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ISOENZYME POLYMORPHISM IN NATURAL POPULATIONS OF THE GENUS *BAPTISIA* (LEGUMINOSAE)*

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Abstract—Natural populations of three species of *Baptisia* (Leguminosae) were examined by acrylamide gel electrophoresis for the presence of molecular polymorphism with respect to the isoenzyme patterns of leucine aminopeptidase, β-galactosidase and indophenol oxidase. No species-specific patterns were observed and considerable intraspecific polymorphism was detected.

INTRODUCTION

NUMEROUS investigations have been performed which have demonstrated that isoenzyme staining patterns are dependent upon several variables. Within a single species, the pattern is a function of the tissue chosen,[1–3] the stage of development of the organism,[4, 5] the age of the tissue[6] and even the subcellular organelle examined.[7] In interspecific comparisons, many patterns are reported to be species-specific and of diagnostic utility.[8–10] The earliest, and to date most, work in this research area has been done with animal tissues. A few zoologists have investigated the variation in isoenzyme staining pattern within populations of a single species.[11–13] The most extensively studied organism with respect to populational variation in protein constitution is man, because of the clinical value of such information.[14–16] Numerous authors have expressed the desirability of a more complete knowledge of the extent of genetically based protein variations in natural populations in order to insure proper usage of isoenzyme pattern data for systematic purposes.[17–20]

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[1] J. G. SCANDALIOS, *J. Heredity* **55**, 281 (1964).
[2] Y. CHU, *Japan J. Genet.* **42**, 233 (1967).
[3] M. D. UPADHYA and J. YEE, *Phytochem.* **7**, 937 (1968).
[4] C. L. MARKERT and F. MOLLER, *PNAS* **45**, 753 (1959).
[5] C. MANWELL, *Comp. Biochem. Physiol.* **17**, 805 (1966).
[6] D. RACUSEN and M. FOOTE, *Can. J. Botany* **44**, 1633 (1966).
[7] S. K. MUKERJI and I. P. TING, *Biochim. Biophys. Acta* **167**, 239 (1968).
[8] H. M. SCHWARTZ, S. BIEDRON, M. VON HOLDT and S. REHM, *Phytochem.* **3**, 189 (1964).
[9] R. L. CLEMENTS, *Phytochem.* **5**, 243 (1966).
[10] C. R. BHATIA, M. BUITTI and H. SMITH, *Am. J. Botany* **54**, 1237 (1967).
[11] C. F. NADLER and C. HUGHES, *Comp. Biochem. Physiol.* **18**, 639 (1966).
[12] J. M. BURNS and F. JOHNSON, *Science* **156**, 93 (1967).
[13] S. GUTTMAN, *Comp. Biochem. Physiol.* **23**, 871 (1967).
[14] L. BECKMAN, G. BJORLING and C. CHRISTODOULOU, *Acta Genet. Statist. Med.* **16**, 122 (1966).
[15] D. A. HOPKINSON and H. HARRIS, *Ann. Human Genet.* **30**, 167 (1966).
[16] R. G. DAVIDSON, *Ann. Human Genet.* **80**, 355 (1967).
[17] C. R. SHAW, *Science* **149**, 936 (1965).
[18] D. BOULTER, D. THURMAN and B. L. TURNER, *Taxon.* **15**, 135 (1966).
[19] C. F. NADLER and C. HUGHES, *Comp. Biochem. Physiol.* **18**, 639 (1966).
[20] C. MANWELL, C. BAKER, P. ASHTON and E. CORNER, *J. Marine Biol. Assn. U.K.* **47**, 145 (1967).

Plants, especially the genus *Baptisia*, are well-suited for populational studies. Since plants are immobile, the extent of the entire population can be established by visual inspection. Since *Baptisia* is a perennial herb, year-to-year sampling of the same plant is possible. In addition, the systematics of the genus is well-established both chemically and morphologically.[21] Natural hybrids of the various species are also available for analysis. The present work is an investigation of molecular polymorphism with respect to isoenzyme staining pattern in natural populations of plants. Three species of the genus *Baptisia* (Leguminosae) were studied: *Baptisia leucophaea*, *B. sphaerocarpa* and *B. nuttaliana*.

RESULTS

The *Baptisia* species under examination were screened with respect to a large number of enzymes and the following were found in detectable amounts: glycerol, malate, formate and glutamate dehydrogenases, polyphenol oxidase (tyrosinase), indophenol oxidase, peroxidase, acid and alkaline phosphatases, α-esterase, β-galactosidase and leucine aminopeptidase. Most of these enzymes exhibited vague, diffuse banding patterns which were not suited to this type of study. Five of the above twelve enzymes gave sharp bands and exhibited intraspecific polymorphism with respect to zymograms, viz. malate dehydrogenase (MDH), α-esterase, indophenol oxidase, β-galactosidase and leucine aminopeptidase. The last three of these gave patterns consisting of relatively few bands which could be unequivocally scored for and these were chosen for intensive study. It was decided to omit MDH and α-esterase from further study because of difficulty in accurate scoring of their complex patterns consisting of numerous bands and the fact that esterases, like phosphatases and peroxidases, are probably families of enzymes with broad substrate specificities.

