CYTOTAXONOMIC STUDIES IN *GALIUM PALUSTRE* L.

BY

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SUMMARY

Cytological investigations within *Galium palustre* L. showed the occurrence of three cytotypes, a diploid with 2n=24 chromosomes, a tetraploid with 2n=48 and an octoploid with 2n=96. Comparative morphological investigations, together with transplantation and crossing experiments confirmed the complexity of the species. The cytotypes are here considered to be subspecies of *Galium palustre* L.

INTRODUCTION

Galium palustre L. is a common plant of marsh habitats. It occurs in the whole of Europe, Asia Minor and the Caucasus. Within this species the chromosome numbers 2n=24, 48, 88 and 96 are known. (2n=24 – FAGERLIND, 1934, 1937; HAGERUP, 1941; HANCOCK, 1942; CLAPHAM, 1949; PIOTROWICZ in SKALIŃSKA et al. 1961; GADELLA and KLIPHUIS, 1963; HAMET-ATHI and VIRRANKOSKI, 1970), (2n=48 – HANCOCK, 1942; KLIPHUIS, 1962), (2n=96 – FAGERLIND, 1934, 1937; HANCOCK, 1942; KLIPHUIS, 1962).

The number 2n=88 was reported by PIOTROWICZ (l.c.) for plants collected in Poland; this author sees this karyological divergence as an indication of the occurrence of two basic chromosome numbers, X=11 and X=12.

The diploids and the octoploids seem to be the most common cytotypes; they are distributed throughout the whole area. Tetraploids are known only from Britain (HANCOCK, 1942) and Portugal (KLIPHUIS, 1962). The diploid is a plant of damp places which dry out in summer, the octoploid grows in permanently damp zones often bordering upon water (FAGERLIND, 1937; HANCOCK, 1942). The English tetraploid was found in Devon in an intermediate habitat: often submerged in winter and damp in summer, but without having the water table constantly near the surface (HANCOCK, 1942; CLAPHAM, 1949).

The ecological preferences of the diploid and octoploid *Galium palustre* do not always seem to be strictly associated with the chromosome number. HANCOCK (l.c.) described a community with *Galium palustre* near Oxford, England, consisting of both diploids and octoploids, occurring under more or less intermediate circumstances, which were perhaps slightly favourable

for the diploids. He suggests that the octoploid is rather more tolerant in its ecological requirements than the diploid.

Galium palustre is a polymorphic species. The differences in morphology are correlated to a certain degree with the level of ploidy. The diploid can be distinguished from the octoploid by being smaller in general, especially with smaller leaves, flowers and fruits. The tetraploid is intermediate; its characters are variable and there is a certain amount of overlap with the diploid and the octoploid (HANCOCK, 1942, CLAPHAM, 1949). CLAPHAM (1949) considered the differences between the plants with 2n = 24, 2n = 48 and 2n = 96 of such importance that he treated these cytotypes as sub-species. He identified the octoploid as subspecies elongatum (C. Presl) Lange (incl. var. *lanceolatum* auct. ang., p. max. p.), the diploid as subspecies *palustre* (incl. var. *witheringii* Sm. and var. *angustifolium* auct. ang., p. max. p.); he described the tetraploid as a new subspecies *tetraploideum* Clapham.

No morphological or ecological date are known from the 2n = 88 cytotypes reported by PIOTROWICZ (1961) whereas the taxonomic position of this cytotype remains unclear as well.

The present author included *Galium palustre* within the framework of a general cytotaxonomic investigation of different *Galium* species. Cytological and morphological studies were made on plants from different geographical locations which were cultivated during several years in the experimental garden of the University of Utrecht.

MATERIAL AND METHODS

The material included living plants collected in the wild and grows at Utrecht, as well as plants grown from seed which was also collected in the field.

The methods employed for the cytological studies and for the crossing experiments are described in a previous paper (KLIPHUIS, 1970). Measurements of leaves, flowers, fruits and stomata were made on living material; they were processed with normal statistic methods for calculating the mean ($\bar{X}$), the standard deviation (SD) and the standard error of the mean (SE).

RESULTS

I. Cytology

a. Chromosome numbers

The results of the chromosome counts are given in an appendix at the end of this paper. The material is arranged alphabetically by country of provenance. Diploids (2n = 24) tetraploids (2n = 48) and octoploids (2n = 96) were observed. Diploids and octoploids are most common. These cytotypes occur throughout the whole area of the species in Europe. Tetraploids were found only in Portugal.

b. Chromosome portrait

The chromosome portraits of the 2n = 24, and 2n = 48 and 2n = 96 plants are regular (see fig. 1). No B-chromosomes or satellites are present.

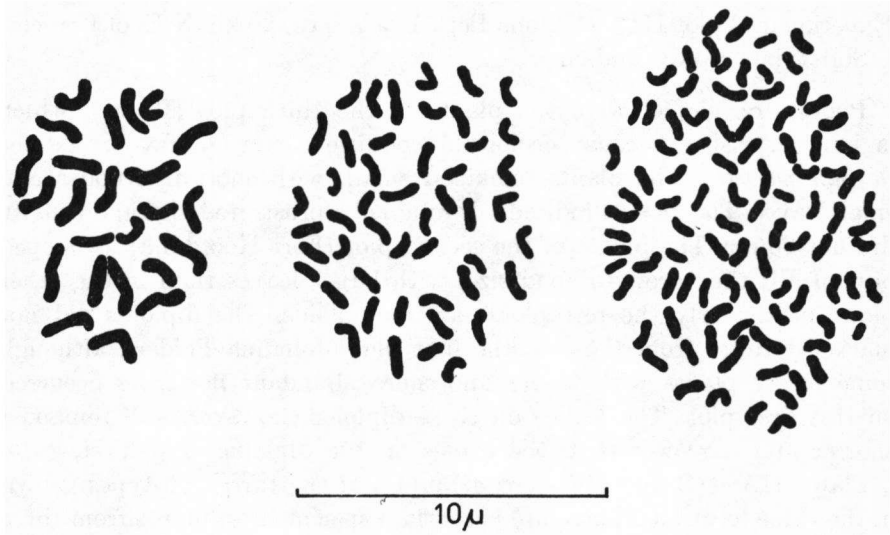


Fig. 1. Mitotic metaphase plates from roottips of *Galium palustre* L. Left a diploid (2n = 24) from Oost Voorne, the Netherlands; right an octoploid (2n = 96) from Monnikendam, the Netherlands, and in the middle a tetraploid (2n = 48) from Gorgolão, Portugal.

II. Morphology

The diploid plants can be distinguished from the octoploids by the above-mentioned morphological differences provided they are cultivated under similar conditions. The position of the 2n = 48 plants is much more unclear. They are intermediate between the diploids and octoploids, but closer to the octoploids than to the diploids. Plate I shows herbarium specimens of the three cytotypes: on the left a diploid plant found near Monnikendam, province of Noord-Holland, the Netherlands (K 381), on the right an octoploid from Loosdrecht, province of Utrecht, the Netherlands (K 736) and in the middle a tetraploid from Sesimbra, Estremadura, Portugal (K 127). It is, however, not always possible to obtain such a clear picture of the three cytotypes, due to overlap in the variation of the characters.

In order to assess the plasticy of the characters, plants were cultivated under different environmental conditions. Clones from a number of plants were transplanted to three different experimental gardens with different soil types where they were cultivated during several years.

Experimental plot I: "Johanna Polder", 6 km S.E. of Utrecht. Soil type: river clay, moderately dry to dry in summer. Very wet or submerged in winter.

Experimental plot II: "Fort Hoofddijk, ca. 4 km from the previous plot. Soil type: Sandy, containing also a little clay passing into heavy clay at a depth of about 80 cm. Humid and well-permeable throughout the year.

