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POPULATION GENETIC STRUCTURE OF WILD *PHASEOLUS LUNATUS* (FABACEAE), WITH SPECIAL REFERENCE TO POPULATION SIZES[1]

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To set up an in situ conservation strategy for *Phaseolus lunatus*, we analyzed the genetic structure of 29 populations in the Central Valley of Costa Rica. Using 22 enzyme loci, we quantified the proportion of polymorphic loci (P_p), the mean number of alleles per locus (A), and the mean effective number of alleles per locus (A_e), which equaled to 10.32%, 1.10, and 1.05, respectively. The total heterozygosity (H_T), the intrapopulation genetic diversity (H_S), and the interpopulation genetic diversity (D_{ST}) were 0.193, 0.082, and 0.111, respectively. The genotypic composition of the analyzed populations showed a deviation from the Hardy-Weinberg proportions ($F_{IT} = 0.932$). This disequilibrium was due to either genetic differentiation between populations ($F_{ST} = 0.497$) or nonrandom mating within populations ($F_{IS} = 0.866$). From the level of genetic differentiation between populations and the private alleles frequencies estimates, gene flow was calculated: $Nm_W = 0.398$ and $Nm_S = 0.023$, respectively. The results suggested that wild Lima bean maintains most of its isozyme variation among populations. Significant positive correlation was observed between population size and P_p, A, and H_o (observed heterozygosity), whereas no correlation was observed with the average fixation index of population (F). The loss of genetic variability in populations was attributed to inbreeding and the bottleneck effects that characterized the target populations. In situ conservation and management procedures for wild Lima bean are discussed.

Key words: Costa Rica; Fabaceae; gene flow; genetic diversity; in situ conservation; isozymes; Lima bean; *Phaseolus lunatus*; population size.

The conservation of genetic resources using complementary ex situ and in situ methods has attracted growing public and scientific interest and support. Wild relatives of cultivated crops receive special attention because of their poor representation in gene banks and their high value as large stores of genetic variation (Frankel, 1974; Brown, 1978; Marshall, 1990). However, wild materials are often difficult to maintain in germplasm collections (Marshall, 1990; Debouck et al., 1993). In situ conservation facilitates the continuing evolution of the gene pool and is a way of circumventing some of the difficulties of ex situ conservation (Altieri and Merrick, 1987; Brown et al., 1997).

Detailed studies of the ecology, population biology, genetics, and reproductive biology of a target species are essential for successful conservation (Hawkes, 1971; Lande, 1988). With regard to genetics, for example, ecological factors and life history traits may affect the distribution of genetic diversity within and among plant populations (Loveless and Hamrick, 1984; Hamrick and Godt, 1990). Genetic variability is also known to increase fitness in populations of many plant and animal species (Hamrick et al., 1979; Barrett and Kohn, 1991).

Population genetic theory predicts the loss of genetic diversity in populations that remain small for several generations (genetic drift), in populations initiated from a low number of colonists (founder effect), and in populations that suffer rapid declines in size (population bottleneck), particularly if recovery is slow or if size fluctuations are frequent (Barrett and Kohn, 1991). However, divergent results have been reported on the relationships between plant population genetic variability and population size for several species (van Treuren et al., 1993; Widén, 1993; Dolan, 1994; Oostermeijer et al., 1994; Raijmann et al., 1994; Weidema et al., 1996; Montgomery et al., 2000).

With the aim to develop a strategy for in situ conservation, we initiated a study in the Central Valley of Costa Rica to understand mechanisms controlling the genetic structure and population dynamics of the wild Lima bean, *Phaseolus lunatus* L. Such material represents a very important genetic reservoir for the improvement of the various *Phaseolus* bean cultigens (Maquet and Baudoin, 1997). *Phaseolus lunatus* was also used as a plant model because of its alternating outbreeder-inbreeder behavior. Lima bean is a self-compatible annual or short-living perennial species with a mixed-mating system; that is, it is predominantly self-pollinating (Baudoin et al., 1998). Wild individuals are characterized by an indeterminate, climbing, vigorous growth habit, a prolonged flowering period (mid-November to mid-February), and a heavy pod load. Around 400 wild *P. lunatus* populations have been recorded in collaboration with the University of San José (Costa Rica) in the target area, which covers 2100 km^2, in variants of premontane and lower montane humid forests, with altitudes ranging from

