astley, 1992; Franklin, 1993; Ingram & Williams, 1984; Jain, 1975). Another key factor to make a success of an *in situ* conservation programme is to monitor as thoroughly as possible the population dynamics (Brown, 1990; Ennos, 1990; Kresovich & McFerson, 1992; Lande, 1988; Ritland, 1990). This requires the study of all the underlying causal agents of variation in the target regions, classified as follows: the environmental parameters (such as latitude, altitude, climate, soil, geology, pests and diseases, pollinators), the demographic parameters (such as population density, shape and size, seed dormancy, plant growth rates and mortality, overlap of generations, flowering periodicity, variation in seed ripening, plant age structure), the gene flow parameters (such as breeding systems, floral biology, reproductive output, pollen or gamete dispersal, seed dispersal), the social parameters (shape of landscapes and agrosystems, their use by human communities, deforestation, urbanization, habitat fragmentation, agriculture intensification). All these factors interact to control the population dynamics and should therefore be examined in an integrative approach.

A case study: Phaseolus lunatus *L. in the Central Valley of Costa Rica*

As a model to develop a strategy for *in situ* conservation, a study is conducted on the dynamics of the wild Lima bean (*Phaseolus lunatus* L.) populations. The Central Valley of Costa Rica was selected as the geographic site due to the presence of numerous natural *P. lunatus* populations and to their risk of extinction as a result of growing urbanization and intensive agriculture (Debouck, 1987). The wild populations in this area belong to the Mesoamerican gene pool currently recognized in this species, the second being the Andean gene pool (Maquet et al., 1990). According to Baudoin (1991), the Lima bean is a self-compatible species with a mixed mating system, e.g. predominantly self-pollinating but with a fair amount of outcrossing (0 to 48%) at least for the cultivated forms. Studies on breeding systems have however been carried out only

with the cultivated material and in sites far away from the natural distribution area of the wild material. The latter is considered as short-living perennial, behaving more like an outbreeder or an inbreeder according to genetic and ecological factors.

A first component of this study is the inventory, mapping and characterization of the Lima bean populations in the target area (the Central Valley of Costa Rica). The second component is the assessment of the genetic diversity among and within the wild populations disseminated in this area through isoenzyme electrophoresis and DNA analysis. The third component concerns the setting up of field experiments to evaluate some relevant parameters of population dynamics. The fourth component is the use of computer to integrate several mechanisms controlling the genetic structure and evolution of these populations into simulations of population dynamics.

This work concerns the second component dealing with the evaluation of genetic diversity in the wild Lima bean populations by isoenzyme electrophoresis.

Material and methods

Environment and wild populations of Lima bean

The Costa Rican collaborators from the University of Costa Rica at San José carried out in 1992–1993 an ecogeographical survey in the target area (2100 km^2). They mapped 402 wild populations. A population is here defined as any group of plants isolated (regardless of its size) at least 500 meters from other plants of the same species. The populations are found mostly in variants of premontane and lower montane humid forests and at altitudes ranging from 500 to 1800 m a.s.l. The region is also characterized by a mean annual rainfall varying between 1200 and 3000 mm, with dry and humid seasons. The wild *P. lunatus* populations are usually found in open and disturbed areas with grasses and scattered trees or bushy thickets or in more woody habitats. The Lima bean colonizes the coffee plantation from long-living fences (usually *Erythrina* and euphorbs) bordering the plots. Two major factors reduce drastically the occurrence of Lima bean in the target area: the severe grazing and seasonal fires in pasture lands and sugar cane plantations and the replacement of traditional small-scale coffee plantations intermixed with legume trees by modern high input demanding plantations.

From the numerous wild populations of the Central Valley, we selected 20 populations according to their size and to their ecological and geographical distributions (Table 1 and Fig. 1).

