EXPERIMENTAL TAXONOMY IN THE GENUS *AMELANCHIER* I: A NEW LOOK AT THE CHROMOSOME NUMBERS OF THE *AMELANCHIER* SPECIES GROWING IN THE NORTHEASTERN UNITED STATES

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Only a limited number of chromosome studies have included the *Amelanchier* species of the northeastern United States. These studies by Moffett (1931), Sax (1931), Cruise (1964), and Löve and Löve (1966) have resulted in conflicting conclusions that have yet to be resolved. Several factors may have contributed to this confusion. Changes in nomenclature and diverging taxonomic opinions could have led to errors in specimen identification. In addition, the formation of diploid gametes and autopolyploidy, not uncommon in the Maloideae, may also occur in *Amelanchier*. Finally the small size of the chromosomes and their multivalent chromosome associations make it difficult to discern with absolute accuracy the number of chromosomes.

The authors are aware of the efforts of our Canadian colleagues, McKay (1973) and Landry (1975), to simplify *Amelanchier* taxonomy by utilizing infraspecific categories. In this paper, however, the taxonomy of Fernald (1950) has been followed since it affords the greater number of species, and knowledge of their chromosome numbers may eventually contribute to a reevaluation of the validity of these species.

MATERIAL AND METHODS

Chromosome counts were made from buds collected from native plants which had been transplanted to an experimental garden located in Monroeville, Allegheny County, Pennsylvania. The collecting of live material of *Amelanchier* for cultivation was guided by its growth habits. Both stoloniferous and fastigiate forms will tolerate the removal of a portion of the clone or base, with the segregate and parent plants continuing good growth.

[1]This paper is based on a dissertation completed in the Department of Biological Sciences, University of Pittsburgh, Pa., in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Table I. **Chromosome Numbers of the *Amelanchier* Species of the Northeastern United States**

Species	Gametic Chromosome Number	Possible Ploidy Level	Locality and Collection Numbers[c]	
A. arborea (Michx. f.) Fern.	17[b]	2*x*	Jefferson Co., W. Va.	R 93
=*A. oblongifolia* (Torr. & Gray) Roemer	17		Fayette Co., Pa.	R108
A. bartramiana (Tausch) Roemer	16[a]	2*x*	McKean Co., Pa.	R168
*Putative hybrid				
=*A. bartramiana* × *A. laevis*	25[a]	3*x*	Forest Co., Pa.	R167
A. canadensis (L.) Medic	17	2*x*	Ocean Co., N.J.	R113
	17[b]		Burlington Co., N.J.	R114
	17		Ocean Co., N.J.	R115a
	17		Middlesex Co., N.J.	R116
	17[b]		Norfolk Co., Mass.	R157
	17		Henrico Co., Virg.	R159
Putative hybrid	16[a]	2*x*	Ocean No., N.J.	R115b
=*A. canadensis* × *A. laevis*	17		Passaic Co., N.J.	R117
A. humilis Wieg.	33[b]	4*x*	Monongalia Co., W. Va.	Davis=R132
	31[b]	4*x*	Propagated specimen from R132	R133
	34[b]		Penobscot Co., Maine	R148

Table 1 (continued)

**A. intermedia* Spach	17	2*x*	Pike Co., Pa.	R119
	28[b]	3*x*	Rutland Co., Vt.	R144
A. laevis Wieg.	15[a]	2*x*	Washington Co., Md.	R109
	18[b]	2*x*	Randolph Co., W. Va.	R161b
**A. nantucketensis* Bickn.	34[a]	4*x*	New London Co., Conn.	R156
**A. obovalis* (Michx.) Ashe	17	2*x*	Lackawanna Co., Pa.	R122a
	24[b]	3*x*	Lackawanna Co., Pa.	R122b
	25[b]	3*x*	Luzerne Co., Pa.	R123
A. sanguinea (Pursch) D.C.	20[a]	3*x*	Pendleton Co., W. Va.	R128b
A. stolonifera Wieg.	28[a]	3*x*	Waldo Co., Maine	R149
**A. wiegandii* Nielsen	36	4*x*	Waldo Co., Maine	R150b