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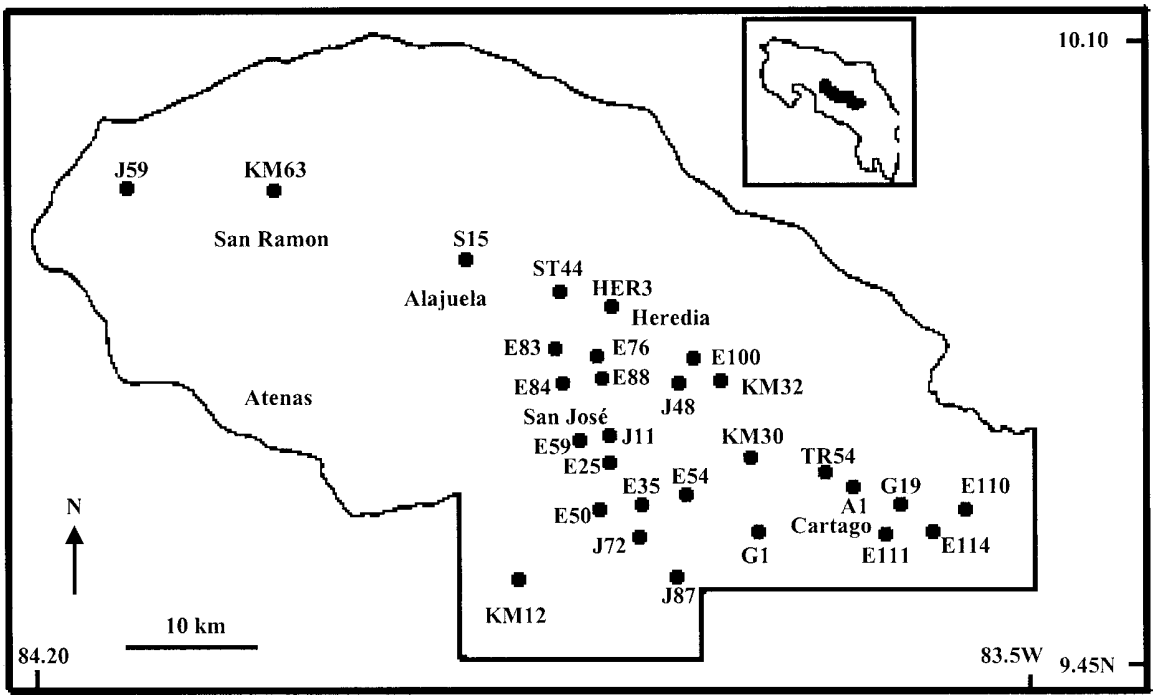

Fig. 1. Location of the 29 wild *Phaseolus lunatus* populations sampled in the Central Valley of Costa Rica.

500 to 1800 m a.s.l. These wild populations are usually found in open and disturbed areas with grasses and scattered trees or bushy thickets; they also colonize coffee plantations from the long-living fences (usually *Erythrina* and euphorbs) bordering the plots. Each year, some wild Lima bean populations are eliminated by land management (Rocha et al., 1997).

The number of plants per population reaching reproductive age differs markedly among years, varying from 1 to 50 plants but only 16% of the populations contained more than five pod-bearing plants. Reproductive individuals can bear several racemes (around 400 each) with 1–20 pods per raceme, each pod containing 1–5 seeds. In the soil seed bank, Degreef et al. (2002) found 3–5 seeds/m^2, and they estimated the annual germination rate ranging from 70 to 86%. In a study of allozyme polymorphisms in Lima bean, Maquet et al. (1996, 1997) estimated genetic structure parameters using 10 putative enzyme loci and 20 wild populations. To refine estimates of population parameter, 12 additional enzyme loci were resolved and their genetic basis established (Zoro Bi et al., 1999). Optimum sampling strategies integrating criteria of efficiency relevant to multilocus and many target populations also have been investigated, in particular the number of plants and the number of seeds to be sampled (Zoro Bi et al., 1998). For the present study specifically, our goals were (1) to estimate the amount of genetic diversity within populations; (2) to determine the degree of genetic differentiation and gene flow among populations; and (3) to examine the relationship between the size of wild Lima bean populations and their genetic variability.

MATERIALS AND METHODS

Plant materials and sampling method—Twenty-nine wild Lima bean populations distributed in the Central Valley of Costa Rica (Fig. 1) were sampled during January to March of 1995, 1996, 1997, and 1998, corresponding to the date of plant physiological maturity. Each population was followed during a complete season so that all individuals bearing pods during this season were sampled. A population is here defined as any set of individuals regardless of size that live in the same habitat patch and therefore interact with each other and are isolated by at least 500 m from other plants of the same species. In each selected population, we sampled all pod-bearing plants, resulting in sample sizes of 1–60 plants per population and 4–6 racemes per plant. One seed was randomly chosen per raceme for electrophoretic analysis, resulting in sample sizes ranging from 4 to 334 seeds per population. The selected populations were representative of the four ecological zones in which the majority of the populations were located (Degreef, 1998) and were identified by several alphanumeric codes (Rocha et al., 1997).

