A Biosystematic Study of *Polygonum ramosissimum* and *Polygonum tenue*[1]

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Abstract

Plants collected in Wisconsin during September 1970 were identified as *Polygonum ramosissimum* and *P. tenue* according to the taxonomic character established by Styles for European species of genus *Polygonum* and adapted for North American species of this genus by Mertens and Raven. The somatic chromosome numbers for these two species were determined to be 60 for *P. ramosissimum* and 30 or 32 for *P. tenue*. These counts were compared with chromosome numbers of species of section *Polygonum*, all of which have a somatic number of either 40 or 60. Chromatographic analysis of free amino acids and secondary substances further suggests that *P. ramosissimum* has amino acids similar to those of other species in section *Polygonum*. Chromatographic data for *P. tenue* is somewhat less conclusive in assigning this species to a section of genus *Polygonum*.

Genus *Polygonum*, section *Polygonum* (*Avicularia*) consists of a number of morphologically similar plant species. Individuals within each species vary morphologically according to local edaphic and climatological conditions. This has led to a general confusion among taxonomists who have been attempting to identify field and herbarium specimens, on the one hand, and to systematists, on the other, who have been attempting to assign natural rank to the various species of *Polygonum*. This study reports on an attempt to establish the natural relationships of two *Polygonum* species to section *Polygonum* by using cytological and chemical parameters.

Research in the 1960's brought taxonomic clarification to some species within section *Polygonum*. Styles (9) defined the four species of the *Polygonum aviculare* aggregate found in Britain (*P. aviculare*, *P. arenastrum*, *P. rurivagum*, and *P. boreale*), and three other species found elsewhere in Europe (*P. maritimum*, *P. raii*, and *P. oxyspermum*) in terms of fruit and perianth characteristics and chromosome number. He found achene texture, color, shape and size, and inflorescence position to be stable and conservative traits. Mertens and Raven (5), using the biosystematic approach of Styles (9), studied the *P. aviculare* complex in North America and were able to devise a workable key for eight species of section *Polygonum*. Savage and Mertens (7) studied six species of *Polygonum* found in Indiana and Wisconsin by using the parameters established by Styles. Moore, Mertens, and Highwood (6) summarized cytotaxonomic data on various species within this same section. These workers compared chromosome number with the taxonomic characters decided upon by Styles and later researchers, to determine if indeed a plant with a certain combination of fruit and perianth characteristics consistently displayed the same chromosome count.

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The present investigation is concerned with examining *Polygonum ramosissimum* and *P. tenue* to determine if they are to be included in section *Polygonum*. The two species, collected in Wisconsin, were keyed according to the following taxonomic couplet established by Savage and Mertens (7):

Achene surface predominantly smooth and shiny but may have stippled or striated edges; inflorescences appear to be terminal, the flowers being more-or-less clustered at the ends of stems among reduced leaves or bracts; perianth tightly oppressed to the achene, which has three equal concave sides.

Achene surface completely smooth, achenes dark reddish brown, sharp angled; calyx yellowish green, plant large [3-10 dm], much branched . . . . . *P. ramossissimum*

Each face of black, trigonous achene smooth and shiny but bordered with striated or stippled margin; plant slender [1-4 dm], stiff or wiry; leaves linear . . . . . *P. tenue*

Materials and Methods

All plant material was collected in Wisconsin at two sites along the Wisconsin River on September 27, 1970. Both species were collected in a sandy beach habitat. *Polygonum ramosissimum* was found 2¼ miles due west of Lone Rock in Richland Co., Wisconsin. *Polygonum tenue* was gathered in Upham Woods in Juneau Co., Wisconsin.

Viable achenes were removed from the plants and germinated on moist filter paper in petri dishes. Chromosome counts were made of root tip and apical meristem cells treated with the mitotic poison, oxyquinoline, and stained with aceto-orcein.

Chromatographic patterns of the two plants under study were compared with patterns made by other species of *Polygonum*, section *Polygonum*. Single dimensional paper chromatograms testing for the qualitative distribution of free amino acids and secondary substances among the compared plants were run to see if a related pattern developed. It was assumed that related species would have similar, but not necessarily identical, chromatographic patterns, and that different plants of the same species would consistently display the same patterns.

