Genetics and Molecular Biology, 28, 2, 277-286 (2005)

www.sbg.org.br

Research Article

Genetic relationships among American species of *Prosopis* (Leguminosae) based on enzyme markers

Cecilia Bessega[1,2], Beatriz Ofelia Saidman[3] and Juan César Vilardi[3]

Facultad de Ciencias Exactas y Naturales, Departamento de Ecología, Genética y Evolución, Buenos Aires, Argentina.

Abstract

In the present work, isoenzyme electrophoresis was used to analyze the variability and phenetic relationships among seven American species of genus *Prosopis* belonging to three different sections: *P. argentina* (Monilicarpa), *P. glandulosa, P. velutina*, *P. flexuosa*, *P. ruscifolia, P. kuntzei* (Algarobia), and *P. reptans* (Strombocarpa). The genetic variability in *P. argentina*, *P. reptans*, and *P. kuntzei* was significantly lower than in the rest of the species analyzed. The species belonging to different sections are highly differentiated, but the relationships retrieved among species belonging to the section Algarobia suggested that the series of this section are not natural groups. *P. kuntzei* is as differentiated from the remaining species of Algarobia as from *P. reptans* or *P. argentina*, suggesting that this species might be included in a different section. The series within section Algarobia are not supported by the clusters retrieved in the phenogram based on isoenzymatic data. The results suggest that the two North American species (*P. velutina* and *P. glandulosa*) would have originated in different founder events.

Key words: Prosopis, isoenzymes, genetic variability, phenetic relationships.

Received: April 26, 2004; Accepted: November 11, 2004.

Introduction

Prosopis is a primitive legume genus that includes shrubs and trees that exhibit a high economic and ecological potential in semiarid areas. They are a component of the climax community, but they also have the ability to colonize new habitats as pioneer species. Many of its species are used in numerous countries to recover arid and semiarid regions and are considered multipurpose trees because all of their biomass can be used. Pods have high carbohydrate and protein content and are used as forage and human food. These trees have wood of high quality and adapt well to silvopastoril and agroforestal production (Roig, 1993). *Prosopis* involves about 44 species grouped in five sections: Prosopis and Anonychium, distributed in Africa and Asia; Strombocarpa and Algarobia, distributed in North and South America; and the monotypic section Monilicarpa, restricted to Argentina.

The section Strombocarpa includes nine species clearly differentiated morphologically from each other, and interspecific hybridization is infrequent (Burkart, 1976). Isoenzymatic studies in five of them (*P. torquata*, *P. pubescens*, *P. strombulifera*, and *P. reptans*) showed low intraspecific variability and yielded many species-specific diagnostic loci (Saidman *et al*., 1996).

Based on morphological grounds the section Algarobia has been divided into six series (Burkart, 1976), but the relationships among species of this section are under debate. Studies based on species of the series Pallidae, Ruscifoliae, and Chilenses indicated that natural hybrids frequently occur in zones of sympatry even between species belonging to different series (Hunziker *et al*., 1986). However, no interserial hybrids have been recorded involving species of the series Sericanthae. The information about hybridization involving species of the two remaining series, Denudans and Humiles, is scarce.

The species of Ruscifoliae and Chilenses studied so far exhibit high levels of variability within populations, but low biochemical and genetical differentiation among species (Saidman and Vilardi, 1987, 1993; Saidman *et al*., 1997, 1998a,b). The relationships observed among species in isoenzymatic (Saidman and Vilardi, 1993; Bessega *et al*., 2000a,b) and molecular (Ramírez *et al*., 1999; Saidman *et al*., 1998a,b; Saidman *et al*., 2000) analyses are not consistent with the series.

Previous works (Hunziker *et al*., 1986; Saidman *et al*., 1996) indicated that the sections Algarobia and

Send correspondence to Cecilia Bessega. Facultad de Ciencias Exactas y Naturales, Departamento de Ecología, Genética y Evolución, Buenos Aires, Argentina. E-mail: cecib@bg.fcen.uba.ar.
[1]Present Address: Instituto de Biotecnología CICVyA - CNIA - INTA - CC 25 - 1712 – Castelar, Argentina.
[2]Fellow of Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET).
[3]Member of CONICET.

Strombocarpa are highly differentiated. The speciation process in these groups as well as their adaptive strategies seem to be quite different.

The Section Monilicarpa is represented by only one isolated species, *P. argentina* Burkart endemic to Argentina. According to Burkart (1937, 1976) its origin and affinities of either Algarobia or Strombocarpa are uncertain. Its preference for sand dunes is in marked contrast to that of the common species of section Algarobia, which prefer heavier soils, sometimes salty clay on lower plateaus and riverbanks (Burkart, 1976). There are only a few biochemical (Burghardt and Palacios, 1997) and molecular (Ramirez *et al*., 1999) studies on *P. argentina* and the population genetic variability has yet to be studied.

In the present work we analyzed, by means of isoenzyme electrophoresis, the variability and phenetic relationships among *P. argentina* and six species belonging to the remaining American sections of *Prosopis*: *P. glandulosa* (Torr) and *P. velutina* Wooton from USA, *P. flexuosa* DC, *P. ruscifolia* Gris., *P. kuntzei* Harms (Algarobia), and *P. reptans* Bentham (Strombocarpa) from Argentina. Parameters of genetic variability and differentiation were estimated in populations of these species. This information is discussed with reference to the expected relationships according to the available morphological evidence and geographical distribution.

Materials and Methods

Species, populations and sampling methods

The present work involved two populations of *P. argentina* ("algarobilla"), one of *P. reptans* ("retortuño"), one of *P. kuntzei* ("itín"), four of *P. ruscifolia* ("vinal"), two of *P. flexuosa* ("algarrobo amarillo"), four of *P. glandulosa* ("honey mesquite") and one of *P. velutina* ("velvet mesquite") (Table 1, Figure 1).

The species and populations sampled cover a wide range of the distribution of the genus *Prosopis* in America (Burkart, 1976). *P. argentina* (sect. Monilicarpa) is endemic in Northwestern Argentina in the Andean provinces of Catamarca, La Rioja, San Juan and northern Mendoza

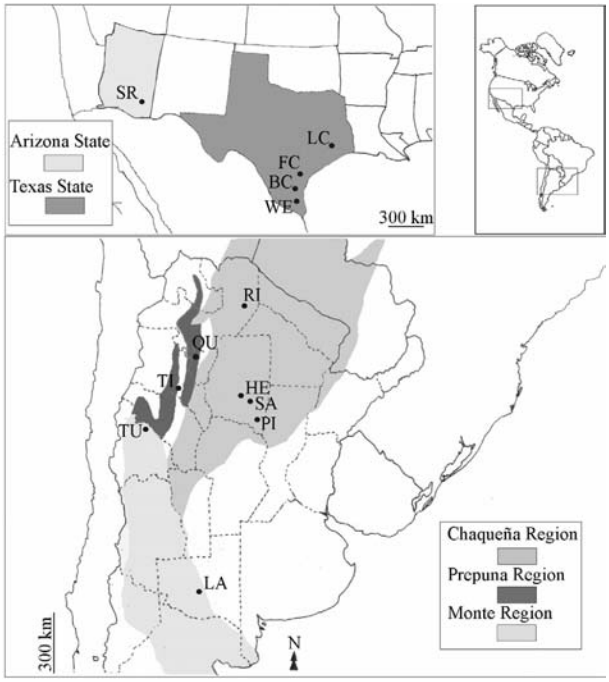

Figure 1 - Map indicating the location of the sampled populations. For references see Table 1.

Table 1 - List of the sections, series, *Prosopis* species and populations studied, collectors and codes used to identify populations in Figure 1.

