

Analysis of the genetic similarities among seven species of *Prosopis* (Leguminosae: Mimosoideae)

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Summary. *Prosopis* (mesquite) is a commercially promising plant genus that has received increased attention lately. Allelic frequencies at 25 enzyme loci in seven species from the section Algarobia were transformed in matrices of genetic distance using three methods. The indices give highly correlated results; only in the cluster *Prosopis alba*–*P. hassleri* were minor discrepancies evident. The phenetic relationships observed agree with other biochemical evidence (chromatography of phenol compounds, electrophoresis of seed proteins, etc) but not with morphological groupings. The present data support the hypothesis that the species belonging to the section Algarobia would be equivalent to sub- or semi-species; the community of such sympatric subspecies constitutes a syngameon.

Key words: *Prosopis* – Isoenzymes – Genetic distance – Genetic variability – Syngameon

Introduction

Commercially interesting plants can be bred to produce higher yields by exploiting the genetic variation present in natural populations. Mesquite (genus *Prosopis*) includes species that possess economic promise as they can produce legume crops in arid environments where other plants fail (Felker and Bandurski 1977, 1979; Leakey and Last 1980; Habit et al. 1981). A basic elucidation of genetic diversity is instrumental in designing experiments that aim at utilizing the genetic resources present in these plants.

About 45 *Prosopis* species have been described using morphological criteria; they have been divided into five sections and eight series (Burkart 1976; Schinini 1981).

The distribution area is vast, covering the Americas, Africa and Western Asia. Several species are protogynous (Burkart 1937, 1940, 1952, 1976), strict outcrossers and self-incompatible (Simpson 1977; Simpson and Solbrig 1977).

The taxonomy of *Prosopis* remains, however, a problem and various authors (Burkart 1940, 1952, 1976; Johnston 1962; Schinini 1981) have suggested different subdivisions of the complex. Probably the major difficulty in morphological classification stems from some diploid species of the section Algarobia being sympatric in the Chaco phytogeographic region (NE Argentina and Paraguay) and forming through hybridization and introgression new phenotypes that defy attempts at morphological analysis. Chromosome studies (Hunziker et al. 1975, 1977) fail to show differences between species. Biochemical analysis, based on a variety of techniques (seed proteins: Burghardt and Palacios 1981; Burghardt 1982; chromatography of phenols: Carman 1973; Bragg et al. 1978; Palacios and Bravo 1981; Naranjo et al. 1984; chromatography of fatty acids: Madriñan- Polo et al. 1976; studies of free aminoacids: Carman et al. 1974; immunological properties: Cohen et al. 1967) showed that the similarity within a section was higher than expected for well-established species; no species diagnostic marker compounds were found.

Some diploid species of the genus have been studied with enzyme electrophoresis (Solbrig and Bawa 1975; Whitmore and Bragg 1979; Saidman and Naranjo 1982; Saidman 1985, 1986; Hunziker et al. 1986). The main result has again been the lack of diagnostic loci as species criteria.

In the following we describe the phenetic relationships between species of the section Algarobia, using mainly the genetic distance measure of Nei (1972, 1975;

