3. Repeat 2 until all the required links are completed.

Results

Table 2 indicates some results produced by the program. The algorithm attempts to include the same number of ramets from each clone and to avoid duplication of links. In practice, the number of ramets from each clone may vary by one, and some links may be repeated. The design for 10 clones is given in *Figure 2*. It can be seen *(Table 3)* that few links are repeated, and that all clones are represented an (approximately) equal number of times.

The encircled ramet in *Figure 2* is redundant, and may be removed from the design to reduce the repetition of links. As such redundancy occurs infrequently in the designs produced, no checks for this are carried out be the program, so as to keep the program simple.

Application to Forest Tree Breeding

The GRO attempts to attain the maximum number of notional crosses among all clones of interest in a breeding population. This can also be achieved through half-diallel mating or polycross schemes, but provided the identity of the pollen parent is not required, the GRO is an economical alternative.

The design takes no account of the direction of the link between neighbours, and may prove unsatisfactory where steady prevailing winds occur during pollination. More serious problems concern practicalities such as location (Brown and Eldridge 1983), flowering, cone and pollen production (Griffin 1982, Koski 1975, Sweet 1975), fertility, self-incompatibility and seed viability (Giertych 1975, Squillace and Goddard 1982), growth rate, crown size and shape (Giertych 1975), isolation (Denison and Franklin 1975, Griffin 1980, Koski 1975, Werner 1975), and pest control (Miller 1983). Unless these and other practical problems are satisfied, it is unlikely that GROs will be useful.

The algorithm may also be applied with success to orchards with an irregular or non-hexagonal shape, but a greater number of trees will be required to complete the necessary links.

Conclusion

This paper has demonstrated that satisfactory designs for gene recombination orchards can be computed without difficulty. However, the utility of these designs may be limited by practical problems.

Acknowledgements

Thanks are due to Dr. D. G. Nikles for suggesting the problem and encouraging me to publish this solution. I also wish to thank Drs. R. D. Barnes, J. Burley, P. Cotterill, R. L. Eisemann and D. G. Nikles for valuable discussions on the topic.

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Isoenzymatic Studies of Alcohol Dehydrogenase and Glutamate Oxalacetate Transaminase in four South American Species of Prosopis and their Natural Hybrids

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Abstract

The diploid species *Prosopis ruscifolia, P. alba, P. hassleri* and *P. vinalillo* have two genes coding for the dimeric enzyme alcohol dehydrogenase. Each gene specifies a polypeptide with different migration mobilities, and the species have variants resulting from non allelic interaction.

The locus Adh-2 is monomorphic and has a single allele present in *P. alba, P. ruscifolia* and *P. hassleri*. The locus

Adh-1 have two alleles: Adh-1[1] fixed in *P. ruscifolia* and Adh-1[2] fixed in *P. alba* and *P. hassleri*.

Seeds from hybrid trees showed different band patterns. The progeny of putative *P. alba* × *P. hassleri* hybrids showed bands with the same mobilities as in their parents. Seeds from *P. ruscifolia* × *P. hassleri* presented three bands patterns similar to that of either *P. ruscifolia* or *P. hassleri*, or six bands patterns produced by allelic and non allelic interaction.

The multiple forms of glutamate oxalacetate transaminase are controlled by three gene loci, each with two alleles. All the alleles are codominant and the heterozygote produces a more darkly stained enzyme with intermediate mobility between the bands corresponding to each homozygote suggesting that the enzymes have a dimeric subunit structure. The hybrid patterns are similar to those of the parents. Allelic frequencies were different between *P. alba-P. hassleri, P. ruscifolia* and *P. vinalillo*. Though *P. vinalillo* has differential characteristics, it showed patterns that suggest a possible hybrid origin involving *P. alba* and *P. ruscifolia*.

On the basis of the results of ADH and GOT, *P. alba* and *P. hassleri* are more similar to each other than to *P. ruscifolia*. These findings do not entirely agree with the latest taxonomic treatment.

Key words: Alcohol Dehydrogenase, Glutamate Oxaloacetate Transaminase, Isoenzymes, Natural Hybrids, *Prosopis*.

Zusammenfassung

Die diploiden Arten *Prosopis ruscifolia, P. alba, P. hassleri* und *P. vinalillo* haben zwei Gene, die für die dimere Enzym Alcoholdehydrogenase kodieren. Jedes Gen spezifiziert ein Polypeptid mit verschiedener elektrophoretischer Wandergeschwindigkeit, und die Arten weisen Varianten auf, die von nicht allelischen Wechselwirkungen herrühren.

Der Locus Adh-2 ist monomorph und hat ein einziges Allel bei den Arten *P. alba, P. ruscifolia* und *P. hassleri*. Der Locus Adh-1 hat zwei Allele: Adh-1[1] in *P, ruscifolia* fixiert, und Adh-1[2] in *P. alba* und *P. hassleri* fixiert.

Samen von Artbastarden wiesen verschiedene Bandmuster auf. Die Nachkommen möglicher Artbastarde von *P. alba* × *P. hassleri* zeigten Bänder mit den gleichen Wanderungsgeschwindigkeiten der Eltern. Bei Samen von *P. ruscifolia* × *P. hassleri* finden sich drei Bandmuster, die denen von *P. ruscifolia* oder *P. hassleri* ähnlich sind, oder sechs Bandmuster, die das Ergebnis allelischer und nichtallelischer Wechselwirkung sind.

