A Taxonomic Study of Genus *Polygonum* Employing Chromatographic Methods[1]

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Abstract

The taxonomy of genus *Polygonum* is confused because the morphological characteristics of the different species are not distinct. This study examined the possibility of using paper chromatography as a means of distinguishing the various species and sections of the genus. The chromatographic patterns for free amino acids and secondary substances of the various species were compared. Eight different species of genus *Polygonum* belonging to three different sections were studied. Five of these species are included in section *Polygonum*: *P. arenastrum, P. aviculare, P. erectum, P. Fowleri,* and *P. allocarpum.* Two species belong to section *Persicaria*: *P. orientale* and *P. pennsylvanicum;* and one species, *P. tenue,* is assigned to section *Duravia.* The chromatographic patterns for the free amino acids were similar for all species studied and were of little value in distinguishing the different species. The chromatographic patterns for the secondary substances were of taxonomic value. Each species studied had a distinct chromatographic pattern for secondary substances and some similarities were noted in the chromatograms of members of the same section.

Earlier studies of genus *Polygonum,* section *Polygonum,* done in the second author's laboratory (5, 7), employed morphological and cytological data as a means of clarifying taxonomic relationships in this group of plants. In the present study various species of *Polygonum* were identified on the basis of morphology and their chromatographic characteristics were then investigated. Plants employed in this study included herbarium specimens and newly collected material from the Canadian Atlantic Provinces, Nova Scotia and New Brunswick. Eight species belonging to three different sections of genus *Polygonum* were studied chromatographically: Five species are included in section *Polygonum: P. arenastrum, P. aviculare, P. erectum, P. Fowleri,* and *P. allocarpum.* Two species belong to section *Persicaria: P. orientale* and *P. pennsylvanicum;* and one species, *P. tenue,* may be assigned to section *Duravia.*

Morphological characteristics examined in making identification of *Polygonum* species included leaf size and shape; and achene color, texture, shape and size; and floral characters including the number of stamens, stigmas, and sepals. From previous studies of section *Polygonum,* the most useful taxonomic characteristics were achene and perianth characters (4, 5, 7).

Several species investigated are similar to each other on the basis of morphological characters. For example, *Polygonum aviculare*

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and *P. arenastrum* are considered to be one species by Fernald (2) and Gleason (3), although more recent investigations have treated them as separate species (4, 8). Styles (8) and Mertens and Raven (4) found that *P. aviculare* has larger achenes with a shape different from those of *P. arenastrum.* The same investigators report that specimens of *P. aviculare* are more heterophyllous than specimens of *P. arenastrum.* Collections of these species made by the present workers in Nova Scotia and New Brunswick and housed in the Ball State University Herbarium generally agree with the published descriptions of Styles (8) and Mertens and Raven (4).

Similarly, on the basis of morphological characters Gleason (3) and Mertens and Raven (4) consider *Polygonum Fowleri* and *P. allocarpum* to be synonymous. In this study specimens of *P. Fowleri* collected in Canada were compared to specimens of *P. allocarpum* and *P. Fowleri* from the Butler University and University of Wisconsin herbaria and were found to have similar morphological characters. The most noticeable difference between these two species is a difference in the size of their achenes. The achenes of *P. allocarpum* were slightly larger than those of *P. Fowleri.* Bi-convex achenes, as opposed to trigonous achenes, occur more frequently in specimens labeled *P. allocarpum.*

Polygonum tenue was also studied because there is a lack of agreement as to the section of genus *Polygonum* to which it belongs. On the basis of pollen structure and plant morphology, *P. tenue* was included in section *Duravia* by Mertens and Raven (4). Fernald (2) and Gleason (3), however, include *P. tenue* in section *Polygonum (Avicularia).* We noted that the leaves of *P. tenue* are greatly reduced in size as compared to those of plants in section *Polygonum. Polygonum tenue* has flowers aggregated near the ends of the branches and has distinctive, shiny, black achenes. The numerous specimens investigated were collected in Indiana by A. D. Savage (7) and are housed in the Ball State University Herbarium.

