BIOCHEMICAL ANALYSIS OF SOME MEMBERS OF ASPIDIACEAE AND ATHYRIACEAE

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ABSTRACT

In the present investigations some members of the families Aspidiaceae and Athyriaceae have been examined for isoenzymes of peroxidase by polyacrylamide gel electrophoresis. These studies reveal that one or more isoperoxidase bands may be pointed out as genus/family markers. The four genera of family Aspidiaceae, *Polystichum, Dryopteris, Cyrtomium* and *Hypodematium* are heterogenous with regard to their isoperoxidase band pattern, whereas *Athyrium* and *Diplazium* belonging to Athriaceae are enzymatically akin. But the two families have nothing in common. Our previous investigations (unpublished) on the phenolic compounds and free amino acid pool of these ferns substantiate the above findings.

INTRODUCTION

Ferns are a large and exceptionally varied group of plants which despite a long and abundant fossil record present a considerable challenge to taxonomists. Quite a few classifications have been proposed (Alston, 1956; Ching; 1940; Christensen, 1938; Copeland, 1947; Holttum, 1947; Nayar, 1970; Pichi-Sermolli, 1958; Reimers, 1954; Sledge, 1973). There is no general agrement as to the correct status of the various subdivisions. A

few serological and electrophoretic studies have been made in ferns (Oliver, 1972).

The morphological differences among the species are the result of the extent of genetic divergence. Numerous taxonomic methods have been used to measure these differences (Gottlieb, 1971). Protein and isoenzymatic analysis by gel electrophoresis is a useful technique for measuring genetic variations at molecular level (Hubby and Lewontin, 1966). The exploitation of primary constituents like enzymes which regulate pathways for the formation of secondary constituents as phenols, alkaloids, terpenoids, fats, carbohydrates etc. would perhaps be more tenable to find interrelationships between plants than the secondary constituents themselves which are actually the function of enzymes. An enzyme on the other hand is the immediate expression of a gene (Transcription and Translation) and on the other hand regulates metabolic pathways leading to the formation or otherwise of so called secondary metabolites.

Peroxidases are widely distributed among ferns and are of immense physiological interest because of their association with numerous catalytic functions. Most important among these is their ability to oxidize indole -- acetic acid, thus playing a key role in regulating its endogenous level. This enzyme may also be involved in the regulation of several morphogenetic processes. The broad substrate specificity of peroxidases may be indicative of diverse functional roles for the various forms of peroxidase. So, because of these mertis of isoenzymes, their common occurrence and the ease of their detection, they have been investigated more than any other plant isoenzyme to date.

The objective of this study was to determine whether variations found in peroxidase isoenzyme banding patterns from the vegetative fronds would substantiate the recognised

classification of the ferns and provide evidence on the ancestory.

MATERIALS AND METHODS

Collections for the current studies were made from Mussoorie and Simla hills. Occasionally fresh fern materials were collected from Kasauli and Botanical Gardens of Panjab University. Fertile fronds of the species under study were washed in distilled water and homogenized in a chilled glass pestle and mortor with a pinch of acid washed sand and crude enzyme extracted in 0.67 M phosphate buffer (pH 7.0). These extracts centrifuged at 2000 g for 2-3 min to remove debris. The supernatant again centrifuged at 15000 g for 5-7 min and stored at 0-7°C. The isoperoxidases were resolved on 10% acrylamide gels, prepared according to the method described by Davis (1964) and Ornstein (1964) with slight modifications. Samples were run in Tris-glycine buffer (pH 8.3) by subjecting them to a current of 3mA/tube for a period of two hours. Gels for isoperoxidases were stained by the method (Mitra et al, 1970). Blue bands indicate the position of peroxidase isoenzyme. Rf value given in the text is the average of three replicates.