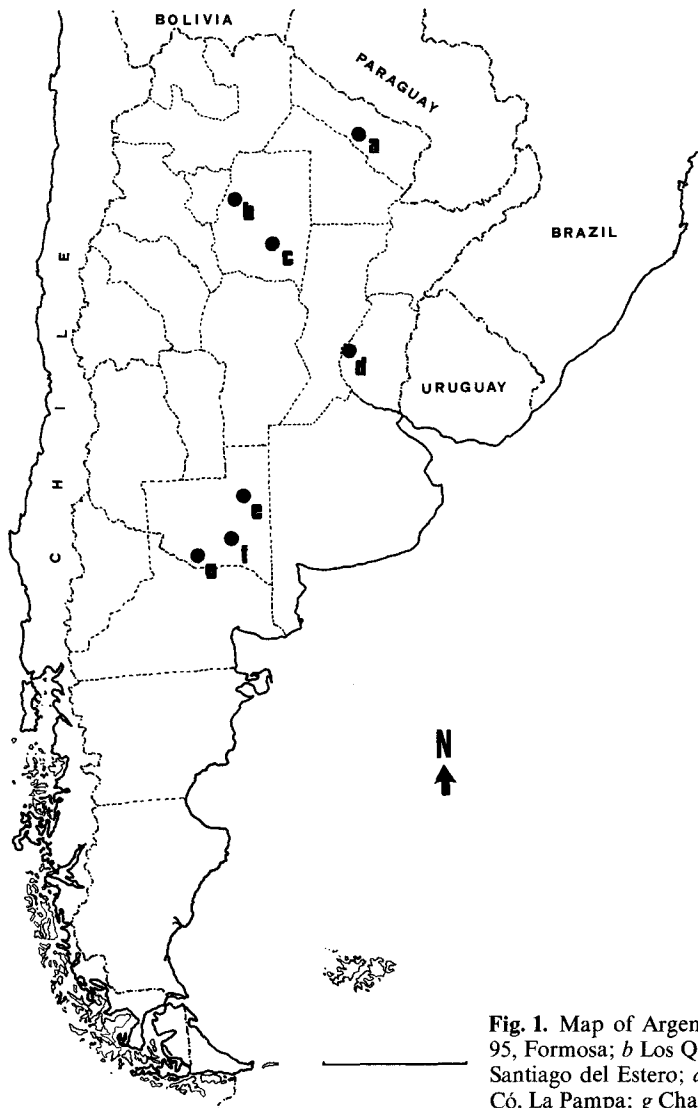


Fig. 1. Map of Argentina indicating the populations sampled; *a* National Road 95, Formosa; *b* Los Quiroga Dam, Santiago del Estero; *c* Avellaneda Department, Santiago del Estero; *d* San Martín, Entre Ríos; *e* Santa Rosa, La Pampa; *f* Cura-Có, La Pampa; *g* Chacharramendi, La Pampa. Bar = 500 km

Nei and Chakraborty 1973) and compare the results with those using the measures of Rogers (1975) and Thorpe (1979). The study is based on the data on 25 enzyme loci coding for 7 enzymes (Saidman 1985, 1986; Saidman and Naranjo 1982). An attempt is made to correlate the results with views on speciation of these species.

Materials and methods

The populations and species studied are listed in Table 1 and the collection sites are indicated in Fig. 1. Collection methods, herbarium numbers, germination and electrophoretic techniques are described elsewhere (Saidman and Naranjo 1982; Saidman 1985, 1986; Hunziker et al. 1986).

Genetic similarity and distance following Nei (1972), Rogers (1972) and Thorpe (1979) and cluster analyses were calculated according to the program of Vilardi (1987).

Table 1. Populations and species studied. Number of individuals given in brackets

Series	Species	Origin and no. of individuals
Chilenses	<i>P. alba</i>	National Road 95, Formosa (82)
	<i>P. nigra</i>	Los Quiroga Dam, S. del Estero (84)
		San Martín, Entre Ríos (79)
		Chacharramendi, La Pampa (94)
	<i>P. alpataco</i>	Chacharramendi, La Pampa (94)
	<i>P. caldenia</i>	Santa Rosa, La Pampa (86)
<i>P. flexuosa</i>	Cura-Có, La Pampa (96)	
Ruscifoliae	<i>P. ruscifolia</i>	National Road 95, Formosa (56) Avellaneda, S. del Estero (125)
	<i>P. hassleri</i>	National Road 95, Formosa (77)

Table 2. Gene frequencies at the 25 loci studied in the nine populations analysed