Finally, one or two specimens of *Polygonum pennsylvanicum* and *P. orientale* from the Ball State Herbarium were studied as representatives of section *Persicaria.*

Paper chromatography was used to study the free amino acids and secondary substances as possible taxonomic characters which might be used to distinguish the different species or to limit the different sections of genus *Polygonum.* A study of free amino acids was of benefit in determining the taxonomic relationships of certain members of the family Iridaceae (6). In similar studies among *Cassia* species paper chromatography of free amino acids and secondary substances was also used as a criterion for classification (1).

The free amino acids referred to in this study occur freely in the cell and are not a part of the protein structure of the cell. The secondary substances include phenolic compounds such as alkaloids, terpenoids, and polyphenols (1). Chromatographic characteristics were

correlated with the morphological characters to interpret taxonomic relationships.

Materials and Methods

Plant extracts that were to be compared by paper chromatography were prepared from freshly collected plant material and from older herbarium specimens. A mixture of 0.5 g of plant material consisting of equal amounts of stems and leaves was mascerated in a mortar. The ground-up material was transferred to a vial containing 4 ml of absolute methanol. A drop of 1 N hydrochloric acid was added to the vial and it was corked and allowed to stand at room temperature for 24 hours before it was used in streaking the chromatography paper.

Chromatograms were run using large (46 x 57 cm) sheets of Whatman No. 1 chromatography paper. All chromatograms were one dimensional and developed by the descending method. The extracts were applied to the paper with a 5 μl pipette in 0.5 inch streaks which were 2.5 inches from the top of the paper. Twenty-five separate applications were applied to the paper for most species.

The chromatograms were developed for 24 hours in a solvent consisting of 12 parts n-butyl alcohol, 3 parts glacial acetic acid, and 5 parts distilled water, then air dried.

Those chromatograms in which free amino acids were to be detected were sprayed with a solution of ninhydrin prepared by dissolving 3 g of ninhydrin in 100 ml of n-butyl alcohol and 3 ml of glacial acetic acid. After spraying with ninhydrin, the chromatograms were air dried for 12 hours before observations were made and recorded.

In detecting secondary substances, chromatograms were viewed under long wave ultraviolet light in the presence of ammonia vapors which intensified the colors. The colors of the different bands were recorded and their Rf values were determined.

Chromatographic data reported in this paper were obtained from the following collections:

Polygonum allocarpum. Washington Co., Maine. C. H. Knowlton, August 3, 1936. Butler University Herbarium.

Polygonum arenastrum. Little Narrows—Iona, Victoria Co., Nova Scotia. D. M. Jones LN2, August 18, 1969. Ball State University Herbarium.

Polygonum aviculare. Great Bras d'Or, Frankaleen Beach, Cape Breton Co., Nova Scotia. D. M. Jones NSJ21, August 17, 1969. Ball State University Herbarium.

Polygonum erectum. Porter Co., Indiana. A. D. Savage 58-30, August 29, 1966. Ball State University Herbarium.

Polygonum Fowleri. Murphy's Cove, Halifax Co., Nova Scotia. D. M. Jones MCS3, August 21, 1969. Ball State University Herbarium.

Polygonum Fowleri. Ship Harbour, Halifax Co., Nova Scotia. D. M. Jones MC4, August 20, 1969. Ball State University Herbarium.

Polygonum Fowleri. Deer Island, Charlotte Co., New Brunswick. T. R. Mertens NB36, August 19, 1968. Ball State University Herbarium.

Polygonum Fowleri. Deer Island, Charlotte Co., New Brunswick. T. R. Mertens NB38, August 19, 1968. Ball State University Herbarium.

Polygonum Fowleri. Riviere Du Loup, Quebec. J. F. Collins and M. L. Fernald 198. August 31, 1904. University of Wisconsin (Madison) Herbarium.

