EVIDENCE FOR MULTIPLE ISOZYMES IN *HYMENOPAPPUS SCABIOSAEUS* GLUTAMATE DEHYDROGENASE HETEROZYGOTES

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Summary

GDH isozyme patterns from field collected plants of *Hymenopappus scabiosaeus* are examined. Some of the apparent heterozygous patterns consist of one broad band while others contain five discernible sharp bands. Resolution of these bands apparently depends upon the distance separating the parental phenotypes on the gel. These observations support a model proposed by Pryor (1974) which states that GDH in maize may be composed of multiple subunits but that the resulting isozyme bands do not resolve on a starch gel because of their close proximity.

1. Introduction

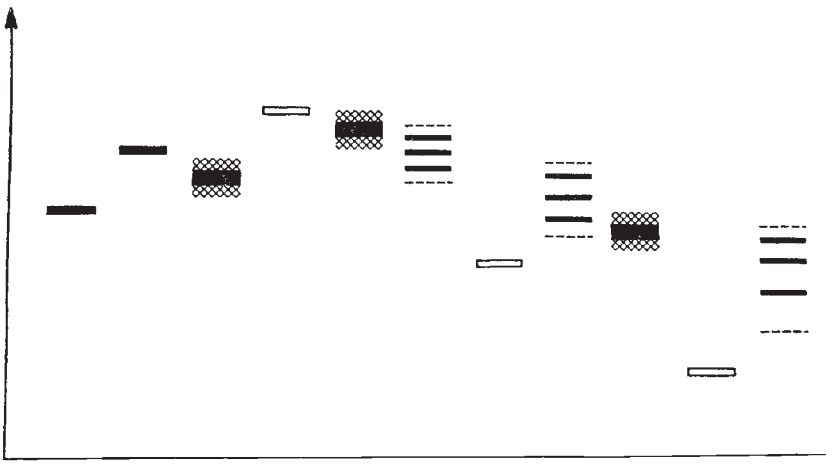
Recently Pryor (1974) reported the occurrence of a single electrophoretic band in maize plants heterozygous for any two of three glutamate dehydrogenase (GDH) alleles. This unusual phenomenon was investigated and three models proposed by Pryor to account for the single band. Model 3 states that "... the single broad GDH isozyme band seen in heterozygotes could be an unresolved mixture of isozymes in which the contribution of the parental homo-hexamers is so small that it is not visible on the starch gel zymograms". Pryor notes that in some animals GDH has been shown to be a complex enzyme composed of six or more identical subunits. GDH has been isolated in different plants and extensively purified and characterised in peas (Pahlich and Joy, 1971). From work with zinc activation and inactivation Joy (1973) suggests that GDH from peas is composed of subunits; however the exact number has not been determined. Observations will be presented here from GDH starch gel patterns in the plant *Hymenopappus scabiosaeus* which support model 3 and suggest that this enzyme is composed of six subunits.

GDH isozymes from *H. scabiosaeus* have recently been used with other isozymes as genetic markers in a population study (Babbel and Selander, 1974). The GDH patterns of the field collected plants from this study fall into three categories: (1) a single sharp band, (2) a single broad diffuse band, (3) multiple sharp bands. The second two categories are believed to represent heterozygote patterns with the third category supporting model 3.

2. Materials and methods

Whole plants of *H. scabiosaeus* were collected from several sites in central Texas. Soluble protein extracts were obtained by homogenising leaf tissues in an ice-chilled glass tissue grinder with sufficient buffer (0·1M HEPES, *p*H 7·0 with 0·05M β-mercaptoethanol) and polyvinylpolypyrrolidone to yield a thick slurry. Slurries were centrifuged at 49,000 × *g* at 4°C

for 20 minutes. Samples of the resulting supernatants were then submitted directly to electrophoresis. GDH isozymes were separated in a 12½ per cent starch gel (Electrostarch, Lot 171) using a gel buffer composed of 0·076M Tris and 0·005M citric acid, *p*H 8·7 and a 0·3M borate-NaOH, *p*H 8·2 tray buffer. Electrophoresis was carried out in the cold at 250 V for about 3 hours. GDH activity was detected on the gel by staining with a reaction mixture containing 40 ml of 0·2M Tris-HCl buffer (*p*H 8·0) with 0·05M L-sodium glutamate, 20 mg NAD, 20 mg nitro blue tetrazolium, and 5 mg phenazine methosulfate.



