

## Cytogenetic relations of *Geum macrophyllum* Willd. with *G. perincisum* Rydb. and *G. oregonense* Rydb.

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A species of *Geum* from the *Eugeum* subgenus grows in North America and has a wide distribution area from Alaska to California. It was first described by Willdenow in 1809, as *Geum macrophyllum*. However, on the enormous areas of the North American continent the species is greatly differentiated and as a result some lower systematic units were distinguished in it. Various workers according to their personal opinion distinguished within the species varieties, subspecies and even separate related species.

It is characteristic for *Geum macrophyllum* that the terminal leaflet of the caudical leaves is large, round or reniform, often with three lobes, and shallowly serrate. *G. perincisum* Rydb. deviates markedly from this type. It was described in 1913 in the North American Flora by Rydberg, who classified it as a new species. *G. perincisum* Rydb. has more elongated leaves and deeply incised terminal leaflets with deeply incised and sharply serrate lobes. It can be seen on the accompanying maps that the distributional area of this species is more continental than that of *G. macrophyllum*. Finally, it was also Rydberg who in 1898 described and classified as a separate species *G. oregonense*. In this species the shape of the leaves is as if intermediate between that of *G. perincisum* and *G. macrophyllum*. *G. oregonense* grows on high mountain meadows of the Pacific coast of America.

These species classified separately by Rydberg met with controversial opinions of later authors. For instance R a u p (1931) thinks that *G. perincisum* is only a variety of *G. macrophyllum* and that *G. oregonense* is a synonym for *G. perincisum*, finding no sufficient reason for distinguishing between the two. H u l t é n (1945) in his Flora of Alaska and the Youkon on the ground of their different distributional areas, considers *G. perincisum* to be a subspecies of *G. macrophyllum*. He makes no distinction between *G. oregonense* and *G. perincisum*, treating them synonymously. F e r n a l d in the last edition of Gray's Manual seems to find no diffe-

rence between *G. oregonense* and *G. perincisum*, while A b r a m s (1944) in his Flora of the Pacific States of the U.S.A. mentions only *G. macrophyllum*. On the other hand, B o l l e (1933) in his monograph of the genus *Geum* distinguishes as a separate species only *G. oregonense*, while *G. perincisum* he describes as a form of *G. macrophyllum*.

This short and incomplete review makes apparent the controversies on the systematic positions of the three taxa and discloses the lack of decided opinions on the subject in taxonomic literature.

The distributional area of *G. macrophyllum* spreads from Alaska through the Aleutian Islands to the Asian continent, where the species can be found in Kamtchatka, on the Commander Islands, north Kurile Islands, and probably also on north Sakhalin. On the south Kurile Island, Sakhalin, Yezo, and in the mountains of north and central Honshu in Japan grows a species closely related to *G. macrophyllum* and described as *Geum Fauriei* by Leveillé in 1910. Morphologically this species is related on the one hand to *G. macrophyllum* and on the other to *G. japonicum* T h u n b. which grows in Japan, China, and Korea. Because of this the Japanese botanists classified this species together with and as a variety of either *Geum japonicum* or *G. macrophyllum*. For instance K o i d z u m i classified it with the first species as *G. japonicum* var. *sachalinensis* K o i d z u m i in Bot. Mag. Tokoyo 29:157 (1915) and H a r a classified it lately with the latter as *G. macrophyllum* Willd. var. *sachalinensis* (K o i d z u m i) H a r a comb. nov. As in my cultures I have the species *G. Fauriei* I was able to ascertain that it is dodecaploid with  $2n = 84$  and differs from *G. macrophyllum* and *G. japonicum* both with  $2n = 42$ . *G. Fauriei* displays the characters of both *G. macrophyllum* and *G. japonicum* and its distributional area lies between the regions occupied by the two other species. It seems very likely that *G. Fauriei* is an amphiploid the parent forms of which were *G. macrophyllum* and *G. japonicum*.

The fact that I have in my cultures *G. macrophyllum*, *G. perincisum*, and *G. oregonense* led me to an investigation on the mutual cytogenetic relations of the three species. Such an investigation may throw a new light on the nature of these taxa and help to establish their proper systematic rank as well as to explain the occurrence of the intermediate forms in natural conditions. The really slight differences between these taxa and the intermediate forms which sometimes appear in Nature may render the task of distinguishing between them solely on morphological grounds very difficult. In such conditions cytogenetic studies may be an aid in establishing the rank of these systematic units. The present paper is nothing more than an introductory study on this group, and as my work was carried out far from the territories where the plants appear, I could not fulfil the numerous conditions necessary for a full study of the pro-

blem. Thus, it was impossible for me to investigate on the spot the geographical distribution of the plants, their ecological requirements, and the possible appearance of hybrids in natural conditions. I was also unable to obtain a sufficient amount of live material from various points of the enormous area occupied by the plants. It is of course obvious that all these points are essential for a detailed investigation, but nevertheless I decided to publish this report on the results which I obtained in the course of a wider study on the whole genus. To such a decision I was led by the hope that this paper will bring the attention of American botanists to this very interesting group of plants. If this aim will be achieved the publication of this report will, I am convinced, prove its utility.

#### I. DESCRIPTION OF THE SPECIES

The three taxa referred to in this investigation are morphologically very closely related and vary mainly by the shape of their caudical and

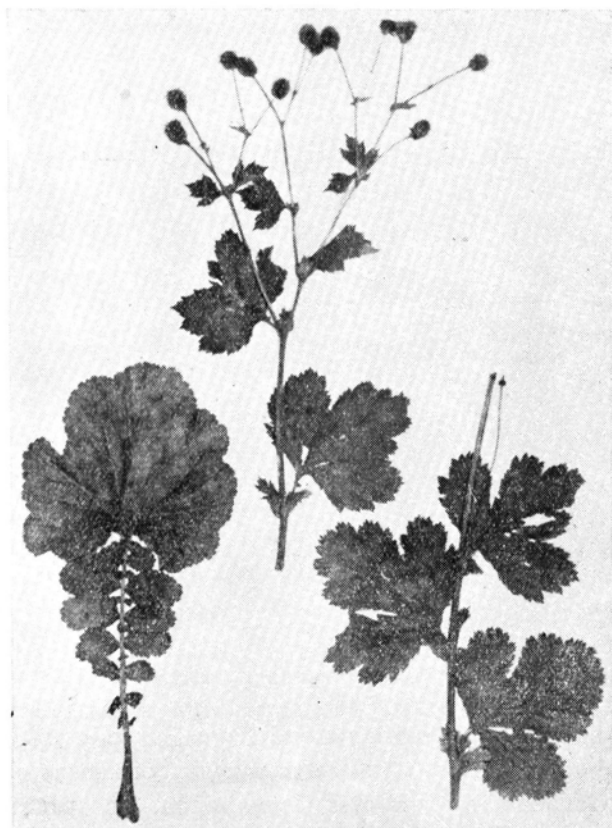


Fig. 1. *Geum macrophyllum* Willd.

stem leaves. There are also slight differences in the degree of pubescence, the size of the petals, rostrum length etc. For B o l l e the presence of glandular hairs on the floral peduncles in *G. oregonense* and their absence in *G. macrophyllum* is presumably, a distinguishing feature between the two species. However, in some specimens of both *G. macrophyllum* and *G. perincisum* glandular hairs do appear also on the floral peduncles. When describing these plants it is difficult to define the features which distinguish them from each other, but when one sees them growing in cultures, one beside the other, there can be no doubt that they are three various taxa.