*Indicates first chromosome report for taxon
[a]Chromosome number determined from a somatic count
[b]Univalents, bivalents, and multivalents observed in meiotic material
[c]Collection numbers preceded by R were made by Robinson

The following procedure for the preparation of the material for cytological examination partly resembles some work of Zielinski and Thompson (1967) with *Pyrus* but contains several modifications. As the time for microsporogenesis approached, from late February through April in southwestern Pennsylvania, daily squashes of fresh material in acetocarmine were made to judge the correct time for proper collection of buds. Collected buds were partially opened and transferred into 0.5% colchicine for approximately three hours. This technique had been noted by Namboodiri (1973) as an aid in the resolution of small chromosomes. The colchicine was washed out with several changes of water. The buds were then fixed in Carnoy's fluid (3 parts absolute ethyl alcohol to 1 part glacial acetic acid) for 24 hours and stored in 70% ethyl alcohol at −4° C. In the slide preparation the anthers were removed from the buds, macerated with a tissue grinder, washed with water, and placed in a 2.0% pectinase solution (Macerase: Calbiochem, San Diego, Calif.) pH 6.0 at 30° C. for two hours. The cells were washed free of pectinase with 50% ethyl alcohol and stained with an alcoholic hydrochloric acid-carmine solution (Snow, 1963). The cells could remain in this solution for a week with no harm, but five days seemed to be sufficient for a good stain. The slides were made semipermanent by squashing the cells in a small amount of 45% acetic acid and mounting in Hoyer's mounting medium (Cunningham, 1972). Camera lucida drawings and photographs were made utilizing an oil-immersion phase contrast system with a Wild M-20 Microscope. Voucher specimens and photographs were deposited in the herbarium of the Carnegie Museum of Natural History, Pittsburgh, Pennsylvania (CM).

RESULTS

Chromosome counts were obtained from 25 specimens representing 11 taxa and three putative hybrids. The results are detailed in Table I. With $n = 17$ considered to be the basic chromosome number in the Maloideae, the apparent ploidy level of the specimens is also indicated. To the authors' knowledge those counts preceded by an asterisk are being reported for the first time. Both recent

literature and the following surveys of chromosome numbers have been reviewed: Darlington and Wylie (1956), Ornduff (1967–69), Moore (1970–72), and Federov (1969).

Photographic evidence is presented in figures 1–3 for three taxa: *Amelanchier arborea, A. humilis,* and the putative hybrid *A. canadensis* × *A. laevis.* The remaining counts can be substantiated from photographs in Robinson (1978) and photographs accompanying the voucher specimens.

As the specimens listed in Table I grew in the experimental garden, routine morphological observations were made. Except for *Amelanchier bartramiana* with its distinctive single or few flowered raceme, the specimens appeared to fall into two morphological complexes. The features of these complexes are detailed in Table II. This division, in part, resembles an earlier attempt by Blanchard (1907) to divide *Amelanchier* taxa into two classes.

If one accepts that a taxon does not have to fulfill every single criterion to be a member of a complex, those growing in the northeastern United States could be divided as follows:

Canadensis complex: *Amelanchier arborea, A. canadensis, A. intermedia, A. laevis,* putative hybrid *A. canadensis* × *A. laevis.*

Sanguinea complex: *A. humilis, A. nantucketensis, A. obovalis, A. sanguinea, A. stolonifera,* and *A. wiegandii.*

In our observations the morphological traits appeared to be correlated with a change in ploidy level, the Canadensis complex representing diploid taxa and the Sanguinea complex triploid and tetraploid taxa. The only taxa observed to be exceptions to this hypothesis were *A. intermedia* and *A. obovalis,* in which both diploid and triploid specimens have been identified. Both of these taxa have been noted for their marked variability (Wiegand, 1920; Fernald, 1941). Their origins may be the result of interspecific hybridizations between the two complexes. Additional breeding studies are presently being conducted to confirm this hypothesis.

