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THE USE OF GENETIC MARKERS TO ASSESS POPULATION STRUCTURE AND RELATIONSHIPS AMONG SPECIES OF THE GENUS PROSOPIS (LEGUMINOSAE)*

BEATRIZ O. SAIDMAN[1,2], CECILIA F. BESSEGA[1,3], LAURA I. FERREYRA[1],
NORMA JULIO[4], and JUAN C. VILARDI[1,2]

Summary: The genus *Prosopis* constitutes a promissory resource for reforestation of arid and semiarid regions of the world. Its main diversity center is located in South America. These plants are also an interesting model for evolutionary studies, involving mating system and population structure analyses as well as the evaluation of genetic differentiation among species. This work is an up-to-date revision of the information about genetic structure of populations and the relationships among several species of *Prosopis*. Some preliminary results and the perspective of future work using modern molecular tools are commented.

Key words: *Prosopis*, isoenzymes, RAPD, cpDNA, RFLP, DNA sequencing, mating system.

Resumen: El uso de marcadores genéticos para evaluar la estructura poblacional y las relaciones entre especies del género *Prosopis* (Leguminosae). El género *Prosopis* constituye un recurso promisorio para la reforestación de regiones áridas y semiáridas del mundo. Su principal centro de diversidad está situado en Sudamérica. Estos vegetales son también un modelo interesante para estudios evolutivos, incluyendo análisis del sistema de apareamiento y la estructura poblacional, como así también la evaluación de la diferenciación genética entre especies. Este trabajo es una revisión hasta la actualidad de la información acerca de la estructura genética de las poblaciones y las relaciones entre varias especies de *Prosopis*. Se comentan además algunos resultados preliminares y las perspectivas de trabajos futuros usando herramientas moleculares modernas.

Palabras clave: *Prosopis*, isoenzimas, RAPD, cpDNA, RFLP, secuenciación de ADN, sistema de apareamiento.

INTRODUCTION

The genus *Prosopis* includes about 44 species grouped in five sections. Some sections are subdivided making a total of eight series. This genus comprises shrubs and trees that exhibit a high economic and ecological potential in semiarid areas of the Americas. Some of them are considered multipurpose natural resources because they may be used for reforestation of arid and semiarid regions, production of wood, charcoal, forage, human food, etc. Unfortunately, the extensive exploitation of some species of this group is leading to the destruction and total eradication of *Prosopis* forests (Hunziker *et al.* 1986).

The rational use of promissory species and the development of programs of germplasm conservation and protection of endangered species require a deep knowledge of their biological characteristics, adaptive strategies, and evolutionary relationships.

Decisions on the most efficient methods for sampling and improving profitable characteristics of these species should be largely based on information of biological characteristics. Studies on taxonomy, demography, and distribution of genetic variability are very important in this respect.

The methodology applied to population genetic studies provides genetic markers useful for taxonomy. They also allow analyzing reproductive system and population structure, which constitute important information for demographic studies.

In a wide sense, a genetic marker is any morphological, chromosomal, biochemical, or molecular trait that can be accurately identified in any individual of the population and is genetically determined. Historically, morphological traits are the most used in taxonomic studies for they usually reveal different adaptive responses linked to the

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[1]Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 1428 Buenos Aires, Argentina. e-mail: vilardi@bg.fcen.uba.ar.

[2]Member of Carrera del Investigador Científico, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

[3]Fellow of CONICET.

[4]Genética de Poblaciones y Evolución, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba.

speciation process. Chromosomal characteristics are also frequently associated to selective effects, the rearrangements may affect the cell cycle and the gametogenesis, and are associated to position effects of the genes they bear. Among biochemical and molecular markers both neutral and selective variants can be found. The usefulness of each of these classes depends on the kind of analysis to be performed.

Neutral markers typically vary randomly in such a way that differentiation among populations is related to divergence time and degree of isolation (gene flow). The distribution of genotypic frequencies of these markers within populations mainly depends on the reproductive system and population structure. In contrast, selective markers reveal adaptation to different environmental conditions and/or adaptive strategies. Sometimes the distinction between these two groups is complicated because of physical linkage among essentially neutral genes with others of selective importance. In such cases, neutral markers may be useful to select indirectly beneficial traits whose expression is affected by the environment or occurs in late stages of development.