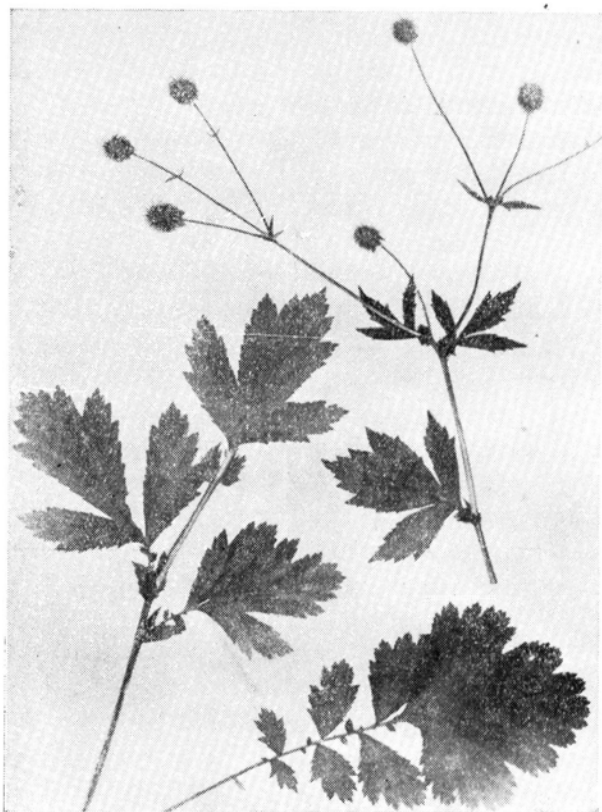


Fig. 2. *Geum oregonense* R y d b.

The plants in question were obtained from the following sources:

1. *G. macrophyllum* W i l l d. was raised from seeds obtained from the Botanical Gardens in Kew and Copenhagen. The plants were very much alike and fully corresponded to the description of the species. Unfortunately none of the plants came from their natural habitats. Plants raised



in gardens were exactly like herbarium specimens collected on the natural habitats. As all the hybrids of *G. macrophyllum* with the other *Eugeum* species are sterile it can be assumed that, in spite of their garden origin, the plants were of a „pure“ species.

2. *G. oregonense* Ryd b. was grown from seeds collected on a herbarium specimen, the origin of which was defined as Lost Creek Bridge, Haydn division, Medicine Bow Forest, Carbon County, Wyoming, Elev. 8000 ft. 5 Aug. 1946 — The Rocky Mountains Herbarium, University of

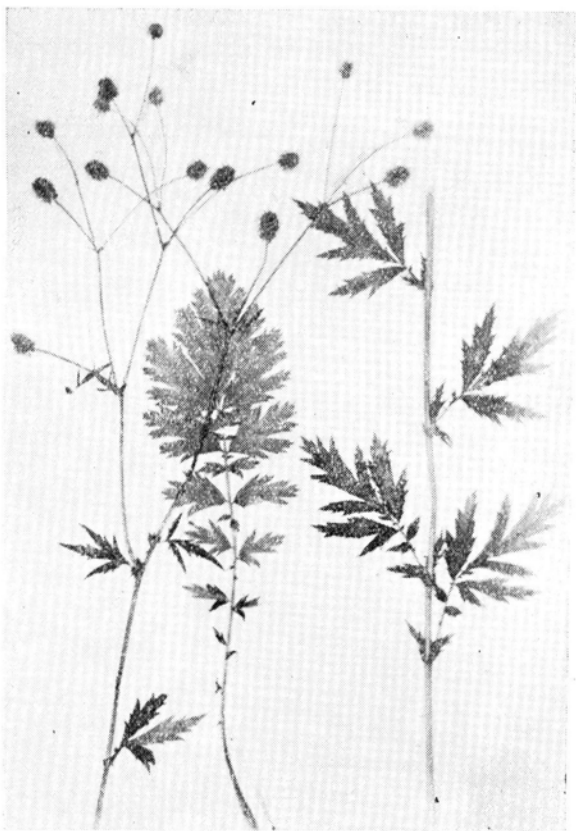


Fig. 3. *Geum perincisum* Ryd b.

Wyoming, Laramie, Nr. 4085 Collect. et determ. C. L. Porter. The seeds were collected in 1948 from the herbarium at the University of Uppsala and their germination was very good. In the herbarium the plant was classified as *G. macrophyllum* though it was a very characteristic specimen of *G. oregonense*.

3. *G. perincisum* Ryd b. was grown from seeds collected on natural habitats and obtained from the Botanical Garden in Montreal. The

seeds were collected in Canada from habitats 1) in Yellowknife on the shores of Great Slave Lake and 2) near Grand Lac de l'Ours. The appearance of plants from these two habitats is very characteristic for *G. perincisum*, their leaves are deeply incised and the differences between them are negligible.

The differences in the outward appearance of the plants from the three species are well illustrated by the photographs of the whole plants (Figs. 1, 2, 3) and of the caudical leaves (Figs. 7, 8, 9). The *G. perincisum* raised in my cultures and represented in Fig. 3 may be considered as belonging to the typical variety with deeply incised leaves. In some herbaria it is classified as *Geum perincisum* var. *perincisum* (Plantes de la Saskatchewan, Canada District de North Battleford No 6814, Lac Makawa, leg.:

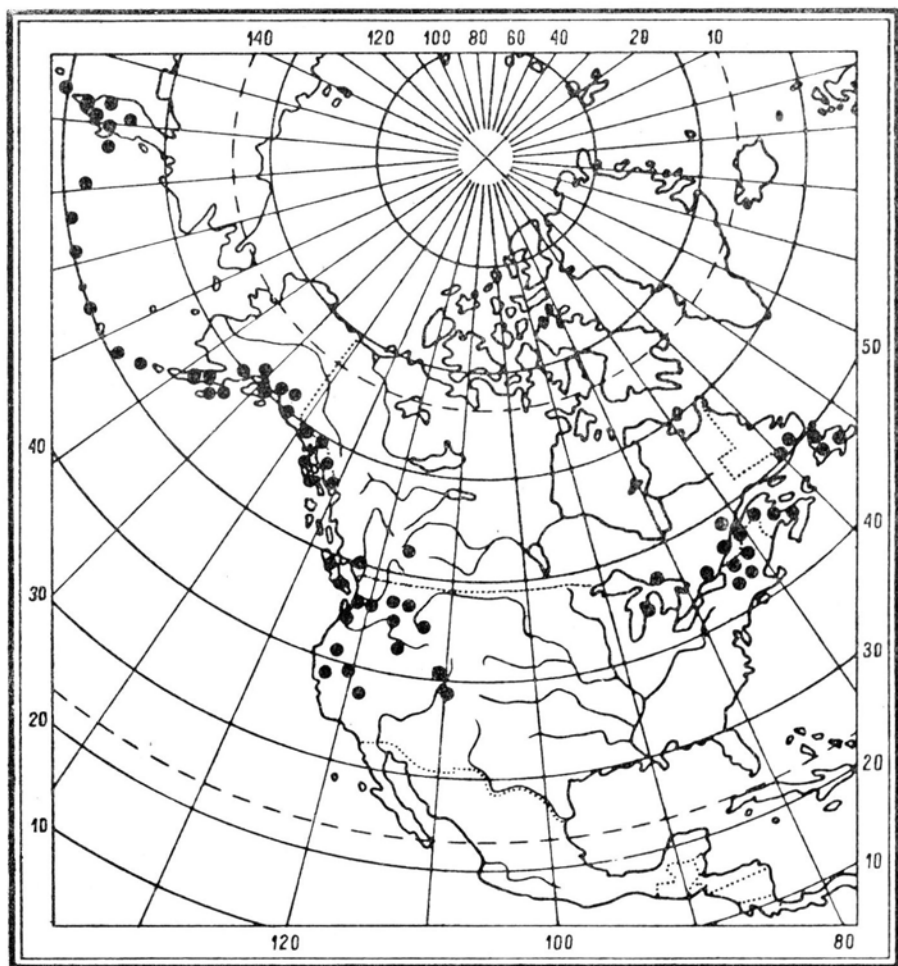


Fig. 4. Distribution map of *G. macrophyllum* Willd.