RESULTS AND DISCUSSION

Family Aspidiaceae is presently represented by eight species classified into four genera. The genus *Polystichum* includes four species, i.e. *P. stimulans, P. obliquum, P. squarrosum* and *P. discretum.* Only one electrophoretic band with Rf 0.78-0.84 is common to all the four species (Table 1, and Fig. 1A) representing generic specificity, but genus marker is not contained

Table I

Name of Family	Genus	Species	RF Value of Band No.						
Aspidiaceae		stimulans	.04-.06	.08-.12	.26-.30	.44-.46	.64-.66	.78-.84	-
		obliqum	.16-.20	.32-.34	.44-.46	.56-.56	.80-.82	-	-
	Polysticum	squarrosum	.16-.20	.24-.29	.49-.51	.58-.61	.79-.83	-	-
		discretum	.20-.21	.25-.27	.43-.46	.80-.82	-	-	-
	Dryopteris	odontoloma	.04-.09	.10-.17	.30-.31	.33-.34	.53-.54	.63-.66	.99-1.0
		marginata	.03-.06	.08-.12	.30-.34	.35-.37	.65-.68	.98-1.0	-
	Cyrtomium	caryotaedium	. 0-.02	.20-.24	.48-.50	.64-.66	.84-.86	-	-
	Hypodermatium	cumenatum	.02-.06	.38-.42	.53-.57	.74-.77	.98-1.0	-	-
Athyriaceae	Athyrium	schimperi	.05-.07	.22-.25	.43-.48	.67-.70	.95-.97	-	-
		pectinatum	.05-.07	.15-.17	.30-.33	.43-.47	.98-1.0	-	-
	Diplazium	esculentum	. 0-.03	.15-.18	.30-.33	.43-.47	.57-.60	.92-.95	-
		polypodoides	.05-.07	.20-.22	.30-.33	.42-.47	.58-.63	.67-.70	.98-1.0
		spectabile	.04-.06	.20-.22	.30-.34	.42-.44	.58-.60	.82-.84	.98-1.0

in any other genera of the family. Within the genus *Polystichum* a band with Rf value 0.44-0.46 is common to *P. stimulans, P. obliquum* and *P. discretum*. However, one band with Rf value 0.16-0.20 is common to *P. obliquum* and *P. squarrosum* and another band with Rf value 0.24-0.29 is common to *P. squarrosum* and *P. discretum* (Fig. 1A). The above observations clearly indicate the genus to be homogenous and there is no distinction between the pinnate and decompound species. In *Dryopteris odontoloma* and *D. marginata* quite a few peroxidases are common with each other. Two of these bands with Rf values 0.04-0.09 and 0.98-1.00 are distinctly common whereas one band with Rf value 0.30-0.34 of *D.marginata* overlaps two bands with Rf value 0.30-0.31 and 0.33-0.34 of *D. odontoloma* (Fig. 1B). The two genera *Cyrtomium* and *Hypodematium* have five isoenzymes each. None of the band is common to the other taxa of the family currently analysed. From the foregoing observations it becomes clear that each of the genera stands out chemically with regard to their isoperoxidases, thereby indicating that the family Aspidiaceae is heteroisoperoxidasic. It is pertinent to note here that we have made similar observations on the basis of their analytic studies of free amino acid pools and phenolic compounds.

Five species of family Athyriaceae have been analysed. The genus *Athyrium* is represented by two species viz. *A. schimperi* and *A. pectinatum*. Both these species contain five bands each, two of them are common and may be considered as genus marker. Three species of *Diplazium* contain 6-7 bands. Three of these bands with Rf values 0.30-0.34, 0.43-0.48 and 0.57-0.63 are common to all the sps. (Table 1. and Fig. 1C). So they may be considered genus specific.

