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Evidence for the duplication of PGI genes in *Dipcadi serotinum* L. (Liliaceae)

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Analysis of electrophoretic phenotypes for phosphoglucoisomerase isozymes (PGI, EC 5.3.1.9) in Spanish populations of *Dipcadi serotinum* L. shows that this diploid species has three gene loci coding for PGI isozymes. The PGI-1 locus specifying the plastid isozymes has two alleles and is probably duplicated since the three banded phenotypes represents a fixed heterozygote which does not segregate among progenies. PGI-2 and PGI-3 loci specifying the cytoplasmic isozymes have three and five alleles respectively and they produce complex non-overlapping phenotypes with the formation of interlocus heterodimers between polypeptides specified by the PGI-2 and PGI-3 loci. These results suggest that the two loci coding for the cytosolic isozymes have arisen by the duplication of an ancestral gene.

INTRODUCTION

The use of electrophoresis in diploid plants to identify the number of gene loci that specify particular enzyme systems has revealed that, in most higher plants, the minimum number and subcellular location of isozymes of many assayed enzymes are highly conserved (Gottlieb, 1982). Nevertheless gene duplication in diploid plants has played an important role in increasing this minimum isozyme number. Gene duplication has been extensively reported in several species of diploid plants, for example, for genes coding for phosphoglucoisomerase (PGI) in *Clarkia* (Gottlieb, 1977; Gottlieb and Weeden, 1979) and for other enzyme systems, in *Stephanomeria* (Roose and Gottlieb, 1980), *Clarkia* (Gottlieb, 1974; Pichersky and Gottlieb, 1983; Soltis *et al.*, 1987), *Heliantus* (Torres, 1974), *Sorghum* (Ellstrand *et al.*, 1983), *Hordeum* (Kahler *et al.*, 1981) and *Layia* (Warwick and Gottlieb, 1985).

In this paper we present evidence of PGI duplication in the diploid species *Dipcadi serotinum* L. Medic. With $2n = 8$ chromosomes (Ruiz Rejón *et al.*, 1980). This species is the only representative of the Genus *Dipcadi* present in Spain (Heywood in Tutin *et al.*, 1980). In a previous report (Oliver *et al.*, 1983) the four zones of PGI activity present in this species were considered as products of four different genes, and due to the complexity of the zymograms by the appearance of additional anodal bands originated by postranslational modifications, some of the PGI isozymes seemed to be coded by duplicated genes. The modification of our electrophoresis techniques simplifies the zymograms leading to match our results with the PGI patterns present in other diploid species.

MATERIAL AND METHODS

Plants of *Dipcadi serotinum* L. ($2n = 8$) were collected from seven Spanish natural populations: locations and sample size of each population are listed in table 1.

Identification of the subcellular locations and number of gene loci which specify PGI isozymes was initially conducted by a comparative examination of extracts of diploid somatic tissues (leaves), and soaked pollen of individual plants (following Weeden and Gottlieb, 1979, 1980*b*). A formal genetic analysis was then conducted on seeds derived from single outcrossed plants sampled from natural populations and also on the progenies derived from experimental self-pollination of different individuals.

Enzyme extracts were absorbed onto paper wicks and subjected to horizontal starch gel electrophoresis, with LiOH/borate *p*H 8·1 electrode buffer and Tris/citrate *p*H 8·3 gel buffer

Table 1 Populations of *D. serotinum* examined for PGI

Location	Sample size
Sitio de Calahonda, Marbella, Malaga	24
Cerro Gorde, Almuñecar, Granada	52
Calahonda, Granada	52
Viator, Almería	39
Balcón de Canales, S. Nevada, Granada	71
Cerro del Toro, S. Nevada, Granada	36
Padul, Granda	25

(Selander *et al.*, 1971). EDTA 5 mM was added to the gel and electrode buffers in order to inhibit calcium dependent proteases. The addition of EDTA together with the use of extracts for electrophoresis prepared in suitable buffers (Weeden and Gottlieb, 1979, 1980*b*) simplifies the zymograms, eliminating additional bands that are resolved under other technical conditions as crude extracts obtained by crushing the plant material in deionised water (Oliver *et al.*, 1983). Gels were stained for PGI activity as described by Wendel (1980).

