Cytogenetic investigations on *Anemone* L. II. Hybrids among *Anemone virginiana*, *A. silvestris* and *A. multifida*

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I. Introduction.

The present paper is a continuation of the previous work on *Anemone* hybrids (W. Gajewski 1946). In the first paper of this series the triploid hybrid between *Anemone silvestris* (diploid) and *A. multifida* (tetraploid) was described. The second generation of this hybrid consisted only of hexaploid plants.

Anemone virginiana blooms distinctly latter as *A. silvestris* and *A. multifida* and the hybrids among them are difficult to obtain. After many failures I have succeeded to obtain only the hybrids with *A. virginiana* as a mother plant; the attempts to obtain reciprocal crosses proved always a failure.

A. virginiana is a diploid plant with $n = 8$ like *A. silvestris*. The chromosomes of both these species are morphologically very similar. The hybrid between these two species, described by Janczewski (1892), was quite sterile. It was thus interesting to test the behaviour of the chromosomes at meiosis in this hybrid. The second hybrid between *A. virginiana* and the tetraploid *A. multifida* is a triploid like the hybrid between *A. silvestris* and *A. multifida*. The comparison of the cytological and morphological features of these two hybrids presents some interest. This work was carried in the years 1938—39 in the Botanical Garden of the University in Warsaw and after the beginning of the war in my garden in Piastów near Warsaw.

II. Material and methods.

Plant material of *A. multifida* and *A. virginiana* was raised from seeds obtained from various Botanical Gardens and *A. silvestris* was cultivated from plants of the Podolian steppes. *A. virginiana* from different sources shows considerable range of variation. To the crosses

described above was chosen a variety with little greenish sepals, with the receptaculum moderately elongated and with the most early period of flowering.

The cytological studies of the parental species were made on permanent preparations. The root tips were fixed in Navashin's fluid (Langlet's modification) and stained with gentian-violet. The floral buds were forfixed for 1—2 minutes in Carnoy fixative and then fixed in Langlet fixative and stained by Newton's gentian-violet technique. The thickness of the root tips preparations was 20 μ , and of the floral buds preparations — 15 μ .

The hybrids bloom firstly in the year 1940 during German occupation. As the Germans have closed the University it was impossible to use the laboratory and I could not make permanent preparations. In these conditions I had only the possibility to study the meiosis in P.M.C. in acetocarmine smear preparations. Before staining the floral buds were fixed in Farmer's fluid. Drawings were made with camera lucida at the magnification of 3000 or 1700.

III. The description of parental species and their hybrids.

The detailed description of *A. silvestris* and *A. multifida* was done in the previous paper of this series. In the present paper I would describe only *A. virginiana* in comparison with other two species and their hybrids. *Anemona multifida* used to the crosses was the variety *magellanica*.

The chief distinguishing morphological traits that were examined in the parents and their hybrids were as follows:

1. Period of flowering. In the year 1940 the beginning of the flowering was as follows:

<i>A. silvestris</i>	begun to flower at 19 May
<i>A. multifida</i>	" " " " 30 May
<i>A. virginiana</i>	" " " " 20 April
F ₁ <i>A. virginiana</i> x <i>A. silvestris</i>	" " " " 27 May
F ₁ <i>A. virginiana</i> x <i>A. multifida</i>	" " " " 2 April

The time of flowering of the two examined hybrids is intermediate between parental species but more like to the earlier flowering parent.

2. The presence of adventitious buds on the roots. The adventitious buds on the roots are present by *A. silvestris* and are absent by *A. multifida* and *A. virginiana*. In the hybrid *A. virg.* x *A. silv.* the roots are ramping and like by *A. silvestris* produce many adventitious buds.

3. The size and shape of basal and involucreal leaves. The size and shape of the leaves of *A. silvestris* and *A. virginiana* are very similar and does not form any differential trait. The leaves of the hybrid *A. virg. x A. mult.* are intermediate between the parental species and like to the leaves of the hybrid *A. silv. x A. mult.* described in the first paper of this series.