Die vielfältigen Formen von Glutamat-oxalacetat-transaminasen werden von drei Loci kontrolliert, jeder mit zwei Allelen. Alle Allele sind codominant und die Heterozygoten produzieren ein dunkler gefärbtes Enzym mit einer Wanderungsgeschwindigkeit, die zwischen der der Homozygoten liegt. Das deutet daraufhin, daß das Enzym eine dimerische Struktur hat. Die Bandmuster der Artbastarde ähneln denen der Eltern. Die allelischen Frequencen von *P. alba - P. hassleri, P. ruscifolia* und *P. vinalillo* waren verschieden. Wenn *P. vinalillo* auch abweichende Eigenheiten aufweist, lassen die Bandmuster die Entstehung eines möglichen Artbastardes zwischen *P. alba* und *P. ruscifolia* vermuten.

Anhand dieser Ergebnisse von Adh und GOT ist die Ähnlichkeit zwischen *P. alba* und *P. hassleri* untereinander größer als die Ähnlichkeit beider mit *P. ruscifolia*. Diese Resultate stimmen nicht ganz mit der taxonomischen Einteilung überein.

Introduction

The genus *Prosopis (Leguminosae, Mimosoideae)* includes about 44 species (Burkart, 1976). Its distribution area comprises Middle East, Africa and, predominantly, America from South West U.S.A. to Patagonia (Argentina).

The species included in this genus are very important because of their high economic and ecological potential. They may be used to revegetative arid areas with the aims of controlling wind, stabilizing shifting dunes, controlling water run off and its consequent erosion, etc. (Leakey and Last 1980). The legumes of some species have high carbohydrate and protein content with a nutritious value equivalent to that of maize and barley (Habit *et al.* 1981) and the wood from species of Section *Algarobia* is used in furniture manufacture (Habit *et al.* 1981).

The legumes fulfill an important ecological role for the species, allowing the endozoic dissemination. They are eaten by herbivorous animals and the hard seeds germinate freely after passing through the digestive tracts of cattle, sheep, goats and wild animals. Furthermore, the seeds are highly adapted for survival and dispersal (Hauman 1947a, b; Morello 1951; Humphrey 1958).

Most of these species are protogynous (Burkart 1976), outcrosser and autoincompatible (Simpson 1977; Simpson and Solbrig 1977). In Argentine species, namely those referred to in the present, the pollen would not spread over great distances, and trees isolated by just a few hundreds of meters do not fructify (Palacios, pers. comm.).

The taxonomy of *Prosopis* constitutes a very hard problem. Since 1875, Bentham and other authors (Burkart 1940, 1952, 1976; Johnston 1962, etc.) have proposed different and controversial subdivisions for this complex.

As recently as in 1976 Burkart published his monograph based on a very profound study of morphologycal differences between species, proposing a new classification for this genus. This work constituted the starting point of a new series of studies which were not possible before because

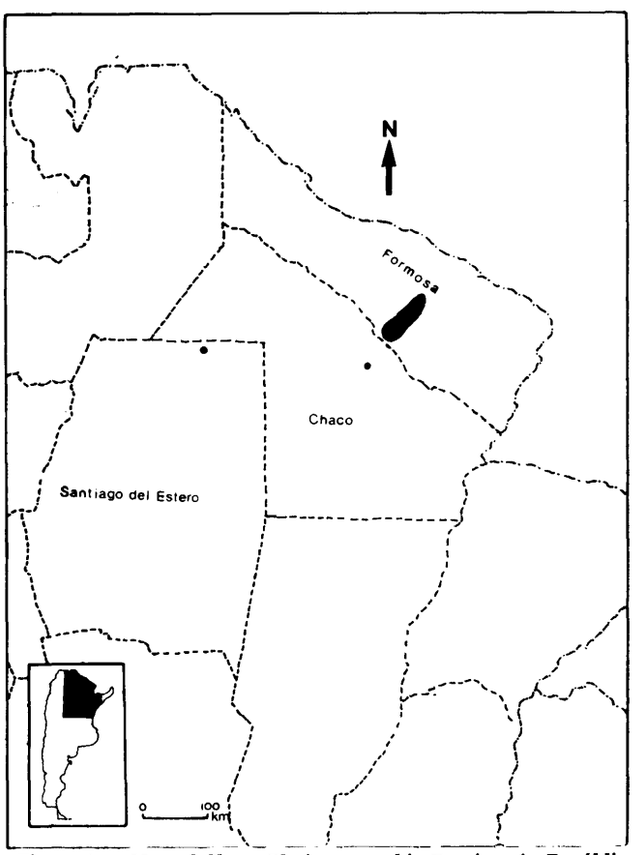

Figure 1. — Map of Chaco Phytogeographic Province in República Argentina indicating the collection sites in Santiago del Estero, Chaco and Formosa Provinces.

of the incertitude in the determination of the species.

The main causes of disagreements in the classification based on morphological traits proceed from the fact that some diploid species belonging to Section *Algarobia* are sympatric in the Chaco Phytogeographic Region *(Fig. 1)* (N. E. Argentina and Paraguay) and hybridization and introgression are frequent between them. These processes create new phenotypes which make the morphological determination very difficult. The hybrids are fertile and introgression could be a source of genetic variation allowing the production of new genotypes which might enable the species to occupy new habitats. Notwithstanding the high intra and interspecific morphological variation existing within the Section *Algarobia*, there is a considerable uniformity for other characteristics. The chromosome number for all diploid species is 2n = 28 (Hunziker *et al.* 1975, 1977). Electrophoretic studies of protein seeds showed high similarity between species (Burghardt and Palacios 1981) and the same occurred in chromatographic studies (Palacios and Bravo 1981; Naranjo *et al.* 1984) where higher similarities than the expected for well differentiated species were found. Therefore, no marker compounds were found which were not shared by two or more species allowing the unequivocal identification of the entities.