Bernard Boivin). Another specimen which I have seen had also been collected in Saskatchewan and was classified by Boivin as *G. perincisum* Ryd b., var. *intermedium* var. nov. (Plants of Saskatchewan No. 4249, Cypress Hills Park, Sask.). The leaves of this specimen were less incised, it resembled some of the hybrids obtained from crosses of *G. macrophyllum* with *G. perincisum*, and indeed it may be descended from such a hybrid.

The three investigated taxa differ considerably in their ecological requirements as well as in their distributional areas. *Geum macrophyllum* usually grows in thickets and forests and is widely spread in the plains and at low altitudes in the mountains. *G. perincisum* grows on low lying plains, mainly in the northern areas of North America, while *G. oregonen-*

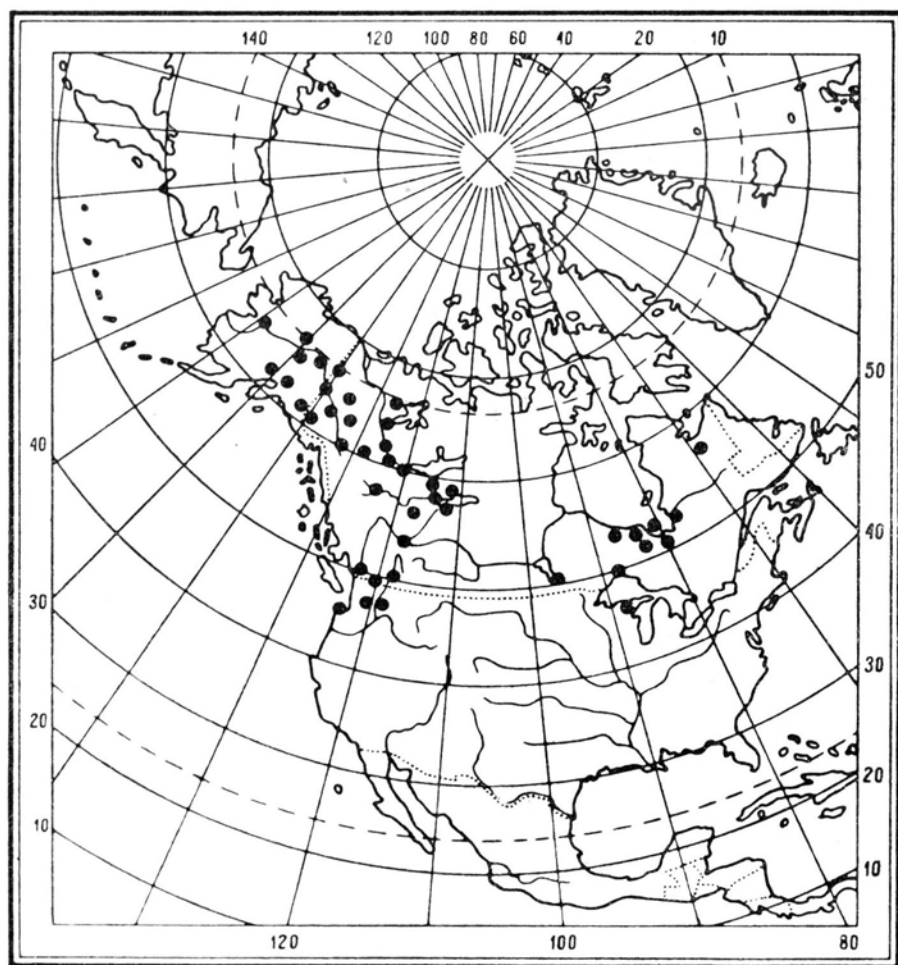


Fig. 5. Distribution map of *G. perincisum* Ryd b.

se seems to be entirely a mountain species growing on mountain meadows at altitudes of more than 2000 m.

Because of the controversial opinions it is very difficult to establish the distribution of the three species. The accompanying distribution maps (Figs 4, 5, 6) are not fully complete. The *G. perincisum* distribution map I owe to Dr Marcel Raymond from the Botanical Garden in Montreal who very kindly sent me a point map marking the habitats of the species in Canada and Alaska. The more southern habitats in the Pacific States of the United States are after R a u p (1931) who published a distribution map of "*G. macrophyllum* var. *perincisum*". However, R a u p makes no distinction between *G. perincisum* and *G. oregonense*

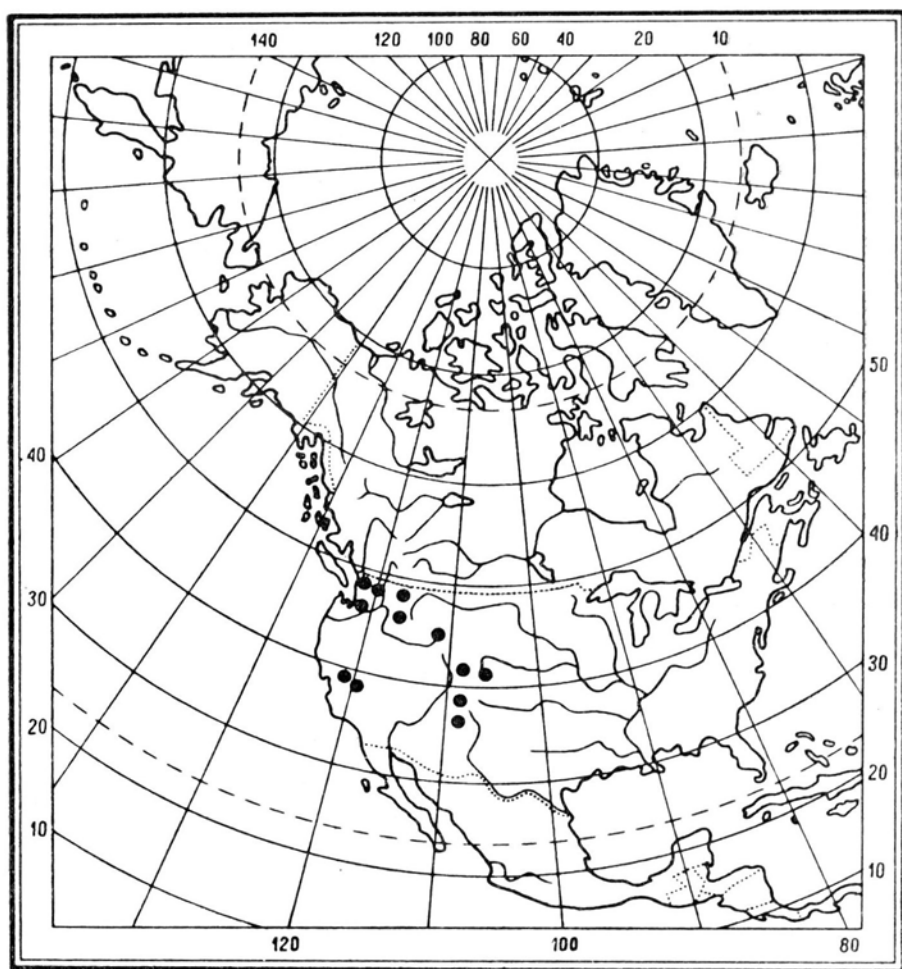


Fig. 6. Distribution map of *G. oregonense* R y d b.

and it seems therefore very likely that these southern habitats refer rather to the latter than to the former of these species. Among the many herbarium specimens which I examined in the herbaria of Uppsala, Lund, Stockholm, Goteborg, Copenhagen, Kraków, Wrocław and Warszawa I found not one specimen of *G. perincisum* which had been collected in

