

CYTOTAXONOMIC NOTES ON SOME GALIUM SPECIES GALIUM BOREALE L. I

BY

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SUMMARY

Cytological investigations within *Galium boreale* L. showed the occurrence of tetraploids ($2n=44$) as well as hexaploids ($2n=66$) in Europe.

Comparative morphological studies failed to demonstrate any differences in characters between the two cytotypes. Crosses between the tetraploid and hexaploid were unsuccessful, due to the occurrence of a strong and effective barrier between the two levels of ploidy.

From a taxonomical point of view the two cytotypes are considered as to belong to the same taxon.

INTRODUCTION

Galium boreale L. is a circumpolar collective species. The distribution on the various continents is as follows:

EUROPE: North-, Central- and East Europe, including the North of the British Isles and Iceland.

South Europe: eastern France as far as the Alpes Maritimes and Plateau Central, the North of Italy, the Balkan peninsula (Montenegro), and the Caucasus.

In West- and South-West Europe (The Netherlands, Belgium, the West of France, Portugal and Spain) only in some localities.

ASIA: Asia minor, Armenia, Central and East Asia (Kamchatka, Sakhalin, Manchuria, Korea, The North of Mongolia and the North of China, the Japanese Isle Hokkaido).

AMERICA: From Alaska to Gaspé in Quebec, sparingly distributed in the North- and North-East of Canada.

South to the North of California and New Mexico. South of the big lakes into Virginia.
Greenland.

Galium boreale was first described by LINNAEUS in his "Species Plantarum" (1753) from material collected in northern Europe. ("Habitat in Europae borealis pratis"). It is exceedingly polymorphic; there is a great variability in the height of the plants, the ramification, the shape of the

panicle, length and width of the leaves, and the shape of the bracts and the petals. The indument also shows great variation, notably on the fruits.

Many investigators have tried to find ways towards a satisfactory classification within this complex of variable characters. The first to offer a clear example was DE CANDOLLE (1830) whose subdivision utilizes hair shape and density of the indument on the fruits.

De Candolle placed *Galium hyssopifolium* Hoffmann, described as a species in 1800, as a variety under *Galium boreale*, characterized by its fruits having no indument. (a. - hyssopifolium - fructu glaberrimo). Plants with hairy fruits fall under two other varieties, viz. b. - intermedium (fructu subscabro, setis subapressis) and c. - scabrum, (fructu setis confertis apice subuncinatis hispidis) = var. typicum Beck 1893 = *G. boreale* L. var. boreale.

This subdivision of *Galium boreale* into three varieties, was applicable to European representatives in the first place. ("in uliginosis montosis Europae totius").

Galium septentrionale ROEMER et SCHULTES (1818), from North-America, was still treated as a species by De Candolle. ("In Am. bor. and Canadae lacus Nov.-Eboracum). Later authors, including Roemer and Schultes, however, considered *Galium septentrionale* as identical with *Galium boreale*. Ever since the species has been treated in a broad sense with a large distribution, although this does not seem very satisfactory when one looks at the great complexity of morphological characters. Such is particular the case with plants from eastern Asia: these differ widely among each other in length and width of the leaves and stand apart from plants elsewhere in the world in having a different indument, which also shows great variation. It is not surprising, therefore, that botanists who studied this wealth of morphological diversity within eastern Asian *Galium boreale* arrived at a larger number of varieties.

Thus CUFODONTIS, in his revision of *Galium* in China (1940), described ten varieties. This was not the real solution of the problem: as pointed out by Cufodontis (l.c.) himself. Morphological characters may occur in almost any combination in the area studied. About 25 % of the Chinese *Galium boreale* could not be assigned to one of the ten varieties (URSCHLER 1955). NAKAI (1939) who also studied material from eastern Asia distinguished six varieties, mainly based on foliar characters. HULTEN, (1930), notes the frequent occurrence of a variety with broadly ovate leaves and a strongly aberrant indument. This variety is identical with var. *kamchaticum* MAXIMOWICZ (1895).

