

A Study of Variation in Leaf Anatomy with Reference to Habitat in two Species of *Polypodium*

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Introduction

Although ecological anatomy has been extensively investigated in higher plants, comparatively few investigations have been made of anatomical variation as related to habitat in ferns. Two species of *Polypodium*, both occurring in Indiana, exhibit a rather conspicuous habitat diversity which suggested a study of such variation. *P. virginianum* L. normally occurs terrestrially or growing upon rocks, but is rarely an epiphyte. *P. polypodioides* (L.) Watt. var. *Michauxianum*, on the contrary, is usually an epiphyte, being commonly non-epiphytic only near the northern limit of its range, which extends into southern Indiana.

The typically non-epiphytic occurrence of *P. virginianum* is normal for temperate zone vascular plants. The species is, according to Fernald (9), chiefly a northern form, extending from Newfoundland and Manitoba as far south as the mountains of northeastern Georgia. *P. polypodioides*, however, is a tropical form whose close relatives extend throughout South America. Weatherby (15) lists the variety as ranging from Maryland, Illinois, and Missouri southward to Florida, Texas, and Guatemala.

In Indiana, Deam (8, pp. 57-58) reported *P. virginianum* as occurring in fifteen counties exclusively in the western portion of the state and extending its complete length in the north-south direction. Its distribution appears to be correlated with the occurrence of outcrops of sandstone, where it is commonly found near rock ledges. No report of its epiphytic occurrence was given. *P. polypodioides* has been found locally in nine Indiana counties, chiefly in the southern portion of the state in counties bordering the Ohio River. It is roughly limited to the unglaciated area, and has been found only as far north as Monroe County. This fern occurs most frequently on cliff faces and rock surfaces, with only one epiphytic occurrence having been reported.

Studies of variation in leaf anatomy in response to environmental factors have been made by many investigators, including Clements (7), Hanson (10), Pool (14), and Cain and Potzger (5), (6). Although the details of procedure and the plants investigated differ widely in these studies, they are in general accord in finding that leaves from increasingly xeric conditions show a reduction in total leaf size, an increase in thickness, increased development of palisade mesophyll,

denser vein network, and usually an increase in stomatal number per unit area. In addition, Cain and Potzger (6) have emphasized the variability encountered between different leaves on the same plant, and even at different locations on the same leaves, with the consequent necessity for the application of statistical techniques to this type of data.

Notations of the rare occurrence of *P. virginianum* as an epiphyte have been made by Fernald (9), Brown (3), and Johnson (11). When epiphytic, it commonly occurs at rather low levels, and usually in relatively protected situations, such as wooded gorges. *P. polypodioides*, on the contrary, has been studied as a typical vascular epiphyte by Andrews (1) and Pessin (13). It occurs on a wide variety of trees and often in relatively unshaded locations. The most favorable substratum is a rough, soft bark with high capacity for water absorption and a low rate of water loss.

In an investigation of microclimatic variation, Baum (2) found the vertical temperature gradient between the 0.5 and 6 foot levels in the summer in the northeastern United States to be the equivalent of a horizontal north-south difference of about 380 miles at the 6 foot level. He suggests that this difference should have an important ecological effect upon vegetation. Terrestrial and epiphytic occurrences of the same species would presumably be influenced by this microclimatic difference.

Material and Methods

Specimens of *P. virginianum* examined in this study were collected at Fern Cliff, Putnam County, 9 miles southwest of Greencastle, Indiana, except for one set obtained along the south bank of the White River in Martin County, approximately 3 miles west of Shoals, Indiana. All specimens of *P. polypodioides* were also obtained from the locality near Shoals with the exception of a single set which was collected at St. George, Dorchester County, South Carolina.

The physical features of the two Indiana localities are remarkably alike. At Fern Cliff, the plants were growing on the cliff face, bordering the south bank of a west fork of Little Walnut Creek. This steep and irregular bluff was cut through the Mansfield sandstone, which outcrops prominently on its face. Since it faces north and is densely wooded, it is relatively moist and heavily shaded. *P. virginianum* is common both on the ground and on the surfaces of the sandstone outcrops. It was, however, found growing epiphytically on only a single tree.

West of Shoals, erosion through the Mansfield sandstone has produced a similar line of north-facing bluffs, near the base of which flows the White River. These are also wooded, and show a comparable luxuriant development of ferns. *P. polypodioides* as well as *P. virginianum* occurs abundantly as dense mats on the surfaces of sandstone outcrops. Only a single tree, however, bore *P. polypodioides* epiphytically.

Growths of foliose lichens were associated with the epiphytic occurrences of both species. The ferns were, however, growing upon the rocks without close associates.