Furthermore it is observed from the band patterns that the band with Rf value 0.98-1.00 is common to *D. polypodoides* and *D. spectabile*, on one band and *A. pectinatum* on the other hand. Curiously enough the genus marker for *Athyrium* i.e. the band with

Rf value 0.05-0.07 is common with the two sps. of *Diplazium*, i.e. *D. polypodoides* and *D. spectabile* and one of the genus marker for *Diplazium*, the band with Rf value 0.30-0.34 is also present in *A. pectinatum*. Thus it is obvious that the band with Rf value 0.43-0.48 is common to all the five taxa represented in the two different genera of the family. Hence this band may conveniently be considered as family marker. So we can safely say that the family as a whole represents a natural assemblage of the taxa included.

Nayar (1970) proposed a new classification of homosporous ferns, giving also a schematic representation of the inter-relationships among them. He included members of the family Aspidiaceae and Athyriaceae in a family Dryopteridaceae. Recently Sledge (1973) has pointed out that the separation of the two groups of genera belonging to Aspidiaceae and Athyriaceae as distinct families, when the characters of these groups overlap so completely with each other, cannot be justified. But on the contrary, present observations reveal that the family Athyriaceae is a natural assemblage on chemical basis, whereas Aspidiaceae has nothing in common with this family. Furthermore the various constituent genera of the family Aspidiaceae stand out separately so that even a marker is absent. However, it may be pointed out at the end that what is needed is much more chemotaxonomic work. Such work would be of interest not only to phytochemists but to all who are concerned with the phylogeny of this large group of plants which present a considerable challenge to taxonomists.

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REFERENCES

Alston, A.H.G.

1956 "The subdivision of the Polypodiaceae", *Taxon, 5:* 23-25.

Ching, R.C.

1940 "On natural classification of the family Polypodiaceae", *Sunyatsenia, 5:* 201-306.

Christensen, C.

1938 "Filicineae." In F. Verdoorn (Ed.). *Manual of Pteridology.* The Hague: M. Nijhoff.

Copeland, E.B.

1947 *Genera Filicum.* Waltham, Mass.: Chronica Botanica Inc.

Davis, B.J.

1964 "Disc electrophoresis II Method and application to human serum proteins." *Ann. N. Y. Acad. Sci., 121:* 404.

Gottlieb, L.D.

1971 "Gel electrophoresis; new approach to the study of evolution. *Bioscience," 21:* 939-944.

Holttum, R.E.

1947 "A revised classification of leptosporangiate ferns." *J. Linn. Soc.* (Bot.), *53:* 123-158.

Hubby, J.L., and Lewontin, R.C.

1966 "A molecular approach to the study of genic heterozygosity in natural population I. The number of alleles at different loci in *Drosophila pseudoobscura.*" *Genetica, 54:* 577-594.

Mitra, R., D.R. Jagannath and C.R. Bhatta

1970 "Disc electrophoresis of Analogous enzymes in *Hordeum. Phytochem., 9:* 1843-50.

Nayar, B.K.

1970 "A phylogenetic classification of the homosporous ferns." *Taxon, 19:* 229-236.

Oliver, J.C.

1972 "Preliminary systematic studies of the Oak ferns. Chromatography and electrophoresis." *Amer. Fern Jl. 62:* 16-20.

Ornstein, L.

1964 "Disc electrophoresis I Background and theory." *Ann. N. Y. Acad. Sci., 121:* 321.

Pichi Sermolli, R.E.G.

1958 "The higher taxa of the Pteridophyta and their classification." *Uppsala Univ. Arrskr., 6:* 70-90.

Reimers, H.

1954 "Pteridophyta." In H. Melchior and *E. we*rdermann (Eds.). *Engler's syllabus pflanzenfamilien, I* Bakterien bis Gymnospermen: 269-311 Berlin: Borntreaeger.

Sledge, W.A.

1973 "Generic and family boundaries in Aspidiaceae and Athyriaceae." In *Phylogeny and Classification of Ferns.* Jermy, A.C. Crabbe, J.A. and Thomas, B.A. (Eds.) 203-210.

