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CYTOTAXONOMIC STUDIES ON GALIUM PALUSTRE L. MORPHOLOGICAL DIFFERENTIATION OF DIPLOIDS, TETRAPLOIDS AND OCTOPLOIDS

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SUMMARY

Three cytoptypes of *Galium palustre* L., i.e. diploids (2n = 24), tetraploids (2n = 48) and octoploids (2n = 96), were subjected to a morphological investigation using pattern detection methods. Diploids and octoploids form two well distinguishable groups. However, it is impossible to differentiate three separate groups. This is mainly due to the intermediate position of the tetraploid cytotype, the characters of which may overlap with those of diploids and octoploids, but to a larger degree with the latter.

1. INTRODUCTION

Galium palustre comprises a polyploid complex with diploids (2n = 24), tetraploids (2n = 48), octoploids (2n = 96) and dodecaploids (2n = 144). Diploids and octoploids occur throughout Europe. The first, however, have a more northerly distribution, the latter a more southerly one. Tetraploids are known from the Balkans (ANČEV 1974; TEPPNER et al. 1976; KLIPHUIS 1986), but they are most frequently occurring in the Sub-Mediterranean – Atlantic area (KLIPHUIS 1983, 1984) Dodecaploids have been reported from two scattered localities, one in Austria and one in Turkey by TEPPNER et al. (1976).

As a rule diploids are plants that grow in damp places that dry out in summer; octoploids prefer permanently damp zônes, often bordering upon water (FAGERLIND 1937; HANCOCK 1942; CLAPHAM 1949; KLIPHUIS 1974). Tetraploids are plants of a habitat intermediate between that of diploids and octoploids (HANCOCK 1942; KLIPHUIS 1983). The ecological preferences, however, are not always found to be strictly associated with the chromosome number. In this respect tetraploids and octoploids are more tolerant than diploids.

Morphologically diploids can be distinguished from octoploids when they are growing under favourable conditions. This is the reason why both cytotypes are often considered as separate species (e.g. EHRENDORFER et al. in *Flora Europaea* 1976). However, the differences are mainly of a quantitative nature, and they may be influenced by the environment in such a way that it is not always possible to identify the cytotype without knowing the chromosome number (KLIPHUIS 1974). The picture is complicated by the occurrence of the tetraploid. Its characters may be influenced by the environment as is the case with the di-

ploids and tetraploids (KLIPHUIS 1974).

When cultivated under uniform conditions, the tetraploids show a variability in their morphological characters which is much larger than that found in the diploids and octoploids. Sometimes distinct vegetative characters are reminiscent of those found in *Galium debile* Desv. (KLIPHUIS 1984). This species stands close to *Galium palustre* L. Its distributional area is about the same as that of the tetraploid *Galium palustre*. It is a diploid that also has the basic chromosome number X = 12 instead of X = 11 (which is the number found for most species of the genus *Galium*). These data point to an allopolyploid origin of the tetraploid. Almost certainly *Galium debile* should be regarded as one of the parental sources (KLIPHUIS 1984).

The suggestion that the tetraploid could have an allopolyploid origin is in contradiction with the opinion of TEPPNER et al. (1976). These authors did not succeed in demonstrating any difference in the morphology of the diploids and tetraploids available for their study. In view of this they suggest an autopolyploid origin for the tetraploid. Their different interpretation is also expressed in the classification of the cytotypes concerned. They included the diploid and tetraploid in one species (e.g. *Galium palustre* L.) without further taxonomic recognition and consider the octoploid as a well delimited, allopolyploid species (e.g. *Galium elongatum* (C. Presl) Lange), clearly separable from the diploid and tetraploid cytotypes. However, in the opinion of HANCOCK (1942), CLAPHAM (1949) and later on also of KLIPHUIS (1974), the diploids, tetraploids and octoploids should be considered as taxa of equal rank, of which the classification into subspecies seems to be the most appropriate one. These two opinions are so contradictory that it seemed to be worthwhile reassessing the evidence using pattern detection methods.