	<i>P. ruscifolia</i> Formosa	<i>P. ruscifolia</i> Avellaneda	<i>P. nigra</i> S. Est.	<i>P. nigra</i> E. Rios	<i>P. alba</i> Form.	<i>P. hassleri</i> Formosa	<i>P. alpataco</i> Cha'mendi	<i>P. flexuosa</i> Cura-Có	<i>P. caldenia</i> S. Rosa
Est-2 ¹	0.00	0.09	0.07	0.14	0.38	0.10	0.41	0.17	0.25
Est-2 ²	0.78	0.56	0.52	0.62	0.39	0.58	0.42	0.51	0.46
Est-2 ³	0.22	0.35	0.41	0.24	0.23	0.32	0.17	0.32	0.29
Est-3 ⁰	0.77	0.73	0.61	0.36	0.69	0.73	0.96	0.78	0.73
Est-3 ¹	0.23	0.27	0.39	0.64	0.31	0.27	0.04	0.22	0.27
Est-4 ⁰	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.41
Est-4 ¹	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.59
Est-5 ⁰	0.55	0.55	0.72	0.75	0.91	0.86	0.87	0.75	0.77
Est-5 ¹	0.45	0.45	0.28	0.25	0.09	0.14	0.13	0.25	0.23
Got-1 ¹	0.08	0.19	0.00	0.00	0.02	0.06	0.00	0.00	0.04
Got-1 ²	0.92	0.81	0.42	0.70	0.98	0.94	0.72	0.44	0.96
Got-1 ³	0.00	0.00	0.58	0.30	0.00	0.00	0.28	0.56	0.00
Got-2 ¹	0.79	0.07	0.24	0.61	0.22	0.23	0.11	0.29	0.00
Got-2 ²	0.21	0.77	0.76	0.39	0.78	0.77	0.89	0.71	0.97
Got-2 ³	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.03
Got-3 ¹	1.00	0.91	0.75	0.34	0.19	0.19	0.43	0.39	1.00
Got-3 ²	0.00	0.09	0.25	0.66	0.81	0.81	0.57	0.61	0.00
Prx-1 ⁰	0.90	0.98	0.92	0.83	0.58	0.69	0.34	0.73	0.61
Prx-1 ¹	0.10	0.02	0.08	0.17	0.42	0.31	0.66	0.27	0.39
Prx-2 ⁰	0.35	0.00	0.32	0.68	0.51	0.42	0.75	0.75	0.59
Prx-2 ¹	0.65	1.00	0.68	0.32	0.49	0.58	0.25	0.25	0.41
Prx-3 ⁰	0.00	0.00	0.65	0.50	0.00	0.00	0.00	0.53	0.56
Prx-3 ¹	1.00	1.00	0.35	0.50	1.00	1.00	1.00	0.47	0.44
Amp-1 ¹	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Amp-2 ¹	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00
Amp-3 ¹	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Amp-4 ¹	0.12	0.09	0.25	0.32	0.30	0.21	0.27	0.25	0.23
Amp-4 ²	0.70	0.71	0.48	0.35	0.64	0.68	0.51	0.57	0.51
Amp-4 ³	0.18	0.20	0.27	0.33	0.06	0.11	0.22	0.18	0.26
Adh-1 ¹	1.00	1.00	1.00	1.00	0.00	0.00	0.81	0.64	0.66
Adh-1 ²	0.00	0.00	0.00	0.00	1.00	1.00	0.12	0.25	0.28
Adh-1 ²³	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.11	0.06
Adh-2 ¹	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
6-Pgd-1 ¹	0.00	0.00	1.00	1.00	0.00	0.00	1.00	1.00	0.00
6-Pgd-2 ¹	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6-Pgd-3 ¹	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
6-Pgd-4 ¹	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Sod-1 ¹	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sod-2 ¹	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sod-3 ¹	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sod-4 ¹	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sod-5 ¹	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Results

Gene frequencies

The data on allele frequencies at 25 loci in the nine populations have been published (Saidman and Naranjo 1982; Saidman 1985, 1986). The compilation given in Table 2 shows high overall similarity. Only at a few loci

have different alleles been fixed in different species groups. Only 6 loci out of the total of 25 studied showed alleles characteristic for groups of species, namely: Amp-2¹, present in *P. alpataco*, *P. flexuosa* and *P. caldenia*; Amp-3¹, characteristic of *P. caldenia*; Adh-1¹, absent in *P. alba* and *P. hassleri* and monomorphic in *P. nigra* and *P. ruscifolia*; Adh-1², monomorphic in *P. alba*

Table 3. Mean heterozygosity (\bar{H}) and percentage of polymorphic loci (P) observed in the populations studied

Species and populations	H \pm SD	P (%)	No. of loci
<i>P. ruscifolia</i> Formosa	0.13 \pm 0.19	38	21
<i>P. ruscifolia</i> Avellaneda	0.13 \pm 0.20	38	21
<i>P. nigra</i> Sgo. del Estero	0.21 \pm 0.24	48	21
<i>P. nigra</i> Entre Ríos	0.22 \pm 0.25	48	21
<i>P. alba</i> Formosa	0.17 \pm 0.23	45	20
<i>P. hassleri</i> Formosa	0.17 \pm 0.21	45	20
<i>P. alpataco</i> Chacharramendi	0.17 \pm 0.23	45	22
<i>P. flexuosa</i> Cura-Có	0.23 \pm 0.24	50	22
<i>P. caldenia</i> Santa Rosa	0.20 \pm 0.25	48	23

and *P. hassleri*; 6Pgd-1¹, only present in *P. nigra*, *P. alpataco* and *P. flexuosa*; 6Pgd-2¹, characteristic of *P. ruscifolia*; 6Pgd-4¹, characteristic of *P. caldenia*.

Genetic variability

As noted above, *Prosopis* species seem to be obligate outbreeders. The percentage of polymorphic loci (P) ranged from 38% in *P. ruscifolia* (Formosa and Avellaneda) to 50% in *P. flexuosa* (Cura-Có) (Table 3), with an average of 45%. The lowest value of heterozygosity per locus per individual (\bar{H}) was also that of *P. ruscifolia* (0.13) and the highest was again that of *P. flexuosa* (0.23) (Table 3), the average for all populations being 0.18.

