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GENETIC VARIABILITY AND PLOIDY LEVEL IN SPECIES OF *PROSOPIS* (*LEGUMINOSAE, MIMOSOIDEAE*)

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Summary *Genetic variability and ploidy level in species of* Prosopis (Leguminosae, Mimosoideae). Polyploidy is an uncommon phenomenon in the genus *Prosopis*, where the only recorded cases are limited to some tetraploid (2n= 56; X= 14) varieties of *P. juliflora* from Haiti, Colombia and Venezuela. *P. juliflora* belongs to Section Algarobia which is characterized by low genetic differentiation among the species so far studied isoenzymatically with heterozygosity (H) estimates varying from 0.13 to 0.20 and P values ranging from 30 to 50%. The low incidence of interspecific genetic differentiation, lack of vegetative reproduction, and high hybrid fertility are the causes for the low incidence of polyploidy in this section. In the present work seven isoenzymatic systems (ADH, EST, GOT, SOD, PRX and 6PGD) were characterized in two Colombian populations of *P. juliflora* and compared with three Argentinian populations belonging to two species, *P. ruscifolia* and *P. caldenia*. Ploidy levels were also determined, 4x for *P. juliflora* and 2x for the other two species. The 20 studied loci were homologous in all species. Some duplicated loci (Got-1 and Got-2) were observed in *P. juliflora* with fixed heterozygosity. The variability in *P. juliflora* populations was similar to or lower than that of diploid species. Cytophotometric studies revealed that the DNA content per genome in *P. juliflora* is lower than in the diploid species. The significance of the results of isozymal and cytological studies with respect to hypotheses on the origin of polyploidy is discussed.

Resumen *Variabilidad genética y nivel de ploidía en especies de* Prosopis (Leguminosae, Mimosoideae). La poliploidía es un fenómeno poco frecuente en el género *Prosopis*, en el cual los únicos casos registrados están limitados a algunas variedades tetraploides (2n= 56; X= 14) de *P. juliflora* de Haití, Colombia y Venezuela. *P. juliflora* pertenece a la Sección Algarobia la cual se caracteriza por la baja diferenciación genética entre las especies hasta ahora estudiadas isoenzimáticamente, con estimaciones de la heterocigosis que varían entre 0,13 y 0,20 y valores de P entre un 30 y un 50%. La baja incidencia de diferenciación genética interespecífica, la falta de reproducción vegetativa, y la alta fertilidad de los híbridos son las causas de la baja incidencia de la poliploidía en esta sección. En el presente trabajo siete sistemas isoenzimáticos (ADH, EST, GOT, SOD, PRX y 6PGD) fueron caracterizados en dos poblaciones colombianas de *P. juliflora* y comparadas con tres poblaciones argentinas pertenecientes a dos especies, *P ruscifolia* y *P. caldenia*. Se determinaron también los niveles de ploidía, 4x para *P. juliflora* y 2x para las otras dos especies. Los 20 loci estudiados fueron homólogos en todas las especies. Se observaron algunos loci duplicados (Got-1 y Got-2) en *P. juliflora* con heterocigosis fijada. La variabilidad en las poblaciones de *P. juliflora* fue similar o menor que en las especies diploides. Los estudios citofotométricos revelaron que el contenido de ADN por genoma fue menor en *P. juliflora* que en las especies diploides. Se discute el significados de los resultados isoenzimáticos y citológicos en relación con la hipótesis sobre el origen de la poliplodía.

INTRODUCTION

It is a known fact that hybrids between well differentiatied races and species show higher viability and fisiological homeostasis. However, these hybrids are usually unable to breed effectively. On the one hand they may be sterile, restricted to asexual reproduction; and if fertile, their valuable heterotic and homeostatic properties are lost through the subsequent sexual generations. The only way to preserve these characteristics is by amphyploid genetic systems which combine hybrid genotypes and effective mating ability.

