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Population Structure in *Gliricidia sepium* (Leguminosae) as Revealed by Isozyme Variation

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Abstract

Gliricidia sepium (JACQ.) WALP. is a woody legume native to seasonally dry sites in Meso-America. It has been introduced to many other parts of the tropics, where it is utilised as a source of fuelwood, living fences, animal fodder and green manure by rural communities. These introductions have, however, been founded on a narrow, or unknown, genetic base, and poor growth performance has been reported at a number of locations. There is, therefore, a need to diversify the genetic base of this species in domestication, and to explore its population structure as a basis for this diversification. Here we report the use of isozyme markers to investigate the distribution of genetic diversity within and among populations of *G. sepium*. Marked differentiation between populations ($F_{ST} = 0.172$) was observed, although most variation occurred within populations. Averaged over all populations, there was a mean number of alleles per locus (A) of 2.0, a mean percentage polymorphic loci (P) of 60% and a mean observed heterozygosity (H_o) of 0.238, values which suggest a rather higher level of genetic diversity than those reported from other comparable species. The values

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of these variables, however, differed considerably between populations, those believed to be native maintaining higher levels of variation than those believed to be naturalised or introduced. Some of the most genetically diverse populations have also been recommended for forage and fuelwood production, indicating that there is a sound basis for the genetic improvement of this species.

Key words: Gliricidia sepium, isozyme variation, population structure, genetic diversity.

FDC: 165.3; 165.5; 176.1 *Gliricidia sepium.*

Introduction

Gliricidia sepium (JACQ.) WALP. is a medium-sized leguminous tree which is found abundantly throughout the dry, deciduous forest of Central America and Mexico (HUGHES, 1987). The species has been transported to, and used extensively in, many tropical and sub-tropical countries, originally for the provision of shade in cocoa, coffee and tea plantations. Today, it is more widely used to provide fuelwood, green manure and animal fodder, and is important for soil stabilisation. Domestication of the species has been in progress for several centuries, with landrace populations developing in many exotic locations (SIMONS and DUNSDON, 1992). These landraces have often been the result of selection for arboreal types suited to the provision of shade. This selection pressure, together with a vegetative mode of propagation allowing clonal lines to predominate, suggests landraces may have suffered a loss in genetic diversity during domestication. Moreover, such selection may have favoured types unsuited to the uses to which *G. sepium* is now being put.

There is, therefore, considerable scope for the improvement of *G. sepium* by means of new introductions from its native range, but information on which the choice of material for introduction might be based is at present very limited. The aim of the investigation reported here was to determine the extent and distribution of genetic variation in populations of *G. sepium* from Central America and Mexico. Within this broad aim, there were more specific objectives. The first was to compare the level of genetic variation in *G. sepium,* with that in other tropical tree species. The second was to determine the extent of genetic divergence between populations, and to determine whether the genetical features of particular populations – their genetic distance from other populations, and the extent and pattern of variation within the population – could be explained in terms of their geography or history. The third objective was to determine whether the pattern of genetic variation observed was consistent with that found by other studies of *G. sepium*.

Seed was collected by the Oxford Forestry Institute (OFI) from 1984 to 1992 across the entire native range of *G. sepium* (SIMONS and DUNSDON, 1992). An extensive collection of populations was therefore available, and the present investigation was based on the isozyme analysis of samples from this collection. Four other studies, also based on the OFI collection, have given some indication of the extent and structure of genetic variation in *Gliricidia.* The first utilised chloroplast DNA (cpDNA) to analyse the intraspecific phylogeny of the genus (LAVIN et al., 1991), which was shown to comprise 2 lineages, one corresponding to *G. sepium,* the other to *G. maculata* RYDB. The latter species is much less common than *G. sepium* and is confined to the Yucatan Peninsula of Mexico, Belize and Guatemala. A number of sublineages were also identified within *G. sepium,* which suggests that there is a high level of intraspecific divergence within this species. The second study was an assessment of population growth performance in a number of field trials world-wide (SIMONS and DUNSDON, 1992). Considerable variation between populations in wood and leaf traits was identified. Significant differences for the timing and quantity of flowering were found between 3 *G. sepium* populations grown at the same site, and substantial between-family differences in flowering within each population were also identified. Between-population variation was also detected in the seed:ovule ratio. The third study involved the use of randomly amplified polymorphic DNA (RAPDs) to provide molecular markers and monitor genetic variation in *Gliricidia* (CHALMERS et al., 1992). Extensive genetic variation was detected between the two species, and within *G. sepium,* 60% of the variation occurred between populations. This is a surprising finding, since recent reviews suggest that tropical trees generally maintain most of their isozyme variation within populations (HAMRICK, 1989; LOVELESS, 1992). The fourth study utilised seed proteins, isozymes and plant morphology to measure genetic diversity in four possibly-naturalised populations of *G. sepium* (CHAMBERLAIN and GALWEY, 1993). Differentiation between populations was also apparent in this study, but a qualitative assessment of the data indicated that most variation was within populations. *G. sepium* has gone through extreme disturbance in its native range, primarily through human intervention, so that it is quite possible that the partitioning and distribution of genetic variation may be more complex in this species than in other comparable species. The present study was expected to shed more light on this question.