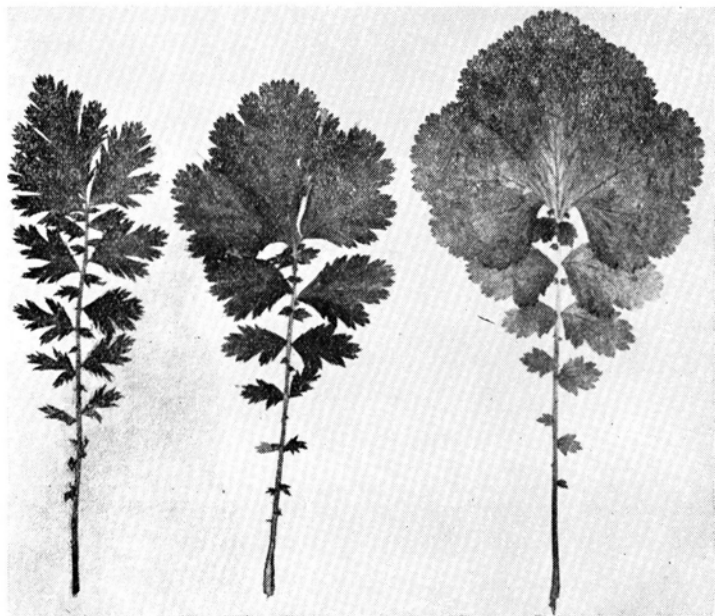


Fig. 7. Basal leaves of *G. macrophyllum* (right), *G. perincisum* (left) and of  $F_1$  hybrid (centre).

the Pacific States of the U.S., all the herbarium specimens collected in these states were either *G. macrophyllum* or *G. oregonense*. It seems that in the United States *G. perincisum* grows only in the States of Michigan, Washington and Montana, and nowhere else. On the other hand I found in the herbaria numerous specimens of *G. perincisum* collected in Canada in the provinces of British Columbia, Alberta, Ontario, Quebec and Saskatchewan.

The distribution map of *G. macrophyllum* was plotted from that made by R a u p (1931) and from the data on the distribution of this species in Alaska and on the Kurile Islands assembled by H u l t é n (1930, 1946) in his floras of Kamtchatka and Alaska and Youkon. Moreover I added to the map the positions of numerous habitats from which the specimens in the above listed herbaria were collected. The territories from which *G. macrophyllum* specimens in the herbaria were obtained, were: in Canada, the provinces of Quebec, Ontario, British Columbia and Newfoundland;

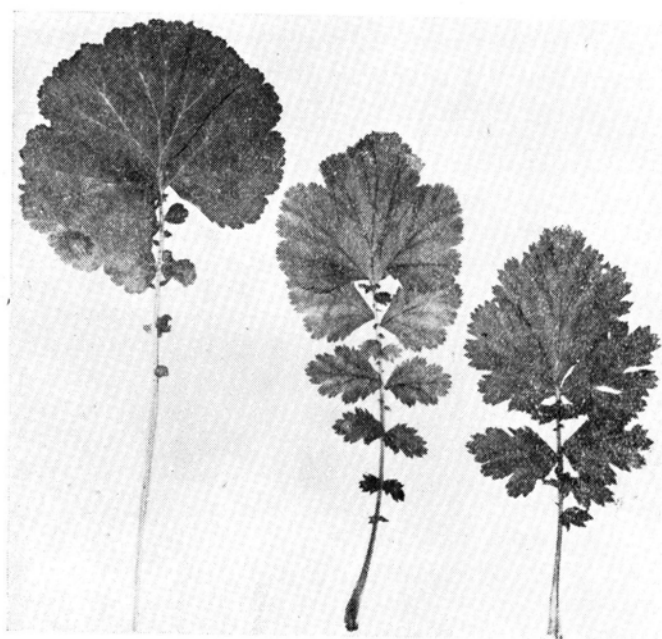


Fig. 8. Basal leaves of *G. macrophyllum* (left), *G. oregonense* (right) and of *F1* hybrid (centre).

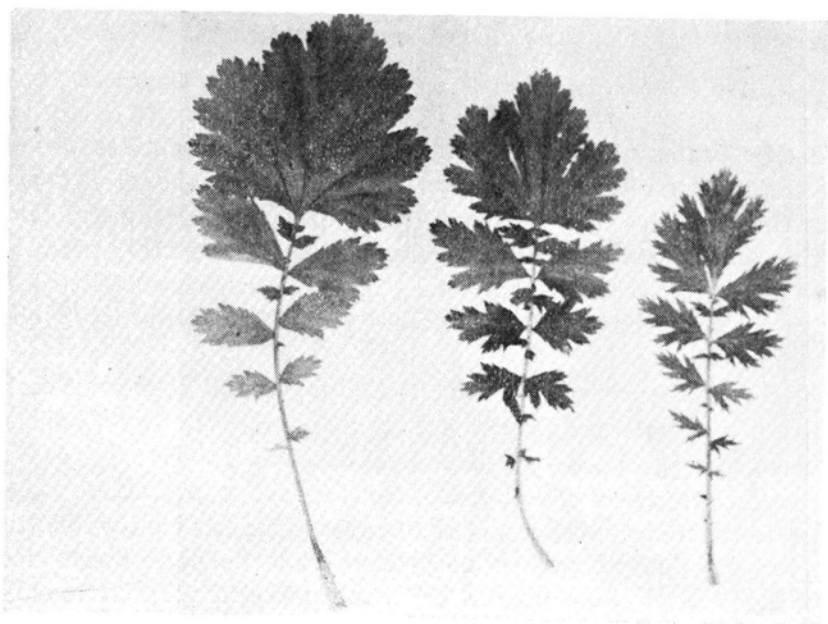


Fig. 9. Basal leaves of *G. oregonense* (left), *G. perincisum* (right) and of *F1* hybrid (centre).

in the United States, the States of New Hampshire (White Mts), New York (Essex Co.), Washington (Spokane Co., Tucoma Co., Seattle, Lincoln Co., Chehalis Co., King Co., Klickital Co., Vancouver Isl.), Montana (Beaverhead Co., Missoulu Co.), Wyoming (Teton Co., Sheridan Co.), Oregon (Sunvie's Isl.), Idaho (Krotinai Co., Latch. Co.), Utah (City Creek Cañon), Colorado (Larimer Co., Feta Pass), Nevada (Washoe Co.) and California (Esmeralda Co., Siskiyou Co.). Finally numerous *G. macrophyllum* specimens from Alaska, Kamtchatka and the Behring Island are to be seen in the herbarium in Stockholm. All these habitats from North America lie approximately within the distributional area as given by Raup in his map.

The distribution of *G. perincisum* is far more continental than that of *G. macrophyllum*. The latter species occupies a large disjunctive area one part of which extends over the Pacific coast of North America from California to Alaska and through the Aleutian Islands to north-eastern Asia and the other forms a distribution island on the Atlantic coast of America, stretching from Newfoundland to the State of New York and to the Great Lakes.

The most difficult to establish is the distribution of *G. oregonense*. It extends probably over a large territory in the Rocky Mountains from Colorado and California to the States of Washington and Montana and maybe to the Rocky Mountains in Canada. Hultén (1946) reports that specimens of *G. oregonense* may even be found in Alaska. However, as this species was not distinguished from *G. perincisum* there is no sufficient data on its distributional area. The map here reproduced is based only on the habitats as given by herbaria. In the U.S. these habitats are situated in the States of: Washington (Okanogan Co., Spokane Co., Ferry Co.), Montana (Blankinship, Boreman), Wyoming (Yellowstone Nat. Park, Albany Co., Laramie Co., Carbon Co.), Nevada (Washoe Co.) and Colorado (Beaver Creek). Moreover, Bole (1933) reports on herbarium specimens of this species collected in California (Plumas Co.).

