A Fertile Amphipolyploid Hybrid of Geum rivale with G. macrophyllum

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I. Introduction

My previous studies on the *Geum* genus (W. Gajewski 1948, 1949, 1952) have shown that within the *Eugeum* subgenus the basic chromosome number is x = 21. As the basic number within the whole *Geum* and other related genera is x = 7, this number in the case of the *Eugeum* subgenus is hexaploid. On the basis of cytological analysis of hybrids of species from the subgenus *Eugeum* and *Geum montanum* from the *Oreogeum* subgenus (W. Gajewski 1942), it can be established that in the hexaploid species of the *Eugeum* subgenus the three genomes with seven chromosomes are composed of two genomes homologous with two *G. montanum* genomes. It is possible that the *Eugeum* subgenus developed by means of an amphidiploid change from a hybrid of the tetraploid *G. montanum* (or other related species) with a diploid species which may have disapeared since, or has not been identified so far. If it were so the amphidiploid change would cause the appearance of this new *Geum* type. This new type has epizoochore fruits; it has shown itself to be very expansive, and has divided into many species.

The *Eugeum* subgenus has approximately 26 species (F. Bolle 1933) dispersed widely throughout Europe, Asia, both Americas, and one appears also in South Africa. Of these 26 species I have submitted to cytological studies 21, and I have found that 16 (*G. rivale, urbanum, molle, hispidum, albarraciense, aleppicum, silvaticum, canadense, laciniatum, macrophyllum, perincisum, oregonense, japo-*

nicum, coccineum, virginianum and *boliviense)* were hexaploid with 2 n = 24, 4 *(G. pyrenaicum, G. magellanicum, G. riojense, G. Fauriei)* were dodecaploid wiht 2 n = 84, while one *(G. Quellyon)* was decaploid with 2 n = 70.

The numerous hybrids, which I obtained from crossing different hexaploid species, had usually in most PMC's 21 bivalents, while in the remaining PMC's the number of univalents varied from 2 to 10, and the number of bivalents was proportionately reduced. These hybrids showed a varied feritility which ranged from nearly normal, to almost complete sterility. The hybrids of hexaploid with dodecaploid species had in meiosis 21 bivalents and 21 univalents. This indicates that the dodecaploid species arose from crossing hexaploid species and the doubling of chromosome number in the hybrids.

The normal conjugation of chromosomes between the hexaploid species of the *Eugeum* subgenus shows that the main factors differentiating the species of this subgenus are genic mutations, and perhaps also, small structural changes in chromosomes, which do not cause deep changes in the general chromosome structure of these species. The genetic analysis of fertile hybrids (W. G a j e w s k i 1950) shows them to differ considerably in the number of genes, some of which give fairly simple Mendelian segregations in the second generation.

Owing to the rather slight disturbances in meiosis the separation of chromosomes is more or less normal, and there are usually 21 chromosomes in the gametes. It does not follow that all these gametes have functional abilities, and the feritlity of hybrids depends mainly on which of the species have been crossed.

There remains the problem of how do the dodecaploid species arise (undoubtedly the youngest philogenetic ones) if the now existent hexaploid species (and these can be assumed to be the ancestors) had homologous chromosome groups. It is a well known fact that in natural conditions the amphidiploids originate usually from species possessing chromosomes which either are not or are only in a small degree, homologous. The amphidiploid has a normal chromosome conjugation, and its fertility is greater, whereas in meiosis of F_1 hybrids univalents prevail. Moreover, due to the presence of univalents in meiosis of F_1 hybrids, restitution nuclei and consequently gametes with unreduced chromosome numbers are formed, which is the main source of amphidiploids in nature.

The solution of the problem was obtained from the analysis of *G. rivale* and *G. macrophyllum* hybrids. The hybrids obtained from both these hexaploid species are nearly completely sterile, and their meiosis develops quite differently than in the case of other *Eugeum* hexaploid hybrids (W. G a j e w s k i 1949). In metaphase of the first PMC division no, or few, bivalents can be seen (1—7). As a result of disturbances during the seperation of chromosome restitution neuclei are formed, and from these pollen grains with unreduced chromosome numbers form after the second division. This course of events is indicated both by cytological investigations and the appearance of amphiploid plants in F_2.

The reason for the lack of conjugation between *G. macrophyllum* and *G. rivale* chromosomes must here be elucidated. The simplest hypothesis, according to which the lack of conjugation was caused by the lack of homology between the chromosomes of these two species, proved to be wrong. By crossing both *G. rivale* and *G. macrophyllum* with *G. aleppicum* or *G. canadense,* hybrids are obtained, and in these a normal conjugation with 21 bivalents can often be seen in meiosis. It follows that the structure of the chromosomes of these two species must be similar, and the chromosome are homologous. There must be, therefore, some other factor for the lack of chromosome cojugation than the structural differences. In one or both parent species there must be some genic factors (physiological) which, though they do not influence the conjugation within the species, cause the lack of it in metaphase of the hybrid of these species. Probably, it is not a question of an absolute lack of conjugation, but rather of premature desynapsis. As I did not investigate the stages of prophase prior to diakinensis this problem could not be solved here.

Observations, made during the last few years, of the hybrid *G. aleppicum* × *G. urbanum* have shown that in different flower buds of the same plant an almost normal chromosome conjugation and complete asyndesis could be found. In some buds the disturbances are so early that meiosis does not take place, and the whole archeosporium degenerates. Undoubtedly the normal development of chromosome conjugation is not exclusively dependent on the chromosomes being homologous, which condition is necessary but unsufficient. The general physiological state of cells has also an important influence on the normal course of division. There are numerous factors — e. g. numerous genes, the threshold of susceptibility to outward conditions — on which the physiological state of cells depends.

Undoubtedly in a hybrid of two species, in which in the protoplasm of the mother organism two different genomes are joined, the mutaul action of genic factors and of the protoplasm may be discordant, as a result the threshold of susceptibility to outward conditions is lowered, and the conjugation of even homologous chromosomes ceases.

This problem is well known and has often been discussed by numerous writers. However, there is relatively little data on the chromosome conjugation in poliploids resulting from doubling of chromosome number in those asyndetic plants in which asyndesis is not due to the lack of structural homology of chromosomes, but is caused by geno-physiological factors. It is difficult to foresee whether factors, which cause asyndesis before the chromosome number is doubled, will act also after the doubling of the chromosome number. In the case of the hybrid under consideration it appeared that they did not.

II. Description of parent species and hybrids

I obtained the first hybrid from *G. rivale* with *G. macrophyllum* in 1938. During the 5 years from 1946 to 1950 this hybrid was obtained several times by crossing different varieties of *G. rivale* and *G. macrophyllum*. The two species can easily be crossed reciprocally and the seed setting is from 47 to 72%. When properly cultivated, numerous hybrids, which develop beautifully are obtained. The F_1 generation is, as a rule, fairly uniform. Reciprocal hybrids when compared displayed no marked differences in morphology and fertility.

The *Geum rivale* used for crosses was obtained from a natural habitat near Warsaw. This is a very typical plant with much anthocyanin. Alsa *var. pallidum*, a variety lacking completely in anthocyanin, was used. This variety was obtained from the Botanical Garden in Copenhagen.

Geum macrophyllum was grown from seeds received from the Kew Botanical Garden. In other crosses the *pernicisum* R y d b. variety (in fact a separate, though, very closely related species), grown from seeds gathered in Yellow Knife near the Great Slave Lake in Canada, was used.

As further hybrid generations were obtained only from crosses of the typical form of *G. rivale* with *G. macrophyllum* from Kew, I shall describe here only these parents and the hybrid obtained by

crossing them. The hybrids from the other varieties displayed the same fertility and cytological conditions, and differed only in morphological details depending on the variety used for crosses. Below is a description of the main characters distinguishing the parent species, and their appearance in the hybrid.

Growth and shape of plants: *G. rivale* has a long overground rhizomatous stalk (a kind of caudex) on the top of which a rosette of caudical leaves is formed annually. The height of stalks reaches 50 cms, and they usually have 3—5 flowers. *G. macrophyllum* has no typical caudex, and has only a thick root base, from which adventitious roots grow and on which, during several years, basal leaf rosettes form in spring. The floral stalks are not numerous, and they reach 90 cms in height. The stalks are topped with an inflorescence composed of numerous (8—15) flowers.

The F_1 plant is similar to *G. rivale* in that it has typically shaped caudex. The numerous ramose flower stalks have 9—29 flowers, and often are higher than both parent species.

