The hybrids between two subgenera of Geum L.

W. GAJEWSKI
(Botanic Garden, Warsaw)

entered 10. X. 51.

Introduction

During my investigations of interspecific *Geum* hybrids I have been especially interested ,for many years, in reciprocal relationship between the species of the subgenus *Oreogeum* (Ser.) F. Bolle and the species of the subgenus *Eugeum* Focke. These two subgenera differ in many essential traits, both morphologically and geographically. Therfore, these two subgenera are considered by some taxonomists as seperate genera and species from subgenus *Oreogeum* are distinguished as genus *Parageum* or *Sieversia*, the species from the subgenus *Eugeum* are included to the genus *Geum* L. only.

To the subgenus *Oreogeum* belong species distributed in mountains and with the achenes tipped with a long, pennately haired style forming a typical anemochoric organ, like in *Pulsatilla* or *Clematis* (Fig. 1). This type of achenes is to be found in many genera of the *Rosaceae* family like *Dryas, Cercocarpus, Chamaebatia, Cowania, Purshia, Fallugia* and in very near related to *Geum* genera *Sieversia* Willd, *Neosieversia* F. Bolle and *Erythrocoma* E. L. Greene.

To the subgenus *Eugeum* belong species with chiefly lowland distribution and with the achenes tipped with a style which is divided in two parts by a semi-circular loop. The lower part of the style called rostrum rests on the achenes forming a hook-like apparatus by means of which the fruits are distributed in an epizoochoric way. The upper part of the style (called stigmatal part) falls when the achenes are ripe (Fig. 1). This kind of fruit is not to be found in any other genus of the *Rosaceae* family.

It seems very probable that the type of long straight styles represents a type phylogenetically older and that the epizoochoric type of styles is a secondary one developed much later in the subfamily *Dryadoidae* as special eolutionary line. Many facts from geographical distribution, morphology and cytology point to this conclusion. The species from the subgenus *Eugeum* show many peculiarities both in morphology and distribution, which indicate that they are less primitive and phylogenetically more advanced than the species from the subgenus *Oreogeum*. The more primitive species of the subgenus *Oreogeum*, have comparatively small and often disrupted areals in European mountains, the shoots are usually with one or more flowers and with few and small bracts-like leaves. The calyx is with a distinct basal tube and with one or two conpicuous circles of bractlets. They styles are long and straight as has been already mentioned above. The species of the subgenus *Eugeum* show the following traits which can be regarded as advanced: hugh, compact areas on the plains, often on the youngest geological terrains, shoots big, branched with many flowers and well developped leaves, the calyx often cut down to the base and with recurved sepals, the bractlets sometimes very reduced and unique in the whole family mode of style structure.

To the subgenus *Oreogeum* belong only species growing in the European mountains. These are:

1. *Geum montanum* L. This species has the widest areal from the Spanish mountains in the west, through Plateau Central, Corse, Alpes, Carpathians to the Balcan mountains in the east. Its vertical distribution is also the largest, ranging from upper limit of forest to about 2300 m, it occurs there in various different plant associations. This species is tetraploid with 2n = 28 (the basic number for the whole genus *Geum* is 7 as in many other genera in the *Rosaceae* family).

2. *Geum reptans* L. is known only in the Alps, Tatra, Transsilvanian Alps and from some mountains of the Balcan Penninsula. Its vertical distribution is also restricted. It is known only in alpine zone (ab. 1700 to 2700) where it grows chiefly among stones and in rock fissures. This species is hexaploid with 2n = 42.

3. *Geum bulgaricum* Panč. This species is known only in a few places in high mountains of the Balcan Peninsula. It is a very high polyploid with 2n = ± 70 (the precise number is not yet ascertained).

F. Bolle (1933) counts to this subgenus also a fourth species *Geum andicola* Reiche growing in Chilean Andes. Having not studied this species and considering it having a different distribution I leave open the question of its close relationship to other European species of this group.

To the subgenus *Eugeum* belong about 30 species occuring chiefly in temperate zones of the north hemisphere but some species grow in South America from Argentine and Chile to Brasil and one species is known from South Africa. From these species I have cultivated and studied cytologically 18 species from which 13 are hexaploids with 2n = 42 *(G. rivale, urbanum, coccineum, molle, hispidum, aleppicum, silvaticum, canedense, macrophyllum, oregonense, laciniatum, perincisum* and *virginianum),* one species *G. Quellyon* is decaploid with 2n = 70 and 4 species *(G. pyrenaicum, magellanicum, Fauriei* and *riojense)* are dodecaploid with 2n = 84. So we see the lowest degree or ploidity known in this subgenus is hexaploid.

The only second tetraploid species of *Geum* is a relic mediterranean species with disjunctive areal, namely *G. heterocarpum* Boiss. This species, with yet another, very curious structure of the styles, belongs to subgenus *Orthostylus.* This subgenus besides *G. heterocarpum* comprises only one species very nearly related to it. Diploid species are till now not known in the genus *Geum.* Diploid number of the chromosomes was found by me only in one species of the genus *Waldsteinia* (*Waldsteinia geoides,* unpubl.). The genus *Waldsteinia* belongs, according to Bolle (1933), to the same subfamily *Dryadoideae* to which *Geum* belongs, but is very distinct from it. Its morphology and distribution indicate a rather ancient origin of arctoteriary type.

Reasuming, it must be stated that the lowest chromosome numbers diploid and tetraploid are not known in *Eugeum* but only in other subgenera or related geenra which bear morphological traits and show geographical distribution indicating more ancient origin.

As notwithstanding their different structures the species from both subgeenra can be crossed rather easily and some hybrids are even partialy fertile, I have crossed many of them. The analysis of genetical and cytological relations between these different groups can throw, I hope, some new light on the question of the evolution of the epizoochoric species of *Geum* from more primitive ones. Some of these crosses are described below.

I. Material and methods.

The living plant material was gathered from seeds or plants collected where possible from natural stations. The culture of the species from the sugenus *Eugeum* is rather easy but the mountain species are very difficult to cultivate under the climatic conditions of Warsaw. The easiest to cultivate is *G. montanum* which I have brought from the Tatra mountains as living plants. I have also grown it from seeds obtained from the Tatra and from the Botanical Gardens in Edinburgh and Sofia. Plant from different sources differ only in minor traits chiefly in the shape of leaves. For the croses I used only specimens from the Tatra mountains.

G. reptans is very difficult to cultivate. I have tried many times to cultivae it from seeds, runners or taking whole plants from the Tatra but they always die sooner or later. Only once I had flowering specimens in my cultures. To some crosses I have used pollen from flowers collected in Tatra and brought to Warsaw. Still more difficult to cultivate in Warsaw is *G. bulgaricum*. I obtained seeds from the Rila mountains in Bulgaria from prof. Stojanoff three years ago. They have germinated very well but from a great many of seedlings I have after three years only 4 plants and none have flowered. The hybrids of *G. montanum* as one of the parents with any species of the subgenus *Eugeum* grow very abundantely, but even hybrids with *G. reptans* as one of the parents are more easy to cultivate than the pure species. *Geum rivale* used in these crosses was from Piastów near Warsaw. Other species from the subgenus *Eugenum* which were crossed with *G. montanum* and *G. reptans* will not be described as they are hardly mentioned in this paper.

The selfing of hybrids was done in isolation cages in which many F_1 plants were grown. The bagging of single shoots in celophane or pergamine paper gave very unsatisfactory results due to pronounced selfsterility.