Polyploidy is a phenomenon characteristic of the plant kingdom, being amphiploidy the most widespread of its variants. Polyploidy is promoted by the combination of three main factors (Grant, 1971): 1st) the occurrence of natural hybridization, 2nd) the presence of different genome or subgenomes in the hybridizing diploid species, 3rd) the occurrence of perennial growing increasing the possibility of somatic duplication or, in annual

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plants, autogamic reproductive system to increase the probability of union of non reduced gametes from the same hybrid. The lack of any of this characteristc group makes polyploidy rare or absent. There are other external polyploidy promoting factors of secondary importance such as, disturbed habitats and physiological stress favouring environments.

There are also secondary factors imposing restrictions to polyploidy. One of them depends on nuclear architecture: a large chromosome/cell size ratio (produced by chromosome duplication) determines a mechanical limitation to proper chromosome segregation. Another factor is the presence of genes preventing failures during meiosis: they reduce the probability of occurrence of non reduced gametes, which are a first necessary step for genomic duplication

The genus *Prosopis* includes about 44 species distributed in arid and semiarid zones of North and South America, Africa and Asia. One of its sections, Algarobia, involves tree and shrubby long living species unable to reproduce vegetatively. Natural hybridization among its species is frequent in zones of sympatry in disturbed environments. However, these related hybridizing species virtually do not differ with respect to chromosomal rearrangements, with the exception of indirect evidences of cryptic structural heterozygosity reducing pollen viability in interspecific hybrids (Naranjo et al. 1994).

This lack of chromosomal differentiation, similarly to what happens in other angiosperms as *Quercus, Ceanotus, Ribes*, etc., may have been one of the main causes of the rarity of polyploidy in this group where the only known species showing diploid and tetraploid races is *P. juliflora*. Populations of this species from Venezuela and Haiti (Hunziker et al., 1977) constitute the only examples of polypolidy so far described in *Prosopis*.

MATERIAL AND METHODS

Population samples

Two samples of *Prosopis ruscifolia* were obtained: ARGENTINA. Formosa Province: Patiño Department, National Road 95, 7-I-1972, Palacios 307-313-321-332-335-478-550-554 (BAFC); and ARGENTINA. Santiago del Estero Province: Avellaneda Department, Herrera, I-1984, Saidman 146-165 (BAFC). One population sample of *P. caldenia* was obtained from ARGENTINA. La Pampa Province: Santa Rosa, 19-III-1981, Saidman and Enus-Zeiger 118-126 (BAFC). Two Colombian populations of *P. juliflora* were sampled at: COLOMBIA. Cundinamarca Departament: Altamira, 27-XI-1981, Hunziker 10039 (SI) and COLOMBIA. Bolivar Department: Cartagena, 27-XI-1981, Hunziker 10040 (SI). The samples consisted of 10 to 15 mother plants separated 50-100 m from each other.

Cytological studies

Mitotic studies

Root tips from germinating seeds were pretreated at 18-22° C for 3 1/2 hours in 0.02 M 8-hydroxiquinoleine solution. The fixations were made in a 3:1 mixture of absolute alcohol and glacial acetic acid for more than 12 h at 4° C. After hydrolysis in 5 N HCl at 20-22° C for 30 min. staining was carried out with Feulgen (2 h) and the material was squashed in 45% acetic acid. The coverslip was removed after freezing with CO_2 and the material was dehydrated in absolute alcohol and mounted in Euparal.

DNA content determinations

DNA content was measured in telophase nuclei (2 C) of the root apex of germinating seeds. Roots of 0.5-1 cm length were fixed in 4% formaldehyde in phosphate buffer for 2 h at 4° C, then washed 24 h in distilled water and transferred to 3:1 (absolute ethanol : acetic acid) for 1-4 days.