Genetic similarities and distances

Genetic distances and identities of all pairs of populations were calculated according to Nei (1972) (Table 4). Cluster analyses were performed from this matrix using three different methods (simple linkage, complete linkage and WPGMA). All of them showed very good concordance with the original matrix, the cophenetic correlation coefficients being in all cases highly significant ($P < 0.01$). However, the phenograms obtained by WPGMA (Fig. 2) and complete linkage were congruent to each other, but showed slight differences to that from simple linkage. The discrepancies are due to the fact that in the latter the cluster formed by the populations of *P. ruscifolia* (Formosa and Avellaneda) associates with *P. nigra*–*P. alpataco*–*P. flexuosa* before the cluster *P. alba*–*P. hassleri*, while the opposite occurs with the other two methods.

In order to compare these results with those obtained with different methods, genetic distance matrices were also calculated according to Rogers (1972) and Thorpe (1979). The matrix with the widest range of distances was the Thorpe's, varying from 0.034 for the pair *P. alba*–*P. hassleri* to 0.310 for *P. ruscifolia* (Formosa)–*P. caldenia*. Rogers' matrix showed the narrowest range, with extreme values 0.032 and 0.257 for the same pairs of populations, while Nei's was intermediate. The correlations between Nei's identity and Roger's or Thorpe's similarities were studied by regression analysis. In both cases the linear regression was highly significant with very similar determination coefficients (R^2), fitting the following expressions:

$$\text{Rogers: } S = 0.0562 + 0.8969 I \quad (R^2 = 0.968)$$

$$\text{Thorpe: } S = -0.1566 + 1.1180 I \quad (R^2 = 0.986),$$

where S is Roger's or Thorpe's similarity as a function of Nei's identity (I).

The phenograms obtained from Rogers' matrix gave the same results as Nei's when using simple linkage and

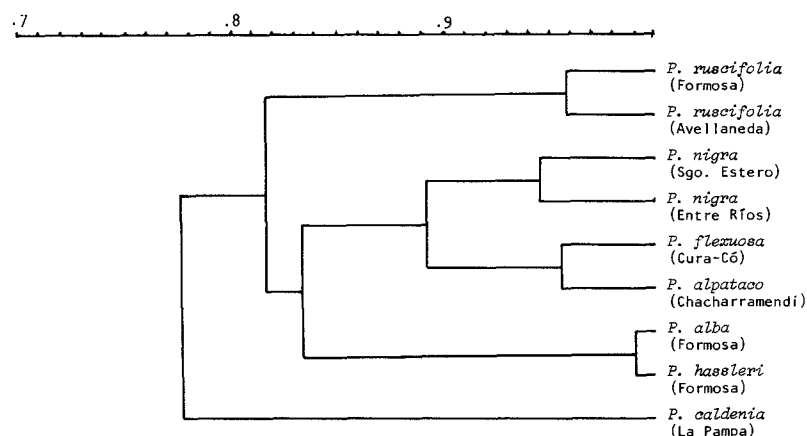
**Fig. 2.** Phenogram obtained from Nei's identities using the WPGMA clustering method

Table 4. Matrix of genetic distances obtained employing Nei's (1972) formula

	<i>P. ruscifolia</i> Formosa	<i>P. ruscifolia</i> Avellaneda	<i>P. nigra</i> S. Est.	<i>P. nigra</i> E. Ríos	<i>P. alba</i> Form.	<i>P. hassleri</i> Formosa	<i>P. alpataco</i> Cha'mendi	<i>P. flexuosa</i> Cura-Có	<i>P. caldenia</i> S. Rosa
<i>P. ruscifolia</i> Formosa	–								
<i>P. ruscifolia</i> Avellaneda	0.042	–							
<i>P. nigra</i> S. del Estero	0.183	0.161	–						
<i>P. nigra</i> Entre Ríos	0.186	0.222	0.056	–					
<i>P. alba</i> Formosa	0.206	0.188	0.206	0.171	–				
<i>P. hassleri</i> Formosa	0.182	0.163	0.188	0.167	0.010	–			
<i>P. alpataco</i> Chacharramendi	0.263	0.251	0.150	0.134	0.165	0.183	–		
<i>P. flexuosa</i> Cura-Có	0.252	0.251	0.083	0.087	0.184	0.181	0.045	–	
<i>P. caldenia</i> Santa Rosa	0.279	0.256	0.240	0.275	0.251	0.253	0.202	0.192	–

WPGMA but they are like Thorpe's for complete linkage. The three phenograms obtained from Thorpe's matrix were congruent, but differed from those of Nei in that *P. ruscifolia* populations associate with *P. alba*–*P. hassleri* before *P. nigra*–*P. flexuosa*–*P. alpataco*.