At Fern Cliff, *P. virginianum* was collected from the following three habitats: (1) growing terrestrially in the humus, (2) growing as dense mats on the vertical faces of sandstone outcrops, (3) growing epiphytically at the base and extending upward to a height of three feet upon the north and northeast sides of the trunk of a single beech tree, *Fagus grandifolia*. For purposes of comparison with material from a different area, this species was also obtained from dense mats of plants (4) growing upon the vertical surfaces of north-facing outcrops of sandstone at the Shoals locality. In the table following, these habitats are named as follows: (1) ground, (2) rock-fern Cliff, (3) tree base, (4) rock-Shoals.

TABLE I. Significant Differences¹ Between Means of Measurements of *P. virginianum* Leaves from Different Habitats.

Item Measured	Habitats			
	Rock-Fern Cliff—Ground	Tree base—Rock-Fern Cliff	Tree base—Ground	Rock-Shoals—Rock-Fern Cliff
Length			—	+
Width	—		—	—
Stomatal Number	—		—	+
Upper Cuticle	—	—	+	+
Lower Cuticle			+	
Upper Epidermis				
Lower Epidermis				+
Mesophyll Total Thickness				+

¹ + indicates that a significant difference exists, and that the first listed habitat of the compared pair is larger than the second. — indicates that a significant difference exists, the second habitat being larger than the first.

P. polypodioides was collected from the following habitats at the previously described locality near Shoals: (1) growing in dense mats occurring on the vertical surface of sandstone outcrops, (2) growing epiphytically upon the east and south-east sides of the base of the trunk of a single *Fagus grandifolia*, (3) growing epiphytically at a level of approximately fifteen feet upon the east and southeast sides of the same beech tree referred to in (2). *P. polypodioides* was also obtained from a location geographically more typical for the species, at St. George, Dorchester County, South Carolina. The occurrence was, (4), on the southeast side of the trunk of a large live oak, *Quercus virginiana*, in a relatively unshaded location. Material was collected from the trunk

at a height of approximately six feet. A dense growth of moss was present about the roots of the plants. These habitats are named in the following table as (1) rock, (2) tree base, (3) tree-15', (4) tree-S. C.

Measurement of gross dimensions was made on sets of 25 leaves from each habitat selected at random insofar as possible. All material was collected in the late fall or winter, so that fully developed leaves were obtained. Length was measured from rhizome to leaf tip, width at the basal lobes of the deeply incised leaf where this dimension was at a maximum.

Stomatal frequency was determined by examination of 25 leaves of each species, selected at random from each habitat. The pair of lobes second from the base of each leaf was arbitrarily selected for study; the number of stomata was counted in each of 4 microscopic fields distributed from the base to the tip of left and right lobes, alternately, of this pair, giving a total of 100 fields examined per habitat. The lower epidermis was removed from leaves of *P. virginianum* for study, while examination was made of strongly illuminated whole mounts of lobes of *P. polypodioides*.

For the study of leaf tissues, samples of 25 leaves were chosen at random from each habitat for each species. In order to obtain comparable samples from each leaf, the pair of lobes second from the base of the leaf was again arbitrarily selected. Samples were obtained by cutting small sections alternately from the left and right lobes of this pair, and perpendicular to the midvein at a distance of approximately 1 cm. from the rachis.

TABLE II. Significant Differences¹ Between Means of Measurements of *P. polypodioides* Leaves from Different Habitats.

Item Measured	Habitats			
	Tree base Rock	Tree-15'— Tree base	Tree-15'— Rock	Tree S.C.— Tree-15'
Length	—	—	—	+
Width		—	—	+
Stomatal Number	—	+	—	
Upper Epidermis	+	—		
Lower Epidermis	+	—		
Palisade Tissue				+
Spongy Mesophyll			—	+
Total Thickness				—

¹ + indicates that a significant difference exists, and that the first listed habitat of the compared pair is larger than the second. — indicates that a significant difference exists, the second habitat being larger than the first.

The samples so obtained were killed and fixed in formalin-acetic-alcohol, embedded in paraffin, sectioned at 10 microns, and stained with safranin and fast green. Tissue thicknesses were measured at right angles to the upper leaf surface at a distance of approximately 250 microns from the midvein. Measurements were made by means of an ocular micrometer.

The data thus obtained were treated by standard statistical procedures. For each set of measurements, the mean, standard deviation, standard error, and coefficient of variation were calculated. In comparing sets of measurements, the difference, standard error of the difference, and significance ratio were determined. Differences were considered significant at the 2% level of confidence, corresponding to a significance ratio of 2.49.

Measurements at regular intervals of the physical factors of the environment such as light, temperature, rainfall, and humidity for each habitat over an extended period are desirable for a study of this nature. Because of the distances to and between the localities involved, and the necessity for such measurements to be made during the spring and summer months, this data could not be secured. This limitation must be considered in examining the results of this study.

