Determination of the Somatic Chromosome Number of *Polygonum tenue*

An Honors Thesis (ID 499)

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Introduction

Plant taxonomists have disagreed for years concerning the section of genus *Polygonum* to which *P. tenue* should be assigned. Although the species has been traditionally assigned to genus *Polygonum*, section *Avicularia* (*Polygonum*), several researchers have proposed that *P. tenue* is actually a member of genus *Polygonum*, section *Duravia*. This proposal is based on the fact that several traits of *P. tenue* are contradictory to those characteristic of section *Avicularia* as a whole.

Hedberg (6) established the limits of genus *Polygonum*, section *Avicularia* on the basis of pollen morphology. This eliminated most North American species from section *Avicularia*, and placed them in section *Duravia*. Biosystematic studies done in the 1960s clarified the positions of several of those North American species assigned to section *Avicularia*. Styles (13) performed a detailed biosystematic study of section *Avicularia* in the British Isles which resulted in establishing species limits based on flower and achene characteristics. Styles also correlated these traits with plant habit, leaf characteristics, habitat, and chromosome number. Raven and Mertens (9) found *P. tenue* to be morphologically similar to four other members of section *Avicularia* with respect to fruit and perianth characteristics, but found the pollen to be of the type found in plants of section *Duravia*.

An investigation by Savage and Mertens (11), using Styles' methods, revealed that P. tenue had an achene stippling pattern dissimilar to that of achenes of other members of section Polygonum.

Jones and Mertens (7) investigated the chromatographic patterns of free amino acids in members of section Aviculariа, including P. tenue, and found that P. tenue lacked a particular pink band characteristic of the other species studied. Brooks and Mertens (1), reporting on a similar study, also found P. tenue to be lacking this band.

Attempts to determine the chromosome number of species in section Aviculariа have been made by several researchers. Several species in section Aviculariа have been reported to have somatic chromosome numbers of 2n=40 or 2n=60. Species with such chromosome numbers include P. erectum (2n=40), P. arenastrum (2n=40), P. fowleri (2n=40), P. aviculare (2n=60), P. ramosissimum (2n=60), and P. marinense (2n=60) (1, 9, 10, 13). Löve and Löve (8) reported the chromosome number of P. tenue to be 2n=20. However, Brooks and Mertens (1) found the somatic chromosome number of P. tenue to be 2n=ca. 30, which, along with pollen morphology and chromatographic data, cast doubts on the placement of P. tenue in section Aviculariа. The present investigation was performed in order to determine a definite somatic chromosome number for Polygonum tenue, so that assignment of the species to an appropriate section of the genus Polygonum could be suggested.

Methods and Materials

Polygonum tenue specimens were collected on August 27 and October 2, 1977, at the same site near Rochester, in Fulton County, Indiana. The achenes gathered in October were the ones used for this experiment, since they were more mature.

The achenes were soaked in water for 24 hours, after which the pericarp was removed and the achene placed on moist filter paper in a petri dish.

The acheres were then refrigerated at 2-5 ^{o}C for at least two weeks, in order to cold shock the embryos. After the cold shock, the petri dishes were placed in an incubator at 23 ^{o}C until the germinated root tip was about 5 mm long. The root tips were then placed in a 0.002 molar solution of oxyquinoline for three hours to cause the chromosomes to contract. The root tips were fixed in a solution of two parts 95% ethyl alcohol to one part glacial acetic acid for fifteen minutes and left in the fixative until microscope preparations were made.

