CYTOTAXONOMICAL STUDIES ON THE GENUS GALIUM. A PRELIMINARY REPORT.

BY

E. KLIPHUIS

(Communicated by Prof. J. LANJOUW at the meeting of April 14, 1962)

Introduction

Cytological studies on the *Rubiaceae* with special references to the genus *Galium* have been done by HOMEYER (1936) and FAGERLIND (1937). EHRENDORFER (1949, 1954, 1955, 1956) described the phylogeny of the section *Leptogalium*. More detailed cytological and cytotaxonomical investigations appeared by HANCOCK (1942) (*Galium palustre* L., *Galium debile* Desv. and *Galium uliginosum* L.), CLAPHAM (1949) (*Galium palustre* L.), EHRENDORFER (1949, 1953) (*Galium pumilum* MURT.) 1955 (*Galium rubrum* L. and *Galium pusillum* L.) and of *Galium boreale* L. by LÖVE and LÖVE (1954) and more recently by RAHN (1961).

FAGERLIND (1937) and, previous to him, HOMEYER (1936) determined the chromosome numbers of many *Galium* species. Later investigations by EHRENDORFER (1949, 1955, 1956, 1961), LÖVE and LÖVE (1954, 1956), PIOTROWICZ (1958), POUQUES (1949), RAHN (1960, 1961) and REESE (1957) confirmed and supplemented this list of chromosome numbers. Many investigators have paid attention to the genus *Galium*. However, their studies have concerned only with some critical species or groups. Many taxonomical problems remain concerning the genus. SCHUMANN (1891) in ENGLER and PRANTL „Die Natürlichen Pflanzenfamilien" divided the genus in 14 sections which are very distinct morphologically. However, within these sections it is often very difficult to define exactly the morphological differences between the species.

It seemed appropiate to initiate taxonomical research in the genus, incorporating cytotaxonomical evidence. Therefore, in 1959, cytotaxonomical investigations in the genus *Galium* were started at the Botanical Museum and Herbarium in Utrecht. Primarily, an investigation of the European species was intended supplemented, as a matter of course, with material from other continents. For investigations of this kind it is necessary to use as many plants as are available from as many areas as possible. Seeds collected in the field were obtained upon request from many botanical institutions. Living plants were collected as well.

The seeds were germinated, and the seedlings, together with the col-

lected plants, were grown in an experimental plot in the Botanical Garden of the State University of Utrecht, "Cantonspark" in Baarn.

Chromosome studies were made from a number of plants. For this purpose roottips were fixed in Karpechenko and embedded in paraffin; microtome sections of 15 micron were stained according to Heidenhain's haematoxylin method, this proving to be the most successful procedure. Microtome sections gave much better results than squashing, This preliminary report will deal with the results obtained so far.

Cytological results:

From a number of plants from different parts of Europe, chromosome numbers were determined.

Voucher specimens have been deposited in the Botanical Museum and Herbarium, Utrecht.

(Asterisks indicate: precise locality not known)

Species	2n	No. plant	Origin
Galium anisophyllum Vill.	44	108	Austria, Vienna, Alpengarten Belvedere
G. aparine L.	44	287	Portugal, near Lissabon
	44	333*	Canada, Montreal, Bot. Garden
	63	200	Denmark, Grönnehave near Nykobing, Seeland
	64	103	Denmark, near Copenhagen
	64	293	France, Dep. du Finistère, Perhassidy
	66	324*	Austria, Klagenfurt
	66	203	Denmark, Kvärkeby near Humleore Seeland
	66	322	England, Liverpool, Anglesey-Newborough Warren
	66	323	England, Liverpool, Steyning
	66	293	France, near Nantes
	66	301	Italy, Udine, vallis Tellinae
	66	33	Netherlands, Weerdinge, prov. Drenthe
	66	338*	Portugal, Coimbra, Bot. Garden
	66	276*	Sweden, Bot. Garden Lund
G. arenarium Lois.	66	295	France, Lescouit, Dept. du Finistère
	66	312	France, near Nantes
G. aristatum L.	44	216	Switzerland, near Gandria, Lake Lugano
	44	218	Switzerland, near San Dominico Lake Lugano
	44	219	Switzerland, near Gandria, Lugano
G. boreale L.	44	211	Denmark, Blasmark, North of Varde Jutland
G. broterianum Bss. et Reut.	22	105*	Portugal, Bot. Gard. Sacavem