After fixation the roots were rinsed for 30 min. in distilled water. Hydrolysis was carried out with 5 N HCl at 20° C. Different times of hydrolysis were investigated and the optimum period was found to be 55 min. They were then given three washes in distilled water for 10 or 15 min., staining for 120 min. in Schiff's reagent pH 2.2 (Teoh and Rees, 1976). The material was then rinsed three times in SO_2 water for 10 min. each, kept in distilled water (5-15 min.) and squashed in 45% acetic acid. The coverslip was removed after freezing with CO_2. The slides were dehydrated in absolute alcohol and mounted in Euparal. The amount of Feulgen staining per nucleus, expressed in arbitrary units, was measured at wavelength of 570 nm using the scanning method in a Zeiss Universal Microspectrophotometer.

The DNA content expressed in pg was calculated using *Allium cepa* var. Ailsa Craig as a standard (2C= 33.55 pg; Bennett and Smith, 1976).

Electrophoresis

Seven isoenzymatic systems: esterase (EST), alcohol dehydrogenase (ADH), glutamate oxalacetate transaminase (GOT), 6-phosphogluconate

dehydrogenase (6PGD), aminopeptidase (AMP), peroxidase (PRX) and superoxide dismutase (SOD), were studied by means of horizontal electrophoresis on starch and polyacrylamide gels, according to the techniques described in (Saidman, 1985).

Depending on the isoenzymatic system soaked seeds (ADH) or cotyledons of seven days old seedlings (remaining systems) were employed, according to Saidman (1985). The number of individuals studied per locus are indicated in Table 3.

Statistical methods

The DNA content of the three species was compared by analysis of variance (nested) of the 2 C values expressed in arbitrary units. Means were compared by Scheffé's method (Scheffé, 1959). The confidence interval for the ratio between the DNA content of *P. juliflora* to that of the other two species was calculated according to Bliss (1967), after previous estimation of the components of the total variance.

Mean heterozygosities (H) and their errors as well as Nei's distances and identities were estimated through the methods proposed by Nei (1987). These estimates were obtained employing the program GENIND (Vilardi, 1992). The cluster analysis from the genetic identity matrix was made by the unweighted pair group method (UPGMA) (Crisci and López Armengol, 1983).

RESULTS

Cytology and DNA content

Prosopis ruscifolia and *P. caldenia* are diploid with 2n= 28 while populations of *P. juliflora* studied here are tetraploid with 2n= 56 (Fig. 1). Chromosomes in all species are very small and no major morphological traits can be described.

The DNA content (2C) was estimated in all species (Table 1) and the results were analysed by ANOVA (Table 2). The ratio of the DNA content of *P. juliflora* with each of the other two species was obtained with their confidence interval at the 5% level (Table 1). Both are significantly lower than the expected value (Table 1) for the tetraploid/diploid DNA ratio. This means that the DNA content per genome (Table 1) is significant lower in *P. juliflora* than expected according to the DNA content of diploid species.

Isozyme studies

Electrophoretic patterns of *P. juliflora* (Fig. 2) were compared with those observed in the species of the Section Algarobia previously studied by Saidman, (1985) and Saidman and Vilardi (1987). In the species studied here 24 isoenzymatic loci could be identified. Almost all of them are homologous in all species. The bands corresponding to the allozymes of *Got-1* and *Got-2* in the diploid species are fixed in *P. juliflora* (Table 3). This suggests that these loci would be duplicated in this species, allowing a permanent heterozygous condition at the enzymatic level. The bands corresponding to the loci *Est-3, Est-4* and *Est-5* stained so lightly in the population of Altamira of *P. juliflora* that they were almost non detectable and their allelic frequencies could not be properly estimated. These loci were excluded from the analysis of genetic variability and from the genetic distance between this population and the rest. The same occured with the loci coding for AMP in the population from Cartagena.

In *P. juliflora* from Altamira only one locus, *Amp-4*, is polymorphic, with three alleles, two active and a null one. The remaining loci in this population usually show the allele most frequent in the other species of the section. Consequently, its genetic variability, estimated by the expected mean frequency of heterozygotes per locus (H= 0.05) and the percentage of polymorphic loci (P= 13%) was very low. However, in the Cartagena population three esterase and three peroxidase loci were polymorphic and therefore the values obtained for this population were similar to those observed in *P. ruscifolia* and *P. caldenia* (Table 4).

