Isozyme Studies on Hybrids Swarms of Prosopis caldenia and Sympatric Species

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Summary

The Section Algarobia of genus *Prosopis* includes a group of species which hybridize and possibly introgress in zones of sympatry, the hybrids being highly fertile. The isolating mechanisms are not known so far. The system glutamate oxalacetate transaminase (GOT) was studied in *P. caldenia, P. alpataco* and *P. flexuosa* in samples obtained from pure parental populations and from sympatry zones where hybrid swarms occur (Chacharramendi, La Pampa, República Argentina). Three GOT loci were detected showing qualitative and quantitative differences between *P. caldenia* and the other two species. The analysis of samples from hybrid swarms demonstrate that the pollen of *P. caldenia* is able to fertilize *P. alpataco* and *P. flexuosa* producing hybrid seeds. However, no adult hybrids involving *P. caldenia* were found in the field and no hybrid seeds were obtained from *P. caldenia* mother plants. These evidences suggest that the hybrids between *P. caldenia* and the other two species do form but can not develop and that *P. caldenia* is not fertilized by alien pollen. This information indicates the existence of an incipient isolation mechanism in the locality studied.

Key words: P. caldenia, P. flexuosa, P. alpataco, hybrid swarm, isozymes.

Introduction

The section Algarobia of genus *Prosopis* includes a group of species which hybridize and possibly introgress in zones of sympatry, the hybrids being highly fertile.

The reproductive systems and isolation mechanisms were studied in only a few species so far. The group is apparently characterized by protogyny (Burkart, 1976), and some species, like *P. chilensis, P. velutina* and *P. flexuosa,* have autoincompatibility systems, which indicate that they would be obligate outcrosser (Simpson, 1977; Simpson and Solbrig, 1977). Though direct evidences are lacking for the rest of the species, this condition is assumed to be of general occurrence in the group. Supporting this assumption is the fact that trees isolated by just a few hundred meters do not form fruits (Ing. Agr. Palacios, pers. comm.). On the other hand the isolating mechanisms related with species differentiation are not known, excepting the observation of a reducing in pollen viability in hybrids between *P. affinis* and *P. nigra* (Naranjo *et al.*, 1984).

Isoenzyme studies at 25 loci in 8 species of Algarobia (Saidman, 1985; Saidman and Vilardi, 1987) revealed an overall high genetic similarity among them, *P. caldenia* being the most easily identifiable species on the basis of its isozyme patterns. The electrophoretically most similar species to *P. caldenia* were *P. flexuosa* and *P. alpataco.* These three species are clearly separated morphologically (Burkart, 1976), living sympatrically in La Pampa province (Argentina).

One of the systems which showed clear-cut differences between *P. caldenia* and the other two species was glutamate oxalacetate transaminase (GOT). In the present work, the patterns of this system were analysed in seeds from parental populations and hybrid swarms, with the aim of detecting which of these species are able to participate in interspecific hybrids.

Materials and Methods

The material used in this study is composed by samples obtained at three localities (*Table 1, Figure 1*) involving three parental populations and a hybrid swarm. The collection sites are located in La Pampa Province (República Argentina). In the locality of Chacharramendi two areas were sampled, one of them contained only *P. alpataco* shrubs and the other had a hybrid swarm with the three species (*P. alpataco, P. flexuosa* and *P. caldenia*) coexisting. The parental trees sampled near the hybrid swarm were very near to one another in such a way that branches from different plants (belonging to different species) were in contact. This condition increased the probability of collecting seeds of hybrid origin.

In the hybrid swarm, the mother plant was identified both morphologically and chromatographically by Dr. Carlos A. Naranjo and Lic. Silvia Enus-Zeiger in order to determine to which species (or hybrid combination) it belonged.

The number of shrubs or trees sampled in the parental populations averaged (8 to 10) was estimated by Vilardi *et al.* (1988) as an ideal in order to get good estimations of the variability of the species belonging to Sect. Algarobia. Samples were obtained from plants approximately 40 m to 50 m separated from each other.

Electrophoretic procedures, development and notation of bands, genes and alleles are described elsewhere (Saidman, 1985, 1986).