Description

The leaves of *P. virginianum* are simple but deeply pinnatifid, being divided into opposite to sub-opposite, linear-oblong lobes which have entire or slightly toothed margins. The lobes vary in number from approximately 5 to 50, depending on the length of the leaf.

The leaves are somewhat coriaceous and are evergreen. Under conditions of moisture deficiency they undergo curling which leaves the lower, stomata-bearing surface exposed.

Both upper and lower leaf surfaces appear glabrous to the naked eye, but microscopic examination shows that a limited number of hairs are present on the lower epidermis. These hairs are typically multicellular, being composed of a narrow, elongate basal cell and a bulbous terminal cell. The hairs are about 250 microns long. Stomata are present only on the lower epidermis, which is loosely attached to the mesophyll. A continuous cuticle is present on both upper and lower leaf surfaces. No palisade is differentiated.

The leaves of *P. polypodioides* are also simple and deeply pinnatifid, with linear-oblong lobes extending nearly to the petiole. The leaves are coriaceous and evergreen. The lobes have entire margins, and are typically both thicker and smaller than those of *P. virginianum*. A range in total number of lobes per leaf of from 3 to 33 was observed from leaves of varying lengths. Stomata are borne only on the lower epidermis. The mesophyll is differentiated into both spongy and palisade tissue.

While the upper leaf surface is glabrous, the lower surface and the petiole are in large part covered with brownish, orbicular to deltoid-

ovate, peltately attached scales. These scales are multicellular, and consist of a basal portion sunk into the lower leaf surface, an outwardly tapering stalk, and a terminal plate-like portion. The scales are variable in size, but combine to cover a major portion of the lower surface, imparting to it a grayish-brown color.

The cuticle displays a seeming lack of regularity, no cuticle being apparent on some portions of the epidermis, while on others it is well-developed. It is not possible to report this as a typically occurring situation on the basis alone of the examination made in connection with this study. However, the cuticle could not be reliably measured for this species in the material examined, and therefore has not been included.

Under dry conditions, the leaves of *P. polypodioides* undergo a characteristic curling, unrolling when moisture again becomes available. The margins of each lobe are folded inward, and at the same time the lobe rolls inward toward the petiole from the end. The entire leaf also curls over toward its base. All curling is in a manner which leaves the lower, scale- and stomata-bearing surface outwardly exposed.

Results and Discussion

Although it is not possible to present here complete tables of the data for each leaf dimension measured¹ the occurrence of significant differences between means of the various measurements from the habitats compared are compiled in Table I for *P. virginianum*, and in Table II for *P. polypodioides*.

Of the *P. virginianum* habitats at Fern Cliff, decreases in both length and width occur in the same order of habitats, ground, rock, and tree-base. Significant differences occur in widths in two habitat pairs, while differences in length are significant only between the tree-base and ground habitats. There seems to be a tendency toward reduction in leaf size between the ground and tree habitats, with the rock location intermediate in this respect.

Comparison of the similar rock habitats at the separate localities for this species, however, reveals a significant difference in length, 6.24 cm., which is nearly three times as great as the difference of 2.38 cm. existing between the most highly contrasting habitat pair, tree base—ground, at Fern Cliff. The opposite is true of the width, since the mean measurement from the rock-Shoals location, 3.97, is smaller than that from any of the Fern Cliff habitats, and significantly so in comparison with the rock habitat there. This apparent anomaly is difficult to explain in terms of ecological variation though real enough, for the leaf presents a long, slender appearance which is distinctive even to casual observation. The species tends to be polytopic in Indiana, since the outcrops of sandstone on or near which it is found are discontinuous. The possibility of slightly differing characters occurring in separate locations quite apart from ecological variation would thus seem to

¹ For complete data, see unpublished thesis, DePauw University, Greencastle, Indiana.

exist. At any rate, the total leaf area, the important factor to which both length and width relate, is increased by the large increase in length to a degree which renders the width decrease comparatively unimportant, whatever its cause.

The leaf of *P. virginianum* is thus seen to show significant variation in both length and width, and consequently in total leaf area. These size variations are conspicuously smaller between contrasting habitats at the same location than they are from two similar habitats at different locations.

Leaves of *P. polypodioides* from contrasting habitats at Shoals also show a regular order of decrease, rock, tree-base, and tree-15', in both length and width. The differences are significant between all pairs for length, and for all but tree base—rock for width, and indicate a decrease in total leaf size with the listed order of habitat change. The epiphytic *P. polypodioides* from South Carolina is significantly both longer and wider than either of the Shoals epiphytes.

As was the case with *P. virginianum* at Fern Cliff, *P. polypodioides* at Shoals shows variability in size of leaf between contrasting habitats. Size differences between occurrences similarly in being epiphytic, but geographically separate, are, however, greater for width and as great for length than those from the same geographic location.