Nei's genetic identities and distances were estimated between all pairs of populations (Table 5). The similarities observed between conspecific populations were about 0.97, while the identities between species were significantly lower ($p < 0.05$), ranging from 0.73 to 0.83. In the corresponding phenogram (Fig. 3) South American diploid species are more related to each other than to *P. juliflora*, though these differences were not statistically significant.

DISCUSSION

P. juliflora grows in Colombia and Venezuela in arid, isolated valleys protected from rains. These dry pockets, surrounded by mountains and isolated from each other by several hundred kilometers, are of post-pleistocene origin and most of these populations would be less than 10,000 years old (Solbrig and Bawa, 1975).

Allozymic variation at four systems (MDH, ADH, EST and AMP) in three populations of this species, one from Colombia and two from Venezuela, was previously studied by Solbrig and Bawa

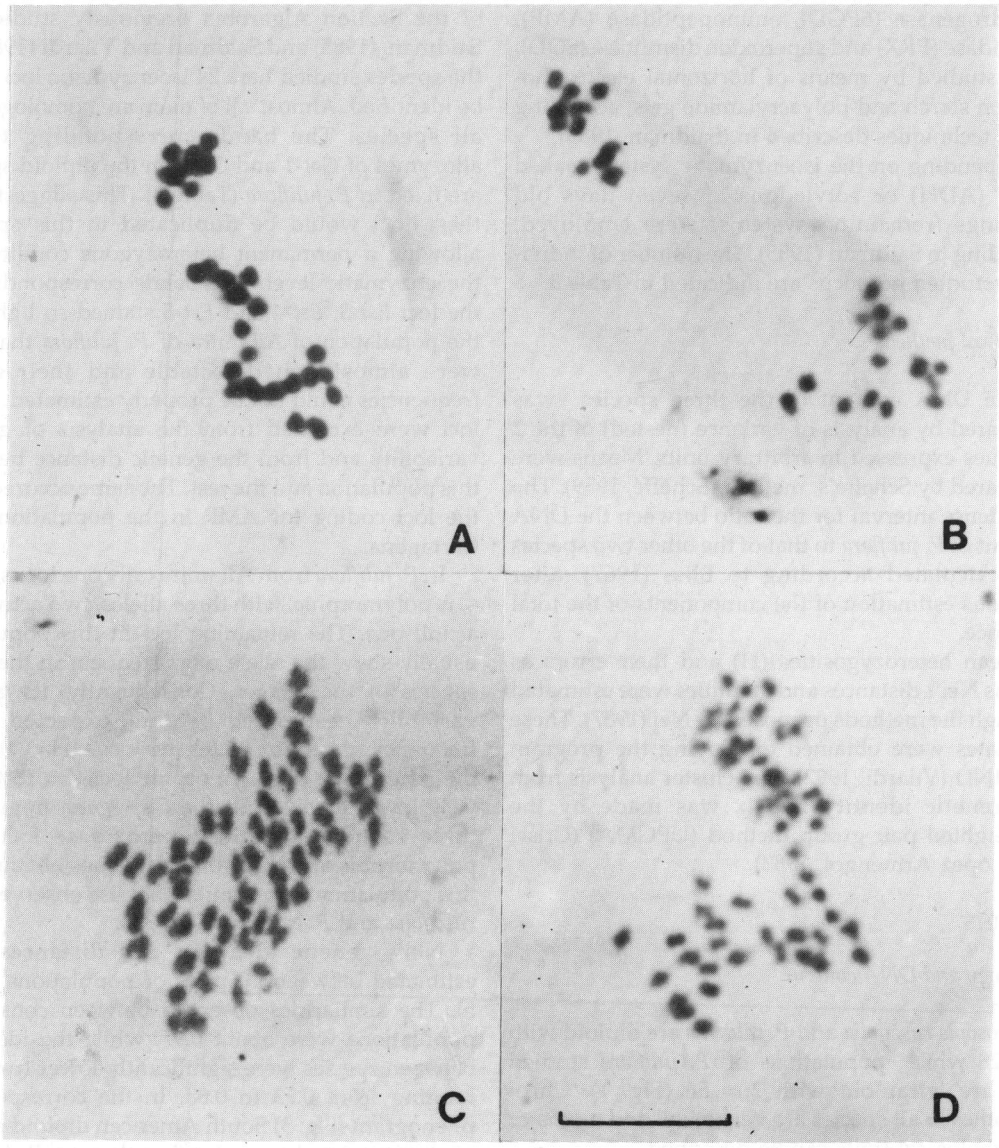

Fig. 1.– Metaphase cells of root tips. *P. ruscifolia* (A) and *P. caldenia* (B) show 2n= 28 chromosomes, while *P. juliflora* (C and D) has 2n= 56. Bar: 10 um.

Table 1.– Chromosome number (2n), total DNA content (2C), DNA content per genome for the studied species and ratio between the DNA content of *P. juliflora* and that of the diploid species

Species	2n	DNA (2C)(pg)	DNA/gen	*P.j./**	(conf.int.)
P. caldenia	28	0.93 +/- 0.04	0.465	1.64	(1.83-1.47)
P. ruscifolia	28	0.90 +/- 0.05	0.450	1.70	(1.93-1.51)
P. juliflora	56	1.53 +/- 0.05	0.383	–	

*(P.j./** : ratio between DNA content of *P.juliflora* and that of the diploid specie).

