ACCEPTING THE CHROMOSOME NUMBER
OF POLYGOUM BUXIFOLIUM SPECIMENS

by

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INTRODUCTION

The taxonomy of genus Polygonum, section Polygonum (Ayiculera) is quite confused despite several major investigations of the species included within the section. Taxonomists disagree on both classification and identification of the species, particularly within the Polygonum aviculare aggregate. The present study focused on three members of the aggregate, P. aviculare L. sensu stricto, P. angustatum Cov., and P. buxbaum Small with the intent of making distinctions between them on the basis of morphological characteristics and chromosome counts.

LITERATURE REVIEW

Genus Polygonum, section Polygonum includes a group of weedy plants, many of Eurasian origin, known as "knotweed" or "knotgrass." Members of the section are distributed all over the world except in Antarctica, and are common weeds in North America. The enlarged nodes coupled with hyaline outgrowths called ocrea which occur at the nodes constitute the major identifying characteristics of knotweed and give the plant a knotty or jointed appearance. Leaves of most plants are simple and entire, ranging from .3 to 5.5 cm long and .1 to 1.8 cm wide (8). Characteristic leaf shape is narrowly lanceolate to oblong-lanceolate and oxeate. The plants typically have obscure flowers which develop in the axils of the leaves, and very small fruits (chenes) which develop from tricarpellary ovaries. There is a tendency in some
species for flowers to aggregate at the upper nodes of the plants. Plants may grow either prostrate or erect.

The history of the taxonomic study of *Polygonum*, section *Polygonum* has involved many researchers with many conflicting opinions as to how the members should be identified and classified. This has led to a wide array of confusing names for questionable species. A brief review of the historical development of the section will illustrate this point. Linnaeus, one of the earliest taxonomists concerned with the identification and classification of *Polygonum*, divided the genus into several sections, including a section he called *Polygonum*. Species Linnaeus included in the section were *P. aviculare* sensu lato (supposedly the type species for the genus), *P. erectum*, *P. articulatum*, and *P. divaricatum*. This section was later (1815) named Centinoide by DeCandolle and then in 1825 *Avicularia* by Koehne. Linnaeus divided *P. aviculare* L. sensu lato into four varieties which have been impossible to relate to any species subsequently described in the *P. aviculare* aggregate (8).

Since Linnaeus' study (1753) of the wide variety of plants included in this genus, attempts to clarify the classification system have resulted in considerable confusion. The first species to be distinctly separated from *P. aviculare* L. s. lato was *P. littorale*, cited in 1800 by H. F. Link in a letter (8). The only distinguishing characteristics he described were thick leaves and branches completely covered with sheaths. Persoon subsequently published the epithet *littorale* in 1805, citing Link's plant and attributing to it broad, fleshy leaves and a woody stem (8). The status awarded *P. littorale* by succeeding researchers ranged
from variety to species level, but a type specimen is unavailable
and the earliest available specimen, annotated by Koch as
P. aviculare variety littorale, is, according to P. J. Styles (8),
P. aviculare s. l. sensu stricto. Thus, P. littorale is considered
by Styles to be a nomen dubium.

Persoon (1805) named other plants in the P. aviculare
aggregate P. menscellense. This is the earliest name for the
species P. aviculare L. s.s. (8).

Sesse (1822) followed with a description of a species,
P. nervilcatum. His writing has been interpreted in several ways
by different taxonomists. Four treatments of this species have
been suggested: 1) that it is a synonym of P. arenarium Waldst.
& Kit., 2) that it is an early name for P. rupestrum Jord.
(suggested by Boreau, 1817), 3) that it should be a full species,
and 4) that it should be included pro parte under P. arenarium
Waldst. & Kit. and pro parte under P. aviculare var. angustissimum
Heism. as suggested by Heism in 1836 (8).

In 1837 the P. aviculare aggregate was credited with five
new species by Boreau: P. arenatissimum, P. humifusum, P. micro-
spicatum, P. rupestrum, and P. arenarium. The first four of
these were based on the research of Jordan. Boreau added
P. arenarium and elevated two varieties to specific level (8).
Styles cites P. arenarium and P. rupestrum as appropriate species
for British flora (8).

Lindman's studies in 1904 and 1912 resulted in the division
of the P. aviculare group into three species with many varieties -
P. olateum (1904), P. heterophonium (1912), and P. aculea (1912).
Subsequent taxonomists have had difficulty distinguishing between *P. calceatum* and *P. sectuale*. Styles considers both to be synonymous with *P. arenastrum* (6). *P. heterophyllum* is more easily discerned by the heterophyllous characteristic, branch leaves being smaller than stem leaves, and the dull, equally-trigonoous fruits. Lindman described five varieties under this species, one of which is *P. ruivaram*, a taxon which will be referred to later. Lindman also contended that hybrids occur between all of the species of the *P. avicularia* group, an idea unaccepted by most present-day taxonomists.