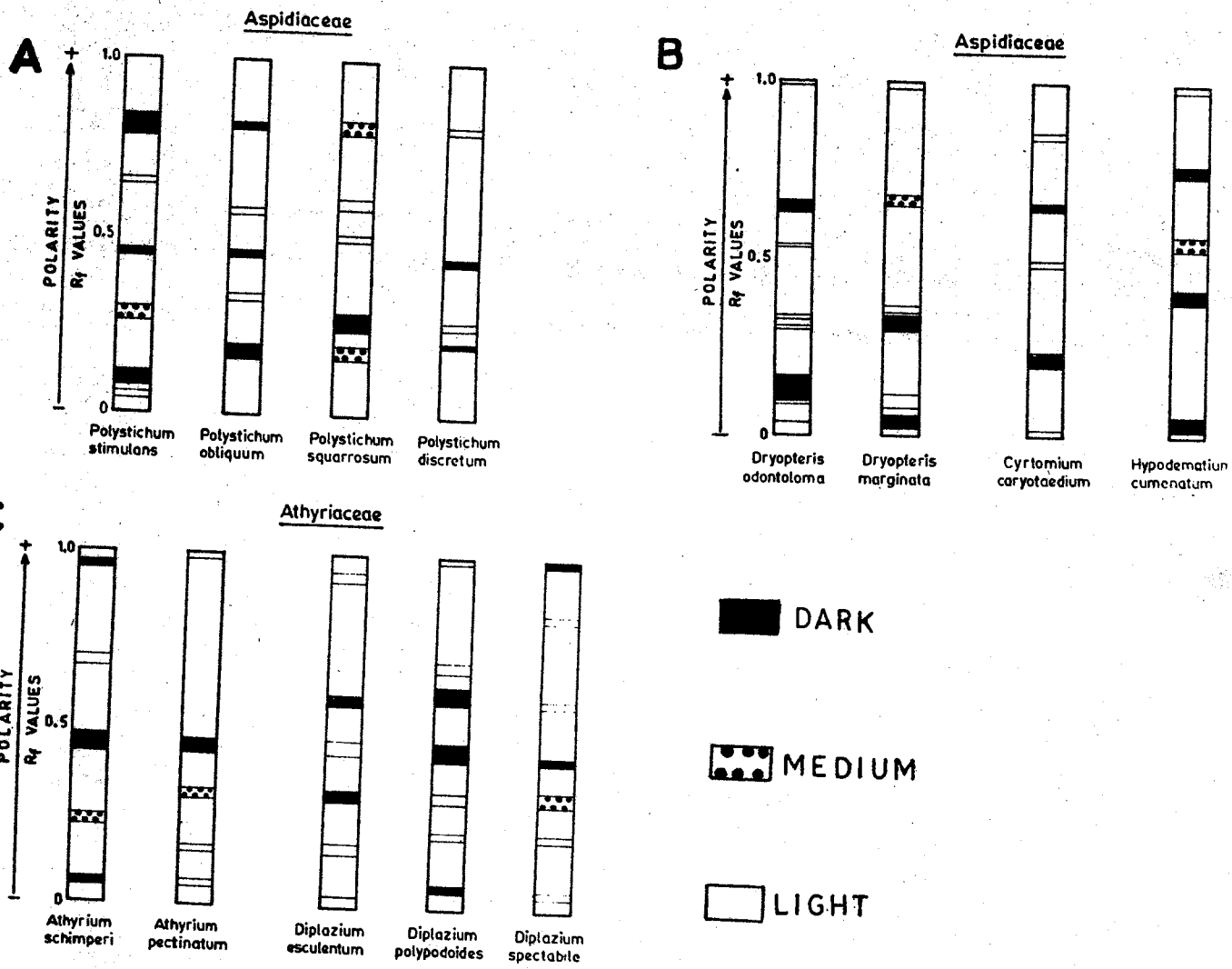

Fig. 1. A-C. Zymogram showing the band pattern of isoperoxidases in Aspidiaceae and Athyriaceae.