Materials and Methods

Seed samples

Eight populations, distributed throughout the native range of *G. sepium,* were chosen from the OFI seed collection for study. Within each population, seed of a number of families was analysed (*Table 1*). Each family comprised seed progeny from a single maternal parent, and a minimum distance of 50 m separated each parent tree to prevent bias due to co-ancestry.

Table 1. – Collection site data for the 8 populations of *Gliricidia sepium* studied.

Population	Country	Latitude (N)	Longitude (W)	OFI No.	Altitude (m)	Annual Rainfall (mm)	Number of families	Number of seeds per family
Monterrico	Guatemala	13°54'	90°29'	17/84	5	1650	20	12
Vado Hondo	Guatemala	14°44'	89°30'	59/87	450	830	20	8
Retalhuleu	Guatemala	14°33'	91°39'	31/92	330	3500	20	8
Ocosito	Guatemala	14° 32'	91°46'	99/92	340	3500	20	8
Masaguara	Honduras	14°16'	87°58'	25/84	825	1100	14	8
San Mateo	Mexico	16°13'	94°58'	35/85	10-30	950	20	8
Pedasi	Panama	7°32'	80°04'	13/86	0-20	850	10	8
Belen Rivas	Nicaragua	11°37'	85°48'	14/86	75	1650	20	8

Sample preparation

250 mg of leaf tissue was harvested from seedlings grown in a glasshouse maintained at 27 °C, and ground in 0.3 ml of extraction buffer comprised of 50 ml gel buffer, 40 mg KCl, 100 mg $MgCl_2$, 18 mg EDTA (disodium salt), 2.0 g PVP-40, 0.5 ml Triton-X-100 and 2 ml 10% DTT. Extracts were centrifuged at 13000 rpm for 2 min., and the supernatant transferred to a clean Eppendorf tube and frozen under liquid nitrogen. Samples were then stored at –70 °C for up to a month without any detectable loss of enzyme activity.

Preparation of polyacrylamide gels

Vertical polyacrylamide gel electrophoresis was carried out in a dual-cooled slab unit (SE 600 series, Hoeffer Scientific) on 7.5% acrylamide. One of 2 gel/electrode buffer systems were used: (i) electrode buffer which comprised 1.2 g lithium hydroxide and 11.9 g boric acid per litre distilled water (pH 8.3), and gel buffer which comprised 5.4 g Tris and 1.28 g citric acid per litre distilled water; (ii) electrode buffer which comprised 15.1 g Tris and 7.3 g citric acid per litre distilled water (pH 7.5) and gel buffer which comprised 8.36 g histidine HCl and 0.03 g EDTA (disodium salt) per litre distilled water. 10 µl of sample was loaded per sample well, and a constant current of 20 mA per gel applied for 3.5 h to 4.0 h.

Enzyme stains

When electrophoresis was complete, standard staining protocols (HAMES and RICKWOOD, 1981; SOLTIS et al., 1983) were used to resolve the isozymes. The enzyme systems used in the analysis were: aspartate aminotransferase (AAT; E.C. 2.6.1.1), esterase (EST; E.C. 3.1.1.-), isocitrate dehydrogenase (IDH; E.C. 1.1.1.42), leucine aminopeptidase (LAP; E.C. 3.4.11.1), phosphoglucose isomerase (PGI; E.C. 5.3.1.9), phosphoglucose mutase (PGM; E.C. 5.4.2.2), and shikimic dehydrogenase (SDH; E.C. 1.1.1.25).