In summary, three facts were relevant: (1) All Thorpe's phenograms were congruent in contrast to discrepancies among those of Nei and Rogers, as shown by the clustering method; (2) Rogers' and Nei's phenograms were congruent using WPGMA and simple linkage methods, but differed from Thorpe's; (3) the differences among the phenograms largely reside in the relationships of the cluster *P. alba*–*P. hassleri*, the other species being the same.

Discussion

Diagnostic loci

As previously described, only 6 out of the 25 loci analysed showed alleles characterizing different groups of species. *P. caldenia* is the most identifiable species on the basis of Amp-3 and 6Pgd-4 patterns, and *P. ruscifolia* can be identified by the band corresponding to locus 6Pgd-2. The rest of the species cannot be definitely identified because their zymograms are very similar, but groups of species (e.g. *P. alpataco*–*P. flexuosa* or *P. alba*–*P. hassleri*) may be formed according to Adh-1 and 6Pgd-1 patterns. *P. nigra* is similar to *P. alpataco*–*P. flexuosa* in the 6Pgd-1 locus but differs from them in Amp-2.

The low number of diagnostic loci and the high similarity of the enzyme patterns of these species make species identification difficult using electrophoretic zymograms only.

Genetic variability

The mean value of the percentage of polymorphic loci (\bar{P}) for all populations studied (45%) is considerably lower than those obtained by other authors in shrubs and trees with characteristics similar to *Prosopis* (i.e. long life cycle, outcrossing and high fecundity) with \bar{P} values around 75.3% (see Hamrick et al. 1979). However, in other groups of trees where an appreciable number of loci were studied, \bar{P} values were similar or even lower than that of *Prosopis*. Thus, in *Pinus pungens* (cited by Hamrick 1979) \bar{P} (15 loci) was 40%, in *Bulnesia arborea* and *B. carrapo*, studying 21 and 22 loci respectively, Hunziker and Schaal (1983) obtained \bar{P} = 28.6% and 22.7%, and in the octoploid *B. bonariensis* the same authors studied 26 loci and obtained \bar{P} = 46.1%. The \bar{P} obtained for the species cited by Hamrick (1979) and Hamrick et al. (1979) was calculated, in many cases, using a few loci only per species. This is evident in *Eucalyptus obliqua*, where only 3 genes were analysed, and the same occurred in *Picea abies* and *Pinus sylvestris*, each showing a \bar{P} of 100%. This may cause an overestimate of the real percentage of polymorphic loci in trees. In the review by Selander (1980) on \bar{P} values in exogamous plants, \bar{P} was approximately 46%, which agrees with that found for the *Algarobia* section.

Similarly, the values of the mean frequency of heterozygotes per locus (\bar{H}) found in the species of *Prosopis* studied here (0.181) were similar to those compiled by Selander (1980) for exogamous plants, where $\bar{H}=0.17\pm 0.031$, but very different from the value obtained by Hamrick (1979) for tree species ($\bar{H}=0.354$).

Indices of genetic similarity and genetic distance

Genetic distance among all populations studied here (Table 3) were very low. The values of genetic identity and distance between species obtained employing Nei's formula ranged within the interval expected for semi- or subspecies or even conspecific populations according to the studies on *Drosophila willistoni* (Ayala et al. 1974). This is particularly evident in the cases of *P. alba*-*P. hassleri* ($D=0.010$; $I=0.990$) and *P. flexuosa*-*P. alpataco* ($D=0.045$; $I=0.956$) pairs which are more similar to each other than the two conspecific populations of *P. ruscifolia* or *P. nigra*.

Correlations between the degree of relationship and the index of genetic similarity are not so well defined for Rogers' and Thorpe's measures because they are not as widely employed as Nei's. However, as shown by the analysis of regression, all these indices are highly linearly correlated, at least within the interval of similarities here observed. The analysis of similarities among populations with several indices simultaneously using computer programs such as that proposed by Green (1979) or Vilardi (1987) would give a scale similar to that proposed for Nei's distance. This would relate the degree of relationship between two populations with the expected value for Thorpe's, Rogers' or even other indices. This information would be of importance because the relative efficiency of each index for taxonomic purposes may vary according to the range of similarities involved. This last point was analysed here by comparing the phenograms from the different similarity indices.

Thorpe's index produced congruent phenograms while differences were found using Rogers' and Nei's indices. Thorpe's similarities varied more widely than Rogers' and Nei's. Phenograms constructed with different methods differed virtually only in the relationships of the species pair *P. alba*-*P. hassleri*.