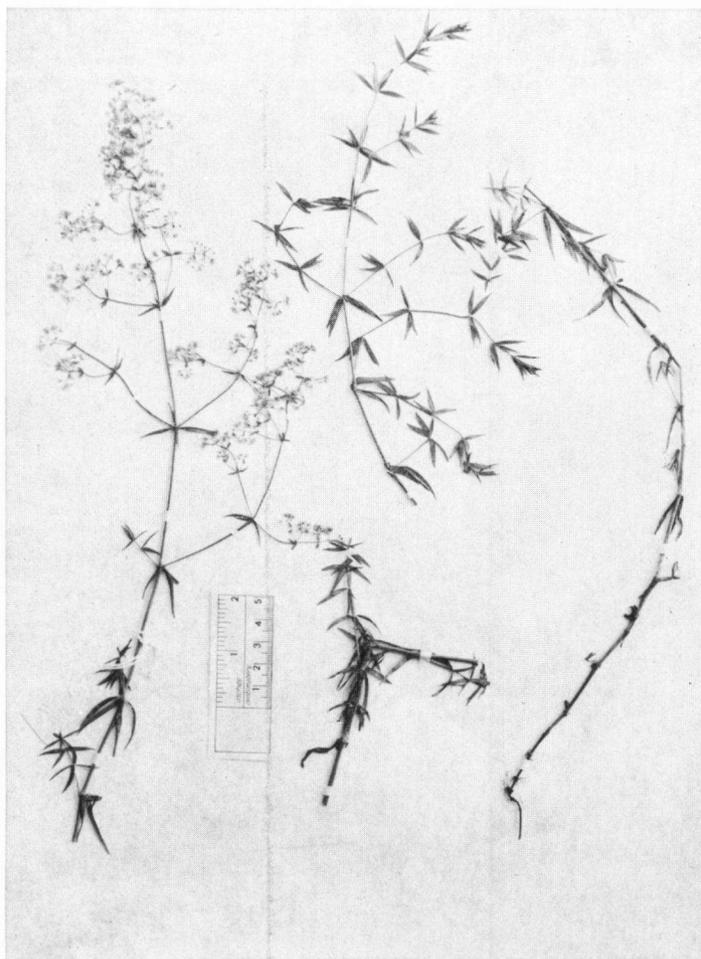
In Europe and America the variability is much less pronounced, but nevertheless one can detect differences in the description of vars. *boreale* and *intermedium* when comparing floras of Europe with floras of America. Thus FERNALD, in Gray's Flora Americana (1953), states for *Galium boreale* var. *boreale*: "fruits villous hirsute with long hairs" and for var.

PLATE 1



Galium boreale L., nr. K 237, plant from Denmark with a narrow pyramidal panicle.
Sixth year of cultivation.

PLATE 2



Galium boreale L., nr. K 607, plant from Czechoslovakia with a broad pyramidal panicle. Sixth year of cultivation.

PLATE 3

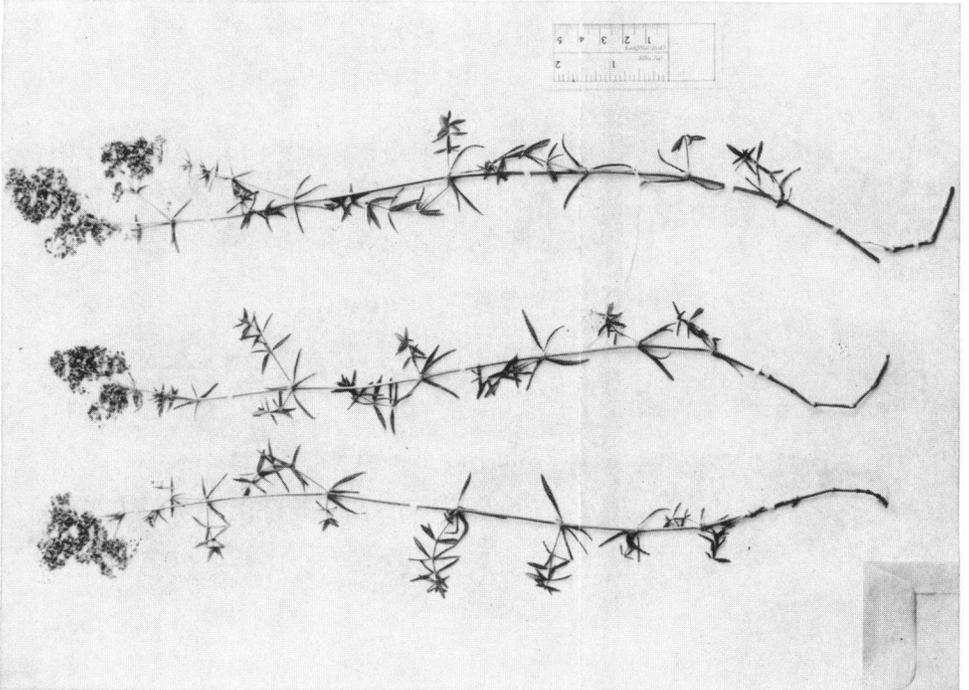
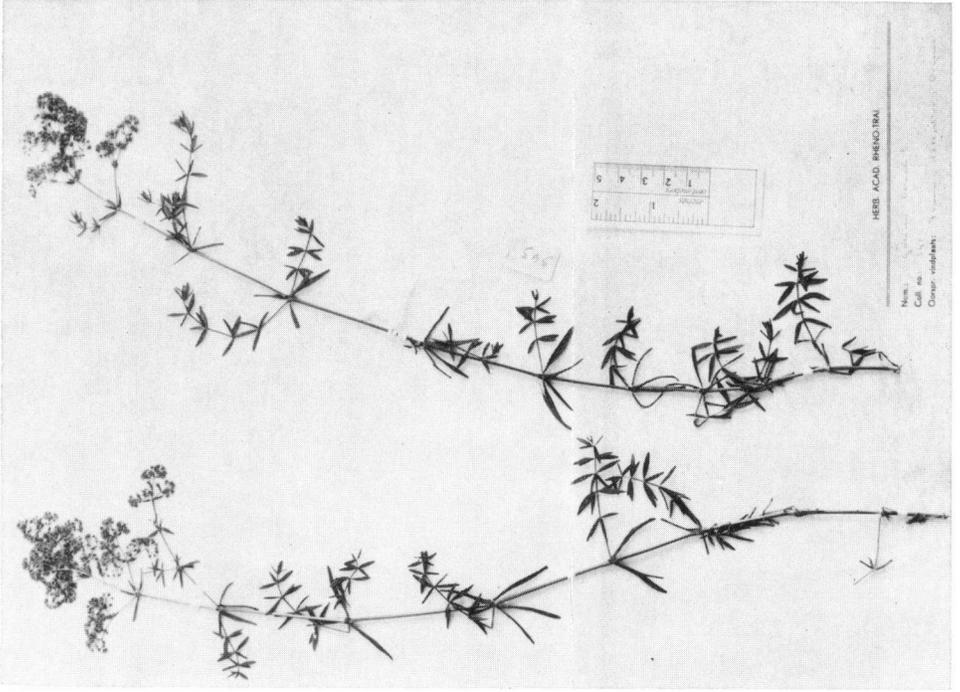