Staining was done with aceto-orcein stain prepared by dissolving 1 gram of orcein in 45 ml of hot glacial acetic acid and filtering the cooled solution. The stain was diluted at the time of use by putting 10 drops of distilled water in a watch glass and adding 10 drops of stain. After putting the root in the diluted stain, the solution was heated over an alcohol lamp until it boiled. The mixture was allowed to cool for one minute, then 5 drops of 1 molar hydrochloric acid were added, and the mixture heated to boiling again. A fresh drop of stain and a drop of distilled water were put on a slide, and the root tip transferred to the slide. The last 1 mm of the root tip was removed with a clean razor blade, and the rest of the root discarded. A cover slip was placed over the root tip, and the root tip cells separated by tapping the cover slip gently with a dissecting needle while watching through a dissecting microscope. The slide was then inverted on a paper towel on a glass plate, and the area over the cover slip was rubbed with the handle of a dissecting needle to squash the cells, rupture the nuclear membrane, and flatten the chromosomes. The slide was examined under a phase contrast microscope with a low power objective giving total magnification of 200x; when a cell with chromosomes was found, it was studied using the oil immersion objective at 1000x total magnification.

One cell was photographed using a Kodak 35 mm back and Kodak photomicrography color film 2483. The cell was under the 100x oil objective, with a zoom factor of 1.2, giving a total magnification of 1200x. Exposure time was 50 seconds with the light at maximum intensity and using phase contrast optics.

Data and Discussion

Definite chromosome counts were obtained for Polygonum tenue from two seeds:

> P. tenue, Rochester, Fulton Co., Indiana, October 2, 1977. Somatic count of 34 (Figure 1).
>
> P. tenue, Rochester, Fulton Co., Indiana, October 2, 1977. Somatic count of 34 (Figures 2, 3).

Photographs were taken and diagrammatic line drawings made in order to show the chromosomes as they appeared in the cells.

Obtaining root tips for squashes was difficult for several reasons. Mold often grew on the seeds, making those root tips unusable; many seeds simply did not germinate at all; and of those seeds that did germinate, many root tips were not growing fast enough to have many cells dividing, thus making it difficult to find cells with chromosomes. Many of the cells with chromosomes visible did not have the nuclear membranes ruptured, so that the chromosomes were not spread apart sufficiently, or else the chromosomes were not flattened enough, also making it impossible to make an accurate count.

Several keys have been constructed for P. tenue, all of which include it in section Avicularia. Of these keys, the most recent is one by Savage and Mertens (11), which uses achene characteristics to position the different species of section Avicularia. Savage and Mertens included P. tenue in the key for section Avicularia for convenience, although they felt that P. tenue belonged in section Duravia. Their key is as follows:

> 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

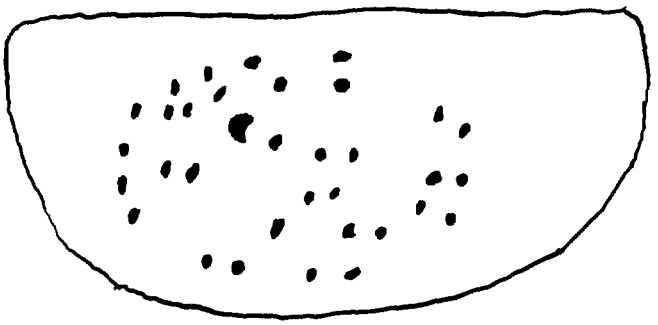

Figure 1. Diagrammatic line drawing of cell of Polygonum tenue. Chromosome number 2n=34 (1200x magnification).

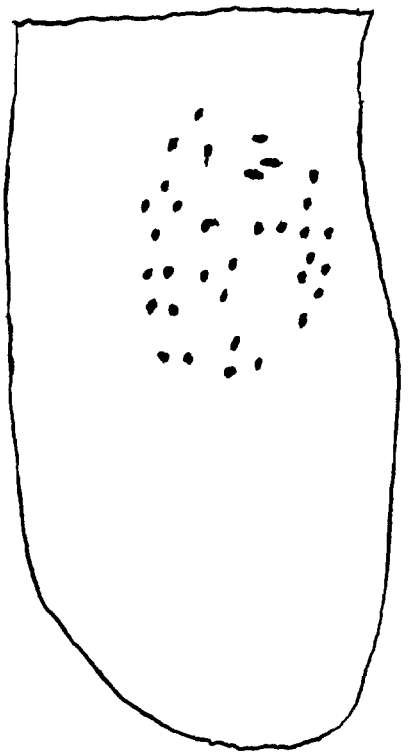

Figure 2. Diagrammatic line drawing of cell of Polygonum tenue. Chromosome number 2n=34 (1200x magnification).