Experimental plot III: "Cantons Park Baarn", ca. 20 km N.E. of Utrecht. Soil type: Sandy and dry.

Poorest developed were the plants of the third plot (Baarn), which is not surprising because ecological conditions were unfavourable for *Galium palustre*. The plants remained small, with short internodes and small leaves. The 2n = 24 individuals retained a prostrate habit and usually did not flower. The plants of the second plot (Fort Hoofddijk) developed best of all; they grew to good size, with larger leaves than in the other plots, particularly the tetraploids and octoploids. The diploids did not markedly differ from those of the first plot (Johanna Polder), although some larger plants with longer and more abundant flowering occurred on this first plot. The leaves on these diploids, however, still remained shorter and narrower than the leaves on the diploids of plot II.

Plates IIA, IIB and IIC show examples of the three cytotypes grown in the three locations. These are herbarium specimens of plants from three parent plants, each belonging to one of the three cytotypes.

They were grown (left to right) in plots I (P), II (F) and III (B). Plate IIA: diploids from Monnikendam, the Netherlands (K 381); IIB: tetraploids from Gorgolão, Portugal (K 326) and IIC: octoploids from Loosdrecht, the Netherlands (K 736).

A comparative morphological study was made of the three cytotypes cultivated under these different conditions. Attention was paid to the stature of the plant, the shape of the panicle, the length of the internodes, the length and width of the leaves, the diameter of the corolla and the size of the fruits.

Stature

Diploids: stems decumbent (especially the non-flowering ones) to ascending, flowering stems often supporting each other, up to 60 cm long when grown under favourable conditions (plot II). Plants remaining prostrate under unfavourable conditions (plot III).

Tetraploids: stems more or less erect, rather firm, sometimes falling down or tending to do so as the season passes.

Octoploids: stems stout but weak, much branched, more or less decumbent and intertwined, up to 150 cm long under favourable conditions (plot II).

Panicle

Diploids: plants from the three experimental plots showed lax oblong panicles, with erect or ascending flowering branches, but more or less

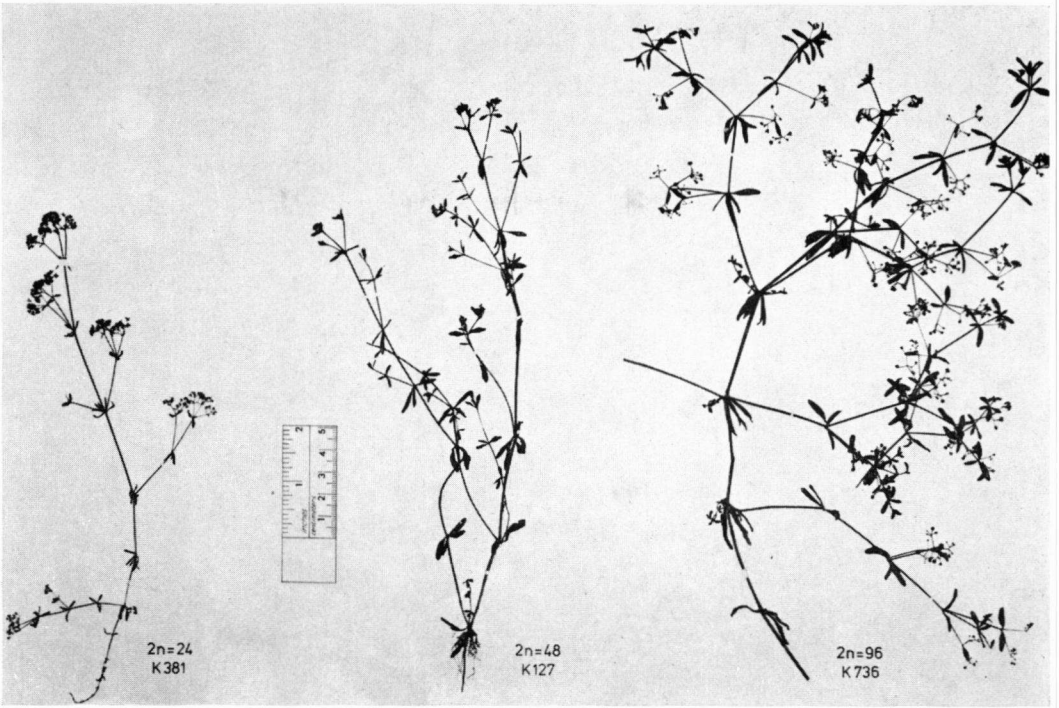
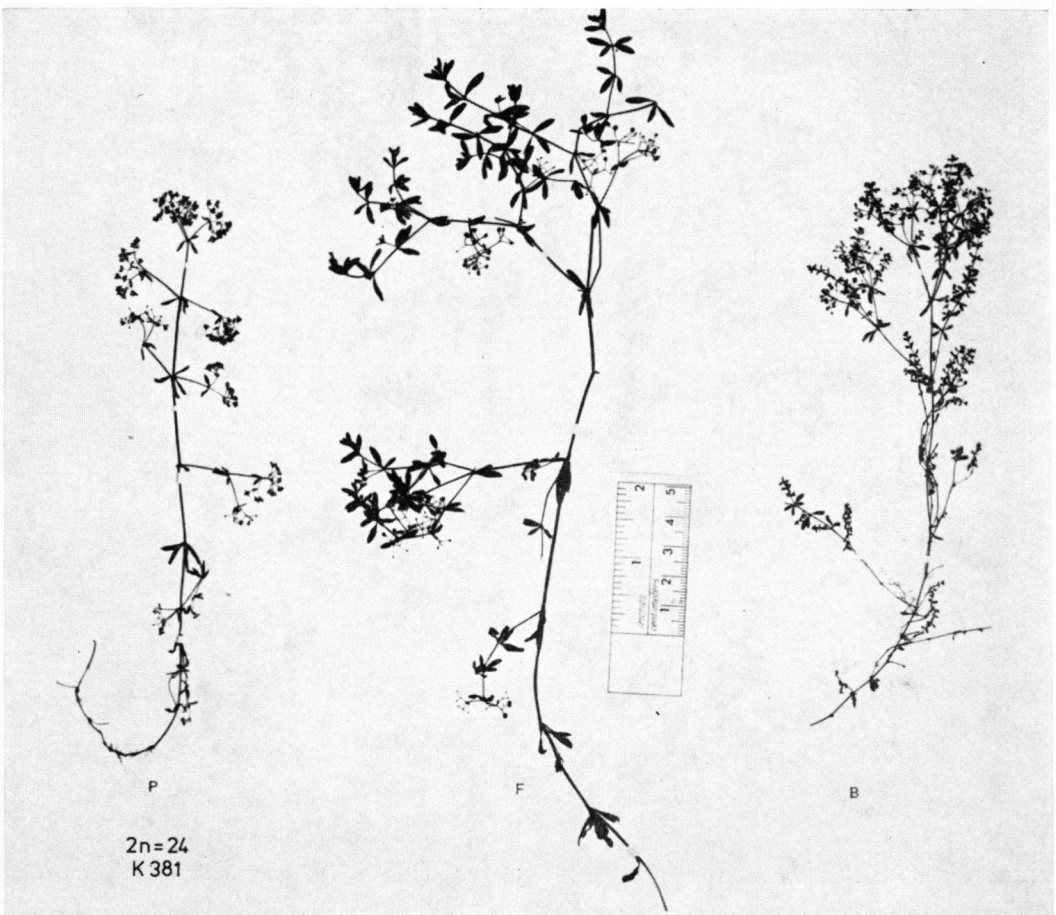


PLATE I. Diploid, tetraploid and octoploid plants of *Galium palustre*, cultivated under the same environmental conditions. For explanation see text.