4. The distribution of the stomata on the epidermis of the leaves.

	No. of stomata lower side on 1 sq. mm	No. of stomata upper side on 1 sq. mm	No. of epid. cells lower side on 1 sq. mm	No. of epid. cells upper side on 1 sq. mm	Stomatal index	
					Lower side	Upper side
<i>A. silvestris</i> . . .	168,7	0,0	537,6	544,8	23,8	0,0
<i>A. virginiana</i> . . .	78,6	0,0	486,2	512,4	13,9	0,0
<i>A. multifida</i> . . .	71,5	42,9	361,7	378,9	16,5	10,1
<i>F₁ virg. x mult.</i> . . .	72,9	21,4	404,6	466,1	15,2	4,3
<i>F₁ virg x silv.</i> . . .	12,9	0,0	364,6	383,2	23,6	0,0

From this table it is to be seen that the number of stomata on the lower side of the leaves of the both examined hybrids is intermediate between their parents. The number of stomata on the upper side of the leaves of the hybrid *A. virginiana x A. multifida* is exactly the half of the number of stomata on the upper side of the leaves of *A. multifida*. It is interesting that the stomatal index of the *A. virg. x silv.* is quite identical with paternal species and the stomatal index of the second hybrid is intermediate between the parental species.

5. The height and mode of ramification of the floral shoots. *A. silvestris* and *A. multifida* have the flowers only of the first and second order, but *A. virginiana* has flowers also of the third order and sometimes even of the fourth. The numbers of flowers on one floral shoot were as follows:

<i>A. silvestris</i>	from 1 to 2	mean 1,1
<i>A. multifida</i>	„ 2 „ 5	„ 2,7
<i>A. virginiana</i>	„ 2 „ 11	„ 4,1
<i>F₁ virg. x silv.</i>	„ 2 „ 4	„ 3,1
<i>F₁ virg. x mult.</i>	„ 3 „ 5	„ 3,3

The height of the floral shoots by the hybrids with *A. virginiana* as a mother plant shows no heterosis and is intermediate between the parental species. The height of the floral shoots in the year 1940 was as follows (mean values):

<i>A. silvestris</i>	35,5 cm.
<i>A. multifida</i>	25,5 cm.
<i>A. virginiana</i>	51,2 cm.
F ₁ <i>virg. x mult.</i>	28,1 cm.
F ₁ <i>virg. x silv.</i>	39,1 cm.

6. Erect or dropping floral peduncles. *A. silvestris* has floral buds on dropping peduncles, *A. virginiana* and *A. multifida* have erect peduncles. The erect floral peduncles of *A. virginiana* dominate completely over the hanging peduncles of *A. silvestris* in the hybrid between these two species.

7. Number, size and colour of the sepals. *A. virginiana* has 5 small, coriaceous, acute and concave sepals of pale greenish colour, *A. silvestris* has 5 large, obtuse sepals of snow-white colour and *A. multifida v. magellanica* has 6—8, small pale yellow sepals. The hybrid *A. virginiana x A. multifida* has 5—7 (mean 6,7) pale yellow sepals of somewhat acute shape. The hybrid *A. virginiana x A. silvestris* has 5 acute and white sepals. The dimensions of the sepals are as follows (mean values):

<i>A. virginiana</i>	length 10,3 mm
	breadth 5,4 mm
<i>A. silvestris</i>	length 28,2 mm
	breadth 19,8 mm
<i>A. multifida</i>	length 15,9 mm
	breadth 7,8 mm
F ₁ <i>virg. x silv.</i>	length 14,6 mm
	breadth 8,5 mm
F ₁ <i>virg. x mult.</i>	length 12,0 mm
	breadth 6,5 mm

8. Size and form of pollen grains. The pollen grains of *A. silvestris* and *A. virginiana* are of similar texture, with three furrows, globular, ca. 19_μ in diameter. The pollen grains of *A. multifida* are greater (29_μ in diameter) and with twelve furrows. The hybrid *A. virginiana x A. silvestris* has the pollen completely bad and all the grains are empty and shrunken. In the hybrid *A. virginiana x multifida* the pollen is generally bad and the grains are shrunken but some rare grains are viable, full of protoplasm and with two nuclei. The viable grains are with twelve furrows 30 to 33_μ in diameter, greater as by both parental species.

9. The fertility. The three parental species are quite fertile, but the hybrids *A. virginiana x silvestris* and *A. virginiana x multifida*

are quite sterile and produce no good achenes. The absolute sterility of both these hybrids shows that probably all the megaspores are unviable. Janczewski has observed that by the hybrid *A. virginiana* x *silvestris* some rare achenes begun to develop but the development is early stopped and the hybrid never form ripe achenes. Janczewski supposes that these achenes arise from the pollination by paternal species. This fact indicates that some rare megaspores of this hybrid are able to be fertilised but the embryos are unable to further development.