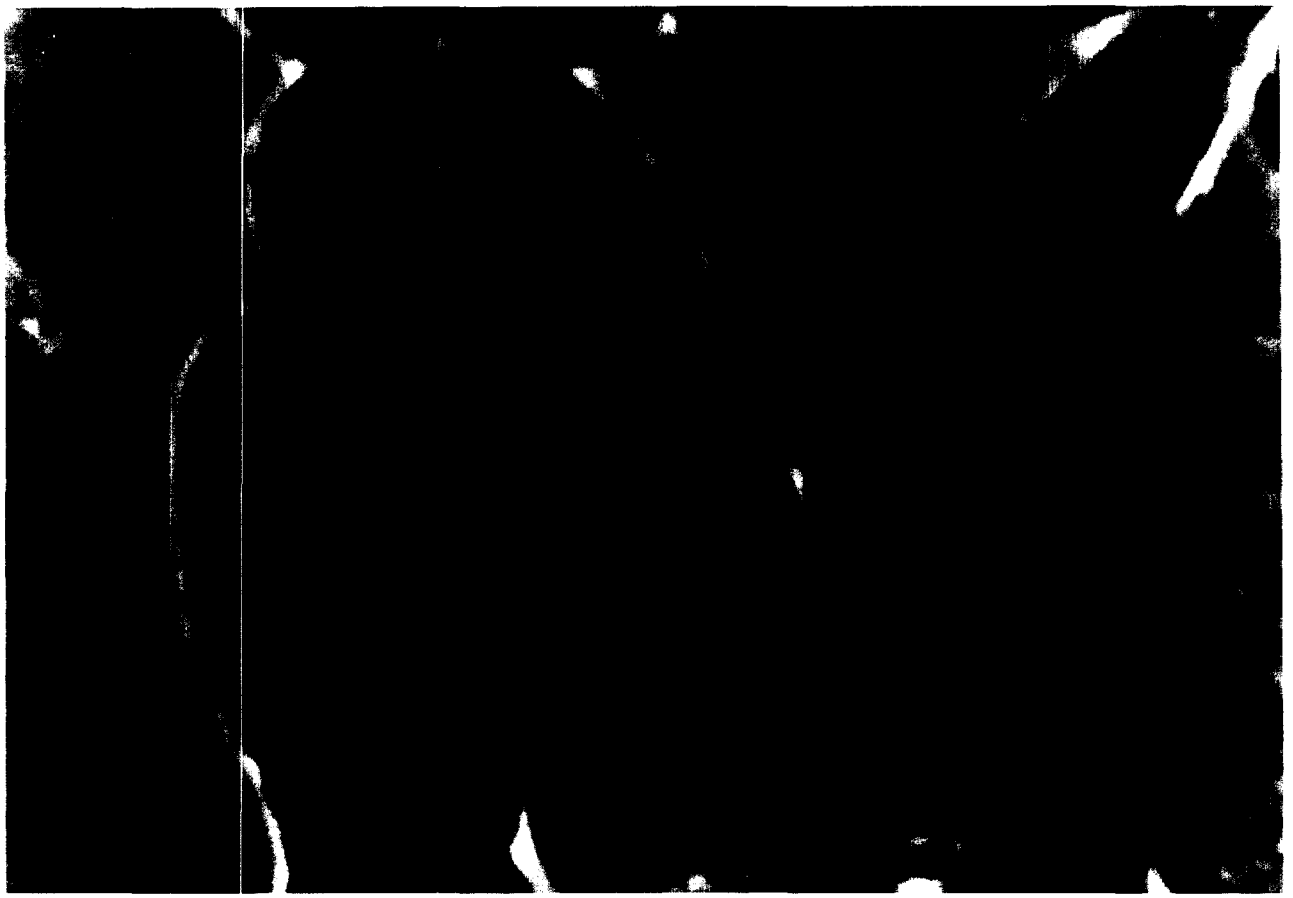

Figure 3. Photograph of chromosomes of cell of Polygonum tenue, as shown in Figure 2. Chromosome number 2n=34 (1200x magnification).

or bracts; perianth tightly oppressed to the achene, which has three equal concave sides.

Each face of black, trigonous achene is smooth and shiny but bordered with striated or stippled margin; plant slender, stiff or wiry; leaves linear . . . . . P. tenue

Savage and Mertens (11) also described the habitat of the plant, finding it sandy and hilly (Figures 4,5). The key by Correll and Johnston (2) deals with P. tenue in the same manner as does that by Savage and Mertens. Keys by other authors, however, use different characteristics. Deam (3) developed a key which differentiates P. tenue on the basis of the stem and branches (strongly angled and erect) and the leaves (linear, sharply pointed, minutely ciliolate). Deam (3) also described the habitat of P. tenue, observing that this species prefers slightly acid soil, and is found in exposed areas where few or no other plants are growing (Figures 4,5). Fernald (4) uses the same stem and branch traits as does Deam, but also uses the terminal inflorescence position, plicate leaves, and erect flowers to describe P. tenue. Gleason (5) keys P. tenue in the same manner Fernald does; however, pedicel traits and the serration of leaves are also used by Gleason in his key for P. tenue. A key by Small (12) uses the following traits to separate P. tenue from the other species in section Avicularia: plants erect; achenes included, never completely exserted; flowers in axillary clusters; stem branched mainly from the base; branches erect or ascending; small plants; pedicels erect; leaves linear or