Section	Series	Species	Collection localities	Collector	Code
Monilicarpa		*P. argentina*	Tinogasta, Catamarca, Argentina	P.Villlagra[1]	TI
			Tucunuco, San Juan, Argentina	P.Villlagra[1]	TU
Strombocarpa	Strombocarpae	*P. reptans*	Herrera, S. del Estero, Argentina	BOS-JCV.[2]	HE
Algarobia	Sericanthae	*P. kuntzei*	Herrera, S. del Estero, Argentina	BOS-JCV.[2]	HE
	Ruscifoliae	*P. ruscifolia*	Herrera, S. del Estero, Argentina	BOS-JCV.[2]	HE
			Rivadavia, Salta, Argentina	BOS-JCV.[2]	RI
			Pinto, S. del Estero, Argentina	BOS-JCV.[2]	PI
			Sarmiento, S. del Estero, Argentina	BOS-JCV.[2]	SA
	Chilenses	*P. flexuosa*	La Amarga, La Pampa, Argentina	BOS-JCV.[2]	LA
			Quilmes, Tucumán, Argentina	BOS-JCV.[2]	QU
		P. glandulosa	Weslaco, Texas, USA	J.Evans.[3]	WE
			La Copita, Texas, USA	J.Evans.[3]	LC
			Bell Co., Texas, USA	J.Evans.[3]	BC
			Frio Co., Texas, USA	J.Evans.[3]	FC
		P. velutina	Santa Rita, Arizona, USA	J.Evans.[3]	SR

[1]IADIZA= Instituto Argentino de Investigaciones en Zonas Áridas (Mendoza Argentina). [2]UBA = Universidad de Buenos Aires (Buenos Aires, Argentina). [3]GRS-USDA = Grassland Research Station USDA/ARS (USA).

(Argentina). *P. reptans* var. *reptans* (Strombocarpa) grows in central Argentina and Peru. From the Algarobia section, *P. glandulosa* and *P. velutina* ranges involve southwestern United States and Mexico. The other three species are restricted to South America: *P. flexuosa* can be found in Prepuna and Monte regions (northwestern and central Argentina); *P. ruscifolia* occurs in the Chaqueña region (Santiago del Estero, Chaco and Formosa provinces, Argentina); and *P. kuntzei* can be found in the Gran Chaco of northern Paraguay and eastern Bolivia to central Argentina.

Sampling methods for all Argentinean populations, except for *P. argentina* and *P. reptans* were those described in previous works (Vilardi *et al*., 1988). At least ten mother plants were sampled in each population. The samples from United States were collected by Dr. J. C. Evans (Grassland Research Station USDA/ARS). They included five or six mother plants per population. In all cases (North and South American species) the sampled trees were separated at least 50 m from each other. This distance between sampled mother plants reduces the probability that they interbreed. About 50 pods were collected from each mother plant. The seeds from each tree were stored in different bags. Similar numbers of seeds from different trees (bags) from each population were sampled for the isozyme analysis. Voucher specimens of each mother tree were taxonomically identified and deposited in the Herbarium of the Laboratorio de Genética, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina. *P. argentina* (kindly donated by Dr. P. Villagra, IADIZA, Mendoza, Argentina) and *P. reptans* population samples involved pods from about 30 mother plants collected in a single bag and only one voucher specimen representative of the whole population was prepared. The rationale for this sampling method is that populations of these species form a continuum where individual shrubs can not be identified. The number of seeds analyzed for each population are listed in Table 2.

Isoenzymatic techniques

Nine systems were studied using horizontal electrophoresis on polyacrylamide gels: esterase (EST), glutamate oxalacetate transaminase (GOT), amino peptidase (AMP), alcohol dehydrogenase (ADH), superoxide dismutase (SOD), 6-phosphogluconate dehydrogenase (6PGD),

Table 2 - Allelic frequencies and number of seeds analyzed (N) of loci showing variation either within or among populations in the studied species of *Prosopis*. One additional loci. *Amp-1,* did not show variation and is not included in the list.

	Adh-1					*Adh-2*				*Skd-1*					*Idh-1*									*Idh-2*	
	(N)	1^{30}	1^{28}	1^{24}	1^{29}	(N)	2^{20}	2^{17}	2^{14}	(N)	1^{24}	1^{22}	1^{19}	1^{17}	(N)	1^{75}	1^{70}	1^{63}	1^{60}	1^{86}	1^{84}	1^{82}	1^{80}	(N)	2^{43}
P. ru HE	68	1	0	0	0	68	0	1	0	66	0.15	0.85	0	0	63	0	0.42	0.58	0	0	0	0	0	63	0
P. ru SA	53	1	0	0	0	53	0.01	0.99	0.00	55	0.07	0.93	0	0	22	0	0.57	0.43	0	0	0	0	0	22	0
P. ru RI	62	1	0	0	0	61	0.06	0.92	0.02	59	0.08	0.92	0	0	55	0	0.39	0.61	0	0	0	0	0	55	0
P. ru PI	41	0.93	0.07	0	0	41	0	1	0	102	0.14	0.28	0.58	0	97	0	0.61	0.37	0.02	0	0	0	0	97	0
P. fl LA	100	0.64	0.25	0.11	0	100	0	1	0	48	0.33	0.60	0.07	0	40	0.06	0.10	0.81	0.03	0	0	0	0	40	1
P. fl QU	49	0.49	0.39	0.12	0	49	0	1	0	46	0	0.58	0.21	0.21	95	0.01	0.12	0.85	0.02	0	0	0	0	96	1
P. gl WE	39	0.94	0.06	0	0	39	0	1	0	43	0.09	0.65	0.26	0	56	0.05	0.92	0.03	0	0	0	0	0	56	0
P. gl LC	40	0.95	0.05	0	0	40	0	1	0	42	0.19	0.43	0.38	0	47	0.04	0.63	0.33	0	0	0	0	0	36	0
P. gl BC	40	0.95	0.05	0	0	40	0	1	0	44	0.25	0.66	0.09	0	43	0.38	0.60	0.02	0	0	0	0	0	44	0
P. gl FC	30	1	0	0	0	30	0	1	0	36	0.17	0.75	0.08	0	45	0.21	0.61	0.18	0	0	0	0	0	46	0
P. ve SR	41	0.78	0.22	0	0	42	0	1	0	32	0.53	0.47	0	0	42	0.83	0.17	0	0	0	0	0	0	42	0
P. ku HE	39	0	1	0	0	39	1	0	0	50	0	1	0	0	50	0	0	0	0	0	0	0	1	50	0
P. ar TI	64	0	0	0	1	64	1	0	0	47	0	0.29	0.52	0.19	47	0	0	1	0	0	0	0	0	47	1
P. ar TU	64	0	0	0	1	64	1	0	0	47	0	0.16	0.65	0.19	47	0	0	1	0	0	0	0	0	47	1
P. re HE	50	0	0	0	1	50	0	0	1	40	0	1	0	0	29	0	0	0	0	0.22	0.45	0.33	0	29	0