Results

A) *P. alpataco, P. flexuosa and P. caldenia from parental populations*

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. Therefore, it was assumed that GOT is a dimeric enzyme and that each zone is coded

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Table 1. — List of the species and hybrids analyzed, herbarium number of the mother plants and number of seeds studied. Vouchers of all the materials studied are kepts in the Herbarium of Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires

Species	Herbar. Number	Collection Site	No. of Seed Aanalysed
P. caldenia Burkart		La Pampa: Santa Rosa, Ecculea Agrotécnica	
	118		8
	119		8
	120		8
	121		8
	123		8
	124'		8
	125		7
	126		5
Hybrid swarm		La Pampa: Dept. Utracán, Chacharramendi	
	104		20
	115		20
	116		20
	117		20
P. flexuosa De Candolle		La Pampa: Dept. Cura-Có, Salar La Amarga	
	127		8
	128		5
	129		8
	131		8
	132		6
	133		9
	135		8
	136		8
Hybrid swarm		La Pampa: Dept. Utracán, Chacharramendi	
	100		20
	103		20
	105		20
	106		20
	107		20
P. alpataco Philippi		La Pampa: Dept. Utracán, Chacharramendi	
	101		9
	102		9
	108		9
	109		9
	111		9
	112		10
	170		5
Hybrid swarm		La Pampa: Dept. Utracán, Chacharramendi	
	171		50
	172		50
P. flexuosa × *P. alpataco*		La Pampa: Dept. Utracán, Chacharramendi	
	110		20
	113		20
	114		20

by one gene with two alleles. The band showing intermediate mobility would be the allodimer formed by random association of two monomers coded by different alleles of an heterozygote.

On the basis of this assumption the bands were named as indicated in *Figure 2*. *P. alpataco* and *P. flexuosa* show-

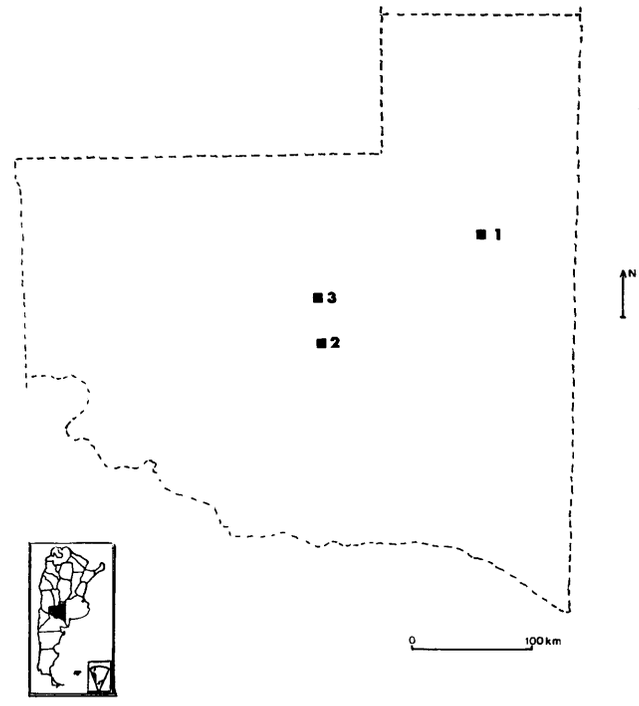

Figure 1. — Map of La Pampa Province in República Argentina indicating the collection sites. 1: Esculela Agrotécnica. 2: Chacharramendi, 3: Salar La Amarga.