To establish the exact distributional area of *G. oregonense* a critical study on a large number of herbarium specimens is needed, and special attention should be given to *G. perincisum* x *G. macrophyllum* hybrids which may resemble morphologically *G. oregonense*.

From the data now available it seems that the distributional area of *G. oregonense* lies within that of *G. macrophyllum* though it is very likely that considerable differences exist in the vertical distribution of the two species. On the whole *G. perincisum* is the most isolated geographically though all the three species have distributional areas more or less overlapping in many places.



The three taxa in question differ by their ecological requirements and their distributional areas. They also differ morphologically mainly by the shapes of the caudical and stem leaves. In *G. macrophyllum* in caudical leaves the terminal leaflet is big, round or reniform, has three shallow incisions and a cordate, shallowly and widely serrate base. The much smaller lateral leaflets are numerous, close together and also rounded. The caudical leaves of *G. oregonense* have a deeply and sharply serrate terminal leaflet smaller than in *G. macrophyllum* with a rhomboid base and usually three deep incisions. The lateral leaflets are fewer, their shape is that of an ovoidal rhomboid and they are sharply and deeply serrate at the ends. The *G. perincisum* caudical leaves have tripartite terminal leaflets with deeply incised, sharply serrated lobes. Similar variations are to be observed in the lateral leaflets. In the case of all these three species a strong leaf rosette appears in the first year of plant life. During the next year numerous floral stems grow out from the leaf axils on the caudex. Though these are perennial plants, they usually perish in culture conditions after flowering for 3—4 seasons. The floral stems are robust, thick and terminated by multifloral inflorescences. Stem and leaves are covered equally by dense, prickly bristles. The floral stems of *G. perincisum* are markedly longer than those of the two other species. The floral stem lengths were during seeding in 1951:

<i>G. macrophyllum</i> ,	maximum length	85 cm,	mean length	80 cm
<i>G. oregonense</i> ,	„ „	100 „	„ „	92 „
<i>G. perincisum</i> ,	„ „	115 „	„ „	98 „

In all the three species the stems are multifoliate. The lower leaves are multifoliate, while the middle and the upper ones are trifoliate, all have stipules. The lower parts of the stems are covered with outstanding bristles and on the upper parts of stems there are many short, soft hairs. Glandular hairs often grow on the floral peduncles and on the styles. Glandular hairs are much more common on the floral peduncles in *G. oregonense* than in *G. perincisum* and *macrophyllum*. The anthocyanin is much more abundant in the stems of *G. perincisum* than in the other two species. The stems of all three taxa are branched. Usually the inflorescence of *G. macrophyllum* and *G. oregonense* is composed of 13—15 flowers and that of *G. perincisum* of 7—9 flowers. In *G. macrophyllum* at anthesis the floral peduncles are very short and the inflorescence forms a tight pseudo-umbellum; it is not till when the seeds begin to set that the floral peduncles elongate to the length of 4—5 cm. In *G. perincisum* and *G. oregonense* the floral peduncles begin to grow out already at anthesis and at fruiting their lengths are 7—8 cm and 10 cm respectively.



The flowers of all the three taxa are very much alike and they vary only by the size and the colour of petals which have a yellow tint more pale in *G. oregonense* than in *G. macrophyllum* and *perincisum*. The dimensions of the petals are:

Length in mm	4	5	6	7	8	9
<i>G. macrophyllum</i>	4	19	3			
<i>G. perincisum</i>		4	50	4		
<i>G. oregonense</i>		4	13	19	3	
Breadth in mm						
<i>G. macrophyllum</i>	21	4				
<i>G. perincisum</i>	9	31	9			
<i>G. oregonense</i>			4	27	8	

It appears from the table that the petals of *G. oregonense* are the largest (though Bolle states that they are smaller than those of *macrophyllum*), and that *G. macrophyllum* petals are the smallest. However, these differences are not considerable and it appears from herbarium specimens that within the species *G. macrophyllum* there are various forms with different petal size.

The receptaculum of all the three species is long and cylindrical, it grows out directly from the flower or from a short, 0.3—0.5 mm long gynophore. The receptaculum length in *G. macrophyllum* and *G. oregonense* is 9—10 (12) mm, *G. perinsicum* it averages at 10—11 mm. To the receptaculum numerous small achenes are attached. In *G. macrophyllum* the number of achenes is 150—200 and the average number is approximately 180, while in *G. perincisum* and *G. oregonense* the achenes are more numerous and there are up to 230 or an average 200 of them on one receptaculum. The ripe infructescence is usually elongated, oval or ovoid, in *G. perinsicum* it is usually more elongated than in the remaining two taxa. The achenes have an elongated base, they are spindle-shaped, covered with numerous bristles and short hairs, and approximately 3 mm long. At the top of the achenes there is a hooked rostrum from which the stigmatic part tends to fall off. The length of the stigmatic part is approximately 1.5 mm and is more or less equal in all the three taxa. The rostrum length varies considerably in the three kinds of plants and is in:

<i>G. macrophyllum</i>	from 3	to 4.5 mm	mean 4.1 mm
<i>G. perincisum</i>	„ 2	„ 3.5 mm	„ 2.8 mm
<i>G. oregonense</i>	„ 3.5	„ 5.0 mm	„ 4.6 mm

The fertility of the three taxa cultivated in Warsaw is high and usually surpasses 90%.

## II. $F_1$ AND $F_2$ HYBRIDS OF *G. MACROPHYLLUM*, *PERICISUM* AND *OREGONENSE*

### The $F_1$ generation

During the years 1948 to 1950 I made repeated crosses between the three taxa and I obtained the three possible hybrids in either direction. There are no significant differences between the reciprocal hybrids and no distinct intersterility barriers between the three taxa. The drop in seed setting after cross pollination is small and seed setting is at 75–80% of normal. The aspect of the hybrid seeds is normal, they are full, well grown and have a high and quick germination rate of 80% and more. The hybrid plants are very vigorous throughout their lives from the young seedling and rosette stage to the later developmental stages, they blossom and seed abundantly. The  $F_1$  show the very characteristic hybrid vigour and often surpass the parent forms by the size of leaves, the height of stems and the number of flowers on the stems. For instance, the length of a caudical leaf in the  $F_1$  *G. macrophyllum*  $\times$  *perincisum* hybrid may be as much as 40 cm and the terminal leaflet is 18 cm long and 20 cm broad, while  $F_1$  *G. macrophyllum*  $\times$  *oregonense* hybrids have caudical leaves which may reach 48 cm in length with terminal leaflets 12 cm long and 20 cm broad. The length of the stems is also much greater than the length of stems in the parent forms, e. g. in 1951 the length of the stems in the  $F_1$  *G. macrophyllum*  $\times$  *perincisum* hybrids ranged from 120 to 135 cm, the average being 130 cm. In this last case the number of flowers on each stem was 21–25.

The shape of the caudical and stem leaves in the hybrids as shown in Figs 7–9 is approximately intermediate between the two parent forms. The photographs in Figs 7, 8 a. 9 show that the leaves of the  $F_1$  *G. macrophyllum*  $\times$  *perincisum* hybrid are shaped somewhat like the leaves of *G. oregonense*. The size of the petals in the hybrids is given by the following table:

Length in mm	4	5	6	7	8	9	10
$F_1$ <i>macrophyllum</i> $\times$ <i>oregonense</i>		2	8	10	22	1	
$F_1$ <i>macrophyllum</i> $\times$ <i>perincisum</i>		9	43	7			
$F_1$ <i>oregonense</i> $\times$ <i>perincisum</i>				14	18	17	
Breadth in mm							
$F_1$ <i>macrophyllum</i> $\times$ <i>oregonense</i>			8	23	12		
$F_1$ <i>macrophyllum</i> $\times$ <i>perincisum</i>	4	30	21	3			
$F_1$ <i>oregonense</i> $\times$ <i>perincisum</i>			10	25	9		

When the dimensions of the petals in the hybrids and in the parent species are compared it appears that in crosses of *G. macrophyllum* with *G. oregonense* or *G. perincisum* the petal size is more related to the latter two species. The shape and the size of the achenes, the rostrum and the stigmatic part are in the hybrids intermediate between the parent forms. There is a marked drop in pollen and achene fertility in all the three hybrids.