This fact deserves particular attention. The species *P. alba* and *P. hassleri* are so different morphologically as to be placed by Burkart (1976) in different series within the section Algarobia (Table 1). Moreover, they are also different ecologically since *P. alba* is widely distributed along almost all Chaco phytogeographic region (NE Argentina and Paraguay) while *P. hassleri* is restricted to Formosa Province (República Argentina). On the other hand, studies on chromatography of phenol compounds (Palacios and Bravo 1981), like the present isoenzymatic data, showed a high affinity between these

species, and field observations by Palacios (personal communication) reveal the highest degree of hybridization between them, with production of highly fertile hybrids. Thus, the available information contradicts previous concepts of the relationships between these species. In addition, the species most similar to *P. alba* after *P. hassleri* is *P. alpataco*, belonging to series Chilenses, while *P. hassleri*, as expected according to Burkart (1976), is isoenzymatically more similar to *P. ruscifolia* of the series Ruscifoliae.

There is another discrepancy between the relationships of *P. caldenia* suggested by the present data and by the morphological classification. According to Burkart (1976) this species is related to *P. nigra*, *P. alpataco*, *P. flexuosa* and *P. alba* (series Chilenses), while the electrophoretic data suggests it is isolated from the rest of the Algarobia species here studied. Our conclusions seem, however, correct on the basis of several observations.

(1) *P. caldenia* is the only species of Algarobia (studied enzymatically so far) which showed an incipient isolating mechanism. This is shown by the following. (a) Although its pollen is able to fertilize sympatric species (*P. alpataco* and *P. flexuosa*) in Chacharramendi (La Pampa Province, Argentina), it is apparently not fertilized by alien pollen, according to the GOT patterns observed in its seeds (Saidman 1985). (b) No morphohybrids with *P. caldenia* as a parent were found in this locality (Naranjo and Enus-Zeiger 1983).

(2) *P. caldenia* can grow on sandy but not on saline soils while other species of the same series (*P. alpataco* and *P. flexuosa*) grow on both (Burkart 1976).

(3) *P. caldenia* is the species most easily identifiable using isoenzymes.

Genetic distances and degree of speciation in Algarobia

The genetic distances obtained here were short. This agrees with other biochemical studies. On the other hand, there are clear morphological differences which allowed Burkart (1976) to consider the taxa analysed here as "good taxonomic species". Discrepancies between morphological and molecular data have been observed in other animal and plant groups (King and Wilson 1975; Mastenbrock et al. 1981; Doebley and Goodman 1984; Falkenhagen 1985). Several hypothesis may explain this disagreement:

The high genetic similarity may be a consequence of weak reproductive barriers. This hypothesis is supported by extensive species hybridization occurring in overlapping regions. In such areas there may be a tendency towards "homogenization of gene frequencies", though this process would not be enough to determine a loss of identity of species. These hybridization areas may be the consequence of modification in the environment as

a result of human activity or climatic and geographical phenomena characteristic of the Chaco phytogeographic region (drought, inundations, desiccation of inlets, etc.). All these processes produce open habitats (Anderson 1949) favouring hybridization and introgression. There is little evidence in species of this section for isolating mechanisms such as GOT patterns and the absence of hybrids of *P. caldenia* with sympatric species and the observation of a reduction in pollen viability in hybrids between *P. affinis* × *P. nigra* (Naranjo et al. 1984).

Another explanation may be different evolutionary rates of morphological and molecular traits. Algarobia species may be comparable to the fish genus *Cyprinodon*, for which Turner (1974) proposed that genes could evolve in two ways: (1) the group of genes determining most of the morphological characters would have evolved quickly as a response to selective and stochastic factors produced by ecological and habitat differences and (2) the second group of genes would not be responsive to the ecological differences and include most of the loci surveyed by electrophoresis.

Turner (1974) also suggested that adaptation to large and frequent fluctuations in critical environmental parameters would demand very strong coadaptation among the enzymes of important physiological pathways. Such fluctuations are common in *Prosopis* populations. A corollary of this hypothesis would be that speciation within the section Algarobia had not involved changes in the genome as a whole but in just a portion of it. This hypothesis agrees with Templeton (1979) who proposed that speciation via the founder principle would not require a "genetic revolution" (Mayr 1954, 1970) but would involve only a relatively small portion of the genome.

Neither hypothesis can be discarded as an explanation of the lack of electrophoretically detectable genetic differentiation among these species, though the relative importance of each process and the actual level of the reproductive barrier remains unsolved. Also, the isoenzymes analysed represent a very small part of the genome and thus the possible and perhaps more important changes in regulatory mechanisms are not taken into consideration (Soulé 1980).

Finally, the data presented here support the hypothesis of Palacios and Bravo (1981) that the species would be biologically equivalent to semi- or subspecies and that the community of these sympatric semispecies would constitute a syngameon (Grant 1957, 1977, 1981). Though the reproductive interactions within this syngameon mean that it behaves as a biological species, the complex of Algarobia semispecies appears to be stable despite the sympatry. This would not be expected in subspecies of an ordinary species.

