A Systematic Study of the Oak Fern[1]

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Abstract

The oak fern has been placed in several different genera. It has been placed in the genus *Dryopteris;* however, cytological studies do not support this interpretation. Some have considered the fern as a member of the genus *Thelypteris;* others have included it in *Phegopteris* with the beech ferns. Currently, most fern taxonomists favor the genus *Gymnocarpium* as redefined by Ching.

In this study biochemical comparisons were made of the oak fern and representatives of genera in which it has been placed. Paper chromatographic separations of free phenolic compounds and cellulose-acetate electrophoresis patterns of oak fern extracts were quite distinctive. Biochemical evidences indicate a separate generic classification for the oak fern.

The oak fern (Fig. 1) has been placed in many different genera. This has resulted in much confusion in the literature and in field guides. In fact, Manton (5) states that the common name of this distinctive plant is more uniformly and easily recognized than the various Latin combinations.

Some authors have placed this fern in the genus *Dryopteris* primarily on the basis of a comparable number and arrangement of stipe bundles (3, 6). Fée included the beech ferns and the oak in the genus *Phegopteris* (6). Newman described the genus *Gymnocarpium* as including basically the same ferns. These plants have in common an elongate creeping rhizome and exindusiate sori (6). Slosson placed the beech ferns and the oak ferns in the cosmopolitan genus *Thelypteris* along with the marsh ferns (11). Recent biochemical studies have indicated differences between the beech and the marsh ferns (7).

In 1933, Ching proposed that the oak fern be placed in a distinct genus and revived the name *Gymnocarpium* for it (2). Morton chose to follow this combination in his treatment of the ferns in the *New Britton and Brown Illustrated Flora* (4).

Early studies emphasizing the morphology and anatomy of the oak fern have failed to clarify its relationship. Cytological studies have shown a similarity in chromosome size to the beech ferns; however, the number differs. A base number of 80 was found in the oak fern. The broad beech fern and the long beech fern showed numbers of $n = 30$ and $n = 90$ respectively (1, 5).

The members of true *Dryopteris* consistently showed a base number of 41 chromosomes (9, 10). These chromosomes are larger than those of either the beech or the oak ferns.

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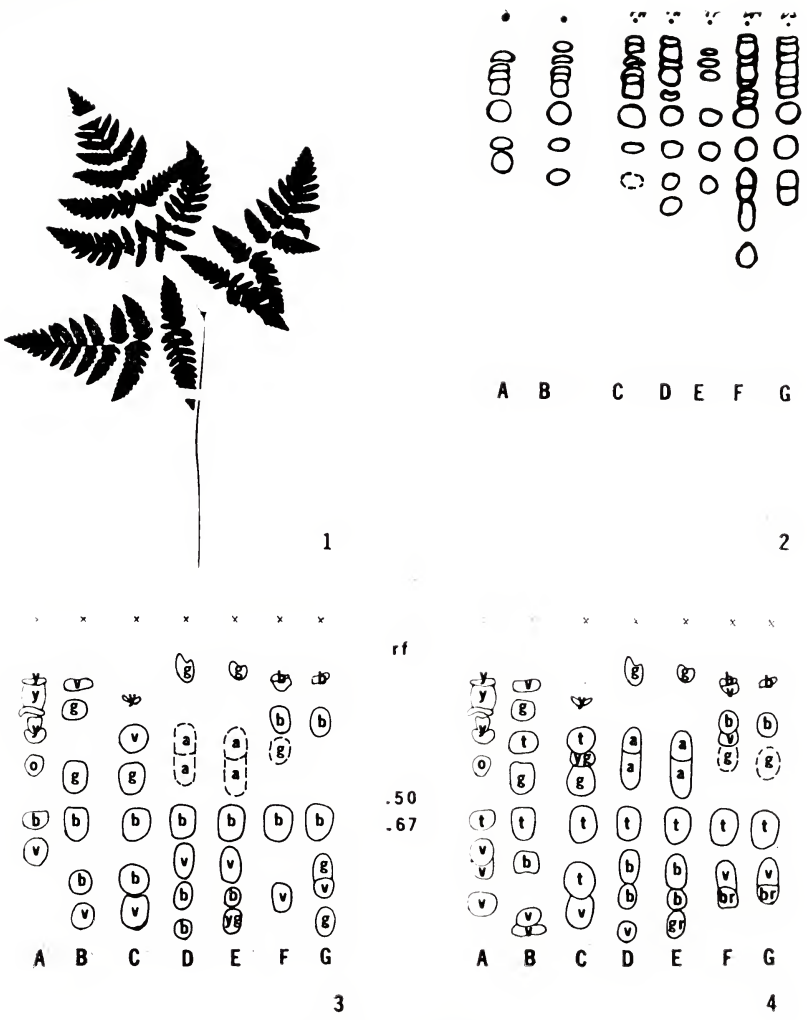