The isozyme studies allow the quantification of genetic differences between related species and, in many cases, they have helped in the evaluation of the systematic relationships between species within a genus. This is due to the fact that even in the absence of diagnostic markers of species, the data obtained from this technique may be quantified by means of suitable coefficients which allow the evaluation of genetic distances between different entities. Therefore, this technique might give important information about the phylogenetic relationships between *Prosopis* species, though only a few reports (Solbrig and Bawa 1975; Whitmore and Bragg 1977; Saidman and Nabanjo 1982) are available so far.

There is in Argentina a morphological polymorphism center with an important number (c. a. 28) of species, 13 of wich are endemic. As a part of a wide research plan, isozyme studies were carried out in these species, with the purposes of:

a) Estimating the number of genes and alleles involved in the production of the isoenzymes and their gene frequencies.

b) Detecting, if any, the diagnostic *loci* for species identification.

c) Determining the genetic variability and the degree of speciation attained within different Sections of the genus.

This paper presents the results obtained from the study of two isozyme systems, ADH and GOT, in the species of Section *Algarobia* indicated in *Table 1* and natural interspecific hybrids.

P. ruscifolia and *P. vinalillo* are particulary interesting for different reasons. The former is a colonizer and invader of areas altered by human activity, causing damage to grasslands, decreasing rangeland productivity by competition and exacerbating soil erosion, these being characteristics not shared by the rest of *Algarobia* species (Rodríguez Rebollar 1947, Morello 1970; Morello *et al.* 1971; Morello and Adamoli 1974; Gómez *et al.* 1973). *P. vinalillo*, on the other hand, is a controversial species which is thought to be a hybrid originated from a cross between *P. alba* var. *panta* and *P. ruscifolia* (Burkart 1976). Its morphology is intermediate between its putative parents and meiotic studies did not show significant differences in the mean number of bivalents, chiasmata per cell and chiasmata per bivalent between the parental species and this hybrid whose fertility is almost normal (Hunziker *et al.* 1975).

The objectives of the present work were:

a) To obtain information about the genes involved in the production of the mentioned isozymes.

b) To find species characteristic allozymic markers.

c) The study of hybrid trees involved:

i) Identifying them with certainty on the basis of the isozyme patterns of their offsprings.

ii) Determining through the observation of the different segregating isozyme phenotypes in the offspring, the probable genetic mechanism involved in the production of such isozymes.

Materials and Methods

The sampling areas of Chaco and Formosa Provinces were separated approximately 60 km. from each other and 250 and 300 km. respectively from Santiago del Estero Province sample sites *(Fig. 1).*

The shrubs in all localities are forming impenetrable wild forests of several hundreds of kilometers large. Since all species are strict outcrosser and their pollen does not travel over distances larger than a few hundreds of meters (see Introduction), the shrubs sampled were selected within areas of about 100 ha, being approximately 500—600 m. separated from each other.

The hybrid trees from Formosa Province were collected in peripheral zones where species overlap and enough distant (more than 500 m.) from the sites of collection of non hybrid ("pure") trees.

The number of mother plants sampled was not too high because of the great distances between collection sites and the difficult accesibility to them during the short and unpredictable fructuation period. Nevertheless, Burghardt and Palacios (1981) have observed no differences in the results of electrophoretic studies based on sampling of many trees from those based on seeds from just 5 to 10 mother plants. Furthermore, taking into account the distribution and the reproductive characteristics of the species (see Introduction) the sampling sizes are considered representative of the populations studied.

The origin, herbarium number and amount of seeds analyzed from each tree are indicated in *Table 1.*

Sampling and Germination Techniques

Approximately 30 seeds per species (not included in *Table 1)* were used for essaying different electrophoretic conditions and buffers and also different organs and developmental stages including 24—48 h, seedling and cotyledons, hypocotyles and epicotyles from 7 (or more) days old plants.

The same patterns of GOT bands were observed in all stages and organs while the best results for ADH were attained in 24 h. seedlings, this being the chosen stage to study both systems. Nevertheless, different buffers (see Electrophoresis Techniques) were used for ADH and GOT and therefore, different seeds had to be analyzed for each system.

10 to 40 seeds randomly chosen from several legumes were analyzed from each tree, making a total of 100—120 seeds per species. Approximately half of them were used for GOT and the rest for ADH. The seeds were scarified and germinated in sterilized Petri dishes under continuous light at 25—30° C. Under such conditions roots become apparent in approximately 16 h.. In all species more than

Table 1. — List of the species and hybrids analyzed, herbarium number of the mother plants and number of seeds studied. Materials were collected and taxonomically identified by Prof. R. A. Palacios. Vouchers of all the materials studied are kept in the Herbarium of Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

SERIES	SPECIES	HERBAR. NUMBER	COLLECTION SITE	NO.OF SEEDS
Ruscifoliae	P.ruscifolia Griseb.		Santiago del Estero Copo Department Monte Quemado	
		571		20
		572		20
		573		20
		576		20
		577		20
	P.hassleri Harms		Formosa Patiño Department Estancia La Primavera	
		327		10
		316		10
		322		10
		311		20
		325		20
		320		20
		462		20
Chilenses	P.alba Griseb.		Formosa Patiño Department Natl. Road 95 Bermejo River	
		489		20
		330		20
		329		20
		315		20
		464		20
	P.vinalillo Stuckert		Chaco Güemes Department Natl. Road 95 2 km North El Asustado	
		500		40
		497		40
		483		40
HYBRIDS	P.alba x P.hassleri		Formosa Patiño Department Natl. Road 95	
		469		20
		312		20
	P.ruscifolia x P.hassleri		Formosa Patiño Department Natl. Road 95	
		479		20
		326		20

95% of seeds germinated, but the zymograms of some of them were not clear enough to be included in the statistical analysis.