2. MATERIAL AND METHODS

2.1. Material

27 diploids, 55 tetraploids and 17 octoploids of *Galium palustre* and one specimen of *Galium debile* were used in this study. The plants were dug up from the field or were grown from seed collected in nature and they were cultivated under uniform conditions in an experimental plot for several years. The diploid and octoploid plants of *Galium palustre* were collected in different parts of Europe. The tetraploid plants all came from the north western parts of the Iberian Peninsula, with the exception of one plant (no. 1343), which was found in the Zlatibor Mountains in Yugoslavia. *Galium debile* was found in the vicinity of Roudar, in the province of Lugo in Spain.

2.2. Observation methods

Chromosome counts are based on metaphase plates of root-tip mitosis. For that purpose root tips were fixed in Karpechenko's fixative, embedded in paraffin wax, sectioned at 15 micron and stained according to Heidenhain's haematoxylin method. The morphological data were obtained from living material. In *table 1* the characters investigated are listed.

Table 1. Investigated characters of diploid, tetraploid and octoploid plants of *Galium palustre* L.

1. Stature:	a) erect b) decumbent to ascending c) decumbent
2. Stem:	a) length in cm b) diameter in mm c) shape, quadrangular or not d) with or without ribs
3. Internodes:	length of the longest internode in mm
4. Panicle:	a) divaricately branched or not b) narrowly oblong to broadly oblong, or not c) broadly pyramidal or not d) interruptedly pyramidal or not
5. Leaf:	a) Length and width in mm Five of the largest leaves of each plant investigated were measured at the bases and at the top of the longest internode and the mean and SD were calculated b) Shape, three types were distinguished: 1) linear to linear-lanceolate, 2) narrowly to broadly-lanceolate, 3) broadly oblanceolate c) apex: being obtuse or somewhat acute d) margin: 1) smooth or rough due to retrorse prickles, 2) flat or revolute e) position: 1) erecto patent, 2) deflexed, 3) more or less patent f) number of leaves in the whorls: 1) four, 2) five or 3) six
6. Corolla:	a) for each plant investigated, the diameter of five of the largest flowers was measured in 0.1 mm, and the mean and SD were calculated b) for each flower whose diameter was measured, the width of the largest lobe was measured in 0.1 mm and the mean and the SD were calculated
7. Pedicel	a) the length of the pedicels of the flowers used for measuring the diameter of the flowers was measured in 0.1 mm b) position of the pedicel in fruit: 1) divergent or 2) convergent
8. Fruit:	a) the length and the width of five of the largest fruits of each individual were measured in mm and the mean and SD were calculated b) structure of the epidermis: 1) distinctly tuberculate, 2) smooth 3) smooth to tuberculate
9. Flowering period:	The beginning of the flowering period was recorded for a number of plants in two successive years of cultivation, I and II respectively. The beginning of the flowering period was recorded by opening of the first bud.

2.3. Pattern detection methods

Both supervised and non-supervised pattern detection methods were used. In the supervised approach the polyploidy of the specimens was used as the classification criterion (yielding three classes: diploids, tetraploids and octoploids) and recognition criteria of these groups were generated on the basis of the morphological characters. In the non-supervised approach no apriori classification is used, but the specimens, as characterized by their morphological characters, are clustered. The thus generated classes can be used for supervising supervised methods. By using both approaches one obtains a more complete picture of

the separability of the groups. In the non-supervised approach the similarity between specimens was defined as the mean city block distance from the range normalised characters.

Clusters were generated by agglomerative cluster analysis. WARD's (1963) clustering criterion was used because it is known that the structure in the data is weak and therefore the strong pattern filtering capacity of this method is needed (HOGEWEG 1976; HOGEWEG & HESPER 1981). Additionally the UPGMA clustering criterion was used to assess the extend of the deformations caused by the strong space dilating properties of WARD's method (which cause aberrant species to be included in early stages in clusters with which they have little in common).

Optimum splitting levels were calculated using the criterion of HOGEWEG & HESPER (1978) and the clusters were characterized monothetically (by using statistics of all characters over the clusters) as well as oligothetically (i.e. by searching a small set of characters which are sufficient to classify the specimens into the clusters). These same characterisation techniques were used on the apriori defined ploidy classes. Multivariate characterization methods are less appropriate in the present context. In order to assess the relative distances between ploidy classes, the dataset was projected into two dimensions using principal coordinate analysis of the mean city block distances (GOWER, 1966).

