A Biosystematic Study of the Narrow-leaved Spleenwort, *Athyrium pycnocarpon*[1]

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Abstract

The systematic position of the narrow-leaved spleenwort has been in question due to various interpretations of morphological data. Some workers have placed it in either the genus *Asplenium* or the genus *Athyrium* on the basis of spore and soral characteristics. Gross form of the pinnae differs from that characteristic of either of these genera. This difference has led some workers to suggest a separate genus.

In this study paper chromatography and cellulose-acetate electrophoresis were used to investigate the secondary constituents of the narrow-leaved spleenwort. Profiles were compared with those of *Athyrium thelypteroides* and *Asplenium platyneuron*. Results indicate a close affinity between the narrow-leaved spleenwort and *Athyrium thelypteroides*.

Until recently, fern taxonomy has been based largely on morphological criteria. Because of various interpretations of these observations, the systematic position of the narrow-leaved spleenwort has been in question. Some workers have placed it in either the genus *Asplenium* or the genus *Athyrium*, primarily on the basis of spore and soral characteristics (4, 7, 8, 11, 12, 13). Gross form of the pinnae differs from that of *Asplenium* and *Athyrium*. This difference led Sprengel to place the narrow-leaf spleenwort in a separate monotypic genus *Homalosorus* (8). Karyologically the narrow-leaf spleenwort resembles *Athyrium* (12). Ferns of the genus *Athyrium* have a chromosome number of $n = 40$; whereas, members of the *Asplenium* complex have a base number of $n = 36$ (7, 11).

In this preliminary study one-dimensional paper chromotography and cellulose acetate electrophoresis were used to investigate the secondary constituents of the narrow-leaved spleenwort. Profiles were compared with those of *Athyrium thelypteroides* and *Asplenium platyneuron* which were accepted as valid representatives of their respective genera. Biochemical information may help clarify relationships among these ferns.

Methods and Materials

Extensive collections of the three species were made in Indiana, North Carolina, and Tennessee. Voucher specimens were deposited in the Ball State University Herbarium. Morphological characteristics including the shape, orientation, and attachment of the sori were examined. Photographs were made to record distinguishing characteristics (Figs. 1, 2, 3).

[1] The authors acknowledge the National Science Foundation for making this study possible. Thanks are extended to Dr. Jeanette C. Oliver for her assistance in this investigation.

FIGURES 1-3. *Photographs of herbarium specimens. 1)* Asplenium platyneuron *(L.) Oakes, ebony spleenwort; 2)* Athyrium thelypteroides *(Michx.) Desv., silvery spleenwort; 3)* Athyrium pycnocarpon *(Spreng.) Tidest., narrow-leaved spleenwort.*
FIGURE 4. *Ninhydrin positive substances separated by one-dimensional paper chromatography. A.* Athyrium thelypteroides, *B.* Athyrium pycnocarpon, *C.* Asplenium platyneuron.

Frond extracts were prepared from randomly selected materials. Extracts were prepared by soaking powdered fronds in methanol (99%) and 1 drop of concentrated hydrochloric acid for 24 hours (1, 6, 9). The extract was prepared in the same manner for chromatographic and electrophoretic runs.

Fifty μl of each sample were applied to Whatman #1 paper using the spot technique. These were developed as one-dimensional descending chromatograms using butanol : acetic acid : water (12 : 3 : 5 v/v) as the solvent (3, 6).

Dried chromatograms were visualized in the presence of long wave ultraviolet light and subsequently sprayed with ninhydrin. These techniques were intended to reveal phenolics and free amino acids, respectively (1, 3, 5, 6, 10).

Cellulose-acetate strips were streaked with 5 μl of extract. The sample was applied at the anode and the secondary constituent migrated toward the cathode. Electrophoretic runs were made in a double veronal buffer salt of pH 8.6. All runs were conducted at 200 v and 5 ma. At the end of each run the strips were air dried. These were examined under ultraviolet light and photographed for a permanent record.

Observations

The use of ninhydrin positive compounds in taxonomic studies has a valid and valuable role as evidenced by the work of Alston and Irwin and others (1, 2, 5, 10). The free amino acids identified on chromatograms of the ferns under consideration have been examined and reproduced in Figure 4. Patterns A and B (*Athyrium thelypteroides* and *A. pycnocarpon*, respectively) bear a striking identity as to number and location of separated free amino acids. When compared with Pattern C *(Asplenium platyneuron)* the degree of coincidence of spots is small. Five of the free amino acids were common to both genera.

Examination of chromatograms in the presence of ultraviolet light provided the fluorescence patterns reproduced in Figure 5. Analysis of the ultraviolet patterns points to a high degree of identity of secondary constituents in *Athyrium thelypteroides* and the narrow-leaved spleenwort. The compounds of *Asplenium platyneuron* were quite dissimilar. These patterns were duplicated in 12 trials.