In 1956, J. K. Small (7) named a species *P. buxiforme* which he had previously (1895) described under the name *P. littorale* Link, but its existence as a distinct entity is disputed. F. N. Raven, for example, suggested that *P. buxiforme* may be a small-fruited form of *P. avicularia* L. s. s. (Personal communication, 1966). However, Savage and Mertens have established a number of morphological features which seem to consistently differentiate these two species (6).

In 1962, Tutin treated *P. avicularia* as an "aggregate species" which included *P. avicularia* L. s. s. (Lindman's *P. heterophyllum*), *P. littorale* Link, *P. ruivaram* Jord. ex Cor., *P. sectuale* Lindl., and *P. calceatum* Lindm. (9).

Taylor and Milligan (9) have concluded that all of the proposed species in the *P. avicularia* aggregate should be treated collectively as one aggregate species, since sharp distinctions between the types on the basis of morphological characteristics are very difficult if not impossible to make. They contend that the morphological characteristics given by Styles for British
plants cannot always be correlated with the chromosome numbers of North American plants. Their conclusions are based on a study of plants collected on the Queen Charlotte Islands, off the northwest coast of British Columbia. Raven (Personal communication, 1968) supports such conclusions particularly in reference to North American species, in light of the present problems in distinguishing between the members of the *P. aviculare* aggregate.

Besides the disorder evidenced in the *P. aviculare* aggregate, taxonomists also disagree as to how to treat members of the entire section. For example, *P. raii* Bab. was named by Babington in 1836. This species has since been confused with several others, including *P. oxyaspernum*, *P. robertii*, and *P. maritimum*. Webb and Chater (10) considered *P. raii* to be a subspecies of *P. oxyaspernum*.

*P. oxyaspernum* is a species described by Meyer & Junge (1824). It was placed in section *Rhotonema* by Heiser. According to Gleason (1) and Mertens and Raven (5), it is synonymous with *P. raii* Bab. Fernald named similar plants *P. scabiense* (1914), a species which was found to be conspecific with *P. oxyaspernum* by Samuelsson in 1951 (8).

In 1827 J. Coutard collected plants later assigned the name *P. robertii* by Loiseleur-Deslongchamps which has since been considered a dubious species (8). Rouy (1910) distinguishes *P. robertii* from *P. aviculare* L. by its smaller, shinier fruits and the plants' being biennial or perennial. However, British botanists have called it a synonym of *P. raii* Bab. Webb and Chater (10) conclude that *P. robertii* must be synonymous with *P. raii*, although they could find no type specimen and no complete description of *P. robertii*. Babington would not comment on this possibility, not
having been able to examine any specimen annotated *P. Robertii*. C. F. Tritton contends that it is not a synonym of *P. Reyi*, but that it is a distinct species. Styles found that specimens from Paris labelled *P. Robertii* were not *P. Reyi* but were a form of *P. aviculare* L. s. lat. Lindman (1904), after examining Stockholm specimens labelled *P. Robertii*, concluded that they were "decidedly *P. aviculare*, s.s." Styles concludes on the basis of this confusion with respect to *P. Robertii* and the fact that no authentic specimens of this species exist, that *P. Robertii* is a nomen lubium (6).

*P. boreale* was first described by Lange (1886) as *P. aviculare* var. *boreale* and was later elevated to specific level by J. H. Small. Druce named similar plants *P. aviculare* var. *grandiflora*; Styles found these plants to correspond to *P. boreale* (6).

Hedberg (6) established the limits of *Polygonum*, section *Polygonum* on the basis of pollen morphology. Most species native to North America are excluded by Hedberg from section *Polygonum* and referred to section *Lunaria*.

A. and O. Löve (7) delimited the section on the basis of cytological evidence. They offered little morphological description of the species they included, making it difficult to compare their species with others. They retain *P. heterophyllum* Lindm., *P. aviculare* L. (stating that it is equal to *P. secundale* Lindm.), *P. furivorum* Ford., and *P. neglectum* Bess. *P. caelestum* is considered only a subspecies of *P. heterophyllum*. Löve and Löve recorded chromosome counts for their species, several of which are not consistent with reports of Styles and others (6).