Isoenzyme analysis

In each selected population, we examined several individuals coming from a random sample of collected seeds, the number of seeds ranging from 14 to 51 according to population size. The procedure adopted for the electrophoretic analysis is described in Maquet et al. (1994). To evaluate the genetic variability of the wild Lima bean populations, we selected seven enzymes obtained from the cotyledons: Alcohol dehydrogenase (ADH), Cytosol amino-peptidase (CAP), Glucose-6-phosphate isomerase (GPI), Isocitrate dehydrogenase (IDH), Malate dehydrogenase (MDH), Phosphogluconate dehydrogenase (PGDH) and Phosphoglucomutase (PGM). The electrophoretic analysis was conducted on a 10% starch gel support with the histidine-citrate pH 5.7 buffer system. From each plant of a population, a cotyledon extract was obtained from a single 5-day old imbibed seed placed in Petri dishes. Cotyledons were ground in a potassium phosphate pH 7.0 buffer as described by Hussain et al. (1988). The homogenate was centrifuged at 17,000 *g* for 30 minutes and the sample was conserved at −70°C until evaluation. The homogenate (12 μl), absorbed onto paperwicks, was subjected to electrophoresis following the method of Hillis & Moritz (1990). This histochemical staining procedures used are those of Hussain et al. (1988) for CAP, GPI and MDH and those of Hillis & Moritz (1990) for ADH, IDH, PGDH and PGM.

Locus and allele designations are assigned following previous studies on common beans made by Koenig & Gepts (1989 a,b): loci are labelled sequentially with those migrating closest to the anodal end designated as number 1. The first and the last stack correspond to the accession G25221, a Mexican wild form, considered as a standard for our analysis. The allele from this genotype is designated as 100 and all other allozymes are measured by their relative distance from the standard.

Genetic parameters

To assess allozyme variation at the species and within and among population levels, several genetic parameters were calculated with reference to the studies of

Table 1. Ecological characteristics of the selected natural Lima bean populations collected during 1993 in the Central Valley of Costa Rica

POP[a]	Site	Alt.	Tm	RHm	Rm	ECO	Land use
E25	Alajuelita, La Cruz	1550	17.5	80.0	1900	B	PC
E27	Escazú	1350	20.0	80.0	2000	C	PC
E28	Piedades Santa Ana	1550	17.5	80.0	1900	B	PC
E29	Colón	1150	20.0	80.0	1700	B	F
E50	San José, Aserrí, Piedra de Aserrí	1550	17.5	80.0	1900	B	PC
E76	La Aurora, Heredia	1000	21.6	77.5	1700	A	PC
G20	Cartago, Paraiso	1500	17.5	85.0	1500	A	AC
J29	San Luis	1250	19.2	87.5	2500	B	PC
J48	San José, Barrio del Socorro	1250	19.2	87.5	2500	B	PC
KM30	Tres Rios	1300	18.2	85.0	2000	B	PC
KM40	Santo Domingo	1150	20.0	85.0	2000	A	PC
KM57	Alajuela, San Ramón, Alto La Cima	1050	20.0	80.0	2000	A	PC
P1	San Joaquín, Barrantes	1050	20.0	80.0	1850	A	PC
S7	Sarchi Norte	950	20.0	80.0	2500	B	PC
S27	Guácima	750	23.8	75.0	2000	A	OL
S32	Atenas, escuela de ganadería	400	25.8	75.0	2250	F	OL
SR8	Alajuela, San Ramón	1050	17.5	82.5	2250	A	PC
SR10	Alajuela, San Ramón	1050	17.5	82.5	2250	A	PC
SR16	Alajuela, San Ramón, Bajo Tajos	1150	18.8	82.5	2250	A	PC
T11	Santa Bárbara	1250	17.5	85.0	2500	B	PC

[a]Pop.: Identification number of the wild population; Alt: Altitude (m.a.s.l.); Tm: Mean annual temperature (°C); RHm: Mean annual relative humidity (%); Rm: Mean annual rainfall (mm); Eco.: Ecological zone (A: premontane moist forest, B: premontane very humid forest, C: Low montane very humid forest, F: transitional premontane moist forest); Land use: PC – perennial crops, AC – annual crops, F – forest, OL – open land.