	Idh-2		*Est-1*					*Est-2*			*Est-3*			*Est-4*			*Est-6*		*Est-7*		*Est-8*		*Est-9*		*Est-10*
	2^{26}	2^{45}	(N)	1^{93}	1^{92}	1^{91}	1^{90}	(N)	2^{87}	2^{0}	(N)	3^{85}	3^{0}	(N)	4^{81}	4^{0}	(N)	6^{90}	(N)	7^{86}	(N)	8^{82}	(N)	9^{78}	(N)
P. ru HE	1	0	77	0.01	0.73	0.25	0	71	0.65	0.35	75	0.49	0.51	76	0.24	0.76	77	__	77	__	77	__	77	__	77
P. ru SA	1	0	68	0	0.89	0.11	0	69	0.61	0.39	69	0.79	0.21	73	0.30	0.70	68	__	68	__	68	__	68	__	68
P. ru RI	1	0	44	0	0.89	0.11	0	48	0.31	0.69	49	0.87	0.13	49	0.59	0.41	44	__	44	__	44	__	44	__	44
P. ru PI	1	0	69	0	0.93	0.07	0	89	0.88	0.12	89	0.70	0.30	89	0.09	0.91	72	__	72	__	72	__	72	__	72
P. fl LA	0	0	96	0.17	0.52	0.32	0	82	0.91	0.09	87	1	0	87	0.88	0.12	96	__	96	__	96	__	96	__	96
P. fl QU	0	0	84	0	0.83	0.16	0.01	49	0.78	0.22	49	0.78	0.22	50	0.50	0.50	84	__	84	__	84	__	84	__	84
P. gl WE	1	0	64	0.09	0.47	0.44	0	32	0.75	0.25	32	0.39	0.61	31	0.18	0.82	64	__	64	__	64	__	64	__	64
P. gl LC	1	0	30	0.07	0.80	0.13	0	34	0.75	0.25	34	0.16	0.84	34	0.03	0.97	56	__	56	__	56	__	56	__	56
P. gl BC	1	0	76	0	0.26	0.74	0	38	1	0	48	0.43	0.57	38	0.03	0.97	76	__	76	__	76	__	76	__	76
P. gl FC	1	0	43	0.50	0.35	0.01	0.14	52	0.67	0.33	52	0.43	0.57	52	0.13	0.87	43	__	43	__	43	__	43	__	43
P. ve SR	1	0	31	0.10	0.74	0.16	0	38	0.35	0.65	38	0.24	0.76	38	0.49	0.51	31	__	31	__	31	__	31	__	31
P. ku HE	1	0	50	0	1	0	0	47	0	1	47	0	1	47	0	1	50	__	50	__	50	__	50	__	50
P. ar TI	0	0	32	__	__	__	__	32	__	__	32	__	__	32	__	__	32	__	32	__	32	__	32	__	32
P. ar TU	0	0	32	__	__	__	__	32	__	__	32	__	__	32	__	__	32	__	32	__	32	__	32	__	32
P. re HE	0	1	50	__	__	__	__	50	__	__	50	__	__	50	__	__	50	1	50	1	50	1	50	1	50

Table 2 (cont.)

	Idh-2		*Est-1*					*Est-2*			*Est-3*			*Est-4*			*Est-6*		*Est-7*		*Est-8*		*Est-9*		*Est-10*
	2^{26}	2^{45}	(N)	1^{93}	1^{92}	1^{91}	1^{90}	(N)	2^{87}	2^{0}	(N)	3^{85}	3^{0}	(N)	4^{81}	4^{0}	(N)	6^{90}	(N)	7^{86}	(N)	8^{82}	(N)	9^{78}	(N)
P. ru HE	1	0	77	0.01	0.73	0.25	0	71	0.65	0.35	75	0.49	0.51	76	0.24	0.76	77	__	77	__	77	__	77	__	77
P. ru SA	1	0	68	0	0.89	0.11	0	69	0.61	0.39	69	0.79	0.21	73	0.30	0.70	68	__	68	__	68	__	68	__	68
P. ru RI	1	0	44	0	0.89	0.11	0	48	0.31	0.69	49	0.87	0.13	49	0.59	0.41	44	__	44	__	44	__	44	__	44
P. ru PI	1	0	69	0	0.93	0.07	0	89	0.88	0.12	89	0.70	0.30	89	0.09	0.91	72	__	72	__	72	__	72	__	72
P. fl LA	0	0	96	0.17	0.52	0.32	0	82	0.91	0.09	87	1	0	87	0.88	0.12	96	__	96	__	96	__	96	__	96
P. fl QU	0	0	84	0	0.83	0.16	0.01	49	0.78	0.22	49	0.78	0.22	50	0.50	0.50	84	__	84	__	84	__	84	__	84
P. gl WE	1	0	64	0.09	0.47	0.44	0	32	0.75	0.25	32	0.39	0.61	31	0.18	0.82	64	__	64	__	64	__	64	__	64
P. gl LC	1	0	30	0.07	0.80	0.13	0	34	0.75	0.25	34	0.16	0.84	34	0.03	0.97	56	__	56	__	56	__	56	__	56
P. gl BC	1	0	76	0	0.26	0.74	0	38	1	0	48	0.43	0.57	38	0.03	0.97	76	__	76	__	76	__	76	__	76
P. gl FC	1	0	43	0.50	0.35	0.01	0.14	52	0.67	0.33	52	0.43	0.57	52	0.13	0.87	43	__	43	__	43	__	43	__	43
P. ve SR	1	0	31	0.10	0.74	0.16	0	38	0.35	0.65	38	0.24	0.76	38	0.49	0.51	31	__	31	__	31	__	31	__	31
P. ku HE	1	0	50	0	1	0	0	47	0	1	47	0	1	47	0	1	50	__	50	__	50	__	50	__	50
P. ar TI	0	0	32	__	__	__	__	32	__	__	32	__	__	32	__	__	32	__	32	__	32	__	32	__	32
P. ar TU	0	0	32	__	__	__	__	32	__	__	32	__	__	32	__	__	32	__	32	__	32	__	32	__	32
P. re HE	0	1	50	__	__	__	__	50	__	__	50	__	__	50	__	__	50	1	50	1	50	1	50	1	50

	Est-10	*Est-11*		*Est-12*		*Est-13*		*Est-14*		*Est-15*		*Amp-1*		*Amp-2*				*Amp-3*				*Sod-1*		*Sod-2*	
	10^{57}	(N)	11^{46}	(N)	12^{40}	(N)	13^{30}	(N)	14^{20}	(N)	15^{18}	(N)	1^{100}	(N)	288	276	270	(N)	377	374	371	(N)	191	(N)	2^{83}
P. ru HE	__	77	__	77	__	77	__	77	__	77	__	41	1	41	0.48	0.40	0.12	41	__	__	__	50	1	50	1
P. ru SA	__	68	__	68	__	68	__	68	__	68	__	95	1	95	0.25	0.52	0.23	95	__	__	__	50	1	50	1
P. ru RI	__	44	__	44	__	44	__	44	__	44	__	62	1	62	0.39	0.41	0.20	62	__	__	__	50	1	50	1
P. ru PI	__	72	__	72	__	72	__	72	__	72	__	29	1	29	0.33	0.55	0.12	29	__	__	__	50	1	50	1
P. fl LA	__	96	__	96	__	96	__	96	__	96	__	105	1	105	0.25	0.57	0.18	105	__	__	__	50	1	50	1
P. fl QU	__	84	__	84	__	84	__	84	__	84	__	44	1	44	0.45	0.55	0	44	__	__	__	50	1	50	1
P. gl WE	__	64	__	64	__	64	__	64	__	64	__	30	1	30	0.42	0.47	0.11	30	__	__	__	50	1	50	1
P. gl LC	__	56	__	56	__	56	__	56	__	56	__	37	1	37	0.41	0.39	0.20	37	__	__	__	50	1	50	1
P. gl BC	__	76	__	76	__	76	__	76	__	76	__	44	1	44	0.25	0.45	0.30	44	__	__	__	50	1	50	1
P. gl FC	__	43	__	43	__	43	__	43	__	43	__	42	1	42	0.38	0.55	0.07	42	__	__	__	50	1	50	1
P. ve SR	__	31	__	31	__	31	__	31	__	31	__	34	1	34	0.79	0.21	0	34	__	__	__	40	1	40	1
P. ku HE	__	50	__	50	__	50	__	50	__	50	__	30	1	30	0	0.72	0.28	30	__	__	__	31	1	31	__
P. ar TI	__	32	1	32	1	32	1	32	1	32	1	16	1	16	1	0	0	11	0	0.27	0.73	32	1	32	1
P. ar TU	__	32	1	32	1	32	1	32	1	32	1	16	1	16	1	0	0	14	0.07	0.72	0.21	32	1	32	1
P. re HE	1	50	__	50	__	50	__	50	__	50	__	80	1	80	1	0	0	80	0.24	0	0.76	50	1	50	1