3. RESULTS

3.1. Cluster analysis

Fig. 1 shows the dendrogram of the specimens investigated of the *Galium palustre* group. It is clear that the diploid *Galium palustre* (2n = 24) form a well separated cluster. Only two "lost" tetraploids are interspersed in the cluster of diploids and there are no diploids (except *Galium debile*, which was included as reference only) outside this cluster. By contrast the other main cluster (the optimum splitting level is into two clusters) consists of tetraploids and octoploids. The tetraploids are in the majority. The octoploids are scattered in small groups all over the cluster. Thus cluster analysis revealed no distinction between tetraploids and octoploids even when Ward's clustering criterion was used.

When the UPGMA clustering criterion was applied the results were similar, although the dendrogram is much less structured. Again the diploids (plus the same two tetraploids) form a cluster. This cluster is, however, not one of the two main clusters. *Galium debile* (which in the Ward dendrogram occurred in an inconspicuous place in the tetra-octoploid cluster) is now shown to be aberrant. Also some of the tetraploids show up as very different from the others.

Principal coordinate analysis (*fig. 2*) further elucidates these results. The tetraploids occupy a relatively large area in the middle of the plot. On one side the diploids form a coherent group only overlapping the tetraploids to a very small extent. On the other side the tetraploids are flanked by and overlap with the octoploids. *Galium debile* is well separated from the rest. With respect to the first principal coordinate *Galium debile* is similar to the diploids (it is itself a diploid), but is far out on the second axis and several tetraploids are not far away.