Hybrids	Pollen fertility %	Achene fertility %
F <sub>1</sub> <i>macrophyllum</i> × <i>perincisum</i>	53,1	32,1
F <sub>1</sub> <i>macrophyllum</i> × <i>oregonense</i>	54,6	40,3
F <sub>1</sub> <i>perincisum</i> × <i>oregonense</i>	47,3	46,2

This percentage data were computed from numerous countings during several seasons.

### The F<sub>2</sub> generation

In 1954 I had F<sub>2</sub> generations derived from each of the F<sub>1</sub> crosses by mutual pollination of several specimens in each F<sub>1</sub> group. In the F<sub>2</sub> there is in respect to F<sub>1</sub> an appreciable drop in the viability and the vigour of

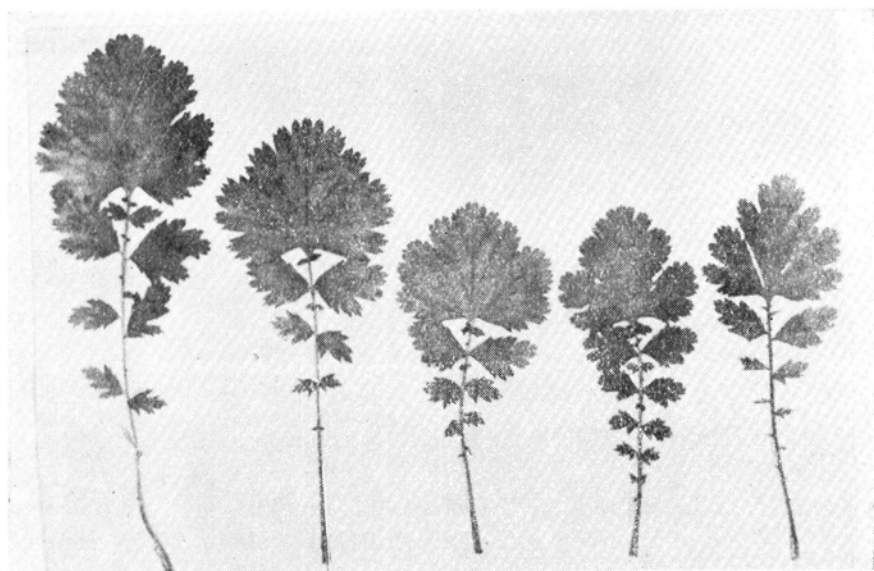


Fig. 10. Basal leaves of some F<sub>2</sub> hybrids *G. macrophyllum* x *perincisum*.

the plants. Some 10—15% of the plants developed feebly and finally perished at various developmental stages. However, among those which survive numerous specimens resemble the  $F_1$  plants in their vigorous development and abundant flowering and seeding. In  $F_2$  there is a mark-

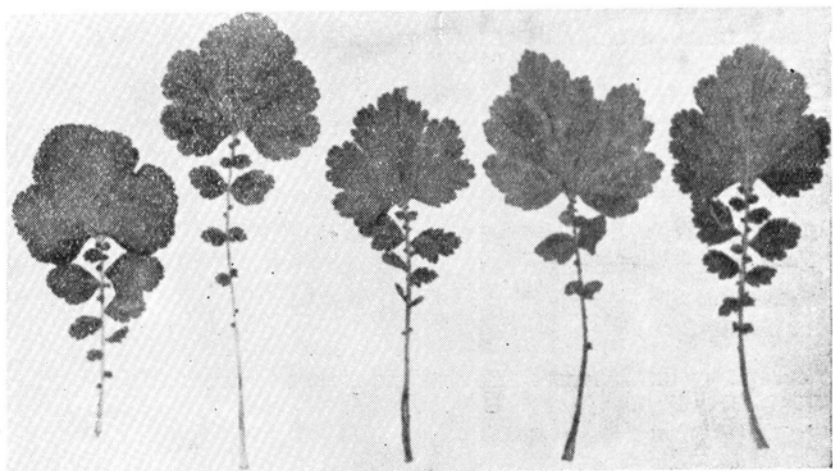


Fig. 11. Basal leaves of some  $F_2$  hybrids *G. macrophyllum* x *oregonense*.

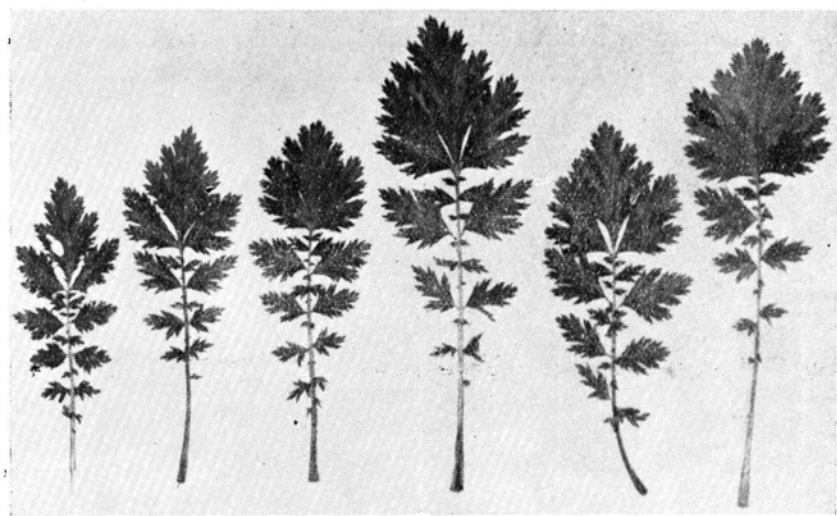


Fig. 12. Basal leaves of some  $F_2$  hybrids *G. oregonense* x *perincisum*.

ed segregation in respect to the shape of caudical and stem leaves. In spite of the small number of  $F_2$  individuals — about 50 in each of the three groups — some of them resemble greatly the parent forms; e. g. 2 out of the 42  $F_2$  plants derived from a cross between *G. oregonense* and

*G. perincisum* had leaves very like the leaves of *G. perincisum*. The nature of the segregation in the  $F_2$  leaf shape is represented in Figs 10—12. In  $F_2$  plants segregation within the limits of parent forms is also apparent in respect to such characters as the length of stems, blossoming date, petal size and the shape of the achenes.

The pollen and achene fertility in  $F_2$  may be judged from the table below:

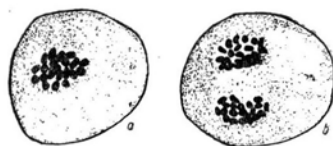
Fertility %		10	20	30	40	50	60	70	80	90
$F_2$ <i>macroph.</i> × <i>perincisum</i>	pollen			3	11	6	10	14	5	5
	achenes			5	5	4	10	17	11	2
$F_2$ <i>macroph.</i> × <i>oregonense</i>	pollen	1	3	4	10	16	7	5	2	
	achenes	4	2	4	10	13	11	2	2	
$F_2$ <i>oregonen.</i> × <i>perincisum</i>	pollen			3	5	12	6	7	9	
	achenes			2	7	3	15	9	6	

It is apparent from this table that pollen and achene fertility of most plants in the  $F_2$  progeny of each of the crosses is higher than in the corresponding  $F_1$  hybrids. In some of these plants the fertility is almost normal, above 80%. There seems to be no correlation between the higher seed fertility and the general appearance of plants more related to the parent type. For instance in the  $F_2$  progeny of *macrophyllum* × *perincisum* from a group of 10 individuals with an achene fertility over 70% only two had leaves resembling the *perincisum* type, the leaves on the remaining plants were of the  $F_1$  type. On the other hand, among 14 plants in which the achene fertility was 20—40% two had leaves related to *perincisum* and the leaves on the remaining plants were related to  $F_1$ .