BIOCHEMICAL AND MOLECULAR MARKERS

The raw data for population genetic studies consist of the number of individuals in the samples with a certain phenotype or genotype. Phenotypic or genotypic frequencies are inferred from this information for the populations as a whole.

Allozyme markers are usually easy to analyze and in many cases are codominant. As genotypes can be inferred directly from the observed band patterns, they are especially useful to study population genetic structure. However, there may be some shortcomings associated with isozymic analysis. Although allozymic variation has been largely considered essentially neutral (see Kimura, 1982), there are examples of enzyme polymorphisms where selective effects have been demonstrated (McDonald & Kreitman, 1991). In such cases, inferences on genetic structure are not reliable. Another limitation of isozyme analysis is that the expression of many isoenzyme systems varies throughout ontogenetic stages and sometimes the environment also affects it.

There are several techniques for the analysis of genetic markers based on DNA polymorphisms. Usually they can be detected independently of developmental or environmental conditions. Polymorphisms for the length of restriction fragments (RFLP) are most often codominant, but the technique is rather laborious and sometimes their study requires previous genetic information of the species under study, which are not always available. Markers based on the polymerase chain reaction (PCR), mainly RAPDs, are dominant, but the technique is simple and does not require previous genetic knowledge. This technique is useful to analyze closely related populations or species.

The most direct approach to genetic variation is DNA sequencing. However, its application at population level is expensive. Depending on the relationship between the populations to be compared, more variable or more conserved regions of the genome should be chosen for the study, in order to provide useful markers for evolutionary studies.

In species of genus *Prosopis*, isozyme, RFLP of mitochondrial and chloroplast DNA, RAPD, and DNA sequencing techniques are currently under use to accomplish different purposes involving:

1. Estimation of genetic distances and cluster analysis.
2. Analysis of genetic structure and distribution of genetic diversity at different levels: individuals, populations, regions, and species.
3. Analysis of mating system parameters.
4. Phylogenetic analysis.

GEOGRAPHICAL DISTRIBUTION, TAXONOMIC PROBLEMS, AND POPULATIONS SAMPLED

The genus *Prosopis* is distributed in all arid and semiarid regions of the world. Its distribution involves South East Asia (3 native species), tropical Africa (1 species), and America (40 species). The highest number of species occurs in South America, in the biogeographic provinces (Cabrera & Willink, 1980) of Monte, Espinal, and Chaco, that belong to the Chaqueño Dominion of the Neotropical Region. These provinces are important centers of species differentiation (Burkart, 1976; Hunziker *et al.*, 1986). The genus occupies most of the Argentinean territory from the annual isohyetal line of 1500 mm in the north up to the 12°C isotherm as the South distribution limit (Hunziker *et al.*, 1986). The Chaqueño Dominion (Argentina, Bolivia, and Paraguay) constitutes a main center of morphological polymorphism of *Prosopis* with about 28 species, 13 of which are endemic (Burkart, 1976).

There is another important center of polymorphism in North America, the Mexican-Texan, with nine species, three of them endemic (Burkart, 1976).

Species belonging to two of the five sections of *Prosopis*, Algarobia and Strombocarpa, have been studied using isoenzyme electrophoresis and DNA marker techniques (Saidman & Vilardi, 1987, 1993; Saidman *et al.*, 1996, 1998a,b).

The section Algarobia involves the most important species from the economic and ecological points of view, and is the most deeply studied genetically. The taxonomy of this group is complicated because, despite the important morphological differences, the species are very similar biochemically and genetically. (Saidman, 1985, 1986, 1993; Saidman & Vilardi, 1987, 1993; Saidman *et al.* 1997, 1998a,b), and interspecific hybridization is very frequent in zones of sympatry (Hunziker *et al.*, 1986). The distinction of some of the series proposed by Burkart (1976) may be questioned on the grounds of different lines of evidence, including morphology, natural hybridization, chromatography of phenol compounds, seed protein, and isoenzyme electrophoresis (see Hunziker *et al.*, 1986), and RAPD (Saidman *et al.*, 1998b; Ramírez *et al.*, 1999; Bessega *et al.*, 2000a).