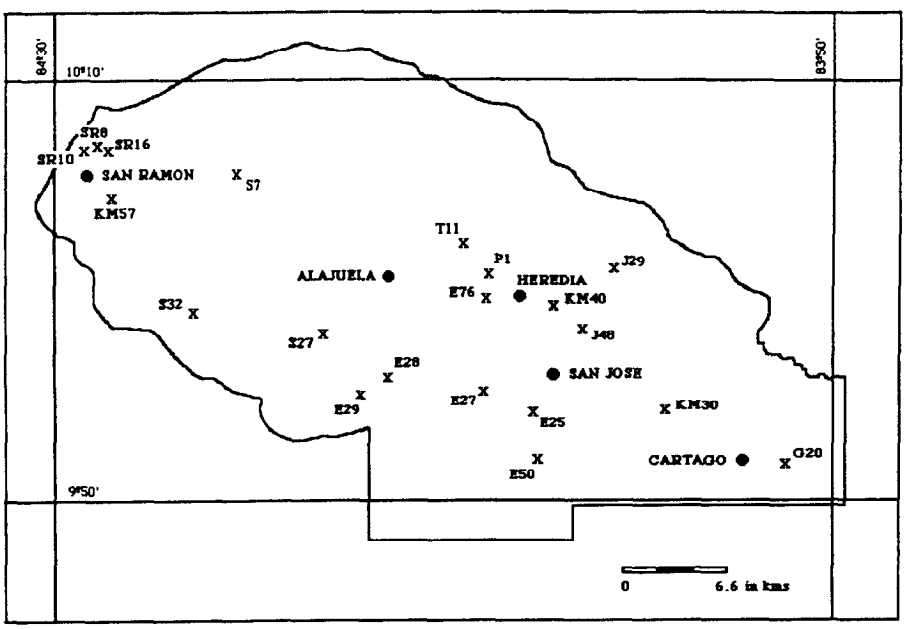

Fig. 1. Geographic distribution of the selected natural Lima bean populations collected during 1993 in the Central Valley of Costa Rica

Hoelzel (1992), Brown et al. (1990), Ollitrault (1987), Pasteur et al. (1987), Lucotte (1983) and Nei (1973, 1987). At the species level, the percentage of polymorphic loci (P) was determined by dividing the number of such polymorphic loci by the number of loci analyzed. The mean number of alleles per locus (A) was obtained by summing the total number of alleles observed over all loci and dividing by the number of loci. These two parameters are influenced by the sample size. Genetic diversity (H) was calculated for each locus from the equation $H = 1 - \sum p_i^2$, where p_i is the mean frequency of the i^{th} allele. Mean genetic diversity was obtained by averaging the H values over all loci. The effective number of alleles (Ae) was calculated by the ratio $1/(1 - H)$. At the within population level, the percentage polymorphic loci (P) was the proportion of loci polymorphic in each population average over all populations. The number of alleles per locus (A) was determined for each population and a mean value was calculated by averaging over all populations. Genetic diversity (H) was determined for each locus and each population using the same equation $H = 1 - \sum p_i^2$. The mean value of H was obtained for each locus by averaging over all populations and an overall mean of H was obtained by averaging over all loci. The effective number of alleles (Ae) was calculated again from the ratio $1/(1 - H)$, H being the overall mean of genetic diversity. Variation among populations is characterized by the total genetic diversity (Ht) and the mean diversity within populations (Hs). These parameters were calculated for each polymorphic locus on the basis of Nei's (1973) genetic diversity statistics. The difference between Ht and Hs gives the genetic diversity due to variation among populations (Dst). The proportion of variation residing among populations (Gst), also called coefficient of gene differentiation, was obtained from the ratio Dst/Ht.

The genetic parameters of the wild Lima bean populations in the Central Valley were compared with the values reported by Hamrick & Godt (1990) in a work of reference covering 473 species of different characteristics (among others: life forms and breeding systems).

For each analyzed population, fitness with the Hardy-Weinberg equilibrium was verified by means of χ^2 test with the correction of Yates:

$$\chi^2 = \sum \left(\frac{(|Ho - Hth| - 0.5)^2}{Hth} \right)$$

where Ho and Hth are, respectively, the observed and expected percents of heterozygotes; Hth being obtained from the theoretical absolute frequencies of the phenotypes (Pasteur et al., 1987). For each population, we also calculated the weighted average fixation index over all loci (F) and the mean autogamy rate: $s = 2F/(1 + F)$. The fixation index is estimated by the method of Nei (1987).

Results and discussion

The electrophoretic results from the 20 wild populations are given in Table 2. The data are presented for the 10 loci used in routine accompanied with their alleles and allelic frequencies. A gene locus is here considered to be polymorphic if the most common allele is present at a frequency less than 0.99.

The loci: CAP, IDH, MDH2, PGDH2, PGM1 are monomorphic. Seven populations (E27, E29, G20, J29, J48, P1 and SR10) possess fixed alleles for all their loci, three others (KM57, S7 and S27) have only one polymorphic locus, seven populations (E25, E28, E50, E76, S32, SR8 and SR16) have two polymorphic loci and the last three (KM30, KM40 and T11) possess three polymorphic loci. Even if the population size cannot be estimated accurately due to the climbing behaviour of the plants, this characteristic is apparently not correlated with the number of polymorphic loci inside a population: for example, a small population like SR16 possesses two polymorphic loci while a large natural population like J29 is completely fixed at least for the tested enzymatic systems.

