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Relationship between Isozymic Variability and Environmental Conditions in the Lichen *Xanthoria parietina*.

By

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With 3 Figures

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Summary

REYES A., LOPEZ-BILBAO M. G. & MOLINA M. C. 1996. Relationship between isozymic variability and environmental conditions in the lichen *Xanthoria parietina*. – Phython (Horn, Austria) 36 (2): 265–275, 3 figures. – English with German summary.

Relationships among lichenized fungus populations of *Xanthoria parietina* collected from different Spanish areas are establised using six isoenzymatic systems: alcaline phosphatase, acid phosphatase, esterases, isocitrate dehydrogenase, phosphoglucoisomerase, phosphoglucomutase and superoxide dismutase. A total of 55 bands were resolved, four of which were constant in all thalli through all sampling data. UPGMA dendrogram and principal component analysis show the existence of groups correlated with climatic conditions. In the case of saxicolous thalli, the substrate also plays an important role.

Zusammenfassung

REYES A., LOPEZ-BILBAO M. G. & MOLINA M. C. 1996. Zusammenhänge zwischen der Variabilität von Isoenzymen und den Umweltbedingungen in der Flechte *Xanthoria parietina*. – Phyton (Horn, Austria) 36 (2): 265–275, 3 Abbildungen. – Englisch mit deutscher Zusammenfassung.

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Beziehungen zwischen Populationen der Flechte *Xanthoria parietina* von verschiedenen Gegenden Spaniens wurden unter Verwendung von 6 Isoenzymsystemen erfaßt: Alkalische – und saure Phosphatase, Esterasen, Isozitrat-Dehydrogenase, Phosphoglucoisomerase, Phosphoglucomutase und Superoxid-Dismutase. Insgesamt konnten 55 Banden aufgetrennt werden, von denen 4 in allen Thalli bei allen Aufsammlungen immer gleich waren. UPGMA-Beziehungen und eine Analyse der Hauptkomponenten weisen auf die Existenz von Gruppen, die mit klimatischen Bedingungen verknüpft sind, hin. Im Fall steinbewohnender Thalli spielt das Substrat ebenfalls eine wichtige Rolle.

Introduction

The analysis of diversity is a critical point in the study of evolutionary processes. Thus, many studies have been carried out in order to describe the amount of variability in a great deal of plant species. In this way, lichens have been widely analysed from a morphological point of view, however, little is known about genetic variability in lichenized species.

Protein electrophoresis is a common method to show whether evolutionarily significant variation exists or not in natural populations. Nevertheless, the interpretation, from the classic genetic point of view, of electrophoretic banding patterns of lichens is not possible for different reasons: 1) the enzymes of a thallus can be produced by both symbionts (ESCRIBANO & LEGAZ 1984, FAHSELT 1985, LEGAZ & VICENTE 1983, VICENTE & LEGAZ 1988); 2) it can be a consequence of the interaction between both symbionts (MARTIN 1973); 3) it can be aditive of multiple enzymatic patterns as a single lichen thallus may be derived from several different propagules (OTT 1987), and therefore the enzymatic pattern from one thallus does not necessarily represents a single genotype.

Among proteins, enzyme forms are used to analyse variability because they are primary gene products. Isoenzyme studies have been widely adopted in species of high plants. In algae however, only few investigations exist (DEMPSEY & DELCARPIO 1971). In this way, KILIAS & al. 1988 isolated phycobionts and carried out isoenzyme analysis because biochemical characters seem more promising in providing a taxonomic base. Several enzymatic systems were also analysed in lichen thalli by using electrophoretic techniques in order to obtain information related to different aspects of lichenized fungus biology.