Pubescence: The stalks and leaves of *G. rivale* are covered with short, soft, straight hairs, while the upper part of the stalk and the flower peduncles are covered with numerous glandular hairs. In *G. macrophyllum* the leaves and the whole stalk are covered with hard, protrusive, bristly hairs (cell walls of which are very thick), between which there are shorter, straight, soft ones. In the F_1 hybrid the pubescence resembles that of *G. macrophyllum*, there are no glandular hairs, and the bristly hairs are fewer and softer.

Shape of caudical and stalk leaves: All the species from the *Eugeum* subgenus have pinnately divided leaves with a big terminal leaflet. The terminal leaftet in *G. rivale* is rhomboid, with a pointed base, whereas lateral leaflets are, relatively to it, fairly big and dentate. *G. macrophyllum* has a big round or reniform terminal leaflet. The leaflet has 3—5 shallow lobes and cordate base, the lateral leaflets are few and small. The leaflet margins are dentate and crenate. The first spring leaves of the F_1 hybrid resemble in shape those of *G. macrophyllum* whereas the later ones have the terminal leaflet divided into 3 rhomboid leaflets. The later leaves are intermediate in shape between the parent species. However, as all the Geum species are highly hterophyllous, and as successively developping caudical leaves sphape differently, it is very difficult to compare the shapes of leaves accurately. This phe-

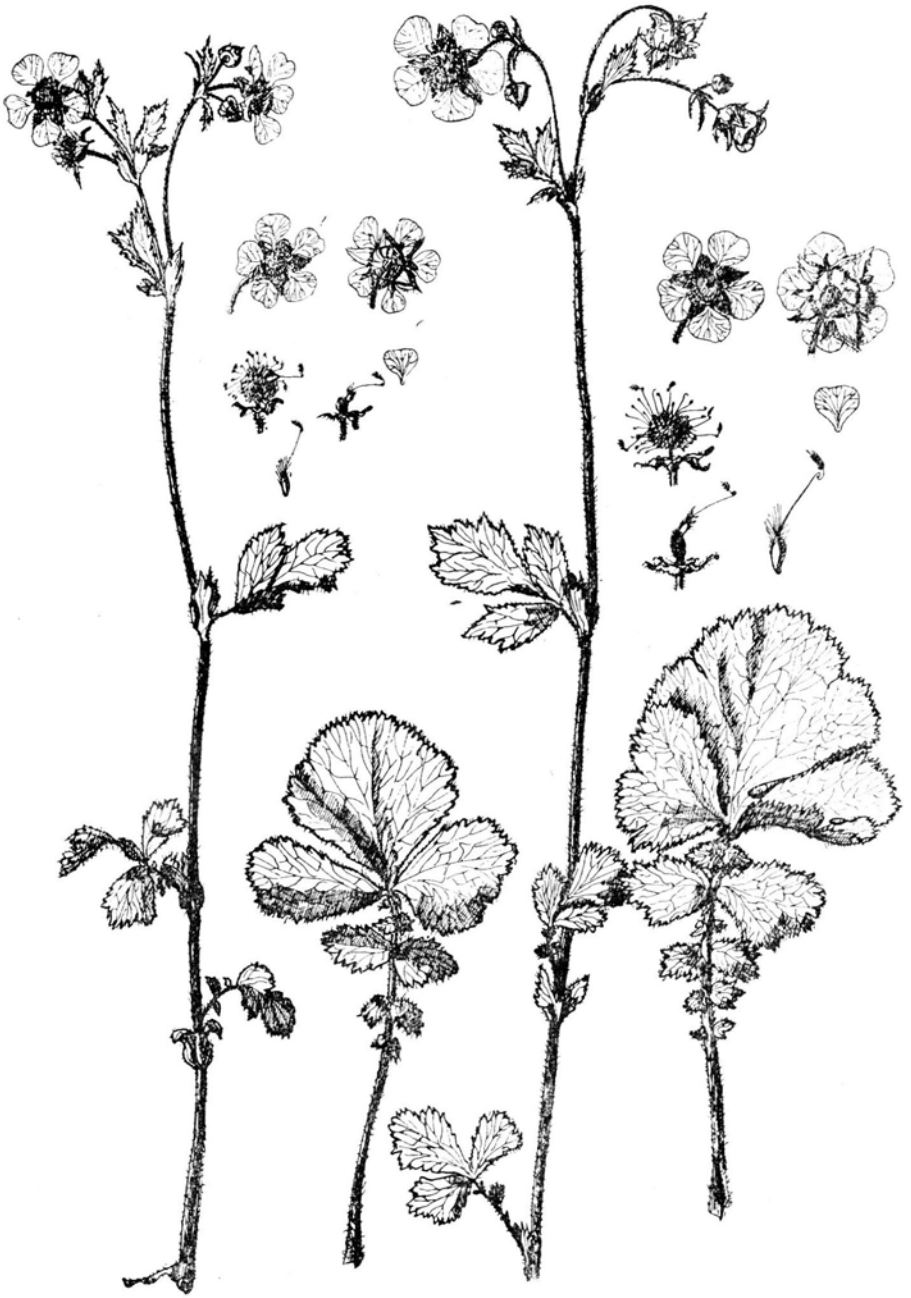

Fig. 1. The F_1 hybrid *G. rivale* × *macrophyllum* (left) and its amphiploid derivative 370-1 from F_2 generation (right) drawn to the same scale.

nomenon is still more pronounced in stalk leaves, where leaves situated on two contiguous internodes are never of the same shape. Leaves of the hybrid are approximately intermediate in dimensions between the parent species and their shape is illustrated in Fig. 1.

Anthocyanin: In *G. rivale* in the upper part of the stalk and in the calyx there is much anthocyanin. This causes the stalk and the calyx to be of a brownish red colour. In *G. macrophyllum* there is no trace of anthocyanin. In the hybrid anthocyanin is dominant though there is less of this pigment here than in *G. rivale*.

Drooping of flower peduncles: The flower peduncles of *G. rivale* droop strongly during flowering, and do not straighten up until the seeds begin to ripen. In *G. macrophyllum* the peduncles are straight and stiff from the very beginning of flowering. The drooping peduncle character dominates partly in the hybrid, in which flowers hang down somewhat less and the peduncles straighten up sooner than in *G. rivale*.

Arrangement of calyx and corolla: In *G. rivale* both calyx and corolla face upwards during flowering, and flowers are bell shaped. In *G. macrophyllum* the calyx is reflexed downwards, sepals adhere to the peduncle, and the petals spread ronud horizontally. The F_1 hybrid has usually a widely spreading calyx with horizontal or sometimes partly reflexed sepals, while the petals stand up only a little, and so the flower is opened out widely. In the hybrid the arrangement of sepals and petals is intermediate between the parent species.

Shape and pigment of petals and sepals: Sepals of calyx and epicalyx in *G. rivale* are coloured red with anthocyanin. Their lengths are approximately 15 mm and 5—6 mm respectively. *G. macrophyllum* calyx and epicalyx are green, with no anthocyanin, their lenghts are 5—6 mm and 1—2 mm respectively. The hybrid has a calyx tinted red with anthocyanin, though less intensively than the parent, and the lengths of its calyx and epicalyx are 7—8 mm and 2—3 mm respectively. In *G. rivale* the petal is emarginate at the top. Its broad upper part narrows abruptly into a long, narrow claw. In *G. macrophyllum* the petals are ovoid and pointed, narrowing gradually at the base, without claw or notch at the apex. The F_1 hybrid has emarginate petals similar to those in the *rivale* parent, with traces of a small claw at the base (Fig. 1). The petals were of the following dimensions (mm):

G. rivale	length	10,5 ± 0,07		width	8,7 ± 0,07	
G. macrophyllum	„	7,8 ± 0,03		„	6,9 ± 0,03	
F_1	„	8,5 ± 0,04		„	8,6 ± 0,04	

It appears from the above that petals and sepals of a calyx and epicalyx are intermediate in size between the parent species. The petals of *G. rivale* are cream coloured, very often however, this is supressed by the red anthocyanin which forms a red net along the veins, or even covers the whole surface of petals. The *macrophyllum* petals are yeollow. The petals of the hybrid are yellow, but similarly to *G. rivale* this may be partly supressed by the red anthocyanin. In the hybrid of *G. rivale var. pallidum* is no anthocyanin, and the yellow colour of the petals is clearly visible, and is not discoloured.