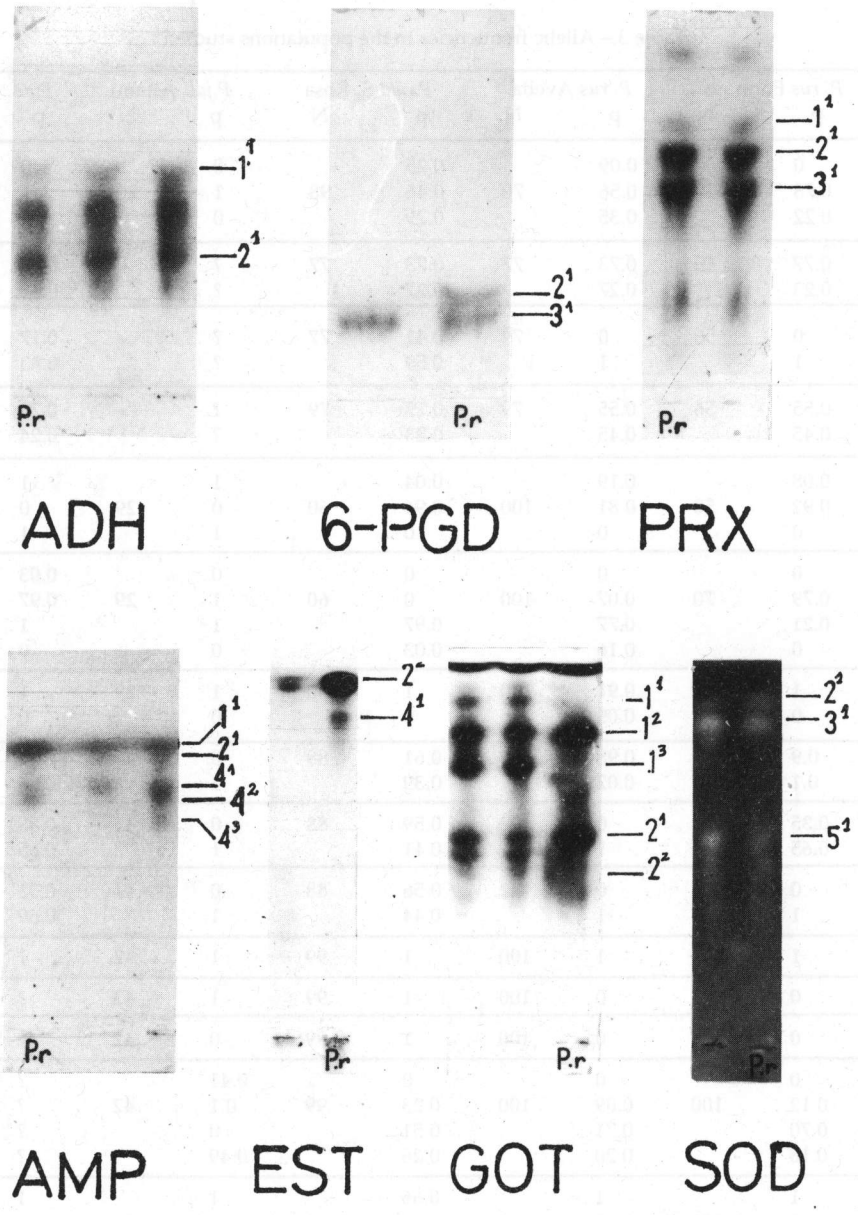

Fig. 2.– Zymograms of the seven systems analyzed in *P. juliflora* (P. r.= individuals of *P. ruscifolia* used as control for band identification).

Table 2.– Analysis of variance to test the differences in total DNA content among species

Source of variation	Degrees of Freedom	Sum of Squares	Mean Squares	F	p
species	2	215.14	107.57	54.47	$<10^{-9}$
individuals	28	55.29	1.97	19.95	$<10^{-9}$
cells	441	43.65	0.09		
total	471	314.08	–		

Table 3.– Allelic frequencies in the populations studied

	P. rus Formosa		*P. rus* Avella.		*P.cald* S. Rosa		*P.jul.* Altami.		*P.jul.* Cartag.	
	p	N	p	N	p	N	p	N	p	N
Est-2^1	0		0.09		0.25		0		0	
Est-2^2	0.78	30	0.56	70	0.46	98	1	20	1	36
Est-2^3	0.22		0.35		0.29		0		0	
Est-3^0	0.77	45	0.73	77	0.73	77	?	–	0.47	36
Est-3^1	0.23		0.27		0.27		?		0.53	
Est-4^0	0	56	0	79	0.41	77	?	–	0.17	36
Est-4^1	1		1		0.59		?		0.83	
Est-5^0	0.55	56	0.55	79	0.77	79	?	–	0.76	36
Est-5^1	0.45		0.45		0.23		?		0.24	
Got-1^1	0.08		0.19		0.04		1		1	
Got-1^2	0.92	50	0.81	100	0.96	60	0	29	0	48
Got-1^3	0		0		0		1		1	