	C	B	BC	A	AB	AC	D	BD	CD	E	CE
o	134	9	48	0	1	1	0	2	2	0	2
e	129·4	6·0	55·7	0	0·3	1·6	0	0·7	3·2	0	1·6

FIG. 1.—Electrophoretic patterns of glutamate dehydrogenase observed among field collected plants of *Hymenopappus scabiosaeus*. The observed number (*o*) of plants expressing each pattern and the expected number (*e*) based on the genetic interpretations explained in the text and Hardy-Weinberg expectations are given below each pattern.
$\chi^2_{3d.f.} = 7{\cdot}2$, $P > 0{\cdot}05$; $\chi^2_{3d.f.}$ with Yates' correction $= 3{\cdot}2$, $P > 0{\cdot}1$.

3. Results and discussion

Among the 199 plants sampled eight different GDH isozyme patterns were observed (fig. 1). The following genetic interpretation of the observed patterns requires breeding data for direct confirmation. In the absence of such data, however, the interpretations can be checked by calculating gene frequencies from the data and then, assuming a situation which approaches panmixis among the collection sites (*H. scabiosaeus* is a predominant outcrosser), comparing observed genotypic numbers with those predicted by Hardy-Weinberg equilibrium. The close agreement seen between expected and observed numbers (see fig. 1) provides indirect evidence for the genetic interpretations.

By far the most common observed pattern is C. This phenotype was observed in 134 plants. BC and B were the next most common. BC consists of a single rather broad diffuse band and is similar to the heterozygous patterns reported by Pryor in maize. This band migrates to a position nearly inter-

mediate to B and C, although somewhat closer to B and appears to represent the heterozygote between B and C (assuming that B and C represent homozygotes). CD and AB also display broad diffuse bands and are believed to be heterozygotes involving B or C and one or the other of 2 alleles not observed in the homozygous condition, A and D.

The remaining three patterns (BD, AC, and CE) also appear to represent heterozygote configurations, but again they involve alleles not found in the homozygous condition (A, D, and E). The predicted electrophoretic positions of these three alleles in the homozygous state is shown in fig. 1. BD, AC, and CE are different in that each is composed of five discernible sharp bands. The difference between the two types of putative heterozygote patterns can most easily be explained by invoking Pryor's third model. Namely, GDH is a multimeric enzyme, perhaps a hexamer and in a heterozygous individual seven different combinations of parental bands are possible. The intensity of the resulting isozymes can be predicted by the terms of the binomial $(a+b)^6$ which give the probability of occurrence of each combination. For example, 6B subunits = 1/64; 5B, 1D = 6/64; 4B, 2D = 15/64; 3B, 3D = 20/64; 2B, 4D = 15/64; 1B, 5D = 6/64; and 6D = 1/64. Accordingly, we might predict for a hexamer a heterozygote pattern containing seven bands with the centre three being the darkest and of about equal intensity. On either side of these three a considerably lighter band will appear and finally a very light band at each end should appear in the same position as the band of the respective parents. This band, however, will be very faint compared to the other bands and may not be distinguishable. Patterns BD, AC, and CE fit this description. However, in patterns BC, CD, AD, and FN, NS, and FS in maize as reported by Pryor, the individual bands are not resolved. The failure to resolve may simply be because the bands are so close to each other. The resulting phenotype is, therefore, a single broad diffuse band which is darkest in the centre.

The resolution then, of the isozymes associated with different heterozygote configurations of *Gdh* in *H. scabiosaeus* appears to be a function of the relative distance separating the parental bands. The patterns observed in individuals heterozygous for widely separated bands support the hypothesis that GDH is a multimeric enzyme composed of perhaps six subunits.

4. References

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