RESULTS AND DICUSSION

Analysis of different electrophoretic phenotypes of PGI from leaves of *Dipcadi serotinum* reveals that there exist four clearly separated zones of PGI activity (fig. 1). They are in decreasing order of anodal mobility, PGI-1, PGI-2, PGI-2/3 and PGI-3. The PGI-2/3 bands represent interlocus hybrid enzymes formed between the subunits coded by the genes PGI-2 and PGI-3 (explanation given below).

A comparison of zymograms of leaf and soaked pollen extracts shows that they are distinguished by the absence from leachate pollen of the isozymes of the PGI-1 zone (fig. 2). This suggests that the PGI-1 isozymes are located in the plastids as also noted in other plant species (Schnarrenberger and Oeser, 1974, Schnarrenberger *et al.*, 1975; Weeden and Gottlieb, 1979, 1980*a*, 1980*b*, Gottlieb and Weeden, 1981; Gottlieb, 1982; Lack and Kay, 1986). The PGI-1 zone is weakly stained in relation to the other zones and was not well resolved in the majority of individuals analysed; for this reason data from only 40 individuals are available. In this initial survey only two different phenotypes were found in the PGI-1 zone; 36 individuals had a single banded pattern designated 1A (the most anodal band)—these are considered to be homozygous for PGI-1A; the remaining four individuals show three electrophoretic bands (fig. 3), having the same 1A band plus two less anodal isozymes designated 1AB and 1B—this is the typical pattern for a heterozygous state of a dimeric enzyme. As all the seeds produced by these heterozygous plants were also three banded in the PGI-1 zone, it would seem that in these four plants the PGI locus is duplicated and this has given rise to a state of fixed heterozygosity at the PGI-1 locus (Allendorf *et al.*, 1975; Gottlieb, 1976; Oliver *et al.*, 1983). The single banded pattern exhibited by the majority of individuals, therefore, may be due to each duplicated gene of PGI-1 possessing identical alleles or alternatively one of the duplicated genes codes for a null allele. Additional data (*e.g.* studies of dosage effects, *etc.*) are required before a firmer conclusion is reached on this point.

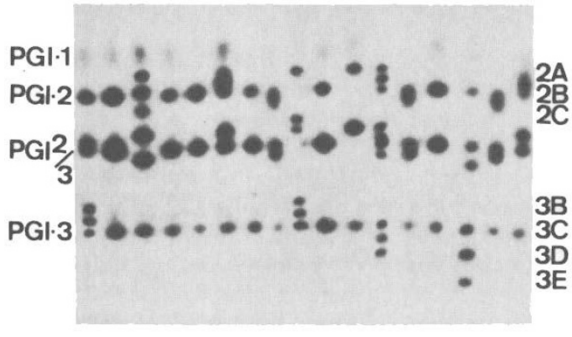

Figure 1 Electrophoretic phenotypes of PGI enzymes from some individuals of *D. serotinum*. The four PGI zones are indicated at the left and the homodimeric enzymes of cytoplasmic isozymes at the right, the homodimeric enzyme 3A is absent in these individuals and is not shown.

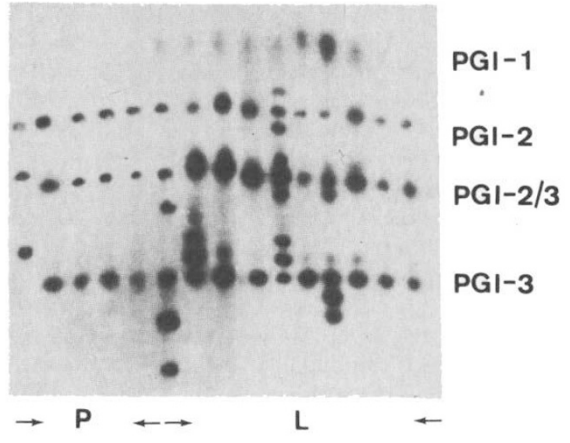

Figure 2 Comparative analysis of soaked pollen and leaf extracts that shows the absence of PGI-1 or plastid PGI isozymes and the presence of intergenic heterodimers (PGI-2/3) between the cytosolic isozymes PGI-2 and PGI-3.