The frequent interspecific hybridization creates intermediate phenotypes that difficult morphological determination, and the consequences of this phenomenon in Algarobia are not clear. Palacios & Bravo (1981) proposed that a group of sympatric species of this section that undergo frequent hybridization would constitute a syngameon, as defined by Grant (1981).

The understanding of causes and genetic consequences of hybridization in this group requires the use of genetic markers.

BIOCHEMICAL AND MOLECULAR STUDIES IN SPECIES OF *PROSOPIS*

Isoenzyme electrophoresis was applied to species belonging to two sections, Algarobia and Strombocarpa. Important differences were detected between these sections regarding several population genetic parameters.

The species of Algarobia exhibited a higher genetic variability within populations, measured in terms of percent of polymorphic loci (mean P=51.5) and heterozygosity (mean H=0.21) than those of Strombocarpa (P=15.31; H=0.06) (Table 1). The differentiation among species within section Algarobia measured through Nei's (1972) distance was relatively low with values ranging within the expected according to Ayala *et al.* (1974) for sub to semispecies.

The genetic similarities among species of section Algarobia obtained from isoenzyme data do not agree with the expected according to the morphological evidence. The phenograms obtained from genetic distance matrices (Saidman & Vilardi, 1993; Saidman *et al.*, 1998a) are not consistent with the series defined by Burkart (1976).

The lack of consistence of molecular and morphological data was also observed in a RAPD analysis by Ramírez *et al.* (1999), in which the phenetic relationships among species do not support Burkart's (1976) series.

In section Strombocarpa species are clearly differentiated by isoenzymes, with many diagnostic loci. The differentiation between the sections Algarobia and Strombocarpa at the isoenzymatic level was so strong that the ancestral homology of band patterns could be observed in only one (SOD) of the seven systems analysed (Hunziker *et al.*, 1986; Saidman *et al.*, 1996).

RFLP analysis of mitochondrial (mtDNA) and ribosomal DNA (rDNA) analyzed by Southern blot, and the different sizes of the transcript spacer of rDNA obtained by PCR yielded similar conclusions about the differentiation between these sections (Saidman *et al.*, 1998a). The phenogram based on both mtDNA and rDNA variation were consistent and showed higher differentiation among species belonging to Strombocarpa than within Algarobia. The species of Algarobia associate in a single cluster, and distances between species within this section are lower than any recorded distance between species belonging to different sections. The distances between species within Strombocarpa are in some cases as high as those observed between species belonging to different sections.

Table 1. List of *Prosopis* species and populations analyzed so far for allozymic and/or DNA variation, along with their estimated variation. **H**= mean heterozygosity; **P**= percent of polymorphic loci; $F_{IS=}$ fixation index; *****= not determined.