PLATE 4



3c.

Galium boreale L., plant from France, showing the constancy of the shape of the panicle over three successive years of cultivation. 3a the fourth year, 3b the fifth year, 3c the sixth year.

intermedium: "fruits covered with short appressed or incurving hairs". No mention is made of uncinata hairs in *Galium boreale*, a character found in every European Flora. Var. *intermedium* is described in European Floras as having fruits covered with short, firm, appressed hairs.

The complex of *Galium boreale* was again, and more thoroughly, investigated by LÖVE and LÖVE (1954). These authors included experimental observations and tried to find a correlation between morphology, cytology and geographical distribution. Comparative morphological study of living material from Iceland, Scania (Sweden), and Manitoba (Canada), as well as herbarium material from the whole area, led them to the conclusion that, in fact, there are two taxa, each of them defined by a distinctive combination of characters; these characters, when taken individually, are not necessarily restricted to only one of the two taxa and may occur in both. The two taxa are an Eurasiatic one, from Europe, including Iceland, to central Siberia, and an American-Asiatic one which occurs in America and East Asia, penetrating deeply into central Asia. The former has nodes glabrous or with scattered hairs, circular-ovate and obtuse bracts, the color of the petals is creamy. The panicle is less divaricated as the panicle from the American Asiatic type.

From this latter type are the nodes clearly puberulent, the bracts oblong, acute and the color of the petals is bright white. According to the results of biometric observations there is a difference in the length and width of the corolla, in the length of the fruits, and the anthers in the two taxa. In cytological investigations only tetraploids were found in material from Europe, and only hexaploids in material from North-America. The combination of characters mentioned above that define the two taxa, according to Löve and Löve, are clearly and strictly associated with the two cytotypes.

The occurrence of tetraploids and hexaploids in *Galium boreale* was known. HOMEYER (1935) reported $2n=44$ and $2n=66$ in material from unknown origin; these results, therefore, do not provide any information on geographic distribution. FAGERLIND (1934, 1937) found $2n=44$ in plants from Sweden, TURESSON (1938) $2n=66$ in plants from North China (Shansi), POUQUES (1949) $2n=44$ in plants from France, whereas BÖCHER and LARSEN (1950) reported $2n=44$ for material from Denmark and Iceland, and $2n=66$ for material from Alaska.

Geographical distribution, morphology, but particularly cytology, according to Löve and Löve provide sufficient arguments to consider the two taxa as different species. The Eurasiatic taxon should be identical with the *Galium boreale* described by LINNAEUS, (1753) and the American-Asiatic taxon with the *Galium septentrionale* described by ROEMER and SCHULTES, (1818). When the views of Löve and Löve are accepted, the position of the varieties *boreale*, *intermedium* and *hyssopifolium* needs a revision. URSCHLER shortly afterwards (1955) examined herbarium specimens collected in America, Europe and Asia. She could demonstrate that

denseness and length of the hairs on the fruits has every form of transition from one extreme into the other and that, consequently, it was impossible to segregate a variety with closely appressed hairs. The hairs of all European and of nearly all Asiatic herbarium plants were curved and uncinatate at the tip. In America and eastern Asia, besides plants with glabrous fruits and those with fruits covered with curved hairs, there are also plants with straight-haired fruits.