Thus, it has been described by LARSON & CAREY 1986 that the largest thalli of *Umbilicaria vellea* and *U. mammulata* showed higher degree of polymorphism whereas the smallest ones are mainly monomorphic as a consequence of physiology and developmental changes or as an accumulation of hyphae from different sources. Also differences in isozymes has been detected between rhizine and upper thallus in umbilicate lichens (FAHSELT & HAGEMAN 1994). On the other hand, FAHSELT 1986 found genetic differences in *Cladonia cristatella* for esterases and alkaline phosphatases

that could be explained by meiotic recombination between polymorphic loci in these populations. Moreover, a higher degree of variability has been detected in umbilicate lichen with sexual reproduction when compared with those asexual ones (FAHSELT 1989).

Isozymes have also been used to describe both seasonal and geographical variability. In relation to geographical differences, it has been found that banding patterns of particular enzymes can remain constant within, and even between, lichenized fungus species (FAHSELT & KROL 1989, HAGEMAN & FAHSELT 1986). On the other hand, isozymic differences have been described between geographically separated populations of a single species (HAGEMAN & FAHSELT 1984).

The lichen *Xanthoria parietina* (L.) Th.Fr. is an ubiquitous species of temperate regions, able to develop on different organic and inorganic substrates (LAUNDON 1992). As the aim of the present work is to determine whether isozymic variation is geographical or substrate correlated, *X. parietina* is the ideal biological material.

Materials and Methods

Samples of *Xanthoria parietina* were collected from different Spanish geographical areas and substrates, both organic and inorganic (Table 1).

Information about minimum temperature is shown in Figure 1, and the distribution of the annual mean rainfall is presented in Table 2. Both data were provided by the Instituto Nacional de Meteorología.

For each substrate, a random sampling from an approximately 5 m^2 meter area was carried out to eliminate intrasubstrate variation, i.e., lichen thalli growing on identical substrates in that area were taken, mixed and then analyzed.

Samples of about 1.5 g of fresh thalli were macerated in a mortar with 1.5 ml 0.1 M Tris-HCl buffer, pH 7.1, at 4 °C. Sheets of 0.5 cm × 1.5 cm Whatman 3MM paper were impregnated in different homogenates and applied on a layer of 12% (w/v) starch gel. Horizontal electrophoresis was carried out in a discontinuous system consisting of 76 mM Tris 5 mM citrate, pH 8.65, for the gel and 300 mM boric acid 60 mM sodium hydroxyde, pH 8.1, for electrodes. Electrophoresis was carried out for 2h 30 min at 4 °C and 240V (POULIK 1957).

Phosphoglucomutase (PGM) EC 2.7.5.1; esterase (EST), EC 3.1.1.1; superoxide dismutase (SOD), EC 1.15.1.1; isocitrate dehydrogenase (IDH) EC 1.1.1.42; phosphoglucoisomerase (PGI), EC 5.3.1.9; and acid phosphatese (ACPH), EC 3.1.3.2, activities were stained according to plant protocols (BREWER & SING 1970). Enzymatic reactions were carried out in the dark, at 37 °C for variable time periods according to each particular enzyme. Reactions were stopped with abundant distilled water and, later, with methanol:acetic acid:distilled water (70:15:70 v/v).

After gel staining, banding patterns for each enzyme were constructed. Later, these band patterns for the six analyzed systems were compared for the different samples, two-by-two, and Jaccard coefficient (J) were calculated to estimate overall similarities among samples as follows: $J = N_{AB} / (N_T - D)$, where N_{AB} is the number of common bands between both samples, N_T the total number of analyzed bands and D the different ones.

Table 1

Spanish regions and location where the samples of *Xanthoria parietina*, from different substrates were collected.The samples are named by a letter and so they will be named in the text.