The morphological traits of the triploid hybrid *A. virginiana* x *multifida* are very like to the triploid hybrid *A. silvestris* x *multifida* already described in the first publication of this series. Both hybrids present intermediate size and shape of leaves, sepals and stomata and intermediate number of flowers on floral shoots, sepals in the flower and stomata on surface unity. In both hybrids the yellow colour of sepals of the *multifida* parent is dominant over the white and greenish colour of *A. silvestris* and *A. virginiana*. The diploid hybrid *A. virginiana* x *silvestris* is also intermediate between parental species, only from *silvestris* parent dominates the presence of adventitious buds on ramping roots and from *virginiana* parent — the erect peduncles. Both hybrids described in this paper present no heterosis.

IV. The cytology of the investigated plants.

The cytology of the diploid *A. silvestris* and of the tetraploid *A. multifida* was described in the previous paper. It must be remembered that the 32 somatic chromosomes of *A. multifida* form four sets of eight chromosomes very similar to the double sets of eight chromosomes of *A. silvestris*. The course of meiosis in these two species is quite normal.

A. *Anemone virginiana* L.

1. The somatic complement. The metaphasal plate from root tip is shown on the fig. 1 a. The somatic chromosomes of *A. silvestris* are represented for the comparison on the fig. 1 b. We see that the chromosomes of both these diploid species are very similar.

2. The meiosis in P. M. C. The prophasal stages of meiosis in P. M. C. were studied only in diplotene when the bivalents are visible and the loops between chiasmata are large, fig. 1 c. The total number of bivalents is difficult to count exactly as in diplotene the chromosomes are crowded together. In diakinesis many P. M. C. showed clear pic-

tures of eight bivalents distributed in the whole enlarged nucleus, one pair being attached to the one nucleolus present, figs. 1 d, e. The bivalents in diakinesis as in the following metaphase are always eight

Fig. 1. *Anemone virginiana*: a — somatic metaphase plate, b — somatic metaphase from *A. silvestris*, c — diplotene, d, e — diakinesis, f, g — metaphase I, h — metaphase II, i — anaphase II.

in number. They form crosses with unequal arms or rings with loose ends, figs. 1 f, g. The course of anaphase I is quite normal and two groups of 8 chromosomes are formed. Also the homeotypic division is quite normal and at the metaphase II two plates with 8 chromosomes are formed, fig. 1 h. - After the second anaphase 4 groups of 8 chromosomes are formed, fig. 1 i. The tetrads are normal with four nuclei of equal size. The pollen grains are in 98% normal.

B. *F₁ A. virginiana* x *A. silvestris*.

Both parental species have the same haploid chromosome number. The hybrid being completely sterile, it was highly probable, that the meiosis should be abnormal, but in P. M. C. the course of meiosis was found to be as normal as by the two parental species. The somatic complement of the chromosomes in this hybrid was not studied. In metaphase of the first meiotic division in P. M. C. 108 cells were analysed and in 106 eight bivalents were found, figs. 2 a, b, c, d and only in 2 cells I have found 6 bivalents and two univalents lying on both sides of metaphasal plate, fig. 2 e. The shape of the bivalents is very similar to those of parental species; the most frequent were the bivalents in form of crosses with unequal arms. At anaphase I two regular groups of 8 chromosomes were found in the majority of studied P. M. C. In 42 analysed P. M. C. at the anaphase I only in one cell I have

found two groups of 10 chromosomes and in one another 8 and 9 chromosomes in two anaphasal groups, in the remaining 40 cells two groups with 8 chromosomes were present. At metaphase II two plates of eight chromosomes appear, figs. 2 h,i. The course of anaphase II is in general normal and as a result of anaphasic movements four groups of 8 chro-

Fig 2. *F₁ Anemone virginiana x silvestris*: a, b, c-metaphase I from polar view, d-metaphase I from side view, e-metaphase I from side view with two univalents, f, g-anaphase I, h,i-metaphase II, j-anaphase II, k-anaphase II with two chromosomes lost, l-telophase, k-tetrad.