Electrophoresis Techniques

Electrophoresis was carried out in 2 mm. thick horizontal gels on 105 ml. trays. ADH was studied in 13% starch gel (Connaught Laboratories). Tray buffer was Tris-citrate pH 7 and gels were prepared with tray buffer diluted 1:14 according to Shaw and Prassad (1969) technique. GOT was analyzed in 5% polyacrilamide. Tray buffer was Lithium-borate (pH 8.3; 0.2 M). Gel buffer was composed of Tris-citrate (pH 8.3; 0.2 M): Lithium-borate (pH 8.3; 0.2 M) (9:1) (Scandalios 1969).

Seedlings were squashed in 0.05 ml. buffer gel. The homogenate, absorbed in a 2 × 4 mm. piece of No. 3 Whatman filter paper, was run according to techniques described by Cordeiro (1974). Electrophoresis was carried out at 10 V/cm for approximately 4 h. at 4° C.

ADH was developed according to Shaw and Prassad (1969) while GOT was developed lowering the gel in a solution composed of 0.15 g. fast violet B salt, 0.001 g. pyridoxal-5-phosphate, 0.226 g. L-aspartic acid, 0.037 g. α-ketoglutaric acid and 0.125 g. P. V. 40 in 50 ml. phosphate buffer (pH 7.5; 0.2 M).

Development of both ADH and GOT was carried out at 37° C until the bands appeared. The gels were then fixed in methanol:destilled water:acetic acid (5:5:1). GOT geles were photographed immediately after the development because the bands vanish quickly.

Notation for Bands, Genes and Alleles

Bands were named with the name of the corresponding enzymatic system in capital letters and numbered according decreasing electrophoretic mobilities. Ex. gr. ADH-1, ADH-2, etc.

Loci were named with the name of the electrophoretic system with a first capital letter followed by small letters and numbered according decreasing mobilities of their products. Ex. gr. Adh-1, Adh-2, etc.

Alleles were named with the same symbol of their corresponding loci and an index according decreasing mobilities of their products (bands). Ex. gr. Adh-1^1, Adh-1^2, etc.

Results

ADH

I) *P. alba, P. hassleri and P. ruscifolia*

All individuals showed a pattern composed of three bands which were named ADH-1, ADH-1-3 and ADH-3 in *P. ruscifolia* and ADH-2, ADH-2-3 and ADH-3 in *P. alba* and *P. hassleri.* ADH-3 is present in the three species. ADH-1-3 and ADH-2-3 always have intermediate mobility between ADH-1 and ADH-3 and ADH-2 and ADH-3 respectively *(Fig. 2,* phenotypes 1 and 2; *Fig. 3A).*

Other bands which appeared in some individuals above ADH-1-3 and ADH-3 were not analyzed because their patterns were not constant.

The three bands pattern shown by these species is similar to that found in other species of this genus, *P. glandulosa* var. *glandulosa* and *P. pallida* (Whitmore and Bragg 1979).

II) *P. ruscifolia* × *P. hassleri*

Individuals with three or six bands patterns were found. The former were similar to that of either *P. ruscifolia* or *P. hassleri.* The latter had five bands which showed the same relative mobilities as ADH-1, ADH-2, ADH-1-3,

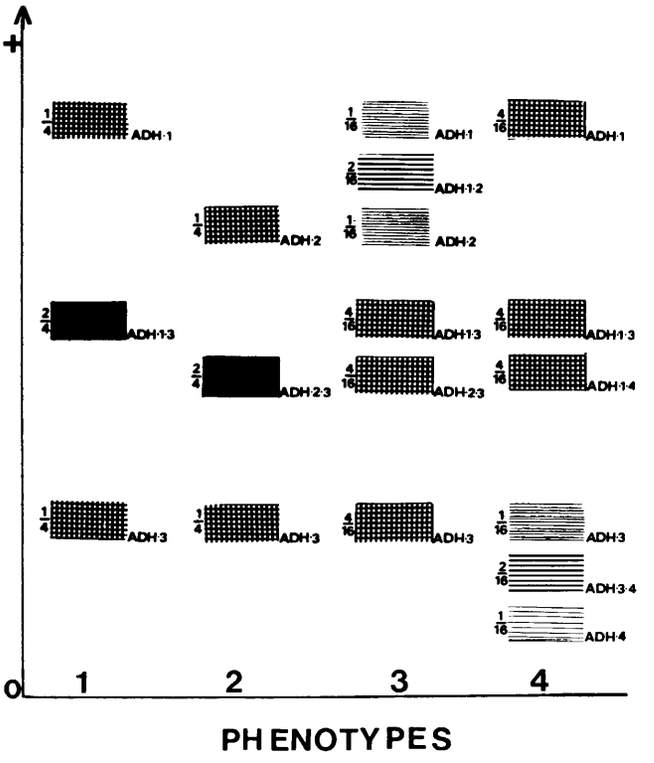

Figure 2. — Scheme of the different phenotypes of ADH found in *Prosopis* species and hybrids. The numbers at the left side of the bands indicate relative band intensities.