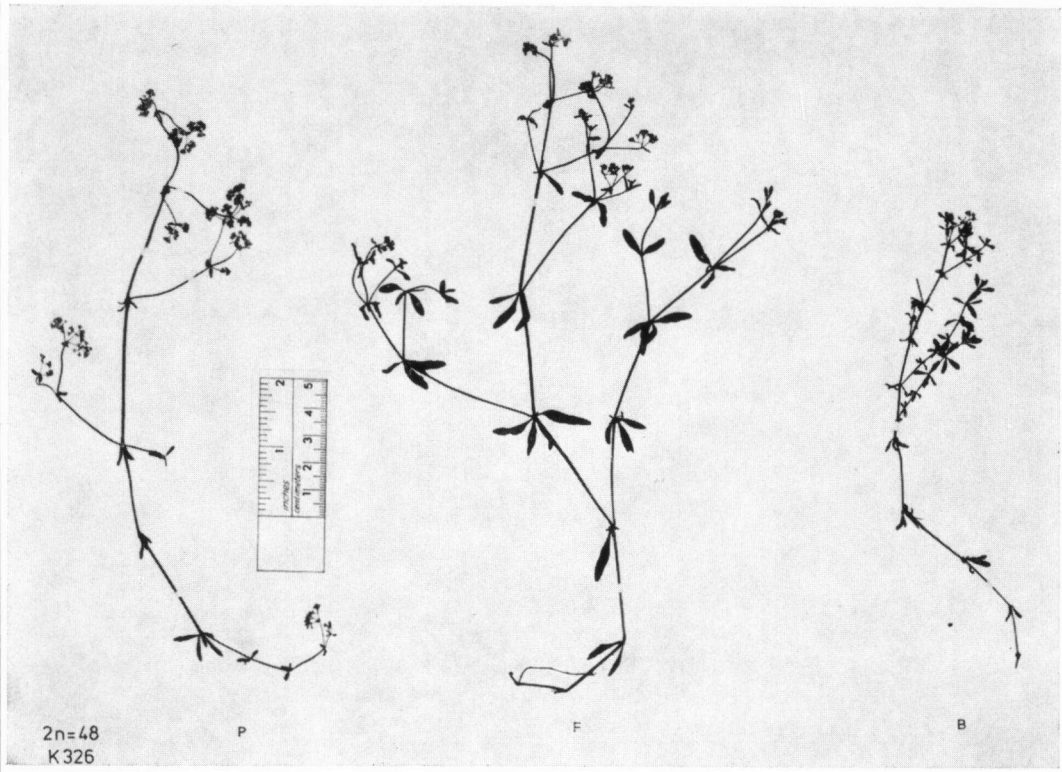


PLATE IIb. Three plants, clones from a tetraploid *Galium palustre*, cultivated on different soils. For explanation see text.

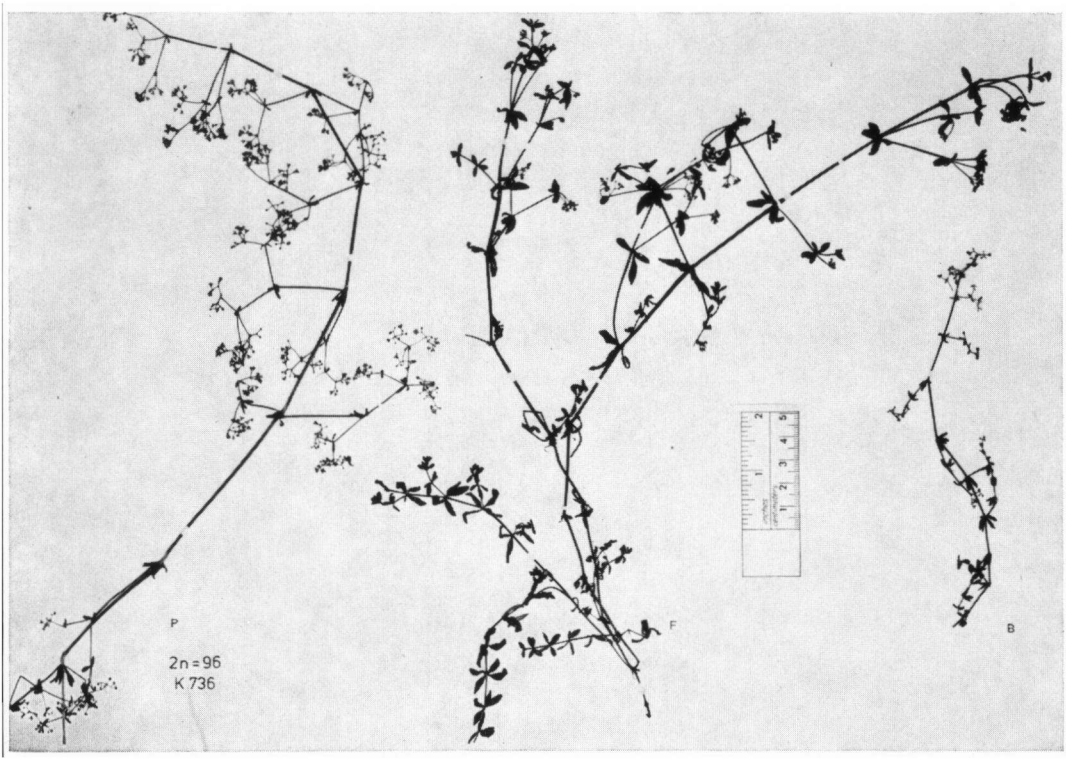


PLATE IIc. Three plants, clones from an octoploid *Galium palustre*, cultivated on different soils.

reflexed when in fruit. The shape varied from narrowly oblong to broadly oblong. This may be different in the same individual from year to year and was observed on all three plots.

Octoploids: panicles large, lax and pyramidal with erect flowering branches which become spreading, but not reflexed, in fruit.

Tetraploids: panicle shape more or less intermediate between those of the other cytotypes, although closer to that of the octoploids. Fruiting branches more or less reflexed like those in the diploids. No differences were observed between plants cultivated under different circumstances.

Internodes

The length of the internodes is variable in all three cytotypes, even within a single individual. The great amount of overlap makes separation impossible.

Leaves

Leaves in whorls of mostly four, sometimes six, obtuse to acute, never acuminate or mucronate, single-veined, margins entire and rough because of retrorse prickles.

The shape of the leaves varied in the three cytotypes. Leaves of the diploids in plot I and III were linear to lanceolate, usually broadest just above the middle; in plot II they were clearly lanceolate. The tetraploid and octoploid plants of plot I had oblanceolate or occasionally linear leaves. Those of plot II had broadly oblanceolate to sometimes narrowly elliptical to ovate leaves; in plot III the plants had small, oblanceolate leaves.

Both length and width of the leaves of the three cytotypes varied considerably. In order to see whether there is a correlation between this variability and the differences in chromosome number of the cytotypes cultivated on the different soils, we measured the length and width of (mostly) 50 leaves of each cytotype from each plot. The mean ($\bar{X}$), the standard deviation and the standard error of the mean were calculated and the results are given in table I.

The table shows clearly a certain overlap in length as well as in width of the leaves of the three cytotypes. On each plot the diploids and octoploids differ, the first always having shorter and narrower leaves. The values found in the tetraploids are intermediate between those of the two other cytotypes, with exception of the plants grown in plot III, where the tetraploids had the longest and widest leaves of all. In plot I the leaf-width in the tetraploids equalled or almost equalled the leaf-width of the octoploids. The spreading of the computed values within the cytotypes on the three experimental plots is shown when these values are arranged according to plants for each experimental plot. Table IIA and table IIB show the data for the length and width of the leaves.