A mixture of 0.3 g of plant root, steam, and leaves was pulverized in a motar and then transferred to a vial with 2 ml of 80% methanol. After a drop of 1 N hydrochloric acid was added, the vial was corked and allowed to stand at room temperature for 24-48 hours before the extract was used to streak the chromatography paper.

Single dimensional chromatograms were developed by the descending method using 46 X 57 cm Whatman No. 1 chromatographic paper. The plant extracts were applied to the paper with a 10-μl pipet. Generally 100 μl of plant extract were needed to get readable results. The chromatograms were developed for 24 hours, using a solvent mixture, described by Smith (8), consisting of 12 parts n-butyl alcohol, 3 parts glacial acetic acid, and 5 parts distilled water.

The chromatograms in which the free amino acids were to be detected were sprayed with a ninhydrin aerosol bomb. The chromatograms were dried in the dark for 24 hours before observations were made. Secondary substances were detected by placing unsprayed chromatograms under long-wave ultraviolet light in the presence of ammonia vapors.

Data

Definite chromosome counts were obtained for *Polygonum ramosissimum* from these plants:

P. ramosissimum Michx., Richland Co., Wisconsin, Sept. 27, 1970, G. M. Brooks No. 89 (BSU). Somatic count of 60.

P. ramosissimum Michx., Richland Co., Wisconsin, Sept. 27, 1970, G.M. Brooks No. 75 (BSU). Somatic count of 60.

Photographs of the chromosomes found in cells of these two plants were taken (Fig. 1). Diagramatic line drawings of the cells counted were made because all chromosomes do not appear equally well at the focal level from which the photomicrographs were taken (Fig. 2).

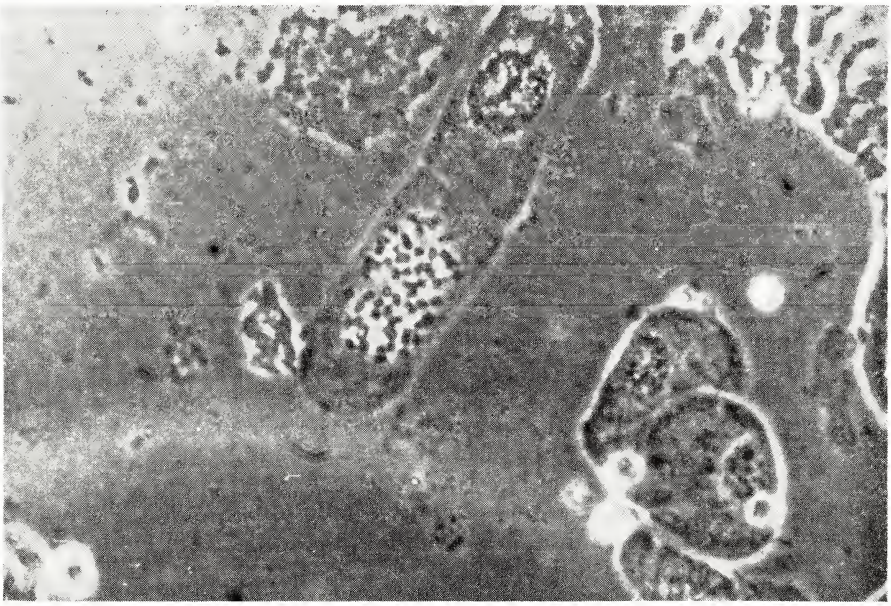

FIGURE 1. *Photograph of chromosomes from* Polygonum ramosissimum, *G. M. Brooks No. 89. Chromosome number 2n=60 (1000 X Magnification).*

Tentative counts were obtained from the following specimens of *Polygonum tenue*:

P. tenue Michx., Juneau Co., Wisconsin, Sept. 27, 1970, G.M. Brooks No. 209 (BSU). Somatic count of ca. 30.

P. tenue Michx., Juneau Co., Wisconsin, Sept. 27, 1970, G.M. Brooks No. 216 (BSU). Somatic count of 30 or 32.

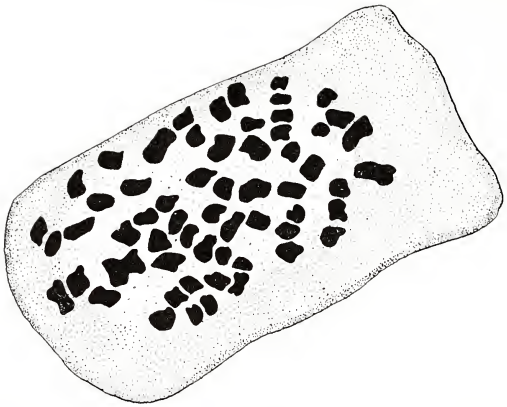

FIGURE 2. *Diagramatic line drawing of cell of* Polygonum ramosissimum *shown in Figure 1. Chromosome number 2n=60.*

The chromatographic procedure described previously was repeated with at least five different specimens of each species studied. The specimens of each species were found to display consistent chromatographic patterns. The following specimens were used as representative samples in preparing the chromatograms from which the data in Tables 1 and 2 were determined:

P. aviculare L., Great Bras d'Or, Nova Scotia, August 17, 1969, D. Jones No. NSJ 15 (BSU).

P. erectum L., Porter Co., Indiana, August 29, 1966, A.D. Savage No. 58-30 (BSU).

P. ramossissimum Michx., Richland Co., Wisconsin, Sept. 27, 1970, G. M. Brooks No. 89 (BSU).

P. tenue Michx., Fulton Co., Indiana, August 20, 1966, A.D. Savage No. 50-1 (BSU).

TABLE 1. *R_f values of the qualitative distribution of free amino acids for four species of* Polygonum.

Color	*P. aviculare*	*P.erectum*	*P.ramosissimum*	*P.tenue*
Pink	.06	—	.06	—
Purple	.12	.11	.12	.10
Purple	.14	.14	.14	.15
Purple	.19	.18	.18	—
Purple	.26	.24	.26	.26
Purple	.31	.31	.32	.34
Yellow	.36	.35	.36	.37
Purple	.43	.40	.40	.40
Purple	—	.47	.46	—
Purple	.52	.52	.53	.53
Purple	.58	.59	.60	—
Purple	.65	—	.66	—

A single dimensional paper chromatogram was developed for *P. aviculare, P. erectum, P. ramosissimum,* and *P. tenue.* It was sprayed with ninhydrin to develop the free amino acids which had migrated down the paper from the sites of origin. A second single dimensional chromatogram was developed with the same four plants and was placed under long-wave ultraviolet light in the presence of ammonia vapors to highlight the secondary substances. Table 1 gives the R_f values of the qualitative distribution of the free amino acids for the four plants; Table 2 shows the same for the secondary substances.

TABLE 2. *R_f values of the qualitative distribution of secondary substances for four species of* Polygonum.

P. aviculare R_f Color	*P. erectum* R_f Color	*P. ramosissimum* R_f Color	*P. tenue* R_f Color
.37 yellow	.47 blue	.53 green	.45 yellow
.46 blue	.58 yellow	.60 yellow	.55 yellow
.55 yellow	.69 yellow	.70 yellow	.62 blue
.68 yellow	.82 blue	.85 blue	.69 yellow
.77 yellow			.80 blue

Discussion

Species of genus *Polygonum* section *Polygonum* generally give somatic chromosome counts of 40 or 60. Styles (9) determined the somatic number 40 for *P. arenastrum* and 60 for *P. aviculare,* while Mertens and Raven (5) determined the somatic number of 60 for *P. marinense.* Löve and Löve (4) reported that *P. erectum* and *P. fowleri* each has a somatic number of 40, and these counts have been confirmed by Moore, Mertens, and Highwood (6). It may be that the basic chromosome number in this group of plants is $x=10$, and that those species with 40 chromosomes are of tetraploid origin, while those with 60 are hexaploids. This suggests that *P. ramosissimum* might also have a somatic chromosome count of 40 or 60 if it is to be included in section *Polygonum.* The determination that *P. ramosissimum* indeed does have a somatic count of 60 agrees with this cytotaxonomic requirement. Löve and Löve (4) published a somatic chromosome number of 20 for this species collected in Manitoba. However, voucher specimens were not kept for cross-checking purposes, thus calling their finding to question.