SAMPLE	SUBSTRATE	LOCATION	REGION
A	*Populus nigra*		
B	*Prunus amygdalus*		
C	*Ficus carica*		
D	*Cytisus scoparius*	ATAJATE	SOUTH
E	*Quercus ilex*		
F	*Olea europaea*		
G	Calcareous Rock		
H	*Populus alba*	BADAJOZ	SOUTH-WEST
I	*Catalpa bignonioides*		
J	*Robinia pseudoacacia*	TOLEDO	CENTRAL
K	Basaltic Rock		
L	*Fraxinus excelsior*	MONTEJO	
M	*Robinia pseudoacacia*	DE LA	CENTRAL
N	*Populus nigra*	SIERRA	
O	*Robinia pseudoacacia*	PEDRAZA	CENTRAL
P	Calcareus Rock	SANTANDER	NORTH

Table 2

Minimal temperature and rainfall from sampling areas.

	MINIMAL TEMPERATURE °C	RAINFALL mm
ATAJATE	8–10	1000–1500
SANTANDER	8–10	1000–1500
BADAJOZ	8–10	300–500
TOLEDO	4–6	300–500
MONTEJO DE LA SIERRA	4–6	300–500
PEDRAZA	4–6	300–500

The data matrix obtained using these coefficients was analyzed by using the UPGMA cluster analysis in the NTSYS program (ROLF 1990), which show the genetic distance among the analysed samples.

A principal component analysis (PCA) was also carried out based upon the presence-absence protein bands by means of the NTSYS program (ROLF 1990) to determine trends of variation.