Three zymograms were observed when gels were stained for leucine aminopeptidase (LAP, L-leucyl-peptide hydrolase: EC 3.4.1.1). These patterns and the R_P values associated with each band are shown in Fig. 1. The frequencies at which these patterns were observed in inter- and intraspecific comparisons are indicated in Table 1. Since the two species, *B. sphaerocarpa* and *B. nuttaliana*, exhibit only a single zymogram (pattern A), the LAP pattern cannot be used to distinguish between these two species. *B. leucophaea* exhibited all three patterns in different frequencies among the populations examined.

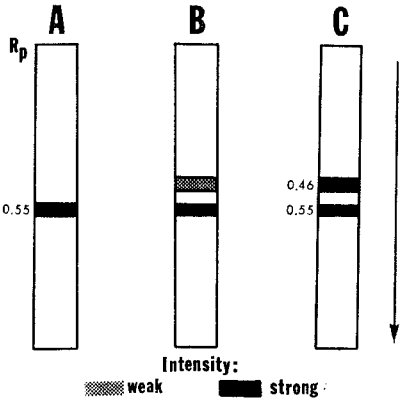

FIG. 1.

[21] R. E. ALSTON and B. L. TURNER, *Am. J. Botany* **50**, 159 (1963).

TABLE 1. FREQUENCY OF OCCURRENCE OF LAP PATTERNS A, B AND C IN *Baptisia* SPECIES

Species	Population	Freq. A	Freq. B	Freq. C	Number of plants analyzed
Baptisia leucophaea	1	0·54	0·41	0·05	56
	2	0·63	0·31	0·06	49
	3	0·87	0·00	0·13	30
	4	0·91	0·04	0·04	47
	5	0·66	0·33	0·00	21
	6	0·57	f(B)+f(C)* = 0·43		9
	7	0·50	f(B)+f(C) = 0·50		10
	8	0·33	f(B)+f(C) = 0·67		12
	9	0·34	f(B)+f(C) = 0·67		12
B. sphaerocarpa	10	1·00	0·00	0·00	18
	11	1·00	0·00	0·00	8
	12	1·00	0·00	0·00	9
B. nuttaliana	13	1·00	0·00	0·00	22

* f(B) and f(C) are the frequencies at which the patterns B and C were observed respectively.

Two zymograms were found with respect to β-galactosidase (β-D-galactoside galactohydrase: EC 3.2.1.23). A single band is either present or absent at R_P 30. Both *B. leucophaea* and *B. sphaerocarpa* are polymorphic with respect to β-galactosidase. The frequencies at which the band was observed for populations of each of these species are indicated in Table 2. β-Galactosidase was not detected in any of the twenty-two members of the single *B. nuttaliana* population which was examined.

TABLE 2. FREQUENCY OF OCCURRENCE OF β-GALACTOSIDASE PATTERN A IN *Baptisia* SPECIES

Species	Population	Freq. A
Baptisia leucophaea	1	0·83
	2	0·57
	3	0·60
	4	0·27
	5	0·80
	6	0·63
B. spaerocarpa	10	0·67
	12	0·69
B. nuttaliana	13	0·00

Indophenol oxidase (ferrocytochrome c: oxygen oxidoreductase: EC 1.9.3.1), like esterase, peroxidase and phosphatases, is a family of similar enzymes with broad substrate specificities. The bands present when stained for indophenol oxidase are located in two regions of the gel. At the bottom (anodic end) of the gel is a region in which may be found a rapidly migrating single band which is polymorphic in that it may be present or absent. Near the top of the gel is a region in which four possible banding patterns were observed as illustrated in Fig. 2. The banding patterns of the two regions of the gel assort randomly as was shown by a statistical analysis of the results from a large population (forty-seven plants of *B. leucophaea*). The result revealed random assortment with a chi-squared value of 0·719 (probability = 0·96 for $n = 5$). The two regions of the gel may, therefore, be considered independently. Presumably

they result from two separate families of enzyme molecules. The frequencies at which the various indophenol oxidase patterns occurred among the populations studied is indicated in Table 3. No correlation was observed between any morphological polymorphism or ecological condition and the isoenzyme pattern exhibited.