Genetic characterization—For electrophoretic variation, we analyzed 22 readable and reproducible enzyme loci resolved from 15 enzymatic systems: aconitate hydratase (ACO, E.C. 4.2.1.3), alcohol dehydrogenase (ADH, E.C. 1.1.1.1), diaphorase (DIA, E.C. 1.8.1.4), endopeptidase (END, E.C. 3.4.-.-), fluorimetric and colorimetric esterases (fEST and cEST, E.C. 3.1.1.-), glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.9), glutamate dehydrogenase (GDH, E.C. 1.4.1.2), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), leucine aminopeptidase (LAP, E.C. 3.4.11.1), malate dehydrogenase (MDH, E.C. 1.1.1.37), phosphoglucomutase (PGM, E.C. 5.4.2.2), phosphogluconate dehydrogenase (PGDH, E.C. 1.1.1.44), shikimate dehydrogenase (SKDH, E.C. 1.1.1.25), and superoxide dismutase (SOD, E.C. 1.15.1.1). Enzymes were extracted by grinding 5-d-old cotyledon tissues in a potassium phosphate buffer, pH 7.0, containing 20% sucrose (Sigma S-8501 [Bornheim, Flanders, Belgium]), 5% PVP-40, 0.05% triton X-100 (Sigma T-8532), 14 mmol/L 2-mercaptoethanol (Sigma M-6250), and 0.1 mol/L KH_2PO_4. The pH value was adjusted to 7.0 with a solution of 5 mol/L NaOH. Electrophoresis was performed using a horizontal 10% starch-gel (Sigma S-4501) containing 3% sucrose. Two buffer systems were employed: continuous histidine-citrate, pH 6.1 (Kazan et al., 1993) for ADH GPI, IDH, LAP, MDH, and PGDH, and discontinuous lithium-borate, pH 8.1/Tris-citrate, pH 8.4 (Murphy et al., 1990) for ACO, DIA, END, cEST, fEST, GDH, PGM, SKDH, and SOD. The techniques for gel electrophoresis and histochemical staining procedures are those reported elsewhere (Zoro Bi et al., 1999).

TABLE 1. Population acronyms (alphanumeric codes), sample sizes, estimates of genetic diversity, average fixation index (*F*), and summary of results of tests for deviations of genotypic frequencies from Hardy-Weinberg equilibrium in 29 wild populations of *Phaseolus lunatus*.

	Sample size		Genetic diversity indices						H-W deviations			
Population	*m*	*n*	P_p	*A*	A_e	H_o	H_e	*F*	Tests	HE	HD	NS
A1	3	12	5	1.05	1.01	0	0.008	1	1	0	1	0
E25	39	144	19.05	1.19	1.05	0.005	0.032	0.84	4	0	3	1
E35	4	16	0	1	1	0	0	—	0	0	0	0
E50	18	86	14.29	1.14	1.08	0.010	0.051	0.80	3	0	3	0
E54	21	82	9.52	1.10	1.06	0.014	0.037	0.62	2	0	2	0
E59	4	16	0	1	1	0	0	—	0	0	0	0
E76	10	58	23.81	1.24	1.08	0.008	0.057	0.86	5	0	5	0
E83	5	20	9.52	1.10	1.08	0.012	0.026	0.54	2	0	1	1
E84	19	109	23.81	1.24	1.11	0.012	0.066	0.82	5	0	5	0
E88	21	151	19.05	1.19	1.15	0.033	0.083	0.60	4	0	4	0
E100	60	334	18.18	1.18	1.08	0.012	0.050	0.76	4	0	4	0
E110	5	16	4.76	1.05	1.05	0	0.033	1	1	0	1	0
E111	1	4	0	1	1	0	0	—	0	0	0	0
E114	2	8	4.76	1.05	1.05	0	0.025	1	1	0	1	0
G1	38	137	13.64	1.14	1.06	0.009	0.034	0.74	3	0	3	0
G19	3	12	0	1	1	0	0	—	0	0	0	0
HER3	2	8	13.64	1.14	1.14	0	0.073	1	3	0	3	0
J11	4	16	4.55	1.05	1.03	0.011	0.020	0.45^{ns}	1	0	0	1
J48	51	202	18.18	1.18	1.04	0.005	0.024	0.79	4	0	4	0
J59	7	27	4.55	1.05	1.01	0	0.007	1	1	0	1	0
J72	3	11	9.09	1.09	1.02	0.016	0.015	-0.07^{ns}	1	0	0	1
J87	26	102	5.26	1.05	1.02	0.005	0.017	0.71	1	0	1	0
KM12	3	12	18.18	1.18	1.05	0.008	0.038	0.79	2	0	2	0
KM30	28	109	14.29	1.14	1.04	0.002	0.025	0.92	3	0	3	0
KM32	6	24	4.55	1.05	1.06	0	0.012	1	1	0	1	0
KM63	8	31	4.55	1.05	1	0	0	—	0	0	0	0
S15	4	16	4.55	1.05	1.05	0	0.023	1	1	0	1	0
ST44	3	12	18.18	1.18	1.11	0.008	0.065	0.88	3	0	3	0
TR54	21	137	14.29	1.14	1.12	0.007	0.064	0.99	3	0	3	0
Mean	17	78	10.32	1.10	1.05	0.006	0.030	0.79				
SE	17	83	7.30	0.07	0.04	0.007	0.024	0.24				

Notes: m, the number of pod-bearing plants; *n*, the number of seeds collected; P_p, the percentage of polymorphic loci; *A*, the mean number of alleles per locus; A_e, the effective number of alleles per locus; H_o, the observed heterozygosity; and H_e, the heterozygosity expected under Hardy-Weinberg equilibrium. Tests indicate the number of loci for which tests could be performed: HE, the number of loci with a significant excess of heterozygotes; HD, the number of loci with a significant deficiency of heterozygotes; and NS, the number of loci with nonsignificant inbreeding coefficients. SE is the standard error. ^{ns} indicates a nonsignificant value.