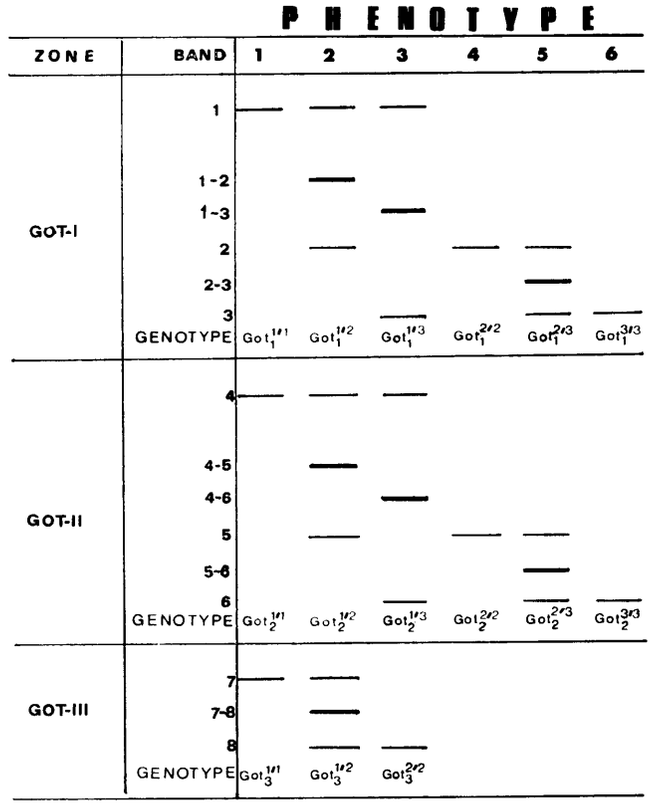

Figure 2. — Scheme of different phenotypes of GOT found in the species and hybrids analyzed.

Figure 3. — Zymograms showing the isoenzymatic variants of GOT in different species and hybrids. a: *P. alpataco*, f: *P. flexuosa*, fxa: *P. flexuosa* × *P. alpataco*.

Figure 4. — Zymogram of *P. caldenia* and seeds collected in the hybrid swarm. C: P. *caldenia*. Hybrid swarm, a: *P. alpataco*, f: *P. flexuosa*, c: *P. caldenia*.

ed the patterns (phenotypes) 4, 5 or 6 for GOT I; 1, 2, or 4 for GOT II and 1, 2 or 3 for GOT III. *P. caldenia* presented 2 or 4; 4 or 5 and 2 for GOT I, GOT II and GOT III respectively (*Figure 2*).

Each zone was thought to be coded by one gene (respectively Got-1, Got-2 and Got-3). Got-1 and Got-2 would have three alleles, Got-1^3 and Got-2^1 are present only in individuals of *P. alpataco* and *P. flexuosa*, while Got-1^1 and Got-2^3 were found only in *P. caldenia*. Got-3 was monomorphic in *P. caldenia* showing only the allele Got-3^2 and polymorphic in the other two species, where Got-3^1 was also found (*Figures 2, 3, 4*). The bands GOT-6 and GOT-7 produced respectively by Got-2^3 and GOT-3^1 have the same mobility hence, if both were present in the same individual (see the section about hybrid swarm), they would yield a very deeply staining band.

Table 2. — Allelic frequencies of Got-1, Got-2 and Got-3 loci in *P. alpataco*, *P. flexuosa* and *P. caldenia*.

Alleles	*P. alpataco* (60)[a]	*P. flexuosa* (60)[a]	*P. caldenia* (60)[a]
Got-1^1	0.000	0.000	0.042
Got-1^2	0.715	0.442	0.958
Got-1^3	0.285	0.558	0.000
Got-2^1	0.110	0.285	0.000
Got-2^2	0.890	0.715	0.967
Got-2^3	0.000	0.000	0.033
Got-3^1	0.425	0.392	0.000
Got-3^2	0.575	0.608	1.000

a) No. of seeds analysed

Allelic frequencies of GOT I, GOT II and GOT III are shown in *table 2*.

B) P. alpataco × *P. flecuosa* (*Natural Hyprids*)

Since the hybrids between *P. alpataco* and *P. flexuosa* showed the same patterns as the parental species, it was assumed that the same loci and alleles are present in them (*Figure 3*).

C) P. alpataco, P. flexuosa and P. caldenia sampled in the hybrid swarms

The seeds collected from trees identified on morphological and cromatographic grounds as *P. alpataco* and *P. flexuosa* showed all the patterns described in *Figure 2*, that is, they include also bands characteristic of *P. caldenia* (*Figure 4*). On the other hand, when analysing the patterns of seeds from *P. caldenia* trees, only the bands characteristic of this species were found.