Polygonum orientale. Delaware Co., Indiana. N. H. Woodruff, July 17, 1941. Ball State University Herbarium.

Polygonum pennsylvanicum. Delaware Co., Indiana. Ethel B. Finster, August 19, 1924. Ball State University Herbarium.

Polygonum tenue. Fulton Co., Indiana. A. D. Savage 50-1, August 20, 1966. Bal State University Herbarium.

Data and Discussion

The chromatograms that were sprayed with ninhydrin to reveal free amino acids had very similar patterns (Fig. 1). With the exception of *Polygonum erectum* which had 10 bands, the chromatograms of species of section *Polygonum* had 9 bands (Table 1). Great difficulty was encountered in obtaining readable results from

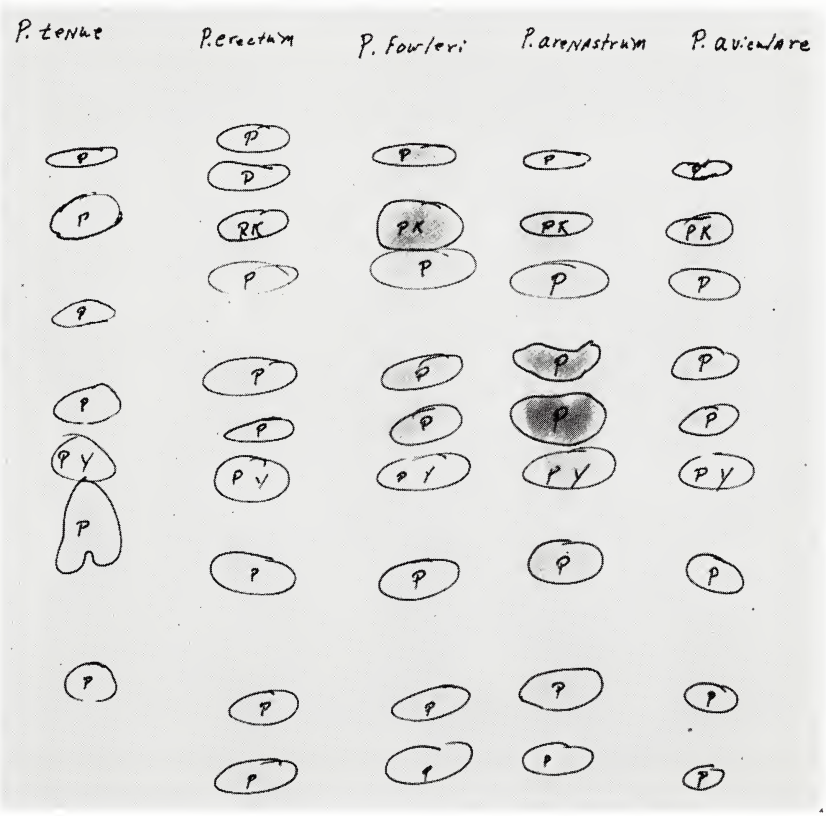

FIGURE 1. *A chromatogram of the free amino acids of plants of sections* Polygonum *and* Duravia. *The species of section* Polygonum *included were* P. arenastrum, P. aviculare, P. Fowleri, *and* P. erectum. *One species from section* Duravia, P. tenue, *was chromatographed. Letters refer to bands of distinctive color, as follows: P = purple; PK=pink; PY=pale yellow.*

extracts of *P. tenue*. In all other species 25 separate applications of extract to the paper gave readable results, but 40 separate applications were necessary to obtain readable chromatograms for *P. tenue*. Within section *Persicaria*, chromatograms of *Polygonum pennsylvanicum* had ten principal bands and those of *P. orientale* had nine bands.