FIGURE 1. *The oak fern.*

FIGURE 2. *One-dimensional descending chromatograms showing ninhydrin positive compounds. A. Oak fern, B.* Phegopteris polypodioides *Fee, C.* Phegopteris hexagonptera *(Michx.) Fee, D.* Thelypteris noveboracensis *(L.) Nieuwl., E.* Thelypteris palustris *Schott, F.* Dryopteris marginalis *(L.) Gray, G.* Dryopteris spinulosa *(Mull.) Watt.*

FIGURE 3. *One-dimensional descending chromatograms as visualized in the presence of long wave ultra-violet light. A. Oak fern, B.* Phegopteris hexagonoptera *C.* Phegopteris polypodioides, *D.* Thelypteris noveboracensis, *E.* Thelypteris palustris, *F.* Dryopteris marginalis, *G.* Dryopteris spinulosa. (*a=avocado, b=blue, g=green, o=orange, v=violet, y=yellow*).

FIGURE 4. *One-dimensional descending chromatograms showing compounds seen in the presence of ultra-violet light and ammonia vapor. A. Oak fern, B.* Phegopteris hexagonoptera, *C.* Phegopteris polypodioides, *D.* Thelypteris noveboracensis, *E.* Thelypteris palustris, *F.* Dryopteris marginalis, *G.* Dryopteris spinulosa. (*a=avocado, b=blue, br=brown, g=green, gr=grey, o=orange, t=turquoise, v=violet, y=yellow*).

This study was undertaken in an attempt to clarify the status of the oak fern by the consideration of biochemical characteristics. Chromatographs and electrophoretographs of the oak fern were compared with those of representatives of the beech ferns, the marsh ferns, and the dryopteroids.

Methods and Materials

Extensive collections of the ferns were made throughout Indiana, Ohio, and Wisconsin. Voucher specimens were deposited in the Ball State University Herbarium.

Extracts were prepared by powdering dried fronds and soaking the materials in methanol:water:hydrochloric acid (7.9 : 2 : 0.1) for 48 hours. Some comparisons were made by paper chromatography. Fifty μl of each sample were applied to Whatman #1 paper using the spot method. The chromatographs were run descendingly using butanol: acetic acid: water (12 : 3 : 5) as the solvent.

Dried chromatograms were examined in the presence of ultraviolet light. The chromatograms were sprayed with ninhydrin for the detection of free-amino acids and related substances, and alkaline silver nitrate, a general reagent for the detection of phenolic compounds (8).

Cellulose acetate electrophoresis studies were made using a Buchler migration chamber and Buchler cellulose acetate strips measuring 1 by 6 inches. Ten μl of extract were applied with a streaking pipette at the center of each strip and migrations were allowed to proceed for 1 hour. The electrophoretic runs were conducted at 200 v and 5 ma in a double veronal buffer salt of pH 8.6. Electrophoretic patterns were observed in the presence of long wave ultraviolet light.

Results

Extracts of the oak fern were compared chromatographically and electrophoretically with those of *Phegopteris polypodioides*, *Phegopteris hexagonoptera*, *Thelypteris noveboracensis*, *Thelypteris palustris*, *Dryopteris marginalis*, and *Dryopteris spinulosa*.

Chromatographs examined in the presence of long wave ultraviolet light and ultra violet light in combination with ammonia vapor revealed little similarity between the oak fern and the others with which it has been grouped. The fluorescent substances were indicated by their positive reactions with alkaline silver nitrate to be phenolic in composition. One compound with a rf value of 0.67 was common to all species (Figs. 3, 4). Several compounds appeared to be genus specific.

Five compounds, rf 0.16, 0.32, 0.40, 0.80, and 0.89 were specific to the genus *Dryopteris* (Figs. 3, 4). Compounds with rf values of 0.14, 0.38, 0.48, 0.80, and 0.89 were characteristic of *Thelypteris* (Figs. 3, 4). The genus *Phegopteris* was characterized by spots with rf values of 0.35, 0.48, and 0.91 (Figs. 3, 4).

A few species specific compounds were noted in all ferns considered.

Ten compounds were detected in the oak fern. None were shared with other genera with the exception of rf 0.67 which was found in all samples.