It should be noted that only one specimen each of *Amelanchier sanguinea* and *A. stolonifera* has been cytologically examined in this study. Each has been identified as a triploid, but more specimens should be examined before the chromosome status of these taxa can be established.

Table II. **Morphological Features of the Sanguinea and Canadensis complexes**

Characteristic	Sanguinea complex	Canadensis complex
winter bud color[a]	maroon	russet brown
flexion of sepals	sepals recurving from middle, giving calyx plus floral cup urceolate appearance	sepals not recurving from middle
flexion of flowering raceme	erect, resembling miniature candelabrum	flexion of raceme various
average $\frac{\text{length}}{\text{width}}$ mature leaves[b]	=or less than 1.5	greater than 1.5
top of ovary[b]	densely appressed pilosity	glabrous to varying amts. of pilosity
shape of fruit	pear shaped	round
anthesis	3–4 days later than Canadensis complex	3–4 days earlier than Sanguinea complex

[a]Color observed was the darkest portion in the body of the bud scales. Color Harmony Manual, Container Corp. of Am., 1958.
Maroon: 7pi, 71/2pi, 71/2pg, 8pi
russet brown: 4pi, 4pg, 5pg, 5pi
[b]Additional data can be found in Robinson (1971).

Since the work of Sax (1931), there had been no substantiation of diploid specimens of *Amelanchier* from North America prior to this study, although Favarger and Correvon (1967) identified a diploid race of the common *Amelanchier* of Europe, *Amelanchier ovalis* Medikus. The evidence for diploid, triploid, and tetraploid specimens of *Amelanchier* helps to explain the variation in this genus. The amazing potential for variation and propagation in this genus makes the taxonomic confusion quite understandable. We feel that any revision of *Amelanchier* taxonomy would be premature until further chromosome and breeding studies have been conducted. The present studies will be extended beyond a regional basis and hopefully will include a fuller complement of *Amelanchier* species. In this regard the senior author (Dr. W. Ann Robinson, 4264 Northern Pike, Monroeville, Pa. 15146) would be most pleased to receive any semi-dried fruit from documented sources with the possibility of propagation of the taxa.

Figure 1. Camera lucida drawing (not to scale) and optical sections of diakinesis from microsporogenesis in *Amelanchier arborea*, specimen *R93*. Dashed line indicates nucleolus. 2_{I}, 13_{II}, 2_{III}.