### III. CYTOLOGICAL CONDITIONS WITHIN THE GROUP

All the three taxa are hexaploid with  $2n = 42$  and in Figs 13 a. 14 may be seen metaphase plates of the first PMC division with 21 bivalents from

Fig. 13. *G. perincisum* Rydb.: a — metaphase I with 21 bivalents, b — anaphase I with 21 chromosomes in both groups.



*G. perincisum* a. *G. oregonense*. The course of meiosis in *G. macrophyllum* was already published (W. Gajewski 1949, 1953). The course of

meiosis in the parent species is absolutely normal and only in 2—3% of *G. oregonense* PMC's one or two chromosome bridges are visible at anaphase. They may be the result of inversions. Also in the PMC's of the  $F_1$  hybrids meiosis develops very normally, only in some PMC's a few univalents may sometimes be found at metaphase I. Among 165 PMC's of the  $F_1$  *macrophyllum* x *perincisum* hybrid at the M I stage only 3 cells with 2 univalents and one cell with 4 univalents were found, in the remaining 161 PMC's the course of meiosis was as normal as in the parent species. In the  $F_1$  *G. macrophyllum* x *oregonense* hybrid only in 6 out of 65 PMC's 2 or 4 univalents were found at M I and A I. Finally in the  $F_1$  *G. oregonense* x *perincisum* hybrid the number of univalents in 80 PMC's at the M I stage was 2 in 3 PMC's, 4 in 12 PMC's, 6 in 4 PMC's, and 8 in 1 PMC,



Fig. 14. *G. oregonense* R y d b.: a — metaphase I with 21 bivalents, b — metaphase II with 21 chromosomes in one plate, c — anaphase I with a „bridge“.

i. e. somewhat higher than in the other cases. In anaphase at least some of the univalents lag in the spindle and do not move to the poles till after the bivalents have separated. Usually in very few cells only single chromosomes lag at late anaphase and telophase and are not included in the daughter nuclei. The course of the second meiotic division and the appearance of the tetrads is in all the three  $F_1$  hybrids almost normal. Nevertheless, nearly half of the young pollen grains degenerate.

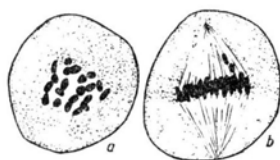


Fig. 15.  $F_1$  *G. macrophyllum* x *perincisum*: a — metaphase I with 21 bivalents, b — metaphase I with 2 univalents

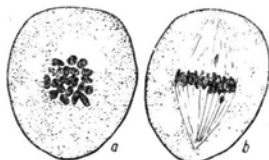


Fig. 16.  $F_1$  *G. macrophyllum* x *oregonense*: a — metaphase I with 21 bivalents, b — metaphase I with 2 univalents.

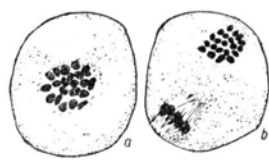


Fig. 17.  $F_1$  *G. oregonense* x *G. perincisum*: a — metaphase I with 21 bivalents, b — metaphase II with 21 chromosomes

In  $F_2$  *G. macrophyllum* x *perincisum* and  $F_2$  *G. macrophyllum* x *oregonense* root tips were examined in several plants for the chromosome

number. Root tips were obtained from 12 young seedlings of the two hybrids (5 and 7 respectively) and it was found that the somatic chromosome number was always 42. Meiosis of  $F_2$  plants was examined in aceto-carmin smears and it was found that in most plants meiosis was either normal or with very few univalents in some rare PMC's. The marked segregation of fertility in  $F_2$  cannot be correlated with the disturbances in meiosis.

#### IV. SEXUAL ISOLATION OF THE GROUP FROM OTHER SPECIES IN THE SUBGENUS *EUGEUM*

Mention has already been made of the almost complete isolation of *G. macrophyllum* from all other species of the subgenus *Eugeum* the isolation which is made evident by the all but perfect sterility and the frequent very low viability of the hybrids (W. G a j e w s k i 1953). It is now apparent that in this respect *G. perincisum* and *G. oregonense* behave in the same way as *G. macrophyllum*. The fertility of crosses of the three taxa with some other species from the subgenus *Eugeum* is illustrated by the following computation:

Achene fertility of $F_1$ hybrids in %							
Hybrid with:	<i>G. rivale</i>	<i>G. urbanum</i>	<i>S. aleppicum</i>	<i>G. coccineum</i>	<i>G. laciniatum</i>	<i>G. vernum</i>	<i>G. silvaticum</i>
<i>G. macrophyllum</i>	0,0	0,0	0,0	0,0	0,0	0,05	0,05
<i>G. oregonense</i>	0,1	0,0	0,02	0,01	0,0	0,05	0,05
<i>G. perincisum</i>	0,1	0,0	0,01	?	0,01	0,05	?

This evidence shows the almost complete sterility of all the hybrids from crosses with 6 species in the subgenus *Eugeum* and with *G. vernum* from the subgenus *Stylipus*. Crosses of *G. aleppicum* and *G. urbanum* with the three taxa from the *macrophyllum* group show a marked drop of viability, have yellow green leaves and deformed flowers, and usually perish before blossoming.

Some time ago I was able to demonstrate that crosses of *G. macrophyllum* with all but a few *Geum* species gave hybrids with meiosis of an asyndetic type in which at metaphase of the first PMC meiotic division univalents were predominant. Cytological studies have shown that also in crosses of *G. oregonense* and *G. perincisum* with the other species from the subgenus *Eugeum* meiosis was of the same asyndetic type. This I was able to demonstrate on crosses of: *G. rivale* with *G. oregonense* and *perin-*

*cisum*, *G. urbanum* with *G. oregonense* and *perincisum*, *G. laciniatum* with *G. oregonense* and *perincisum*, *G. silvaticum* with *G. oregonense*, *G. vernum* with *G. perincisum* and *oregonense*, and finally *G. aleppicum* with *G. oregonense* and *perincisum*.

I described in detail in an earlier paper (W. G a j e w s k i 1949) the course of meiosis in the *G. macrophyllum* x *rivale* hybrid. Now it appears that in crosses of *G. rivale* with *G. oregonense* and *perincisum*

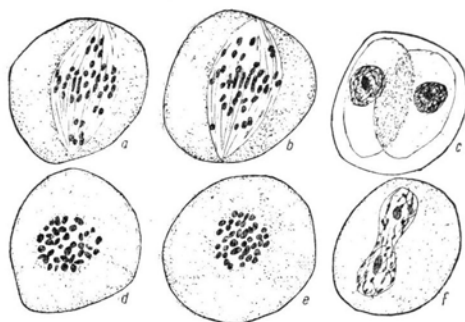


Fig. 18. *F*<sub>1</sub> *G. perincisum* x *rivale*: a — metaphase I with 3 bivalents and 36 univalents, b — polar view of metaphase I with ca 5 bivalents and ca 32 univalents, d — metaphase I with 5 bivalents and 32 univalents, e — polar view of metaphase I with ca 4 bivalents and ca 34 univalents, f — telophase I — restitution nucleus, c — a dyad.