Concerning the geographic distribution, alleles of GP1, MDH1 and PGM2 are present throughout the whole Central Valley while $ADH2^{61}$ is mainly located around the Heredia region with an extension from this region to the Northeastern borders of the Valley. $PGDH1^{86}$ is only represented in the population E28 which is located in the Southwestern part of the Valley.

Genetic parameters evaluated at species, within and among population levels are shown in Table 3. At the species level, the percentage of polymorphic loci (P) and the mean genetic diversity (H) are similar to the values found by Koenig & Gepts (1989 a,b) in wild populations of common bean (P = 45.0 and H = 0.132) and by Hamrick & Godt (1990) analyzing data from 473 plant species. More specifically, our values are closer to those reported by Hamrick & Godt (1990) for the species having an annual life form (P = 50.7 and H = 0.161) and an outcrossing-animal breeding system (P = 40.0, H = 0.124). The

Table 2. Allelic frequencies observed among 20 wild populations of *P. lunatus* proceeding from the Central Valley of Costa Rica

	n[a]	ADH2	CAP	GPI1	IDH	MDH1	MDH 2	PGDH1	PGD H2	PGM 1	PGM2
E25	50	61 (0.14) 100 (0.86)	100	100	100	100 (0.74) 140 (0.26)	100	100	100	100	85
E27	50	100	100	100	100	100 (0.01) 140 (0.99)	100	100	100	100	85
E28	50	100	100	100	100	100 (0.86) 140 (0.14)	100	86 (0.83) 100 (0.17)	100	100	85
E29	50	100	100	100	100	100	100	100	100	100	85
E50	50	100	100	100	100	100 (0.98) 140 (0.02)	100	100	100	100	85 (0.39) 100 (0.61)
E76	50	61 (0.01) 100 (0.99)	100	100	100	100 (0.86) 140 (0.14)	100	100	100	100	85 (0.61) 100 (0.39)
G20	51	100	100	96	100	100	100	100	100	100	85
J29	50	61	100	100	100	140	100	100	100	100	85
J48	50	61 (0.01) 100 (0.99)	100	100	100	140	100	100	100	100	100
KM30	50	61 (0.02) 100 (0.98)	100	96 (0.08) 100 (0.92)	100	100 (0.06) 140 (0.94)	100	100	100	100	85
KM40	50	61 (0.80) 100 (0.20)	100	100	100	100 (0.77) 140 (0.23)	100	100	100	100	85 (0.57) 100 (0.43)
KM57	49	100	100	100	100	100 (0.98) 140 (0.02)	100	100	100	100	85
P1	51	100	100	100	100	140	100	100	100	100	85
S7	14	100	100	96 (0.07) 100 (0.93)	100	140	100	100	100	100	85
S27	50	100	100	100	100	140	100	100	100	100	85 (0.17) 100 (0.83)
S32	20	100	100	96 (0.63) 100 (0.37)	100	140	100	100	100	100	85 (0.70) 100 (10.30)
SR8	50	61 (0.97) 100 (0.03)	100	100	100	100 (0.97) 140 (0.03)	100	100	100	100	100
SR10	49	100	100	100	100	140	100	100	100	100	85
SR16	40	61 (0.03) 100 (0.97)	100	100	100	100 (0.97) 140 (0.03)	100	100	100	100	100
T11	50	61 (0.97) 100 (0.03)	100	96 (0.04) 100 (0.96)	100	100 (0.09) 140 (0.91)	100	100	100	100	85

[a]n: number of plants evaluated.

mean number of alleles per locus (A) found in the wild Lima bean populations is less than the value reported by Hamrick & Godt (1990) for the several reviewed species (A = 1.96). Our A value is closer to that of a short-lived perennial species (A = 1.70) and a selfing (A = 1.69) or an animal-pollinated mixed-mating (A = 1.68) species. On the other side, at the within population level, the P, A and H values of Lima bean are lower than the three reported by Hamrick & Godt (1990) for all the reviewed species (P = 34.2, A = 1.53, H = 0.113). The lower values obtained with Lima bean are confirmed whatever the life form and breeding systems of the species surveyed by the two authors.