Gynophore and receptacle (recaptaculum): In *G. rivale* the pistils are placed on a 4—5 mm long receptacle, which is covered with fairly long hairs, and which has 110—130 spirally arranged carpels. While the seeds ripen an 8—10 mm gynophore grows out between the base of the calyx and the base of the receptacle, this causes the ripe achenes to be wholly above the elevated sepals of the calyx. In *G. macrophyllum* the ripe achenes are placed on a cylindracal or flattened receptacle which is 10—12 mm long. The receptacle is covered with scarce, short hairs, and there on it 200—240 carpels. There is no gynophore. In the hybrid the receptacle is 4—5 mm long. It is covered with long hairs, and there are 150—170 carpels on it. The gynophore is short and its length is 1—2 mm, 1,47 ± 0,5 mm on the average.

Shape of pistils and achenes: *G. rivale* belongs to the *Gmeliniana* section, and has stalks with few and usually big flowers and a long filiform stigmatal part of the pistil which is usually longer than a half of the rostrum. *G. macrophyllum* belongs to the *Murrayana* section, and has stalks with numerous small flowers of which the stigmatal part of the pistil is short, comalike and not longer than 1/3 of the rostrum length. In *G. rivale* the achene, the rostrum and the stigmatal part of the pistil are longer and also more thickly covered with longer hairs than in *G. macrophyllum*. The lengths of achenes, the rostrums and stigmatal parts of the parent species and the hybrid are the following:

G. rivale achene 5,8 mm, rostrum 8,5 mm, stigmatal part 5,0 mm,

G. macrophyllum achene 2,8 mm, rostrum 4,0 mm, stigmatal part 1,5 mm,

F_1 achene 4,2 mm, rostrum 7,7 mm, stigmatal part 3,3 mm.

It appears from the above that in the hybrid the achene and stigmatal part are intermediate in length between the parent species, while the rostrum resembles more that in the *G. rivale* parent. In *G. rivale* the achene and 3/4 of the rostrum are covered with long, straight hairs and with glandular ones. In *G. macrophyllum* straight hairs can be found only on the achene, whereas on the rostrum there are scarce glandular ones. The pubescence of achenes and pistil in the hybrid is similar to that of the *macrophyllum* parent.

F l o w e r i n g t i m e: *G. rivale* begins to flower 10—12 days before *G. macrophyllum* and the hybrid begins flowering carlier than both parent species. E. g. in 1952 *G. rivale, G. macrophyllum* and their hybrid began to flower on May 1-st, May 12-th and April 28-th respectively.

F e r t i l i t y: The fertility of pollen and seeds in both parent species was high and ranged from 88 to 96% of good pollen grains, and nearly 100% of well grown achenes. The F_1 hybrids shows a very high degree of sterility. The fertility of the pollen in different preparations ranged from 0,2 to 0,8%, on the average 0,5%.

The aspect of a pollen shows a considerable diversity (the pollen was examined in a 1 : 1 mixture of acetocarmine and glycerin), and among the bad grains which do not stain with acetocarmine some very small ones, the diameter of which was 5—7 μ, were found, whereas the average diameter of bad grains was 20 μ. Good grains which stain with acetocarmine are filled with protoplasm, have 2 nuclei and their diameter is approximately 32 μ; Often however, big grains with a diameter up to 40 μ are found. The fertility of hybrid seeds is even lower. Usually, most flowers of a hybrid never form good achenes, and overblown flowers quickly wither. Single (rarely 2 or 3) achenes develop in 1 out of 10 or 12 flowers, which makes approximately 0,06% of all pistils. Seeds from this hybrid were obtained only in the course of free polination. Neither artificial selfpolination, nor polination with pollen of a parent species have produced results so far.

III. F_2 and other filial generations

The second generation was grown from seeds of F_1 parents obtained in the course of free and uncontrolled polination. In 1946, 47 seeds were gathered and on September 28-th they were sown out. Of these seeds, 5 came up in Autumn of the same year, and 12 in

Spring of 1947. From the seedlings, in 1948, 10 flowering plants were obtained. The general appearance of the plants was not uniform. As the polination of F_1 hybrid was free, and as it grew near many *Geum* species and hybrids, most of these plants were probably obtained from polination of the hybrid by the pollen of the other *Geum* plants. This is indicated by both, morphological features, and their almost complete sterility. However, three plants indicated by numbers 370-1, 370-2, and 167-3 were particularly remarkable, because morphologically they greatly resembled the F_1 plant. Especially, in plants 370-1 and 167-3 the different parts, such as leaves, sepals, and petals, were enlarged, and both plants were remarkable by the intense dark green. A description of these plants will be mainly a repetition of the description of the F_1 plants, and so, I shall not give it here. Some idea of their appearance can be obtained from Fig. 1 which depicts F_1 plant and No 370-1 of F_2. To demonstrate the enlarged dimensions of organs of F_2 plants, measurements of length and width of 50 petals of F_1 and F_2 plants are given:

Length of petals

mm	5	6	7	8	9	10	11	12	M	m
F_1			4	11	17	13	5		8.58	0.04
F_2 (370-1)					5	16	21	8	10.14	0.01

Width of petals

mm	5	6	7	8	9	10	11	12	13	M	m
F_1			4	10	14	18	4			8.66	0.04
F_2 (370-1)					1	5	18	22	4	10.94	0.01

Nos 167-3 and 370-1 resemble each other greatly, wheras No. 370-2 differs little from F_1 plants, though its petals and leaves are somewhat bigger. In spite of their different organs being enlarged the F_2 plants are lower than the F_1 ones, and their average height is 40—58 cms.

A very remarkable problem is that of fertility of these plants. In number 370-1 the percentage of good pollen was 59,3. In each flower out of the 140—160 pistils 30—40 good achenes developped, which made approximately 23,3%. As in the F_1 plant one achene developped from about 10 flowers, which was appr. 0,06%, the fertility of F_2 in relation to F_1 increased approx. 400 times. The fertility of No 167-3 was also high and was 39,2% and 21% of pollen and seeds respectively (in two flowers out of 328 pistils 69 good achenes developed).

The third plant, No 370-2, was completely sterile both in pollen and in seeds. The remaining 7 plants of the F_2 generation were

not examined cytologically. They were highly sterile and probably developed from polination with alien pollen. Cytological examination of the first three plants — which will be described in detail in part IV of this paper shows that Nos 370-1 and 167-3 are dodecaploid. They form, in metaphase of the first PMC division, 42 bivalents and a varying from 2 to 12 — number of univalents. No 370-2 is 9-ploid, and in metaphase is has 21 bi- and 21 univalents. It is, therefore, very probable that the first two plants originate from the union of two unreduced gametes of the hybrid, whereas the third plant originates from the union of one reduced and one unreduced gamete.

No 370-1 was isolated from the other *Geum* plants and in 1950 from the gathered seeds a F_3 generation of 25 individuals was obtained (No 3701). Morphologically the F_2 generation resembled greatly the parent plant and its population was fairly uniform. This generation shows however a slight segregation of such characters as pubescence, flowering time, size of petals and, especially, the length of gynophore which is absent in 7 and 1—4 mm long. in the remaining 18 plants. Measurements of petals (mean values of 50 measurements) of each of the plants were:

No	Length	Width	No	Length	Width	No	Length	Width
1	11,5	11,8	10	10,1	10,8	19	12,7	12,5
2	12,2	12,5	11	12,1	12,5	20	10,5	10,6
3	10,3	10,7	12	10,5	10,2	21	12,7	12,6
4	12,2	12,3	13	10,4	11,2	22	10,2	10,5
5	11,8	11,7	14	11,1	11,3	23	10,3	10,4
6	11,2	11,6	15	11,6	12,1	24	10,7	10,7
7	10,5	10,7	16	10,0	10,7	25	12,1	12,5
8	10,7	9,9	17	11,9	11,8			
9	12,1	12,2	18	10,7	10,8			

As it apperas from the table the lengths of petals vary from 10,0 to 12,7 mm and their widths from 9,9 to 12,5 mm and plainly surpass the limit of variability of the F_2 plant, which shows that in F_3 the factors determining the dimension of petals segregate. Similar results were obtained in measurements of calyx and epicalyx lengths. In spite of differences in their size there are no fundamental differences in the shape of petals of all F_3 plants.

However, the F_3 plants have shown the greatest differences in fertility. In this generation there are plants which, in compa-

rison to F_2 No 370-1, have both a lower and an almost normal fertility. The fertility of individual plants of F_3 is as follows:

No	Pollen fert.	Seed fert.	No	Pollen fert.	Seed fert.
1	12,0	14,2	14	14,4	17,2
2	24,1	18,7	15	0,7	0,0
3	84,2	28,7	16	24,2	18,8
4	41,3	29,4	17	64,4	35,2
5	47,6	32,6	18	85,7	52,3
6	20,5	8,4	19	19,3	14,7
7	61,3	37,4	20	81,2	49,3
8	47,1	33,0	21	62,0	17,2
9	49,2	21,2	22	75,1	44,4
10	65,3	25,2	23	84,4	29,4
11	17,1	7,7	24	78,8	19,3
12	14,2	8,4	25	17,2	14,3
13	52,3	23,2			

If it is remembered that in No 370-1 pollen and seed fertility is 59,3% and 23,3% respectively, it must be ascertained that approximately 20% of F_2 plants have a higher fertility than the mother plant. On the other hand, besides much more fertile plants, there was one which was almost completely sterile.