Section	Series	Species	Population	Nº loci	H	P	FIS	Reference
Algarobia	Ruscifoliae	*P. ruscifolia*	Herrera, Sgo. del Estero	24	0.232	52.2	0.29	Montoya et al. (1994)
			Sarmiento, Sgo. del Estero	24	0.223	60.9	0.33	Montoya et al. (1994)
			Rivadavia, Salta	24	0.222	60.9	0.3	Montoya et al. (1994)
			Pinto, Sgo. del Estero	24	0.239	60.9	0.48	Montoya et al. (1994)
		P. vinalillo	Tucumán	24	0.253	62.5	0.31	Ferreyra et al. (1998)
		P. hassleri	Dept. Patiño, Formosa	24	0.170	45.0	*	Saidman (1985)
	Chilenses	*P. nigra*	La banda, Sgo. del Estero	24	0.210	48.0	*	Saidman (1985)
			Paraná, Entre Ríos	24	0.220	48.0	*	Saidman (1985)
			La Merced, Salta	24	0.244	58.3	0.54	Montoya et al. (1994)
			Huilla catina, Sgo. del Estero	24	0.298	66.7	0.18	Ferreyra et al. (1998)
		P. caldenia	Santa Rosa, La Pampa	23	0.200	48.0	*	Saidman (1985)
		P. flexuosa	Quilmes, Tucumám	24	0.261	60.9	0.29	Bessega et al. (1996)
			La Amarga, La Pampa	24	0.222	60.9	0.1	Ferreyra et al. (1998)
		P. alpataco	Chacharramendi, La Pampa	24	0.170	45.0	*	Saidman (1985)
		P. alba	Trancas, Tucumán	24	0.140	41.7	0.31	Montoya et al. (1994)
			Chicoana, Localidad	24	0.169	50.0	0.41	Montoya et al. (1994)
			La Merced, Salta	24	0.163	37.5	0.33	Montoya et al. (1994)
			Sumalao, Salta	24	0.168	41.7	0.41	Montoya et al. (1994)
			Burruyacu, Tucumán	24	0.190	50.0	0.24	Ferreyra et al. (1998)
		P. glandulosa	Weslaco, Texas	27	0.217	52.2	0.38	Bessega et al. (2000a)
			La Copita, Texas	27	0.222	52.2	0.16	Bessega et al. (2000a)
			Bell Co, Texas	27	0.183	47.8	0.27	Bessega et al. (2000a)
			Frio Co., Texas	27	0.210	52.2	0.07	Bessega et al. (2000a)
		P. velutina	Santa Rita, Arizona	27	0.127	33.3	0.31	Bessega et al. (2000a)
		P. chilensis	Villa Dolores, Córdoba	17	0.248	58.8	0.29	Julio (2000)
			Patquía, La Rioja	17	0.262	64.7	0.25	Julio (2000)
			Belén, Catamarca	17	0.296	64.7	0.16	Julio (2000)
			Las Talas, La Rioja	17	0.252	64.7	-0.12	Julio (2000)
			Talampaya, La Rioja	17	0.198	52.9	0.62	Julio (2000)
			La Higuerita, San luis	17	0.219	52.9	0.3	Julio (2000)
			Media Naranja, Córdoba	17	0.276	64.7	0.31	Julio (2000)
			Chacabuco Santiago, Chile	17	0.258	64.7	0.26	Julio (2000)
			Monte Patria, Limari, Chile	17	0.241	52.9	0.15	Julio (2000)
			Soto, Córdoba	17	0.195	41.2	0.28	Julio (2000)
			Conlara, Córdoba	17	0.262	58.8	0.1	Julio (2000)
			Astica, San Juan	17	0.258	52.9	0.03	Julio (2000)
		P. juliflora	Cartagena, Colombia	20	0.130	30.0	*	Saidman et al. (1997)
			Altamira, Colombia	20	0.050	13.0	*	Saidman et al. (1997)
	Sericanthae	*P. kuntzei*	Tacana, Tucumán	18	0.139	33.3	*	Saidman et al. (1998a)
Strombocarpa	Strombocarpae	*P. strombulifer*	Copacabana, Catamarca	23	0.050	13.0	*	Saidman (1985)
			Conesa, Río Negro	23	0.020	8.0	*	Saidman (1985)
		P. reptans	Herrera, Sgo. del Estero	23	0.090	17.4	*	Saidman et al. (1996)
			Icaño, Sgo. del Estero	23	0.090	21.0	*	Saidman et al. (1996)
		P. torquata	Famatina, La Rioja	17	0.080	18.0	*	Saidman (1985)
		P. pubescens	Bell Co, Texas	14	0.036	7.1	*	Saidman et al. (1996)
	Cavenicarpae	*P. ferox*	Los Cardones, Salta	22	0.088	22.7	*	Saidman et al. (1996)

ISOENZYMATIC AND MOLECULAR STUDIES IN THE SECTION ALGAROBIA

Algarobia is the best studied section from the population genetic standpoint. The studies conducted in species of this section involve the distribution of genetic variability, population structure, mating system, genetic differentiation among populations and species, and relationships between genetic differentiation and geographic isolation.

Population structure in species of Algarobia

Population structure was analyzed by means of Wright's (1951) F statistics in 31 populations of 8 species: *P. nigra*, *P. alba*, *P. flexuosa*, *P. chilensis*, *P. ruscifolia*, *P. glandulosa*, *P. velutina*, and *P. vinalillo* (Table 1).

Observed and Hardy-Weinberg expected genotypic frequencies were compared through the F_{IS} coefficient (Table 1). In all populations but one, mean F_{IS} estimates were positive. These results indicate a general trend towards significant homozygote excess within populations, which might be due to a certain degree of endogamy.

Since these species were largely considered obligate outcrosser (Burkart, 1976; Simpson, 1977; Simpson *et al.*, 1977), the explanation of this result required an analysis of the mating system.