Variation among populations were determined by the three parameters (Ht, Hs and Gst) from the polymorphic loci (ADH2, GPI1, MDH1, PGDH1 and PGM2) in order to compare our results with those of Hamrick & Godt (1990). The total genetic diversity in Lima beans is very similar to that of short-

Table 3. Levels of allozyme variation for 20 wild populations of the Central Valley of Costa Rica

Level	P	A	Ae	H
Species	50.0	1.50	1.17	0.146
Population	13.0	1.14	1.03	0.029
	Ht	Hs	Gst	
Among population	0.292	0.058	0.803	

P: percentage of polymorphic loci; A: number of alleles per locus, Ae: effective number of alleles per locus; H: mean genetic diversity; Ht: total genetic diversity; Hs: mean diversity within populations; Gst: coefficient of gene differentiation.

lived perennial species (Ht = 0.300) and animal-pollinated mixed-mating species (Ht = 0.304). On the other hand, the intrapopulation diversity is very low, markedly inferior to values reported for both annual (Hs = 0.200) and short-lived perennial forms (Hs = 0.222) and for selfing species (Hs = 0.149). Indeed 80% of the total diversity are observed between the wild Lima bean populations, as expressed by Dst (Ht – Hs = 0.234). Consequently the coefficient of gene differentiation obtained from our material is largely superior to that of an annual (Gst = 0.357) and an autogamous (Gst = 0.510) species, on the basis of Hamrick & Godt's (1990) data.

From these first results, no definitive conclusion can be drawn concerning the breeding system of the wild Lima bean populations in the Central Valley of Costa Rica. Several genetic parameters range between those reported for a selfing and mixed-animal mating system but reveal a tendency for a predominantly selfing mating system.

Table 4 indicates the heterozygote percent, the fixation index, the autogamy rate and the test of the Hardy-Weinberg equilibrium for the 20 wild Lima bean populations. Results show a lack of heterozygotes. The probability indicating a significant difference between observed and expected heterozygotes at one locus must be inferior to 0.05, which correspond for one degree of freedom (number of phenotypes minus number of alleles) to a χ^2 above 3.8 (Pasteur et al., 1987). Except for two populations: S7 and the GP11 locus (but with a very low number of tested individuals: 14) and E76 at the MDH1 locus, all populations give a χ^2 adjusted higher than 3.8. Therefore the analyzed material does not fit with the Hardy-Weinberg equilibrium. This implies that the weighted average fixation index (F) and the mean autogamy rate(s) are overestimated as those parameters are only correctly assessed if the sample has been pooled from one subpopulation. Negative F values are reported for the two populations: E27 and J48 but these ones are not significantly different from 0. Such values are the consequence of an inadequate sampling as fixation indices were calculated from a single non-polymorphic locus according to our definition. In spite of this fact, the mean fixation index (F = 0.790) is very close to the value reported by Brown (1979) for the predominant inbreeders (F > 0.900). The mean autogamy rate is 0.95, giving an allogamy rate of 5%, ranging from 0 to 21% (Table 4).

Considering the case of the wild Lima bean populations in the Central Valley, several factors could explain the departure from the Hardy-Weinberg equilibrium:

- *A first factor* concerns the genetic determination which guided our zymogram analyses: it was mainly deduced from previous studies made on *P. vulgaris* by Koenig & Gepts (1989 a,b) and Weeden et al. (1989). Pedigree tests are underway to check the allelic inheritance in some populations of *P. lunatus*.
- *A second factor* might be the presence of subpopulations in a single natural population. In this situation, sampling could have been made with individuals from subpopulations between which no natural crosses occurred randomly. Such populations might also have different allelic frequencies, giving rise to the departure from Hardy-Weinberg equilibrium. The heterogeneity observed in some populations is related with the environment and the resulting selection pressure while in others limited pollen or seed dispersal is mainly responsible of the patchy genetic structure. The particularly narrow distribution of some alleles (such as $PDGH1^{86}$) could support the first assumption: selection from environment. Although isozymes are usually neutral (Kimura, 1990), several cases of alleles adapted to a specific environment are known (Allard, 1988; Ennos, 1990). On the other hand, the high Gst value observed in the Central Valley could support the second assumption: the limited pollen or seed dispersal (Varvio et al., 1986).
- *A third factor* might be the founder effect generated by the regular weedings made by local farmers of the Central Valley. By doing so, a few plants are allowed to survive along fences in some populations, with a recolonization of the natural site relying therefore only from these plants