Root tips were fixed in Karpetchenko, floral buds were prefixed in Carnoy for 2—5 minutes and then fixed in Karpetchenko. They were embedded in paraffin and cut 15—20 μ thick. Permanent preparations were stained with cristal-violet according to Newton's method.

II. Hybrids between *G. montanum* and *G. rivale*.

From numerous hybrids obtained between *G. montanum* and different, species of the subgenus *Eugeum* I will describe here in detail only hybrids with *G. rivale.* This hybrid was obtained already in the year 1948 and has been most thoroughly studied. Since the war I have crossed both parental species in both directions and in total I have had in culture ab. 250 F_1 plants from different crosses. The parental species can be crossed very easily and seed setting in both directions is very high, ranging from 65 to 87% (in average 81,5%). The seeds germinate quickly, the mortality of young seedlings is moderate and they grow to robust F_1 plants with luxuriant growth and flowering.

This hybrid has been found many times in natural places among parental species on the Alps, Plateau Central, Carpathians Mountains and on the Balcan Peninsula. It was described there under the names *G. inclinatum* Schl., *G. sudeticum* Tausch., *G. tirolense* Kern. and others. I have seen typical F_1 plants and segregants probably from back-crosses in the Tatra mountains in the Rozpadlina and the Kościeliska valleys.

The parental species differ more or less in all vegetative and floral parts. Some characters of parental species and F_1 hybrids are tabulated below:

Character	G. rivale	F_1	G. montanum
Height of the shoots (cm)	40—60	30—60	10—20
Beginning of the flowering	30.IV	24.IV	22. IV
Anthocyanin in stems and peduncles	abundant	rather abundant	traces
Anthocyanin in sepals and petals	abundant	abundant	lacking
Colour of the petals	cream	yellow	yellow
Dimensions of petals			
Length x breadth (mm)	10,1 x 8,0	14,2 x 12,5	16,6 x 15,7
Presence of the claw on the base of the petals	long	short	absent
Position of sepals and petals	erect	intermediate	horizontal
Nodding of the peduncles	pronounced	intermediate	absent
Length of gynophore (mm)	10	2—4	absent (1 mm)
Structure of the styles	hook-like	intermediate	straight, plumose
Pollen and seed fertility	normal	ab. 15—20%	normal

Fig. 1. Table showing the inheritance of the structure of the styles in the hybrid *G. montanum* × *rivale*. First row: parental species *G. rivale* (left) and *G. montanum* (right). Second row: three flowers from the F_1 hybrid with different structures of the styles, type III left, type II centre and type I right. Third row: 5 plants from the second generation with different kinds of the styles. Fourth row: two heads on the left are from the back-cross *G. rivale* × F_1 *montanum* × *rivale* and the two heads to the right are from the back-cross *G. montanum* × F_1 *montanum* × *rivale*.

Besides the traits listed above the parental species differ in size and shape of leaves, mode of branching of shoots, type and degree of hairness and in other traits. More accurately I will discuss only the structure of the styles and the fertility.

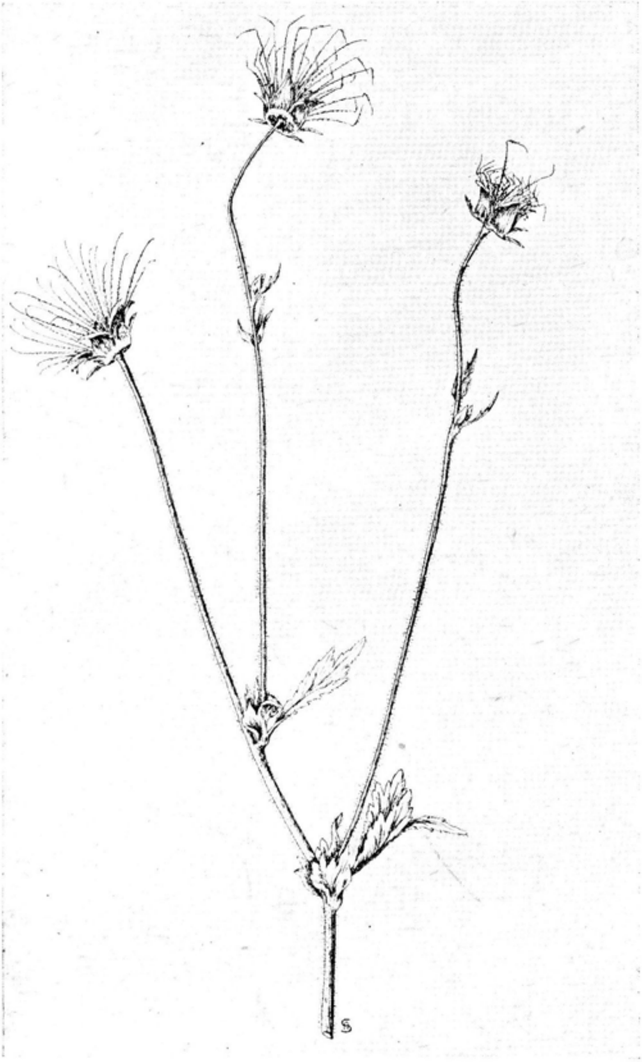


Fig. 2. A floral shoot from F_1 hybrid *G. montanum* × *rivale* with the first flower with straight styles. The styles in the second and third flowers are distinctly bent.

The average dimensions of the achenes and styles in parental species are as follows:

G. rivale achenes 3,5 mm, rostrum 9,1 mm, stigmatal part 5,5 mm.

G. montanum achenes 3,8 mm, pennate style from 29 to 33 mm (av. 29,1 mm) long.

The structure and the dimensions of the styles in F_1 hybrids show great variation. According to their structure they can be divided into three types:

Type I — the styles are straight, undivided more or less pennately haired. The stigmatal part persists on the styles.

Type II — the stigmatal part of the styles is separated from the lower one by a distinct and sometimes semi-circular curvature. During the ripening of the achenes the stigmatal part falls off but the rostrum is not tipped with a hook.

Type III — Betwen the stigmatal part and the rostrum a typical hooked curvature is formed. After the falling of the stigmatal part the rostrum is tipped with a more or less distinct hook.

The different types of styles structure of this hybrid are illustrated in figs. 1 and 2.

But what is most interesting in this hybrid is that these types of styles can be found in one the same flower. Different proportions

TABLE I

The distribution of different types of styles in 10 F_1 plants fruiting in the year 1950.

No. of plant	No. of shoot	No. of flower	Percentage of types of styles			No. of plant	No. of shoot	No. of flower	Percentage of types of styles			No. of plant	No. of shoot	No. of flower	Percentage of types of styles		
			I	II	III				I	II	III				I	II	III
1	1	1	56,5	39,8	3,6	2	1	1	4,9	14,7	80,3	4	1	1	18,9	14,8	66,2
		2	68,6	22,3	8,9			2	0,0	12,1	87,9			2	5,8	13,7	80,3
		3	23,5	31,7	44,8			3	0,0	3,3	96,7			3	—	—	—
	2	1	12,3	28,5	59,2	3	1	1	1,9	18,4	79,6	5	1	1	7,8	14,1	78,2
		2	6,5	14,2	79,2			2	0,0	1,1	98,9			2	2,6	6,6	90,7
		3	—	—	—			3	0,0	0,0	100			3	—	—	—
6	1	1	26,6	29,7	43,6	8	1	1	70,3	29,6	0,0	10	1	1	5,4	8,7	85,8
		2	1,7	8,7	89,4			2	46,4	49,4	4,1		1	2	2,3	5,9	91,6
		3	—	—	—			3	—	—	—			3	0,0	6,3	93,7
7	1	1	44,9	48,3	6,7	9	1	1	94,2	5,7	0,0		2	1	—	—	—
		2	1,0	8,6	90,3			2	80,9	19,0	0,0			2	0,0	7,0	93,0
		3	0,0	0,0	100			3	89,3	10,6	0,0			3	0,0	0,0	100

of the three types of styles are formed in successively developing flowers of one and the same plant. This curious phenomenon can be seen on table I.