linear-lanceolate; achenes ovoid. The general morphological descriptions of P. tenue by all authors do not vary greatly. Unfortunately, none of the descriptions or keys for section Avicularia which include P. tenue also include chromatographic data or chromosome numbers for the species involved. Tutin, et al. (14) do, however, use chromosome numbers in their descriptions of Polygonum spp. which are found in Europe and assigned to section Avicularia. This, however, does not help in assigning P. tenue to a particular section, since the species

Figure 4. Photograph of Polygonum tenue.

Figure 5. Photograph of Polygonum tenue, as found at site of collection near Rochester, Fulton Co., Indiana.

is found only in North America.

Differences between sections Avicularia and Duravia are described mostly by Hedberg and Small. Hedberg (6) found that sections Duravia and Avicularia could be differentiated on the basis of their pollen, although there were some species in section Duravia whose pollen grains were very similar to grains of species in section Avicularia. Duravia-type pollen differed from Avicularia-type pollen in the following respects: 1) the pollen grains in section Duravia were always tricolporate, while section Avicularia pollen grains could be tri- or tetracolporate or hexarugate; 2) the furrow length in section Duravia was shorter than that of section Avicularia, section Duravia having a furrow length no longer than one-half the polar axis, while furrows in pollen in section Avicularia were from one-half to four-fifths the length of the polar axis; and 3) section Duravia pollen was found only in plants from the New World, while section Avicularia pollen was found in plants from most parts of the world.