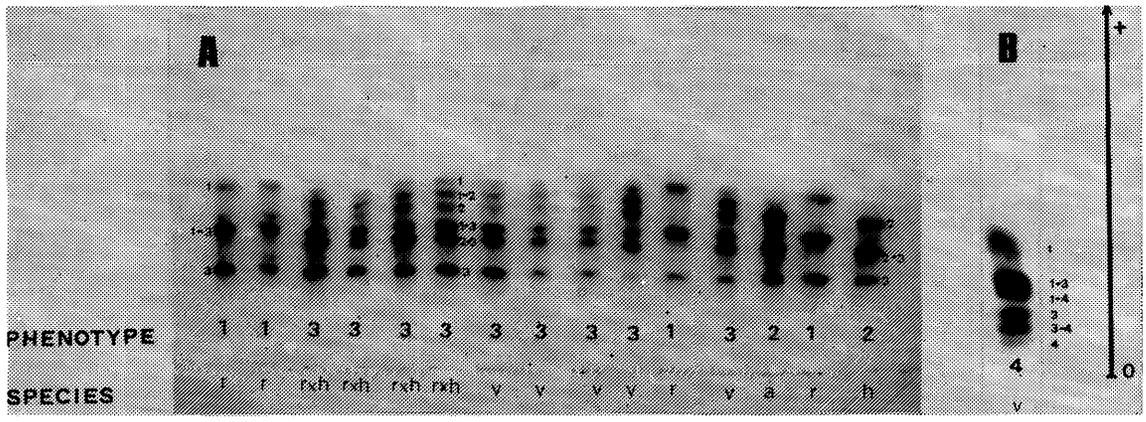

Figure 3. — A) Zymogram showing isoenzymatic variants of ADH found in different species and hybrids. B) Zymogram of an individual of *P. vinalillo* heterozygous for Adh-2 gen. The numbers near the origin (phenotypes) are referred to those of *Fig. 2.* r: *P. ruscifolia,* a: *P. alba,* h: *P. hassleri,* v: *P. vinalillo,* r × h: *P. ruscifolia* × *P. hassleri.*

ADH-2-3 and ADH-3 and a new band which migrated between ADH-1 and ADH-2 and was named ADH-1-2 *(Fig. 2,* phenotype 3; *Fig. 3A).*

III) *P. alba* × *P. hassleri*

All individuals showed the bands ADH-2, ADH-2-3 and ADH-3 characteristic of *P. alba* and *P. hassleri,*

IV) *P. vinalillo* (putative hybrid between *P. alba* and *P. ruscifolia)*

The individuals showed either three or six bands patterns. Some of the former were similar to that of *P. ruscifolia* and others to that of *P. alba* (or *P. hassleri).* Most of the six bands phenotypes were like the ones observed in the hybrids *P. ruscifolia* × *P. hassleri.* Nevertheless, the pattern was different in three individuals which showed the bands ADH-1, ADH-1-3, ADH-1-4, ADH-3, ADH-3-4 and ADH-4. Three of these bands are similar to those found in *P. ruscifolia* (ADH-1, ADH-1-3, ADH-3); the band ADH-4 is slower than ADH-3; ADH-1-4 and ADH-3-4 have intermediate mobility between ADH-1 and ADH-4 and ADH-3 and ADH-4 respectively *(Fig. 2,* phenotype 4; *Fig. 3B).*

GOT

I) *P. alba, P. ruscifolia* and *P. hassleri*

Three zones with GOT activity were detected. They were named in order of decreasing anodic mobility GOT I, GOT II and GOT III. Every individual showed one or three bands in each zone. When three bands were present, the one with intermediate mobility was more intensely stained than the others *(Fig. 4* and *5).* Therefore, it was assumed that each zone was coded by one gene with two alleles which produces a dimeric enzyme. The band showing intermediate mobility would be the allodimer formed by random accociation of two monomeres coded by different alleles of an heterozygote.

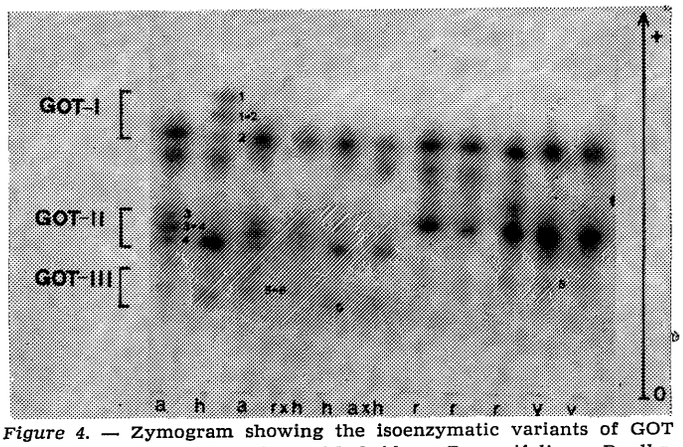

Figure 4. — Zymogram showing the isoenzymatic variants of GOT found in the different species and hybrids. r: *P. ruscifolia,* a: *P. alba,* h. *P. hassleri,* v: *P. vinallilo,* a × h: *P. alba* × *P. hassleri,* r × h: *P. ruscifolia* × *P. hassleri.*

On the basis of this assumption the bands were named: GOT-1, GOT-1-2, GOT-2, GOT-3, GOT-3-4, GOT4, GOT-5, GOT-5-6 and GOT-6. In some individuals GOT III zone was so slightly stained that its study was very difficult and sometimes band intensity ratio departed from the expected for a dimeric enzyme (1/4 GOT-5 : 2/4 GOT-5-6 : 1/4 GOT-6). Besides these bands, several others appeared immediately above and below the first group. These were not analyzed because their pattern was not clear enough. Each zone was thought to be coded by one gene (respectively Got-1, Got-2 and Got-3) each with two alleles (Got-1^1, Got-1^2 and son on).