In this table the shoots and the flowers on them are enumerated according to the time of developing. We see that diffirent plants show great individual differences in the amount of different types of the styles. It is a general tendency to develop more of the III-rd type of the styles on the shoots and flowers which develop later. At the end of the vegetation seanson the general appearence of fruits heads is more uniform than at the beginning. This phenomenon could be observed year after year on the same plants.

The styles of all three types are haired through their whole length. Their length varies according to the type: it is the longest in type I, the shortest in type III. The means of 20 measurements in each type are given below. Only styles from well developed achenes were measured. The styles on the poor achenes are much shorter, as their growth stops shortly. The differences concern chiefly the length of the rostrum, the length of the stigmatal part is in all types nearly the same. The length of the styles (rostrum + stigmatal part)

are:		
	G. montanum	29,1 mm
	G. rivale	14,5
	F_1 Type I	18,5
	F_1 Type II	16,6
	F_1 Type III	16,0

Together with the formation of the rivale-like structure of the styles they also shorten and have a more stiff consistence.

Besides with *G. rivale* I have also crossed *G. montanum* with many other species from the subgenus *Eugeum*. In hybrids of *G. montanum* with *G. coccineum, G. urbanum, G. aleppicum* and *G. macrophyllum* I have observed the same types of styles as in the hybrid with *G. rivale*. In hybrids of *G. montanum* with *G. molle, G. hispidum, G. canadense* and *G. silvaticum* most of the styles were straight of type I and few only of type II. All species mentioned above are hexaploids. If however *G. montanum* is crossed with dodecaploid species of the subgenus *Eugeum* like *G. pyrenaicum* or *G. magellanicum* all styles are uniform of the hooked type.

In F_2 66 plants were analysed as to their structure of the styles, and 56 had styles of more or less rivale-like type, 4 had straight pennate styles like montanum parent and the remaining 6 had mixed styles of different structure like in F_1 plants. In the back-cross to

G. rivale 43 plants had styles of *rivale* type and only 3 were of F_1 type with different kinds of styles.

The back-cross to *montanum* consisted unfortunately only of 8 plants all with straight styles related to *montanum* type. As the plants of F_2 and the back-crosses have different numbers of the chromosomes and the numbers are ascertained only in some of them, I leave a detailed discussion of them for the future when the whole cytological analysis will be completed.

Any differences were stated between the reciprocal F_1 hybrids. In the year 1947 I sowed separately seeds of type I and type III which were taken from one F_1 plant, unfortunately not from selfed flowers. In two small F_2 families obtained from these seeds, plants with different kinds of styles were found in each family. This seems to indicate that the differences among the styles of F_1 plants are rather of a phenotypical nature. This will be discussed later.

Now, considering the fertility of this hybrid, I was quite surprised with its high degree considering the great differences in taxonomical position and in the chromosome number of the parental species.

The percent of good pollen grains was calculated from preparations in acetocarmine with glycerine. Different preparations varied greatly. In the year 1949 the polen fertility was found from 27,4% to 46,5% and in average was 34,5%. The preparations from May show in average lower fertility than those from April. In the year 1950 the pollen fertility from different preparations ranged from 22,9% to 51,4% and in average was 31,6%.

Seed fertility was ascertained by counting filled well and plump achenes and those that were shrunken and without embryos. Both classes are rather sharply separated, only few achenes being in midway with small not well developed seeds. Seed fertility was studied only from plants that were open pollinated. The seed fertility from baged flowers is always much lower, due to selfsterility. The percent of good achenes in different flowers may be very different. Unsually the first flowers are quite sterile but in flowers that appear later well developed achenes are much more numerous. The average fertility of the achenes raises during the season and is greater at the end than at the beginning:

Average seed fertility from early spring shoots (1949) . .	3,44%
,, ,, ,, ,, summer shoots (1949) . . .	18,37%
,, ,, ,, in the year 1950	16,55%
,, ,, ,, in the year 1951	15,32%

Fig. 3. Somatic metaphasal plates from root tips of *G. montanum* with 2n = 28 (left), F_1 *G. montanum* × *rivale* with 2n = 35 (centre) and *G. rivale* with 2n = 42 (right).

The range of variation in particular flowers is very large, for instance in the year 1951 besides quite sterile flowers there were also flowers with 32% of good achenes. In general the seed fertility is distinctly lower in comparison with pollen fertility.

The fertility of hybrids of *G. montanum* with other species of the subgenus *Eugeum* is much lower, in spite of the fact that the course of meiosis was in all these hybrids very similar. For instance the seed fertility of the hybrid *G. montanum* x *G. urbanum* was ab. 0,6%, *G. montanum* x *G. lacinianum* — ab 1,5%, *G. montanum* x *G. aleppicum* — ab. 2,5%. Only the hybrids of *G. montanum* with *G. coccineum* and *G. canadense* were more fertile (8—18%).

The cytological investigations. The metaphasal plates from root tips are drawn on fig. 3. We see that the chromosomes are small and nearly of the same length. In *G. montanum* there are 28 chromosomes and in *G. rivale* — 42 chromosomes. The course of meiosis in P.M.C. is in both species quite normal. The plates of I-st and II-nd metaphases of both species are on fig. 4.

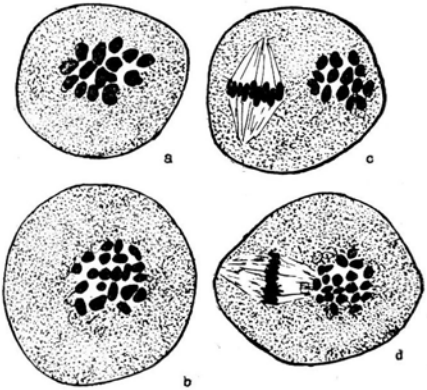


Fig. 4. I-st and II-nd metaphases in P. M. C. from *G. montanum* (upper row) and from *G. rivale* (lower row).

Several somatic metaphasal plates of the F_1 hybrids showed always 35 chromosomes as was to be expected (Fig. 3). The course of meiosis in P.M.C. has been already described (W. Gajewski 1949). I remember only that in the I-st metaphase 14 bivalents and 7 univaletns were formed in most of the P.M.C., in other cells only 13 or 12 bivalents were found and a corresponding higher numbers of univalents. (Fig. 5). At the metaphase the univalents are scattered on the whole spindle and at the anaphase they are segregated at random to both poles usually at the same time as the bivalents separate. The anaphasic groups contain from 14 to 21 chromosomes. Rarely some univalents in late anaphase move to the equator where they divide or pass undivided to the poles. In spite of the irregularities at meta — and anaphase the interkinesis and second division are nearly normal. Very seldom chromosomes lost in cytoplasm or small supernumerary nuclei could be found. The appearance of the tetrads is normal with four microspores, but sometimes of different size, evidently due to uneven distribution of the chromosomes. Small supernumerary microspores are very rare

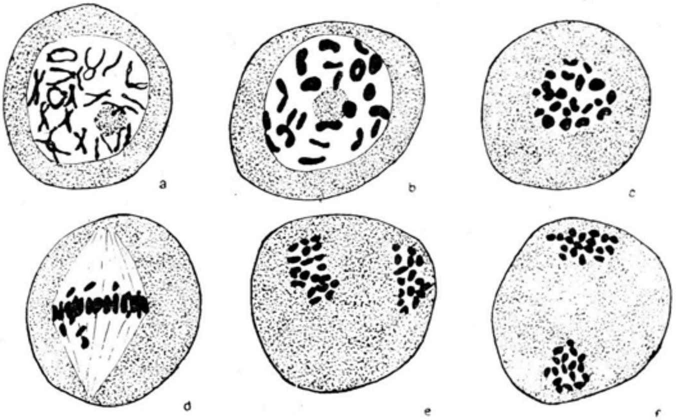


Fig. 5. The course of meiosis in P. M C. of the hybrid G. *montanum* × *rivale*. a — early diakinesis, b — late diakinesis with $14_{II} + 7_{I}$, c —I-st metaphase with $14_{II} + 7_{I}$, d —— profile view of I-st metaphase with univalents on the spindle, e — and f — I-st anaphase with 17 and 18 chromosomes in two groups.

