CYTOTAXONOMIC NOTES ON SOME SPECIES OF THE GENUS GALIUM L. (RUBIACEAE) COLLECTED IN THE NORTH-WESTERN PARTS OF SPAIN

E. KLIPHUIS

Department of Biosystematics, University of Utrecht, Transitorium III, W 406, Padualaan 8, Utrecht, the Netherlands.

(Recibido el 28 de febrero de 1982)

Resumen. En esta nota se incluyen una serie de observaciones sobre la citotaxonomía de siete especies de Galium (Rubiaceae) del NW de España. Se indican los números cromosómicos siguientes: G. mollugo L., 2n = 22; G. album Miller, 2n = 44; G. fruticans Cav., 2n = 44; G. uliginosum L., 2n = 44; G. rivulare Boiss. & Reuter, 2n = 22 y 2n = 44; G. harcynicum Weigel, 2n = 22 y 2n = 44; G. debile Desv., 2n = 24; y G. palustre L. 2n = 48 y 2n = 96.

Summary. This is a contribution to the cytotaxonomy of seven species of Galium from NW Spain. The chromosome numbers for the following taxa are reported: G. mollugo L., 2n = 22; G. album Miller, 2n = 44; G. fruticans Cav., 2n = 44; G. uliginosum L., 2n = 44; G. rivulare Boiss. & Reuter, 2n = 22 and 2n = 44; G. harcynicum Weigel, 2n = 22 and 2n = 44; G. debile Desv., 2n = 24, and G. palustre L., 2n = 48 and 2n = 96.

This paper has been projected as a contribution to the knowledge of the chromosome numbers of Spanish plants as a part of a cytotaxonomic study of the genus Galium in the Iberian peninsula. It includes karyologic and taxonomic observations of different populations of seven species of this genus, collected in Santander, Asturias and Galicia.

**Chromosome number.** \(2n = 22\).


*Galium mollugo* L. belongs to the *Galium mollugo* group. This complex comprises a number of closely related taxa of diploid and tetraploid level. Some of these are described as species or subspecies. Their taxonomic position, however, is not always clear and it is questionable whether all of these can be maintained. This is due to the variability of the distinguishing characters, which often overlap to a large extent.

*Galium mollugo* is one of the diploid representatives of the complex. In spite of its polymorphy it can be defined by a distinctive combination of characters. These are: a lax, much branched, narrow to broadly ovoid panicle; oblong to broadly oblanceolate leaves, abruptly narrowing towards the apex; flowers up to 4 mm. in diameter with lobes less than 1 mm. in width and pedicels 1.5 - 4.5 mm. in length, strongly divaricate in fruit. In the experimental garden these characters remain constant during the years of cultivation. It is mainly the combination of these characters, rather than the characters themselves, which distinguish *Galium mollugo* from the other taxa within the mollugo group.

The distributional area of *Galium mollugo* extends from the South of England across Europe (North of France, Central Germany, Poland) into the central parts of European Russia. It comprises the whole of Southern Europe, including the Crimea, except the South - East of the Balkans. It occurs sympatrically with the more extensive area of the closely related tetraploid representative of the group: *Galium album* Miller. In view of the similarities and the differences in morphology, this tetraploid must be of allopolyploid origin, and the diploid *Galium mollugo* must be one of the sources.

No hybrids are known of *Galium mollugo* and other diploid taxa within the group or with diploids of related groups (p. e. *Galium lucidum* group). The position of *Galium mollugo* is furthermore underlined by the existence of a strong and effective reproductive barrier between the diploid and tetraploid level within the group. Despite repeated attempts we were unable to obtain
hybrids between *Galium mollugo* and *Galium album*. In this respect the experiments by FAGERLIND (1973) were fully confirmed. Taking everything into account we feel justified in considering this diploid taxon as a separate species as did KRENDL (1967).

*Galium mollugo* was collected in four localitites. All the material investigated turned out to be diploid with $2n = 22$ chromosomes. The chromosome portrait is regular without satellites or additional chromosomes. The number $2n = 22$ has also been reported by PIOTROWICZ (1958) for plants from Poland, from the South of Switzerland (Ticino) by KLIPHUIS (1962), for Austria and Yugoslavia by KRENDL (1967), for Czechoslovakia by KLIPHUIS (1962), KRENDL (1967) and MAJOVSKY (1974), and for France by LABADIE (1976).