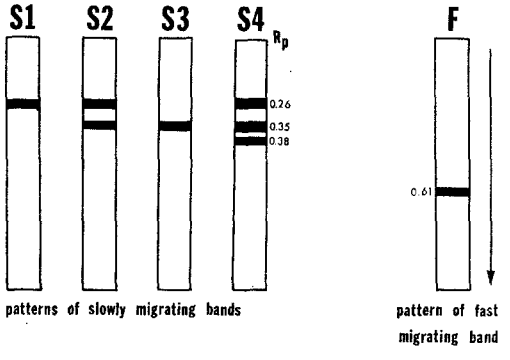

FIG. 2.

TABLE 3. FREQUENCY OF OCCURRENCE OF INDOPHENOL OXIDASE PATTERNS IN *Baptisia* SPECIES

Species	Population	Freq. S1*	Freq. S2	Freq. S3	Freq. S4	Freq. F
Baptisia leucophaea	1	0·15	0·20	0·65	0·00	1·00
	2	0·04	0·71	0·23	0·02	0·86
	3	0·03	0·87	0·10	0·00	0·63
	4	0·11	0·47	0·43	0·00	0·66
	5	0·05	0·65	0·20	0·10	0·91
	6	0·00	0·22	0·79	0·00	1·00
	7	0·30	0·50	0·20	0·00	1·00
	9	0·00	0·58	0·42	0·00	0·75
B. sphaerocarpa	10	0·06	0·56	0·00	0·39	0·06
	11	0·00	0·50	0·00	0·50	0·25
	12	0·00	0·62	0·00	0·38	0·69
B. nuttaliana	13	0·00	0·55	0·32	0·14	0·00

* Pattern designations refer to those zymograms illustrated in Fig. 3.

DISCUSSION

No species-specific patterns which would be of diagnostic utility as taxonomic characters were observed in the *Baptisia* species examined. Certain characteristic bands were noted which, while they do not uniquely identify a species, are consistently present or absent in all members which are examined of a given species. For example, β-galactosidase is consistently absent in *B. nuttaliana*. Similarly, the single, fast-migrating band in the indophenol oxidase pattern is consistently absent in *B. nuttaliana*. The slowly migrating band of the LAP pattern is absent in *B. sphaerocarpa* and *B. nuttaliana*.

The present work demonstrates that within a single tissue of a given species in a defined physiological state, there may exist a genetically based polymorphism with respect to isoenzyme pattern. There is therefore, no way to predict, *a priori*, the possible taxonomic or physiological implications of a given isoenzyme pattern until possible intraspecific variation has been evaluated.

EXPERIMENTAL

Collection

Leaves for analysis were collected from a total of 303 plants at thirteen population sites distributed throughout the eastern half of the state of Texas. Populations were generally chosen of a size such that about one-half of the plants present were sampled. All plants were sampled at the same developmental phase, viz. the immediate onset of flowering. For analysis, basal leaves from the second to forth nodes were collected and refrigerated.

Extraction

Five to seven leaves from a single plant (0·6 to 0·8 g wet weight) were ground to a fine powder in a mortar containing liquid N_2. This powder was transferred to a TenBroeck homogenizer and 2·0 ml of chilled 20% sucrose (w/v) was added. After manual homogenization for about 30 sec, the sample was decanted and centrifuged at 1500 × *g* for 15 min to remove particulate debris. The opalescent supernatant was withdrawn and about 50 μl layered directly on the stacking (large pore) gel as the experimental sample. Use of a sample gel was precluded by the fact that some material present in the sample prevented polymerization of the standard gel preparation solution.

Separation

Enzymes were separated using a Canalco acrylamide gel disc electrophoresis apparatus and constant current power supply. Samples were analyzed at 3 mA per gel tube for 50–60 min to provide a running distance of 35–40 mm as revealed by the bromophenol blue front.

Staining

Leucine aminopeptidase. Gels were incubated in a solution buffered to pH 4·5 of L-leucyl-β-naphthylamide and Fast Black K.[22] *β-Galactosidase.* Gels were incubated in a solution of 6-bromo-2-naphthyl-β-D-galactopyranoside in phosphate-citrate buffer. After 2–3 hr the gels were washed and placed in a solution of tetrazotized *o*-dianisidine adjusted to pH 7·8 with solid $NaHCO_3$.[23] *Indophenol oxidase.* Gels were incubated in a solution buffered to pH 6·5 of α-naphthol and dimethyl-*p*-phenylenediamone.[24]

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[22] L. BECKMAN, *Genetics* **50**, 899 (1964).
[23] H. BERGMEYER (editor), *Methods of Enzymatic Analysis*, p. 948, Academic Press, New York (1963).
[24] H. A. DAVENPORT, *Histological and Histochemical Techniques*, p. 363, W. B. Saunders, Philadelphia (1964).