III. Hybrids between *G. montanum* and *G. reptans*.

This hybrid, before it was obtained artificialy, was studied by me from natural stations in the Tatra mountains. The parental species, in spite of their different vertical distribution in the mountains, meet in several places. Such places where both these species grow together are usually on the basal parts of big fields of stones debris (piargi) on southern slopes. There between the pure species also typical F_1 hybrids are to be found. They are easy to discern by their interpediate shape of the leaves (Fig. 6). In the Polish Tatra I have observed this hybrid above lake Morskie Oko below the Mięguszowiecki, on the southern slope above lake Czarny Staw, in the valley Pięć Stawów on the slopes of Miedziane, in the valley Pańszczyca and on the Krzyżne Pass. The hybrids transplanted to pots and brought to Warsaw grew well, flowering abundantely through many years.

In the year 1947 I pollinated 2 flowers of *G. montanum* with the pollen of *G. reptans* cultivated in Warsaw. I have obtained 86 achenes. They have germinated well but the majority of the young seedlings died. I have grown to maturity only 10 F_1 hybrids which were big plants richly flowering. They were quite identic with natural hybrids from the Tatra. Like natural hybrids they were highly sterile having only ab. 0,7% of good pollen. During the last five years both the natural and artificial hybrids have yielded only 6 good achenes from which I have not obtained any F_2 plant. By pollination of *G. montanum* with the pollen of F_1 hybrids I obtained last year 12 good achenes. They have all germinated and I have now 8 young plants which look rather well and show segregation of the shape of the leaves. This indicates that backcrossing is possible. Also some hybrids collected in the Tatra have leaves intermediate between *G. montanum* and F_1 hybrids and represent perhaps plants from this backcross. Unfortunately they were not studied cytologically.

The hybrids between *G. montanum* and *G. reptans* were also found in the Alps and described under the name *G. rhaeticum* Bruegg. From seeds obtained under this name from the Botanical Garden in Edinburgh I have obtained plants that were rather uniform and similar to the back-cross *G. montanum* x F_1 *montanum* x *reptans*. These plants were fertile with ab. 80% of good pollen and with up to 45% of good achenes in some of the flowers. The number of the chromosomes in two of these plants was ab. 40 and they represent probably some of more or less stabilisised segregants from this back-cross.

In their morphology the parental species, especially in floral structures are rather similar, both being of the same subgenus *Oreogeum*. They differ distinctly in shape of the leaves: in *G. montanum* the terminal leaflet is big, circular with cordate base and in *G. reptans*

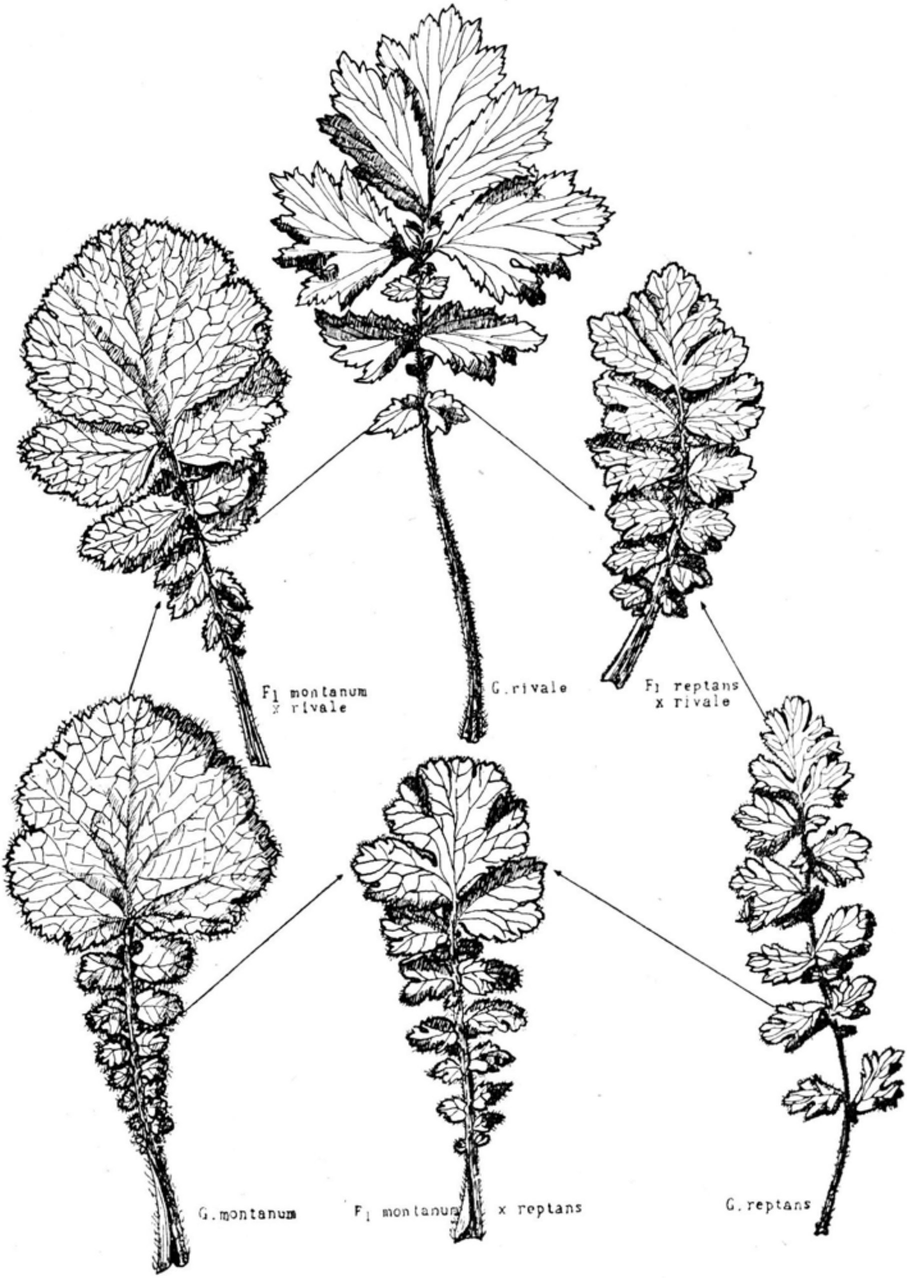


Fig. 6. Table showing the shape of the leaves of different hybrids in comparison with parental species.

the terminal leaflet, like the lateral ones, is deeply cut in linear segments. Besides, *G. reptans* is the only one known species of *Geum* which forms long runners that creep on the earth and on the tip form adventitious roots. In this way *G. reptans* propagates vegetatively. *Geum reptans* forms very long and strong central root wheras *G. montanum* forms a creeping rhizome-like stem with many small lateral roots. In F_1 hybrids the runners are absent and a rhizome-like stem as in *G. montanum* is formed. Other small differences in anthocynin, in dimensions of sepals and petals, in the time of flowering are inherited in an intermediate form.