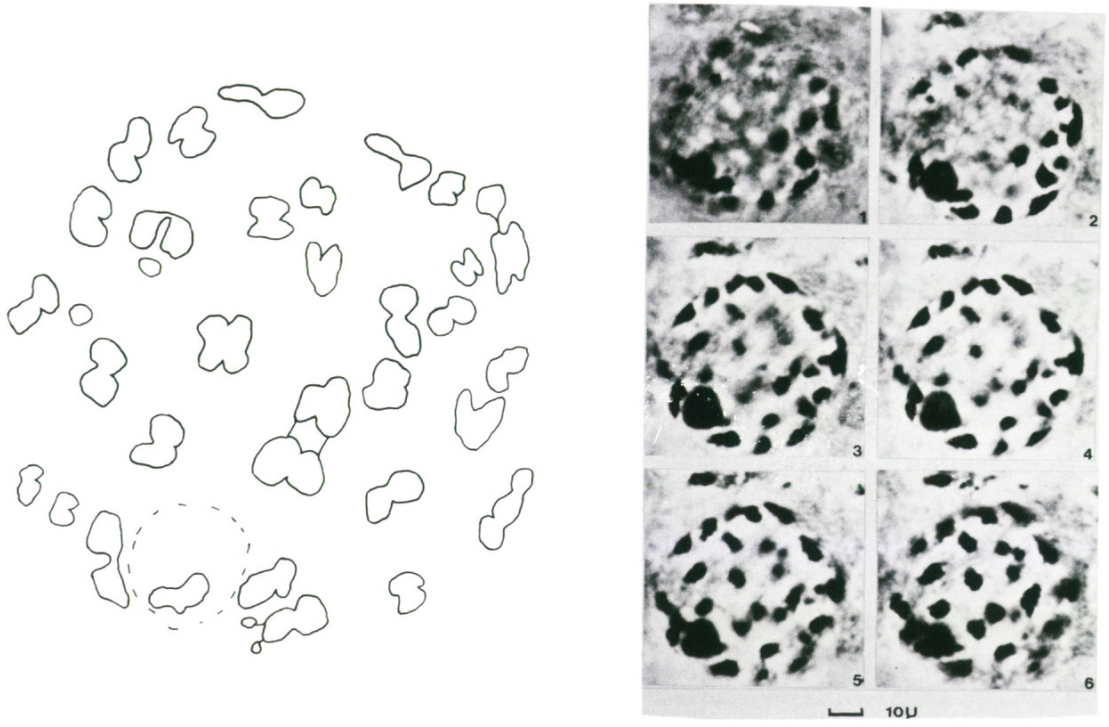

Figure 2. Camera lucida drawing (not to scale) and optical sections of diakinesis from microsporogenesis in *Amelanchier humilis*, specimen *R148*. Dashed line indicates nucleolus. 2_{I}, 28_{II}, 2_{III}, 1_{IV}.

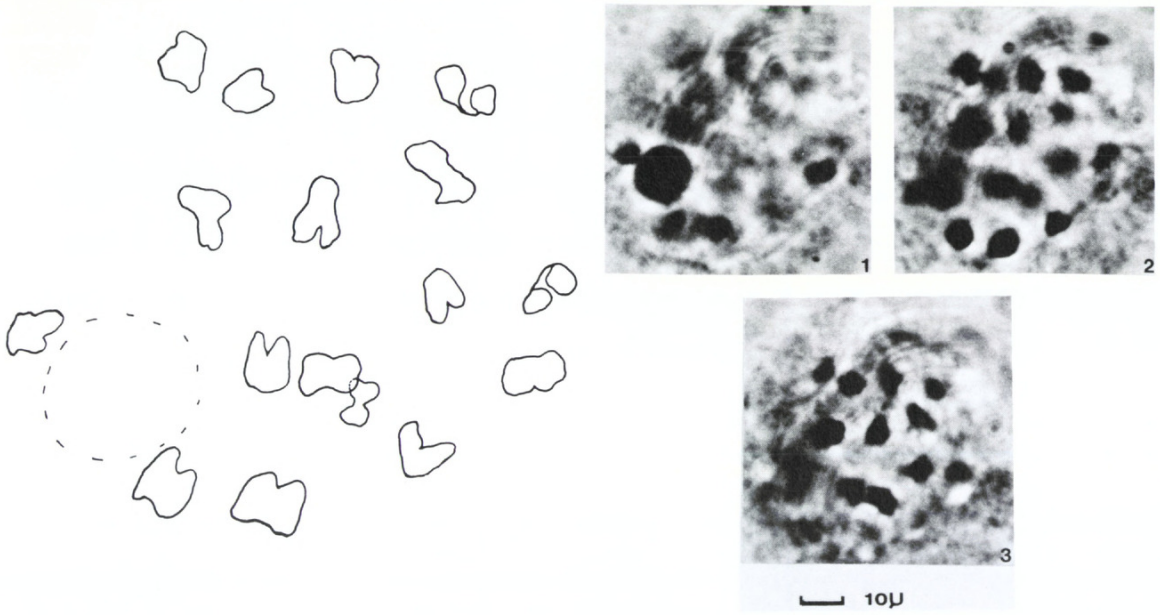

Figure 3. Camera lucida drawing (not to scale) and optical sections of diakinesis from microsporogenesis in the putative hybrid *Amelanchier canadensis* × *A. laevis*, specimen *R117*. Dashed line indicates nucleolus.

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