Analysis of isozyme polymorphisms

As full-sib progeny tests were not possible, and as the trees sampled were assumed to be open-pollinated, genetic interpretations of the isozyme gel, or zymogram, banding patterns were based on the evaluation of isozyme polymorphisms in other well-documented investigations (WENDEL and WEEDEN, 1989). These interpretations have been corroborated by genetic analysis, and the sub-unit structure of many isozymes is known (SHIELDS et al., 1983). In most cases, the banding patterns implied a simple diploid genetic model and could be interpreted in terms of loci and alleles. A genotype was assigned to each seed on this basis, and the allele frequencies for each enzyme system in each family were calculated (summary in *Table 2*) and entered into BIOSYS-1 (SWOFFORD, 1989). In addition to the parameters offered by BIOSYS-1, SHANNON's index of diversity $H = -\Sigma p_i \log_2 . p_i$, where p_i is the genotype frequency (SHANNON and WEAVER, 1949), was also employed as a means of quantifying genetic variation.

Results

Enzyme descriptions

With the exception of a few zones of enzyme activity in which the banding pattern was not sufficiently clear to interpret, all the zymogram bands could be interpreted as the products of polymorphic loci. The alleles that are inferred to exist at each locus are identified by letters; each zymogram band, and the genotype to which it corresponds, is then identified by a combination of letters indicating which alleles contribute to it (*Figure 1*). Three bands were observed on AAT gels, 2 parental homodimers (A and B) and an additional product of intermediate mobility, the heterodimer. This isozyme was polymorphic for all populations except that at Pedasi. Of the 2 polymorphic zones of activity observed on EST gels, Est-1 and Est-2, the latter gave a somewhat inconsistent pattern and proved difficult to score, and was therefore omitted from the data analysed. For the Est-1 locus, 3 bands were observed, a fast (C), an intermediate (B) and a slow (A), with a maximum of only 2 bands appearing in any one sample. Hence, Est-1 was presumed to be a monomer encoded by a tri-allelic locus. All the allozymes were present in every population, with the exception of that at Pedasi, which was monomorphic for the B allozyme. Two zones of activity were exhibited on IDH gels and produced a correlated zymogram. The banding zones can, therefore, be considered as a single locus with a mobility polymorphism. A maximum of 3 bands was observed, with neither the intermediate nor the slow band (A) occurring on their own. The isozyme was therefore considered a dimer encoded by a bi-allelic locus. The only 2 populations to exhibit the heterodimer were Monterrico and San Mateo.

A polymorphic zone of activity observed on LAP gels, Lap-1, exhibited up to 4 bands. As with Est-1, only 2 of these bands appeared in any one sample, indicating a monomer encoded by a locus with 4 alleles. The fastest moving allozyme (D) was the least common, occurring only at Belen Rivas. One zone of banding was observed on PGI gels. The complex pattern of bands exhibited in this zone was typical of 2 dimer-producing loci with overlapping ranges of mobility. Masaguara and Pedasi were monomorphic for the slower moving isozyme, Pgi-1, but polymorphic at Pgi-2, whereas all other populations were polymorphic at both loci. Three zones of activity could be distinguished on PGM gels. Poor resolution of the first 2 band-

Table 2. – Summary allele frequencies for 8 polymorphic loci among populations of *Gliricidia sepium*.

Population	Locus/ Allele																				
	Aat-1		Est-1			Idh-1		Lap-1				Pgi-1		Pgi-2		Pgm-3			Sdh-1	
	A	B	A	B	C	A	B	A	B	C	D	A	B	A	B	A	B	C	A	B
Belen Rivas	0.53	0.47	0.14	0.82	0.04	0.00	1.00	0.44	0.10	0.44	0.02	0.94	0.06	0.07	0.93	0.00	0.06	0.94	0.20	0.80
Masaguara	0.88	0.12	0.38	0.47	0.15	0.00	1.00	0.56	0.34	0.10	0.00	1.00	0.00	0.22	0.88	0.56	0.19	0.25	0.03	0.97
Monterrico	0.44	0.56	0.08	0.58	0.34	0.02	0.98	0.18	0.75	0.07	0.00	0.73	0.27	0.20	0.80	0.58	0.11	0.31	0.10	0.90
Ocosito	0.12	0.88	0.05	0.57	0.38	0.00	1.00	0.70	0.28	0.02	0.00	0.82	0.18	0.30	0.700	0.56	0.02	0.42	0.03	0.97
Pedasi	0.00	1.00	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	1.00	0.00	0.005	0.95	0.00	0.01	0.99	0.00	1.00
Retalhuleu	0.78	0.22	0.12	0.55	0.33	0.00	1.00	0.63	0.34	0.03	0.00	0.91	0.09	0.19	0.81	0.65	0.00	0.35	0.05	0.95
San Mateo	0.74	0.26	0.41	0.43	0.16	0.07	0.93	0.61	0.15	0.24	0.00	0.81	0.19	0.16	0.84	0.90	0.00	0.10	0.02	0.98
Vado Hondo	0.12	0.88	0.04	0.57	0.39	0.00	1.00	0.78	0.22	0.00	0.00	0.95	0.05	0.12	0.88	0.49	0.00	0.51	0.00	1.00