TABLE I

Length and width in mm of the leaves of diploid, tetraploid and octoploid plants of Galium palustre cultivated on different soils. $\bar{X}$ is the mean, SE the standard error of the mean, SD the standard deviation and N the number of observations.

Size of leaves in mm	I Polder				II Fort				III Baarn			
	$\bar{X}$	±SE	SD	N	$\bar{X}$	±SE	SD	N	$\bar{X}$	±SE	SD	N
length												
2n=24	8.20	0.07	1.64	500	10.85	0.10	2.04	400	5.19	0.06	1.24	400
48	11.25	0.16	2.92	350	14.26	0.20	2.48	150	9.11	0.24	2.92	150
96	12.35	0.11	2.42	500	17.15	0.18	3.39	350	7.30	0.08	1.56	400
width												
2n=24	1.12	0.02	0.36	500	1.96	0.03	0.62	400	0.93	0.01	0.24	400
48	1.94	0.03	0.29	350	2.47	0.06	0.77	150	1.73	0.03	0.38	150
96	1.89	0.02	0.52	500	2.67	0.04	0.68	350	1.20	0.02	0.37	400

These tables show clearly the great variability both in length and width of the leaves within each of the cytotypes cultivated on the different fields.

In order to compare the leaf-length and -width of the 2n=48 cytotypes with those of the 2n=24 and 2n=96 plants, the Standard Error of Difference (SED) from plants from each of the fields was calculated.*

The observed differences between the mean length of the leaves of the cytotypes 2n=48 versus 2n=24 and 2n=96 versus 2n=48 are in all cases greater than twice the standard error of differences, except for those concerning the tetraploids from plot III. The same holds for the width of the leaves except that the values computed for the tetraploids and diploids of plot I are about the same.

In spite of a great amount of overlap, the tetraploids have longer and broader leaves than the diploids and shorter and narrower than the octoploids, with exception of those cultivated on plot III. Here the tetraploids have larger leaves than both diploids and octoploids. The tetraploids and octoploids from plot I could not be separated on the basis of the width of their leaves. SED computations for leafsize of diploids grown under favourable conditions (plot II) and octoploids cultivated under unfavourable circumstances (plot III) showed that under such circumstances the diploids have significantly longer leaves than the octoploids.

From these data it is clear that the size of the leaves is determined by external factors to such an extent that it is useless as a diagnostic morphological character.

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*) $SED = \sqrt{\frac{(SD_a)^2}{N_a} + \frac{(SD_b)^2}{N_b}}$

(SD_a) and (SD_b) are the standard deviations of the cytotypes; N_a and N_b are the numbers of observations.

TABLE IIA

Length of the leaves of a number of 2n=24, 48 and 96 cytotypes of *Galium palustre* L., cultivated on different soils. The first column gives the plant number. $\bar{X}$ is the mean, SE the standard error of the mean, SD the standard deviation; $X_{min.}$ and $X_{max.}$ are the minimum and maximum values observed, and N is the number of observations.

		I Polder					II Fort					III Baarn				
Leaf length		$\bar{X} \pm$ SE		SD	$X_{min.}$-$X_{max.}$	N	$\bar{X} \pm$ SE		SD	$X_{min.}$-$X_{max.}$	N	$\bar{X} \pm$ SE		SD	$X_{min.}$-$X_{max.}$	N
2n=24	37	6.82	0.10	1.23	4.00– 9.60	50										
	39	9.68	0.18	1.26	4.80–12.00	50										
	154	7.33	0.20	1.41	4.80– 9.60	50	9.60	0.24	1.70	6.40–12.80	50	4.58	0.10	0.71	3.20– 5.60	50
	155	7.38	0.21	1.48	5.60– 9.60	50	9.50	0.32	2.23	6.40–12.80	50	4.46	0.07	0.51	4.00– 5.60	50
	205	8.34	0.25	1.77	5.60–11.20	50	10.64	0.27	1.89	8.00–16.00	50	5.15	0.11	0.84	4.00– 5.60	50
	560	7.39	0.19	1.36	6.40–10.40	50	12.03	0.27	1.90	8.80–15.20	50	4.06	0.09	0.63	4.00– 5.60	50
	625	8.83	0.17	1.17	5.60–11.40	50	11.12	0.25	1.78	8.80–16.00	50	5.82	0.13	0.90	4.80– 8.00	50
	629	9.70	0.21	1.50	6.40–12.80	50	10.51	0.30	2.08	8.00–16.00	50	5.97	0.24	1.72	4.80–11.20	50
	643	7.92	0.13	0.92	6.40– 9.60	50	10 85	0.22	1.56	8.00–13.60	50	6.21	0.14	1.00	4.00– 8.80	50
	644	8.59	0.20	1.41	5.60–12.00	50	12.34	0.25	1.76	9.60–16.00	50	5.24	0.10	0.74	4.00– 8.80	50
2n=48	127	9.25	0.29	2.43	4.80–16.00	70										
	285	10.33	0.25	2.20	6.40–16.00	80	14.69	0.37	2.61	9.60–21.60	50	9.63	0.20	2.72	5.60–16.00	50
	326	13.47	0.28	2.78	8.00–20.00	100	15.33	0.32	2.28	8.80–20.80	50	7.84	0.18	1.29	5.60–11.20	50
	503	11.17	0.24	2.39	5.60–16.00	100	12.75	0.25	1.75	8.80–16.50	50	9.87	0.29	2.06	6.40–16.00	50
2n=96	210	11.68	0.20	1.39	9.60–16.00	50						6.78	0.17	1.23	4.80– 8.80	50
	242	14.19	0.30	2.10	11.20–21.60	50	17.41	0.41	4.14	10.40–32.00	100	7.76	0.24	1.69	4.00– 9.60	50
	403	14.27	0.30	2.04	11.20–20.20	50						6.51	0.17	1.21	4.00– 9.60	50
	405	10.42	0.17	1.20	8.00–12.00	50	15.49	0.31	2.20	9.80–20.00	50	7.36	0.31	2.22	4.00–12.00	50
	715	10.84	0.17	1.32	8.00–12.80	50										
	736	13.92	0.33	2.30	8.80–19.20	50	17.97	0.54	3.81	12.00–28.00	50	7.81	0.23	1.62	5.60–12.00	50
	740	10.72	0.25	1.77	6.40–14.40	50	16.40	0.42	3.00	11.20–24.00	50	7.79	0.15	1.08	4.00– 9.60	50
	741	10.43	0.30	2.17	6.40–16.20	50	17.01	0.42	2.94	12.80–24.00	50	7.90	0.24	1.70	4.80–12.00	50
	742	14.16	0.24	1.68	11.20–16.20	50										
	760	12.84	0.26	1.86	9.60–16.80	50	18.40	0.32	2.28	12.80–21.60	50	6.74	0.15	1.06	4.80– 8.80	50

TABLE IIB

Width of the leaves of a number of 2n=24, 48 and 96 cytotypes of *Galium palustre* L., cultivated on different soils. The first column gives the plant numbers. $\bar{X}$ is the mean, SE is the standard error of the mean, SD is the standard deviation; $X_{min.}$ and $X_{max.}$ are the minimum and maximum values observed, N is the number of observations.