Got-2^0	0		0		0		0		0.03	
Got-2^1	0.79	70	0.07	100	0	60	1	29	0.97	48
Got-2^2	0.21		0.77		0.97		1		1	
Got-2^3	0		0.16		0.03		0		0	
Got-3^1	1	70	0.91	100	1	60	1	29	1	48
Got-3^2	0		0.09		0		0		0	
Prx-1^0	0.9	100	0.98	22	0.61	88	0	41	0.44	41
Prx-1^1	0.1		0.02		0.39		1		0.56	
Prx-2^0	0.35	100	0	22	0.59	88	0	41	0.35	41
Prx-2^1	0.65		1		0.41		1		0.65	
Prx-3^0	0	100	0	22	0.56	88	0	41	0.31	41
Prx-3^1	1		1		0.44		1		0.69	
Amp-1^1	1	100	1	100	1	99	1	42	?	–
Amp-2^1	0	100	0	100	1	99	1	42	?	–
Amp-3^1	0	100	0	100	1	99	0	42	?	–
Amp-4^0	0		0		0		0.41		?	
Amp-4^1	0.12	100	0.09	100	0.23	99	0.1	42	?	–
Amp-4^2	0.70		0.71		0.51		0		?	
Amp-4^3	0.18		0.20		0.26		0.49		?	
Adh-1^1	1		1		0.66		1		1	
Adh-1^2	0	100	0	100	0.28	69	0	28	0	20
Adh-1^{23}	0		0		0.06		0		0	
Adh-2^1	1	100	1	100	1	69	1	28	1	20
6Pgd-2^1	1	100	1	100	0	100	0	20	0	20
6Pgd-3^1	1	100	1	100	1	100	1	20	1	20
6Pgd-4^1	0	100	0	100	1	100	0	20	0	20
Sod-1^1	1	100	1	100	1	100	1	20	1	20
Sod-2^1	1	100	1	100	1	100	1	20	1	20
Sod-3^1	1	100	1	100	1	100	1	20	1	20
Sod-4^1	1	100	1	100	1	100	1	20	1	20
Sod-5^1	1	100	1	100	1	100	1	20	1	20