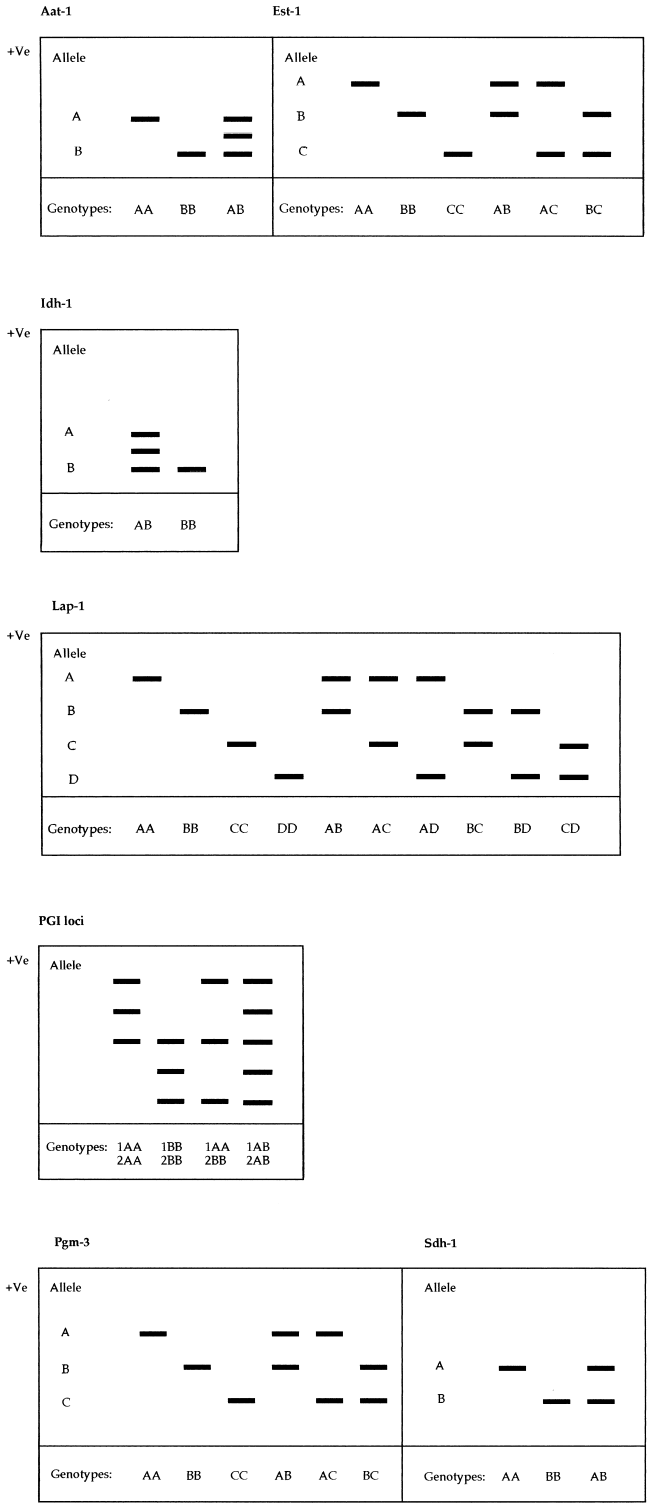

Figure 1. – Schematic illustration of the zymogram banding patterns for the putative polymorphic enzyme loci Aat-1, Est-1, Idh-1, Lap-2, Pgi-2, Pgm-3 and Sdh-1 found in *Gliricidia sepium*. The letters refer to the genotype designations used in the text.

ing zones made them impossible to score, but the third putative locus (Pgm-3) exhibited up to 3 bands, a slow (A), an intermediate (B) and a fast (C). Only 2 bands occurred in any one sample; hence the isozyme was presumed to be a monomer encoded by a tri-allelic locus. The intermediate allozyme was the least common. On SDH gels, 1 zone of activity was observed, with 2 bands being apparent, a fast (B) and a slow (A). The isozyme was presumed to be a monomer encoded by a bi-allelic locus, and was polymorphic within all populations except those at Pedasi and Vado Hondo.