Loci were labeled sequentially, with those migrating closest to the anodal end designated as number 1. Accession G25221 from the collection of the Centro Internacional de Agricultura Tropical (CIAT, Cali, Colombia), a Mexican wild form, was used as the control for our analyses. The allozyme from this genotype was designated 100, and all other allozymes were assessed according to their relative migration distance. The genetic control and the quaternary structure of the analyzed enzyme systems have been discussed previously (Zoro Bi et al., 1999).

Data analysis—Most of the following genetic variability indices were calculated using the computer programs GENSURVEY (Vekemans and Lefèbvre, 1997). Statistical analyses of correlations were done using the SAS statistical package version 8.2 (SAS Institute, 1990).

Genetic diversity and population-level homozygosity—To estimate population-level genetic variability, the allozyme multilocus genotype data were used to calculate the proportion of polymorphic loci (P_p; 99% criterion), the mean number of alleles per locus (*A*), the effective number of alleles per locus (A_e), and the observed (H_o), and expected (H_e) heterozygosity corrected for small sample size (Nei, 1987).

Wright's F [$F = (1 - H_o/H_e)$], the inbreeding coefficient, measures the deviation of population genotypic composition from Hardy-Weinberg (H-W) expectations. If inbreeding is avoided, $F = 0$; negative F indices are usually from selection in favor of the heterozygotes whereas positive values indicate that the considered population has an inbreeding system of mating. The inbreeding coefficient was calculated at each polymorphic locus and tested for significant deviation using χ^2 tests (Li and Horvitz, 1953). The average fixation indices were also calculated for each population and tested for significant difference from zero.

Genetic structure and gene flow—The partitioning of total genetic diversity into within- and among-population components was examined using Nei's (1973, 1987) genetic diversity statistics. For each polymorphic locus, total gene diversity (H_T) was partitioned into diversity within populations (H_S) and diversity among populations (D_{ST}) as $H_T = H_S + D_{ST}$. A measure of genetic differentiation among populations relative to the total genetic diversity (G_{ST}) was calculated at each polymorphic locus ($G_{ST} = D_{ST}/H_T$). Theoretically, G_{ST} ranges from zero (all genetic variation maintained within populations) to one (all genetic variation maintained among populations). The genetic structure of the studied populations was also analyzed in term of F statistics (F_{IT}, F_{IS}, and F_{ST}) following Weir and Cockerham (1984). G_{ST} and F_{ST} were tested for significant difference from zero using a χ^2 test (Workman and Niswander, 1970). The number of migrants into a population per generation (Nm) was estimated using Wright's (1951) equation as modified by Crow and Aoki (1984) and the private alleles method (Slatkin, 1985; Barton and Slatkin, 1986).

Genetic diversity and population size—To examine the relationships between population size and genetic variation encoded by isozyme loci, Spearman rank correlation coefficients (r) were calculated and tested for significant difference from zero. The population size was expressed as both sampled pod-bearing plants number, *m*, and the total number of seeds collected per popu-

TABLE 2. Nei's (1973) genetic diversity indices, *F* statistics, and estimates of interpopulation gene flow.

	Nei's genetic diversity indices				*F* statistics			Gene flow	
Locus	H_T	H_S	D_{ST}	G_{ST}	F_{IT}	F_{IS}	F_{ST}	Nm_W	Nm_S
Adh-2	0.331	0.139	0.192	0.580	0.860	0.776	0.383	0.169	
Dia-1	0.023	0.019	0.005	0.210	0.924	0.874	0.217	0.877	
cEst-2	0.343	0.163	0.180	0.524	0.991	0.897	0.919	0.212	
fEst-2	0.011	0.009	0.002	0.159	0.914	0.899	0.152	1.233	
Gpi-1	0.067	0	0.067	1	1	1	0.999	0.000	
Mdh-2	0.488	0.203	0.285	0.584	0.814	0.761	0.217	0.166	
Pgdh-1	0.050	0.013	0.037	0.743	1	1	0.747	0.081	
Pgm-2	0.343	0.131	0.212	0.618	0.909	0.617	0.763	0.144	
Skdh	0.083	0.062	0.021	0.250	0.973	0.971	0.081	0.699	
Mean	0.193	0.082	0.111	0.519	0.932	0.866	0.497	0.398	0.023
SE	0.181	0.077	0.106	0.273	0.066	0.128	0.358	0.430	