Species	2n	No. plant	Origin
G. cruciata (L.) Scop.	22	68	Netherlands, near Arcen, prov. Limburg
G. hercynicum Weig.	22	106*	Portugal, Bot. Garden Sacavem
	22	244	Portugal, Tras-os-Montes: Lisa do Alvao
	44	165	Germany, Eifel near Schleiden
	44	297*	France, Bot. Garden Rouen
G. mollugo L.	44	157	Belgium, near Hasselt
	44	158	Belgium, Albertkanaal near Canne
	44	195	Bulgaria, near Sofia
	44	102	Denmark, near Copenhagen
	44	204	Denmark, Espe, Grusgrav, Fyn
	44	110*	France, Bot. Garden Toulouse
	44	294	France, Pentrez, Dept. Finistère
	44	138*	Germany, Bot. Garden Erlangen
	44	229	Germany, Göttingen, Hagen
	44	230	Germany, Berlin-Tegel, Bahndamm
	44	231	Germany, Oberfranken, near Pegnitz
	44	239	Germany, Münster, Dortmund-Ems-Kanal
	44	262	Germany, Hamburg-Lehmsdahl
	44	320	England, near Steyning
	44	265*	Russia-Estonia, Tartu
	44	266*	idem
	44	269	Russia, Estonia, Annemois near Tartu
	44	270	Russia, Estonia, Eor Elva
	44	271	idem
	44	214	Italy, route from Portofino to San Fruttuoso, Peninsula of Portofino
	44	18	Netherlands, Dunes near Egmond
	44	58	Netherlands, South-Limburg, Epen
	44	133	Netherlands, Weerdinge, prov. Drenthe
	44	134	idem
	44	135	idem
	44	142	Netherlands, Johannapolder, Utrecht
	44	143	idem
	44	179	Russia, Kapachstan, Alma-Ata
	44	261*	Scotland, Edinburg
	44	330*	Norge, Bot. Garden Bergen
	44	292	Ungarn, near Gödöllo
G. mollugo L. var. *tiroliense* (Willd.) Hayek.	22	130	Switzerland, in mountains near Ascona, Ticino
	22	132*	Yougoslavia, Ljubljuana
G. palustre L.	24	154	Netherlands, near Maarn
	24	155	idem
	24	381	Germany, near Frankfort
	48	127*	Portugal, Hort. Bot. Lissabon
	48	285*	idem

Species	2n	No. plant	Origin
	48	326*	Portugal, Bot. Garden Coïmbra
	96	242	Portugal, Sacavem, Ribatejo Vila Franca de Xira
	24	275*	Sweden, Bot. Garden Lund
G. parisiense L.	44	327*	Portugal, Bot. Gard. Coïmbra
	66	279*	Germany, Hort. Bot. Frankfort
var. *leiocarpum* Tausch	55	296*	France, Normandie
G. purpureum L.	22	290	Austria, Frohnleiten
	22	213	Italy, Peninsula of Portofino, near San Fruttuoso
	22	220	Switzerland, near San Dominico, Lugano, Ticino
	22	222	Switzerland, Mont San Salvatore near Ciona, Ticino
	22	274*	Switzerland, Bot. Garden Neuchâtel
G. pumilum Murr.	44	253	Netherlands, Gaasterland, near Laaxum, prov. Friesland
	44	233	Denmark, Vorgod, Jutland
	88	169	Belgium, near Baelartshoven
	88	170	idem
G. rotundifolium L.	22	289*	Austria, Bot. Garden Wien
ssp. *ovalifolium* Scott	22	309*	France, Isle of Corsica
G. spurium L.	20	269*	Russia-Estonia
	20	318	Russia, Erevan, Armenia, 800 m
G. schultesii Vest.	66	113*	Czecho-Slovakia, Praha
	66	280*	Germany, Bot. Gard. Frankfort
	66	325	
G. silvaticum L.	22	69	Netherlands, Plasmolen, St. Jansberg
	22	70	idem
	22	71	idem
	22	112	idem
G. valantia Web.	22	289*	England, Bot. Gard. Oxford
	22	308*	Germany, Bot. Gard. Marburg
	22	243	Portugal, Sacavem, Estremadura
	22	281*	Portugal, Bot. Gard. Lissabon
	22	291	Ungarn, near Gödöllo
	44	339*	Portugal, Bot. Gard. Coïmbra
G. verum L.	22	111*	France, Bot. Gard. Toulouse
	44	160	Belgium, St. Pietersberg near Canne
	44	15	Netherlands, dunes between Egmond and Bakkum
	44	16	idem
	44	40	Netherlands, Kunraderberg, S. Limburg
	44	41	idem
	44	46	idem
	44	48	idem
	44	50	idem
	44	84	Netherlands, Westkapelle, prov. Zeeland
	44	148	Netherlands, Callantsoog