Small (12) described the morphology of plants assigned to sections Avicularia and Duravia. He found the plants in the two sections to be very similar morphologically; the major differences between the two sections were 1) in section Duravia, the leaves were not articulated to the ocreae, but were more or less articulated in section Avicularia; 2) the calyx in section Duravia was five-parted, but could be five- or six-parted in section Avicularia; and 3) species in section Duravia usually had eight stamens, but species in section Avicularia had from three to eight stamens. Small placed several species in section Avicularia which Hedberg placed in section Duravia on the basis of pollen type, including P. minimum, P. douglasii, P. engelmanni, P. austinae, P. spergulariaeforme, P. nuttallii, P. polygaloides, P. watsoni, P. kelloggii, and P. sawatchense (6, 12). Although Small placed

the above species, as well as P. tenue, in section Avicularia, his descriptions of all the species in question permit assignment of these species to section Duravia as he described the section (12). The more recent keys and descriptions mentioned above also permit assignment of P. tenue to section Duravia.

There is still need for work on the taxonomy of genus Polygonum. An attempt should be made to gather chromatographic data and chromosome numbers for as many of the species currently assigned to sections Avicularia and Duravia as possible, in order to determine which species belong to section Avicularia and which belong in section Duravia; such information would also help to determine whether there is a need to subdivide the genus at all.

Conclusions

1) The somatic chromosome number of P. tenue is $2n=34$.

2) The chromosome number, pollen morphology, chromatographic data, and general morphology of the plant suggest that P. tenue should be assigned to section Duravia of genus Polygonum.

3) Further investigation of chromosome numbers and chromatographic data of species whose taxonomic position in genus Polygonum is disputed (P. douglasii, P. watsoni, etc.) should be done in an effort to clarify the position of these species in genus Polygonum.

4) The writer recommends that section Duravia should be separated from section Avicularia and that a description of the former section be made on the basis of the general morphology of those plants whose pollen is of the Duravia type as described by Hedberg.

Literature Cited

1. Brooks, George R. and Thomas R. Mertens. 1972. A biosystematic study of Polygonum ramosissimum and Polygonum tenue. Proc. Indiana Acad. Sci. 81: 277-283.

2. Correll, Donovan S. and Marshall C. Johnston. 1970. Manual of the Vascular Plants of Texas. Texas Research Foundation, Renner.

3. Deam, Charles C. 1940. Flora of Indiana. Indiana Department of Conservation, Indianapolis.

4. Fernald, M. L. 1950. Gray's Manual of Botany, Ed. 8. American Book Co., New York.

5. Gleason, H. A. 1952. New Britton and Brown Illustrated Flora of the Northeastern United States and Canada, Vol 2. New York Botanical Garden, New York.

6. Hedberg, Olov. 1946. Pollen morphology in the genus Polygonum L. s. lat. and its taxonomical significance. Svensk. Botan. Tidskr. 40: 371-404.

7. Jones, Daniel M. and Thomas R. Mertens. 1971. A taxonomic study of genus Polygonum employing chromatographic methods. Proc. Indiana Acad. Sci. 80: 422-430.

8. Löve, A. and D. Löve. 1956. Chromosomes and taxonomy of eastern North American Polygonum. Can. J. Bot. 34: 501-521.

9. Mertens, Thomas R. and Peter H. Raven. 1965. Taxonomy of Polygonum, section Polygonum (Avicularia) in North America. Madroño 18: 85-92.

10. Moore, Donald N., Thomas R. Mertens, and Joyce E. Highwood. 1970. Cytotaxonomic notes on genus Polygonum, section Polygonum. Proc. Indiana Acad. Sci. 79: 396-400.

11. Savage, Argyle D. and Thomas R. Mertens. 1968. A taxonomic study of genus Polygonum, section Polygonum (Avicularia) in Indiana and Wisconsin. Proc. Indiana Acad. Sci. 77: 357-369.

12. Small, J. K. 1895. A Monograph of the North American Species of the Genus Polygonum. Memoirs from the Department of Botany of Columbia College, Vol 1.

13. Styles, B. T. 1962. The taxonomy of Polygonum aviculare and its allies in Britain. Watsonia 5: 177-214.

14. Tutin, T. G., et al., eds. 1964. Flora Europaea, Vol. 1. Cambridge University Press, Cambridge.