Polygonum tenue collected for this study had a somatic chromosome count of 30 or 32. This departure from a count of 40 or 60 (which numbers seem to be generally characteristic of plants in genus *Polygonum,* section *Polygonum*) may suggest that *P. tenue* belongs to another section of the genus. This would be especially true if the count proves to be 32 rather than 30. Löve and Löve (4) reported this plant to have a somatic count of 20. Again, no voucher specimens were kept to verify their findings for *P. tenue* collected in Minnesota. Mertens and Raven (5) commented that *P. tenue* was morphologically similar, from the standpoint of its fruit and

perianth characteristics, to other species of section *Polygonum*, but that this plant had pollen of the type found in section *Duravia*. (2). Thus, palynological data tend to support the contention that *P. tenue* should be assigned to a section of the genus other than section *Polygonum*.

Alston and Irwin (1) were able to separate closely related species of the genus *Cassia* (Caesalpinia Family) by using paper chromatographic techniques. Jones and Mertens (3), by using the same technique, were able to qualitatively distinguish among a number of species of *Polygonum* section *Polygonum*. The data in the present investigation (Tables 1 and 2) show that *P. ramosissimum* has basically the same qualitative distribution of amino acids as do the two established species of section *Polygonum*, *P. aviculare* and *P. erectum*.

Although the chromatogram of amino acids for *P. tenue* indicated that this species has many spots in common with the other three species studied, certain repeatable differences in the chromatograms were noted:

1) To obtain a readable chromatogram for *P. tenue* specimens, the extract had to be four times as concentrated at the site of origin as for any of the other species investigated.

2) The color intensity of the various chromatogram spots for *P. tenue* was always less than for comparable spots of the other species.

3) Although the size of the streak of origin of the *P. tenue* chromatogram was comparable to that used for the other species, the size of the spots in the chromatogram were consistently smaller than comparable spots for the other species.

It may be argued that the chromatogram of *P. tenue* differs from those of the other three species only in lacking certain spots and that this could be due to less diversity in its genome in response to a more restricted habitat. However, the three points cited above would seem to argue for the uniqueness of the chromatogram of *P. tenue*.

Secondary substances are so termed since thay are not involved in basic energy transfer or catabolic activity of plant tissues. These compounds (alkaloids, terpenoids, and polyphenols) are stable by-products and so can be used as chemical taxonomic parameters.

Although certain spots in the chromatograms of secondary substances of the different species seem to be identical (*e.g.*, .68 yellow in *P. aviculare* and .69 yellow in *P. erectum*), the overall qualitative distribution of secondary substances was unique for each species. This suggests that secondary substance analysis may be used to establish speciation, since each of the four species studied had a different overall chromatographic pattern and various plants within a species displayed a consistent pattern.

Conclusions

1) *Polygonum ramosissimum*, with a somatic chromosome count of 60, seems to be similar to established species of section *Polygonum* all of which have either 40 or 60 as their somatic chromosome number.

2) Since the tentative somatic chromosome counts of 30 or 32 for *P. tenue* were made from collections at one site, and since they do not agree with counts reported in the literature, fresh specimens of *P. tenue* containing viable achenes should be collected and a definite chromosome number determined.

3) Chromatographic studies indicate that the overall qualitative free amino acid distribution for *P. ramosissimum* is similar to the distributions shown by species of *Polygonum* in section *Polygonum*.

4) *Polygonum tenue* has quite a different overall qualitative amino acid distribution due to: a) the greater concentration of extract required in order to get readable chromatograms, b) the much weaker color intensities for the various spots, and c) the smallness of the spots in comparison to comparably colored spots of the three other species at the same R_f locus on the chromatogram.

5) The overall qualititative distribution of secondary substances is unique for each species with various specimens of the same species giving the same pattern.

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