The plants studied are hairy, particularly the stems and nodes of the lower parts. In this the picture agrees with the observation made by KRENDL (1967). According to this author the number of plants with indument increases proportionately southwards. The collection numbers K 1950 and K 1951, moreover, have stems rough owing to retrorse prickles. The margin of the leaves is rough due to antrorse prickles. Before coming into flower, these plants (K 1950 and K 1951) are dark in colour due to anthocyanine. Once in flower the leaves are dark green and the colour of the stem is less intensely reddish-brown.

*Galium album* Miller, *Gard. Dict.* ed. 6 no. 7 (1768).

*Chromosome number. $2n = 44$.*


*Galium album* Miller is the tetraploid representative of the *Galium mollugo* group. It is exceedingly polymorphic. Characters such as the stature and height of the plant, the ramification, the shape of the panicle, and the length and width of leaves and petals may vary to a large extent.

Due to this variability the intraspecific classification is extremely
difficult. According to Schönbek-Temesy & Ehrendorfer (1979) at least four subspecies can be recognized. Two of them, e.g. subsp. album and subsp. pycnotrichum (H. Braun) Krendl have broadly ovoid inflorescences and whitish flowers. The other two, e.g. subsp. prusense (C. Koch) Ehrend. & Krendl and subsp. amani Ehrend. & Schönb.-Tem., have a narrow cylindrical panicle and yellowish flowers. The subsp. album is distinct from the subsp. pycnotrichum by having oblanceolate leaves, gradually narrowing towards the apex instead of oblong broadly oblanceolate leaves, abruptly narrowing towards the apex. Ehrendorfer & Krendl, 1967). Subsp. amani is less polymorphic than subsp. prusense and differs from the latter in having smaller corollas, somewhat narrower leaves and its stem is often tinged with anthocyanine (Schönbek-Temesy & Ehrendorfer, 1979). The report of a finding by Strid & al. (1981) of a diploid of the subsp. prusense on Mount Olympus (Greece) makes the taxonomic position of this subspecies of interest and it requires further investigation.

Galium album Miller is widespread throughout Europe except in the Northernmost parts. It extends eastwards into West Siberia, the Caucasus and Asia Minor. The subsps. pycnotrichum, prusense and amani are plants from the South-Eastern parts of the area. The subsp. album is distributed throughout the whole area except for the East-Southernmost parts.

A cytogeographical and morphological investigation of plants of the Galium mollugo group, in particular of those from West and North-West Europe, showed the tetraploid Galium album Miller subsp. album to be the only representative of the group for the Netherlands, Belgium, the North of France, North-West Germany and Denmark. It may also be the only representative of the group for Scandinavia, Iceland, the Northern parts of Central Russia and most of the British Isles.

In zones of contact the subsp. album hybridizes with the tetraploid Galium verum L. and Galium lucidum All., giving rise to large and variable populations with series of fertile hybrids connecting one parent with the other. Almost any combination of the morphological characters is possible in such hybrid swarms.

Within the subspecies album one can sometimes recognize extremes in one distinct character. However, a continuous bridge between these extremes confuses the picture and makes a taxonomic treatment almost impossible. An exception, perhaps, can be made for plants occurring in the dunes of the North-Sea coasts of West Europe. These plants have a completely prostrate habit, which remains constant throughout the years of cultivation. This cha-
racter, therefore, must be genotypic and these plants should be considered as
ecotypes sensu TURESSON (1922).

**Galium fruticescens** Cav., *Icon Descr.* 3: 3 (1795).

*Chromosome number. 2n = 44.*


In the opinion of EHRENDORFER & KRENDL (1976) this species belongs to the *Galium lucidum* group. This group comprises a number of diploid and tetraploid taxa occurring in Central and Southern Europe. Its distribution is often limited and of an endemic character. The plantas are found mainly in dry to very dry, sunny and warm places on rocky or stony ground.