	Sod-3		*Sod-4*		*Sod-5*		*Sod-6*		*Sod-7*		*Sod-8*		*Pgd-1*					*Pgd-2*				*Prx-1*		
	(N)	3^{75}	(N)	4^{72}	(N)	5^{50}	(N)	6^{70}	(N)	7^{60}	(N)	8^{95}	(N)	1^{30}	1^{26}	1^{23}	1^{0}	(N)	2^{33}	2^{21}	2^{0}	(N)	1^{36}	1^{0}
P. ru HE	50	1	50	1	50	1	50	__	50	__	50	__	101	0.60	0.40	0	0	100	0.30	0.16	0.54	49	0.10	0.90
P. ru SA	50	1	50	1	50	1	50	__	50	__	50	__	62	0.40	0.60	0	0	62	0.21	0.23	0.56	44	0.07	0.93
P. ru RI	50	1	50	1	50	1	50	__	50	__	50	__	74	0.63	0.37	0	0	84	0.26	0.16	0.58	40	0.06	0.94
P. ru PI	50	1	50	1	50	1	50	__	50	__	50	__	78	0.14	0.86	0	0	79	0.19	0.20	0.61	40	0.42	0.58
P. fl LA	50	1	50	1	50	1	50	__	50	__	50	__	41	0.78	0.22	0	0	41	0.06	0	0.94	90	0.87	0.13
P. fl QU	50	1	50	1	50	1	50	__	50	__	50	__	102	0.01	0.99	0	0	68	0.12	0.20	0.68	41	0.32	0.68
P. gl WE	50	1	50	1	50	1	50	__	50	__	50	__	56	0	1	0	0	56	0	0	1	31	0.19	0.81
P. gl LC	50	1	50	1	50	1	50	__	50	__	50	__	36	0	1	0	0	36	0.03	0	0.97	26	0.52	0.48
P. gl BC	50	1	50	1	50	1	50	__	50	__	50	__	46	0	1	0	0	46	0.02	0	0.98	40	0.06	0.94
P. gl FC	50	1	50	1	50	1	50	__	50	__	50	__	32	0	1	0	0	32	0	0	1	38	0.07	0.93
P. ve SR	40	1	40	1	40	1	40	__	40	__	40	__	34	0	1	0	0	34	0	0	1	48	__	__
P. ku HE	31	1	31	1	31	__	31	1	31	__	31	__	26	1	0	0	0	26	0	0	1	47	0.62	0.38
P. ar TI	32	1	32	1	32	1	32	__	32	1	32	1	47	0.94	0.02	0	0.04	47	1	0	0	37	0.84	0.16
P. ar TU	32	1	32	1	32	1	32	__	32	1	32	1	48	1	0	0	0	48	0.99	0	0.01	41	0.72	0.28
P. re HE	50	1	50	1	50	1	50	__	50	__	50	__	50	1	0	0	0	50	0	0	1	50	__	__

	Prx-2			*Prx-3*			*Prx-4*		*Prx-5*				*Prx-6*		*Prx-7*		*Got-1*								
	(N)	2^{29}	2^{0}	(N)	3^{21}	3^{0}	(N)	4^{40}	(N)	5^{39}	5^{38}	5^{37}	(N)	6^{32}	(N)	7^{25}	(N)	1^{72}	1^{69}	1^{61}	1^{71}	1^{70}	$1^{69,5}$	$1^{68,5}$	1^{68}
P. ru HE	49	0.89	0.11	33	0.74	0.26	33	__	33	__	__	__	33	__	33	__	86	0.05	0.95	0	0	0	0	0	0
P. ru SA	44	0.47	0.53	44	0.77	0.23	44	__	44	__	__	__	44	__	44	__	52	0.11	0.82	0.07	0	0	0	0	0
P. ru RI	40	0.72	0.28	40	0.38	0.62	40	__	40	__	__	__	40	__	40	__	49	0.05	0.95	0	0	0	0	0	0
P. ru PI	40	0.27	0.73	40	0.29	0.71	40	__	40	__	__	__	40	__	40	__	38	0.13	0.87	0	0	0	0	0	0
P. fl LA	98	0.88	0.12	96	0.66	0.34	96	__	96	__	__	__	96	__	96	__	60	0.44	0.56	0	0	0	0	0	0
P. fl QU	41	0.72	0.28	41	0.38	0.62	41	__	41	__	__	__	41	__	41	__	64	0.51	0.44	0.05	0	0	0	0	0
P. gl WE	31	1	0	31	0.57	0.43	31	__	31	__	__	__	31	__	31	__	91	0.11	0.38	0	0	0.51	0	0	0

Table 2 (cont.)

	Prx-2			*Prx-3*			*Prx-4*		*Prx-5*				*Prx-6*		*Prx-7*		*Got-1*								
	(N)	2^{29}	2^{0}	(N)	3^{21}	3^{0}	(N)	4^{40}	(N)	5^{39}	5^{38}	5^{37}	(N)	6^{32}	(N)	7^{25}	(N)	1^{72}	1^{69}	1^{61}	1^{71}	1^{70}	$1^{69,5}$	$1^{68,5}$	1^{68}
P. gl LC	26	0.67	0.33	26	0.19	0.81	26	__	26	__	__	__	26	__	26	__	56	0.09	0.46	0	0	0.45	0	0	0
P. gl BC	40	0.84	0.16	40	0.28	0.72	40	__	40	__	__	__	40	__	40	__	34	0.25	0.54	0	0	0.21	0	0	0
P. gl FC	38	0.72	0.28	35	0.11	0.89	35	__	35	__	__	__	35	__	35	__	39	0.20	0.35	0	0	0.45	0	0	0
P. ve SR	48	__	__	48	__	__	48	__	48	__	__	__	48	1	48	1	43	0.12	0.08	0	0.80	0	0	0	0
P. ku HE	47	0.85	0.15	47	0.45	0.55	47	__	47	__	__	__	47	__	47	__	98	0	0	0	0	0	0	0	0
P. ar TI	37	0.84	0.16	37	0.27	0.73	37	__	37	__	__	__	37	__	37	__	27	0	0	0	0	0	0.80	0.20	0
P. ar TU	41	0.60	0.40	41	0.24	0.76	41	__	41	__	__	__	41	__	41	__	30	0	0	0	0	0	0.55	0.45	0
P. re HE	50	__	__	50	__	__	50	1	50	0.42	0.14	0.44	50	__	50	__	100	0	0	0	0	0	0	0	0.05