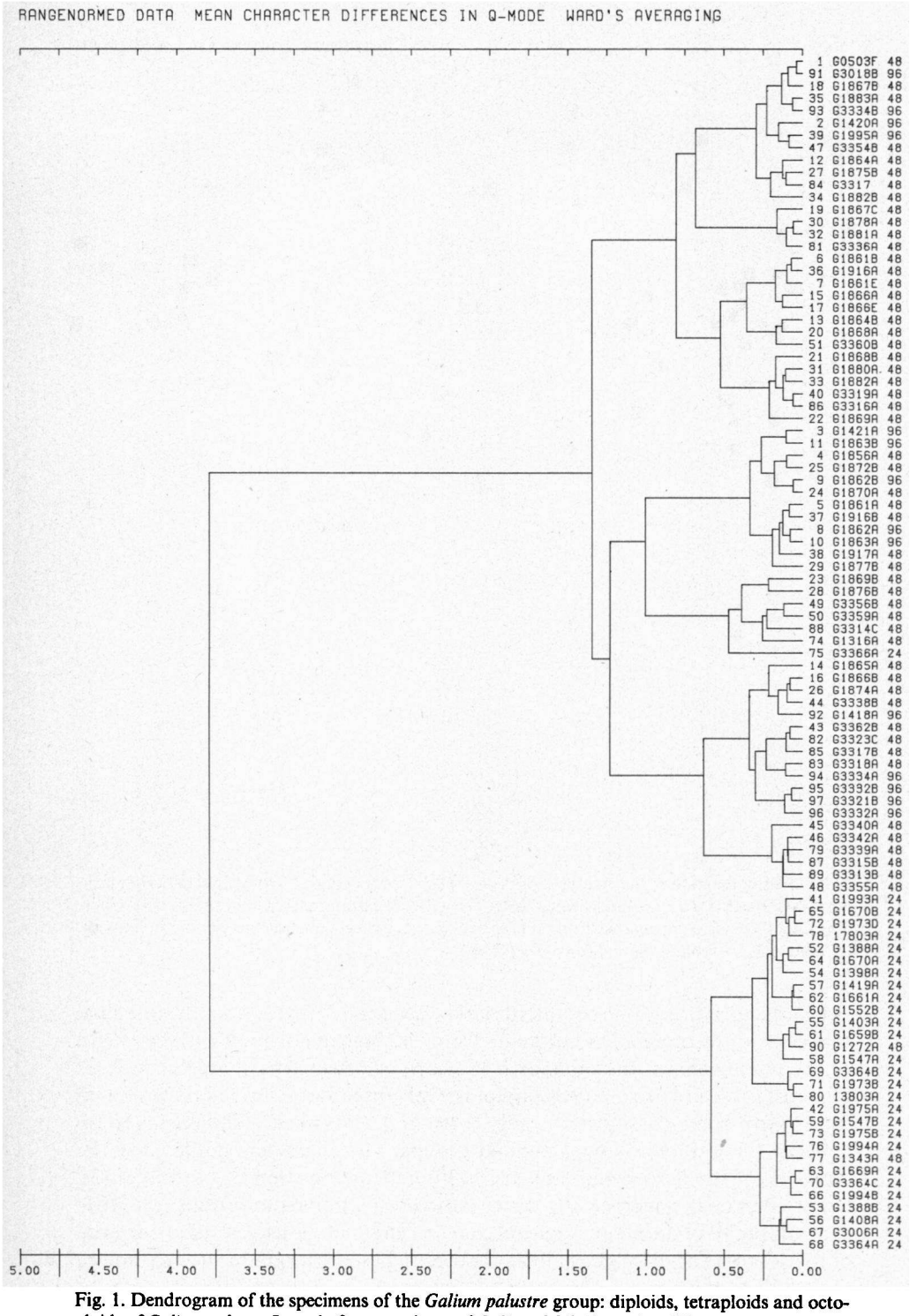


Fig. 1. Dendrogram of the specimens of the *Galium palustre* group: diploids, tetraploids and octoploids of *Galium palustre* L. and of one specimen of *Galium debile* Desv. (no: 75).

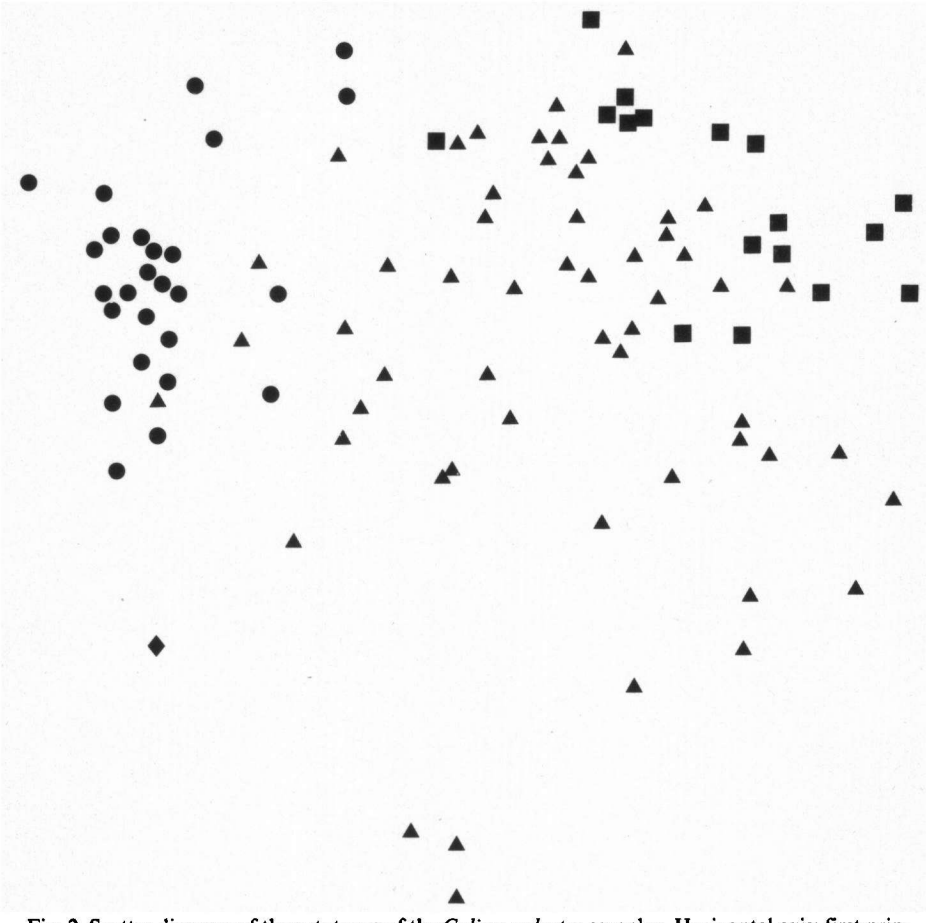


Fig. 2. Scatter diagram of the cytotypes of the *Galium palustre* complex. Horizontal axis: first principal axis; Vertical axis: second principal axis; The axes are calculated by Gower's Principal Coordinate method on mean character differences between the specimens. *Galium palusre*: diploids ●, tetraploids ▲, octoploids ■ and *Galium debile* ◆.