Discussion

Allelic frequencies of *P. alpataco* and *P. flexuosa*, are different from *P. caldenia*. The presence of seeds carrying the alleles characteristic of *P. caldenia* collected from *P. flexuosa* and *P. alpataco* plants (in the hybrid swarm) indicates that the pollen of *P. caldenia* is able to fertilize these sympatric species in the locality of Chacharramendi. However, apparently *P. caldenia* is not fertilized by alien pollen, since no "hybrid seed" (according to GOT patterns) was collected from *P. caldenia* mother plants.

Despite this evidence indicating that, though limited to one direction, hybridization do occur between *P. caldenia* and the other two species, no adult morphohybrids were found in this locality involving *P. caldenia* as one of the putative parents (Naranjo and Enus-Zeiger, 1983). This suggests that plants from hybrid seeds do not reach the adult stage in this locality.

The present results indicate the existence of an incipient isolation mechanism between *P. caldenia* and the other two species. This isolation is mediated by a prezygotic (prevention of heterologous pollination), and postzygotic (prevention of development of hybrid seeds or seedlings involving *P. caldenia*) isolation mechanisms.

This incipient isolation mechanism occurring in the area of sympatry analysed would be reinforced by an ecological one since *P. caldenia* can grow on sandy but not on saline soils, while *P. alpataco* and *P. flexuosa* grow on both (Burkart, 1976). This might be the cause determining that *P. caldenia* is the most easily identifiable species of those studied of Section Algarobia according to the patterns of 25 isozyme loci (Saidman, 1985; Saidman and Vilardi, 1987). However, becauseof of the difficulty of making controlled crosses, the actual (physiological and or genetical) level or reproductive isolation cannot be yet ascertained.

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Literature Cited

BURKART, A.: A monograph of the genus *Prosopis* (Leguminosae sufam. Mimosoideae). J. Arnold Arboret. **57** (3), 219—249; (4), 450—525 (1976). —— NARANJO, C. A. and ENUS-ZEIGER, S.: Cromatografía de fenoles y morfología en especies e híbridos de *Prosopis* de La Pampa. Actas XIX Jornadas Argent. Botánica **32** (1983). —— NARANJO, G. A., POGGIO, L. and ENUS-ZEIGER, S.: Phenol chromatography, morphology and cytogenetics in three species and natural hybrids of *Prosopis* (Leguminosae, Mimosoideae). Plant Syst. Evol. **144**, 257—276 (1984). —— SAIDMAN, B. D.: Estudio de la variación alozímica en el género *Prosopis*. Ph.D. thesis. Fac. Cs. Ex. y Nat. Buenos Aires, Argentina (1985). —— SAIDMAN, B. O.: Isoenzymatic studies of alcohol dehydrogenase and glutamate oxalacetate transaminase in four South American species of *Prosopis* and their natural hybrids. Silvae Genetica **35**, 3—10 (1986). —— SAIDMAN, B. O. and VILARDI, J. C.: Analysis of the genetic similarities among seven species of *Prosopis* (Leguminosae, Mimosoideae). In Press (1987). —— SIMPSON, B. B.: Breeding systems of dominant perennial plants of two disjunct warm desert ecosystems. Oecologia (Berl.) **27**, 203—226 (1977). —— SIMPSON, B. B. and SOLBRIG, O. T.: Introduction. In: SIMPSON, B. B. (Ed.). Mesquite/Its biology in two desert ecosystems. US/IBP. Synthesis. Ser. 4 Dowden, Hutchinson and Ross Inc. Pennsylvania, 1—15 (1977). —— VILARDI, J. C., SAIDMAN, B. O. and PALACIOS, R. A.: Muestreo según variabilidad. In: Prosopis en Argentina — Documento Preliminar. I Taller International sobre Recurso Genético y Conservación de Germoplasma en Prosopis. Univ. Nac. Córdoba — Univ. Nac. Buenos Aires. FAO, 119—124 (1988).