No major differences between species were found for Got-1, since the genotype Got-$1^{1/1}$ (homozygote for Got-1^1) showed the lowest frequency in all of them *(Table 2).* *P. ruscifolia* presented a higher frequency of fast bands for Got-2 and Got-3 while *P. alba* and *P. hassleri* showed a high amount of Got-$2^{2/2}$ and Got-$3^{2/2}$ individuals *(Table 2).* The differences were analyzed by contingency tables for each locus. Got-1 showed no significant difference between species ($\chi^2 = 1.99$; $p = 0.37$) while in Got-2 and Got-3 the differences were highly significant ($p \cong 0$) ($\chi^2 = 70.38$ and 74.75 respectively) *(Table 2).* When comparing *P. alba* with *P. hassleri* the differences were non significant for both Got-2 ($\chi^2 = 1.65$; $p = 0.43$) and Got-3 ($\chi^2 = 0.41$; $p = 0.81$) *(Table 2).*

II) *P. ruscifolia* × *P. hassleri, P. alba* × *P. hassleri* and *P. vinalillo*

The patterns were similar to those found in pure species *(Fig. 4* and *5).*

Discussion

On the grounds of ADH results it may be assumed that this enzyme is dimeric in *Prosopis,* as in the cases of wheat (Hart 1970), *Clarckia franciscana* (Gottlieb 1974), maize (Schwartz 1975) and other species, and it would be coded by two genes. Each gene would produce a different homodimer, and a heterodimer would be produced by non allelic interaction between them *(Fig. 2,* phenotypes 1 and

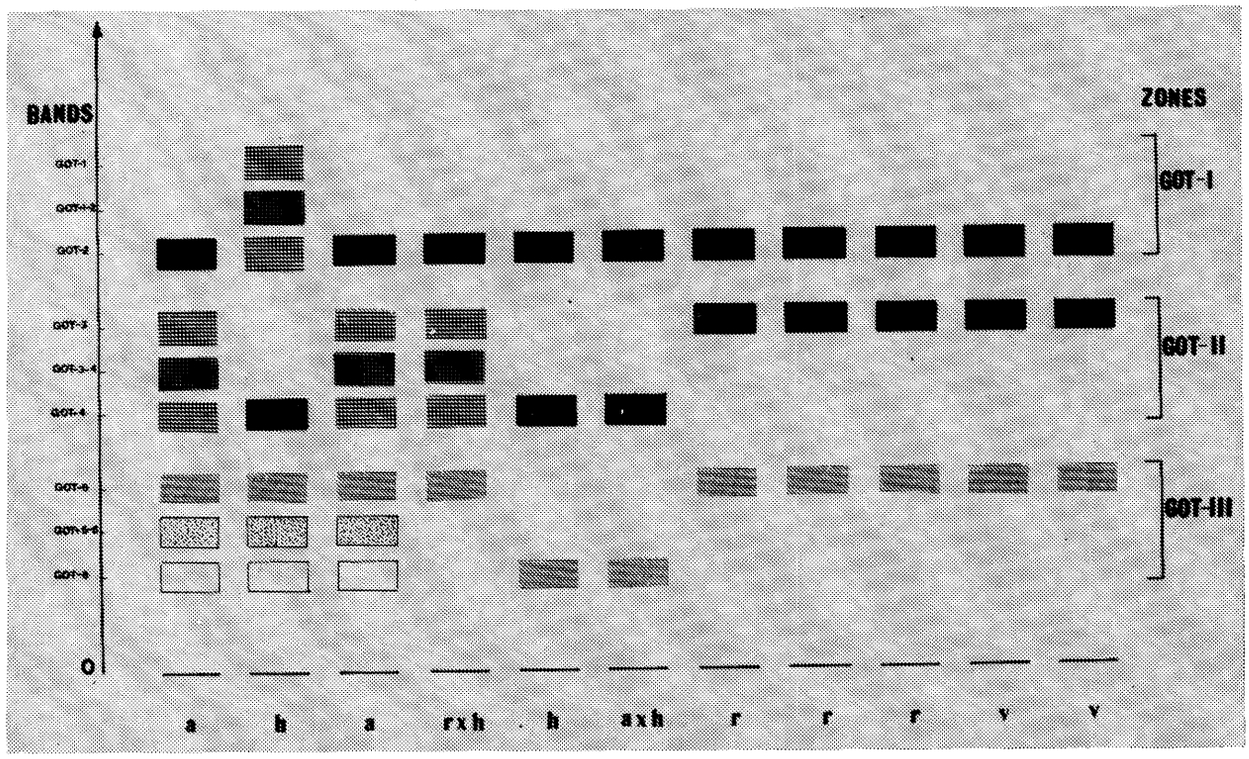

Figure 5. — Scheme of the bands analyzed in the zymogram of *Fig. 4.*

2). These two loci were named Adh-1 and Adh-2 respectively. The former would have two alleles: Adh-1^1 fixed in *P. ruscifolia* and Adh-1^2 fixed in *P. alba* and *P. hassleri.* Adh-2 is monomorphic and common to the three species *Fig. 2,* phenotypes 1 and 2). The relative staining of bands was similar to the expected for a dimeric enzyme in all individuals of *P. alba* and *P. hassleri,* i.e., 1/4 ADH-2 : 2/4 ADH-2-3 : 1/4 ADH-3. In most individuals of *P. ruscifolia* the intensity ratio was also close to the expected (1/4 ADH-1 : 2/4 ADH-1-3 : 1/4 ADH-3), though in several individuals ADH-1 intensity was lower than the expected *(Fig. 3A).*

The hypothesis that ADH is a dimeric enzyme was supported by the results of the study of seedlings from trees taxonomically identified as natural hybrids. Since these seedlings were produced by non controlled crossings they may be the result of 1) back-cross with one of the parents, 2) cross with another hybrid or 3) cross with an individual from a third species. Therefore, individuals showing either hybrid or progenitor like patterns can be obtained from the same tree.