	Got-1		*Got-2*									*Got-3*		*Got-4*		*Got-5*		*Got-6*				*Got-7*		
	1^{67}	$1^{71,5}$	(N)	2^{48}	2^{40}	2^{34}	2^{27}	2^{54}	2^{39}	2^{37}	2^{38}	2^{36}	(N)	3^{49}	(N)	4^{44}	(N)	5^{50}	(N)	6^{25}	6^{26}	6^{0}	(N)	7^{24}	7^{22}
P. ru HE	0	0	61	0.59	0.27	0.08	0.06	0	0	0	0	0	86	__	86	__	86	__	86	0	0	1	86	__	__
P. ru SA	0	0	47	0.80	0.20	0	0	0	0	0	0	0	52	__	52	__	52	__	52	0	0	1	52	__	__
P. ru RI	0	0	44	0.59	0.41	0	0	0	0	0	0	0	49	__	49	__	49	__	49	0	0	1	49	__	__
P. ru PI	0	0	34	0.54	0.40	0.06	0	0	0	0	0	0	38	__	38	__	38	__	38	0	0	1	38	__	__
P. fl LA	0	0	51	0.28	0.72	0	0	0	0	0	0	0	60	__	60	__	60	__	60	0	0	1	60	__	__
P. fl QU	0	0	51	0.31	0.64	0.05	0	0	0	0	0	0	51	__	51	__	64	__	64	0	0	1	51	__	__
P. gl WE	0	0	55	0.63	0.37	0	0	0	0	0	0	0	55	__	55	__	91	__	91	0	0	1	55	__	__
P. gl LC	0	0	53	0.82	0.18	0	0	0	0	0	0	0	53	__	53	__	56	__	56	0	0	1	53	__	__
P. gl BC	0	0	21	0.93	0.07	0	0	0	0	0	0	0	34	__	34	__	34	__	34	0	0	1	34	__	__
P. gl FC	0	0	36	0.90	0.10	0	0	0	0	0	0	0	39	__	39	__	39	__	39	0	0	1	39	__	__
P. ve SR	0	0	34	0	0	0	0	1	0	0	0	0	34	1	34	1	43	__	43	0	0	1	34	__	__
P. ku HE	0	1	82	0	0	0	0	0	0.96	0.04	0	0	100	__	10 0	__	98	1	98	0.23	0.63	0.14	100	__	__
P. ar TI	0	0	39	0.42	0	0.58	0	0	0	0	0	0	31	__	31	__	27	__	27	0	0	1	31	0.84	0.16
P. ar TU	0	0	39	0.81	0.04	0.15	0	0	0	0	0	0	31	__	31	__	30	__	30	0	0	1	39	0.83	0.17
P. re HE	0.95	0.05	100	0	0	0	0	0	0	0	0.27	0.73	31	__	31	__	100	__	100	0	0	1	31	__	__

peroxidase (PRX), isocitrate dehydrogenase (IDH) and shikimic dehydrogenase (SKD). The methods employed for the former seven systems are described in Saidman (1985). For IDH and SKD the method was adapted from Verga (1995). The homogenates were made from five to seven day old cotyledons for all systems but ADH, for which 16-h-old seedlings were analyzed. Since ADH must be analyzed at a different life stage, it was not possible to study this system on the same individuals used for the assays with all the other enzymes.

Statistical methods

The genetic interpretation of isozyme data in *P. argentina* and *P. kuntzei* was based on previous studies on other species of *Prosopis* (Saidman and Vilardi, 1987; Verga, 1995). Standard measures of genetic diversity were calculated for each population from allelic frequency data. The diversity parameters estimated included mean number of alleles per locus ($\hat{A}$), mean number of alleles per polymorphic locus ($\hat{A}P$), percentage of polymorphic loci ($\hat{P}$), and mean expected ($\hat{H}_e$) and observed ($\hat{H}_o$) heterozygosity. They were calculated using BIOSYS 1.7 (Swofford and Selander, 1981). Expected heterozygosity estimates were compared among species by Kruskall-Wallis analysis of variance, using the program Statistix ver. 1.0 (Analytical Software, 1996). Pair-wise comparisons of mean heterozygosities were performed by Wilcoxon test using the program Statistica (Statsoft, Inc., 1995). These test have the advantage of having no assumptions about the distribution of the coefficients to be compared.

In order to discuss bias from Hardy-Weinberg expected frequencies, Wright (1951) $\hat{F}_{is}$ indices were estimated.

Differentiation between populations were estimated by two methods. The first one was based on unbiased Nei's (1978) genetic distances. This method might have some error because the precise homology between genes in species of different sections are uncertain due to the absence of intersectional hybrids. The second approach employs a phenotypic criterion to assess band homologies and to estimate relative associations among species. The bands were considered present when their frequency in the population was equal to or higher than 0.05 and a 0-1 matrix (presence-absence) was constructed. Manhattan distances were estimated from that matrix using the program RAPD (Black IV, 1996). Based on the genetic distances, two phenograms were obtained by UPGMA using the same program. In order to evaluate the reliability of the branches, 100 phenograms were obtained from bootstrapped pseudoreplicates of the respective matrices.

Results

Allelic frequencies

From all species, a total of 46 loci were detected. Some of them were invariant: *Amp-1*, *Sod-1*, *Sod-3*, and

Sod-4. The remaining loci showed variation within or among populations (Table 2).

For every system, the loci were numbered according to the chronological order in which they were described in previous works. Alleles were named with a superscript indicating the relative mobility of the corresponding product. The superscript 0 refers to null alleles. Some loci were species-specific, showing no homology with loci present in other species (Table 2).

Genetic variability and endogamy estimates

Genetic variability and endogamy estimates are shown in Table 3. In all populations $\hat{H}_e$ was higher than $\hat{H}_o$, yielding positive $\hat{F}_{is}$ estimates. This result indicates that some endogamy occurs in all studied populations. There is no significant correlation between genetic variability and the $\hat{F}_{is}$ coefficient (p = 0.45). For most variability estimates *P. flexuosa* (Quilmes) exhibited the highest values, while *P. kuntzei* (Herrera) and *P. reptans* (Herrera) were the less variable populations.

The estimates of $\hat{A}$, $\hat{A}P$, and $\hat{H}_e$ were compared among populations by Kruskall-Wallis statistics (KWS). Non-significant differences were detected for ÂP (KWS = 8.36; p = 0.87), but the differences among populations were significant for both $\hat{A}$ (KWS = 28.477, p = 0.012) and $\hat{H}_e$ (KWS = 22.536, p = 0.047).

When only populations of the same species were included in the analyses of $\hat{A}$ and $\hat{H}_e$, the differences were not significant in any case (KWS = 0.008-0.31, p = 0.93-0.96 and KWS = 0.026-0.43, p = 0.872-0.509 for Â and $\hat{H}_e$, respectively). All populations of each species were pooled and the comparisons were made at species level. The Kruskall-Wallis test indicated highly significant differences for both Â and $\hat{H}_e$ (KWS = 27.89, p = 0.0001 and KWS = 28.20, p = 0.0001 respectively). In general terms the species of section Algarobia resulted in more variation than the rest ($\overline{A}$ = 1.71, $\overline{He}$ = 0.20). However, when Algarobia species were compared to each other the differences were significant (KWS = 12.64, p = 0.013 and KWS = 11.92, p = 0.018 for $\hat{A}$ and $\hat{H}_e$ respectively) as a consequence of the low variability of P. kuntzei (Herrera). When this population was excluded, no significant differences occurred among species of Algarobia (KWS = 2.98, p = 0.39 and KWS = 4.73, p = 0.19 for $\hat{A}$ and $\hat{H}_e$, respectively). When P. kuntzei (Herrera), *P. argentina*, and *P. reptans* (Herrera) were compared, the differences were not significant (KWS = 1.46, p = 0.50 and KWS = 0.84, p = 0.65 for $\hat{A}$ and $\hat{H}_e$, respectively).