Table 4.– Variability estimates in the populations studied

Species and Populations	H	P(%)	N loci
P. ruscifolia R. Nac. 95 (Formosa)	0.13 ± 0.04	38 ± 11	21
P. ruscifolia Avellaneda (S.Est.)	0.13 ± 0.04	38 ± 11	21
P. caldenia Santa Rosa (La Pampa)	0.20 ± 0.05	48 ± 10	23
P. juliflora Altamira (Colombia)	0.05 ± 0.03	13 ± 5	20
P. juliflora Cartagena (Colombia)	0.13 ± 0.05	30 ± 10	20

Table 5.- Genetic identities (below diagonal) and distances (above diagonal) between populations.

	1	2	3	4	5
1) *P. ruscifolia* Formosa	-	0.033	0.210	0.319	0.231
2)*P. ruscifolia* Sgo. Estero	0.967	–	0.192	0.279	0.205
3) *P. caldenia* La Pampa	0.811	0.825	–	0.288	0.202
4) *P. juliflora* Altamira	0.727	0.756	0.750	–	0.024
5) *P. juliflora* Cartagena	0.794	0.815	0.817	0.976	–

(1975), who observed very little differentiation despite the large distances between them. Furthermore, in agreement with what was found here in Altamira, they found little variable patterns though unfortunately the ploidy level was not determined in their material.

When comparing different species of the section, almost all loci are homologous and the same alleles are the most frequent in almost all populations. This situation provides an explanation for the low incidence of polyploidy in the section Algarobia. Given the high interspecific similarity, a significant heterosis through hybridization is not expected. Therefore, polyploidy would not be an efficient mechanism to fix advantageous genic combina-

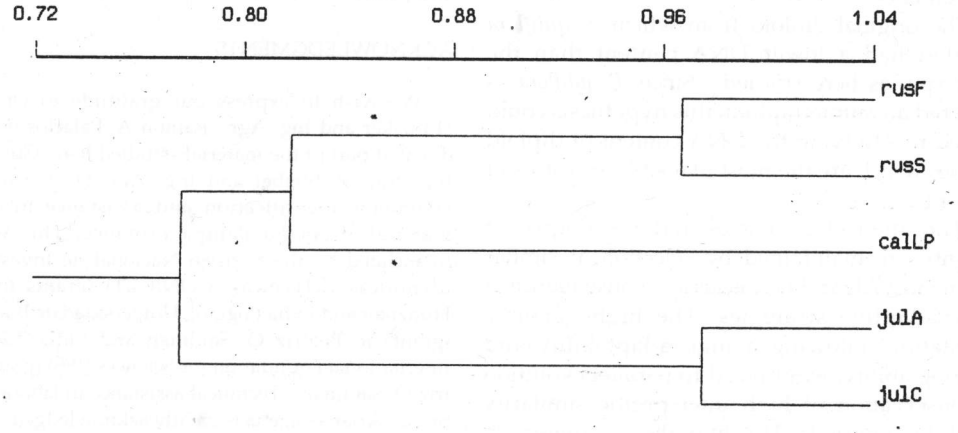

Fig. 3.– Phenogram representative of the relationships among the studied populations as obtained from the genetic identities through UPGMA (rusF: *P. ruscifolia* Argentina. Formosa Province: Patiño Department ; rusS: *P. ruscifolia* Argentina. Santiago del Estero Province: Avellaneda Department, Herrera; calLP: *P.caldenia* Argentina. La Pampa Province: Santa Rosa; julA: Colombia. Cundinamarca Department: Altamira; julC: Colombia. Bolivar Department: Cartagena.).