Table 4. Heterozygote rate, fixation index, autogamy rate and the test of the Hardy-Weinberg equilibrium in natural populations of Lima bean proceeding from the Central Valley of Costa Rica

					χ^2				
Pop[a]	Ho	Hth	F	s	ADH2	GPI1	MDH1	PGDH1	PGM2
E25	0.014	0.062	0.770	0.87	32.56		20.88		
E27	0.002	0.002	−0.010				49.25		
E28	0.000	0.053	1.000	1.00			42.11	35.18	
E29	0.000	0.000							
E50	0.000	0.051	1.000	1.00			12.36		45.85
E76	0.020	0.074	0.728	0.84	49.25		3.46		42.87
G20	0.000	0.000							
J29	0.000	0.000							
J48	0.002	0.002	−0.010		48.25				
KM30	0.000	0.030	1.000	1.00	11.86	38.23	34.01		
KM40	0.009	0.116	0.924	0.96	29.72		32.59		40.98
KM57	0.000	0.004	1.000	1.00			12.36		
P1	0.000	0.000							
S7	0.000	0.013	1.000	1.00		3.58			
S27	0.000	0.028	0.920	0.96					32.61
S32	0.000	0.089	1.000	1.00		15.86			16.43
SR8	0.004	0.012	0.656	0.79	5.18		5.29		
SR10	0.000	0.000							
SR16	0.000	0.010	1.000	1.00	9.36		9.86		
T11	0.004	0.030	0.866	0.93	5.29	28.15	29.35		
Mean			0.790	0.95					

[a]Ho: mean rate of observed heterozygote; Hth: mean rate of expected heteroygote; F: weighted average fixation index; s: mean autogamy rate; χ^2 is calculated for the 5 polymorphic loci and with the correction of Yates.

and from soil seed bank. This could explain why small populations (in which no subpopulations are present) diverge also from Hardy-Weinberg equilibrium (Motro & Thompson, 1982).

– *A fourth factor* could be the overlap of distinct generations occurring at the same period on the same site, a very common fact for such a short-lived perennial species.

Conclusion and prospects

The genetic parameters obtained from the electrophoretic analysis indicate that the wild populations of *P. lunatus* in the Central Valley of Costa Rica behave like a predominant selfing species, with an outcrossing rate less than 10% (Hamrick & Godt, 1990). Very low within-population diversity is observed while a good diversity is found among populations.

The mean number of alleles per locus is not high which could result from the specific nature of the enzymes used in our study: these enzymes require very often a specific substrate and are involved in metabolic cycles. It is therefore intended to increase the number of tested enzymes, giving priority to non-specific enzymes like esterases, peroxidases or acid phosphatases. Another possibility would be to exploit minisatellite markers, allowing the recognition of heterozygotic and homozygotic genotypes (Bruford et al., 1992; Schaal et al., 1991).

In order to understand the lack of heterozygotes within the natural populations, several aspects deserve more in-depth investigations. First, the genetic determination of the enzymatic systems should be precised through a study of segregating hybrid populations involving purified parental genotypes with different fixed alleles. Secondly, more emphasis should be given to analyze the spatial pattern of genetic variation within plant populations. A trial is being conducted in Costa Rica with some populations, where each maternal genotype is precisely located on the field with the help of a grid. The electrophorectic analysis

of each genotype will enable identification of the eventual subpopulations, their equilibrium and the number of migrants per generation within each subpopulation. Thirdly, the plant mating system of the Lima bean populations should be deeply studied in order to estimate the outcrossing rate and its variation within and among populations during several generations. For this purpose it is intended to apply the mixed-mating model as defined by Brown (1990) and to estimate the distribution shape and the dispersal distance of the pollen.

Data concerning population dynamics like demographic parameters, population size, plant age and longevity, length of flowering period, seed production and seed dispersal will be collected during several generations in some selected populations of the Central Valley of Costa Rica. These field observations are necessary to understand better how a population can recolonize a natural site and to determine the variation of the population structure (Wade & McCauley, 1988).

Other studies will involve the relations between the distribution of some specific alleles and the environment (climate, soil) in the target site, the number of populations to be collected in order to capture all the alleles whatever their frequency and the sampling methodology within a population (number of seeds per population and number and location of plants within a population). All these topics will help set up the conservation strategies in the Central Valley of Costa Rica.

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