Incidence of stomata, while at times showing variation to a significant degree in both species, as shown in Tables I and II, does not form an indicative pattern. It increases in number, for example, with increase in leaf size in *P. polypodioides* between the tree-15' and the rock habitats, and increases in number with the decrease in leaf size in the same species between the tree-15' and the tree-base habitats. Consequently, as has been noted by Miller for other plants (12, p. 418), the number of stomata does not appear to be a clear-cut variable of increasingly xeric conditions.

The cuticle, which, as noted earlier, was measured only in *P. virginianum*, regularly shows significant changes between habitats. Because of its high degree of variability within a given habitat (coefficients of variation are about 20%), it seems worthwhile only to note that in general the upper cuticle seems to be influenced more by habitat contrasts than is the lower.

No individual tissues from leaves of *P. virginianum* from contrasting habitats showed differences in thickness great enough to be significant. Although the significance ratio for the sum of the individual leaf tissues, total leaf thickness, was rather high, it did not reach the 2% level of confidence. Consequently the gross leaf measurements, for which significant differences do exist, have been, apparently, more responsive to the environmental contrasts between the habitats at Fern Cliff than were the internal tissues, particularly the mesophyll, which is normally the most variable of the leaf tissues.

That variation can exist in mesophyll thickness in *P. virginianum*, however, is shown by the significant difference in means between the

rock occurrences at the separate Fern Cliff and Shoals localities (Table I). This increase in mesophyll at Shoals is also reflected in a significant difference in total leaf thickness between the two habitats. As was the case for gross measurements, greater variability of both mesophyll and total leaf thickness is found between similar habitats which are geographically separate than between contrasting habitats at the same locality.

Leaf tissues of *P. polypodioides* from the habitats at the Shoals locality, while showing some slight variation, reached the level of significance only for the relatively thin epidermis from two habitat pairs, and for the spongy mesophyll, which was thicker in the rock than in the tree-15' location (Table II).

Internal structure of leaves from the epiphytic occurrence in South Carolina, on the other hand, contrasts greatly with that of leaves from the Shoals locality. There is a greatly increased development of both palisade and spongy mesophyll, resulting in significant increases of thickness, both of these tissues and of total leaf thickness.

As was also found for gross measurements, thicknesses of leaf tissues are more constant for the differing habitats at a single locality than between epiphytic habitats at different localities.

In both species, more variation occurred in gross measurements between differing habitats at the same locality than did in the thicknesses of component leaf tissues. Leaf size, particularly length, probably due to continued apical growth, seems to be more responsive to differences in environmental factors than is the development of leaf tissues.

The relatively smaller amount of variation in leaf anatomy previously noted in each species from contrasting habitats at the same geographic locations, compared to the contrast between plants from similar habitats at separated locations suggests that contrasts in environmental factors are greater due to geographic separation than to vertical distribution in the occurrences studied for each plant. The existence of this situation seems plausible in the present instance, since, it will be recalled, both the Fern Cliff and Shoals localities are rather specially situated and similar north-facing bluffs, moist, shaded, and highly protected. It seems possible that the rare epiphytic occurrence of these ferns so far north is dependent upon the existence of special conditions under which the environmental factors, other than the actual nature of the substratum, are not sufficiently different from those of the normal ground or rock habitats to bring about the ecological variation in anatomy of which the leaves of these plants are capable.

In the absence of measurements of environmental factors, no specific conclusions can be drawn as to the type of conditions producing these variations. However, there is no apparent reason to believe them different from those producing similar responses in other plants. From the data presented, it is apparent that a considerable degree of plasticity exists in the response to environmental conditions exhibited by the two species of *Polypodium* considered. In general, this response is

similar to that typical of higher plants, with decrease in total leaf size accompanied by a relative increase in thickness, principally of the palisade and spongy mesophyll.

Summary

1. In this paper, the variation in leaf anatomy of two fern species, *P. virginianum* and *P. polypodioides*, has been examined. For each species, samples of 25 leaves from each of three different habitats, including both terrestrial and epiphytic occurrences, were obtained from the same location. A comparison was also made for each species to one similar habitat at a geographically separate location.

2. Gross leaf dimensions, stomatal number, and thickness of the various leaf layers were measured for each species. Standard statistical procedures were applied to the data thus obtained.

3. Results indicate that these ferns exhibit variation in leaf anatomy similar in both degree and direction to that previously reported for higher plants. Leaf size, as shown principally by length of leaf, apparently is modified by environmental differences which are not sufficient to affect significantly the thickness of the leaf layers.

4. Variation in leaf anatomy is found to be more pronounced between the ecologically similar but geographically separate habitats, than it is between the contrasting but adjacent habitats which were compared. It is therefore suggested that the normally rare (in Indiana) epiphytic occurrence of these two species may be in this instance related to the location of host trees in special habitats where the normal vertical gradient of increasing xeric conditions is decreased.

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