The two most important representatives with the most extensive area are *Galium corrudifolium* Vill. and *Galium lucidum* All. The first has a Mediterranean distribution, the second is a common species in South-Central and Southern Europe. Diploids as well as tetraploids are found in both species (KLIPHUIS, unpubl.). *Galium lucidum* (44) hybridizes with *Galium album* (44), and in this way it is connected by all possible intermediates with the *Galium mollugo* complex. It is often difficult or even impossible to distinguish the two species where they grow sympatrically. In spite of such large hybrid swarms *Galium album* and *Galium lucidum* are well delimited species and they should be considered ad belonging to two different polyploid complexes. The difference in morphology, the cytological data, the eco-geographical distribution and the relationship with other species within each of the groups concerned suggest this.

*Galium fruticescens* is closely related to *Galium lucidum* and *Galium corrudifolium*. Compared with *Galium lucidum*, *Galium fruticescens* is generally a smaller plant without stolones, with smaller and narrower leaves. The leaf margin is revolute and strongly scabrid instead of more less revolute and scabrid. The pedicels are relatively long and may be as longs as the diameter of the flowers, which may be up to 5 mm.

To distinguish *Galium fruticescens* from *Galium corrudifolium* is often difficult and may be problematical, because in unfavourable circumstances *Galium fruticescens* may show similarities in habit to *Galium corrudifolium*. However, under all circumstances, in nature as well as in the experimental plot, *Galium fruticescens* is characterized by having inflorescences with rigid
branches from near the base upwards. This characteristic is found neither in *Galium lucidum* nor in *Galium corrudifolium*. Furthermore, *Galium corrudifolium* is generally a much smaller plant, with smaller, narrow linear leaves, shorter pedicels and smaller flowers and fruits.

*Galium frutescens* has a more limited area than *Galium lucidum* and *Galium corrudifolium*. It is a plant from rocky, warm and dry places in Central and Central-South parts of the Iberian Peninsula. The material studied was collected in two localities on the Northernmost border of the area of the species. Cytological investigation showed tetraploids with $2n = 44$ chromosomes. This is a new count; up to now the chromosome number was not known.

The chromosome portrait is regular and does not show satellites or B-chromosomes.


*Chromosome number.* $2n = 44$.

*Material investigated.* NAVARRA. Near Erasun, between Santesteban and Leiza, 7.X.1977, Kliphuis K 1852; idem, Kliphuis, K 1936.

*Galium uliginosum* is a plant from marshes, fens and other wet habitats; it is locally frequent and it is distributed throughout Europe, except in the Northernmost parts. It extends eastwards into Siberia and it is very rarely found in the Mediterranean area.

Within *Galium uliginosum* two cytotypes occur, a diploid with $2n = 22$, and a tetraploid with $2n = 44$ chromosomes.

In material from Denmark, Finland, Germany, Norway, Switzerland and the French Alps only diploids were observed. In the literature this cytotype has been reported for Britain (HANCOCK, 1942), Central Europe (EHRENDORFER, 1961), and the Netherlands (KLIPHUIS, 1974a).

The plants from the two localities in Spain turned out to be tetraploids with $2n = 44$ chromosomes. Tetraploids were also found in four localities in the West Pyrenees (Pyrénées Atlantiques, France). In the literature, tetraploids have been recorded for Germany (ROHWEDER, 1937), Iceland (LÖVE & LÖVE, 1956), Czechoslovakia (GARAJOVA, 1959), the Netherlands (2 out of 19 localities) and Belgium (one locality in the Ardennes) (KLIPHUIS, 1974a).

The diploid seems to be the most common cytotype distributed throughout the whole area of the species. The tetraploid seems to have a ten-
tendency towards a more West, South-Western distribution, although the finding of a plant with $2n = 44$ chromosomes reported for Czechoslovakia by Garajova (1959) is not in agreement with this statement.

*Galium uliginosum* is rather uniform in its morphology. Differences, if present, are mainly of a quantitative character, and they are not correlated with the difference in chromosome number (Kliphuïs, 1974a). The differences in morphology may possibly be affected by environmental factors.


*Chromosome number.* $2n = 22$ and $2n = 44$.

**Material investigated:**


Within the material investigated diploids ($2n = 22$) as well as tetraploids ($2n = 44$) could be demonstrated. In the literature only diploids are recorded (Ehrendorfer, 1976).

Diploids and tetraploids may occur intermingled as is shown in the material collected in a very moist habitat on a limited area on the banks of the Rio Enviande (Lugo).