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References

- Anderson E (1949) Introgressive hybridization. John Wiley, New York Chapman and Hall, London
- Ayala FJ, Tracey ML, Barr LG, McDonald JF, Pérez-Sales S (1974) Genetic variation in natural populations of five *Drosophila* species and the hypothesis of the selective neutrality of protein polymorphisms. *Genetics* 77:343–384
- Bragg LH, Bacon JD, McMillan C, Mabry TJ (1978) Flavonoid patterns in the *Prosopis juliflora* complex. *Biochem Syst Ecol* 6:113–116
- Burghardt AD (1982) Estudios electroforéticos en el género *Prosopis* (Leguminosae). 13th Congr Argentino Genet, p 74
- Burghardt AD, Palacios RA (1981) Caracterización electroforética de algunas especies de *Prosopis* (Leguminosae). 12th Congr Argentino Genet, p 11
- Burkart A (1937) Estudios morfológicos y etológicos en el género *Prosopis*. *Darwiniana* 3:27–47
- Burkart A (1940) Una monografía del género *Prosopis* (Leguminosae). *Darwiniana* 4:57–128
- Burkart A (1952) Las leguminosas argentinas, silvestres y cultivadas, 2nd edn. Acme Agency, Buenos Aires, pp 126–143
- Burkart A (1976) A monograph of the genus *Prosopis* (Leguminosae sufam. Mimosoideae). *J Arnold Arbor Harv Univ* 57:219–249 and 450–525
- Carman NJ (1973) Systematic and ecological investigations in the genus *Prosopis* (Mimosaceae), emphasizing the natural products chemistry. Dissertation, University of Texas, Austin
- Carman NJ, Dossaji SF, Mabry TJ (1974) A populational survey of aminoacids in *Prosopis* species from North and South America. *Biochem Syst Ecol* 2:73–74
- Cohen R, Cei JM, Roig VG (1967) Ensayos preliminares con técnicas de precipitinas por difusión en gel de agar sobre afinidades proteínicas en el género *Prosopis*. *Rev Fac Cienc Agrar Univ Nac Cuyo* 13:29–41
- Doebley JF, Goodman MM (1984) Isoenzymatic variation in *Zea* (Gramineae). *Syst Bot* 9:203–218
- Falkenhagen ER (1985) Isozyme studies in provenance research of forest trees. *Theor Appl Genet* 69:336–347
- Felker P, Bandurski R (1977) Protein and aminoacid composition of the legume seeds. *J Sci Food Agric* 28:791–797
- Felker P, Bandurski R (1979) Uses and potential uses of leguminous trees for minimal energy input agriculture. *Econ Bot* 33:172–184