mosomes were present, fig. 2 j. In 182 examined P.M.C. at anaphase II all were normal with four groups of chromosomes. Only in one cell two chromosomes were lost in cytoplasm, fig. 2 k. In 12 P. M. C. I have counted 8 chromosomes in each of four groups and in 15 P. M. C. I have counted 8 chromosomes in two out of four present groups. At the telophase II four nuclei are formed, fig. 2 l. After cell walls formation normal tetrads are formed, fig. 2 m. The tetrads seem to be quite normal but the pollen is bed and all the grains are shrunken, without cytoplasm and nuclei. They must degenerate before or during the mitotic division of pollen nucleus. The cause of this degenerescence must be not the numeric distrubution of the chromosomes, as all the young pollen grains have 8 chromosomes, but the genetic unbalance of the pollen grains which contain in their nuclei 8 chromosomes derived from two different species. Only the grains with all chromosomes derived from one parent should be theoretically viable in absence of interspecific crossing-over. In this hybrid with complete interspecific

chromosome pairing all chromosomes are interchanged and no viable pollen grains are formed. The same phenomenon of complete gametic sterility with normal meiotic divisions was several times observed in various interspecific hybrids e. g. *Ribes sanguineum* x *aureum* (Tischler 1906), *Digitalis purpurea* x *D. ambigua* and *Digitalis lanata* x *D. micrantha* (Hasse-Bessel 1922), *Primula floribunda* x *P. verticillata* (Digby 1912) and in others.

C. F₁ *Anemone virginiana* x *multifida*.

The somatic complement of this hybrid was not studied. The meiosis was investigated in P.M.C. The prophasal stages and diakinesis were not found in my preparations. Many P.M.C. at metaphase I were studied and I have found varying number of uni- and bivalents ranging from 3 bivalents and 18 univalents to 8 bivalents and 8 univalents. In 40 P.M.C. studied at metaphase I have found following configurations:

Configuration	3II + 18I	4II + 16I	5II + 4I	6II + 2I	7II + 0I	8II + 8I
No. of P.M.C.	3	0	11	0	4	2

The configuration with 3 II is represented on the fig. 3a with 4 II — on the fig. 3b, with 5 II — on the fig. 3c and with 7 II — on the fig. 3d. The mean number of bivalents per 1 P.M.C. is 5,30. A very similar number 5,04 was found in the hybrid *A. silvestris* x *A. multifida*. At the metaphase the bivalents form an equatorial plate and the univalents lie on the equator or on the spindle. At the anaphase the bivalents separate and separated partners move to the opposite poles. The univalents rest on the spindle and after the bivalents have reached the poles they begun to move to the equatorial plane of the spindle and split longitudinally. The univalents halves move then to the poles, fig. 3g. This is true for all univalents that have reached equatorial plane of the spindle. The behaviour of the univalents lying outside it is variable. They are either included unsplit to the daughter nuclei or left out in the cytoplasm. The anaphase and telophase I are generally very irregular and after the first division many cells occur with unequal or supernumerary nuclei, figs 3h, i. The number of the chromosomes in the anaphasic groups is very variable, but accurate counts were made only in few cells. In one P.M.C. the two anaphasic groups have 15 and 17 chromosomes, fig. 3h and in other the two large groups possess 12 and 13 chromosomes and 3 chromosomes form a separate „micronucleus“, fig. 3i. At telophase I generally about 30% of P.M.C. show supernumerary

nuclei or possess chromosomes lost in the cytoplasm. At metaphase II two or three plates with variable number of the chromosomes are formed. The attempts to count the exact chromosome number proved

Fig 3. *F₁ Anemone virginiana* x *A. multifida*: a-metaphase I with a configuration $3_{II}+18_{I}$, b-metaphase I with $4_{II}+16_{I}$, c-metaphase I with $5_{II}+14_{I}$, d-metaphase I with $7_{II}+10_{I}$ e, f-metaphase I from profile view, g-anaphase I with dividing univalents, h-anaphase I with 15 and 17 chromosomes in each of two groups, i-anaphase I with 12, 13 and 3 chromosomes in three groups, j-telophase I, k-telophase I with supernumerary nucleus and one chromosome lost in the cytoplasm, l-anaphase II with lagging chromosomes, m-telophase II with supernumerary nuclei, n-triad, o-polyad.