		I Polder					II Fort					III Baarn				
Leaf width		$\bar{X} \pm SE$		SD	$X_{min.}$-$X_{max.}$	N	$\bar{X} \pm SE$		SD	$X_{min.}$-$X_{max.}$	N	$\bar{X} \pm SE$		SD	$X_{min.}$-$X_{max.}$	N
2n=24	37	0.96	0.03	0.20	0.80–1.20	50										
	39	1.47	0.05	0.34	0.80–2.00	50										
	154	0.96	0.04	0.25	0.64–1.60	50	1.46	0.06	0.43	0.80–2.40	50	0.85	0.03	0.19	0.64–1.20	50
	155	0.86	0.01	0.19	0.80–1.20	50	1.48	0.07	0.49	0.80–2.40	50	0.94	0.03	0.24	0.80–1.45	50
	205	0.90	0.03	0.21	0.64–1.20	50	1.62	0.04	0.29	1.20–2.80	50	0.86	0.03	0.21	0.75–1.60	50
	560	1.10	0.05	0.38	0.64–2.00	50	2.02	0.08	0.55	1.20–2.80	50	0.93	0.03	0.24	0.80–1.20	50
	625	1.14	0.04	0.27	0.80–2.00	50	1.85	0.08	0.54	0.80–3.20	50	1.13	0.04	0.25	0.80–1.60	50
	629	1.38	0.05	0.33	0.80–2.20	50	2.33	0.06	0.43	1,60–3.20	50	0.91	0.03	0.19	0.80–1.45	50
	643	1.02	0.04	0.26	0.80–1.60	50	2.59	0.06	0.41	2.00–3.20	50	0.93	0.03	0.18	0.64–1.60	50
	644	1.41	0.04	0.22	0.80–2.00	50	2.32	0.07	0.50	1.20–2.00	50	0.74	0.02	0.16	0.56–1.20	50
2n=48	127	2.01	0.06	0.46	0.80–2.80	70										
	285	2.10	0.04	0.39	1.60–3.20	80	2.97	0.09	0.62	2.00–4.00	50	1.86	0.06	0.41	0.80–2.40	50
	326	2.30	0.05	0.50	1.20–3.20	100	2.82	0.07	0.48	1.60–3.60	50	1.81	0.04	0.31	1.60–2.40	50
	503	1.36	0.03	0.29	0.80–2.00	100	1.64	0.05	0.34	1.20–2.40	50	1.53	0.05	0.34	0.80–2.00	50
2n=96	210	1.66	0.02	0.15	1.60–2.00	50						1.10	0.05	0.38	0.80–2.40	50
	242	2.03	0.06	0.40	1.20–2.50	50	2.22	0.05	0.50	1.20–3.60	100	0.80	0.03	0.19	0.64–1.68	50
	403	1.90	0.06	0.39	1.20–2.40	50						1.04	0.05	0.33	0.64–1.60	50
	405	1.67	0.04	0.31	1.20–2.40	50	2.84	0.06	0.41	2.00–4.00	50	1.31	0.03	0.24	0.80–1.60	50
	715	1.76	0.04	0.28	0.96–2.40	50										
	736	1.63	0.07	0.52	1.20–3.20	50	2.70	0.09	0.61	2.00–4.00	50	1.37	0.03	0.24	0.96–1.60	50
	740	2.36	0.09	0.55	0.80–3.20	50	2.34	0.10	0.73	1.40–4.00	50	1.41	0.07	0.49	0.80–3.20	50
	741	1.98	0.06	0.40	1.40–2.60	50	3.04	0.07	0.46	2.40–4.00	50	1.41	0.04	0.26	0.80–1.60	50
	742	2.01	0.06	0.63	1.20–2.40	50										
	760	1.87	0.04	0.29	1.20–2.40	50	3.23	0.08	0.60	1.60–4.00	50	1.12	0.05	0.32	0.80–1.60	50

TABLE IIIA

Size of the corolla in mm of diploid, tetraploid and octoploid plants of *Galium palustre* L., cultivated on different soils. For explanation see text. $\bar{X}$ is the mean, SE the standard error of the mean, SD the standard deviation, $X_{min.}$ and X_{max} are the minimum and maximum values observed, N is the number of observations.

corolla size in mm	I Polder					II Fort					III Baarn				
cytotype	$\bar{X} \pm$ SE		SD	$X_{min.}$-$X_{max.}$	N	$\bar{X} \pm$ SE		SD	$X_{min.}$-$X_{max.}$	N	$\bar{X} \pm$ SE		SD	$X_{min.}$-$X_{max.}$	N
2n=24	3.43	0.02	0.41	2.20–4.20	500	3.34	0.02	0.37	2.30–4.40	600	3.45	0.01	0.34	2.70–4.40	610
2n=48	3.75	0.02	0.35	2.80–4.50	265	3.83	0.02	0.29	2.70–4.80	240	3.75	0.03	0.32	3.30–4.50	150
2n=96	4.20	0.02	0.45	3.00–4.80	460	4.21	0.02	0.42	3.10–5.30	650	4.04	0.02	0.39	3.00–5.20	435

TABLE IIIB

Flower size of a number of 2n=24, 48 and 96 cytotypes of *Galium palustre* L., cultivated on different soils. The first column gives the plant number. $\bar{X}$ is the mean, SE the standard error of the mean, SD the standard deviation; $X_{min.}$ and $X_{max.}$ are the minimum and maximum values observed, N is the number of observations.

Corolla size in mm		I Polder					II Fort					III Baarn				
		$\bar{X} \pm$ SE		SD	$X_{min.}$-$X_{max.}$	N	$\bar{X} \pm$ SE		SD	$X_{min.}$-$X_{max.}$	N	$\bar{X} \pm$ SE		SD	$X_{min.}$-$X_{max.}$	N
	39	3.55	0.03	0.22	3.00–4.00	50	3.42	0.03	0.30	2.90–4.00	100	3.18	0.03	0.24	2.70–4.00	50
	154	3.59	0.03	0.34	2.60–4.20	100	3.59	0.03	0.32	2.90–4.10	100	3.53	0.04	0.35	2.80–4.40	100
	205	3.10	0.06	0.32	2.40–4.00	25	3.26	0.03	0.32	2.30–4.00	100	3.62	0.03	0.31	2.70–3.90	100
2n=24	625	3.20	0.03	0.26	2.70–4.00	100	3.26	0.04	0.37	2.40–4.00	100	3.16	0.05	0.39	2.80–4.00	60
	629	3.88	0.03	0.25	2.80–4.20	100						3.43	0.03	0.29	2.80–4.00	100
	644	3.40	0.06	0.30	2.80–4.00	25	3.70	0.03	0.29	2.40–4.30	100	3.85	0.03	0.34	1.00–4.30	100
2n=48	285	3.84	0.04	0.31	3.20–4.40	65	3.73	0.03	0.25	2.70–4.50	100					
	326	3.57	0.03	0.31	2.80–4.00	100	3.95	0.05	0.32	3.50–4.80	40	3.90	0.04	0.26	3.50–4.50	50
	503	3.89	0.03	0.33	3.10–4.50	100	3.88	0.03	0.25	2.70–4.50	100	3.68	0.03	0.33	3.30–4.50	100
2n=96	210	4.04	0.04	0.28	3.00–4.60	50	3.96	0.03	0.28	3.00–4.50	100					
	242						4.39	0.04	0.30	3.50–5.00	75	3.96	0.03	0.27	3.20–4.20	100
	715	3.94	0.05	0.33	3.30–4.60	50	3.90	0.03	0.31	3.10–4.70	100					
	736	4.21	0.04	0.37	3.20–4.60	100	4.32	0.07	0.36	3.20–4.70	25	3.88	0.04	0.40	3.00–5.20	100
	740	4.09	0.04	0.34	3.20–4.80	75	4.56	0.04	0.31	4.00–5.20	50	4.45	0.07	0.43	3.00–5.20	35
	741	4.55	0.04	0.42	3.50–5.50	100	4.65	0.02	0.21	4.00–5.30	100					
	742	4.09	0.05	0.29	3.10–4.60	50	3.95	0.03	0.30	3.00–4.50	100	4.01	0.02	0.22	3.50–5.00	100
	760	4.21	0.05	0.30	3.50–4.70	35	4.25	0.04	0.35	3.40–5.00	100	4.18	0.04	0.44	3.10–5.10	100

TABLE IV

Fruit size in mm of 2n=24, 48 and 96 cytotypes of *Galium palustre* L., cultivated on different soils. $\bar{X}$ is the mean, SE the standard error of the mean, SD the standard deviation; $X_{min.}$ and $X_{max.}$ are the minimum and maximum values observed; N is the number of observations.