Characterisation of the polyploid classes (see *table 2*) reveals that the principal character to distinguish the diploids from the higher polyploids is the length of the main stem: in the diploids it is clearly shorter. This character in itself is almost sufficient to classify the diploids. Only three more characters are needed for a fairly good classification, only 5 (= 5%) "mistakes". The mistakes are between the tetraploid and octoploid groups. The characters needed are: the diameter of the corolla (20) and fruit (30) and the position of the leaves (5). Other characters which clearly differ between the polyploid groups (see *table 2*) are the habit of the plant, the shape and the length of the leaves, the occurrence of five leaves in a whorl, the panicle characteristics, the length of the internodes, the width of the corolla, the largest diameter of the fruit and the diameter of

Table 2. Distribution of character values over the polyploid groups.

KW AVAR	No. in Table 1	Character	2n=24 $\bar{X}$	2n=24 SD	2n=48 $\bar{X}$	2n=48 SD	2n=96 $\bar{X}$	2n=96 SD
		Characters which conforming to the distinguishing in cytotypes						
63.9	2.a	Stem, length	30.3	4.8	48.2	7.0	54.5	6.2
60	8.a	Fruit, mean length	1.8	0.2	2.1	0.2	2.4	0.2
58.9	8.a	Fruit, mean width	1.5	0.2	1	0.1	2.3	0.1
51	6.a	Corolla, mean diameter	35.3	4.3	41.2	3.6	47.5	4.2
46.2	3	Internodes, length	41	15.7	78.2	18.4	84.6	23.9
36.5	4.d	Panicle	0.0	0.2	0.7	0.5	1.0	0.0
32.3	1	Stature	1.5	0.6	0.8	0.9	0.0	0.0
29.6	4.b	Panicle	0.9	0.3	0.4	0.5	0.0	0.0
27.2	5.a	Leaf, mean length	13.4	3.5	18.7	14.4	19.1	3.2
26.4	6.b	Corolla, mean width	10.5	1.2	11.8	1.7	11.8	2.9
25.4	2.b	Stem, diameter	10.5	1.7	16.1	3.8	18.9	4.6
18.3	5.b	Leaf, shape	1.7	0.5	1.1	0.6	1.2	0.7
17.3	5.f	Leaf, number in a whorl, 5	0.0	0.2	0.6	0.5	0.6	0.5
16.3	4.c	Panicle	0.2	0.4	0.6	0.5	0.9	0.3
		Other characters						
	9	Flowering period II	–	–	166.0	18.4	166.1	3.5
	5.d.1	Leaf, margin	0.2	0.4	0.4	0.5	0.1	0.3
	5.f	Leaf, number in a whorl, 6	0.0	0.0	0.3	0.5	0.3	0.5
	6.a	Corolla, SD diameter	1.5	0.6	1.9	1.1	2.3	1.4
	5.a	Leaf, mean width	29.7	9.8	29.6	7.1	33.1	7.0
	5.a	Leaf, SD length	1.4	0.5	1.7	1.0	1.8	0.8
	9	Flowering period I	–	–	165.9	4.1	169.3	3.3
	8.a	Fruit, SD length	0.1	0.1	0.1	0.0	0.1	0.0
	5.a	Leaf, SD width	3.2	1.5	3.1	2.2	3.9	2.2
	8.a	Fruit, SD width	0.1	0.0	0.1	0.1	0.1	0.0
	7.a	Pedicel, length	27.9	7.8	26.4	7.4	25.0	10.0
	5.f	Leaf, number in a whorl, 4	1.0	0.2	0.8	0.4	1.0	0.0
	5.e	Leaf, state	2.4	0.6	1.94	0.9	1.8	0.9
	5.c	Leaf, apex	0.0	0.2	0.3	0.3	0.0	0.0
	5.d.2	Leaf, margin	1.0	0.2	0.9	0.3	0.8	0.4
	8.c	Fruit, setting	0.8	0.4	0.9	0.4	0.8	0.4
	8.b	Fruit, epidermis	1.0	0.2	1.0	0.1	1.0	0.0
	6.b	Corolla, SD width	0.9	0.3	2.6	12.0	0.8	0.5
	2.b	Stem, ribs	0.9	0.3	0.9	0.3	0.9	0.2
	4.a	Panicle	0.4	0.5	0.4	0.5	0.4	0.5
	7.b	Pedicel, divaricate	1.0	0.2	1.0	0.0	1.0	0.0
	7.b	Pedicel, convergent	0.0	0.2	0.0	0.0	0.0	0.0