Notes: H_T, the total genetic diversity; H_S, the genetic diversity within populations; D_{ST}, the genetic diversity among populations; G_{ST}, the among-populations gene differentiation coefficient; F_{IT}, the mean inbreeding coefficient of a set of populations; F_{IS}, the fixation index related to nonrandom mating within populations; F_{ST}, the interpopulation genetic differentiation due to genetic draft; Nm_W, the gene flow estimate according to Wright's (1951) equation; Nm_S, the gene flow estimate based on Slatkin's (1985) private alleles method; and SE, the standard error.

lation, *n* (Table 1). The intrapopulation genetic variation considered for this analysis was characterized through the following indices: the proportion of polymorphic loci (P_p), the mean number of alleles per locus (*A*), the mean observed heterozygosity (H_o), and the average fixation index (*F*). These measures of genetic variability are not independent from each other, but all levels were presented here to facilitate comparisons with other studies. To check that the results of correlation could only be attributed to the difference in sample size, we selected the 12 populations with more than 50 seeds (Table 1) for in-depth analyses. From each of the 12 populations, we selected randomly 20 sets of 30 seeds using a numerical resampling method designed with the random numbers generator of FORTRAN. Then we obtained 20 sets of 12 populations, each population containing 30 seeds. With each of these new data sets (where all the populations had the same sample size) we reanalyzed the correlation.

RESULTS

Genetic diversity and population-level homozygosity—From the 22 analyzed enzyme loci and the screening of the 29 populations, nine expressed polymorphisms (at 99% criterion) and a total of 31 alleles were observed, most loci having a common allele. The complete genotypic and allelic frequencies are available from the authors upon request. Three private alleles were observed: *fEst-2*^{110} in E88 with 0.836 frequency, *Gpi-1*^{96} in E114 with 1 frequency, and *Pgdh-1*^{86} in KM12 with 0.750 frequency. From Table 1, the proportion of polymorphic loci (P_p) varied from 0% (e.g., E35) to 23.81% (e.g., E76), with a mean of 10.32%; the mean number of alleles per locus (*A*) and the effective number of alleles per locus (A_e), varied respectively from 1 (e.g., E35) to 1.24 (e.g., E76) with a mean of 1.10 and from 1 (e.g., E35) to 1.15 (e.g., E88) with a mean of 1.05. As shown in Table 1, the average H_o was 0.006, ranging from 0 (e.g., A1) to 0.033 (E88) and the average H_e was 0.030, ranging from 0 (e.g., E35) to 0.083 (E88). These results indicated that in wild Lima bean populations, enzyme loci express a low allelic richness ($A = 1.10$), the polymorphic loci presenting uneven allele frequencies ($A_e = 1.05$).

In general, observed genotype frequencies were significantly different from H-W expectations (Table 1). Indeed, of 59 inbreeding coefficients calculated, only 4 (6.8%) were not significantly different from zero ($\alpha = 0.05$). Such results were obtained from locus *Adh-2* in populations E25 ($F = 0.32$; $P = 0.051$) and E83 ($F = 0.34$; $P = 0.246$) and *Pgm-2* in population J11 ($F = 0.44$; $P = 0.107$). Only locus *cEst-2* in population J72 showed a negative but insignificant *F* index ($F = -0.11$, $P > 0.999$). Accordingly, the average fixation index (*F*) is significantly higher than zero for the analyzed populations, except for J11 and J72 (Table 1).

Genetic structure and gene flow—The estimates of population genetic structure using Nei's genetic diversity statistics are shown in Table 2. The average of total heterozygosity (H_T) and intrapopulation genetic diversity (H_S) were 0.193 and 0.082, respectively. The interpopulation genetic diversity (D_{ST}) and the coefficient of genic differentiation among populations (G_{ST}) varied from 0.002 (*fEst-2*) to 0.285 (*Mdh-2*) and from 0.159 (*fEst-2*) to 1 (*Gpi-1*), with a mean of 0.111 and 0.519, respectively. The results indicated that in wild Lima bean, about 52% of the total genetic diversity is among populations, 48% representing intrapopulation genetic diversity. The high levels of genetic differentiation among populations ($G_{ST} = 0.519$; $\chi^2 = 215.18$, $P < 0.001$) and the interpopulation genetic diversity ($D_{ST} = 0.111$) were probably indicative of low gene flow, which was confirmed by the estimates of the number of migrants per generation based both on Wright's equation ($Nm_W = 0.398$) and Slatkin's method ($Nm_S = 0.023$). Such results corresponded to the occurrence of genetic divergence in wild Lima bean populations, given that genetic drift results in substantial local differentiation if $Nm < 1$ (Wright, 1931; Slatkin, 1987).