Cellulose-acetate electrophoresis patterns of *Athyrium thelypteroides* and the narrow-leaved spleenwort were alike except the band nearest the cathode was blue in the former and yellow in the latter. The pattern obtained from *Asplenium platyneuron* extract was entirely different (Fig. 6). The patterns were repeated without variation in four successive trials.

Summary

On the basis of the biochemical evidence presented in this work, it appears that the narrow-leaved spleenwort is more closely related

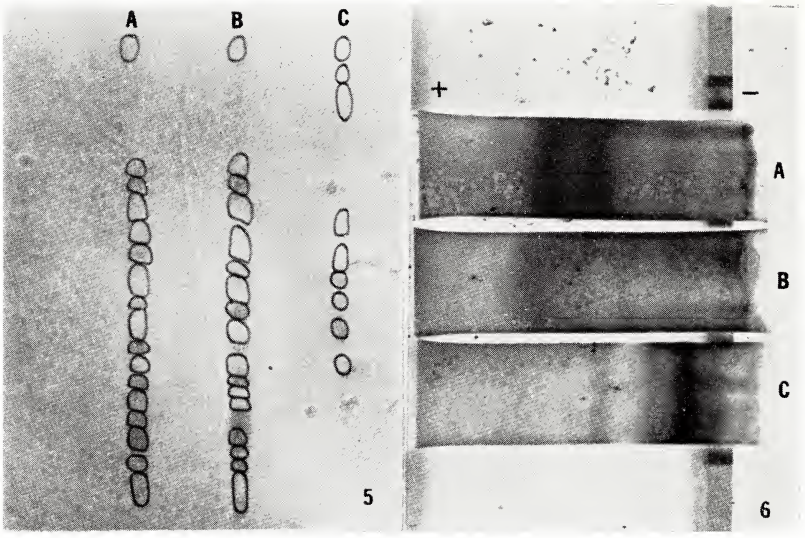

FIGURE 5. *Fluorescent compounds separated by paper chromatography. A.* Athyrium thelypteroides, *B.* Athyrium pycnocarpon, *C.* Asplenium platyneuron.

FIGURE 6. *Cellulose acetate electrophoresis membranes photographed in the presence of ultra-violet light. A.* Athyrium thelypteroides, *B.* Athyrium pycnocarpon, *C.* Asplenium platyneuron.

to *Athyrium thelypteroides* than to *Asplenium platyneuron.* Additional studies of a similar nature involving other species of *Athyrium* and *Asplenium* must be done in order to completely solve the generic problem.

Literature Cited

1. ALSTON, R. E., and H. S. IRWIN. 1961. The comparative extent of free amino acids and certain "secondary" substances among *Cassia* species. Amer. J. Bot. 48:35-39.

2. _____, and B. L. TURNER. 1963. Biochemical systematics. Prentice Hall, Inc., Englewood Cliffs, N. J. 404 p.

3. CHALLICE, J. S., and A. H. WILLIAMS. 1968. Phenolic compounds of the genus *Pyrus*—II. Phytochemistry 7:1781-1801.

4. COBB, BOUGHTON. 1956. A field guide to the ferns. Houghton-Mifflin Company, Boston. 881 p.

5. HEYWOOD, V. H. 1968. Modern methods in plant taxonomy. Academic Press, London. 312 p.

6. MEDINA BLANCO, M., E. PEINAGO LUCENA, A. G. GOMEZ CASTRO, and A. G. ARROYO. 1968. Vegetal taxonomy: A chromatographic study of genus *Trifolium.* Archivos Zootecnia 17:1-25.

7. RADFORD, A. E., H. E. AKLES, and C. R. BELL. 1968. Manual of the vascular flora of the Carolinas. The University of North Carolina Press, Chapel Hill, N. C. 1183 p.

8. SMALL, J. K. 1938. Ferns of the southeastern states. The Science Press Printing Co., Lancaster, Pa. 519 p.

9. SMITH, D. M., and D. A. LEVIN. 1963. A chromatographic study of reticulate evolution in the Appalachian *Asplenium* complex. Amer. J. Bot. 50:952-958.

10. SMITH, IVOR, and J. C. FEINBERG. 1965. Paper and thin layer chromatography and electrophoresis. Shandon Scientific Company Ltd., London. 241 p.

11. WAGNER, W. H. JR., 1954. Reticulate evolution in the Appalachian Aspleniums. Evolution 8:103-118.

12. ———. 1955. Cytotaxonomic observations on North American ferns. Rhodora 57:219-240.

13. WHERRY, E. T. 1942. Guide to Eastern Ferns. The Science Press Printing Co., Lancaster, Pa. 252 p.