Fig. 7. Somatic metaphasal plates from root tips of *G. reptans* with 2n = 42 (left) and of F_1 hybrid *G. montanum* × *reptans* with 2n = 35 (right)

Cytological investigations. The metaphasal plate from root tip of *G. reptans* with 42 chromosomes is on fig. 7a and the metaphasal plate from F_1 hybrid with 35 chromosomes on fig. 7b. The meiosis in *G. reptans* has not been studied.

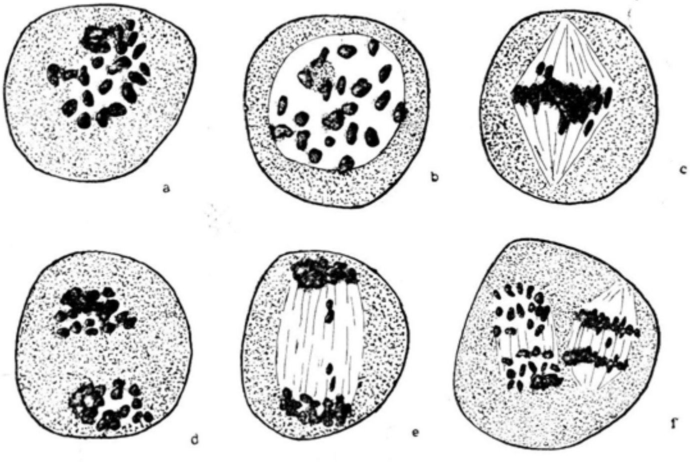


Fig. 8. The course of meiosis in P. M. C. of the hybrid *G. montanum* × *reptans:* a — I-st metaphase with 21 chromosomal bodies, b — diakinesis with 21 chromosomal bodies, c — I-st metaphase from polar view with 7 univalents out of bivalents plate, d — I-st anaphase with 17 and 18 chromosomes in two groups, e — Ist anaphase with two lagging univalents, f — II-nd anaphase.

The course of meiosis in P.M.C. of the hybrid is very similar to the meiosis in the hybrid *G. montanum* x *G. rivale*. (Fig. 8). At diakinesis in most P.M.C. 21 chromosomal bodies could be discerned. They consist o bivalents and univalents but it is difficult to distinguish among them. At I-st metaphase from 32 analysed P.M.C. in 28 I have found 21 chromosomes, in 2 P.M.C. — 23 chromosomes and in 2 P.M.C. — 22 or 23 chromosomes. Metaphasal plates from profile view, clearly show a dense mass of bivalents on the equator and 7 or rarely 9 univalents scattered on the spindle. Most often the majority of the univalents is grouped in the neighbourhood of the bivalents more rarely they lay near the poles. During anaphase the univalents are distributed to the poles together with bivalent halves. Sometimes one or two univalents remain and at the late anaphase they begin to divide. Their division is often abnormal and not completed and they are only stretched and move to the poles as whole long bodies. In a great majority of the P.M.C. at telophase and interkinesis we do not observe any chromosomes that are not included to both daughter nuclei. The number of the chromosomes in both anaphasic groups was counted only in three P.M.C. in which a distribution of 17 and 18 chromosomes was found (Fig. 9). In P.M.C. in which it was possible to count the number of the chromosomes only in one of the two anaphasic groups numbers 17 and 18 were also the most frequent but I have found also such numbers as 15,16 and 20.

The course of the second meiotic division is rather normal. Only in few P.M.C. at meta — or anaphase some chromosomes are delayed lying on the spindle behind the rest of the chromosomes. The telephase II and stage of tetrads look quite regular. Very rarely small additional microspores are to be found. Notwithstanding the apperently normal picture of the tetrads most of the young microspores degenerate and do not develop into functionig pollen grains, which are formed only in ab. 0,8%.

This is a rather a curious fact when campared with the fertility of the hybrid *G. montanum* x *G. rivale*. Both these hybrids have nearly the same course of meiosis. The hybrid of *G. montanum* with *rivale* is between two subgenera while the hybrid *G. montanum* x *reptans* is between two species of the same subgenus. Nowithstanding the taxonomical relatinships the first hybrid is much more fertile than the second. The hybrid sterility is then not related with the systematic position of the parantel species but is rather due to some genic factors present in parental species, which cause the lethality of the hybrid gametes.

IV. The hybrids between *G. rivale* and *G. reptans.*

This hybrid was obtained in the year 1949 by pollinating *G. rivale* with the pollen of *G. reptans* brought directly from the Tatra mountains. Three flowers were pollinated and I have collected 224 achenes (seed setting ab. 70%). Many seeds have not germinated and most of the young seedlings died. To the adult state of flowering and fructication I have raised only 28 F_1 hybrids. In later stages of vegetation the hybrids grew abundantely and flowered from early spring to the first frosts in autumn. The whole F_1 generation was very uniform. Some of the traits of this hybrid in comparison to parental species are tabulated below:

Character	*G. rivale*	F_1 hybrid	*G. reptans*
Height of the shoots (cm)	40—60	15—30	10—15
Beginning of the flowering	30.IV	22.IV	22.IV
Anthocyanin in shoots and penduncles	abundant	abundant	scarce
Anthocyanin in sepals and petals	abundant	abundant	scarce
Colour of the petals	cream	yellow	yellow
Number of petals	5	5—7	5—9
Dimensions of petals			
Length x breadth (mm)	10 x 8	16 x 12	17 x 11
Presence of the claw	long	short	absent
Position of sepals and petals	erect	intermediate	horizontal
The nodding of the peduncles	very pronounced	intermediate	absent
Length of the gynophore (mm)	8—10	2—3	absent or to 1 mm
Structure of the styles	hook-like	straight	straight
Presence of the runners	absent	intermediate	present
Pollen fertility	normal	10—15%	normal
Seed fertility	normal	very low	normal

Very striking is the difference in leaf shape of *G. rivale* and *G. reptans.* The shape of the leaves of the hybrid is exactly intermediate (Fig. 6). The typical long runners of *G. reptans* does not appear in this hybrid but some of the shoots grow abnormally long, with many small bracts-like leaves, they bend down and after a long period of vegetative growth give few flowers on the top. Such shoots are somewhat intermediate between typical runners and floral shoots, but they never take roots. The presence of the runners is in the hybrid of *G. reptans* with *G. montanum* completely recessive.

It is intersting that in this hybrid all achenes are uniform and have only straight, pennate styles of *reptans* type. They are straight from the earliest stage of developement, whereas in the hybrid of *rivale* with *montanum,* even when the adult styles are straight, they pass through a stage at the beginning of their developement when they are distictly bent. The clear dominance of straight styles of *G. reptans,* as compared with incomplete dominance of the same type of styles of *G. montanum,* over the hooked type of the styles of *G. rivale* may be dependent on the hexaploid nature of *G. reptans* and tetraploid — of *G. montanum.*

In spite that ab. 11,5% of good pollen grains are formed in this hybrid the seed fertility is very low. From nearly one thousand of flowers I have collected only 2 seeds which have not yet germinated.

Cytological investigtions. The somatic number of the chromosomes of this hybrid has not been studied, but its hexaploid nature, like of parental species, is clear from the study of meiosis in P.M.C. (Fig. 9). Few cells analysed at the stage of diakinesis show in the nuclei 28 to 30 chromosomal bodies. It is rather difficult at this stage to distinguish between uni — and bivalents, but we have already seen that the small univalents are numerous.