Variation among the Gliricidia populations

The levels of variation at the 8 putative polymorphic loci identified in *G. sepium* were assessed on the basis of the average number of alleles per locus (A), the observed mean heterozygosity (H_o), the expected mean heterozygosity assuming random mating (H_e) and the percentage of polymorphic loci (P) (*Table 3*). The population at Monterrico, Guatemala, was the most variable for all criteria except P, for which it was equalled by the population from San Mateo, Mexico. The other populations from Guatemala, notably those from Retalhuleu and Ocosito, also exhibited high values for P and A. The least variable population, for all criteria and by a considerable margin, was that from Pedasi, Panama.

Table 3. – Measures of genetic variability, averaged over 8 isozyme loci, within populations of *Gliricidia sepium* (standard errors in parentheses).

Population	Mean number of alleles/locus (A)	% polymorphic loci (P)	Mean heterozygosities (H)	
			Observed	Expected
Belen Rivas	2.1 (0.3)	66.7	0.216 (0.074)*	0.260 (0.081)
Masaguara	2.0 (0.1)	60.0	0.213 (0.118)*	0.295 (0.092)
Monterrico	2.3 (0.2)	73.3	0.361 (0.075)	0.364 (0.076)
Ocosito	2.1 (0.3)	66.7	0.304 (0.077)	0.306 (0.075)
Pedasi	1.2 (0.1)	12.5	0.017 (0.004)	0.016 (0.003)
Retalhuleu	2.0 (0.2)	66.7	0.281 (0.076)	0.297 (0.076)
San Mateo	2.2 (0.1)	73.3	0.303 (0.065)	0.308 (0.069)
Vado Hondo	1.8 (0.2)	60.0	0.210 (0.063)	0.234 (0.073)

*) significant deviation from HARDY-WEINBERG expectations at $P < 0.05$.

There was generally good agreement between the observed and expected heterozygosities, except in the populations at Belen Rivas and Masaguara, which had heterozygosities significantly lower than expected. This suggests that these 2 populations were in genetic disequilibrium.

A comparison of SHANNON's index of diversity (H) for each of the polymorphic loci (*Table 4*) reveals a similar pattern of variation across the populations, with Monterrico again having the highest mean level of diversity, and Pedasi the lowest. There is a significant degree of correspondence between the patterns of diversity for the various polymorphic loci: a principal component analysis on the matrix of correlations between the H values for the various loci showed that 57% of the variation present was explained by the first principal component ($\tilde{n}^2$ for equality of latent roots = 72.11, d.f. = 27,

Table 4. – Estimates of SHANNON's diversity index (H; SHANNON and WEAVER, 1949) at 8 isozyme loci within populations of *Gliricidia sepium*.

Population	H								
	Aat-1	Est-1	Idh-1	Lap-1	Pgi-1	Pgi-2	Pgm-3	Sdh-1	Mean
Belen Rivas	0.604	2.338	0.000	3.413	1.249	1.187	1.249	0.796	**1.325**
Masaguara	0.977	1.572	0.000	1.721	0.000	0.714	1.575	1.187	**0.968**
Monterrico	0.609	1.803	1.708	2.025	0.706	0.796	1.705	1.046	**1.355**
Ocosito	0.977	1.965	0.000	2.407	0.831	0.678	2.328	1.536	**1.134**
Pedasi	0.000	0.000	0.000	0.000	0.000	1.323	2.004	0.000	**0.416**
Retalhuleu	0.766	1.663	0.000	2.193	1.087	0.813	0.643	1.323	**1.061**
San Mateo	0.716	1.550	1.187	1.659	0.813	0.872	1.347	1.708	**1.232**
Vado Hondo	0.977	2.051	0.000	0.766	1.323	0.977	0.601	0.000	**0.837**

$P < 0.001$). Some of this correspondence was due to the extreme value of the population from Pedasi at all loci: when this population was omitted from the analysis, the variation due to the first component fell to 42% ($\tilde{n}^2 = 35.41$, d.f. = 20, $P = 0.018$).