Owing to a lack of time and space, the F_4 generation was grown from one of the F_3 plants, i. e. the most fertile No 18. The obtained F_4 generation flowered in 1952 and was composed of 37 individuals. Morphologically this generation was more uniform than F_3 and reproduced fairly exactly the type of the F_2 No 370-1. Its fertility is much higher than that of F_3 generation, and ranges from 60,2% to 87,2% and from 34,2% to 62,1% for pollen and seeds respectively. In 1952 the *Geum* cultures were viciously attacked by parasite fungi which lessened their vigour and possibly their fertility. It may be that, if the conditions were normal, the fertility of at least some of the plants would be higher.

Besides F_3 raised from No 370-1, I had, in 1950, a small F_3 family of 14 individuals raised from No 167-3. These plants were fairly uniform and resembled the mother plant, but their fertility was rather low, i. e. 35,2—62,4% and 13,3—37,2% for pollen and seeds respectively. Farther generations from these plants were not grown.

On the whole, it can be said that the progeny of amphidiploid plants obtained in F_2 bred true, giving a comparatively slight se-

gregation of morphological characters and a fairly considerable segregation in the degree of fertility. In F_3 and F_4 generations plants with fertility much higher than that of the mother plant have appeared, which makes it plausible to presume that an adequate selection may greatly increase the fertility of these amphidiploids.

IV. Cytological examination

Cytological examinations were done mainly on fixed material. Root tips were fixed in Navashin and flower buds were first placed for 4—5 mins. in Carnoy, and then fixed in Navashin. After immersion in parafin and slicing with microtome the preparates were stained with cristal violet according to Newton's method. In some cases meiosis in PMC's was examined in pressed out preparates stained with acetoorcein or acetocarmine.

In both *G. rivale* and *G. macrophyllum* there are 42 chromosomes in the somatic plate. Similarly to the whole genus the chromosomes here are small and hardly differ in size. In both species the course of meiosis in PMC's is absolutely normal. In metaphase of the first division 21 bivalents are formed, and both divisions develop without disturbances. The course of meiosis and the metaphase plate of *G. rivale* are already described (W. G a j e w s k i 1951, 1952), and so in the present paper only several drawings illustrating the course of meiosis in *G. macrophyllum* are given (Figs. 2a-c).

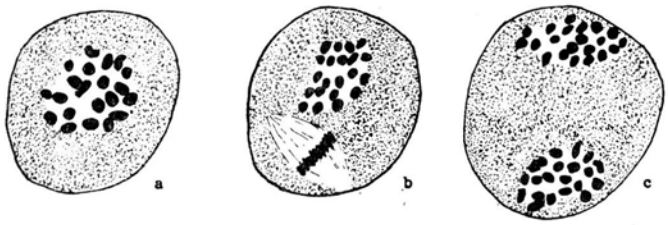

Fig. 2. Meiosis in P.M.C. of *G. macrophyllum*. a-metaphase I, b-c- metaphase II.

The course of meiosis in the F_1 *G. macrophyllum* × *rivale* hybrid was also previously described (W. G a j e w s k i 1949). The results were then given of an analysis of 31 PMC's in the M I stage during which the following arrangements of chromosomes were found:

$0_{II} + 42_I$ in 3 *PMC's*	$4_{II} + 34_I$ in 6 *PMC's*
$1_{II} + 40_I$ in 5 *PMC's*	$5_{II} + 32_I$ in 2 *PMC's*
$2_{II} + 38_I$ in 4 *PMC's*	$6_{II} + 30_I$ in 5 *PMC's*
$3_{II} + 36_I$ in 5 *PMC's*	$7_{II} + 28_I$ in 1 *PMC*

In that paper a detailed description of the course of meiosis in PMC is given (l. c., page 231 and Figs. 38—40 and 57—65). To enable a comparison of the course of meiosis in the F_1 hybrid with that of its amphidiploid progeny several drawings of the course of meiosis in PMC are given here (Figs. 3a-h). During diakinesis (Fig. 3a) four unmistakable bivalents are visible, whereas the rest are univalents. M I as seen from above, with 4 or 5 bivalents and remaining univalents visible, is ilustrated by Fig. 3b. In this drawing only those chromosomes which lie more or less in one plane, or just near it, are drawn in, the remaining ones dispersed in the spindle on either side of the plane, are not. As it can be seen from Fig. 3c and 3d, the chromosomes in M I do not form a regular plate, and only bivalents and some of the univalents lie on the equatorial plane of the spindle. The other univalents are dispersed irregularly throughout the spindle. Often, as a result of disturbances during the anaphase division, chromosomes do not separate to the two daughter nuclei, and then restitution nuclei are formed (Fig. 3 f). Then the restitution nuclei undergo a normal division and dyads are formed (Fig. 3h). The percentage of dyads found among tetrads varied

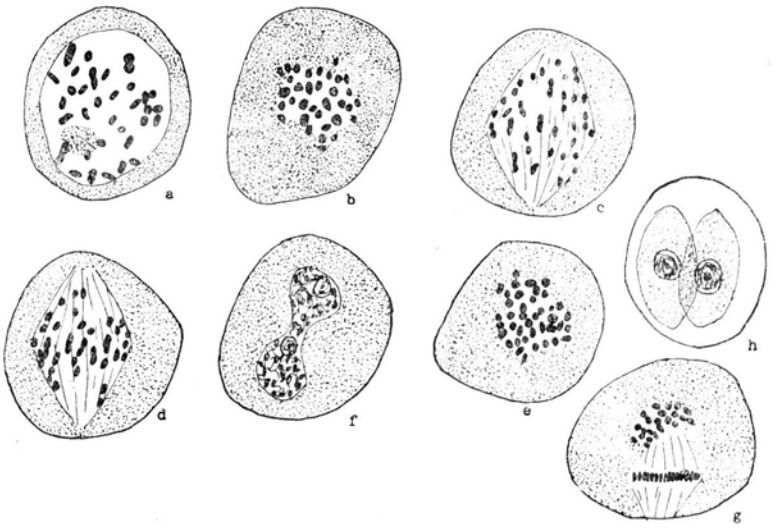

Fig. 3. Meiosis in P.M.C. of F_1 hybrid *G. rivale x macrophyllum*. a-diakinesis with 4 bivalents, b-metaphase I from polar view with ab. 4 to 5 bivalents, only chromosomes lying on the plate are drawn, c-d- metaphase I from side view with plenty of univalents scattered on the spindle, e-metaphase I with 40 chromosomal bodies, f-restitution nucleus, g-metaphase II with 22 chromosomes in one of the plates, h-diad.

in the different preparates from 0,2 to 1,5. In cells in which restitution nuclei do not form, univalents are separated during late anaphase into two groups. Often however, several chromosomes are not included in the daughter nuclei, and lag in the cytoplasm. Daughter nuclei undergo then a homeotypic division, and often in metaphase of the second division the presence of 21 or nearly 21 chromosomes on the plate can be observed (Fig. 3g). This indicates that univalents can be divided almost equally between the two daughter nuclei. As a result of the second division normally looking tetrads composed of 4 microspores are formed. However, most microspores degenerate and only those which have an unreduced, or rarely haploid, number of chromosomes develop into pollen grains. I have found a very similar course of meiosis in the hybrid of *G. macrophyllum* with *G. rivale v. pallidum* and also in the hybrids of *G. macrophyllum v. perincisum* Rydb. with *G. rivale*. The same type of meiosis is found not only in the hybrids *G. macrophyllum* with *G. rivale*, but also in the hybrids of the first species with such species as *G. urbanum, molle, hispidum, silvaticum* and *laciniatum*. Only in hybrids of *G. macrophyllum* with *G. aleppicum* or *G. canadense* the course of meiosis is either normal with 21 bivalents, or with not more than 1—3 chromosome pairs appearing as univalents. The course of meiosis of such hybrids as *G. macrophyllum* × *G. aleppicum* is illustrated in Figs. 4a-f, and of hybrids