Most of the bands were purple when sprayed with ninhydrin but a distinct pale yellow band was present about midway down the paper for all species studied. Plants that are members of section *Polygonum* had a narrow pink band near the top of the paper. Figure 1 demonstrates that the chromatogram of a *P. tenue* plant lacks this pink band. *Polygonum tenue* is considered to be more closely allied to section *Duravia* by some authors (4), and the chromatographic data (Table 1) suggest that *P. tenue* differs considerably from species in section *Polygonum*.

Polygonum orientale and *P. pennsylvanicum* of section *Persicaria* produced chromatograms that were similar, but chromatograms of *P. pennsylvanicum* had one more pale yellow band than did those of *P. orientale*. Neither species had the narrow pink band found near the top of the chromatograms of members of section *Polygonum*.

The Rf values of the free amino acids for the different species of *Polygonum* appear similar, with the exception of the narrow pink band found only in species of section *Polygonum* (Table 1). It has not been established whether this narrow pink band is a distinct characteristic for all species of section *Polygonum*. A distinct pale yellow band with an Rf value of ca. 0.60 was noted for the 2 species in section *Persicaria*, but was not found in the chromatograms of members of sections *Polygonum* and *Duravia*. It has not been determined whether the presence of this additional pale yellow band is characteristic of all species of section *Persicaria*.

In analyzing the chromatograms for the secondary substances distinct variations were noted in the chromatographic patterns for the different species (Table 2). A wide yellow band (Rf = 0.60 to 0.70) was located at a somewhat similar position in all members of section *Polygonum*, but this band was also present in *Polygonum pennsylvanicum* of section *Persicaria*. A distinct narrow bluish-white band (Rf = 0.55 to 0.63) occurred in *P. arenastrum*, *P. aviculare*, and *P. erectum*. This band also occurred at approximately the same place in *P. pennsylvanicum*. A dark blue band (Rf = 0.51 to 0.59) which was located immediately above the bluish-white band also occurred in *P. arenastrum*, *P. aviculare*, and *P. erectum*. Thus, there are some chromatographic similarities among members of the same section, but no definite pattern could be recognized for distinguishing these three sections of genus *Polygonum* on the basis of secondary compounds.

In comparing the chromatographic patterns of secondary substances for the different species, several bands were found at similar locations for *Polygonum aviculare*, *P. arenastrum*, and *P. erectum*.

TABLE 1. *Rf values for chromatograms of free amino acids for different species of* Polygonum.

	Color and Rf Values for *Polygonum* Species						
Color	*P. pennsylvanicum*	*P. orientale*	*P. tenue*	*P. Fowleri*	*P. erectum*	*P. aviculare*	*P. arenastrum*
Purple	—	—	—	—	.14	—	—
Purple	.17	.17	.17	.17	.17	.17	.17
Pink	—	—	—	.23	.22	.23	.22
Purple	.27	.32	.22	.27	.27	.28	.27
Purple	.36	.39	.31	—	—	—	—
Purple	.43	.51	—	.36	.36	.35	.35
Pale yellow	.49	.57	—	—	—	—	—
Purple	.55	.64	.39	.42	.42	.40	.42
Pale yellow	.60	—	.45	.45	.47	.45	.45
Purple	.68	.68	—	.56	.55	.55	.55
Purple	.73	.74	.49	.68	.68	.66	.66
Purple	.79	.80	.65	.73	.74	.74	.73

TABLE 2. *Rf values of chromatograms of secondary substances for different species of* Polygonum.