The proportion of polymorphic loci ($\hat{P}$) differed significantly among populations and among species (χ^2_{14} = 28.84, *P* = 0.011 and χ^2_6 = 28.21, p < 0.0001). The proportion of polymorphic loci was compared among the same population groups as described for $\hat{A}$ and $\hat{H}_e$. The species of Algarobia studied here exhibited significant differences for P estimates (χ^2_4 = 11.92, p = 0.018), but these differences became non-significant when *P. kuntzei* (Herrera) was excluded (χ^2_3 = 3.75, p = 0.29). The *P* estimates did not differ statistically among *P. kuntzei*

Table 3 - Genetic variability and fixation index (F_{IS}) coefficients estimated for each population. N = mean sample size per locus, $\hat{A}$ = mean number of alleles per locus, $\hat{A}P$ = mean number of alleles per polymorphic locus, $\hat{P}$ = % of polymorphic loci (5% criterion), $\hat{H}_o$ = observed heterozygosity, $\hat{H}_e$ = unbiased heterozygosity expected under Hardy-Weinberg. Numbers in parenthesis indicate the standard error.

Population	N	$\hat{A}$	$\hat{A}P$	$\hat{P}$	$\hat{H}_o$	$\hat{H}_e$	$\vec{F}_{is}$
P. argentina Tinogasta	34.4 (1.4)	1.39 (0.63)	2.22 (0.44)	31.03 (8.74)	0.038 (0.019)	0.110 (0.03)	0.415 (0.189)
P. argentina Tucunuco	35.5 (1.4)	1.43 (0.69)	2.33 (0.50)	31.03 (8.74)	0.036 (0.017)	0.116 (0.04)	0.193 (0.165)
P. reptans Herrera	53.9 (2.6)	1.28 (0.62)	2.40 (0.55)	20.00 (8.16)	0.025 (0.014)	0.085 (0.04)	0.209 (0.114)
P. kuntzei Herrera	50.3 (3.2)	1.25 (0.44)	2.00 (0.00)	25.00 (9.03)	0.006 (0.005)	0.094 (0.04)	0.540 (0.115)
P. ruscifolia Herrera	64.4 (2.8)	1.79 (0.83)	2.36 (0.63)	58.33 (10.28)	0.057 (0.022)	0.233 (0.05)	0.294 (0.127)
P. ruscifolia Sarmiento	57.4 (2.3)	1.71 (0.69)	2.21 (0.43)	59.33 (10.28)	0.049 (0.020)	0.226 (0.05)	0.335 (0.087)
P. ruscifolia Rivadavia	49.7 (1.3)	1.75 (0.68)	2.20 (0.41)	62.50 (10.09)	0.052 (0.020)	0.225 (0.05)	0.390 (0.107)
P. ruscifolia Pinto	57.0 (3.1)	1.83 (0.76)	2.33 (0.49)	62.50 (10.09)	0.045 (0.017)	0.234 (0.05)	0.476 (0.091)
P. flexuosa La Amarga	76.4 (3.4)	1.83 (0.87)	2.43 (0.65)	58.33 (10.28)	0.082 (0.027)	0.217 (0.04)	0.096 (0.065)
P. flexuosa Quilmes	60.7 (2.8)	1.87 (0.85)	2.50 (0.52)	58.33 (10.28)	0.056 (0.020)	0.262 (0.05)	0.395 (0.085)
P. glandulosa Weslaco	51.0 (2.5)	1.71 (0.81)	2.42 (0.51)	50.00 (10.42)	0.045 (0.019)	0.209 (0.05)	0.379 (0.102)
P. glandulosa La Copita	44.4 (1.6)	1.79 (0.78)	2.36 (0.50)	58.33 (10.28)	0.056 (0.022)	0.215 (0.05)	0.165 (0.094)
P. glandulosa Bell Co.	50.0 (2.3)	1.71 (0.75)	2.31 (0.48)	54.17 (10.39)	0.049 (0.019)	0.178 (0.05)	0.269 (0.129)
P. glandulosa Frio Co.	41.9 (0.9)	1.75 (0.90)	2.50 (0.67)	50.00 (10.43)	0.062 (0.025)	0.203 (0.05)	0.070 (0.070)
P. velutina Santa Rita	37.9 (0.9)	1.56 (0.82)	2.40 (0.70)	40.00 (10.00)	0.038 (0.016)	0.142 (0.04)	0.312 (0.175)

(Herrera), *P. reptans* (Herrera), and *P. argentina* populations ($\chi^2_2 = 1.38$, p = 0.50).

Genetic distances among populations

Two matrices of genetic distances (Table 4) were obtained respectively from Manhattan and Nei's genetic distances. They were highly consistent according to a Mantel test (r = 0.95; p = 0) based on 500 permutations. The corresponding trees are also highly consistent with minor differences. The phenograms are rather consistent with morphology because the populations of each species cluster together; species belonging to the section Algarobia are clustered in a single group; and *P. kuntzei*, which is morphologically very different from the remaining species studied of Algarobia, is the most phenetically distant species of this section. Excluding *P. kuntzei*, *P. velutina* (Arizona, USA) is the most differentiated of Algarobia, and the cluster formed by the Argentinean species *P. ruscifolia* and *P. flexuosa* with the North American *P. glandulosa* (Figure 2).

The distance between the populations of *P. argentina* and *P. reptans* Herrera was almost as high as that recorded among any of the Algarobia species.

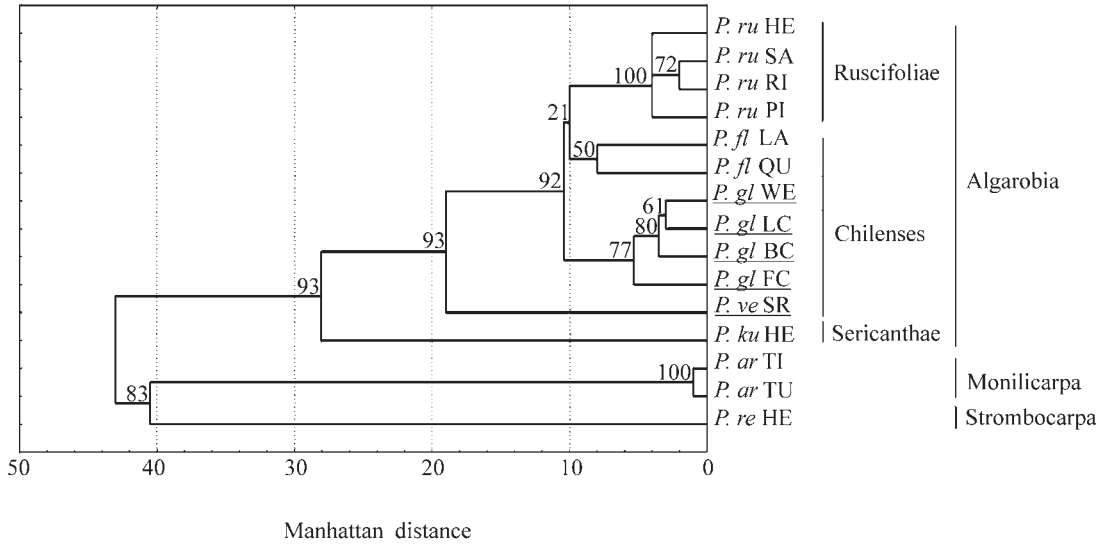

Figure 2 - Phenogram representative of Manhattan distances between populations of the species studied of genus *Prosopis*. Numbers over branches represent bootstrap support for each node. North-American populations are underlined.

Table 4 - Nei (above diagonal) and Manhattan (below diagonal) genetic distance matrix.