F statistics for the 29 populations (Table 2) indicated that the mean inbreeding index was significantly higher than zero ($F_{IT} = 0.932$). Then the genotypic composition of the wild Lima bean showed a deviation from the expected H-W proportions. A high and significant value was also obtained for F_{IS} (0.866), suggesting the occurrence of nonrandom mating system for the studied populations. Although relatively low, the estimate of F_{ST} was significant ($F_{ST} = 0.497$; $\chi^2 = 206.06$, $P < 0.001$).

Genetic diversity and population size—The relationship between population size expressed by the plants number (*m*) and the proportion of polymorphic loci (P_p), the mean number of alleles per locus (*A*), and the mean observed heterozygosity (H_o) as well as the correlation coefficients (*r*) describing this relationship are shown in Fig. 2. Significantly positive correlations were observed between the three genetic diversity indices (P_p, A, and H_o) and the population size expressed as both number of individuals in the population and collected seeds

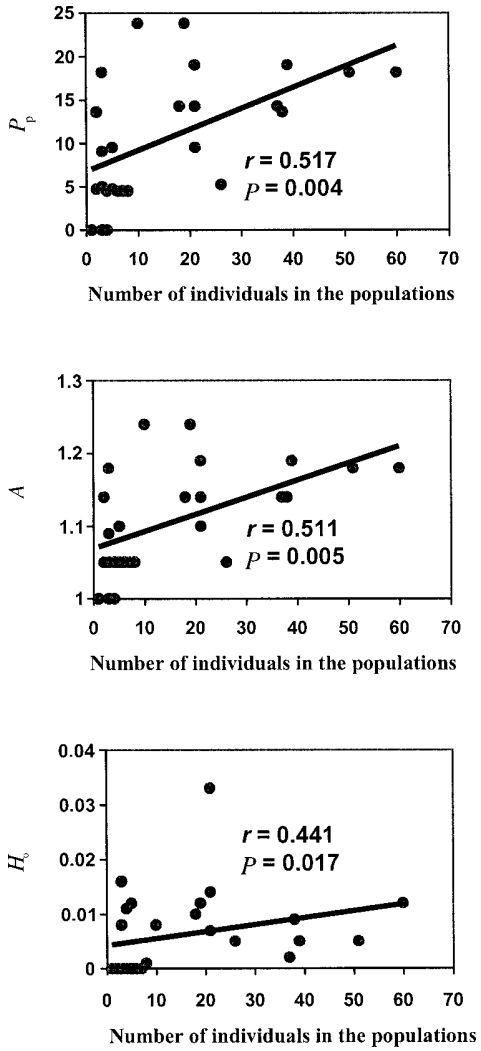

Fig. 2. Relationship between population size and measures of genetic variation (proportion of polymorphic loci; P_p, number of alleles per locus, A; and mean heterozygosity, H_o) examined using a Spearman rank non-parametric correlation for 29 wild *Phaseolus lunatus* populations.

numbers. Considering sample size as the collected seeds number, the following results were obtained: $r = 0.546$ with $P = 0.004$ for P_p, $r = 0.546$ with $P = 0.004$ for A, and $r = 0.449$ with $P = 0.016$ for H_o. A negative but insignificant correlation was highlighted for the average fixation index: $r = -0.317$ with $P = 0.131$ when sample size was expressed as the individuals in the population and $r = -0.266$ with $P = 0.208$ when the collected seeds number was considered as sample size. The observed tendency was confirmed by the results of analyses obtained from the 20 sets of 12 populations with 30 seeds per population (data not shown here). Indeed, of the 20 tests performed, 19 showed significant correlation for P_p, 16 for A, and 15 for H_o whereas no significant correlation was found for F.

DISCUSSION

Genetic diversity and population-level homozygosity—The intrapopulation polymorphism indices estimated in this study were smaller than those reported by Hamrick and Godt (1990) for autogamous plants ($P_p = 20.0\%$, $A = 1.31$, $A_e = 1.10$, and $H_e = 0.074$), short-lived perennial herbaceous plants ($P_p = 28.0\%$, $A = 1.40$, $A_e = 1.12$, and $H_e = 0.096$), and for species with an animal-pollinated mixed-mating system ($P_p = 29.2\%$, $A = 1.43$, $A_e = 1.12$, and $H_e = 0.090$). It should be noted that the reviews of Hamrick and Godt (1990) based on eight life histories and ecological characteristics did not explain more than 30% of the genetic variation observed in plant species (Godt and Hamrick, 1993). Table 3 presents a compilation of statistics on the population genetic variability, estimated using isozymes for some wild and predominantly autogamous plant species. The mean of the data presented in this table was close to the indices for intrapopulation genetic variation of wild Lima bean: $P_p = 13.24$, $A = 1.28$, and $H_o = 0.034$. The low allelic richness and frequent heterozygote deficiency observed in the populations studied could be attributed to a number of different causes: founder effects, a high and somewhat steady selfing rate (Zoro Bi, 1999), assortative mating (homogamy), selection favoring homozygote individuals, and Wahlund effects. As will be discussed more thoroughly later, the founder effect has been highlighted in the studied populations (Rocha et al., 1997). In the Central Valley of Costa Rica, the outcrossing rate of wild Lima bean ranged from 0.027 to 0.268 with a mean of 0.096, indicating that this plant

TABLE 3. Genetic diversity indices of some wild and predominantly autogamous species.