KW AVAR – Kruskal Wallis one way analysis of variance.
$\bar{X}$ – mean value of character in the group.
SD – standard deviation of character in the groups.

the stem. Thus the separation criteria are mostly size related although some shape characters coincide with those of the polyploid groups.

It is interesting to note that is harder to find an oligothetic recognition criterion

for the cluster generated by the cluster analysis than for the polyploid groups. Eight characters are needed to reach 5% misclassifications. This indicates that the cluster analysis "loses itself in n dimensions", picking up ad hoc similarities between specimens and thereby losing the loco-dimensional (major) polyploid pattern. By contrast the necessarily low dimensional representation of the principal coordinate analysis clearly shows this major pattern. The tetraploids are shown to be variable but occupy an intermediate position between the diploids and the octoploids. They are, however, more similar to the octoploids, and in some cases it is even impossible to distinguish between the two.

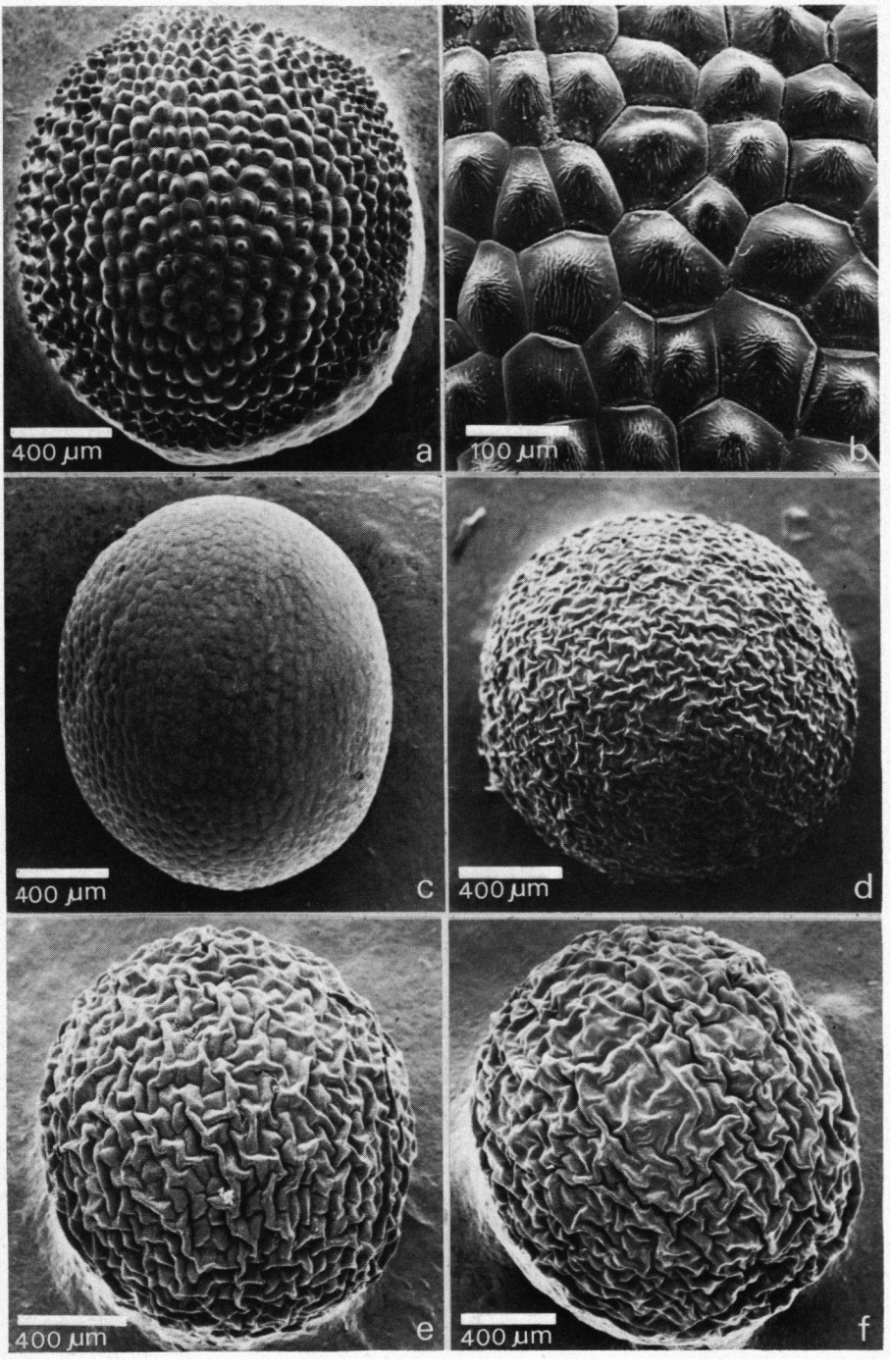
4. DISCUSSION

The results of our study clearly show the isolated position of *Galium debile*. We agree with the opinion of EHRENDORFER et al. (1976) and TEPPNER et al. (1976) that *Galium debile* is a well delimited species. Its morphology varies and particularly the vegetative characters may be strongly influenced by the environment (HANCOCK 1942; KLIPHUIS 1984). *Galium debile* has linear, apiculate leaves which are broadest beyond the middle. It is characterized by having the pedicels convergent in fruit and by having distinctly tuberculate fruits (*fig. 3*). The leaves of *Galium palustre* are narrowly to broadly oblong-lanceolate, obtuse or sometimes slightly subacute. The pedicels are divergent in fruit and the fruits are never tuberculate but they are always smooth to somewhate rugose. In fresh fruits the cuticula of *Galium palustre* is regularly structured. In dry fruits it shrivels and becomes wrinkled (*fig. 3*). Even when the plants were cultivated under uniform conditions it was impossible to make a clear-cut distinction between the three cytotypes of *Galium palustre* on the basis of the characters