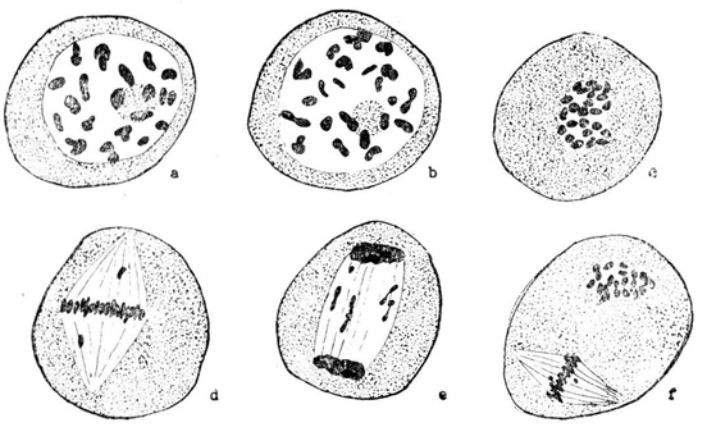

Fig. 4. Meiosis in P.M.C. of F_1 hybrid *G. macrophyllum x aleppicum*. a-diakinesis with 21 bivalents, b-diakinesis with 19 bi-and 4 uni-valents, d-c-metaphase I with 24 chromosomal bodies, d-metaphase I with 2 univalents, e-anaphase I with univalents lagging on the spindle, f-metaphase II with 21 chromosomes in one of the plates.

G. aleppicum × *rivale* in Figs. 5a-f. It can be seen that both these species give with *G. aleppicum* hybrids in which the course of meiosis is normal and chromosome conjugation complete in at least a part of PMC's. It follows that, if chromosomes of *G. macrophyllum* and *G. rivale* were capable of normal conjugation with chromosomes of a third species, they must be homologous. Thus, the complete or nearly complete asyndesis in metaphase of *G. macrophyllum* × *G. rivale* hybrid does not result from a lack of homology between the chromosomes, but probably is caused by a too early desynapsis

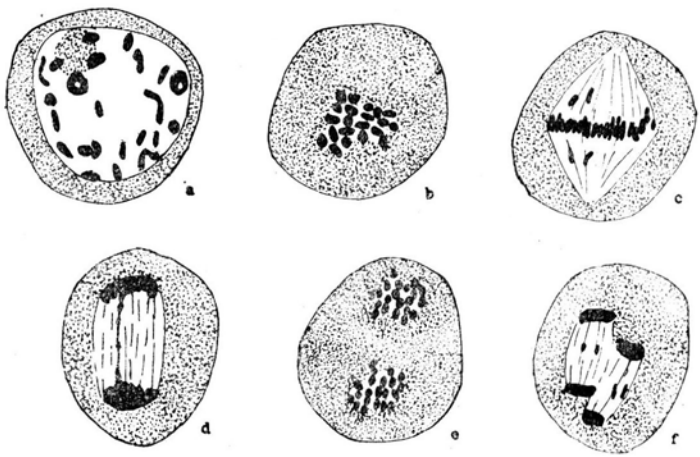

Fig. 5. Meiosis in P.M.C. of F_1 hybrid *G. aleppicum x rivale*. a-diakinesis with 19 bivalents and 4 univalents, b-metaphase I with 21 bivalents, c-metaphase I with 4 univalents on the spindle, d-anaphase I with a bridge, e-metaphase II with 21 chromosomes in each of the two plates, f-anaphase II with lagging chromosomes.

taking place in prophase. However, the lack of adequate preparates from early prophase made the observations of the beginning of desynapsis impossible.

Somatic plates of F_2 plants were not examined. However, the degree of the polyploidy could be judged of with great accuracy from the course of meiotic division in PMC's. In No 370-1 plant, in late diakinesis the number of chromosomes in the nucleus is remarkably great. Both the chromosomes and the nucleus are bigger than in F_1 plants. The great number of chromosomes which overlay each other and the frequent formation of chromosome groups by chromosomes lying very near or even closely touching each other made the counting of them extremely difficult. The chromosomes were counted fairly exactly only in 3 PMC's, and their number was

found to be approx. 42,44 and 39 in the respective cells (Fig. 6a). It is very difficult to distinguish at this stage uni- from bivalents. It is not till in the first division metaphase that the analysis of numerous plates shows them to be bivalents (Figs. 6b and 6c) and not

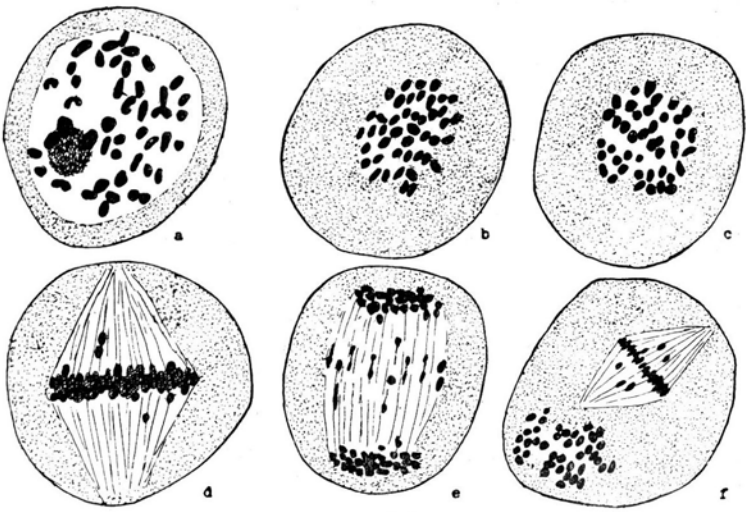

Fig. 6. Meiosis in P.M.C. of the F_2 hybrid *G. rivale x macrophyllum* nr 370-1. a-diakinesis with 45 chromosomal bodies, b-metaphase I with 44 chromosomes, c-metaphase I with 42 chromosomes, d-metaphase I from side view with many bivalents and ab. 12 univalents, e-anaphase I with univalets lagging on the spindle, f-metaphase II with ab. 39 chromosomes in one of the plates.

univalents, as is the case in the F_1 hybrid. In some PMC's all chromosomes are tightly packed in a plate and in a longitudinal view there is no doubt that only bivalents are present. In other quite numerous cells there are, besides the bivalents 2—14 univalents which usually are dispered throughout the spindle and on the plate (Fig. 6d). Altogether 39 metaphase PMC's were analysed, and the following chromosome arrangements were found:

$42_{II} + 0_I$ in 5 *PMC's*	$38_{II} + 8_I$ in 3 *PMC's*
$41_{II} + 2_I$ in 7 *PMC's*	$37_{II} + 10_I$ in 4 *PMC's*
$40_{II} + 4_I$ in 14 *PMC's*	$36_{II} + 12_I$ in 1 *PMC*
$39_{II} + 6_I$ in 4 *PMC's*	$35_{II} + 14_I$ in 1 *PMC*

The course of anaphase differs greatly in different cells, and depends on the number of univalents. When there are none, or only 2—4, anaphase develops quite normally, and in its later stages no chromosomes can be seen on the spindle between anaphase groups. However, if in metaphase the univalents were numerous, then in late

anaphase after the seperation of bivalents some (sometimes even a dozen or more) univalents lag in the spindle, and often arrange themselves in a regular ring on the equatorial plane of the spindle (Fig. 6e). The univalents lying on the equatorial plane of the spindle either divide, and their halves move to the opposite poles, or stretch out along spindle axis, and then each one moves to one of the poles as an elongated hank. This kind of behaviour of univalents may be found also in other Geum hybrids (W. G a j e w s k i 1949). In some PMC's as the result of retarded division and lagging in the movement toward the poles, some univalents are not included in the daughter nuclei. However, in most PMC's there are only two nuclei in telophase and interkinesis, and neither small nuclei nor chromosomes lag in cytoplasm.

The course of the second division is usually fairly normal, though sometimes, in metaphase, some chromosomes lie off the equatorial plane, and in anaphase some of them lag on the spindle. In the M II plate the chromosome number is not always 42, but may vary from 39 to 45 (Fig. 6f). The tetrads look quite normal. Dyads are never observed. However it seems that, due to the considerable number of univalents and their unequal separation, not all microspores are capable to develop, and many degenerate, thus the percentage of well grown pollen grains is only 59,3

The course of meiosis in No 167-3 plant is essentially the same as in No 370-1, though in the first plant the number of univalents is far greater. Not one cell without univalents was found. In 11 PMC's observed in M I stage the arrangements of chromosoms were as follows:

$41_{II} + 2_I$ in 1 *PMC*	$37_{II} + 10_I$ in 2 *PMC's*
$40_{II} + 4_I$ in 1 *PMC*	$35_{II} + 14_I$ in 4 *PMC's*
$38_{II} + 8_I$ in 1 *PMP*	$34_{II} + 16_I$ in 2 *PMC's*

In the late anaphase, after the separation of daughter bivalents, the numerous univalents form on the plane a fairly regular plate. This can be observed in numerous PMC's. The univalents behave in a way similar to that described in the case of the previous plant. In most PMC's, in spite of much lagging, all univalents separate, and are included in the daughter nuclei. In two PMC's, during metaphase of the second division, exactly 42 chromosomes were found. It is probable that the lower pollen fertility of this plant is caused by the presence of a great number of univalents during the first division.