The matrix of NEI's genetic distances (D; NEI, 1978) between pairwise combinations of the 8 *G. sepium* populations is presented in *table 5*. There is little differentiation between populations from Guatemala, Mexico and Honduras, D values ranging from 0.009 to 0.091. This group contrasts sharply with the 2 most southerly populations, at Belen Rivas and Pedasi, which are more distant, both from the northerly group of populations, and from each other. The population at Pedasi is particularly distinct. Among the northerly group of populations, San Mateo is the most distant from both Belen Rivas and Pedasi, not only with respect to D, but also geographically.

Table 5. – Matrix of NEI's genetic distance (NEI, 1978) for pairwise comparisons of 8 populations of *Gliricidia sepium* over 8 isozyme loci.

Population	1	2	3	4	5	6	7	8
1 Belen Rivas								
2 Masaguara	0.083							
3 Monterrico	0.119	0.028						
4 Ocosito	0.095	0.022	0.012					
5 Pedasi	0.186	0.244	0.259	0.200				
6 Retalhuleu	0.120	0.018	0.023	0.018	0.281			
7 San Mateo	0.167	0.027	0.059	0.059	0.356	0.031		
8 Vado Hondo	0.079	0.030	0.041	0.009	0.157	0.044	0.091	

A comparison of WRIGHT's F-statistics (WRIGHT, 1951) for all 8 populations is given in *table 6*. The mean value of F_{IS} over all loci suggests that there is generally little deviation from random mating within populations, though there is a substantial deficiency of heterozygotes at the Est-1 locus. The mean value for F_{ST} indicates a moderate level of differentiation between populations, populations being particularly strongly differentiated for the loci Pgm-3 and Aat-1. SHANNON's index of diversity (H) can be similarly partitioned into within- and between-population components (*Table 7*), to show that, on average, between-population diversity accounted for 35.6% of the total variation – a value twice as high as the mean F_{ST} value of 17.2%.

Table 6. – Summary of WRIGHT's F-statistics (WRIGHT, 1951) at 8 isozyme loci among 8 populations of *Gliricidia sepium*.

Locus	F-statistics		
	F_{IS}	F_{IT}	F_{ST}
Aat-1	0.052	0.237	0.196
Est-1	0.231	0.325	0.122
Idh-1	-0.062	-0.010	0.049
Lap-1	-0.133	0.025	0.139
Pgi-1	-0.101	-0.002	0.090
Pgi-2	0.030	0.133	0.106
Pgm-3	-0.042	0.303	0.332
Sdh-1	0.019	0.095	0.078
Mean	**0.024**	**0.192**	**0.172**

Table 7. – Estimates of SHANNON's diversity index (H; SHANNON and WEAVER, 1949) at 8 isozyme loci partitioned between and within 8 populations of *Gliricidia sepium*.

Locus	H_{pop}	H_{sp}	H_{pop}/H_{sp}	$(H_{sp} - H_{pop})/H_{sp}$
Aat-1	0.703	0.607	1.158	-0.158
Est-1	1.618	1.670	0.969	0.031
Idh-1	0.362	2.004	0.181	0.819
Lap-1	1.773	3.743	0.474	0.526
Pgi-1	0.751	1.046	0.718	0.282
Pgi-2	0.920	0.872	1.055	-0.055
Pgm-3	1.432	1.948	0.735	0.265
Sdh-1	0.950	1.323	0.718	0.282
Mean	**1.064**	**1.652**	**0.644**	**0.356**

Discussion

There is considerable genetic variation within populations of *Gliricidia sepium* as characterised by the percentage of polymorphic loci, the average number of alleles per locus, the mean heterozygosity and SHANNON's diversity index. The population at Pedasi is an exception, showing a low level of variation for all criteria. This population was confined to scattered trees in pasture and fencelines (C. E. HUGHES, pers. comm.[4]). Thus, all the evidence indicates that *G. sepium* is almost certainly not native here and has been established from a very restricted genetic base. A low level of variability within the Pedasi population has also been found using RAPD markers (CHALMERS et al., 1992). The population at Vado Hondo also had lower levels of genetic variation than the others. In addition, low seed:ovule ratios, which are a putative indicator of diversity, have also been recorded at Vado Hondo. This population is separated from those on the Pacific coast by mountain ranges rising to altitudes above 1500 m, and is located in secondary vegetation on fallow agricultural land. The population is therefore likely to be naturalised in this area and not native.