Urschler finally arrives at the following subdivision: for Europe and Asia: *Galium boreale* L. var. *boreale* (fructus pilis uncinatis vel incurvis obtecti) and *Galium boreale* L. var. *hyssopifolium* (Hoffm.) DC. (fructus glabri), and for America and Asia *Galium septentrionale* Roem. et Schult. var. *septentrionale* (fructus pilis rectis patentibus obtecti), *Galium septentrionale* Roem. et Schult. var. *incurvatum* Urschler (fructus pilis leviter incurvis vel uncinatis obtecti) and *Galium septentrionale* Roem. et Schult. var. *glabrum* Urschler (fructus glabri vel glabrescentes).

The splitting of the complex into two species, as by Löve and Löve, is not generally accepted, however. HARA (1956) draws attention to plants from Yezo, the Kurilles and Sakhalin which always have bristly fruits and hairy nodes, but in other morphological characters as brought up by Löve and Löve resemble *Galium boreale*, rather than *Galium septentrionale*. He reduced *Galium septentrionale* to a subspecies of *Galium boreale* and indicated its occurrence in America, also in Greenland, but not in Asia. HULTEN (1958) considers the interrelationships within *Galium boreale*, sensu lato, as so intricate that he rejects both the view of Löve and Löve and that of Hara.

Although Löve and Löve found absolute correlation between the two species and their respective chromosome numbers, later investigators, however, also saw hexaploids besides tetraploids, within the area of the Eurasiatic taxon (PIOTROWIC 1959, 1961; RAHN, 1961). RAHN (l.c.) who carried out morphological investigations on cultivated material from Europe was unable to find any character whereby the tetraploid and hexaploids could be separated. Characters studied included the height of the plants, length and width of the leaves, size of the pollengrains (with the aid of mathematical computations), length of the anthers, and length of the fruits. Neither could Rahn, in studies of herbarium material from the whole area arrive at a subdivision of the complex using the combination of characters provided by Löve and Löve: "Almost any combination of the morphological characters seems to be possible within this species complex".

Galium boreale is a good example of the complexity of a collective species. Despite due attention having been paid to this complex, there still is much that is not clear.

Within the framework of general cytotaxonomic research of various *Galium* species the *Galium boreale* complex was also included. Combined cytotaxonomical and morphological studies were made on plants cultivated

under uniform conditions during several years, together with a morphological and geographical study of herbarium material.

The results of these investigations are presented and discussed below. This study does not pretend to give a final solution to the taxonomical problem of the *Galium boreale* complex, since there was not living material from the whole area available, and also because the author did not have herbarium material from a number of regions—particularly in East Asia, where the variability is greatest—at his disposal.

This paper merely aims at being a contribution towards such a final solution, if there will ever be one.

MATERIAL AND METHODS

Living plants dug out in the field as well as plants grown up from seeds collected in the wild were cultivated during several years in an experimental plot of the Botanical Garden of the State University of Utrecht. Methods used in the cytological investigations as well as in the crossing experiments are described in a previous paper. (KLIPHUIS, 1970). Measurements on the leaves and the corolla were made on living material. For this purpose the leaves were removed from the plant and put on graphpaper. The corolla was removed with the aid of a pair of tweezers and mounted between two strips of cello tape. (This turned out to be an excellent method. The strips can be kept, if desired, during several years).

The measurements of the corolla were made with the aid of a dissecting microscope, magnification $10\times$, (type Hensoldt-Wetzlar).

The statistical treatment of the measurements is in accordance with the common statistical method for calculating the mean (\bar{X}), the standard deviation (SD) and the standard error of the mean (SE). To demonstrate the variation amplitude the minimum and maximum value is also given.

The drawings of the metaphase plates of the roottip cells were made with the aid of an Abbé Camera Lucida, microscope used: Zeiss, type Junior Standard.

RESULTS

I. Cytology

a. chromosome numbers

The results of the chromosome counts are given in table I. In this table the place of origin, and the plant number are given. It may be observed that within the european distributional area tetraploids ($2n=44$) as well as hexaploids ($2n=66$) are found.

b. chromosome portraits

The chromosome portraits of both the tetraploids and the hexaploids show a regular pattern. No additional chromosomes or satellites were observed.

The chromosome portraits of the $2n=44$ cytotype and of the $2n=66$ cytotype are given in figs. 1 and 2 respectively.

TABLE I
Places of origin of the material investigated

Tetraploids (2n=44)

Czechoslovakia: K 607, near Praha; K 712, Bohemia.

Denmark: K 102, Fårup, Jutland; K 211, Blasmark, north of Varde, W. Jutland;
K 235, Sorø, Seeland; K 236, K 241, Ølstykke, Seeland; K 237, Rådvad, Seeland;
K 783, near Copenhagen.

Finland: K 1097, Varsinais, Nausiainen.

France: K 771, Annileau, Ohnenheim, Rieds-Rhénans; K 788, K 1102, near Orgeux
(Dept. Côte d'Or).

Iceland: K 1058, Bisky, Geysir.