In the sterile plant No 370-2 the course of meiosis is quite different. In numerous metaphases, both in polar and longitudinal views, it can be seen that the total number of chromosomes is also 42 (Fig. 7a), but in each cell there are approximately 21 bi- and 21 univalents. In the longitudinal view a compact plate of bivalents and 21-27 univalents are visible. The univalents are dispersed at the sides of a plate and on the spindle (Figs. 7b and 7c). In anaphase,

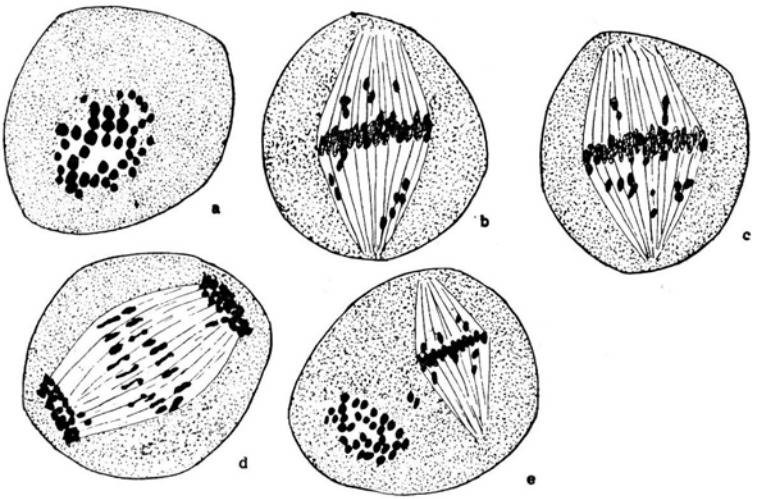

Fig. 7. Meiosis in P.M.C. of the F_2 hybrid *G. rivale* x *macrophyllum* nr 370-2. a-metaphase I with ab. 40 chromosomes, b-metaphase I with 21 univalents, c-metaphase I with 23 univalents, a-anaphase I with a ring of univalents on the equator of the spindle, e-metaphase II.

after the separation of daughter bivalents, the univalents form a ringlike plate on the outer layers of the spindle (Fig. 7d). Usually the number of univalents is less than 21 and varies between 13 and 19. This indicates that some of them separate together with the bivalents to the daughter nuclei. In late anaphase the usually undivided univalents separate to the two poles, though sometimes they divide, and single chromatides move to the poles. In metaphase of the second division the number of chromosome varies. (Fig. 7e). The following numbers have been observed: 32 twice and 31, 28, 35 and 36 once each. Disturbances in the course of the second division are also fairly numerous e. g. chromosomes lie outside the two M II plates, often several chromosomes lie lagging on the spindle. In spite of this tetrads look normal and have four microspores, and dyads have not been found. Nevertheless, nearly all microspores de-

generate, and the number of good pollen grains which stain in carmine is extremely small.

It thus appears that plant No 370-2 is a 9-ploid. It probably arises either from the union of two, one reduced nad one unreduced hybrid gametes of the F_1 hybrid, or perphas from polination of an unreduced egg-cell of the F_1 hybrid with pollen containing 21 chromosomes of some other *Geum* species or hybrid. However, the great morphological resemblance of No 370-2 to F_1 hybrid makes the first assumption more plausible. This may indicate that in F_1 reduced gametes possessing function abilities can be formed.

Ufortunately, the number of plants from the F_3 generations examined cytologically was small. The main reason for this was that in 1950 and 1951 most fixation proved to be defective, and were too weak for exact cytological analysis. In 1952 meiosis was examined in the most fertile No 18 plant from the F_3 generation (No 3701), on smear preparations. It was found that the course of meiosis is on the whole the same as in No 370-1. However, the course of meiosis is far more normal, and PMC's without or with only few univalents are more numerous than in the mother plant. In 22 PMC's in the M I stage the following chromosome arrangements were found:

$42_{II} + 0_I$ in 7 *PMC's* $39_{II} + 6_I$ in 3 *PMC's*
$41_{II} + 2_I$ in 5 *PMC's* $38_{II} + 8_I$ in 1 *PMC*
$40_{II} + 4_I$ in 6 *PMC's*

Furthemore, in 1952 the somatic number in root tips of 5 F_4 generation plants was examined. It was found that in all the 5 plants this number was ab. 84 (Fig. 8a), but owing to the considerable amount of chromosomes and their partial adhesion to each other, the counting was done which an exactitude of $\pm$ 2 chromosomes. Meiosis was examined in smear preparates stained with acetoorcein. It was found that in many plants, the course of meiosis was rather regular, and that in many cells there were no, or only a few, univalents (Figs. 8b and 8c).

It can be said that, on the whole, in F_3 and F_4 generation plants meiosis develops more regularly, and the number of univalents is smaller than in plant No 370-1. As a result of the presence of univalents in meiosis and disturbances in their separation, not only amphidiploid, but also aneuploids may appear. However, this was not established with certainty, as in the diploid chromosome number of 84 it was difficult to count the chromosomes and to discern the

lacking, or additional, one or two. Nevertheless, it is possible that the sterile, or partly sterile, plants from the progeny in the further generations of the amphipolyploid, may be such aneuploids.

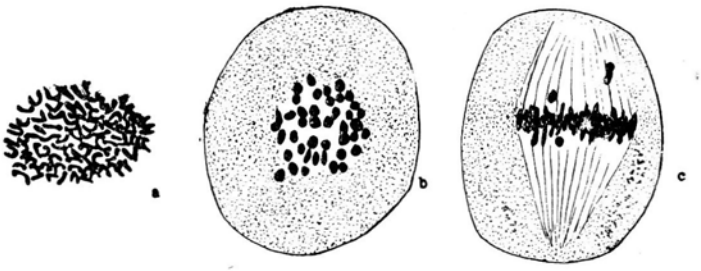

Fig. 8. F_4 *G. rivale x macrophyllum.* a-somatic plate with 84 chromosomes, b-metaphase I with 42 bivalents, c-metaphase I from side view with 4 univalents.

V. Discussion

The present state of observations of the *G. macrophylum* × *G. rivale* amphidiploid indicates that, although its fertility is far from being normal, the plants appearing in successive generations are more and more fertile, and their meioses are more and more regular in comparison to the original amphidiploid. It seems therefore probable that, if this amphidiploid appeared in nature it would be capable of surviving and developping into a new species. The strong selection, which acts in nature of the most fertile individuals would further such a course of events. The two parent species appear together on large territories in USA and Canada, and so the possibility of a cross between them is great. In fact, a hybrid of these two species from the territories of their common habitat has been described, and named *Geum pulchrum* Fernald. It was described by Fernald (1906) as a new species, but Rydberg in his North American Flora recognized it to be a hybrid of *G. macrophyllum x rivale*. The hybrid has been found in the State of Vermont in USA and in the Quibeck and Alberta provinces in Canada. A cytological examination of these natural hybrids is very necessary, and this need should be met by the American botanists.