Table 2. — Contingency table for different genotype frequencies corresponding to the loci Got-1, Got-2, and Got-3 in *P. ruscifolia, alba* and *P. hassleri.*

	P. ruscifolia	*P. alba*	*P. hassleri*	global χ^2	global *p*	*P. al.* vs. *P. hass.* χ^2	*P. al.* vs. *P. hass.* *p*
Got-$1^{1/1}$	0	0	0				
Got-$1^{1/2}$	5	2	5	1.93	0.37	----	----
Got-$1^{2/2}$	35	44	41				
Got-$2^{1/1}$	32	4	2				
Got-$2^{1/2}$	8	11	16	70.38	~0	1.65	0.43
Got-$2^{2/2}$	2	28	26				
Got-$3^{1/1}$	33	3	2				
Got-$3^{1/2}$	8	9	11	74.75	~0	0.41	0.81
Got-$3^{2/2}$	0	27	26				

According to the assumed hypothesis, the hybrids *P. ruscifolia* × *P. hassleri* could show a maximum of six bands in the case of an heterozygous seed for the locus Adh-1. This individual would form: a group of three bands (ADH-1, ADH-1-2 and ADH-2) as a result of allelic interaction between Adh-1^1 and Adh-1^2; other two bands (ADH-1-3 and ADH-2-3) by non allelic interaction between Adh-1^1 and Adh-2 and Adh-1^2 and Adh-2 respectively; and finally the monomorphic ADH-3 band common to the three species. This agrees with what was observed in some seeds from putative hybrids.

In the six bands pattern seeds the locus Adh-1 is in heterozygous condition while Adh-2 is homozygous. Consequently such seeds have one Adh-1^1, one Adh-1^2 and two Adh-2 genes per diploid genome. If polypeptidic synthesis, association and isoenzymatic expression were equivalent for all product of these genes, the expected intensity ratio for the six bands produced in individuals of hybrid origin *(P. ruscifolia* × *P. alba* and *P. ruscifolia* × *P. hassleri)* would be: 1/16 ADH-1 : 2/16 ADH-1-2 : 1/16 ADH-2 : 4/16 ADH-1-3 : 4/16 ADH-2-3 : 4/16 ADH-3 *(Fig. 2,* phenotype 3; *Fig. 3).* Therefore, the confirmation of such relative intensities would allow the assumption that the isozyme regulation is the same in the different species involved in the hybrids studied and that their polypeptidic subunits have the same affinity (Gottlieb 1974). Nevertheless, some individuals of *P. ruscifolia* showed lower intensity of band ADH-1 than the expected *(Fig. 3A).* This fact was also observed in some hybrid seeds where *P. ruscifolia* would have been one of the parents *(Fig. 3A).* This quantitative variation of the Adh-1 product could be caused by the following pheno-

mena: i) changes in the aminoacid sequence of polypeptidic chain without changes in electrophoretic mobility of molecules but causing alterations in the specific activity; ii) different amounts of enzymatic molecules with the same structure; iii) production of enzymes in the same amount and with the same catalytic activity but different factors in cell environment of individuals produce a differential activity of enzymes. Discriminating among these possibilities falls out of the scope of the present paper, but this differential activity, whatever the factor involved, suggests a divergency between the allel fixed in *P. ruscifolia* (Adh-1^1) from that of *P. alba* and *P. hassleri* (Adh-1^2) which did not show such quantitative variation.

The fact that the products of two different genes maintain their high affinity producing heteropolymeres suggests that both loci arose by duplication of a common ancestor gene, and that such duplication would have a relatively recent. origin. As the different evolutive forces operate, differential mechanisms of gene regulation are being developed and the duplicated genes become genes coding for different enzymes with different regulatory systems (Ohno 1970). Perhaps, the species of Section *Algarobia* are in early stage of the gene differentiation of this isoenzymatic system since the loci Adh-1 and Adh-2 maintain yet a high homology.

The presence of a three bands pattern in all individuals suggests an adaptive strategy based on fixation of permanent heterozygosity at enzymatic level. A similar pattern was found in *P. juliflora* (Solbrig and Bawa 1975), a species that could be a permanent heterozygote for ADH. Unfortunately, there is some uncertainty about this case because *P. juliflora* has diploid and tetraploid races (Hunziker *et al.* 1975, 1977) and the actual level of ploidy was not determined in the material used in the mentioned work. If the individuals studied by Solbrig and Bawa (ibid.) had been tetraploid, the heterozygosity could have been a consequence of polyploidy.

Permanent heterozygosity is though to be advantageous because it allows polypeptidic complementation without segregational load, with the possibility that divergence between the polypeptids leads to a differential specialization (Gottlieb 1974). Nevertheless, this strategy may also be restrictive as the species can not respond to changing selective pressures through varying allelic frequencies, and so, the lack of allelic diversity could restrain the adaptability to environmental heterogeneity (Fincham 1972).