Estimation of mating system parameters

Mating system analysis using isoenzymatic data is based on the mixed model and Ritland & Jain (1981) estimation procedure. The method assumes that progeny are derived from either random mating (outcrossing) or self-fertilization.

The sample is subdivided into families. Each family consists of seeds collected from the same mother plant. Consequently, each family involves full and/or half sibs, depending on the origin of the fertilizing pollen grains. The genotype of the female parent of each group can be assessed by the method of Brown & Allard (1970).

Using the program MLTR (the improved version of MLT; Ritland, 1990) multilocus (t_m) and single locus (t_s) outcrossing rates, the correlation of t (r_t) within progeny arrays, the correlation of outcrossed paternity (r_p), and fixation index of maternal parents (F_{ISm}) were estimated in seven species of Algarobia (Fig. 1): *P. glandulosa*, *P. velutina*, *P. chilensis*, *P. nigra*, *P. alba*, *P. flexuosa*, and *P. ruscifolia* (Bessega *et al.*, 2000b).

The estimated values of single and multilocus outcrossing rates varied from about 0.72 to 1.00, indicating that the species are mostly outcrosser but up to 28% of selfing can occur, with an average of 15%. These outcrossing rate estimates were similar to the values obtained by Keys & Smith (1994) for populations of *P. velutina*. The r_t estimates obtained by Bessega *et al.* (2000b) were high, indicating that outcrossing rates vary among trees within populations. Finally, high r_p estimates were also obtained, which means that many individuals (seeds) within a family are full sibs.

The estimated inbreeding coefficients for mother plant genotypes (F_{ISm}) were in all cases significantly lower than the values obtained for the whole seed population (F_{IS}). A possible explanation for such difference might be that selective forces favor heterozygous seeds, reducing the proportion of selfed individuals in the adult population (Bessega *et al.*, 2000b).

An additional observation was that pollen and ovule allele frequencies do not differ significantly. This result is compatible with the hypothesis of limited pollen dispersal.

Partial selfing and limited pollen dispersal would explain satisfactorily the homozygote excess detected in all populations of species of Algarobia studied so far. Selection against homozygotes would be the cause for the relatively high variability in these species (Bessega *et al.*, 2000b).

Isoenzyme differentiation within and between species of Section Algarobia

One of the most remarkable characteristics of the section Algarobia is the high genetic similarity among the species so far studied (Saidman, 1985, 1986, 1993; Saidman & Vilardi, 1987, 1993; Saidman *et al.*, 1997, 1998a,b; Bessega *et al.*, 2000a,b, unpubl.). Almost all alleles of polymorphic isoenzyme loci are shared by all species. Diagnostic loci are mostly absent and species differ only in allelic frequencies.

At first glance this among-species similarity might be the consequence of the high natural interspecific hybridization rates recorded in this section (see Hunziker *et al.*, 1986). However, some evidence are against this interpretation.