investigated. This is due to the intermediate position of the tetraploid. Within this cytotype the variability in morphology is such that there is an overlap with both, the diploids and the octoploids. The overlap with the octoploid is the greatest. Within the tetraploids it is impossible to demonstrate any group with a certain systematic value.

The three cytotypes concerned show differences in ecological preference, but these differences are not absolute. In this respect tetraploids and octoploids have a much greater tolerance than diploids. The conditions in the experimental plot were slightly more favourable to the tetraploids and octoploids than to the diploids. Habit and vegetative characters are strongly influenced by the environment (KLIPHUIS 1974). This may be one reason why the diploids form such a well separated cluster in the cluster analysis. It may also be one of the reasons why the tetraploids and octoploids overlap to such a high degree.

The tendency for ecological and geographical differentiation combined with differences in morphology, particularly in the shape of the panicle and the size

Fig. 3. SEM micrographs of a fresh fruit of *Galium debile* Desv. (a and b) and of a diploid plant of *Galium palustre* L. (c) and of a dry fruit of a diploid (d), tetraploid (e) and octoploid plant (f) of *Galium palustre* L.


400 µm
a
100 µm
b
400 µm
c
400 µm
d
400 µm
e
400 µm
f

of the flowers and the fruits, make it possible to distinguish to a certain degree diploids and octoploids occurring in nature outside the area of the tetraploid. Within the area of the latter this is almost impossible. Unless one knows the chromosome number it is not always possible to identify the cytotype correctly. It is this situation which makes it so difficult to decide about the systematic position of the cytotypes within this polyploid complex. We found no justification for the view of TEPPNER et al. (1976) who considered the diploid and tetraploid together as one species on the one side and the octoploid as a separate species on the other side. Our results point more to an allopolyploid origin for the tetraploid, indicating that *Galium debile* should be seen as one of the parental sources.

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