The isoenzymatic system ADH is very interesting since it helps to differentiate *P. ruscifolia* from *P. alba* and *P. hassleri* and to recognize the parents of putative hybrids or introgressants.

GOT results would indicate that each group of three bands is produced by different genes, each having two alleles coding for a dimeric enzyme. These genes and alleles would be the same for the three species since no new combination (different from the parental ones) was found when analyzing hybrids or introgressants.

The allelic frequencies *(Table 3)* demonstrate that all species are polymorphic using the 1% criterion for Got-1, Got-2 and Got-3. The mean proportion of heterozygous loci per individual ($\overline{H}_i$) (Nei 1975) was calculated for each species using the data from the two systems analyzed in the present paper. Since the proportion of heterozygous loci of an individual is a continuous variable, its mean values ($\overline{H}_i$) for the three species *(P. ruscifolia* = 0.095, *P. alba* = 0.079 and *P. hassleri* = *0.127)* were compared by

Table 3. — Allelic frequencies of Got-1, Got-2 and Got-3 loci in *P. alba* and *P. hassleri.*

	P. ruscifolia	*P. alba*	*P. hassleri*
Got-1^1	0.063	0.022	0.055
Got-1^2	0.937	0.978	0.945
Got-2^1	0.857	0.220	0.227
Got-2^2	0.143	0.780	0.773
Got-3^1	0.903	0.192	0.192
Got-3^2	0.097	0.808	0.808

Table 4. — Analysis of variance for the comparison of $\overline{H}_i$ (mean proportion of heterozygous loci per individual) in *P. ruscifolia, P. alba* and *P. hassleri.*

Source of Variation	Degr.of Freedom	Sum of Squares	Mean Squares	F	p
Species	2	0.052	0.026	0.99	0.37
Error	136	3.317	0.024	----	----
Total	138	3.369	----	----	----

analysis of variance *(Table 4).* The results indicated that $\overline{H}_i$ is not statistically different between species ($F_{2,126} = 0.99$; $p = 0.37$).

P. vinalillo is thought to be a natural hybrid originated from a cross between *P. ruscifolia* × *P. alba* var. *panta* (Burkart 1976). Its morphology is generally intermediate between its putative parents. Nevertheless, no significant differences in meiotic behaviour were found between the parental species and the hybrid and its fertility was almost normal (Hunziker *et al.* 1975). The study of 80 seedlings from two trees (No. 497 and No. 500) showed some patterns similar to those expected for a hybrid between *P. ruscifolia* × *P. alba* (or *P. hassleri)* and others similar to those of *P. ruscifolia.* This leads to the assumption that the trees were hybrids between *P. alba* (or *P. hassleri)* and *P. ruscifolia* and the seeds were originated by introgression with *P. ruscifolia.* In some individuals of *P. vinalillo* with phenotypes having the ADH-1 band, this, as occurred in *P. ruscifolia,* showed lower intensity than the expected. One tree (No. 483) fitted the Burkart's (1976) description of *P. vinalillo* (Palacios, pers. comm.) and its seedlings presented the expected zymograms for *P. alba* (or *P. hassleri), P. ruscifolia, P. ruscifolia* × *P. alba* (or *P. hassleri)* and *P. ruscifolia* × an individual which has a new band (ADH-4), produced by a new allele, Adh-2^2 *(Fig. 2,* phenotype 4; *Fig. 3B).* These findings would indicate: a) the presence of a new mutant, b) an incipient polymorphism in some of these species or c) the new allele came from another sympatric species, not studied in this paper. In any of these cases, the unknown parent would have to show a three bands pattern: ADH-1, ADH-1-4 and ADH-4.

In spite of the evidences about the hybrid origin of *P. vinalillo* from *P. ruscifolia* × *P. alba* (or *P. hassleri),* this species showed characteristics not observed in its putative parents: in some individuals ADH-3 and ADH-4 *(Fig. 3A, B)* were less stained than the expected. This would indicate an incipient divergence for ADH of this species with respect to the possible parents.

Twenty two individuals were heterozygotes (Got-$1^{1/2}$) for GOT I zone and 58 were Got-$1^{2/2}$, the heterozygote frequency being higher than in presumptive parents. Only homozygotes carrying the fast alleles were found for GOT

II and GOT III (i.e. Got-$2^{1/1}$ and Got-$3^{1/1}$ genotypes). Since the 80 seedlings analyzed originated from two mother plants, gene frequencies can not be properly estimated from these samples. Nevertheless, these results indicate that the alleles Got-2^1 and Got-3^1 would have reached fixation in *P. vinalillo*, while the locus Got-1 remains polymorphic as in the other species.

The information obtained in this work could be valuable for comparison of the species analyzed. On the basis of the results of ADH and GOT, *P. alba* and *P. hassleri* are more similar to each other than to *P. ruscifolia*. These findings do not agree with the classification proposed by BURKART (1976) since it places together *P. hassleri* and *P. ruscifolia* in Series *Ruscifoliae* and *P. alba* as belonging to Series *Chilenses*. Disagreements between morphological and molecular data could be due to the fact that genetic distances or similarity indexes are trustworthy only when they are obtained from a high number of loci, and few genes were so far studied. However, similar conclusions were attained studying seed proteins (BURGHARDT and PALACIOS 1981) and phenol chromatography (PALACIOS, pers. comm.) and from field observations (PALACIOS, pers. comm.) where *P. alba* and *P. hassleri* show a high degree of hybridization.

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