At the I-st metaphase I was able to analyse completely 23 P.M.C. and I have found the following configurations:

$14_{II} + 14_{I}$ in 18 P.M.C.
$13_{II} + 16_{I}$ in 3 P.M.C.
ab. $12_{II} \pm 18_{I}$ in 2 P.M.C.

From profile views the metaphasal plates show a dense mass of bivalents at the equator and numerous univalents scattered on the whole spindle. At early metaphase some of the univalents lie near both poles, but in later metaphase most of them are grouped near the equatorial plate. Using constantely the micrometric screw it

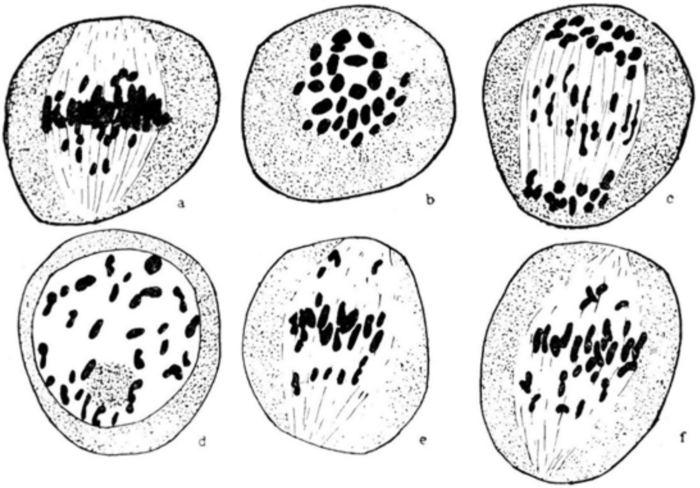


Fig. 9. The course of meiosis in P. M. C. of the hybrid *G. rivale* × *reptans:* a — I-st metaphase from profile view with ab. 15 univalents, b — I-st metaphase from polar view with 28 chromosomes, c — I-st anaphase with 14 chromosomes forming a ring on the equator, d — diakinesis with 28 chromosomal bodies, e and f — I-st metaphase with with 14 and 16 univalents outside of the bivalents plate.

is evident that the univalents are in peripheral zone of the spindle and only rarely in the inner parts.

During anaphase the bivalents separate and the halves move to both poles, forming two anaphasal groups. At this time the univalents rest on the spindle and form more or less regular ring on the equatorial plane of the spindle. Later when the bivalents halves have formed two groups at the poles, the univalents begin to divide. As has been already described by me (W. Gajewski 1949), for other *Geum* hybrids, the division of the univalents is very irregular and in most cases is not completed. Many univalents are only stretched and in form of two beads united by a thin chromosomal thread they move to one of the poles. The division and the movement to the poles of the univalents are much delayed, so, that when they reach the poles, the nuclei are sometimes already in telephasal stage. Such univalentes rest in cytoplasm uninclued to any of the two nuclei. During interkinesis the univalents lost in cytoplasm are resorbed or they form small supernumerary nuclei.

Unfortunately I have not found in my preparations P.M.C. at the II-nd meiotic division. The tetrads consisted usually of four micronuclei. In 98 analysed cells only in 3 cells two supernumerary small microspores were found and in one cell only two microspores were seen. The presence of a dyad indicates that in rare cases restitution nuclei after the first or second division may be formed. The microspores must have different unbalanced chromosomal constitution as most of them degenerate before the first mitotic division.

V. Discussion.

The genetic and cytological relations between the species of the subgenera *Eugeum* and *Oreogeum* show many interesting aspects. The most interesting is the inheritance of the different structures of the styles. They are not only very important from the taxonomical point of view but also they are of utmost importance for the biology of dissimination of the species form two subgenera. The creation of a new, active mode of seed dissimination by epizoochorism was a great evolutionary step in the speciation processes in the whole genus. We see that in Europe the anemochoric species from the subgenus *Oreogeum* represent a conservative and relic group of species occuring only in mountains, whereas the epizoochoric species from the subgenus *Eugeum* possess sometimes big areas which are still now enlarging, as for instance *G. urbanum* in Europe and in America as a plant introduced by man.

It would be very interesting to throw some light on the question how the hooked styles have arisen from the straight ones. Have they arisen through one big evolutionary step or are they evolved gradually by means of numerous small mutations?

The hybrids among *G. montanum* and *G. reptans* with different species from the subgenus *Eugeum* have shown the following interesting features concerning the structure of the styles:

1. The hybrids among *G. montanum* and such hexaploid *Eugeum* species as *G. rivale, urbanum, coccineum, aleppicum* and *laciniatum* have on one individual and even in the same flower different kinds of styles from straight, through intermediate to hooked ones.
2. The hybrids of *G. montanum* with *G. hispidum, molle, canadense* and *silvaticum* have nearly all styles straight or only slightly bent.
3. The hybrids of *G. montanum* with dodecaploid *Eugeum* species like *G. pyrenaicum* or *G. magellanicum* have all styles with hooked curvature.
4. The hybrids of hexaploid *G. reptans* with *G. rivale* and *G. coccineum* have all styles straight.

In the second generation and in the back-crosses of *G. montanum* x *rivale* but also of *G. montanum* x *aleppicum* the structure of the styles shows segregation. In F_2 and especially in back-crosses some plants segregate that possess styles more or less of parental types.

The study of the development of the styles from small primordia to adult stage shows that at certain period of development all young styles of the hybrid *G. montanum* x *rivale* have distinct curvature between the rostrum and stigmatal part. This was even observed in the hybrid *G. montanum* x *hispidum* which later has only straight styles. From this early curvature, in some styles a complete loop may be formed, but in others it may gradually disappear. The comparison with the development of the styles of *G. rivale* indicates that up to a certain moment the development of the styles in the hybrid is nearly the same, but from this moment on, the development of the different styles in the same flower may be different. The later development of the straight or hooked styles shows some correlation with the period of vegetation.

The stigmatal part of the styles in subgenus *Eugeum* has different anatomical structure from the lower part called rostrum as was shown by Iltis (1915). It posses numerous stomata and chloroplasts which are lacking in the rostrum. Between the rostrum and the stigmatal part on the top of the hooked curvature a zone of small thin walled cells is inserted. The stigmatal part during its growth is contorted by some kind of torsional growth. In *G. montanum* the stigmatal part forms only a few milimeters long tip of the to 4 cm long style. The stomata are present not only in the stigmatal part but also in the upper zone of plumose style. Stigmatal part and lower part of the style are not separated by any zone od differently shaped cells. The small stigmatal segment dehisce but do not fall off. In the styles of the hybrids *G. montanum* x *rivale* the primordial curvature arises at the place with anatomical structure typical for rostrum and if in the succeeding stages of the development it disappears, then the structure below and above this place is the same. If however a hooked curvature is formed, a layer of small separating cells develops. The part above this curvature which corresponds to the stigmatal part of *Eugeum* species is not homogenous, its lower part having the structure typical for rostrum without stomata and chloroplasts.