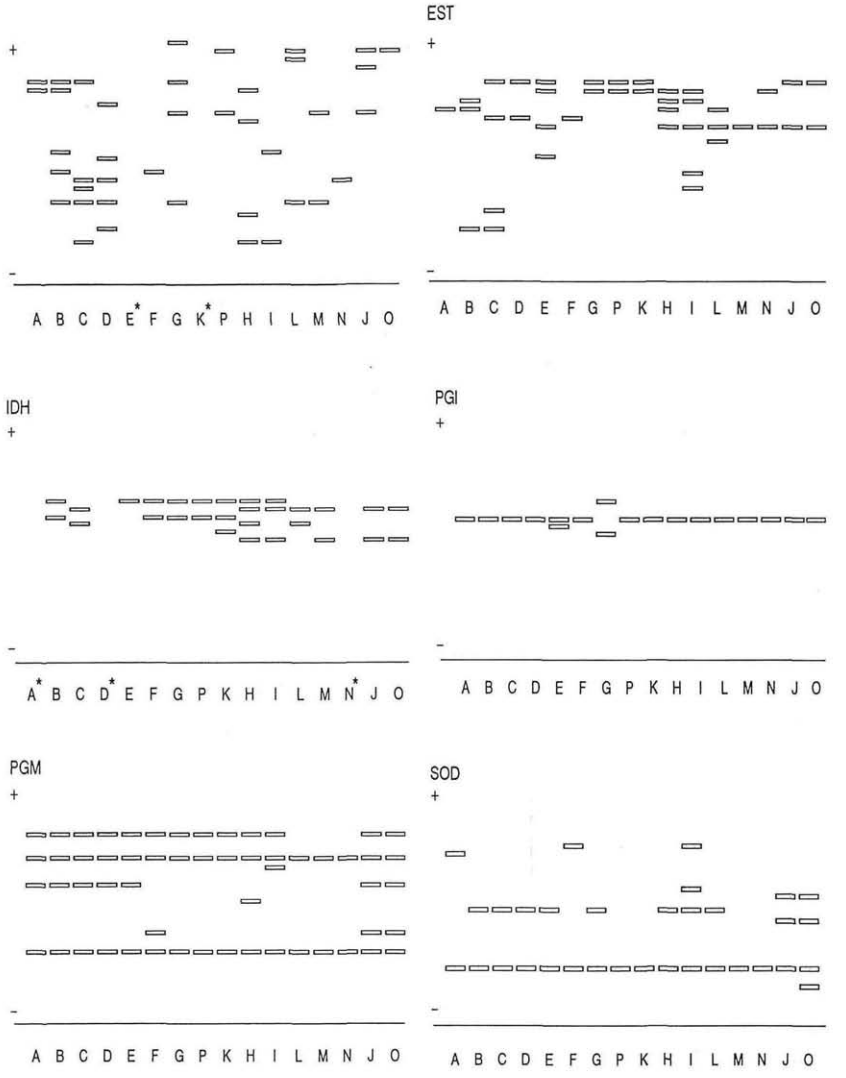

Fig. 1. Banding patterns obtained in the six isoenzymatic systems assayed in *Xanthoria parietina*: ACPH (acid phosphatase); EST (estearase); PGM (phosphoglucomutase); SOD (superoxide dismutase); IDH (isocitrate dehidrogenase) and PGI (phosphoglucoisomerase) in the 16 analysed samples.

A t-Student test comparising two by two was done using the Jaccard similarity coefficients in order to determine whether intragroup similarity was higher than intergroup one in the case of epiphyta.

Results

A total of 55 reproducible bands representing the six enzyme systems were analyzed in the 16 samples. Out of the 55 studied proteins, four of them resulted to be common to all samples, while 27 of them were only present in one locality.

The banding pattern obtained for each system is shown in Figure 1. ACPH and EST are the most polymorphic systems with respect to both number of bands (one to five) and electromorphs (12 and 14 respectively). The systems PGM, SOD and IDH are moderately polymorphic with respect to the number of electromorphs (eight, six, and seven respectively), but less to the number of bands (two to five for PGM, one to four for SOD, and two to four for IDH). Finally, the PGI system showed the most conserved pattern, with three electromorphs and one to two bands.

From these banding patterns, Jaccard coefficients were obtained: ranging from 0.2 to 0.9 when comparing different lichen samples. The mean value for intrageographic comparisons was 0.44. The analysis of these similarity values by NTSYS produced the dendrogram shown in Figure 2. Geographic differentiation among the samples can be clearly observed. Two clusters are separated at a similarity level (genetic distance) of 0.32: in one of them are grouped the Southern populations and the saxicolous group, while in the other are the Central populations. Within the Southern cluster, the samples from Badajoz are separated at a level of 0.33 from the Atajate and rock group. The latter group is separated from the epyphitic ones of Atajate at a level of 0.44. The sample from calcareous rock of Atajate is slightly more similar to the other rocks from the Northern and Central area than to the epiphytics of this area. In the Central region group, the samples from Montejo de la Sierra are separated at a level of 0.36 to the others from Toledo and Pedraza. Moreover, the sample collected from *Robinia pseudoacacia* from Montejo de la Sierra is more similar to the others from this area than to those grown on the same substrate from Toledo and Pedraza.

On the other hand, the first axis extracted by the principal component analysis accounted for 16.87% of the original variation while axis II extracted 14.47%, for a cumulative total of 31.34% of the variation of the total data set. Figure 2 displays a plot of the 16 OTUs on these first two axes. The first axis discriminates the Central region samples (J, K, L, M, N, O) from those samples from the other samples while along the second axis the Atajate samples and the saxicolous group appear separated from the Badajoz and Central region ones. Furthermore, for each locality, the relative positions of the samples is according to its geographical area.

The consistency of the epiphytic groups (Atajate, Badajoz and Central region) was proved by the t-Student test. We obtained a statistically higher value of similarity within locality than between localities ($t_{76} = 4.46$, $p < 0.001$).

Abbreviations: UPGMA: unweighted pair-group method using arithmetic averages; PGM: phosphoglucomutase; EST: Esterase; IDH: isocitrate dehydrogenase; SOD: superoxide dismutase; IDH: isocitrate dehydrogenase; PGI: phosphoglucoisomerase; ACPH: acid phosphatase; Tris-HCl: Tris (Hidroximetil)Aminometano-HCl; Tris-citrate: Tris(Hidroximetil)Aminometano-citrate; NTSYS: numerical taxonomy and systematics; OTUs: operative taxonomic units.