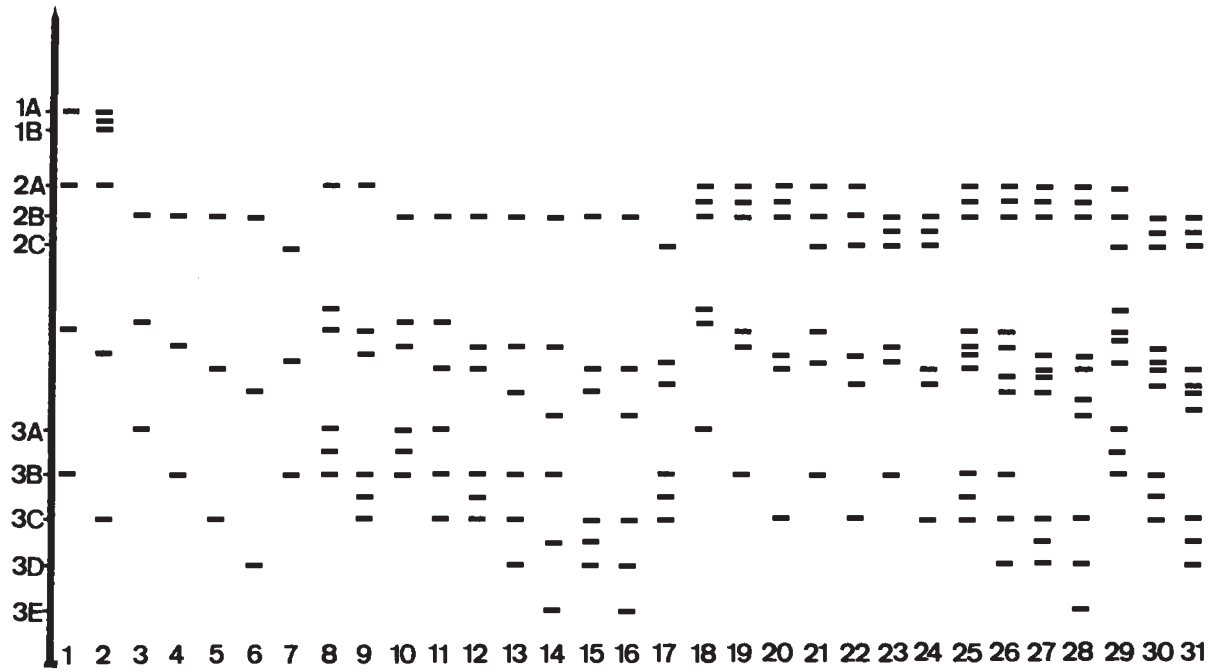

Figure 3 Diagrammatic representation of PGI phenotypes observed in *D. serotinum*. Each phenotype is designated by a number. The homodimeric enzymes are indicated at the left. Intensity of heterodimeric enzyme bands is not indicated in the figure. Only the two found phenotypes for PGI-1 are represented.

The remaining zones of activity PGI-2, PGI-2/3 and PGI-3 were present in both soaked pollen and leaf extracts indicating that their isozymes have a cytosolic location (Weeden and Gottlieb, 1980*b*). For dimeric isozymes postmeiotically expressed in pollen (*e.g.*, the cytosolic PGI isozymes) a comparison between the electrophoretic patterns expressed in pollen and somatic tissues can provide valuable information on the number of gene loci present and their allelic states (Weeden and Gottlieb, 1979).

For the cytosolic isozymes of PGI in *D. serotinum*, individual plants show three to ten isozyme bands in somatic tissues (figs 1 and 3). The individuals with three banded phenotypes (fig. 3; individuals 1 to 7) show no differences between leaf extracts and soaked pollen isozymes (fig. 4), indicating that these dimeric phenotypes are specified by two gene loci (Gottlieb, 1977; Gottlieb and Weeden, 1979; Weeden and Gottlieb, 1979; Lack and Kay, 1986) that we have called PGI-2 and PGI-3.