tions. However, some duplicated loci are observed in *P. juliflora* in which apparently the heterozygosity became fixed at the molecular level (*Got-1* and *Got-2*).

In tetraploids of recent origin the intergenomic diversity (which would be expressed through peptide complementation) is usually higher than the intragenomic (allelic variability = H), while the older tetraploids present a higher intragenomic diversity even more than some diploid species. In *P. juliflora* intergenomic diversity is observed only for GOT loci, but the values estimated for H and P are similar or even lower than those found so far for diploid species in the Section Algarobia. This is compatible with the hypothesis of a recent origin of the tetraploid, which, therefore, would have not yet increased its intragenomic diversity. This is also consistent with the fact that the populations from Colombia and Venezuela of this species are less than 10000 years old. Also in agreement with this assumption is the fact that it is not morphologically distinguishable from diploid cytotypes of the «same species».

Nevertheless, the results from the cytophotometric study are discordant. Recent tetraploids are expected to conserve the same DNA content per genome as the original diploids, while in ancient ones a reduction of this value usually occurs as a response to selective processes tending to fit the cell cycle to the requirements of the species (Bennett and Smith, 1976). In the present work it was found that the diploids *P. ruscifolia* and *P. caldenia* do not differ, while *P. juliflora* shows a reduced amount of DNA per genome. Two hypotheses might be advanced to explain this disagreement.

1) The original diploid from which *P. juliflora* originated had a lower DNA content than the diploid species here studied. Since *P. juliflora* is considered an autotetraploid, this hypothesis could be tested by studying the DNA content of diploid cytotypes which are the most plausible ancestors of tetraploids.

2) The allelic frequencies and the degree of variability are maintained by selection at similar levels in most Algarobia species as a consequence of similar adaptive strategies. The high genomic coadaptation, allowing a high adaptability and colonizing ability, would lead to parallel evolution and conservation of high interspecific similarity through long periods. This hypothesis agrees with the overall high genetic similarity exhibited among the species of this section studied so far. Indeed, the genetic identities among species of Section Algarobia ranged between the values expected for semi- or even subspecies (Saidman, 1985, 1993; Saidman and Vilardi, 1987, 1992). One of the alternative possible explanations for this high similarity among Algarobia species within the Chaco Biogeographic Region (Saidman 1985; Hunziker *et al.*, 1986; Saidman and Vilardi, 1987) is the very frequent hybridization and introgression between them leading to the homogenization of allelic frequencies. This is not a good explanation for the identity (I= 0.73-0.82) here observed between *P. juliflora* and the other two species. These values are about the expected for semispecies (according to Ayala *et al.* 1974) despite the fact that they are completely isolated both genetically (as a consequence of the different ploidy level) and geographically.

These hypotheses are not necessarily exclusive. In both cases, the divergence between *P. juliflora* and Argentine Algarobia species is expected to have occurred during a period longer than that which has elapsed for the divergence within the latter group. Indeed, this divergence must have occurred after polyploidization took place, and the only difference resides in the relative length of these stages. But in both cases, the genetic variability has been maintained with minor changes. In fact the genetic distance between *P. ruscifolia* and *P. caldenia* is not statistically shorter than that between any of them and *P. juliflora*.

Since several facts indicate that in America the principal centre of origin of *Prosopis* species is Argentina (Burkart 1976), *P. juliflora* would have to be recent. The fact that its variability is similar to that of species expected to be older again implies that this degree of variability has been maintained by selection during colonization and species diversification.

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