Color and Rf Values for *Polygonum* Species							
P. aviculare		*P. arenastrum*		*P. erectum*		*P. tenue*	
Pale yellow	.29	Orange	.40	Pale yellow	.34	Blue	.33
Pale blue	.32	Blue	.51	Blue	.59	Yellow	.62
Pale yellow	.50	Bluish white	.55	Bluish white	.63	Pale yellowish-brown	.69
Blue	.58	Yellow	.60	Yellow	.70	Bright Blue	.74
Bluish white	.61	Pale yellow	.65	Brownish yellow	.80	Brownish yellow	.80
Yellow	.68	Pale blue	.69	Blue	.90	Blue	.90
Pale blue	.75	Pale green	.76				
Yellow	.80	Blue	.90				
Yellowish-brown	.90						

Color and Rf Values for *Polygonum* Species							
P. Fowleri		*P. allocarpum*		*P. pennsylvanicum*		*P. orientale*	
Pale yellow	.35	Pale yellow	.25	Pale yellow	.43	Yellow	.43
Orange	.40	Pale yellow	.31	Pale blue	.53	Yellow	.53
Pale blue	.48	Orange	.39	Bluish white	.59	Brownish yellow	.62
Yellow	.60	Pale yellow	.46	Yellow	.70	Pale green	.72
Pale blue	.68	Yellow	.60	Brownish yellow	.83	Pale blue	.78
Bright blue	.75	Pale bluish-white	.68	Bluish white	.89	Brownish yellow	.83
Blue	.84	Yellow	.75	Blue	.93	Blue	.90
		Blue	.84				

Previous morphological and cytological studies indicate that *Polygonum aviculare* and *P. arenastrum* are closely related (8). The chromatographic patterns for these two species support this fact, yet each had certain bands that were not present in the other. This would support Style's (8) conclusion that they are separate species. The similarities of the chromatographic patterns of *P. erectum*, *P. aviculare*, and *P. arenastrum* may indicate that *P. erectum* is more closely related to the other two species than previously thought.

Freshly collected plants tentatively identified as *Polygonum Fowleri* were compared with herbarium specimens of *P. Fowleri* by chromatographing their secondary substances. The chromatographic patterns were nearly identical in color and Rf values for all specimens identified as *P. Fowleri*, even though they were collected at different times and from different areas. Since *P. Fowleri* and *P. allocarpum* are thought to be synonymous, chromatograms were run to compare these two species. The chromatographic patterns of specimens identified as *P. Fowleri* and *P. allocarpum* were distinctly different (Table 2). This difference supports the conclusion that *P. Fowleri* and *P. allocarpum* are distinct species.

The species examined in this study had no distinct differences in the chromatographic patterns of their amino acids. Chromatographic patterns for secondary substances seem to be distinct for each species. Certain species did show some similarities which may indicate that they have a close phylogenetic relationship.

One factor that has not been thoroughly explored in this study or in studies cited in this investigation is the environmental effect on the type of secondary substances that are produced by the plant. The effect of certain chemicals in the soil on the formation of secondary substances has not been established. If the formation of these substances is influenced by soil conditions, the chromatographic pattern for a particular species would be altered. If the latter is true, this procedure would be of limited value as a taxonomic tool.

Summary

Chromatographic patterns of secondary substances described in this investigation appear to be of taxonomic value. Each species of *Polygonum* studied has a distinct chromatographic pattern for its secondary substances.

The chromatographic patterns of the secondary substances of *P. arenastrum* and *P. aviculare* are quite similar and tend to support the conclusion that these species are closely related. However, the chromatograms were not identical to each other and this fact, when considered with their morphological and cytological differences, supports the observation that they are separate species.

Based on the similarity of the chromatographic patterns of their secondary substances, *P. erectum* appears to be closely related to *P. aviculare* and *P. arenastrum*. Because of apparent differences in the

chromatographic patterns of their secondary substances, *P. Fowleri* and *P. allocarpum* would appear to be separate species. However, morphological differences between *P. Fowleri* and *P. allocarpum* are not distinct; therefore, the taxonomic relationship of these species is not clear. The chromatograms of the secondary substances do not appear to be affected by the age of the plant material used in the preparation of the extract.

The chromatographic procedure used in this investigation for the study of free amino acids appears to be of little taxonomic value in separating the different species of genus *Polygonum*. The various species of this genus showed very few differences in chromatographic patterns with respect to free amino acids.

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