The subunits coded by PGI-2 and PGI-3 associate to form interlocus hybrid enzymes PGI-2/3, with mobilities approximately intermediate to those of the respective intralocus homodimers; for

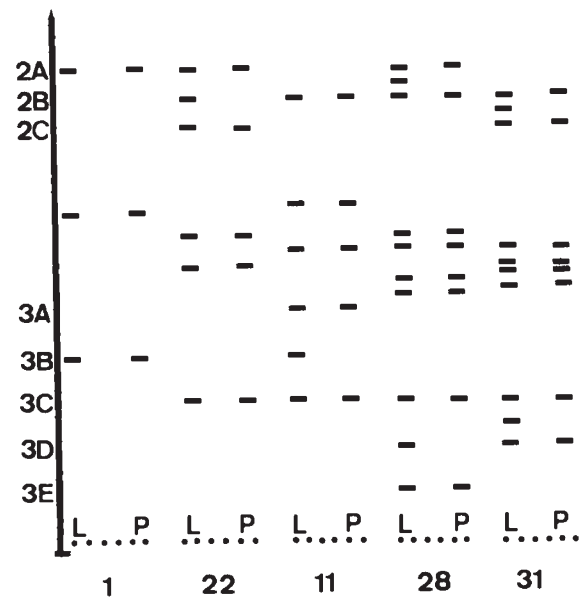

Figure 4 Diagrammatic representation of the comparative analysis of cytosolic PGI patterns in leaves (L) and soaked pollen (P) of different individuals, where the absence of intragenic or allozyme heterodimers in the pollen from individuals heterozygous at either loci can be observed.

this reason the heterodimeric isozymes that have originated by interlocus hybridisation are present in haploid pollen. In the PGI-2 zone three different bands occur; these are the products of the PGI-2 alleles which we have denoted as 2A, 2B and 2C. In the PGI-3 zone four bands were initially found; these are the products of the PGI-3 alleles 3A, 3B, 3C and 3D. In this sense individuals 1–7 (fig. 3) are homozygous at both loci and the following phenotypes and genotypes (between parentheses) have been assigned to them: 2A3B ($2^{aa}3^{bb}$), 2A3C ($2^{aa}3^{cc}$), 2B3A ($2^{bb}3^{aa}$), 2B3B ($2^{bb}3^{bb}$), 2B3C ($2^{bb}3^{cc}$), 2B3D ($2^{bb}3^{dd}$) and 2C3B ($2^{cc}3^{bb}$). Other results can be explained using this model.

When individuals are heterozygous at just one of the two loci proposed, phenotypes with six bands always appear in diploid tissues (figs 1 and 3). So if the PGI-2 and PGI-3 loci are respectively homozygous and heterozygous (fig. 3; individuals 8 to 17) the phenotypes observed contain one band corresponding to the homodimer for the PGI-2 gene, two intermediate bands corresponding to interlocus heterodimers and three bands for the locus PGI-3 including two homodimers and one intermediate intralocus heterodimer typical of dimeric enzymes. If, however, the PGI-2 gene is heterozygous and the PGI-3 gene is homozygous, then there will appear phenotypes with three bands for PGI-2 zone, two bands for PGI-2/3 zone and one band for PGI-3 (fig. 3; individuals 18 to 24). Fig. 4 shows that pollen extracts from all of these individuals express not six but only five bands.

When both loci are heterozygous, ten isozymes may be observed in leaf extracts with the three fastest bands being dimer products of the PGI-2 alleles, the three slowest bands being dimer products of the PGI-3 alleles and the four intermediate bands being heterodimer products of interlocus hybridization (fig. 3; individuals 25 to 31). Pollen extracts of these individuals present not ten but only eight isozymes (fig. 4).