The stigmatal part in *G. rivale* develops first and reaches its final length when rostrum is only 2/5 of its mature dimensions. This

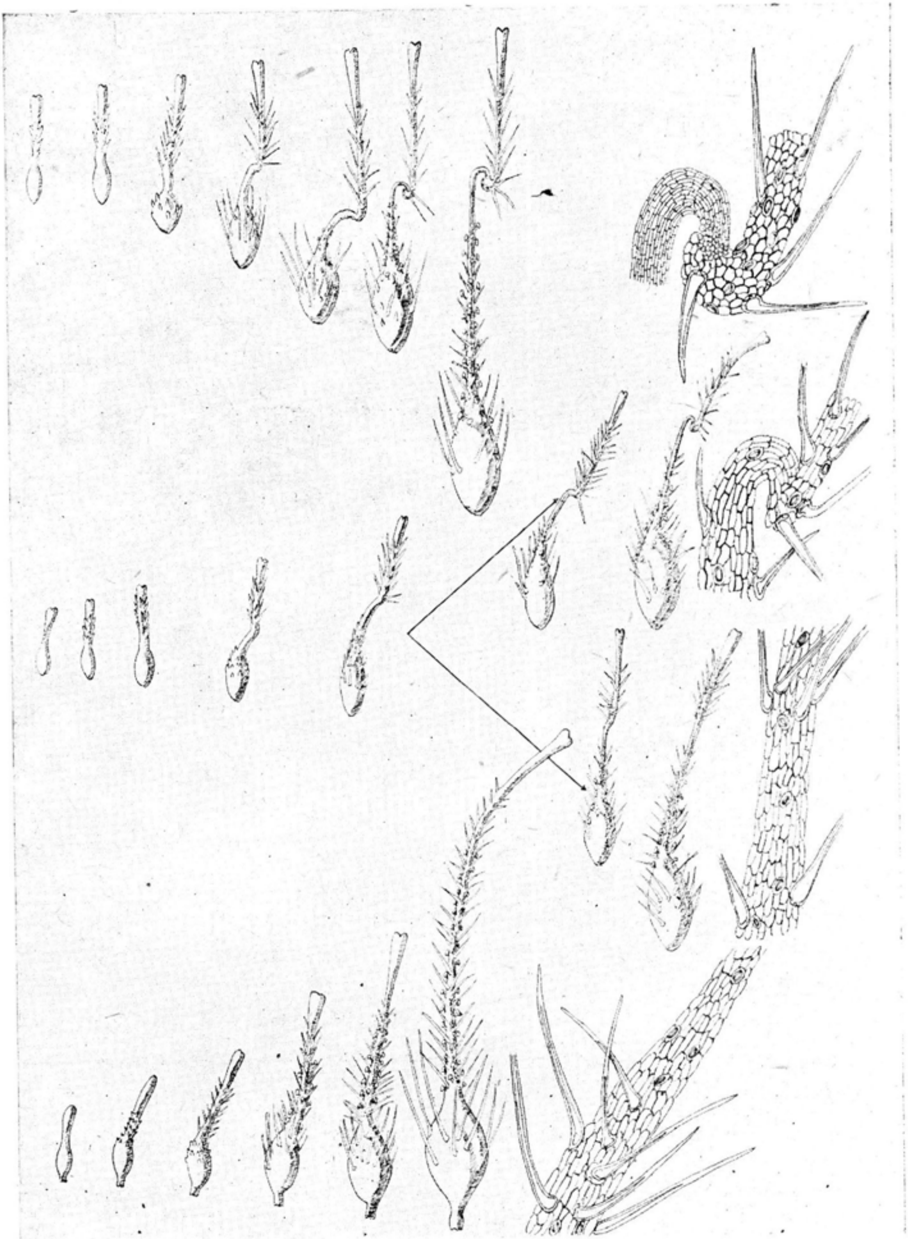


Fig. 10. The development of the styles of *G. rivale* (upper row), of the hybrid *G. montanum* × *rivale* which is the same in the young stages and then different in different styles (middle row) and of *G. montanum* (lower row). On the right side of each row the anatomical structure of the part of the styles between the stigmatal part and the rostrum is presented.

stage is attained somewhat one-two days before the anthesis. After the fertilization rostrum still grows for a relatively long time. At this period the stigmatal part contorts which causes probably the stopping of water transport through the vessels. Then the stigmatal part wither and at last it falls. In the rostrum the cell walls begin to lignify and a stiff hooked organ is formed.

The rythm of growth and the differentiation of the styles of *G. montanum* are different. The structure of the styles is more uniform through the whole length, the period of growth is more prolongated. As we see, the differences between the two subgenera in the structure of the styles do not consist only in the presence or absence of the hooked curvature.

It seems improbable that the loop is simply the result of the torsional gwroth of the styles, as supposed Bolle (1933). If even the torsional growth was the primary cause of the formation of this loop then the later development of the hooked type of the styles was followed by other anatomical and developmental changes which have caused the formation of a perfectly functioning organ of epizoochoric dissimination. These changes consisted in the supression (partial or complete) of the plumose hairness, in different tempo and mode of growth of rostrum and stigmatal part, in the formation of a seperation layer, in the lignification of rostrum cells and so on. The study of F_2 plants shows that all these characters segregate independently. In F_1 hybrids these processes are clearly not coordinated due to the presence of two genomes with genetic factors acting in different or even opposite ways. This causes that the former tendency to the formation of the loop may be stopped and overrun by the opposite tendency toward the formation of straight pennate type of the styles.

It seems that the heterogenity in the structure of the styles in this hybrid is the result of developmental disharmonies and has nothing to do with the cases of somatic segregation or somatic mutations. That this is purely a phenotypical variation has shown the offspring raised from different types of the styles from F_1 plant, which were in general the same from any type of the achenes. It seems the most probable that the hooked type of the styles of *G. rivale* is due to factors that have incomplete dominance and only partial penetrance when united with the factors for straight pennate styles of *G. montanum.*

The results obtained with the hybrids between different *Eugeum* and *Oreogeum* species seem to indicate that these factors are not identical in various species, probably both in number and nature. So, for instance, the hexaploid *G. reptans* causes complete dominance of straight styles while tetraploid *G. montanum* is only partialy dominant with hexaploid *G. rivale* and *G. coccineum.* On the other hand the hooked styles of dodecaploid *G. pyrenaicum* and *G. magellanicum* are completely dominant over the straight styles of *G. montanum.*

It is then possible that the higher degree of ploidity of one parent and thus a higher number of genic factors governing the development of one type of styles structure causes that the genic balance in the hybrid is clearly directed and a complete dominance is observed. Reasuming, it must be stated that the differences now existing between the species of the two subgenera in the structure of the styles seem to be due to many genic factors. But the main question how the hooked styles have arisen from straight ones rests still unanswered. It seems to me hardly probable that the hooked styles have evolved gradually from the straight ones. They could arisen through one big mutation or through crossing and amphidiploidity between an *Oreogeum* type species and others, now perhaps extinct, species with falling styles, like, for instance, now have *Potentilla, Coluria* or *Waldsteinia.* In these genera the styles are homologous to the stigmatal part of the styles of *Eugeum*, the rostrum of *Eugeum* species is rather homologous with the lower part of the styles of *Oreogeum* species. Afterwards in such amphidiploids through a long selectional process the new epizoochoric type of styles was built by the action of many minor modificating genes. In the light of cytological relationships that exist between the two subgenera this hypothesis seems probable.