	I Polder					II Fort					III Baarn				
Fruit size in mm	$\bar{X} \pm$ SE		SD	$X_{min.}$-$X_{max.}$	N	$\bar{X} \pm$ SE		SD	$X_{min.}$-$X_{max.}$	N	$\bar{X} \pm$ SE		SD	$X_{min.}$-$X_{max.}$	N
2n=24	1.21	0.01	0.13	0.80–1.50	150	1.23	0.01	0.10	0.90–1.50	150	1.19	0.01	0.12	0.70–1.40	150
2n=48	1.39	0.02	0.17	1.20–1.60	75	1.41	0.02	0.13	1.20–1.70	75	1.36	0.02	0.16	0.90–1.60	75
2n=96	1.71	0.01	0.16	1.40–2.10	150	1.69	0.01	0.17	1.40–2.20	150	1.62	0.01	0.14	1.20–2.00	150

The corolla

The corolla is white, the four lobes are acute. Measurements of the corolla diameter are given in Table IIIA and Table IIIB.

The tables show different corolla size in diploids, tetraploids and octoploids. Moreover, the corolla size is not, or hardly, influenced by difference in cultivation. The corolla size of the tetraploids is intermediate between those of the diploids and the octoploids. SED computations showed that in all possible combinations the 2n = 48 values can be separated from the 2n = 24 and 2n = 96 values, in spite of a great amount of overlap.

Fruits

The fruits are rugose, becoming black at maturity,

Fifteen fruits were measured from each of ten diploid, ten octoploid and five tetraploid plants. The mean ($\bar{X}$), the standard deviation (SD) and the standard error of the mean (SE) are given in Table IV.

The diploids have the smallest and the octoploids have the largest fruits with the tetraploids again being intermediate. The size of the fruits was apparently not influenced by differences in cultivation. SED computions proved the tetraploid fruits to be larger than the diploid fruits and smaller than the octoploid ones in all possible combinations, despite a certain degree of overlap.

Stomata

The size of 50 stomata was measured of each of ten diploid, ten tetraploid and ten octoploid plants from the experimental plot II. The mean ($\bar{X}$), the standard deviation (SD) and the standard error of the mean (SE) are given in table V.

TABLE V

Stomata size in micron of diploid, tetraploid and octoploid *Galium palustre* L., cultivated on the experimental plot II (for explanation see text). $\bar{X}$ is the mean, SE the standard error of the mean, SD the standard deviation; $X_{min.}$ and $X_{max.}$ are the minimum and maximum values observed; N is the number of observations.

stomata size	$\bar{X}$	± SE	SD	$X_{min.}$–$X_{max.}$	N
2n=24	27.71	0.13	2.88	21.60–36.00	500
2n=48	32.25	0.16	3.50	25.20–39.60	500
2n=96	34.54	0.18	4.40	25.20–43.20	500

The observed differences are such that, although there is an overlap with both the diploids and octoploids (see fig. 2), the tetraploids can be said to have larger stomata than the diploids and smaller than the octoploids.

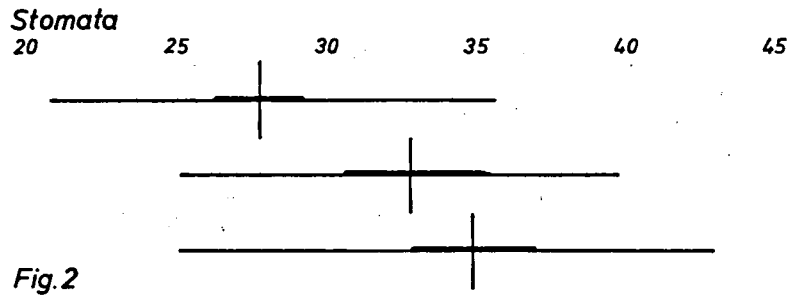

Fig. 2. Ranges of variation in the length in μ of the stomata of diploid, tetraploid and octoploid *Galium palustre* L. The horizontal lines represent the ranges, the vertical lines the means and the solid lines twice the standard deviation of the mean.

III. Ecology

Diploids and octoploids have different ecological preferences. Diploids were found in places which are usually inundated in the winter and wet but not inundated in the summer. The octoploids occur in places inundated throughout the year, or at least near water on very wet soil.

A good example of a habitat of diploids was found near Odoorn in the province of Drenthe, the Netherlands. In winter these grounds, on a sub-soil of loam, are flooded to a high of 10–15 cm. During spring the water disappears, whereafter the vegetation stays dry until late fall. Populations of *Galium palustre* in this area were sampled by taking three plants in 25 spots which were all transferred to the experimental garden.

Excellent examples of both diploid and octoploid types of habitats were met with along the dike of the IJsselmeer between Monnikendam and Hoorn over a length of 30 km. In many places the water stays permanently or the soil is at least soaked all the time. On higher grounds the water disappears during the summer leaving at most a more or less wet soil. Samples were taken from populations at regularly spaced intervals, each sample consisting of 5–7 plants, which were again brought to the experimental garden. All plants from permanently inundated or ever-soaked grounds were octoploids whereas all plants from the temporary dry grounds were diploids.

Species often occurring side by with the diploid *Galium palustre* are:

Angelica silvestris L., *Anthoxanthum odoratum* L., *Cirsium palustre* (L.) Scop., *Filipendula ulmaria* (L.) Maxim., *Holcus lanatus* L., *Lychnis flos-cuculi* L., *Phragmites australis* (Cav.) Trin. ex Steud., *Plantago lanceolata* L., *Potentilla anserina* L., *Prunella vulgaris* L., *Ranunculis acris* L., *Ranunculus repens* L., *Rumex hydrolapathum* Huds., *Symphytum officinale* L., *Trifolium pratense* L., *Valeriana officinalis* L.

Species occurring with the octoploid *Galium palustre*: *Apium inundatum* (L.) Rchb., *Cardamine pratensis* L., *Carex otrubae* Podp., *Cirsium palustre* (L.) Scop., *Hippuris vulgaris* L., *Hydrocotyle vulgaris* L., *Lychnis flos-cuculi* L., *Lysimachia vulgaris* L., *Lysimachia nummularia* L., *Mentha aquatica* L., *Phragmites australis* (Cav.) Trin. ex Steud., *Potentilla anserina* L., *Ranunculus aquatilis* L., *Trifolium pratense* L., *Veronica anagalis-aquatica* L.

IV. Flowering period

The flowering period of the three cytotypes growing in the experimental garden was recorded every seven days during three successive years. The beginning of the flowering period was marked by the opening of the first bud, the full flowering by the whole inflorescence having open flowers. The end of the flowering period could not be registered exactly, due to a more or less irregular second flowering period in which additional inflorescences developed which lasted until the end of the season.

The 2n = 24 cytotype flowers about 10–14 (20) days earlier than the 2n = 48 and 2n = 96 cytotypes and the flowering period was reached earlier by about the same amount of time. The cytotypes 2n = 48 and 2n = 96 appeared to have more or less the same flowering behaviour. Fig. 3 demonstrates the histogram of the percentage of flowering of thirty plants with 2n = 24 chromosomes, twenty with 2n = 48 chromosomes and twenty five with 2n = 96 chromosomes, all flowering during the same summer.