Species	2n	No. plant	Origin
	44	147*	Russia, Bot. Gard. Ashkhabad
var. *praecox* (Lang) Petrak	22	224	Switzerland, mainway Giorno-Ciona Ticino
G. ruthenium Willd.	22	347*	France, Bot. Gard. Straszbourg
	44	259	Germany, Heiligerhafen
	44	303	Italy, Udine, Frinli
	44	174	Russia, Bot. Gard. Moscow
	44	317	Russia, Erevan, Armenia
G. uliginosum L.	22	189	Netherlands, Putten, prov. Gelderland
	22	190	idem
	44	32	Netherlands, Weerdinge, prov. Drenthe
	44	188	Netherlands, Putten, prov. Gelderland

Notes on some critical species.

In the following species different chromosome numbers were counted: *Galium aparine* L., *Galium hercynicum* Weig., *Galium mollugo* L., *Galium palustre* L., *Galium parisiense* L., *Galium pumilum* Murr., *Galium valantia* Webb., *Galium verum* L., *Galium ruthenium* Willd., and *Galium uliginosum* L.

Galium aparine L.

In literature the following chromosome numbers are given: 2n = 22 and 2n = 44 by POUCQUES (1949), and 2n = 66 and 2n = 88 by FAGERLIND (1934). So Galium aparine apparently occurs as a di-, tetra-, hexa- and octoploid. The relation to morphological differences, if present, is not known exactly. The chromosome numbers 2n = 63 and 2n = 64 were found repeatedly in somatic metaphase plates of roottips.

The meaning of these unusual numbers is not known and needs further investigation.

Galium hercynicum Weig.

Up to now, only the tetraploid was known. In this paper, however, diploid plants are also listed. Further research concerning this species is planned.

Galium mollugo L.

This species is extremely variable. It is noteworthy that of all investigated plants the chromosome numbers are exactly the same (2n = 44), with the exception of those belonging to ssp. *tiroliense* (Willd.) Hayek. (2n = 22). This fact, in view of the considerable morphological differences between the plants, indicates that cytological data are of minor importance in order to get a clear picture of the variability of this species.

Galium palustre L.

The chromosome numbers: 2n = 24, 48 and 96 were determined. These numbers are mentioned in literature too. (DARLINGTON et al. 1945.; TISCHLER 1950). According to CLAPHAM (1949), in England *Galium palustre* L. consists of two distinct forms, i.e. a diploid with 2n = 24 chromosomes and an octoploid with 2n = 96. Morphologically, these two species can be separated as well. The plant with the 2n = 24 chromosomes corresponds with *Galium palustre* L. var. *witheringii* Sm. The one with 2n = 96 chromosomes with *Galium palustre* L. var. *elongatum* Sym.

The morphological differences include stomatal size, which appeared to provide a fairly accurate means of identification. (HANCOCK 1942). There has been no evidence of hybridization.

However, besides the diploid and octoploid, a tetraploid has been found. This tetraploid is intermediate and cannot be distinguished with certainty from the two other forms. Therefore, CLAPHAM (1949) suggested that the three forms should be treated as cytological subspecies rather than separate species.