The above summary of the history of section *Polygonum*
and particularly of the *P. aviculare* aggregate indicates the obvious confusion and the need for clarification of the section. The major basis for the resolution of such complexities has been C. T. Styles' publication of a detailed study of genus *Polygonum*, section *Polygonum* in the British Isles (3). Styles based the identity of species within the section on achene and perianth characters, correlated with plant habit, leaf characteristics, chromosome number, and habitat. This technique was later used by Nortens and Raven in a study of North American *Polygonum* (5). Styles separated the complex aggregate "species" *Polygonum aviculare* into four distinct species, following Tutin's primary groups - *P. heterophyllum* Lindm. (*P. aviculare* s.s.), *P. acuale* Lindm. (*P. arenarium* For.), *P. murivum* Jorg. ex For., and *P. boreale* Small (3). Nortens and Raven (5) have published a survey of North American species and have found that many of the British plants correspond to American specimens. They have cited the following species found in North America as belonging to section *Polygonum*: *P. arenarium*, *P. arenocelos*, *P. ramossissimum*, *P. setulosa*, *P. erectum*, *P.孔雀* (which is synonymous with *P. Allocornum* Blake), *P. aviculare* s.s., *P. arenarium*, *P. prolificum*, *P. glaucum*, *P. oxyceras* (including *P. sericeum*), *P. murivum* (a new species), and *P. boreale*. Nortens and Raven found it hard to distinguish between *P. arenarium* and *P. aviculare* at times. They could find no plants corresponding to Styles' *P. murivum* and *P. boreale* among North American specimens. After review of the work by Löve and Löve, Nortens and Raven conclude that what Löve and Löve call *P. heterophyllum* and *P. littoralis* "s.l.* are *P. aviculare* L. s.s.
A. and B. Löve's P. neglectum Less. and P. avicularia L. are probably P. trunnatum.

Hertsen (4) and Savage and Hertsen (6) also recognize P. buxiforme Small, a species which seems to exhibit characteristics intermediate between P. archestrum and P. avicularia. They (6) suggest that the difficulty of making sharp distinctions between P. archestrum and P. avicularia in the study of North American specimens could be resolved by the inclusion of P. buxiforme in the aggregate.

Fruit and flower characteristics were found by Styles to be most significant in making taxonomic distinctions between species of the section. Habit and size of the plant are too variable to be of great taxonomic significance. Leaf shape and size may be used cautiously in combination with other more stable traits. Pericarp characters, chromosome number, and fruit size, color, and texture in combination with vegetative characters are the most dependable taxonomic criteria (6).

MATERIALS AND METHODS

The data obtained in this study were derived from examinations of specimens received from several herbaria and examinations of fresh specimens collected during the extent of this investigation. Seventy-five specimens were received from the New York Botanical Garden, thirty specimens from the Lundell Herbarium in Texas, and forty-five specimens from the University of Texas Herbarium. Fresh specimens were collected in Indiana by A. D. Savage (summer, 1936) and by the author (summer, 1963), and in Nova Scotia and
New Brunswick by J. R. Hartens (summer, 1968). All specimens were studied extensively, and these characteristics (texture, color, shape, and size), inflorescence position, and fruiting pericarp characteristics were noted. Each specimen was then assigned to a species and annotated.

Visible schemes from the recently collected specimens (currently housed at New State University) were used in making chromosome counts of the mitotic cells of germinated root tips. In order to make the counts, normal, mature schemes from the specimens collected by Savage, Hartens, and the author were placed in a petri dish containing moist filter paper for two or three days. The moisture softened the fruit wall or pericarp, which was then easily removed with a razor blade and forceps. The schemes were subsequently cell-shocked at 4°C for at least ten days. The schemes were kept moist during this period. They were then removed from the refrigerator and allowed to germinate in the dark at room temperature. When the root tips were about 5 mm long, they were placed in a solution of oxyzinol (0.002 molar solution of 8-quinolinol in distilled water) for three hours. After that time they were fixed in 3 parts 95% ethyl alcohol: 1 part glacial acetic acid for 15 minutes.

The root tips were then placed in aceto-orcein stain consisting of 1% orcein in 45% acetic acid diluted 1:1 with water as needed. The diluted stain solution containing the root was heated in a watch glass until the stain boiled. After allowing the stain to cool for one minute, several drops of 1M HCl were added and the mixture was again heated until it was just ready to boil. The root tip was then removed to a drop of fresh diluted
stain on a clean microscope slide and the last 1 cm of the root tip was cut off. The rest of the seedling was discarded. A coverslip was added, and the root tip was squashed by first tapping the area of the coverslip which lay over the root tip and then inverting the slide and rubbing the back of the slide with the handle of a dissecting needle. The slide was then heated prior to microscopic examination. Chromosome counts were made using an American Optical phase microscope at 1000x magnification.