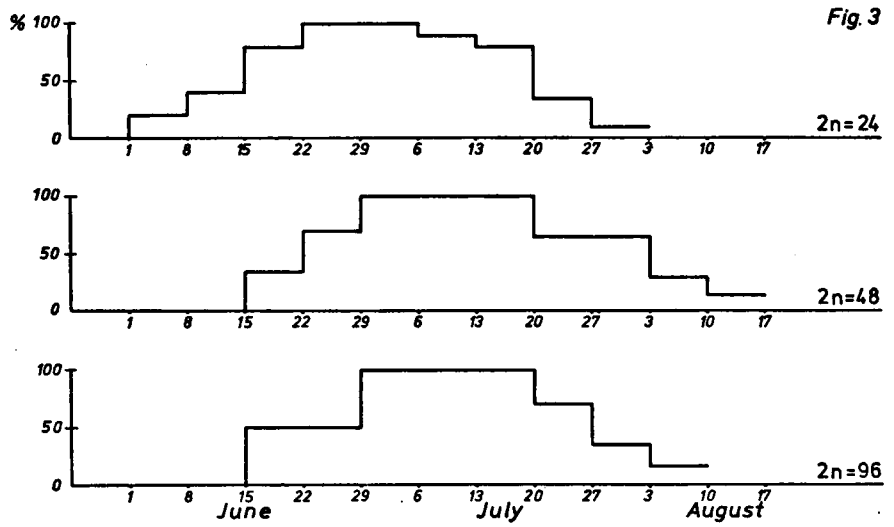

Fig. 3. Histogram of flowering periods of the three cytotypes of *Galium palustre* L. For explanation see text.

V. Crossing experiments

Crossing experiments were made between the three cytotypes in order to understand their interrelationship.

The methods used for these experiments were described in a previous paper (KLIPHUIS, 1970). Active cross pollination between plants of the same cytotype was always followed by setting of fruits. The seeds of these fruits germinated and gave rise to plants with the same chromosome number.

Fruits were never formed when the inflorescences were enveloped by paperbags.

Active self-pollination also gave negative results.

No hybrids could be obtained from any combination of crosses between plants of different ploidy levels.

Discussion

The cytological investigation confirmed the existence of the three cytotypes within *Galium palustre*. The number 2n = 88, reported by Piotrowicz (1961) from plants from Poland, could not be confirmed. The diploids and octoploids are common. Tetraploids, for the first time reported by Hancock (1942) from one locality in England, were observed by us only in material from Portugal. Tetraploids may occur in other parts of the area as well, but apparently this cytotype is rare.

The differences in ecological preferences formerly described by Fagerlind (1934, 1937) for the diploid and octoploid plants in Sweden and later by Hancock (1942), Clapham (1949) and Clapham et al. (1952) for Britain were fully confirmed for material from the Netherlands. Communities were these cytotypes occur side by side as reported by Hancock (l.c.) were not found although this phenomenon may occur under exceptional environmental circumstances.

The comparative morphological study of various characters showed that diploids differ from octoploids. Good distinguishing characters proved to be the panicle shape, the mode of branching with fruit maturation, and the size of flower, fruit and stomata. Plant and leaf size are to a very large extent influenced by environmental factors and there is a time lag in the flowering time as noted by Hancock (1942) and Clapham (1949).

Cytology, ecology and morphology provide sufficient arguments to justify recognition of two taxa which, when looked at the isolation, might be considered as two separate species, as was done repeatedly in the past, namely *Galium palustre* L. s. str. (diploid) and *Galium elongatum* (C. Presl.) Lange (octoploid).

However, the picture is obscured by the presence of a tetraploid which has an intermediate position as regards the characters, with an overlap to both the diploid and the octoploid sides. With this tetraploid bridging the gap, as it were, which would otherwise have existed between the diploid and octoploid, it does not seem correct to assign the rank of species to the diploid and the octoploid. The tetraploids, with their growth habit, leafsize and flowering coinciding more or less with that of the octoploids, seem to stand closest to the latter. This applies also to the size of the stomata. This might lead us to place the diploid as a separate taxon opposite the tetraploid and octoploid. This interpretation would be equally untenable. In the tetraploids, too, plant and leafsize are highly dependent upon the conditions of cultivation. These characters may overlap to such an extent that it is not always possible to identify the cytotype. Octoploids grown under adverse conditions may remain smaller and have smaller leaves than diploids grown under favourable conditions. Tetraploids may overlap with both. Examples are given in Plate IIIa, IIIb and IIIc.

Plate IIIa shows a diploid (K 644) from plot II and an octoploid (K 405) from plot I. Plate IIIb shows tetraploid (K 326) from plot II and an octoploid from plot I (K 715). Plate IIIc gives a picture of the three cytotypes cultivated under unfavourable conditions (plot III).

In addition to their differences the cytotypes have different sizes of corolla and fruit. Despite a certain amount of overlap the tetraploid have definitely smaller flowers and fruits than the octoploids and larger flowers and fruits than the diploids. These differences appeared to be independent from cultivation conditions. The panicle of the tetraploids is partly as that of the octoploids and partly as that of the diploids; it is laxly pyramid-shaped with branches reflexed by fruit-maturing, and it can in this way be distinguished from the two other cytotypes.

In the experimental garden the tetraploids developed erect and firm stems which did not fall down, or which only did so at the end of the season. In the very early season the stout, but weak stems of the octoploids grow like those of the tetraploids, but they soon fall down. The stem development affords an excellent character to distinguish tetraploids from octoploids in the garden; unfortunately, unless noted by the collector, it is lost with drying and cannot be seen in the herbarium material.

Considering all data the conclusion must be that the three cytotypes should be understood as three taxa of equal rank. Which rank should be assigned them, depends upon one's aims in classification such as is shown by DAVIS and HEYWOOD (1969), when they cite the now classic experiments by FAGERLIND (1937) on crossing *Galium mollugo* L. with *Galium verum* L.. FAGERLIND proved that in both these species diploids as well as tetraploids occur, which, within the species concerned, cannot be separated by morphological characters. Intraspecific hybridization on different ploidy level is not possible; interspecific hybridization is possible between tetraploids only. These results by Fagerlind could be reproduced in the experimental garden in Utrecht. The hybrid is morphologically intermediate and has pale yellow flowers. A biosystematic classification would regard the two tetraploids as one species, thus separating them from the diploids (DAVIS and HEYWOOD, 1969).

In the case of *Galium palustre* one would, consequently, have to distinguish three separate species within *Galium palustre* L. s.l., since hybridization, as shown by the crossing experiments, does not occur between different ploidy levels. From the taxonomic point of view this is unsatisfactory. The tetraploid is just too much intermediate in its morphology and a number of characters are too obviously affected by environmental factors. It is often impossible to identify herbarium specimens without cytological data.

In view of this Clapham's solution appears the best. He considered the three cytotypes as sub-species of *Galium palustre* L.: subsp. palustre (2n = 24), subsp. tetraploideum Clapham (2n = 48) and subsp. elongatum (C. Presl.) Lange.