The population at Monterrico was the most variable, and that at San Mateo showed a comparable level of diversity. Both these populations are dense and large (>1000 trees). The populations at Ocosito and Retalhuleu, on the other hand, are much smaller (<200 trees). Their levels of within-population variability are closer to those at Monterrico and San Mateo than might be expected in view of their size, and comparison of their observed and expected heterozygosities indicates that they are in genetic equilibrium. These findings suggest that quite small populations of *G. sepium* can maintain genetic diversity. LAVIN et al. (1991) concluded, on the basis of cpDNA studies, that Guatemala is the ancestral centre of diversity for *G. sepium,* and the high levels of genetic variation detected within these Guatemalan populations lend support to this hypothesis.

The levels of isozyme variation found in *G. sepium* are similar to levels found in a number of other tropical woody species. The observed mean heterozygosity (H_o) averaged over all populations of *G. sepium* is 0.238, a value comparable to that found in a number of common, predominantly outcrossed and entomophilous, woody tropical species on Barro Colorado Island, Panama ($H_o = 0.211$) (HAMRICK and LOVELESS, 1989). In a review of isozyme variation in tropical tree species, however, LOVELESS (1992) noted that shrubby species, and species with shorter stature, generally had significantly lower levels of heterozygosity than did canopy species ($H_o = 0.116$ and $H_o = 0.161$ respectively). *G sepium* is often shrubby in form and rarely reaches heights greater than 10 m, yet its overall level of

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heterozygosity is greater than either of these estimates. A possible reason for this discrepancy is the apparent intolerance of inbreeding exhibited by *G. sepium*. SIMONS and DUNSDON (1992) found that following matings between full- or half-sibs, there was a reduction in the seed:ovule ratio relative to that obtained following matings between unrelated individuals, indicating that inbred progeny are eliminated by ovule abortion. Another possible explanation is the sampling strategy employed. The seed used in the present investigation was collected from trees of *G. sepium* separated by distances of at least 50 m: hence unrelated trees are likely to have been sampled. This method of sampling will bias estimates of genetic diversity upwards relative to estimates from populations in which every tree is sampled. LOVELESS's review, in common with many other studies, does not indicate the breeding system of the species studied, nor the sampling strategies employed, so these hypotheses cannot be tested.

The populations at Masaguara and Belen Rivas show a deficiency of heterozygotes and thus appear to be in genetic disequilibrium. The former population consisted of 3 sub-populations around the village of Masaguara, which lies within an enclosed valley where *G. sepium* is otherwise only found occasionally in fencelines. The largest, most even-aged of the sub-populations was originally established as shade for coffee plantations – hence *G. sepium* is probably not native in this area. The high level of genetic diversity at this site can be explained, however, by the hypothesis that the sub-populations have different ancestral origins – that is, that the population as a whole is based on multiple introductions. This could also account for the deficiency of heterozygotes, gene flow between the sub-populations being restricted. The population at Belen Rivas, on the other hand, appears to be a native population, not introduced or naturalised. It forms a large, extensive stand with many old trees, suggesting its establishment there for a long time, possibly as a remnant of a larger forested area. In some sections of the stand, however, *G. sepium* forms living fences, that are likely to have been established from cuttings. Some of the trees sampled may be progeny of these fenceline trees, and pollen flow from woodlots of *G. sepium* planted in the vicinity of the sampled population, may explain the deficiency of heterozygotes in this population.

NEI's genetic distances among the eight populations of *G. sepium* studied shows that there is only moderate differentiation between the populations from Guatemala, Honduras and Mexico. If Pedasi is excluded, its non-native status and low level of diversity accounting for its great dissimilarity to the other populations, Belen Rivas appears to be the most distinct population. The population from which it is most differentiated is that at San Mateo, and these two populations also have the maximum geographic separation (around 600 km). Studies of plant morphology in field trials (SIMONS and DUNSDON, 1992) and cpDNA analysis (LAVIN et al., 1991), however, do not show such a marked distinction of the Belen Rivas population. In the field trials, the population from Belen Rivas was the only one to show a deep red coloration of the bark, but it was not distinctive for any other morphological traits. The cpDNA analysis indicated that Belen Rivas shared a Central American ancestral cytotype with Monterrico. The other populations were classed as having either a Mexican or mixed Central American/Mexican cytotypes. This difference between the results of the isozyme and the cpDNA approaches may have arisen because these 2 types of marker represent different portions of the genome.