	P. ru HE	*P. ru* SA	*P. ru* RI	*P. ru* PI	*P .fl* LA	*P .fl* QU	*P. gl* WE	*P. gl* LC	*P. gl* BC	*P. gl* FC	*P. ve* SR	*P. ku* TA	*P. ar* TI	*P. ar* TU	*P. re* He
P. ru HE		0.010	0.014	0.036	0.078	0.063	0.031	0.041	0.040	0.041	0.223	0.290	0.491	0.488	0.452
P. ru SA	4.00		0.013	0.025	0.086	0.061	0.036	0.043	0.042	0.038	0.212	0.306	0.541	0.526	0.462
P. ru RI	4.00	2.00		0.040	0.074	0.061	0.051	0.057	0.061	0.048	0.213	0.295	0.509	0.500	0.445
P. ru PI	4.00	4.00	4.00		0.095	0.055	0.038	0.021	0.040	0.037	0.197	0.328	0.532	0.515	0.476
P. fl LA	12.00	10.00	10.00	8.00		0.037	0.104	0.112	0.118	0.119	0.301	0.351	0.500	0.504	0.534
P. fl QU	12.00	10.00	10.00	8.00	8.00		0.068	0.068	0.078	0.072	0.233	0.342	0.482	0.479	0.485
P. gl WE	12.00	10.00	10.00	8.00	8.00	12.00		0.016	0.013	0.015	0.206	0.307	0.543	0.570	0.473
P. gl LC	11.00	9.00	9.00	7.00	9.00	11.00	3.00		0.022	0.015	0.189	0.296	0.503	0.492	0.453
P. gl BC	13.00	11.00	11.00	9.00	11.00	13.00	3.00	4.00		0.015	0.199	0.330	0.565	0.553	0.472
P. gl FC	12.00	10.00	10.00	10.00	10.00	14.00	4.00	5.00	7.00		0.181	0.313	0.537	0.524	0.438
P. ve SR	22.00	20.00	20.00	20.00	20.00	22.00	14.00	17.00	17.00	18.00		0.478	0.760	0.743	0.561
P. ku TA	27.00	27.00	25.00	27.00	31.00	31.00	27.00	26.00	26.00	29.00	33.00		0.667	0.676	0.616
P. ar TI	38.00	40.00	38.00	38.00	38.00	36.00	44.00	41.00	41.00	42.00	52.00	37.00		0.012	0.666
P. ar TU	39.00	41.00	39.00	39.00	39.00	37.00	45.00	42.00	42.00	43.00	53.00	38.00	1.00		0.665
P. re HE	47.00	47.00	47.00	49.00	49.00	49.00	49.00	48.00	46.00	49.00	47.00	40.00	41.00	40.00	

Discussion

According to the differences in genetic variability, two heterogeneous groups of species can be established. The first one, with high variability, involves all species studied of section Algarobia except for *P. kuntzei*. The second one, with low variability, is represented by the remaining species.

The causes for the differences in genetic variability may be related to the reproductive system, the adaptive strategies or the evolutionary history of these species. The species of Algarobia with high variability were largely considered outcrosser, with self-incompatibility system (Solbrig and Bawa, 1975; Solbrig and Cantilo, 1975; Neff *et al*., 1977; Simpson, 1977; Simpson *et al*., 1977; Hunziker *et al*., 1986). Recent studies on the mating system of *P. alba, P. nigra, P. flexuosa, P. glandulosa, P. velutina, P. ruscifolia*, and *P.chilensis* (Bessega *et al*., 2000b) indicated that they are mostly outcrosser, although about 15% selfing can occur. Besides, these species are widely distributed and able to grow in diverse soils (Burkart, 1976). Finally, Bessega *et al*. (2000a) advanced the hypothesis that hybridization may have played a role in the first steps of species diversification of this group promoting reticulate evolution and boosting invasiveness ability. Information about the mating system of the remaining species is lacking. *P. argentina* is endemic and restricted to the western Argentinean provinces of Catamarca, La Rioja, and Mendoza, and, in contrast with most species of Algarobia, *P. argentina* shows a marked preference for soft and sandy soils (Burkart, 1976). Consequently, it can be considered a highly specialized species and its low variability a consequence of this high specialization. The low variability of *P. reptans*, as well as other species of the section Strombocarpa, had been previously recorded by Saidman *et al*., (1996). Selfing in species of Strombocarpa can not be ruled out because at least one species of this section, *P. tamarugo*, appears to self (Hunziker *et al*., 1986). *P. reptans* was shown to be able to undergo vegetative reproduction by means of underground runners (Burkart, 1976; Roig, 1993). The ability to reproduce vegetatively and the possibility of selfing allow the advancement of the hypothesis that the low variability in *P. reptans* might be the result of founder effect associated with colonization and a certain degree of endogamy (Saidman *et al*., 1996). However, the estimated F_{IS} does not suggest a higher tendency to selfing than the rest of the studied species of *Prosopis*.

Populations of *P. kuntzei* are usually dense and widely distributed. Therefore, low population sizes are not a likely explanation for its low variability. Its ability to colonize is similar to that of other species of Algarobia, but unlike the others, *P. kuntzei* apparently is not involved in natural hybridization events. The evolutionary history of this species might be very different from that of the remaining species of the same section. If hybridization did not play a role in the early evolution of this species, its variability might have been rapidly eroded. The actual cause for the low variability in *P. kuntzei* should be addressed through deeper analyses of its mating system.

The analysis of isoenzymatic patterns indicated that *P. argentina*, *P. reptans*, *P. kuntzei*, and *P. velutina* can be differentiated from each other and from the rest of the species studied here. The remaining species, which belong to section Algarobia, exhibit, for most enzyme loci, transspecies polymorphisms (similar to those described for DNA sequences in Klein, 1980; Garrigan and Hedrick, 2003), differing from each other only in allelic frequencies. The similarities obtained from isozyme data agree with the expected relationships among the three sections based on morphological grounds (Burkart, 1976). *P. argentina* (Monilicarpa) and *P. reptans* (Strombocarpa) are clearly differentiated from each other and from species of Algarobia. This result is consistent with studies based on phenolic compounds (Carman, 1973) and seed proteins (Burghardt and Palacios, 1997). The high biochemical differentiation observed among species belonging to different sections supports the hypothesis that these groups are natural and that the sections in the Burkart's (1976) system might be elevated to subgenera as suggested by Hunziker *et al*., (1986), Saidman *et al*., (1996), and Burghardt and Palacios (1997).

Among the species of Algarobia studied here *P. kuntzei* was the most differentiated isoenzymatically. This result is also consistent with morphological data because this species and *P. sericantha* are subaphyllous and horrid trees or shrubs with all branchlets spiny, included in a separate series, Sericanthae. Besides, although natural hybridization is very frequent between species of Algarobia belonging to different series (Palacios and Bravo, 1981; Hunziker *et al*., 1986), no hybrids have been described involving *P. kuntzei*. In the present paper, *P. kuntzei* shows private isoenzymatic patterns, genetic variability significantly lower than the remaining species of Algarobia, and the degree of genetic differentiation with respect to the remaining species of Algarobia is almost as high as those recorded for *P. reptans* (Strombocarpa) or *P. argentina* (Monilicarpa). This evidence suggests that *P. kuntzei* might be included in a section different from that involving the rest of Algarobia species studied here.

According to the present results, the series Chilenses and Ruscifoliae defined by Burkart (1976) would not be natural groups, because the series are not represented in the clusters retrieved. Since the genetic differentiation among these species is low, all of them might be included in a single series rather than two. *P. velutina* is the most differentiated species within this group. This result suggests that this species has no common origin with the other North American species, *P. glandulosa*. In agreement with previous RAPD and isoenzymatic studies (Bessega *et al*., 2000c) and cladistic analyses based on cpDNA and rDNA data (Bessega, 2001), the present results suggest that *P.*

glandulosa and *P. velutina* would have originated from two independent founder events.

Acknowledgments

We wish to express our gratitude to Ing. Hugo Cordo and Dr. J. C. Evans for his invaluable help in obtaining samples from the USA and to Prof. R. A. Palacios for taxonomic identification of Argentinean species. Special thanks to Dr. P. Villagra and Ing. M. Cony who generously provided the samples of *P. argentina*. This work was carried out thanks to the financial support of the Universidad de Buenos Aires (PID Nº X086), Agencia Nacional de Promoción Científica y Tecnológica (PICT Nº 06628) and Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 0722/98).