Ireland: K 1103, Athlone, Co. Roscommon.

Norway: K 593, near Oslo; K 1090, Akers, Akershus; K 1152, K 1153, Rondane;
K 1157, Ørsthynfoss, Nordheimsund, Hordasland.

Poland: K 368, Szcawnica.

Sweden: K 331, near Stockholm; K 538 spontaneous, precise locality unknown;
K 1092, Vartofta Åsaka; K 1099, Kälvene Mäss; K 1101, Södermanland,
Botkyrka.

Switzerland: K 1098, Berner-Oberland, Swiss Alps.

U.S.S.R.: K 263, K 272, Estonia, near Tartu; K 790, near Leningrad.

Hexaploids (2n=66)

France: K 343, Côte d'Or (Dept. Côte d'Or); K 683, near Orgeux (Dept. Côte d'Or).
Roumania: K 1093, Finate.

Sweden: K 537, near Stockholm.

U.S.S.R.: K 590, near Leningrad; K 707, Latviya.

II. Morphology

Comparative morphological investigations were carried out in plants with $2n=44$ and with $2n=66$ chromosomes.

All plants investigated came from the european part of the distributional area.

Comparisons were made among plants cultivated during six successive years. Attention was paid to the shape of the panicle, the shape of the bracts, the leaves and the corolla. The indument, particularly that of the nodes, the leaves and the fruits, was also studied. The results of these investigations are described below:

THE PANICLE

The panicle is pyramidal in shape, varying from very narrowly pyramidal to very broadly pyramidal. Examples of the extreme forms are represented by plate 1 and 2 respectively.

The panicle shape remained constant through the years of cultivation. An example is plate 3 showing herbarium specimens from three successive years from an ancestral plant from France (fourth, fifth and sixth year of cultivation respectively).

The shape of the panicle is variable, but is constant for each individual. This applies to both cytotypes.

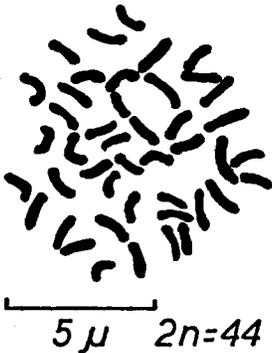


Fig. 1.

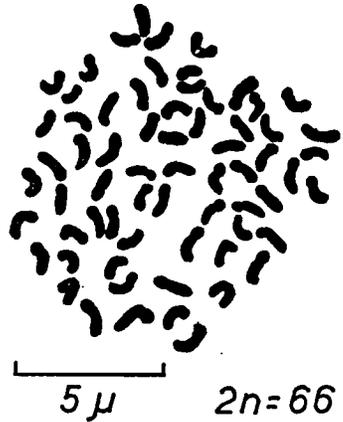


Fig. 2

Mitotic metaphase plates from roottips of *Galium boreale* L. Left: tetraploid ($2n=44$) from Denmark. Right: hexaploid ($2n=66$) from Sweden.

THE BRACTS

Bracts vary from ovate, obtuse to lanceolate, acute with a slight majority tending towards the first.

In both cytotypes the bracts appeared to be variable within each plant, even the two extremes occurring in the same individual.

THE LEAVES

The leaves are in whorls of four. They are lanceolate, attenuate towards both ends, somewhat obtuse and not mucronate at the top, with three parallel nerves, the lesser veins obsolete, with usually revolute margins, these mostly provided with minute antrorse aculii.

There is a great variability both in length and width of the leaves which would seem to render attempts at establishing any correlation with the cytotypes useless. To find out whether or not such a correlation actually exists, statistical methods were employed. Length and width of a number of leaves of both tetraploid and hexaploid plants were measured. The measurements were taken from plants during their fourth year of cultivation and were repeated during the two following years.

Length and width of 25 leaves of each plant was measured. The mean (\bar{X}), the standard deviation (S.D.), and the standard error of the mean (S.E.) were calculated. The results are shown on table II. The first column gives the plant number, the roman numerals in the second column indicate the first, the second and third year of observation (i.e. the fourth, fifth and sixth year of cultivation respectively), the third column lists the means (\bar{X}) and the standard error of the mean (S.E.) of the length and the width respectively. In the last column the minimum and maximum value is mentioned.