The differentiation between populations exhibited by NEI's genetic distances can be further examined utilising WRIGHT's F-statistics. The mean value of F_{ST} over loci, 0.172, reflects a moderate amount of between-population differentiation and is higher than the average value found in other tropical tree species (F_{ST}=0.119 – HAMRICK et al., 1992; G_{ST}=0.109 – LOVELESS, 1992). The differentiation can be seen clearly in the case of Belen Rivas, which exhibits a rare allele at the Lap-1 locus. The naturalised status of the population at Pedasi significantly contributes to the differentiation observed between the populations of *G. sepium*.

The differentiation between populations of *G. sepium* with respect to isozymes confirms the marked variation in morphology observed among populations in field trials, where up to 5-fold differences in growth performance were found (SIMONS and DUNSDON, 1992). Similarly, in a study using RAPDs to detect genetic variation, it was concluded that 60% of the variation present in *G. sepium* occurred between populations, as measured by SHANNON's diversity index (CHALMERS et al., 1992) – a proportion considerably higher than that indicated by the F-statistics obtained in the present investigation. CHALMERS et al., however, sampled only 5 individuals per population, and an investigation of the effects of sample size indicated that this is severely inadequate, and that at least 10 individuals per family and 10 or more families per population are needed (CHAMBERLAIN, 1993). Another study of RAPD variation among *G. sepium* populations, using NEI's genetic diversity indices (SIMONS and DUNSDON, 1992), also concluded that 60% may be an overestimate of the proportion of variation between populations, and confirmed that at least 10 samples per family are required to describe accurately the variation within a given population. Furthermore, CHALMERS et al. employed RAPD phenotype frequencies to estimate and partition SHANNON's diversity index. In this study, genotype frequencies were also used to calculate SHANNON's diversity index. Partitioning of this data estimates that 35% of the total isozyme variation lies between populations, twice the estimate obtained by F-statistics, but still less than that estimated by RAPD markers. This suggests that SHANNON's diversity index will bias between-population estimates upwards in comparison to F-statistics, leading to an overestimation of the level of population differentiation.

Conclusions

This study of isozyme variation within 8 populations of *G. sepium,* combined with information from the collection sites, has led to the identification of populations that are probably native and that maintain high levels of genetic diversity, namely those located at Monterrico, Ocosito, San Mateo, Retalhuleu and Belen Rivas. It is notable that three of these populations have been highly recommended for planting in exotic locations (SIMONS and DUNSDON, 1992). The population at Monterrico is a superior leaf producer, making it particularly suitable for hedgerow intercropping and the provision of animal fodder. The population at Belen Rivas is a superior wood producer, and that at Retalhuleu has the best combined leaf and wood production at all sites at which it was tested. Farmers and research institutes utilising these populations can be sure their plantings have a broad genetic base. Conversely, populations probably resulting from domestication or introductions on a narrow genetic base have also been identified, namely those at Pedasi, Vado Hondo and Masaguara. However, with the exception of the population at Pedasi, their levels of genetic diversity are still higher than those found in a number of other tropical trees (HAMRICK et al., 1992; MORAN et al., 1989) suggesting that they too are suitable for introduction to exotic locations, and that only introductions based on very few trees will suffer due to ultimate fixation of alleles.

Most of the genetic variation found in *G. sepium* occurs within populations, and in each population most variation occurs within families. This is as predicted for a predominantly outcrossing, entomophilous plant species (LOVELESS and HAMRICK, 1984) and is consistent with results obtained in other tropical tree species (HAMRICK et al., 1992; LOVELESS, 1992). Differentiation between populations of *G. sepium* is, however, moderately high in comparison with other similar species. This may be an artefact of sampling, or it may be a result of the adaptation of *G. sepium* to a wide range of environments, ecologically and geographically, both naturally and following domestication. This distribution of genetic diversity has a number of implications for population sampling strategy. In order to obtain a representative sample of the genetic diversity in a given population, e.g. to provide families for a seed orchard, families for progeny trials, or for molecular work, it is critically important to collect widely, and at an even spacing throughout the population. The trees making up the population collections used in this study were collected at 50 m spacings. The maximum distance a seed has been observed to travel from the maternal tree is 37 m (SIMONS and DUNSDON, 1992). Consequently, at 50 m intervals or more there is a low risk of sampling families related on the maternal side. The collecting of populations of *G. sepium* throughout its native range is still important, however, since a significant degree of differentiation between populations, at both the molecular and the morphological level, has been identified.

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