References

Analytical Software (1996) Statistix ver. 1.0: User' s Manual. Analytical Software. Tallahassee, FL.

Bessega C (2001) Estructura poblacional y relaciones filogenéticas (distancia y parsimonia) en especies del género *Prosopis* (Leguminosae). PhD Thesis, FCEyN. Universidad de Buenos Aires, Buenos Aires, Argentina.

Bessega C, Ferreyra LI, Saidman BO and Vilardi JC (2000)a Unexpected low genetic differentiation among allopatric species of section Algarobia of *Prosopis* (Leguminosae). Genetica 109:255-266.

Bessega C, Ferreyra LI, Julio N, Montoya S, Saidman BO and Vilardi JC (2000)b Mating system parameters in species of genus *Prosopis* (Leguminosae). Hereditas 132:19-27.

Bessega C, Saidman BO and Vilardi JC (2000)c Isozyme and RAPD studies in *Prosopis glandulosa* and *P. velutina* (Leguminosae, Mimosoideae). Genetics and Molecular Biology 23:1-5.

Black IV WC (1996) FORTRAN programs for the analysis of RAPD-PCR markers in populations. Colorado State University, Ft. Collins, CO, USA.

Burghardt AD and Palacios RA (1997) Electrophoretic characterization of the American sections of *Prosopis* L. (Leguminosae: Mimosoidae). Bull of the International Group for the study of Mimososidae 20:71-83.

Burkart A (1937) Una nueva especie de *Prosopis* del Monte occidental argentino. Revista Argentina de Agronomía 4:39-42.

Burkart A (1976) A monograph of the genus *Prosopis* (Leguminosae subfam. Mimosoidae). Journal Arnold Arboretum 57:219-525.

Carman NJ (1973) Systematic and ecological investigations in the genus *Prosopis* (Mimosaeae) emphasizing the natural products chemistry. PhD. Thesis, University of Texas, Austin, USA.

Garrigan D and Hedrick PW (2003). Perspective: Detecting adaptive molecular polymorphism: lessons from the MHC. Evolution 57:1707-1722.

Hunziker JH, Naranjo CA, Palacios RA, Poggio L and Saidman BO (1986) Studies on the taxonomy, genetic variation and biochemistry of Argentine species of *Prosopis*. Forest Ecology and Management 16:301-315.

Klein J (1980) Generation of diversity at MHC loci: Implications for T-cell receptor repertoires. In: Fougereau M and Dausset J (eds) Immunology 80, Academic Press, London, pp 239-253.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small numbers of individuals. Genetics 89:583-590.

Neff JL, Simpson BB and Moldenke AR (1977) Flowers-flower visitor system. In: Orians GH and Solbrig OT (eds) Convergent Evolution in Warm Deserts. US/IBP Synthesis Series, Dowden Hutchinson and Ross, Stroudsburg, pp 204-223.

Palacios RA and Bravo ID (1981) Hibridación natural en *Prosopis* (Leguminosae) en la Región Chaqueña Argentina. Evidencias morfológicas y cromatográficas. Darwiniana 23:3-35.

Ramírez L, De La Vega A, Razkin N, Luna V and Harris PJC (1999) Analysis of the relationships between species of the genus *Prosopis* revealed by the use of molecular markers. Agronomie 19:31-43.

Roig FA (1993) Aportes a la etnobotánica del género *Prosopis*. In: Unidades de Botánica y Fisiología Vegetal, IADIZA (eds) Contribuciones Mendocinas a la Quinta Reunión Regional para América Latina y el Caribe de la Red de Forestación del CIID, pp 99-121.

Saidman BO (1985) Estudio de la variación alozímica en el género *Prosopis*. PhD Thesis, FCEyN, Universidad de Buenos Aires, Buenos Aires, Argentina.

Saidman BO and Vilardi JC (1987) Analysis of the genetic similarities among seven species of *Prosopis* (Leguminosae: Mimosoideae). Theoretical Applied Genetics 75:109-116.

Saidman BO and Vilardi JC (1993) Genetic variability and germplasm conservation in the genus *Prosopis*. In: Puri S (ed) Nursery Technology of Forest Tree Species of Arid and Semiarid Regions. Winrock-Oxford & IBH Publishing Co. PVT. LTD., New Delhi, Bombay, Calcuta, pp 187-198.

Saidman BO, Vilardi JC, Pocoví MI and Acreche N (1996) Isozyme studies in Argentine species of the Section Strombocarpa, Genus *Prosopis* (Leguminosae). J Genetics 75:139-149.

Saidman BO, Vilardi JC, Montoya S and Poggio L (1997) Genetic variability and ploidy level in species of *Prosopis* (Leguminosae). Bol Soc Argent Bot 32:217-225.

Saidman BO, Bessega C, Ferreyra LI and Vilardi JC (1998)a Random amplified polymorphic DNA (RAPDS) variation in hybrid swarms and pure populations of genus *Prosopis*. In: Bruns S, Mantell S, Tragärdh C and Viana AV (eds) Recent Advances in Biotechnology for Tree Conservation and Management International Foundation for Sciences. Stockholm, pp 122-134.

Saidman BO, Vilardi JC, Montoya S, Dieguez MJ and Hopp HE (1998)b Molecular markers: A tool for the understanding of the relationships among species of *Prosopis* (Leguminosae, Mimosoidae). In: Puri S (ed) Tree Improvement: Applied Research and Technology Transfer. Science Publishers Inc. USA, pp 311-324.

Saidman BO, Bessega C, Ferreyra LI, Julio N and Vilardi JC (2000) The use of genetic markers to assess population structure and relationships among species of genus *Prosopis* (Leguminosae). Bol Soc Argent Bot 35:315-324.

Simpson BB (1977) Breeding system of dominant perennial plants of two disjunct warm desert ecosystems. Oecologia 27:203-226.

Simpson BB, Neff JL and Moldenke AR (1977) *Prosopis* flowers as a resource. In: Simpson BB (ed) Mesquite: Its Biology in Two Desert Ecosystems, US/IBP Synthesis Series, Dowden Hutchinson and Ross, Stroudsburg, pp 84-107.

Solbrig OT and Bawa KS (1975) Isoenzyme variation in species of *Prosopis* (Leguminosae). Journal Arnold Arboretum 56:398-412.

Solbrig OT and Cantilo PD (1975) Reproductive adaptations in *Prosopis* (Leguminosae, Mimosoisae). Journal Arnold Arboretum 56:185-210.

StatSoft, Inc (1995) STATISTICA for Windows [Computer program manual]. Tulsa, OK: StatSoft, Inc., 2300 East 14th Street, Tulsa, OK, http://www.statsoftinc.com.

Swofford DL and Selander RB (1981) BIOSYS-1.7: A FORTRAN program for the comprehensive analysis of electrophoretic data in populations genetics and systematics. J Hered 72:281-283.

Verga AR (1995) Genetische untersuchungen an *Prosopis chilensis* und *Prosopis flexuosa* (Mimosaceae) im trockenen Chaco Argentiniens. PhD Thesis, Universität Göttingen.

Vilardi JC, Saidman BO and Palacios RA (1988) Muestreo según variabilidad. In: *Prosopis* en Argentina. Documento preliminar elaborado para el I Taller Internacional sobre Recursos Genéticos y Conservación de Germoplasma en *Prosopis*. FCA. UNC-FAO, PIRB, pp 119-124.

Wright S (1951) The genetical structure of populations. Annals eugenics 15:323-354.

Associate Editor: Márcio de Castro Silva Filho