a failure. At anaphase II lagging chromosomes on the spindle were often observed, fig. 3l. At telophase II many multinuclear cells appear, fig. 3m. The tetrads present also very variable picture. Besides normal tetrads with four cells also „polyads“ with 5, 6 and 7 cells are to be found, figs. 3m, n. In two instances cells with only 3 nuclei were met

with, fig 3o and once a diad was found. In 50 P.M.C. at the tetrad stage I have found following numbers of microspores:

No. of microspores	2	3	4	5	6	7
No. of P. M. C.	1	2	30	9	7	1

The presence of a diad suggest the possibility of the formation of unreduced pollen grains in about 0,5%. In the aceto-carmin preparations of the pollen of this hybrid I have found about 1% of apparently good pollen grains which owing to their diameters should be unreduced. The mode of the diad formation is unknown to me.

V. Discussion.

The species, used to the crosses, all belong to the section *Ericephalus* Hook. et Thom. of the genus *Anemone* L. This section contains species with densely wooly haired achenes. Janczewski has divided this section into two subsections: *Oriba* Adams and *Anemonanthea* DC. The species from the subsection *Oriba* differ from these of the subsection *Anemonanthea* in having tuberous stolons, unifloral shoots, colored pollen grains and different mode of germination. Janczewski has stated that species between these two subsections are incompatible and that the species of the subsection *Anemonanthea* intercross relatively easy. The hybrids in the subsection *Oriba* are difficult to study as the seeds germinate only in the second or third year. The results of crossing experiments, Janczewski interpretes as an physiological proof of the correctness of his subdivivision oh the section *Ericephalus* based on morphological researches.

From the cytological point of view the whole section has the same basic chromosome number 8. The chromosome numbers of some species of this section are as follows:

Subsection *Oriba*

- A. caroliniana* $n = 8$ (Moffet 1932)
- A. coronaria* $n = 8$ (Nakajima (1931)
- A. fulgens* $n = 8$ (Moffet 1932)
- A. hortensis* $n = 8$ (Nakajima 1936)
- A. palmata* $n = 16$ (Langlet 1932)

Subsection *Anemonanthea*:

- A. silvestris* $n = 8$ Langlet 1932)
- A. virginiana* $n = 8$ (Langlet 1932)
- A. japonica* $n = 8$ (Moffet 1932)
- A. vitifolia* $n = 8$ (Gajewski unpubl.)

- A. parviflora* $n = 8$ (Langlet 1932)
A. baldensis $n = 12$ (Moffet 1932)
 $n = 16$ (Gajewski unpubl.)
A. multifida $n = 16$ (Moffet 1932)
A. rupicola $n = 16$ (Moffet 1932)
A. Janczewskii $n = 24$ (Gajewski 1946)

We see in the subsection *Anemonanthea* a whole series of chromosome numbers from diploid to hexaploid is present. The triploid number 12 found by Moffet for *A. baldensis* seem to be somewhat doubtful as I have found only tetraploid number 32 in root tips from seeds obtained from two Botanical Gardens.

Are the tetraploid species, like *A. multifida*, amphidiploids arisen from diploid species? It is difficult to answer this question basing on the present experiments. The apparition of new amphidiploid species *Anemone Janczewski* described in the previous paper suggests this possibility.

The complete coniugation among the chromosomes of *A. silvestris* and *A. virginiana* is worth noting. Both species morphologically somewhat related have quite different distributional areas. *A. silvestris* is an euroasiatic species from southern steppe regions and *A. virginiana* is north-american species from forest regions. The complete coniugation of the chromosomes in the hybrid between these two species indicates a probable common descent and relatively not pronounced changes in their chromosomal structure notwithstanding their quite different geographic distribution.

Summary

1. The description is given of two hybrids between *Anemone virginiana* as a mother plant and *A. silvestris* and *A. multifida* as father plants.
2. The hybrid between *A. virginiana* and *A. silvestris* is diploid like parental species, with $n = 8$. At meiosis in P.M.C. 8 bivalents are formed and in general the whole course of meiosis is quite normal. In spite of normal course of meiosis all pollen grains degenerate and the hybrid is completely sterile.
3. The hybrid between diploid *A. virginiana* and the tetraploid *A. multifida* is triploid with $n = 12$. At meiosis in P.M.C. from 3 to 8 bivalents are formed and 18 to 8 univalents. The course of meiosis shows many disturbances and the majority of pollen grains are unviable and only 1% pollen grains are visibly good and probably unreduced. The hybrid is sterile.

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