Evidence obtained so far does not enable the present author to support the view of CLAPHAM. Further investigations on this point still have to be done.

The cytotaxonomical importance of the differences in chromosome numbers within the species *Galium parisiense* L., *Galium ruthenium* Willd., *Galium valantia* Webb, *Galium verum* L. and *Galium uliginosum* L., will also be the objective of investigation.

Galium pumilum Murr.

As a result of extensive cultivation experiments, EHRENDORFER (1949, 1953) distinguishes four ecotypes within this species. According to GOODWAY (1955) five distinct taxa are included in *Galium pumilum* Murr. in England. Additional investigations must prove whether this is correct and whether the same holds for *Galium pumilum* Murr. growing in the Netherlands or not.

Galium boreale L.

This species is highly variable. LÖVE and LÖVE (1954) divided this species in two types, occurring in two distinct areas: an Eurasiatic and an American-asiatic type. The first is tetraploid, the second hexaploid, with 2n = 44 and 2n = 66 chromosomes respectively. The plants with 2n = 44 chromosomes are identical with the Linnaean species *Galium boreale*. The hexaploids correspond with Galium septentrionale *S.* & *R.* GRONTVED (1954) observed the Linnaean species in Greenland. Therefore LÖVE and LÖVE, in their study on the Islandic Flora (1956), described this species as being Bis-Atlantic and not as Eurasiatic. RAHN (1961)

showed the existence of hexaploid forms of Galium boreale in Europe. He could not find any morphological difference with the tetraploid.

This indicates that more than one chromosome number occurs within the Linnaean *Galium boreale*. Further investigations concerning this problem are being performed by RAHN.

Acknowledgement

This investigation was carried out at the Botanical Museum and Herbarium, Utrecht, director Prof. Dr. J. LANJOUW. It was made possible by a grant of the Netherlands Organisation for Pure Scientific Research.

REFERENCES

CLAPHAM, A. R. in WILLMOT, Br. flow. plants and modern syst. methods: 72, (1949).
DARLINGTON, C. D. and A. P. WYLIE, "Chromosome-atlas of flowering plants." London. (1955).
ENGLER and PRANTL, Die natürlichen Pflanzenfamilien IV-4, 149 (1891).
EHRENDORFER, F., Oester. Bot. Zeitschr. 96, 109 (1949).
————, Oester. Bot. Zeitschr. 100, 616 (1953).
————, Rapp. + Comm. VIII congrès intern. de Bot., Paris sect. 4, 82 (1954).
————, Oester. bot. Zeitschr. 102, 195 (1955).
————, Ber. Bayer. Bot. Gesell. XXXI, 5 (1956).
————, Madrono 16–4, 109 (1961).
FAGERLIND, F., Hereditas 19 223 (1934).
————, Acta horti Bergiani 11, 195 (1937).
GOODWAY, K. M., Proc. Bot. Soc. Br. Isles 1, 383 (1955).
GRONTVED, J., Bot. Tidskr. 51, 98 (1954).
HANCOCK, B. L., New. Phytol. 41, 70 (1942).
HOMEYER, H., Planta 18, 65 (1932).
————, Bot. Jahrb. 67, 237 (1936).
JÖRGENSEN, C. A., TH. SÖRENSEN and M. WESTERGAARD, Biol. Skrifter Dansk. Videns Selsk. 9, 97 (1958).
LÖVE and LÖVE, Am. Midl. Naturalist 52, 88 (1954).
————, Acta horti Gotoburgensis XX, 65 (1956).
PIOTROWICZ, M., Acta Biol. Cracov. 1, 159 (1958).
POUCQUES, M. L. DE, Rev. Gen. Bot. 56, 5 (1949).
————, Rev. Gen. Bot. 56, 172 (1949).
RAHN, K., Bot. Tidskr. 56, 117 (1960).
————, Bot. Tidskr. 56, 351 (1961).
REESE, G., Flora 144, 598 (1957).
TISCHLER, G., „Die Chromosomenzahlen der Gefässpflanzen Mitteleuropas" 's-Gravenhage 1950.