Chromosome counts were obtained for plants designated as *E. erectum* L., *E. buxiforme* Small, and *E. crenatum* Bor.

Several problems were encountered in trying to determine the chromosome counts. First, cold frequently killed the scheme before it had germinated. Second, few of the many schemes that had been cold-shocked would germinate, or, more frequently, germinated too slowly to produce many dividing cells in the squash. Third, the staining technique yielded such a variety of results (probably due to the rate of germination of the schemes) that it was difficult to get a good stain every time. Fourth, even the squashes of well-stained dividing cells were often not clear enough to get an accurate count. On a good slide, usually only four or five cells contained well-stained chromosomes well-distributed in a flat cell so that they could be counted with some accuracy. Many times the nuclear membrane did not rupture, preventing the chromosomes from being released and dispersed throughout the cell and therefore making a count impossible. These problems all contributed to many unusable slides, many uncertain counts, and possibly to inconsistent results.
DATA

The results of chromosome counts obtained for *P. buxiforme* Small were as follows:


4) *P. buxiforme* Small, Halifax County, Nova Scotia, August 18, 1968, J. H. Mertens 48-25. (Ball State University). 2n=50, February 21, 1969 (see Figure I).

Two chromosome counts were also made on *Polygynum erectum*, another species in section *Polygynum*. Previous reports of the chromosome number of *P. erectum* include 2n=20, by A. and D. Löve (3), and 2n=ca. 40, by Savage and Mertens (6). The counts reported herein seem to establish the chromosome number of *P. erectum* as 2n=40.


A chromosome count obtained for a *P. arenstrum* specimen confirms previous reports (6,5) of 2n=40.


Greenhouse populations of *P. buxiforme*, *P. aviculare*, and *P. arenstrum* (all from the *P. aviculare* complex) as well as
FIGURE I. Cell from Polygonum biforme plant. Halifax
County, Nova Scotia, August 16, 1969, T. R. Kertes
P. erectum were established from germinated seeds of plants assigned to these species. Flowers from the plants were then examined. Unfortunately, no flowers could be found on the P. buxiforme and P. aviculare plants, thus making it impossible to describe fresh flowers of these species. Results from the other two species showed very few differences between them (Table I). P. erectum flowers differed from P. arenosum flowers in that in most cases two or often three sepals of the flowers of P. erectum seemed to be fused together. Also the sinuses between sepals of P. erectum flowers were shallower than those of P. arenosum.

**Table I.** Data from study of flowers on *Polygonum* specimens grown in the greenhouse.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sepal Number</th>
<th>Stamen Number</th>
<th>Stigma Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. arenosum</td>
<td>4-5</td>
<td>3-6</td>
<td>2-3</td>
</tr>
<tr>
<td>(6 plants)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. erectum</td>
<td>4-5</td>
<td>2-5</td>
<td>2-3</td>
</tr>
<tr>
<td>(10 plants)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A loan was requested from the New York Botanical Garden of the plants that J. K. Small had designated as the type specimen(s) for *Polygonum buxiforme*. Instead, seventy-five specimens of various species were received. Some of these were not even included in section *Polygonum*. Only one or two specimens had even been annotated by Small. However, the plants were extensively studied, the fruits measured, and then the plants were assigned to a species and annotated. Forty-three plants were designated as P. buxiforme, nine as P. buxiforme(?), six as P. arenosum, six as P. aviculare, one as P. erectum, and one was designated P. Fowleri. Great ranges in fruit sizes
were recorded (Table II).

**TABLE II.** Means and ranges in size of fruits of 65 Polygonum specimens. (Data are reported in ocular units; 12 ocular units equal 1 mm.)

<table>
<thead>
<tr>
<th>Species</th>
<th>No.</th>
<th>Fruit in Calyx</th>
<th>Calyxremoved</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Length</td>
<td>Width</td>
</tr>
<tr>
<td><em>P. buxiforme</em></td>
<td>43</td>
<td>24-42</td>
<td>16-27</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>32.55</td>
<td>24.47</td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>34.60</td>
<td>25.20</td>
</tr>
<tr>
<td><em>P. buxiforme (?)</em></td>
<td>9</td>
<td>29-40</td>
<td>16-28</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>34.50</td>
<td>25.20</td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>34.60</td>
<td>25.20</td>
</tr>
<tr>
<td><em>P. arenastrium</em></td>
<td>6</td>
<td>25-32</td>
<td>15-22</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>29.14</td>
<td>19.71</td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>37.25</td>
<td>22.33</td>
</tr>
<tr>
<td><em>P. aviculare</em></td>
<td>6</td>
<td>35-41</td>
<td>20-26</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>37.25</td>
<td>22.33</td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>37.25</td>
<td>22.33</td>
</tr>
<tr>
<td><em>P. erectum</em></td>
<td>1</td>
<td>47</td>
<td>31</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>47</td>
<td>31</td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>47</td>
<td>31</td>
</tr>
<tr>
<td><em>P. Fowleri</em></td>
<td>1</td>
<td>49-51</td>
<td>29-55</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>50.00</td>
<td>32.00</td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>50.00</td>
<td>32.00</td>
</tr>
</tbody>
</table>