Tetraploid *P. juliflora* populations showed high

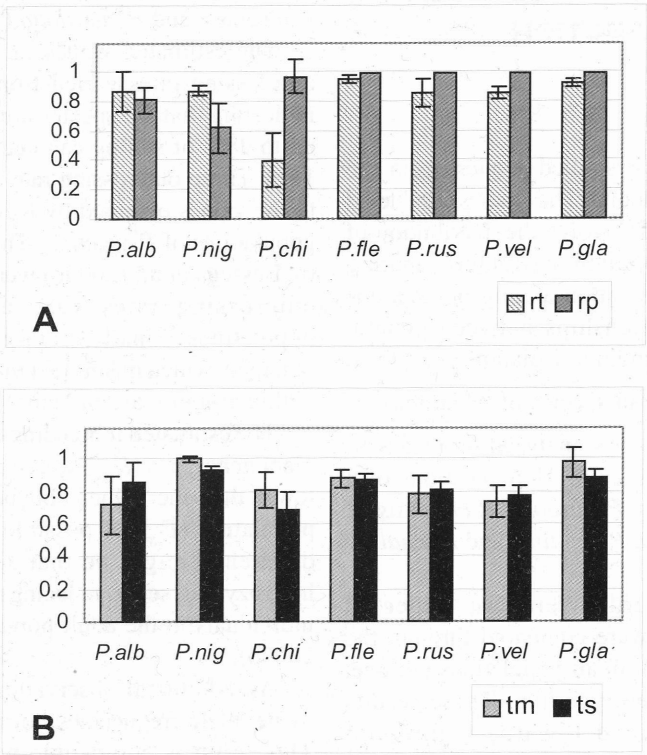

Fig. 1. Parameters of mating system in six species of *Prosopis*. ***P.alb***= *P. alba*, ***P.nig***= *P. nigra*, ***P.chi***= *P. chilensis*, ***P.fle***= *P. flexuosa*, ***P.rus***= *P. ruscifolia*, ***P.vel***= *P. velutina*, ***P.gla***= *P. glandulosa*, **A**= tm and ts denote multilocus and single locus outcrossing rates respectively. B= rt and rp denote correlation of tm within progeny arrays and correlation of outcrossed paternity.

genetic similarity with two allopatric diploid species, *P. ruscifolia* and *P. caldenia* (Saidman *et al.*, 1997). In this case, hybridization and introgression is not a plausible explanation for the genetic similarity since the geographic distribution and the different ploidy level are expected to prevent any genetic exchange between *P. juliflora* and the other two species.

Furthermore, in surveys involving Argentinean populations with different specific status and a wide geographical range indicated that Wright's (1951) F_{ST} estimates among allopatric conspecific populations are much lower than those recorded among parapatric populations of different nominal species that hybridize frequently (Saidman *et al.*, 1998a).

Finally, recent results by Bessega *et al.* (unpubl.) studying North and South American species of Algarobia also indicate that high genetic similarity is independent of the opportunity of hybridization to take place. They found that interspecific genetic distances are not correlated to geographic distances. The analysis of populations of *P. glandulosa* from Texas and *P. ruscifolia* and *P. flexuosa* from Argentina showed that the genetic differentiation between the South American species was not different from the differentiation between any of them and *P. glandulosa.*

The conclusion of these studies is that although hybridization and introgression does occur between species of section Algarobia, the high similarity among species can not be explained solely on the basis of interspecific gene flow. Since most alleles are shared by almost all species and their genetic variability is also very similar, it is conceivable that selection may have played a role in preserving the genetic variability detected isoenzymatically.

Distribution of genetic variability in Prosopis chilensis

Prosopis chilensis is one of the most promissory species of this genus. Burkart (1976) suggested that it should be included in reforestation programs because of its great morphological variability and relatively fast growing. Further studies (Roig, 1993;

Dalmasso, 1993; Cony, 1993; Karlin *et al*., 1997) encouraged the development of selection programs based on the high morphological variability associated to climatic and edaphic diversity and the high fruit productivity of this species.

Rational programs to improve beneficial characteristics of this species require information about the distribution of genetic variability. Recent studies on the genetic structure of this species (Julio, 2000) evaluated the distribution of genetic variability. A hierarchical analysis of population differentiation was performed using the formula of Wright (1978). A total of 12 populations were sampled from three geographical regions: Chaco (Córdoba and San Luis, Argentina) and Monte biogeographical provinces (La Rioja, Catamarca, and San Juan, Argentina), and Valle Central (Chile).

Results indicated that most variation occurs within populations (~87% of total variance). The differentiation among populations within regions was very low (~13%) and no divergence occurs among regions.

MOLECULAR STUDIES

Isozymes proved to be excellent tools to show differences between the sections Algarobia and Strombocarpa and to study population structure. In contrast, the lack of diagnostic loci makes isozymes unsuitable for unequivocal species and hybrid recognition within the section Algarobia.

Diverse molecular techniques are being used to produce markers useful to fulfil these purposes as well as to conduct evolutionary studies. The results of recent analyses based on RAPDs are promissory. Saidman *et al.* (1998b) studied 5 populations involving the species *P. alba*, *P. nigra*, and *P. flexuosa* and natural hybrids. They estimated allelic frequencies of RAPD loci using the method of Lynch & Milligan (1994). A phenetic approach indicated that hybrid populations are not intermediate between their putative parents. Bessega *et al.* (2000a) estimated genetic variability and differentiation between 4 populations of *P. glandulosa* and one of *P. velutina* using both RAPD and isozyme techniques. In this study RAPDs yielded higher genetic distances among populations of *P. glandulosa* than isozymes did.