The now existent 12-ploid species from the *Eugeum* subgenus, such as *Geum pyrenaicum* and *G. Fauriei,* show a close relation and similarity to some 6-ploid species appearing in their neighbourhood. E. g. the 12-ploid *Geum pyrenaicum* from the Pyrenees has probably one chromosome set from the 6-ploid *G. silvaticum* from the

West Mediterranian area and one from an other species related to *G. urbanum*. The hybrids of these two species closely resemble *G. pyrenaicum*, but, so far, I have not succeeded in obtaining a doubling of their chromosome numbers. Also the 12-ploid *G. Fauriei* growing in Japan and in Sacchalin has many features of two hexaploid species *G. japonicum* (a japano-chinese species) and *G. macrophyllum* growing in the Far Fast in Kamchatka, Sachalin, and east Siberia. The dodecaploid species are undoubtedly the youngest in the evolution of the *Eugeum* subgenus, and are younger than the hexaploid ones. The probability, that the parent species of dodecaploids still exist, is great. Detailed studies and attempts of doubling the chromosome number in the hybrids may lead to an experimental raising of these species. Although the amphidiploid hybrid described here has not been found in nature, the understanding of the mechanism of its origin is of importance in studies on amphidiploidy in nature. All hexaploid species from the *Eugeum* subgenus studied so far have homologous chromosome sets. E. g. *G. rivale* has been crossed with 9 other hexaploid species and with all has shown a potentially complete chromosome conjugation in F_1 hybrids. This also is the case with other hexaploid *Eugeum* species with the exception of *G. macrophyllum* (and the closely related to it *G. perincisum* and *G. oregonense*). *G. macrophyllum* in crosses with 7 species gives hybrids, the meiosis of which is asyndetic and such as described in this paper, and with two species only (*G. canadense* and *G. aleppicum*) the hybrids have a potentially complete chromosome conjugation. Cytoplasm seems to have no influence on asynapsis, as no differences in the course of meiosis in reciprocal hybrids are observed.

All this indicates that in *G. macrophyllum* genome there must be some factor, or set of factors, which causes the union of this genome with the genome of some other species to result in a partial, or even complete, deficiency of bivalents in the first division metaphase of the hybrids. A deficiency of bivalents in metaphase does not result from a lack of structural homology of chromosomes, but is caused by factors of a genico-physiological nature. Nothing is known how these factors act. Probably the conjugating chromosomes separate too early, a phenomenon known in the literature of the subject as desynapsis. I found in the hybrid of *G. aleppicum* x *urbanum* in different buds of the same plant, both a normal conjugation and complete asyndesis exactly similar to that described previously. Undoubtedly the continuity of chromosome conjugation, from prophase tili the end of first division metaphase in meiosis, is decisevely

influenced by the psysiological state of cells. This state depends on a harmonious concurrence of genic factors introduced by the two genomes, cytoplasm of the mother organism and all the environmental conditions.

In the *G. macrophyllum* genome there must be some factors which in crosses with most *Geum* species cause, in the hybrid genomes, an abnormal course of prophase and desynapsis. At the same time, these factors cause an almost complete sexual isolation of the *macrophyllum* species from other related hexaploid species. It is interesting that all hybrids, of which one of the parents is *G. macrophyllum,* are almost entirely sterile, even though some of them show a normal chromosome conjugation. Some of these hybrids have a lowered vitality, and disturbances in development as well as flower deformations occur in them. It is impossible to decide at present, whether all these effects are brought about by the same factors or by different ones.

For the present considerations it is of importance that, as a result of asyndesis, the highly sterile hybrids produce functionally capable, unreduced gametes. Owing to this, the hybrids can give a fertile amphidiploid progeny.

From crosses between a number of hexaploid species I obtained numerous fertile hybrids with a normal chromosome conjugation. All these hybrids, in F_2 and further generations, give plants with character combinations different from those of the parent species. The characters show different, more or less complex segregational ratios. If crosses took place in nature, either small hybrid populations form, or a mutual introgression of the two species takes place. The probability of a new amphidiploid arising from these hybrids is extremely slight, because: 1) the hybrid gametes have a reduced chromosome number, and so the doubling can only be somatic, 2) as the chromosomes are entirely homologous the possibility of the amphidiploids being highly fertile is small.

Undoubtedly, the appearance in one of the species of factors, which in hybrids of this species with other ones cause asyndesis, may greatly quicken the evolution of new polyploid types. In two species developped owing to — for instance — geographical isolation and independent, in the two isolated populations, genic and chromosome mutations, the disappearance of homology between sets of chromosomes is usually very slow. On the other hand, it is known that the development of asyndesis may be dependent on one gene and may be caused by one mutation. E. g.: two species from the

Eugeum subgenus, the North American *Geum canadense* and *Geum coccineum* growing in the Balkans and Asia Minor, are isolated from each other since at least the middle of the Tertiary. In spite of this, their chromosomes are homologous and hybrids from crosses between them are fertile. In the literature of the subject there are many examples of chromosome homology and even high hybrid fertility being retained in spite of very long isolation. E. g. the North American *Platanus occidentalis* and the *Pl. orientalis* from the Mediterranean Region give a fertile hybrid *(Pl. acerifolia),* which has a normal meiosis. (S a x 1933). Probably, both these specific populations are isolated from each other since the middle of the Tertiary, i. e., for approx. 30 million years. As we see, in some cases sexual isolation may develop extremely slowly, though undoubtedly, in other cases this may happen much more quickly. Thus, the appearance of genic differences which cause asynapsis, may be the factor bringing about a much earlier sexual isolation between species with homologus chromosome sets. It would be extremely interesting to investigate the nature of factors, on which a normal or abnormal course of meiosis depends. We know that there are numerous genes, which influence the development of chiasmata, the formation of spindles etc. A statement may be risked that each successive stage in division has a specified gene, which regulates it. So far mainly, those genes are known, the reccessive allelomorphs of which in the homozygous state bring about, within the different plant and animal species, disturbances in some definite stages of meiosis. The first such gene has been found in maize (B e a d l e 1930, 1933) and named „asynaptic". It prevents the development of chiasmata between conjugating chromosomes in pachytene. As a result, univalents alone, or almost alone, appear in metaphase. Also other such recessive genes have been found in maize. These do not influnce the conjugating chromosomes, but prevent the formation of the spindle (B e a d l e 1932). Similar genes were also described in numerous other organisms, e. g. *Datura* (B e r g n e r. C a r t l e d g e, B l a k e s l e e 1934), *Crepis* (H o l l i n g s h e a d 1930), *Drosophila melanogaster* (G o w e n 1931) and many others.

The number of known asynapses with a genico-physiological nature appearing in interspecific hybrids is far smaller. The main reason for this is that usually the study of factors that bring about asynapsis is difficult, because the sterility of hybrids makes a genetic analysis impossible. D o b z h a n s k y (1941) thinks that the

behaviour of chromosomes after the doubling of their number is the main criterion for distinguishing whether, the appearance of univalents in an interspecific hybrid is caused by genic factors, or by the lack of structural homology in chromosomes of the two species. He writes (l. c. page 327): „Where chromosome pairing in a diploid hybrid is supressed by the genetic constitution rather than by dissimilarities in the gene arrangements, the same supression should be encountered in the allotetraploid derived from it". To support this statement he describes hybrids of A with B races of *Drosophila pseudoobscura*. In this hybrid when, in the diploid spermatocytes, there are ony bivalents, then in tetraploid spermatocytes there are bi-, tetra- and also tri- and univalents. However, if in hybrids of other strains there are only univalents in the diploid spermatocytes then also in tetraploid spermatocytes the conjugation is unchanged.

In the case of the hybrid here described the situation is different. The nature of asynapsis is not structural. This is indicated by the normal conjugation between chromosomes of each of the parent species with those of other species, such as *G. aleppicum* and *G. canadense*. Nevertheless, after the doubling of chromosome number the amphiploid hybrid shows a normal or nearly normal conjugation. Thus, the doubling of chromosome number supresses almost entirely asyndesis, when it is of genic nature. This reveals itself by a simultaneous and great increase of fertility of amphidiploids in relation to F_1 hybrids.

The disaccord of my results and the results of D o b z h a n s k y very extensive studies on *Drosophila* may be due to the different material used for experimenting. In the first place, undoubtedly the term „genic sterility", as distinguished from „chromosome sterility", introduced by D o b z h a n s k y does not comprise a uniform phenomenon. The term „genic sterility" is synthetical and denotes a number of phenomena, the physiological causes of which are often different. The lack of chromosome conjugation in meiosis of hybrids, may have different causes such as:

1) Chromosomes from the A species do not conjugate with chromosomes from B species, because there is no harmony in the cooperation of the hybrid nucleus with the cytoplasm from the mother species.
2) Chromosomes from the A species do not conjugate with chromosomes from the B species, because, as a result of the bringing together of two physiologically unadjusted genomes there is no chromosome conjugation, and the premature break up in

two chromatids prevents the formation of chiasmata (Darlington's reduced precocity).

3) The threshold of susceptibility to internal and external conditions of hybrids of A with B species is so changed that in conditions, in which meiosis of the parent species is normal, meiosis of the hybrids is subject to many abnormalities. In this kind of hybrids one may expect to find that meiosis, is influenced by internal and external conditions, — such as e. g. age, sex, position of flowers on shoot, temperature etc. — and there may be both normal and asyndetic meioses.

4) Finally, the cause of asyndesis in the hybrid may be the existence of factors in the genome of one species, or in the genomes of both species, which in a specific way prevent the conjugation between the chromosomes from the A and B species.