*Galium rivulare* is rather uniform in its morphology. The height of the plants, the length and width of the leaves, the length of the pedicels and the diameter of stems, pedicels and flowers may vary. Differences in indument may even be considerable. Plants may be glabrous or hairy and may be rough due to retrorse prickles on the ribs of the stems. Neither the quantitative differences (apart from the diameter of the stems and pedicels) nor the differences in indument are correlated with the level of ploidy. In the diploids, the stem is less than 1 mm wide and the pedicels are capillary. In the tetraploids, the stem is over 1 mm wide and the pedicels are thin but not capillary.

*Galium rivulare* is a plant from North and Central Spain and North Por-
tugal. It occurs in damp zones, in hedges, at edges of forests, in open deciduous woods or other shady places, preferably near or on banks of rivulets, brooks and small rivers.

In Flora Europaea (1976) *Galium rivulare* is included in the section *Leptogalium*. However, in our opinion it would be better to place it in the section *Trachygalium*, close to *Galium uliginosum*.


*Chromosome number.* $2n = 22$ and $2n = 44$.

*Material investigated.*


**Galium harcynicum** Weigel belongs to the Section *Leptogalium* Lange, and within this Section it occupies a more or less isolated position characterized by well-defined morphological characters.

**Galium harcynicum** is represented by two cytotypes, a diploid with $2n = 22$ and a tetraploid with $2n = 44$ chromosomes. The diploid is restricted to the North-Western parts of the Iberian Peninsula, whereas the tetraploid is widely distributed throughout the whole European-Atlantic area of the species (KLIPHUIS, 1981).

The cytological and geographical differences are correlated with quantitative morphological differences. Compared to the tetraploid the diploid, in general, is a smaller plant with shorter internodes, fewer flowers per inflorescence, smaller leaves, flowers, fruits and pollen grains.

In the Northern parts of Portugal the diploid is the most common cytotype, in particular on the lower slopes in the vicinity of the Serra de Gerês. It occurs on acid soils, in open grasslands with open woods in an area with much
rainfall and humidity during most parts of the year. In this limited area only diploid plants were found. Southwards, as could be demonstrated for the Serra da Estréla, both cytotypes occur side by side, however, tetraploid plants are most frequent (KLIPHUIS, 1981).

The distribution pattern of both cytotypes in the North-Western parts of the Iberian Peninsula is no very well known. In East Galicia only tetraploids are known from 6 localities 5 of which are in the province of Lugo and one in

Fig. 1.—Localities of the cytologically investigated plants of *Galium harcyricum* Weigel. The diploids are represented by a cross the tetraploids by a black dot. Records published in earlier papers (KLIPHUIS, 1972, 1981) are also included.
the province of Orense. In the South, in the province of Pontevedra near Piñeira, one locality with diploids was recorded (KLIPHUIS, 1981). In West Galicia, two tetraploid plants and one diploid plant were found in the same area between Santiago de Compostela and La Coruña (KLIPHUIS, 1972).

So it seemed of interest to obtain more cytological data from the most North-Western parts of Spain: Galicia. Galicia has to a certain extent a climate comparable to that of Northern Portugal and the ecological circumstances are roughly the same.

Plants obtained directly from the wild as well as plants grown from seed collected in the field were used in this study. The cytological investigation showed diploids and tetraploids. The results of the counts are given in Figure 1. This Figure shows the distribution of the localities of the collections. The diploid is represented by a cross, the tetraploid by a black dot. Records published in earlier papers (KLIPHUIS, 1972, 1981) are also included in this figure. In all, 20 localities have been mapped, 16 of which consist of tetraploids and 4 of diploids. A total of 56 plants were investigated, 48 of which were tetraploids and 8 diploids.

The result of this study shows a distribution pattern for the two cytotypes in Galicia which is comparable to that for the southern parts of the area of the species in Portugal. The picture is in agreement with the situation in the Serra de Estrêla, where diploids occur among a majority of tetraploids.

*Galium harcynicum* is a common plant throughout most parts of North and Central Portugal. It is much rarer in Galicia. In this respect the picture is not comparable. The diploid, in particular, is a plant from North Portugal. The area of the diploid is more southerly, peripheral and limited than that of the tetraploid and it must be considered as the centre of diversification and distribution.