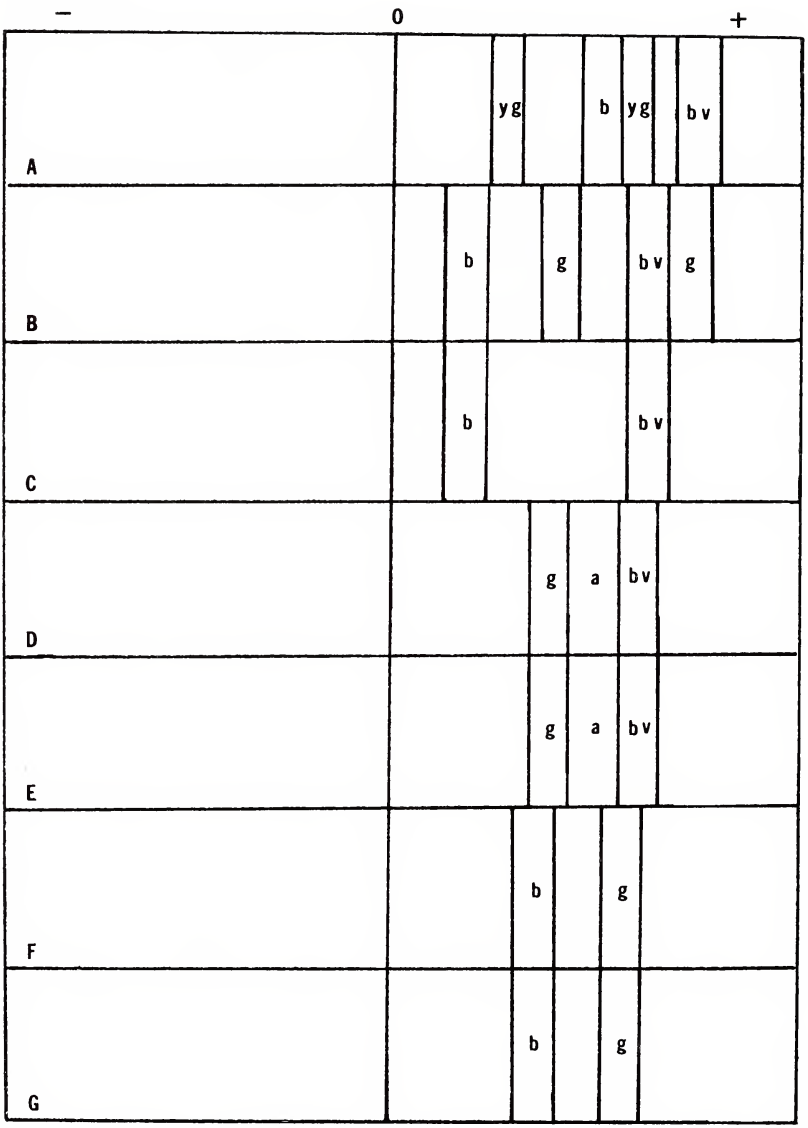

FIGURE 5. *Cellulose acetate electrophoresis patterns. A. Oak fern, B.* Phegopteris polypodioides, *C.* Phegopteris hexagonoptera, *D.* Thelypteris noveboracensis, *E.* Thelypteris palustris, *F.* Dryopteris marginalis,, *G.* Dryopteris spinulosa *(a=avocado, b=blue bv=blue-violet, g=green, y=yellow).*

Some chromatographs were sprayed with ninhydrin. Little could be derived concerning relationships as most ninhydrin positive compounds were shared by all ferns or were species specific (Fig. 2).

Cellulose acetate electrophoresis membranes were observed in the presence of long wave ultraviolet light. All constituents migrated toward the positive pole. The patterns obtained were quite distinctive within each genus.

Dryopteris marginalis and *D. spinulosa* shared two fluorescent bands. Three common bands were noted in *Thelypteris noveboracensis* and *T. palustris*. The genus *Phegopteris* was characterized by two specific bands. Two additional species specific bands were seen in *P. polypodioides* (Fig. 5).

Distinctive blue-violet and yellow bands were seen in the oak fern. These did not correspond with those of the other genera (Fig. 5). No attempt was made to determine the constituency of the bands.

Discussion and Summary

The results of this study indicate the desirability of the separate generic classification of the oak fern. No significant affinities were indicated by chromatographic or electrophoretic patterns with representatives of *Dryopteris*, *Thelypteris*, and *Phegopteris*.

Some workers have suggested a single genus to include the beech and the marsh ferns. This study, however, provides additional evidence for the maintenance of *Phegopteris* and *Thelypteris* as separate genera (7).

Many problems continue to exist among these ferns. Additional studies involving the Roberts fern, *Gymnocarpium robertianum*, which is strikingly similar to the oak fern and studies of the thelypteroids from other parts of the world must be undertaken before a complete taxonomic understanding of this group of ferns can be gained.

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