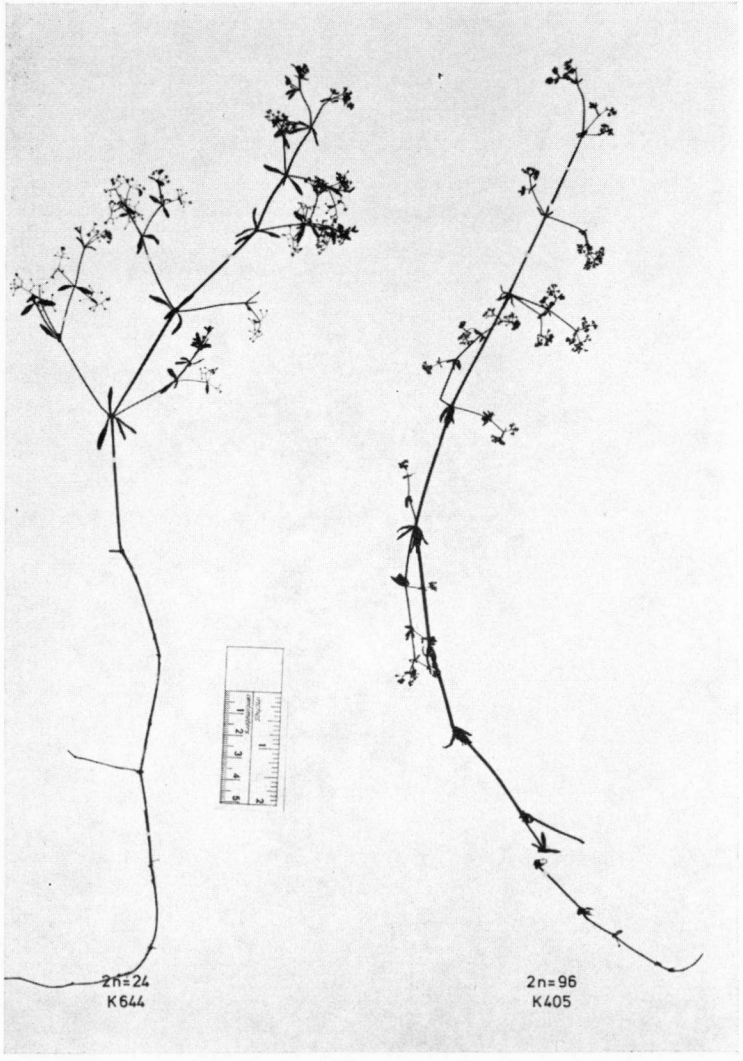

PLATE IIIa. A diploid *Galium palustre* from the Netherlands (K

PLATE IIIb. A tetraploid *Galium palustre* from Portugal (K 326),

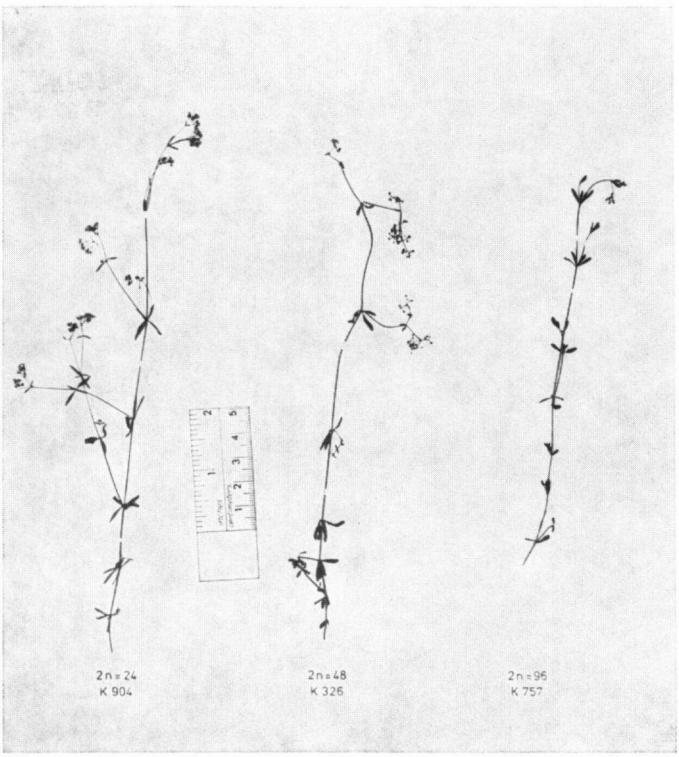

PLATE IIIc. Three cytotypes of *Galium palustre* L. cultivated under unfavourable circumstances. The diploid and octoploid are from the Netherlands, the tetraploid from Portugal.

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APPENDIX

Material used in the cytological investigation of *Galium palustre*.

Diploids 2n=24.

Czechoslovakia: K 729 – Bohemia.

Denmark: K 205 – Sjaelland, Roskilde; K 361 – Sjaelland, near Copenhagen, Skodsborg.

France: K 560, K 703 – Brennilis, Finistère.

Germany: K 1087 – Wietzen, near Hannover.

Netherlands: Province of Brabant, K 1008 – Drunense duinen, between Helvoort and Kromvoort.

Province of Drenthe, K 814 – Odoornerveld; K 818 – Odoorn, west of the village, K 871–K 940 – plants from populations (see text), near Odoorn.

Province of Friesland: K 982 – Isle of Terschelling, Groede; K 988 – Isle of Terschelling, Parabool-duin; K 1189 – Isle of Schiermonnikoog, Reddingsweg.
Province of Gelderland: K 629, K 632 – Leemkoele near Speulderbos; K 644, K 645 – "De Schovenhorst", Putten; K 1003 – between Barneveld and Renswoude; K 1011 – near Lobith.
Province of Limburg, K 37, K 39 – Ravensbos near Houthem.
Province of Noord-Holland; K 381 – near Monnikendam; K 739, K 743 – south of Hoorn; K 855, K 857, K 860, K 865, K 866, K 867 between Monnikendam and Hoorn (see text); K 861, K 862, near Volendam.
Province of Utrecht, K 154, K 155 – near Maarn; K 625, K 626 – between mainroad Utrecht–Arnhem, near Maarn; K 737 – near Loosdrecht; K 977 – Estate of Sandwijck, De Bilt.
Province of Zuid-Holland; K 747–K 753, Oost Voorne, former airstrip; K 754, K 755 – near the Biological Station "Weeversduin"; K 756 – Oost Voorne, in the dunes near the coast aside "de badweg"; K 805, K 806 – Oost Voorne, Kwakjeswater; K 1005, K 1006, Oost Voorne, Equisetum-valley.
Norway: K 1150 – Isle of Boroya, Oslo Fjord.
Portugal: K 1024, K 1027 – Serra do Gerês.
Sweden: K 275 – collected in nature (Lund).

Tetraploids 2n=48.
Portugal: K 127 – Estremadura, near Sesimbra; K 181, K 185 – Estremadura, between Marcodo and Santana; K 285 – Estremadura near Sesimbra; K 326 – Gorgolão, near Coimbra; K 503 – near Oporto.

Octoploids 2n=96.
Czechoslovakia: K 715 – Bohemia.
Denmark: K 201 – N.E. Jutland, Iglesø near Rold Skov; K 210 – Albaek Mose near Tarm.
Ireland: K 1108 – Strokestown, Co. Roscommon.
Netherlands: Province of Drenthe, K 1079 – between Donderen and Norg.
Province of Friesland, K 983, Isle of Terschelling, de Bosplaat; K 984, K 986 – Isle of Terschelling, Herdersplak; K 1212 – Isle of Ameland, near Nes.
Province of Gelderland, K 1013 – near Lobith.
Province of Noord-Holland, populations between Monnikendam and Hoorn, see text: K 740–K 742; K 757–K 760; K843–K 854; K 858, K 863, K 864.
Province of Utrecht, K 736, Loosdrecht; K 997, K 1001 – between Loenen and Loosdrecht; K 868, K 869, K 870 – Fort Hoofddijk, De Bilt; K 942, K 944, K 945 – Eempolder.
Portugal: K 242 – Ribatejo: Villa Franco de Xiro; K 476 – near Sacavem.
Rumania: K 403, K 405 – near Bucarest.