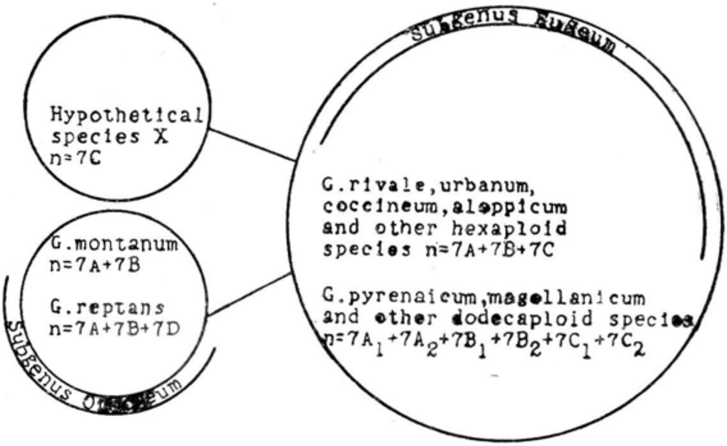
The second question which I would like to point out is the compatibility and hybrid sterility among the species of the two subgenera. It is interesting that even between such distant species as *G. montanum* and the species from the subgenus *Eugeum* the cross-compatibility is very high. As other crosses have shown the compatibility is high in the whole genus between all subgenera, sections and species. The fertility of different hybrids is not strictly correlated witch the taxonomic position of the parents. The fertility may be normal between some of the *Geum* species as I have already described before (W. Gajewski 1950). This is the case when certain species from the same subgenus and with the same chromosome numbers are crossed. Normal fertility is always correlated with normal pairing at meiosis, but other hybrids with normal pairing could also be quite sterile or only partially fertile. The analysis of many interspecific hybrids among hexaploid *Eugeum* species has shown, that the degree of hybrid sterility is not correlated with the systemtaic position of the parental species but it results from the balance of genic factors united together in the hybrid. This is also true for the hybrids between the two subgenera. For instance the hybrids between the tetraploid *G. montanum* and hexaploid *G. rivale* and *G. urbanum* have the same type of meiosis with $14_{II} + 7_{I}$,but the first hybrid has ab. 16% of good achenes and the second only ab. 0,6%. The hybrid *G. rivale* x *G. urbanum* has normal chromosome pairing at meiosis and is highly fertile. The hybrid of *G. montanum* with hexaploid *G. reptans* has the same type of meiosis as the hybrids mentioned above, and is nearly sterile although both parental species belong to the same subgenus.

It rests to discuss the cytological relatonships among the species from the two subgenera. We have found that the tetraploid *G. montanum* gives the same type of configuration —$14_{II} + 7_{I}$ when crossed with *G. rivale* or *G. reptans* which are both hexaploid. When we cross the two hexaploid species we obtain the configuration of $14_{II} + 14_{I}$.

These facts are tentatively explained in the following manner: supposing that the conjugation of the chromosomes indicates their homology and common descent, we can state that *G. rivale* and *G. reptans* have 14 of their 21 chromosomes homologous with 14 chromosomes of *G. montanum*. The remaining 7 chromosomes of *G. rivale* and 7 chromsomes of *G. reptans* are different, nonhomologous. If the two 7-chromosomal sets of *G. montanum* we denote *AB* then the three sets of *G. rivale* will be *ABC* and the three sets of *G. reptans* can be denoted *ABD*. We can suppose that both *G. rivale* and *G. reptans* have arisen by amphidiploidity from hybrids of *G. montanum* (or related species) with two different diploid species which brought the sets *C* or *D*. What were these diploid species we do not know. Perhaps they do not exist now. The hypothetical diploid species with the genome *C* could probably have a decisive role in the evolution of the hooked epizoochoric type of styles of the subgenus *Eugeum*.

The more or less normal conjugation of the chromosomes in hybrids among different hexaploid species of the subgenus *Eugeum* and the same type of conjugation in the hybrids among these species and *G. montanum* indicate that all these species have the same three chromosomal sets *ABC*. They differ mostly in genic composition or perhaps in small chromosomal mutations that have not altered the homology of the *A, B, C* chromosomal sets. The evolution within the subgenus *Eugeum* consisted thus chiefly in gene and chromosome mutations. From time to time the hybrids between compatibile hexaploid *Eugeum* species gave rise to dodecaploid species through the process of amphidiploidity. I have found before (W. Gajewski 1949) that some hybrids among hexaploid species are nearly completely asynaptic due probably to the action of special genic factors. Such hybrids give frequently unreduced gametes. From the union of such gametes 12-ploid F_2 plants are formed as I have found for the hybrid *G. rivale* x *macrophyllum* (unpublished data).

As a working hypothesis a following scheme of the evolutionary steps in the genus *Geum* is proposed:



That such a scheme of evolution is possible we see from numerous examples from other genera. For comparison I will only point at some facts known from the genus *Triticum* and related *Aegilops*. We know that hexaploid wheat species with the genome *ABD* (according to Kihara's denotation) have arisen from tetraploid wheats with the genome *AB* and the diploid *Aegilops squarrosa* with genome *D*. This was proved by McFaden and Sears (1944, 1946) and by Kihara and Lilienfeld (1949) who have obtained from the crosses of *Triticum dicoccoides* and *Aegilops squarrosa*, after the doubling of the chromosome number, amphidiploids very similar to the hexaploid *Triticum spelta*. The different hexaploid wheat species like *Tr. vulgare, spelta, compactum, sphaerococcum,* *macha* and *Vavilovi* have the same genome *ABD* and the hybrids among them show potential complete pairing like in hybrids of hexaploid *Eugeum* species.

The hybrids among hexa- and tetraploid wheat species give the same type of conjugation of $14_{II} + 7_{I}$ as we have observed among tetraploid *G. montanum* and hexaploid *Eugeum* species.

In the genus *Triticum* the synthesis of hexaploid species took place probably in more recent time as in the genus *Geum*. McFadden and Sears suppose that *Tr. spelta* have arisen in relatively early times in East Europe and Western Asia and from this species *Tr. vulgare* has evolved probably even in early historic times. In the genus *Triticum* the highest chromosome number is hexaploid and numerous di- and tetraploid species are still in existence.

In the genus *Geum* the evolutionary state is much more advanced. The diploid species of *Geum* and its nearest relatives do not exist now probably. The tetraploid species are few only. The majority of species are hexaploid or with a higher degree of ploidity which is not known in *Triticum*. The very large distribution of *Eugeum* species on both hemispheres indicates that probably the main types of amphipoliploids were already formed during the Tertiary epoch and the more primitive species have disappeared since that time. It is not excluded, however, that further studies will disclose some missing links in the scheme of the evolution proposed here.

SUMMARY

1. In the hybrid *G. montanum* (14) x *G. rivale* (21) the most common configuration at meiosis in P.M.C. is $14_{II} + 7_{I}$. This hybrid is partially fertile with a very peculiar heteromorphic structure of the styles. In one specimen or even in one flower styles are formed that are straight (like in *G. montanum*), intermediate and with hook-like curvature (like in *G. rivale*).

2. In the hybrid *G. montanum* (14) x *G. reptans* (21) the same mode of chromosome conjugation was found. The hybrid is nearly completely sterile and morphologically uniform.

3. In the hybrid *G. rivale* (21) x *reptans* (21) a configuration of $14_{II} + 14_{I}$ was found in the majority of P.M.C. during I-st metaphase. This hybrid has all styles straight and plumose like in *reptans* parent, and is nearly completely sterile.

4. *G. montanum* and *G. reptans* belong to the subgenus *Oreogeum*, which is characterised by long, straight, pennate styles for-

ming an anemochoric organ of dissemination. G. *rivale* is a representative species from the large subgenus *Eugeum* with a hooked type of the styles adapted to epizoochoric mode of dissemination. There are many reasons to believe that the subgenus *Oreogeum* is a more primitive and that the subgenus *Eugeum* is a more advanced. A hypothesis is presented that *Eugeum* species have arisen from *Oreogeum* species through amphidiploidy and subsequent genic differentiation.

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