Taxon	Genetic diversity indices						References
	P_p	A	H_o	H_T	H_S	G_{ST}	
Arenaria uniflora	17.90	1.09	0.048	0.371	0.103	0.572	Wyatt et al., 1992
Bromus tectorum	4.60	1.05	0.000	0.115	0.046	0.478	Novak et al., 1991
Ceratophyllum demersum	20.00	1.22	0.076	0.211	0.085	0.495	Les, 1991
C. echinatum	7.00	1.07	0.071	0.529	0.255	0.481	Les, 1991
Eichhornia paniculata	7.60	—	0.002	0.060	0.027	0.570	Barrett and Shore, 1989
Phaseolus acutifolius	—	—	—	0.372	—	—	Schinkel and Gepts, 1989
P. lunatus	10.32	1.10	0.008	0.193	0.082	0.519	The present study
P. vulgaris	—	—	—	0.132	0.006	0.952	Koenig and Gepts, 1989
Setaria glauca	13.50	1.54	—	0.099	0.016	0.729	Wang et al., 1995b
S. viridis	25.00	1.86	—	0.108	0.023	0.647	Wang et al., 1995a
Sorghum bicolor	—	—	—	0.262	0.075	0.714	Ollitrault, 1987
Mean	13.24	1.28	0.034	0.220	0.071	0.605	

Notes: P_p, the percentage of polymorphic loci; A, the mean number of alleles per locus; H_o, the observed heterozygosity; H_T, the total genetic diversity; H_S, the genetic diversity within populations; and G_{ST}, the among-populations gene differentiation coefficient. A dash indicates unpublished data.

has a high level of autogamy (Zoro Bi, 1999). Because this author observed also that only 1% of the apparent selfing was due to biparental inbreeding, the hypothesis of homogamy (assortative mating) could be discarded. In a previous work, the actual genetic structure of six wild Lima bean populations was assessed using isozymes electrophoresis (Zoro Bi et al., 1997; Zoro Bi, 1999). Seeds were sampled according to a grid of 4 $\times$ 4 m for bidimensional populations or 4 m apart for linear populations, and the genotypes of mother plants at each node were so determined. The genetic structure in the populations was obvious: alternative alleles at each locus were clustered in opposite parts of the populations, creating a patch structure mainly composed of homozygote individuals. The Wahlund effects could be another explanation of the observed frequent heterozygote deficiency. However for this study, in each population, all pod-bearing plants were sampled during a complete flowering period. This sampling procedure, combined with the fact that the mean seed germination rate within a year was 78% and that 4% of the germinating seeds reach maturity in the same year allowed us to discard the hypothesis concerning Walhund effects. The four insignificant inbreeding coefficients obtained in the studied populations probably had no biological significance, but might have resulted from the method of sampling.

Genetic structure and gene flow—The mean intrapopulation gene diversity index (H_S = 0.082) estimated for the 29 populations was higher than the value reported from a previous study by Maquet et al. (1996): H_S = 0.058. On the other hand, the total heterozygosity (H_T = 0.193), the interpopulation gene diversity index (D_{ST} = 0.111), and the among-populations gene differentiation coefficient (G_{ST} = 0.519) were significantly lower than those reported in that study (H_T = 0.292, D_{ST} = 0.234, and G_{ST} = 0.803). Differences in the two statistics could be attributed to the difference between sampling schemes adopted for these investigations. For the present study, 1–60 plants were selected per population; 4–10 seeds were randomly chosen per sampled plant so that 4–334 seeds were analyzed according to the plant number per selected population. On the other hand, in Maquet et al. (1996), 14–50 seeds were randomly chosen per population, regardless of the number of plants harvested in the target populations. Therefore, the number of analyzed seeds might not have been representative of the total number of plants in each population, leading to an overestimation of H_T, D_{ST}, and G_{ST}.

The extent of genetic heterogeneity among populations as measured by D_{ST} (=0.111) was indicative of the occurrence of several genetic phenomena such as high selfing rate, genetic drift, and limited gene flow. Generally, in short-lived perennial predominantly autogamous species such as wild Lima bean, gene differentiation among populations expressed by G_{ST} is very high (Hamrick and Godt, 1990). Wild Lima bean is a mixed-mating, predominantly autogamous species (Zoro Bi, 1999) that is expected to express high levels of population genetic divergence and low levels of within-population genetic diversity. The estimates of the populations genetic structures indices analyzed in this study were also in accordance with the designated trend: F_{IT} = 0.932, F_{ST} = 0.497, and F_{IS} = 0.866. This mating trait, coupled with founder effects associated with recruitment events in the populations studied (Rocha et al., 1997), could explain the high level of genetic divergence and lower level of genetic diversity in wild *P. lunatus* populations. The authors established after a 7-yr survey of these populations in the target area that the number of wild Lima bean populations containing more than five pod-bearing plants did not exceed 16%, predicting a probable occurrence of genetic drift.