Discussion

The banding pattern of the 16 samples analyzed in this study (Fig. 1) shows the absence of some bands in certain samples. If the samples were genetically identical, then loss of enzymatic activity, differential genic expression and catabolite repression might cause different phenotypes (VICENTE & LEGAZ 1985). Nevertheless, enzyme polymorphism in geographically isolated populations may be due to genetic differences between samples. In this way, several studies describe geographical isozyme variability in different lichen species (FAHSELT 1986, FAHSELT & KROL 1989, HAGEMAN & FAHSELT 1986, SKULT & al. 1986).

The hypothesis that populations are geographically substructured predicts that the similarity between different samples collected from the same area will be higher than that found for samples from the same substrate in different areas. This pattern occurred in our study as demonstrated by the t-Student test, that shows a higher whitin locality similarity than between localities ($t_{76} = 4{,}46$, $p < 0.001$). Thus, lichenized fungus growing on the same substrate from different locations, samples J, M and O from *Robinia pseudoacacia* or samples A, N from *Populus nigra* are less similar to each other than each is to samples from different substrates in the same location (Fig. 1 and 2). However, in the case of lichenized fungus growing on rocks (samples G, K, P), the within substrate similarity is higher than the geographic one (Fig. 2). In the latter case, the most determinative factor is the substrate, while comparing epiphytic samples, geographic area seems to be the most important determinant of genetic similarity. This dichotomy may be due to the fact that the close relationship between the lichenized fungus and its substrate is different in both cases. Saxicolous lichenized fungus are able to degrade and desintegrate their substrate by biochemical and biophysical mechanisms (ASCASO & al. 1990), while epiphytic lichens are able to interact with the tree, interferring with the metabolism of the phorophyte (LEGAZ & al. 1988, MONSO & al. 1993, ORUS & ASCASO 1982). This different behavior could explain the results obtained in the dendrogram (Fig. 1).

The geographical distribution of *X. parietina* samples is correlated with two factors: minimum temperature and rainfall as can clearly be observed in the principal component analysis (Fig. 2). Thus, the first axis

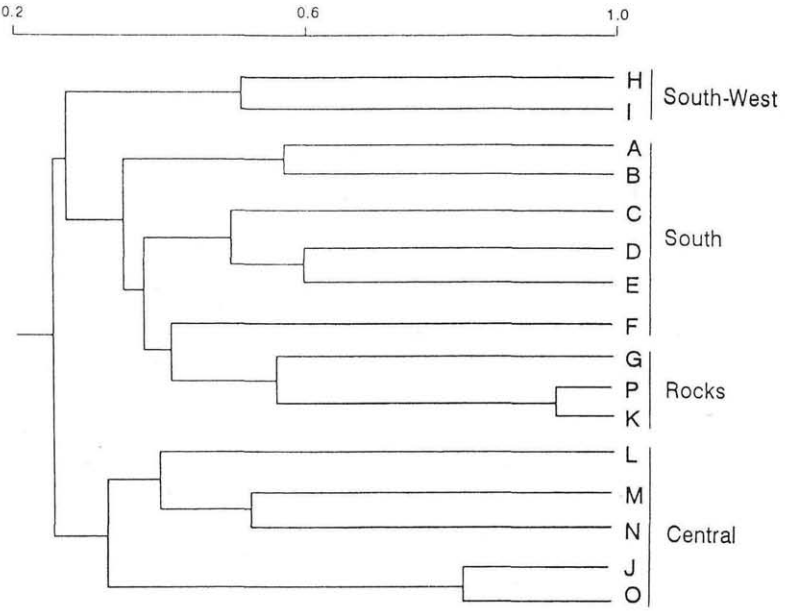

Fig. 2. Dendogram obtained by the NTSYS software by means of a UPGMA method based on a Jaccard similarity coefficient. The designation of the samples are referred in Table 1. Genetic distance is indicated in the upper part of this graphic.

discriminate the regions according to the mean minimum temperature (Table 2), as Atajate, Badajoz and Santander present a temperature of 8–10 °C while 4–6 °C in the Central region. The second axis differentiate the regions depending on the annual rainfall (Table 2), as Santander and Atajate present 1000–1500 mm of annual rainfall and Central region and Badajoz 300–500 mm. Nevertheless, samples collected from rocks cannot be discriminated by this second axis, and this could be due to the substrate importance above mentioned.