**DISCUSSION**

*P. aviculare s.s., P. arenastrium, and P. buxiforme* are all members of the *P. aviculare* aggregate of genus *Polygonum*, section *Polygonum*. Although they are quite similar in appearance, attempts have been made to distinguish between the three on the basis of morphological characteristics. *P. aviculare* plants generally exhibit marked heterophyllly, branch leaves being smaller than stem leaves, and produce relatively large, trigonous achenes with one broad, slightly concave side and two somewhat
narrower, concave sides and are long in proportion to their width. The perianth is divided for nearly three-fourths of its length and is tightly oppressed around the achene. The reported chromosome number for *E. avicularis* s.s. is \(2n=60\) (9).

*E. arenstrum* specimens have much smaller leaves showing no heterophyllly, and tend to grow in flat mats. The trigonous achenes are also smaller, having two equal, convex sides and one narrower, concave side. The tightly oppressed perianth is divided for only one-half its length. The reported chromosome number for *E. arenstrum* is \(2n=40\) (9), which was confirmed in the present study.

*Polygonum buxiforme*, the currently debated species, includes plants which resemble *E. avicularis* but which show little or no heterophyllly. Achenes are again trigonous, dull, and striated, but tend to be intermediate in size between *E. avicularis* and *E. arenstrum*. The achenes are "heart-shaped," being much broader in relation to length than those of *E. avicularis*, and have one very broad, flat side and two narrower, concave sides. The perianth is divided for approximately two-thirds of its length and is characterized by a pronounced papery "flange" or border at its base. Löve and Löve (7) have reported a chromosome number of \(2n=20\) for *P. buxiforme*, but this number has not been confirmed in the present study (see below).

The characteristics described above have been used with some success in attempting to distinguish between the three species of the aggregate. However, some specimens seem to show a few characteristics of one species and a few of another, or different
schemes on the same plant may appear to belong to different species. In fact, the inclusion of P. buxiforme as a separate taxon in the P. aviculare aggregate was suggested by Savage and Kertes (6) in order to resolve the identity of specimens which exhibited morphological characteristics intermediate between P. aviculare and P. arenastrum. However, even the morphological characteristics described for P. buxiforme seem unstable and hard to substantiate from plant to plant. Therefore, it was hypothesized that chromosome numbers might be better indicators of species identity than morphological traits, a theory which prompted the present study.

The immediate purpose of this study was to discover the chromosome numbers for plants designated as P. buxiforme in order to provide additional evidence for or against considering P. buxiforme a distinct entity. Chromosome counts obtained for those plants varied--2n=40 and 2n=60. Evidently the specimens identified as P. buxiforme on the basis of morphological features do not always have the same chromosome number. It may well be that plants identified as P. buxiforme are, in fact, either P. arenastrum or with 2n=40 or P. aviculare L. s.s. with 2n=60. In other words, morphological differences previously used to distinguish between these three species may be unreliable and/or not consistently correlated with chromosome numbers, at least in relation to North American plants. Instead, it may be necessary to consider these three species as one aggregate, P. aviculare L. sensu lato, because of the great difficulties of finding consistent and distinct differences between them. This suggestion has also been proposed by Taylor and Mulligan (9) and by P. S. Raven.
(Personal communication, 1968).

CONCLUSIONS

1) Size and shape of fruit and perianth characters may be used in a general way to help identify specimens of the *P. aviculare* aggregate, but these characters are too variable to be the sole defining criteria.

2) The inconsistent chromosome counts obtained for plants named *P. bicornutum* seem to indicate that *P. bicornutum* cannot be determined as distinct from *P. aviculare* or *P. arnostrum*. This supports the conclusion that the three species should be considered as one aggregate, *P. aviculare* L. sensu lato.

3) The chromosome number of *Polygala cretaea* L. has been established as 2n=40.
LIFE AND DEATH


GENERAL BIBLIOGRAPHY