The estimates of *Nm* based either on Wright's (1951) equation or the Slatkin approach were very low: Nm_W = 0.398 and Nm_S = 0.023. In addition, we noted that Nm_W was approximately 19 times higher than Nm_S. Such difference could be attributed to the low number of observed private alleles (3 out of 31 alleles) and their high frequencies (*Pgdh-1*^{86} in KM12 with 0.750 frequency and *Gpi-1*^{96} in E114 with 1 frequency). These result were in accordance with those obtained from investigations on wild Lima bean intrapopulation gene flow evaluated using pollen dispersal (Hardy et al., 1997), seed dispersal, and vegetative growth (Baudoin et al., 1998). Indeed, we estimated flower and pollen dispersal (through vegetative growth) as well as seed dispersal within some populations by considering the foraging behavior of pollinators and using a technique for labeling and tracking pollen grain and seeds in vivo. From this study, we concluded that the horizontal transfer distance for pollen and seeds did not exceed 6 m (Baudoin et al., 1998). The neighborhood parameter area and the neighborhood size equaled 8.4 and 1.6, respectively. Because this last value was smaller than 20, random local genetic differentiation is expected (Wright, 1931).

Genetic diversity and population size—Except for *F*, we found a significant correlation between the size of the investigated populations and their levels of genetic variation. Thus, our data were consistent with the idea that genetic variation within populations is related to population size. Such results have been observed previously in other plant species, in particular in rare or threatened species (Moran and Hopper, 1983; Karron, 1987; van Treuren et al., 1991; Godt et al., 1996; Routley et al., 1999). Various explanations have been formulated for the correlation between population size and intrapopulation genetic variability indices. In our case, the most likely phenomena to explain the correlation is the inbreeding highlighted in this study and in former studies (Zoro Bi et al., 1997; Zoro Bi, 1999). Inbreeding reveals itself through a higher number of homogygotes than would be expected under panmictic mating. In wild Lima bean from the Central Valley of Costa Rica, an excess of homozygotes was observed and gene dispersal within populations was not sufficient to maintain random union of gametes (Hardy et al., 1997; Baudoin et al., 1998). In smaller populations, we mainly observed fewer alleles and simultaneously found lower levels of heterozygosity (computed as H_o). The lower heterozygosity was mainly due to fixed alleles (Table 1). A correlation between heterozygosity and effective population size is also expected for loci under weak heterozygote advantage in selection when populations are small in size. Heterozygote advantage in finite populations will slow fixation for alleles with intermediate frequencies and accelerate it for rare and very common alleles in small populations. Selection on individual alleles detected by electrophoresis is generally weak, so they are likely to be subject to genetic drift unless population sizes are very large (Montgomery et al., 2000; Hedrick, 2001). Random genetic drift in small bottlenecked populations and founder effects resulting from extinction/recolonization episodes that characterized the studied populations also could have lowered the genetic variability in small populations. Indeed, in the target area, many wild Lima beans are found in coffee plantations, fallow lands, or

along hedges, so that weeding practices contribute to the destruction of plants. Recolonization of the cleared sites could be due to any nearby plants, to new individuals emerging from the soil seed bank, or to human activities (such as seed transportation over longer distances on shoes or tools). The absence of correlation between populations size and the average fixation index was evident since in the majority of the analyzed populations, *F* values were high and significantly different from zero, regardless of their sizes.

Conservation and management implications—Conservation of plant genetic resources aims to maintain as much genetic diversity as possible. In situ conservation planning requires choice of populations, delimitation of sites, and continuous management and monitoring of designated populations (Iwanaga, 1996; Ouédraogo, 1996). Both require ecogeographic and genetic knowledge of the target taxon.

For wild *P. lunatus* populations, data from isozyme electrophoresis indicated genetic variability mainly at the interpopulation level, with low values for allelic richness, expected heterozygosity and interpopulation gene flow. Based on these results, we recommend protecting populations from as many distinctive ecological sites as possible, regardless of their size, because some private alleles were observed in small populations.

Once populations have been selected for in situ conservation, sound management is necessary to preserve a high level of genetic variability. For wild Lima bean populations, an appropriate management method was indicated by demographic studies (Degreef, 1998). An analysis of the sensitivity of life cycle matrices obtained from six populations pointed out the importance of seed production, early germination, and rapid plant lignification to maintain in situ populations in the Central Valley. Because genetic drift appears to be important in the populations studied, the resulting genetic uniformity might directly threaten some alleles. The reintroduction of the threatened genotypes (genotypes having rare alleles) at regular time intervals would allow the maintenance of a substantial level of genetic variability in some endangered populations.

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