In my opinion the results of doubling of the chromosome number in asyndetic hybrids may differ greatly and depend on the nature of factors causing asyndesis. In the case of the first two above mentioned causes of asyndesis it is plausible to suppose that even after the doubling of chromosome number asyndesis will not cease. This happens in Dobzhansky's *Drosophila* hybrids. In the third case no such predictions can be made, though undoubtedly, the polyploid organism will have a different threshold of susceptibility than the diploid organism. In the fourth case it seems that if, in an amphidiploid, homologous chromosomes from one species can conjugate together, then factors preventing the conjugation of chromosomes from two separate species will not act, and the conjugation in the amphidiploid will be normal.

Federley's (1913, 1931) studies on interspecific hybrids of moths from the *Pygaera* genus are in disagreement with Dobzhansky's thesis. The *Pygaera curtula* (n = 24) x *P. nigra* (n = 23) hybrid shows a normal chromosome conjugation in females and complete asyndesis in males. The univalents divide twice, and diploid spermatids are formed. Federley obtained triploid hybrids by back-crossing. These hybrids have two genomes from one and one from the other species. In these triploid hybrids, both in males and females, chromosome conjugation between homologous sets is normal, and the third chromosome set remains composed of univalents. In this case the lack of chromosome conjugation in diploid males is not caused by structural reasons, because the chromosomes from both species conjugate regularly in females. On the

other hand, the conjugations in triploid hybrids shows that factors, which prevent it between chromosomes from separate species, have no influence on the conjugation of chromosomes from one species.

According to investigations made by D o b z h a n s k y and his school, sterility and disturbances in the course of meiosis in spermatogenesis of hybrids of A with B *Drosophila pseudoobscura* races, depend on a number of genes situated in different chromosomes. Within each race there are „week" and „strong" strains, which differ by the degree of disturbances in meiosis in hybrids between them. Rciprocal hybrids differ in the size of testes and the degree of disturbances in meiosis, which proves the influence of plasmatic factors. That is why the doubling of chromosome number may change nothing of importance in the conditions preventing chromosome conjugation.

It is possible that the frequent disturbances of chromosome conjugation in hybrids are the result of a disaccord in the development (in duration) of various physico-chemical and structural changes, which take place in the cytoplasm, the nucleus and the chromosomes of hybrid cells. Within each species all these processes are synchronized, and on this the normal course of meiosis depends. On the contrary, in interspecific hybrids the processes are not synchronised. If there is a disaccord in timing between, for instance, the changes taking place in the nucleus and those in cytoplasm of a diploid hybrid, then the doubling of a chromosome number may have no effect on the course of meiosis in the polyploid. If, however, the lack of conjugation is due to, for instance, a disaccord in timing of changes taking place in prophase between two chromosome sets joined in a hybrid nucleus, then after the doubling of the chromosome sunmber conjugation between the same chromosome sets may be normal, because their developmental processes will be synchronised.

All this is of course a very general and speculative explanation, why the doubling of chromosome number in hybrids may have such different results, when the lack of conjugation is due to genico-physiological reasons. There is no doubt however that the highly complex cycle of biochemical and structural changes, which take place during meiosis, may be disturbed in interspecific hybrids in various ways, and at its different stages. The doubling of chromosome number changes essentially the cell physiology, and this may have various effects, which depend on the nature of disturbances and the stage of meiosis, in which the disturbances in the normal

development occur. In the present stage of knowledge it is impossible to foresee the effects produced by the doubling of the chromosome number, and in each case this must be investigated experimentally.

Summary

1) A hybrid of *G. rivale* (2 n = 42) x *G. macrophyllum* (2 n = 42) was described. In metaphase of the 1-st meiotic division in PMC's of the hybrid only univalents, or few (1—7) bivalents, formed. Disturbances in meiosis caused the formation of resitution nuclei and consequently of unreduced gamets. The fertility of the hybrid was 0.5% and 0.06% for pollen and seeds respectively.
2) In F_2 two amphidiploid, with 2 n = 84, and one 9-ploid, with 2 n = 63, plants were obtained. In the amphidiploids the petals, sepals, and other organs were of greater size than in the F_1 mother plants, though otherwise they resembled them.
3) In F_2 amphidiploids the fertility was much higher than in F_1 and in the case of 1 plant (No. 370—1) it was 59,3% and 23,3% for pollen and seed respectively. The 9-ploid was completely sterile. In the amphidiploids numerous PMC's with 42 bivalents in metaphase of the 1-st meiotic division were found. In other PMC's, besides the bevalents, 2—14 univalents appeared. In the 9-ploid the most common arrangement in M I was $21_{II} + 21_{I}$.
4) The F_3 and F_4 generations obtained from the F_2 plant No. 370—1 showed a slight segregation of some morphological features and a considerable segregation in fertility, which ranged in different plants from almost complete sterility to fertility much higher than in F_2. Cytological examination of fertile F_3 and F_4 plants showed a more normal meiosis and less univalents than in F_2.
5) As hybrids of both *G. rivale* and *G. macrophyllum* with other *Geum species (G. aleppicum* and *G. canadense)* had a normal meiosis with 21 bivalents, chromosomes of *G. rivale* and *G. macrophyllum* must be structurally homologous. The lack of chromosome conjugation was of a genico-physiological and not structural nature. The author's supposition was that it was not a case of a lack of chromosome conjugation but of a premature desynapsis.
6) The doubling of chromosome number in hybrids, in which the lack of chromosome conjugation was due to genic factors,

might cause a normal congujation in the amphidiploids. This was in desagreement with D o b z h a n s k y's observations on *Drosophila*.

7) The author discussed the cause of the different effects, which the doubling of chromosome number in asyndetic hybrids might have on chromosome conjugation in the resultant polyploid hybrids, and which differed in different investigated organisms.

REFERENCES

1. B e a d l e G. W. 1930. Genetical and cytological studies of Mendelian asynapsis in *Zea mays*. Cornell. Univ. Agr. Exp. Sta., 129: 1—23.
2. B e a d l e G. W. 1932. Genes in maize for pollen sterility. Genetics, 17: 413—431.
3. B e a d l e G. W. 1933. Further studies of asynaptic maize. Cytologia, 3: 269—287.
4. B e r g n e r A. D., J. L. C a r t l e d g e and A. F. B l a k e s l e e 1934. Chromosome behaviour due to a gene which prevents metaphase pairing in *Datura*. Cytologia, 6: 19—37.
5. B o l l e F. 1933. Eine Uebersicht über die Gattung *Geum* L. und die ihr nahestehenden Gattungen. Repert. spec. nov. regni veget. Beihft. 72: 1—119.
6. D o b z h a n s k y T. 1941. Genetics and the origin of species. Ilnd ed. Columbia Univ. Press. New-York.
7. F e d e r l e y H. 1913. Das Verhalten der Chromosomen bei Spermatogenese der Schmetterlinge *Pygaera anachoreta, curtula* und *pigra* sowie einiger ihrer Bastarde. Zeitschr. f. ind. Abst. — u. Vererb-Lehre., 9: 1—110.
8. F e d e r l e y H. 1931. Chromosomenanalyse der reziproken Bastarde zwischen *Pygaera pigra* und *curtula* sowie ihrer Rückkreuzungsbastarde. Zeitschr. f. Zellforsch. u. mikr. Anat., 12: 772—816.
9. F e r n a l d M. L. 1906. Rhodora, 8: 11 (cyt. wg. F. B o l l e 1933).
10. G a j e w s k i W. 1948. On the chromosome pairing in six hybrids among four *Geum* species. Acta Soc. Bot. Pol., 19: 245—249.
11. G a j e w s k i W. 1949 On the behaviour of univalents at meiosis in some interspecific *Geum* hybrids. Hereditas, *35:* 221-241.
12. G a j e w s k i 1950. The inheritance of specific traits in the hybrid *Geum coccineum* x *rivale*. Acta Soc. Bot. Pol., 20. 456—476.
13. G a j e w s k i W. 1952. The hybrids between two subgenera of *Geum* L. Acta Soc. Bot. Pol., 21: 489—516.
14. G o w e n J. W. 1931. Genetic non-disjunctional forms in *Drosophila*. Amer. Nat., 65: 193—213.
15. H o l l i n g s h e a d L. 1930. Cytological investigations of hybrids and hybrid derivatives of *Crepis capillaris* and *Crepis tectorum*. Univ. California Publ. Agr. Sci., 6: 55—94.
16. S a x K. 1933. Species hybrids in *Platanus* and *Campsis*. Journ. Arnold Arboretum, 14: 274—278.