- Grant V (1957) The plant species in theory and practice. In: Mayr E (ed) The species problem. Am Assoc, pp 39–80
- Grant V (1977) Organismic evolution. Freeman, San Francisco
- Grant V (1981) Plant speciation. Columbia Univ Press, New York
- Green DM (1979) A BASIC computer program for calculating indices of genetic distance and similarity. *J Hered* 70:429–430
- Habit MA, Contreras DT, González RH (1981) *Prosopis tamarugo*: Arbusto forrajero para zonas áridas. *Estud FAO Prod Protec Veg*, vol 25. pp 1–133 Org Nac Unidas para la Agric y la Alimentación, Roma
- Hamrick JL (1979) Genetic variation and longevity. In: Solbrig OT, Jain S, Johnson GH, Raven PH (eds) Topics in plant population biology. Columbia University Press, New York, pp 85–113
- Hamrick JL, Linhart YB, Mitton JB (1979) Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Ann Rev Ecol Syst* 10:173–200
- Hunziker JH, Poggio L, Naranjo CA, Palacios RA, Andrada AB (1975) Cytogenetics of some species and natural hybrids in *Prosopis* (Leguminosae). *Can J Genet Cytol* 17:253–262
- Hunziker JH, Naranjo CA, Palacios RA, Poggio L (1977) Chromosomal cytology and hybridization. In: Simpson BB (ed) Mesquite. Its biology in two desert ecosystems. UB/IBP Ser 4. Dowden Hutchinson and Ross, pp 56–59
- Hunziker JH, Saidman BO, Naranjo CA, Palacios RA, Poggio L, Burghardt AD (1986) Hybridization and genetic variation of Argentine species of *Prosopis*. *For Ecol Manage* 16:301–315
- Hunziker JH, Schaal BA (1983) Isozyme variation in diploid tropical and octoploid subtropical temperate species of *Bulnesia*. *J Hered* 74:358–360
- Johnston MC (1962) The North-American mesquite *Prosopis*, sect. *Algarobia* (Leguminosae). *Brittonia* 14:72–90
- King MC, Wilson AC (1975) Evolution at two levels. Molecular similarities and biological differences between humans and chimpanzees. *Science* 188:107–116
- Leakey RB, Last FT (1980) Biology and potential of *Prosopis* species in arid environments, with particular reference to *P. cineraria*. *J Arid Environ* 3:9–24
- Madriñan-Polo C, Hunziker JH, Cattaneo P (1976) Aceites de semillas de especies de *Prosopis* y *Prosopidastrum* (Leguminosae). *An Asoc Quim Argent* 64:127–138
- Mastenbrock I, Cohen CE, de West M (1981) Seed protein and seedling isozyme patterns of *Zea mays* and its closed relatives. *Biochem Syst Ecol* 9:179–183
- Mayr E (1954) Change of genetic environment and evolution. In: Huxley J, Hardy AC, Ford EB (eds) Evolution as a process. Allen and Unwin, London, pp 157–180
- Mayr E (1970) Population, species and evolution. Belknap Press Harvard University, Cambridge Mass
- Naranjo CA, Enus-Zeiger S (1983) Cromatografía de fenoles y morfología en especies e híbridos de *Prosopis* de La Pampa. *Actas 29th Jornadas Argent Bot*, p 32
- Naranjo CA, Poggio L, Enus-Zeiger S (1984) Phenol chromatography, morphology and cytogenetics in three species and natural hybrids of *Prosopis* (Leguminosae, Mimosoideae). *Plant Syst Evol* 144:257–276
- Nei M (1972) Genetic distance between populations. *Am Nat* 106:283–292
- Nei M, Chakraborty R (1973) Genetic distance and electrophoretic identity of proteins between taxa. *J Mol Evol* 2:323–328
- Nei M (1975) Molecular population genetics and evolution. North Holland, Amsterdam
- Palacios RA, Bravo LD (1981) Hibridación natural en *Prosopis* (Leguminosae) en la Región Chaqueña Argentina, Evidencias morfológicas y cromatográficas. *Darwiniana* 23:3–35
- Rogers JS (1972) Measures of genetic similarity and genetic distance. *Univ Texas Publ* 7213, pp 145–153
- Saidman BO (1985) Estudio de la variación alozímica en el género *Prosopis*. PhD Thesis, Fac Cs Exactas y N University, Buenos Aires
- Saidman BO (1986) Isoenzymatic studies of alcohol dehydrogenase and glutamate oxalacetate transaminase in four South American species of *Prosopis* and their natural hybrids. *Silvae Genet* 35:3–10
- Saidman BO, Naranjo CA (1982) Variaciones de esterasas en poblaciones de *Prosopis ruscifolia* (Leguminosae). *Mendeliana* 5:61–70
- Schinini A (1981) Contribución a la flora del Paraguay. *Bonplandia* 5:101–108
- Selander RK (1980) Variación genética en las poblaciones naturales. In: Ayala FJ (ed) Evolución molecular. Ed Omega, Barcelona, pp 21–46
- Simpson BB (1977) Breeding systems of dominant perennial plants of two disjunct warm desert ecosystems. *Oecologia (Berlin)* 27:203–226
- Simpson BB, Solbrig OT (1977) Introduction. In: Simpson BB (ed) Mesquite. Its biology in two desert ecosystems. US/IBP Synthesis, ser 4. Dowden Hutchinson and Ross, Pennsylvania, pp 1–15
- Solbrig OT, Bawa KS (1975) Isozyme variation in species of *Prosopis* (Leguminosae). *J Arnold Arbor Harv Univ* 56:398–412
- Soulé M (1980) Variación aloenzimática, sus determinantes en el espacio y en el tiempo. In: Ayala FJ (ed) Evolución molecular. Ed Omega, pp 61–79
- Templeton AR (1979) The theory of speciation via the founder principle. *Genetics* 94:1011–1034
- Thorpe JP (1979) Enzyme variation and taxonomy. The estimation of sampling errors in measurements of interspecific genetic similarity. *J Linn Soc London Biol* 11:369–386
- Turner BJ (1974) Genetic divergence of Death Valley pupfish species: biochemical vs. morphological evidence. *Evolution* 28:281–294
- Vilardi JC (1987) Un programa BASIC para calcular índices de similitud y distancia genéticas y valorar los resultados mediante análisis de agrupamiento. *Mendeliana* (in press)
- Whitmore DH, Bragg LH (1979) Isozymal differentiation between two species of *Prosopis*. *Biochem Syst Ecol* 7:299–302