TABLE II

Length and width of the leaves in mm. The roman numerals indicate the first, second and third year of observation

Plant nr.		Length			Width		
		$\bar{X} \pm$ S.E.	range		$\bar{X} \pm$ S.E.	range	
Tetraploids: $2n=44$							
K 102	I	24.60	0.62	16-28	5.22	0.18	5.0-7.0
	II	19.32	0.52	14-25	3.52	0.13	2.0-5.0
	III	19.96	0.65	15-26	3.41	0.18	2.3-6.0
K 211	I	18.08	0.49	15-22	3.77	0.13	3.0-5.5
	II	21.68	0.47	18-26	3.29	0.11	2.0-4.0
	III	20.12	0.50	16-25	2.20	0.08	1.8-3.0
K 235	I	23.76	0.36	19-27	5.06	0.13	4.0-6.0
	II	19.88	0.69	15-25	3.51	0.14	2.5-5.0
	III	21.96	0.70	16-29	3.82	0.17	2.0-5.0
K 236	I	24.92	0.58	20-30	5.66	0.20	4.0-7.0
	II	20.40	0.47	15-28	4.45	0.10	3.5-5.0
	III	21.20	0.46	17-27	4.48	0.14	3.5-6.0
K 237	I	26.64	0.62	20-32	5.34	0.20	4.0-7.0
	II	23.65	0.56	20-30	4.64	0.10	4.0-6.0
	III	20.68	0.72	15-30	4.08	0.18	3.0-6.0
K 368	I	22.44	0.79	17-30	3.50	0.10	2.7-4.7
	II	20.80	0.49	10-26	2.84	0.12	1.8-3.8
	III	20.24	0.41	18-27	2.92	0.09	2.0-4.0
K 593	I	23.48	0.76	17-30	4.39	0.08	3.7-5.1
	II	20.80	0.49	20-28	5.56	0.17	4.0-7.0
	III	21.56	0.33	19-28	4.74	0.15	3.8-6.0
Hexaploids: $2n=66$							
K 343	I	20.20	0.43	15-25	3.93	0.12	3.0-5.0
	II	19.88	0.49	15-24	3.90	0.15	3.0-5.0
	III	22.36	0.57	16-25	2.38	0.11	1.8-3.5
K 537	I	19.20	0.48	14-26	3.88	0.11	3.0-5.0
	II	19.39	0.47	16-28	3.46	0.07	3.0-4.0
	III	20.92	0.43	15-27	3.32	0.10	3.0-5.0
K 590	I	23.04	0.31	20-32	4.20	0.15	3.0-6.6
	II	22.56	0.53	18-27	4.50	0.09	3.9-5.0
	III	18.56	0.30	16-24	4.36	0.07	4.0-5.0

A variance analysis showed that no correlation could be confirmed between length and/or width of the leaves and cytotype in any year of cultivation in any plant.

THE COROLLA

The corolla lobes are ovate, mucronate at the apex and are white. The diameter and width of one lobe were measured in 50 flowers of each plant (see fig. 3).

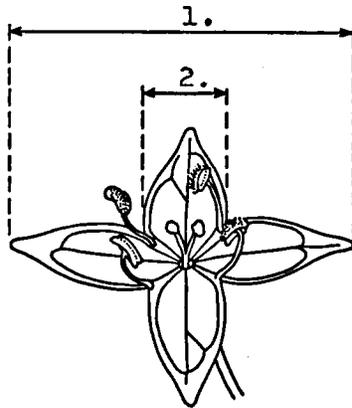


Fig. 3. Method of measurements on flowers of *Galium boreale* L. 1=diameter of the flower, 2=width of one lobe. Both in mm.

Both flowers and leaves (see preceding paragraph) of the same plant were examined. The results are shown in table III. The first column gives the plant numbers; the roman numerals in the second column the first, second and third year of observation (i.e. the fourth, fifth and sixth year of cultivation respectively); the third column the mean and (\bar{X}) the standard error of the mean (S.E.) of the length and width respectively, the fourth column the minimum and maximum value.

From the table there appears a large degree of constancy with minor deviation.

The results of the measurements show that neither the length or the width of the corolla permit a statistical separation of the two cytotypes.

INDUMENT

The manifestly quadrangular and mostly ribbed stems are generally glabrous except for the nodes, and have small retrorse aculii on the ribs. The nodes vary from pubescent (nr. 607) or sparsely pubescent (nr. 241, 368 and 537) to glabrous (the other plants examined).

The surface of the lamina is mostly glabrous, but in some specimens scattered simple hairs, both on the adaxial and on the abaxial side, were found. The midrib is mostly provided with simple hairs on the abaxial side.

The pedicels which measure 1–2 mm in length, are generally glabrous. In a few cases they appeared to be sparsely puberulent with simple hairs. The fruits which measure 1–2.5 mm in diameter, are covered with bristly uncinat simple hairs. The density of the mature fruit indument is variable, even among fruits on one plant. Immature and thus smaller fruits seemingly have a denser indument. Completely smooth fruits were found in plant nr. 343 which, according to descriptions agrees with var. *hyssopifolium*

TABLE III

Diameter of the flower and width of the corolla lobe, both in mm. The roman numerals indicate the first, second and third year of observation.