*Chromosome number. 2n = 24.*

*Material investigated. LUGO. Between Villalba and El Ferrol, near Roudar, 15.X.1979, Kliphuis K 3365; idem, Kliphuis K 3366.*

*Galium debile* Desv. is a plant from South and South-West Europe, occurring into the South of England.

HANCOCK (1942), was the first to report the chromosome number \(2n = 24\) for plants from Devon, England. ANČEV (1974) found the same diploid
number in material from the Struma valley in Bulgaria; TEPPNER & al. (1976) confirmed this number for a plant found near Kilyos, Anatolia in Turkey. Recently STRID & FRANZEN (1981) mentioned it for a plant collected on Mount Olympus in Greece.

*Galium debile* is closely related to *Galium palustre* and it has sometimes been treated as a subspecies of it (e. g. ROUY, 1903). However, *Galium debile* is distinct in its morphology. It is a plant with narrow, sometimes minutely apiculate but never mucronate, linear leaves, and it has relatively long internodes; it is particularly characterized by having the pedicels convergent in fruit and by having distinctly tuberculate fruits.

The chromosome number \(2n = 24\) was confirmed for the material collected in Spain. The chromosome portrait is regular. The chromosomes are small, usually between 1 and 2 microns. No B.-chromosomes or satellites have been observed.


*Chromosome number. \(2n = 48\) and \(2n = 96\).*

*Material investigated.*

Kliphuis K 3322; Rio Grande, between Somozas and Casares, near Abeledo, 16.IX.1979, Kliphuis K 3323; near Espiñaredo, Rio Jubia, 16.IX.1979, Kliphuis K 3324; idem, Kliphuis K 3325; Rio Miñatos, near Burricios, 16.IX.1979, Kliphuis K 3326; Rio Murazo, near Cumbraos, 16.IX.1979 Kliphuis K 3327; Carballo, near Rio Allones, 17.IX.1979, Kliphuis K 3328; idem, Kliphuis K 3329; near Rio Zas, near Jandra, 17.IX.1979, Kliphuis K 3331; near Arca, between Amenal and El Pino, 18.IX.1979, Kliphuis K 3333, idem, Kliphuis K 3335; between Brea and Regas, 18.IX.1979, Kliphuis K 3336: idem, Kliphuis K 3337; idem, Kliphuis K 3338. LUGO. Near Carborrecelle, near Puerto Marin, 18.IX.1979, Kliphuis K 3339, idem, Kliphuis K 3340; idem, Kliphuis K 3341; near Lousadela 18.IX.1979, Kliphuis 3342. PONTEVEDRA. Near Cadrón 3 km from Golada, 18.X.1979, Kliphuis K 3344; idem, Kliphuis K 3346. CORUÑA. Near Rodeiro, 16.IX.1979, Kliphuis K 3345. ORENSE. Rio Osera, near Lousado, 19.IX.1979, Kliphuis K 3347. PONTEVEDRA. Near la Cañiza, 20.IX.1979, Kliphuis K 3348; idem, Kliphuis K 3349; near Rio Miño, near Las Nieves, 20.IX.1979, Kliphuis K 3350; near Lougares, Rio Tea, 20.IX.1979, Kliphuis K 3351. ORENSE between Refojos and Ramirañes, 21.IX.1979, Kliphuis K 3353; 2 km from Ramirañes, 21.IX.1979, Kliphuis K 3354; idem, Kliphuis K 3355; near Quinta, 13 km from Celañova, 21.IX.1979, Kliphuis K 3356; near Freas de Eira, 9 km from Celañova, 21.IX.1979, Kliphuis K 3357; 2 km from Celañova, 21.IX.1979, Kliphuis K 3358; Montes de Bande, 2 km from Verea, 21.IX.1979, Kliphuis K 3359; idem, Kliphuis K 3360; near Baños, 21.IX.1979, Kliphuis K 3361; idem, Kliphuis K 3362; Rio Lima, near frontier with Portugal, 21.IX.1979, Kliphuis K 3363.

b, Octoploids (2n = 96). OVIEDO. Wash-place in small river near Deva (Gijón) 10.X.1977, Kliphuis K 1862; idem, Kliphuis K 1863. CORUÑA. Rio San near Meiras, 15.IX.1979, Kliphuis K 3321; near Louro, 3 km from Muros, 18.IX.1979, Kliphuis K 3332; idem, Kliphuis K 3334.