Correlations between isozymic variability and the geographical area has also been found for *Trebouxia* isolated from different lichen species (KILIAS & al. 1988). In this way, some lichenized fungus species have been described to have shown changes in their isozymic patterns when collected in separated locations, as for example in the case of *Umbilicaria mammulata* (HAGEMAN & FAHSELT 1984, 1986a) and *U. muhlenbergii* (HAGEMAN & FAHSELT 1986b). This geographical differentiation has also been confirmed by the study of total proteins (STROBL & al. 1993) and diverse secondary products (CULBERSON & al. 1990, SKULT 1984). So, correlations between genetic variation and geographic distribution may result from different climatic adaptations among populations of *Xanthoria parietina*.

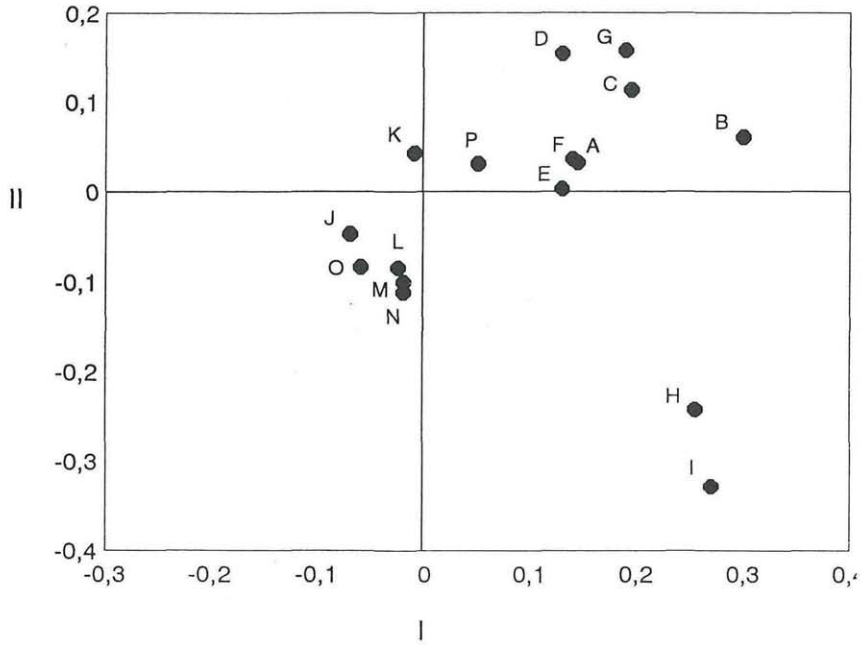

Fig. 3. A plot of 16 OTUs on the first two axes produced by a principal component analysis of isozyme data. The designation of the samples are referred in Table 1.

A great deal of variation was also observed within each locality (Fig. 1, 2), which reveal the existence of a great differentiation in each population. For example, samples from A to G, collected in Atajate from different substrates show a high variability; in these samples, protein polymorphism has also been observed by two-dimensional gel electrophoresis of total proteins (LOPEZ-BILBAO & al. 1996). This variability may be the result of phenomena such as sexual reproduction via recombination (FAHSELT 1985, OTT 1987) and/or to substrate dependent differentiation (LEGAZ & al. 1986, BROWN & al. 1994).

As a conclusion, our results seems to confirm that isozyme polymorphism documents a geographic differentiation correlated with weather conditions, although in the case of saxicolous group the substrate also plays an important role.

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