Plant nr.		diameter			width		
		$\bar{X} \pm$ S.E.	range		$\bar{X} \pm$ S.E.	range	
Tetraploids: $2n=44$							
K 102	I	4.81	0.04	4.3-5.3	1.10	0.02	0.8-1.3
	II	4.85	0.04	4.0-5.0	1.09	0.01	0.8-1.3
	III	4.84	0.04	4.1-5.2	1.07	0.02	0.8-1.3
K 211	I	4.53	0.05	3.8-5.1	0.99	0.02	0.8-1.3
	II	4.14	0.03	3.8-4.6	0.91	0.01	0.7-1.2
	III	4.57	0.05	3.9-5.2	0.95	0.02	0.7-1.3
K 235	I	4.00	0.04	3.5-4.6	0.98	0.01	0.7-1.2
	II	3.88	0.04	3.4-4.6	0.99	0.02	0.8-1.3
	III	3.96	0.04	3.2-4.6	0.97	0.02	0.8-1.3
K 236	I	3.63	0.05	3.0-4.1	0.93	0.02	0.7-1.2
	II	3.89	0.04	3.5-4.5	0.95	0.02	0.7-1.2
	III	3.91	0.04	5.2-5.0	0.98	0.02	0.7-1.2
K 237	I	4.02	0.02	3.8-4.3	0.98	0.01	0.8-1.2
	II	3.94	0.03	3.3-4.5	0.94	0.02	0.7-1.2
	III	4.24	0.03	3.6-4.6	0.96	0.01	0.8-1.2
K 368	I	4.60	0.05	4.0-5.1	1.10	0.02	0.8-1.4
	II	4.66	0.05	4.0-5.1	1.06	0.02	0.8-1.3
	III	4.56	0.05	4.0-5.0	1.02	0.02	0.8-1.3
K 593	I	4.11	0.04	3.7-4.8	0.94	0.02	0.8-1.3
	II	4.10	0.04	3.5-4.8	1.00	0.01	0.7-1.2
	III	4.09	0.05	3.2-4.9	1.01	0.01	0.8-1.3
Hexaploids: $2n=66$							
K 343	I	4.07	0.04	3.2-5.0	0.95	0.01	0.8-1.2
	II	4.26	0.05	3.2-6.0	0.93	0.02	0.6-1.2
	III	4.10	0.05	3.5-4.9	0.92	0.02	0.7-1.2
K 537	I	4.29	0.05	3.5-5.0	1.01	0.01	0.7-1.2
	II	4.02	0.04	3.2-4.8	0.94	0.02	0.7-1.2
	III	4.00	0.05	3.0-4.8	0.95	0.02	0.7-1.2
K 590	I	4.29	0.04	3.5-5.0	1.10	0.02	0.9-1.4
	II	4.02	0.04	3.5-5.0	0.96	0.02	0.7-1.2
	III	4.22	0.04	3.5-5.0	0.96	0.02	0.7-1.2

(Hoffm.) DC. The indument appears to be linked with the individual plants rather than with the cytotype and remains constant over the years of cultivation.

STOMATA

The size of 25 stomata on each of a number of tetraploid and hexaploid plants was measured.

The mean (\bar{X}), the standard deviation (S.D.) and the standard error

of the mean (S.E.) of 150 measurements of the $2n=44$ plants and 125 stomata from the $2n=66$ plants are respectively:

$$\begin{array}{lll} X_{44} = 33.42 \mu & SD_{44} = 3.42 & SE_{44} = 0.28 \\ X_{66} = 34.57 \mu & SD_{66} = 4.12 & SE_{66} = 0.37 \end{array}$$

The $X_{\min.}$ and the $X_{\max.}$ of the tetraploid is 27–39 micron and of the hexaploid is 27–44 micron.

To compare the stomata of the two cytotypes the standard error of differences was calculated. It turned out to be 0,463.

The observed differences between the two cytotypes as regards to the mean stomata length is 1.15; this is more than twice the standard error of differences.

III. Flowering periods

During six successive years, the period of flowering of the plants in the experimental garden was observed.

The flowering period was considered as having started with the opening of the first bud of the inflorescence. The start of this period is irregular in the tetraploid as well as in the hexaploid. It varies in both cytotypes among the individual plants and also from one year to the other.

The earliest beginning of flowering was noted in the last days of May and the first days of June on several plants in different years of observation, in both cytotypes.

The latest start recorded was also in different plants in different years the second part of the first part of July.

Flowering in general begins in the first week of June, full flowering, this is: the whole inflorescence of the plant has open flowers, usually is reached after one week and lasts until the end of July or in some cases until the beginning of August. The mean period was followed by a more or irregular second flowering period due to the development of additional inflorescences and lasting till the end of August or the beginning of September.

IV. Crossing experiments

Crosses were made between the $2n=44$ and $2n=66$ plants. In each experiment 50 flowers were treated on each plant. The experiments were repeated during three years. For these experiments 2400 flowers were emasculated and pollinated. Active cross pollination between plants of the tetraploid stock or between the plants of hexaploid stock was always followed by setting of fruits. Seeds from these crosses always germinated and gave rise to plants with the same chromosome number, $2n=44$ and $2n=66$ respectively.