Galium palustre L. comprises a polyploid complex with the basic chromosome number x = 12. Diploids, tetraploids, octoploids and dodecaploids with 2n = 24, 48, 96 and 144 chromosomes respectively are known.

It is widely distributed in wet places throughout Europe, the North of Africa, and Western Asia, where it extends into the Baikal area, the Caucasus and Turkey. It is also known from the North American Continent.

The cytotypes show a certain degree of geographical and ecological preference. Diploid and octoploids are the most common cytotypes occurring throughout the whole area. In Europe, however, the former have a more
northerly distribution, the latter a more southerly one. Tetraploids are plants from the sub-mediterranean Atlantic area. Dodecaploids are rare and known only from two scattered localities in the Central and South-Eastern parts of the area, e.g. Austria and Anatolia (TEPPNER & al. 1976).

Diploids are plants that grow in damp places which dry out in summer; octoploids are plants of permanently damp zones often bordering upon water (FAGERLIND 1937; HANCOCK, 1942; CLAPHAM, 1949; KLIPHUIS, 1974b).
HANCOCK (1942) who was the first to report a tetraploid, found this cytotype within a habitat intermediate to that of the diploid and octoploid: often submerged in winter and damp in summer, but without the water table constantly near the surface.

Morphologically the diploid differs from the octoploid in characters such as the shape of the panicle, the mode of branching in particular during fruit maturation, the size of flowers, fruits and stomata. In general it is a smaller plant with smaller leaves. The tetraploid is more or less intermediate. Its characters are variable and their is a certain amount of overlap with the diploid and octoploid (HANCOCK 1942; CLAPHAM 1949; KLIPHUIS 1974b). Flower characters are much more stable and are independent of cultivation conditions (KLIPHUIS 1974b). In view of the cytological, ecological and morphological data CLAPHAM (1949) and later on KLIPHUIS (1974b) considered the three cytotypes as taxa of equal rank. In their opinion the classification into subspecies is the most reliable one. This opinion contradicts that of TEPPNER & al (1976). These authors could not demonstrate any difference in the morphology of the diploid and tetraploid plants used in their study. Hence, they included both cytotypes in one species, Galium palustre, without taxonomic recognition. According to these authors the octoploids can be described by a number of well defined characters in such a way that this cytotype too should be considered as a separate species, Galium elongatum. Dodecaploids resemble the octoploids so closely that also an autopolyplloid origin is suggested.

This difference of opinion stems mainly from a difference in the assessment of the position of the tetraploid and may be caused partly through the amplitude in the morphological characters of the tetraploid, partly because up to now less material of this cytotype has been investigated than of the tetraploid for octoploid. So it seemed useful to obtain more material of the tetraploid for cytotaxonomic investigation. For this reason seed of Galium palustre was collected in the North West of Spain, which is a part of the sub-Mediterranean Atlantic area of the complex.

Seed samples were taken from 74 localities. Cytological investigation showed tetraploids in 69 out of these 74 localities. Only in five localities could octoploids be demonstrated. No diploids were found. Figure 2 shows the distribution of localities of the collections. The tetraploids are represented by a black dot, the octoploids by a cross.

The chromosome counts were made from root-tip mitosis of potted plants, which were grown from the seeds collected in the field. From each seed
sample 3 - 5 plants were used for cytological purposes. These plants are being cultivated in an experimental plot. They will be subjected to a comparative morphological investigation, the result of which will be discussed in a forthcoming paper.

The results of the counts show clearly the dominant position and distribution of the tetraploid cytotype in the North-West of Spain.

In the North-West of Spain the climate is influenced by the Atlantic. Relatively dry periods alternate with periods of moderate to high rainfall. Flooding may occur were streams and rivulets cannot drain off all the rainfall quickly enough in the normal way. The tetraploids prefer such wet places, where the water-table is not always near the surface. They were often found near streams and rivulets. The five localities of the octoploids were all situated in a habitat of permanently damp zones with constantly soaked ground. The ecological preference, however, is not always strictly associated with the chromosome number, a phenomenon already noticed by Hancock (1942) for the diploids and octoploids. A certain amount of flexibility in habitat can be observed.

REFERENCES


HANCOCK, B. L. (1942) Cytological and ecological notes on some species of Galium L. Em. Scop. New Phytol. 41: 70-78.