In both cases the observed reduction in the number of bands in soaked pollen extracts (fig. 4) is due to the fact that the intralocus or allozyme heterodimers are absent in haploid tissues since the pollen grains contain only one allele of each heterozygous locus. This fact allows us to determine the allelic state of the different loci and confirm the genotypes proposed.

The number and mobilities of the enzymes intermediate to PGI-2 and PGI-3 are perfectly correlated with the number and mobilities of the particular homodimeric PGI-2 and PGI-3 enzymes of each individual and they suggest, therefore, that these intermediate enzymes are interlocus heterodimers. The results of a genetic analysis of these two loci PGI-2 and PGI-3 based upon the segregation of progeny phenotypes were found to be consistent with the Mendelian inheritance of three and five co-dominant alleles, respectively (table 2). No information has been obtained on any linkage between the two cytosolic loci, since the seed progenies obtained from the double heterozygous individuals were scarce.

In conclusion, the electrophoretic results provide evidence that the plastid and the cytosolic isozymes of PGI in *D. serotinum* are coded by duplicated genes.

Several pieces of evidence reinforce this duplication model: since most diploid plants present only two isozymes for the PGI, one in the plastids and another in the cytoplasm, this reflects a remarkable conservation of metabolic activities in the different subcellular compartments and seems to indicate that two isozymes are the ancestral number for PGI in diploid plants (Gottlieb, 1982). Therefore the presence of additional isozymes of PGI in the same subcellular compartment is evidence of gene duplication for both plastid and cytosolic PGI isozymes. Furthermore, the formation of interlocus hybrids enzymes within each compartment, by the association of subunits coded by different gene loci, provides a strong evidence for their homology, and for the duplicated origin of the gene loci coding for these isozymes. Examples of such gene duplications have been documented in animals and plants (Ingran, 1961; Markert *et al.*, 1975; Avise and Kitto, 1973;

Table 2 PGI phenotypes of progenies obtained from seeds collected from outcrossed plants in nature and from experimental self-pollination* of single individuals for the PGI-2 and PGI-3 genes. This analysis shows allelic segregation for both loci

Parental phenotypes	Progeny phenotypes	Observed frequencies
2A3B	2B3B	10
	2B3A	1
2B3AB	2B3AB	3
	2B3B	6
2C3BD	2C3BC	6
	2BC3CD	6
	2A3B	2
2AC3B	2AC3AB	5
	2C3B	1
2B3C*	2B3C	10
2A3B*	2A3B	8
	2B3B	6
2BC3B*	2BC3B	10
	2C3B	4

Gottlieb, 1977; Pichersky and Gottlieb, 1983; Ellstrand *et al.*, 1983; Warwick and Gottlieb, 1985).

The two loci coding for the cytosolic isozymes of PGI in *D. serotinum* have diverged structurally and must differ significantly in their sequences, because none of the electrophoretic variants at either locus overlap the mobility of any variant at the other. For the plastid PGI isozymes the lack of variability for these enzymes precludes a complete analysis.

On the other hand, the existence of additional isozymes in *D.* serotinum, in contrast to other closely related diploid species, belonging to the tribe Scilae Engler, as *Muscari comosum* and *Scilla autumnalis*, which just have two PGI isozymes (Pascual, unpublished data), permits one to think that gene duplications have occurred during the evolution of *D. serotinum.* This is the same as has happened during evolutive divergence of some diploid species of the genus *Clarkia*, which present two genes for cytosolic PGI (Gottlieb, 1977; Weeden and Gottlieb, 1979, 1980*b*).

Regarding the possible origin of gene duplication for PGI isozymes in *D. serotinum*, cytological analysis shows an asymmetric karyotype with $n = 4$ subteleocentric chromosomes, thus chromosome rearrangements in association with its capability for self-pollination, could have played an important role in the evolution of this species generating gene duplications (Ruiz Rejón *et al.*, 1981). This suggestion is consistent with the existence of other duplicataed isozymes as alcohol dehydrogenase, glutamate-oxalacetate transaminase and esterases (Pascual *et al.*, 1980, 1981; Pascual, 1983; Oliver *et al.*, 1983.

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