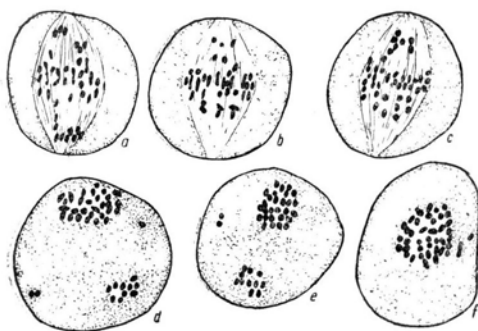


Fig. 19. *F*<sub>1</sub> *G. oregonense* x *rivale*: a — metaphase I with ca 5 bivalents, b — metaphase I with 7 bivalents and 38 univalents, c — metaphase I with 42 univalents, d — very irregular metaphase II, e — metaphase II with abnormal distribution of the chromosomes, f — metaphase II from restitution nucleus.

the course of meiosis is of the same kind. This may be seen from Figs 18 a. 19 and from the table illustrating chromosome configurations in the PMC's of these hybrids at metaphase of the first division:



Chromosome configuration	42I	1II + 40I	2II + 38I	3II + 36I	4II + 34I	5II + 32I	6II + 30I	7II + 28I
<i>G. rivale</i> × <i>macroph.</i>	3	5	4	5	6	2	5	1
<i>G. rivale</i> × <i>oregonen.</i>	2	3	4	3	—	1	—	—
<i>G. rivale</i> × <i>perincis.</i>	8	3	2	5	5	3	1	2

In these hybrids at metaphase and anaphase numerous disturbances occur in the separation of univalents giving frequent restitution nuclei and consequently after the second division dyads are formed in 4—5%.

Very interesting are the reduction divisions in *G. aleppicum* × *oregonense* and *G. aleppicum* × *perincisum* hybrids during which in most PMC's univalents alone are present at metaphase of the first division. The univalents are arranged into a fairly regular plate lying in the equatorial plane of the spindle. However, in some pollen sacs several bivalents usually appear beside the univalents, and then the univalents do not form the metaphase plate but are scattered all over the spindle; it is not till anaphase that the univalents form a more or less regular plate. In a *G. aleppicum* × *oregonense* hybrid I found on an aceto-carmin smear preparation prepared before fixation in Navashin fluid one anther in which meiosis seemed to be normal and in which PMC's with only bivalents at metaphase were predominant. Unfortunately, on fixed slides prepared from buds fixed at the same time and from the same plant I found no other examples of this normal course of meiosis. Presumably the course of meiosis in this hybrid can vary under the influence of some unknown internal or external conditions regulating asynapsis. The further investigations necessary to elucidate this question were impossible as the plants all had a low viability and perished during their second winter season. In an earlier paper I reported that in the cross *G. macrophyllum* × *G. aleppicum* meiosis was of a kind in which bivalents are predominant at metaphase of the first division, now however, I believe it to be very likely that in this hybrid asyndetic meiosis will be found if a sufficient number of buds fixed at various stages and from many plants are examined.

## V. DISCUSSION

The question to be answered now is: how far is it justifiable to decide on the basis of this cytogenetical investigation whether the three taxa concerned form separate species or are only varieties if not subspecies of the species *G. macrophyllum*? The "biosystematic" principles of classi-

fication established by Clausen, Keck and Hiesey (1939, 1945) are based mainly on the genetic barriers which make impossible gene interchange in crosses. On these principles the three taxa in question should be classified as various ecotypes in one ecospecies. However, if these principles of classification are followed strictly, it may happen indeed that whole sections of a genus or sometimes the entire genus will be classified as one species. It seems therefore that the method worked out by Clausen and others cannot always be applied for practical purposes. In Nature there are undoubtedly genera in which the specific differentiation process cannot be regarded as parallel to the process in which physiological intersterility barriers are formed.

When it is considered 1) that often in the subgenus *Eugeum* even so distinct species as, for instance, *G. urbanum* and *G. rivale* give highly fertile and viable hybrids in  $F_1$  and further generations and 2) that mutual crosses between the three taxa in question show a marked drop in pollen and achene fertility, it is reasonable to consider them as three separate, though related, species. Although, the data at my disposal are not complete, they nevertheless seem to indicate that the three species have partly disconnected distributional areas and different ecological requirements.

Undoubtedly it is a question of a fairly recent differentiation in an originally uniform population which took place probably in the pleistocene. The original population differentiated into three distinct populations separated already by the partial hybrid sterility. The common origin of these three species is indicated not only by the morphological similarities and the partial hybrid fertility, but also by their faculty to give asyndetic or sterile hybrids with the other species from the subgenus *Eugeum*. It is most unlikely for each of these species to develop on its own the set of factors which would cause asyndesis or sterility in crosses with the other *Eugeum* species while in mutual crosses between them normal conjugation and a relatively high fertility are retained.

It is possible that *G. macrophyllum*, the distributional area of which spreads along the Rocky Mountains from California to Alaska and through the Aleutian Islands to north east Asia and forms moreover a disjunctive area on the Atlantic coast from the Labrador to the Great Lakes, is the oldest of these species. According to Fernald (1925) some plant species survived through at least a part of the glacial epoch in the refuge regions in Alaska and Labrador and to the south of the pleistocene glaciations. During the glacial and post-glacial migrations conditions favouring isolation might have prevailed which gave rise to such new separate species as *G. perincisum* of which the present distribution in Canada co-

incides exactly with the areas once covered by ice. *G. oregonense* and *G. macrophyllum* have different vertical distributions and it may be that the isolation of the former was caused by a change in the vertical distribution of plants which took place outside the pleistocene glaciation areas. Hultén (1937) considers *G. perincisum* to be a radiant to the north of the Behring Sea area which during the interglacial D (according to his terminology) moved so far to the south that it survived the maximum glaciation and then spread again to the north. It is of course difficult to judge on the accuracy of such suppositions.

However, it is certain that the group investigated here is a very good example of how new species arise in conditions which cause a break-up in the original population and produce little change in the chromosome number and homology, while a gradual genetical differentiation between the isolated populations takes place.

From the point of view of systematic morphology and the well founded principle of not classifying the plants into too many species it would be justifiable to include *G. perincisum* and *G. oregonense* as subspecies of *G. macrophyllum*. On the other hand, when the drop by approximately 50% in hybrid fertility as well as the geographical and probably also ecological isolation of these taxa is considered it becomes apparent that at present they form separate populations. The future evolution of these populations will run independently one of the other and for this reason each of them should now be considered as a distinct, though not yet perfectly separated species.

In south Europe three species from the subgenus *Eugeum* are known; they are *G. rivale*, *coccineum* and *silvaticum* and their chromosome number is also  $2n = 42$ . The crosses between these species are highly fertile and have a normal meiosis with 21 bivalents in most PMC's. However, the differences in their morphology, geographical distribution and ecological requirements are so great that nobody doubts that they are, indeed, three distinctly separate species. It is my opinion that the specific differentiation process through isolation and gradual differentiation is the same in both these specific groups the difference lying only in the various stages in each case. In *G. macrophyllum*, *perincisum* and *oregonense* the process is at present still at an early stage, while in the south European species it is well advanced.

#### SUMMARY

1. *G. macrophyllum* Willd., *G. perincisum* Rydb., and *G. oregonense* Rydb. are three distinct, closely related species with partly separate geographical distributions and various ecological requirements.

2. The hybrids between these species show a drop in pollen and achenefertility by approximately 50%, though the course of meiosis is almost normal.

3. In  $F_2$  both morphological characters and fertility show a considerable and continuous variation and some plants have a much higher fertility than in  $F_1$ .

4. The three species of this investigation are isolated from the other species of the subgenus *Eugeum* by all but perfect sterility of hybrids which have an asyndetic kind of meiosis.

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