Fruits were never formed when the inflorescences were enveloped by paperbags. Even active selfpollination always remained without result.

Seven mericarps were obtained from crosses between tetraploids and

hexaploids and five from crosses between hexaploids and tetraploids.

However, only one mericarp from the latter cross matured, the others shrivelled.

The seeds from this mericarp did not germinate, so that no F_1 generation could be obtained.

DISCUSSION

The results of the cytological investigations show that within the european part of the distributional area of *Galium boreale* both tetraploids with $2n=44$ and hexaploids with $2n=66$ chromosomes occur. This agrees with the results of an investigation by RAHN (1961) and by PIOTROWICZ (1959, 1961). The tetraploids were from: Norway, Sweden, Denmark, France, Ireland, Czechoslovakia and Russia. The hexaploids from: Sweden, France, Roumania and Russia. The tetraploids in Rahn's material were from: Finland, Sweden, Denmark, Germany, England and Ireland, and the hexaploids were from: Sweden, Denmark, Poland and Russia. Piotrowicz's material was from different parts of Poland. Tetraploids and hexaploid plants of *Galium boreale* in Europe seem not to be confined to restricted areas but seem to grow sympatrically.

Thus the idea of LÖVE and LÖVE (1954) that there exist two taxa within the *Galium boreale* complex, an eurasiatic one with only tetraploids and an americanasiatic one with hexaploids only, loses its cytological ground; and it is particularly this difference in chromosome number on which emphasis is placed by Löve and Löve.

While cytological investigations do not confirm Löve and Löve's theory, there is still the morphological aspect to be considered. Löve and Löve pointed out that it is a combination of characters, rather than the characters themselves, that distinguished the two complexes from each other. Comparative morphological examination of both cytotypes, however, has failed to demonstrate such distinctive combinations of characters.

The indument of the stem, the nodes in particular, the leaves, the petioles, the pedicels and the bracts is equally variable in both tetraploids and hexaploids. The same holds for the hairs on the fruits, the one exceptional case being the glabrous fruit in our plant nr. 343. This plant belongs to var. *hyssoipifolium* (Hoffm.) DC. and was found to be hexaploid. Measurements of length and width of the leaves and subsequent statistical analysis did not provide any clue. The size of the corolla was similarly examined and no demonstrable differences between the two cytotypes were observed.

The bractshape is highly variable, even on the same plant, and has appeared totally unsuitable as a discriminating character.

The net result is that the characters examined cannot separate the two cytotypes neither singly nor in combination.

This is in agreement with the conclusions by RAHN (1961), who was equally unable to find distinguishing characters or combinations thereof.

Rahn (l.c.), investigated height of the plants and maximal leaf length. He demonstrated the variability of those characters and furthermore: "neither the diameter of the pollen, the length of the anthers nor the length of the fruits permit a statistical separation of the strains with different chromosome number". Trial probes by the present author on pollen diameter, anther length and fruit size could nothing but confirm Rahn's conclusions.

Flowering periods appeared to vary and were entirely overlapping, i.e. no shift was found when comparing the one cytotype with the other.

Stomatal size proved to be the only character whereby the two cytotypes could be distinguished.

Numerous crosses were undertaken, but it appeared impossible to obtain hybrids this way, which demonstrate a strong and effective barrier of hybrid sterility or incompatibility based on differences in the number or structure of the chromosomes. Therefore, the finding of a pentaploid in nature in Poland by PIOTROWICZ (1961) is very remarkable. As could be shown in other *Galium* species there is a strong and effective barrier between various levels of ploidy, intra- as well as interspecifically. (FAGERLIND, 1934, 1937; EHRENDORFER, 1955; KLIPHUIS, 1970, 1972).

Both tetraploids and hexaploids of *Galium boreale* in Europe do not show morphological differences and in spite of the results of the crossing experiments they must be regarded as belonging to the same taxon which has the following diagnosis:

Perennial herbs to 60 cm, with creeping stock. Stem erect, lignescent at the base, quadrangular with erect ascending often non-flowering branches, ribbed, the ribs with or without minute aculeii, the nodes glabrous or with indument.

Leaves in whorls of four, three nerved, dark green to bluish green when fresh, linear lanceolate to lanceolate elliptical, to 3.5×0.7 cm, widest below the middle, narrowing towards both ends, rounded at the top, the margins and the underside of the midribs with minute retortate aculeii, surface glabrous on both sides or with single hairs.

Flowers in cymes in narrowly to broadly pyramidal terminal panicles. Pedicels short, up to 2 mm long, corolla white, to 4.5 mm in diameter. Fruits ca. 2.5 mm, olive brown, glabrous or densely hispid with uncinat hairs.

The present study has dealt with the European part of the area of the *Galium boreale* complex only. Whether or not morphological characters would permit to separate taxa in collections from outside Europe remains to be investigated.

The results of such investigations now being carried out by the author will be published in a forthcoming paper.

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