Ramirez *et al.* (1999) studied 15 species belonging to five sections (Prosopis, Anonychium, Strombocarpa, Monilicarpa, and Algarobia). In this case DNA obtained from 10 individuals of each species was pooled and used as template for PCR reaction. The RAPD patterns obtained by this method yielded a phenogram that separates species belonging to different sections, but it gives no support to the series defined by Burkart (1976). Since each species was represented by one pooled sample of DNA obtained from different individuals no inference on within species variation were obtained.

Preliminary results by Ferreyra *et al.* (1999) indicate that some combinations of RAPD bands are useful to differentiate individuals of five species (*P. ruscifolia*, *P. alba*, *P. nigra*, *P. flexuosa*, and *P. vinalillo*) and to identify putative parents of natural hybrids.

Other molecular techniques are currently being applied to evaluate phylogenetic relationships among species of *Prosopis*. They involve RFLP (Bessega *et al*., 1998) and sequencing (Bessega *et al*., 1999) of cpDNA fragments.

The probes for southern hybridizations are two contiguous fragments corresponding to the regions IR and SSC. These segments combined make about 17% of the chloroplast genome. The *trnT-trnD* intergenic region was amplified from total genomic DNA by the polymerase chain reaction (PCR) using the universal primers described by Demesure *et al.* (1995). Preliminary results on the relationships among ten species of section Algarobia (*P. ruscifolia*, *P. vinalillo*, *P. alba*, *P. glandulosa*, *P. caldenia*, *P. flexuosa*, *P. alpataco*, *P. nigra*, *P. affinis*, and *P. kuntzei*) and one of section Strombocarpa (*P. reptans)* were obtained by cladistic analysis of both data sets. They show partial agreement. Two remarkable facts may be pointed: *P. reptans* is highly differentiated from the species of Algarobia and *P. kuntzei* separates early from the remaining species of Algarobia. The cladograms obtained do not support Burkart's (1976) series. These preliminary results are consistent with previous biochemical and molecular evidence indicating that the series within section Algarobia should be revised.

Further studies including more species of this group and the study of new regions of DNA, may throw light on the relationships among the species of this genus.

CONCLUDING REMARKS

The analysis of the relationships among species and the distribution of genetic variability at different levels (among species, among populations within species and within populations) in the genus *Prosopis* is very important from theoretical and practical standpoints.

Classical morphological approaches have shown important variation among populations of some species, mainly of section Algarobia, for which varieties and/or ecotypes have been described. Other species have shown important differences in geographic and environmental range. Finally, their multiple uses and ability to grow in arid and semiarid zones have evidenced the potential of these species as natural resources in areas where most traditional forest species are not able to survive.

The studies on the distribution of genetic variability using RAPD and isozyme markers in the most promissory species (section Algarobia) indicated that the majority of within species diversity occurs within populations. This result indicates that few populations are an adequate sample of the whole within species variability. Studies on the association of these markers with selectable traits are yet not developed and they should be encouraged to define the best strategies for improving these species.

The high hybridization rates among several species of section Algarobia has led to the assumption that they constitute a syngameon. In this group species boundaries are not clearly defined. However, the species apparently constitute natural entities effectively isolated from each other. Since the reproductive barriers are incomplete, the species concept that best fit the condition of these entities would be Templeton's (1989) cohesive concept (Montoya *et al.*, 1994; Burghardt, 1995; Saidman *et al.*, 1998a).

RAPD markers are the first molecular tool that allows differentiating qualitatively closely related species of Algarobia: *P. ruscifolia*, *P. alba*, *P. nigra*, *P. flexuosa*, and *P. vinalillo*.

Genetic markers have also contributed to the understanding of the mating system, an important concern for the design of selection programs.

The comparison of species belonging to different sections has shown that they constitute natural well-differentiated groups. The species of Algarobia are more closely related to each other than those of Strombocarpa. By contrast, the biochemical and molecular data suggest that the series should be revised.

The use of cladistic approaches using organelle and nuclear DNA variation will give an insight on the relationships among species with higher differentiation and it will greatly contribute to the understanding of evolution trends in this genus.

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