Die Variabilität der Immissionsresistenz von Fichtenherkünften – ein Beitrag zum IUFRO-Fichtenprovenienzversuch 1964/1968

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Zusammenfassung

Im Jahre 1968 wurden 100 Fichtenherkünfte aus dem europäischen Verbreitungsgebiet der Art *Picea abies* (L.) KARST. auf drei unterschiedlich immissionsbelasteten Flächen angebaut, um die Variabilität der Immissionsresistenz dieser Baumart zu ermitteln und relativ widerstandsfähige Provenienzen für den praktischen Anbau in immissionsbelasteten Gebieten zu selektieren. Jede Fläche enthielt 20 vollständige Blocks mit Einzelbaumparzellen der 100 Herkünfte. Der Versuch zeichnet sich aus durch wiederholte Höhenaufnahmen und Bonitierungen der Benadelung sowie durch die wiederholte Erfassung der Immissionsbelastung mit Hilfe von Nadelanalysen.

Der Bericht enthält Angaben zur biologischen Variabilität der Fichte unter unterschiedlichen Immissionsbelastungen. Dargestellt wird die Entwicklung der Ausfälle, das Höhenwachstum und erstmals die mehrjährige Entwicklung der Benadelung.

Die Pflanzen auf den stärker belasteten Flächen in Hagen hatten fast einen Nadeljahrgang weniger und zeigten auch ein signifikant schlechteres Höhenwachstum als auf der weniger belasteten Fläche Rumbeck. Trotzdem wird auf allen Flächen eine große Variation der untersuchten Parameter gefunden, allerdings auf unterschiedlichem Niveau.

Eine Prognose der weiteren Entwicklung anhand einer Nadelbonitierung im ersten Jahr nach der Pflanzung war nicht möglich, dagegen war die Anzahl der Nadeljahrgänge 5 Jahre nach der Pflanzung deutlich korreliert mit dem weiteren Wachstum und dem späteren Zustand der Benadelung.

Aus den Untersuchungen werden Kenngrößen zur Beurteilung der Anbaueignung am Versuchsort, der relativen Immissionsresistenz und der Anbaueignung in immissionsbelasteten Gebieten abgeleitet. Die Variabilität dieser Kriterien wird untersucht. Zusätzlich wird ein Modell für die Auswertung geordneter kategorieller Daten vorgestellt.

Für das weitere Vorgehen kommt ein Saatgutimport aus SW-Polen, aus dem viele überlegene Herkünfte stammen, oder die vegetative Vermehrung des Versuchsmaterials in Betracht. Der zweite Weg wurde bereits durch den Anbau in alten und neuen Zentren der Waldschäden beschritten. Als Allheilmittel gegen die Waldschäden kommen diese Selektionen aber nicht in Betracht. Sie können nur das Ausmaß weiterer Schäden in besonders belasteten Gebieten verringern.

Summary

The variability of Norway spruce provenances in regard to their tolerance to air pollution — a contribution to the IUFRO-provenance-trial 1964 to 1968

In 1968 100 European provenances of Norway spruce (*Picea abies* (L.) KARST) were planted at three sites with different pollution load in order to investigate the variability of its tolerance to air pollution and to select relatively tolerant provenances for planting in polluted regions of Germany. Every trial contained 20 complete blocks with single tree plots of the 100 provenances. Special features of this experiment are repeated measurements of height growth combined with the survey of needle retention and needle analysis to find out the level of air pollution.

The report contains results of the biological variability of Norway spruce under different pollution levels. The development of dieback, height growth and needle retention is analysed.

The two sites at Hagen with increased levels of many pollutants which originated from mixed industrial and traffic emissions showed significantly lower height growth and poorer needle retention than the third site, Rumbeck, with less pollution. However, a great variation of height growth and needle retention were observed on all sites, only at different levels.

An early indication of future behaviour by assessing the condition of the foliage in the year after planting appeared as difficult, whereas the number of needle sets 5 years after planting was positive correlated with the later development of height growth and foliage.

Three criteria for pollution tolerance are described, site suitability, relative resistance and relative suitability for polluted areas. The variation of these criteria is shown. In addition, a mixed-model for analysing ordered cate-

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