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Biosystematic studies of the *Rumex acetosella* complex (*Polygonaceae*). IV. Pollen morphology and the possibilities of identification of cytotypes in pollen analysis

By

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With 7 figures (3 figs. on 3 plates, 4 figs. in the text)

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Summary

Nijs J. C. M. den, Hooghiemstra H. & Schalk P. H. 1980. Biosystematic studies of the *Rumex acetosella complex* (*Polygonaceae*). IV. Pollen morphology and the possibilities of identification of cytotypes in pollen analysis. — Phyton (Austria) 20 (3—4): 307—323, 7 figures. — English with German summary,

This palynological study was carried out in view of a possible identification of the various cytotypes by pollen analysis and of a karyo-geographic reconstruction of the fossil history of the *Rumex acetosella* agg. An analysis was made of the degree of correlation between some morphological pollen characters and the 2x, 4x and 6x ploidy levels of the *Rumex acetosella* agg. In a series of pollen samples from a wide range of Europe the following details were recorded: diameter, the relation between tricolporate and peri(tetra)colporate grains, and some structural details as visible in SEM photographs. These data were expressed as ratios based on number and size of perforations in the tectum and the number of processes on the tectum.

The mean diameter increases progressively from the 2x to the 4x and to the 6x level. A variation analysis reveals a high degree of inhomogeneity at every ploidy level; the variance of the individual means is so great that only in exceptional cases the diameter as such has any diagnostic significance.

Diploids nearly always have 100% tricolporate pollen grains, tetraploids vary from 50% to 100% tricolporate ones, and hexaploids from 0 to 99% with the mean in the lower percentages. This great variance at the 4x and 6x levels

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is believed to be associated with the regionally hybridogenous structure of the species complex.

On account of their appreciable inconstancy the sculptural characteristics have no diagnostic value.

The present authors are of the opinion that a reconstruction of the former distribution of diploids on the basis of pollen samples from sediments will prove to be practicable.

Zusammenfassung

Nijs J. C. M. den, Hooghiemstra H. & Schalk P. H. 1980. Biosystematische Studien im *Rumex acetosella*-Komplex (*Polygonaceae*). — IV. Pollenmorphologie und die Möglichkeiten der Identifizierung von Cytotypen in der Pollenanalyse. — Phyton (Austria) 20 (3—4): 307—323, 7 Abbildungen. — Englisch mit deutscher Zusammenfassung.

Im Hinblick auf eine mögliche Identifizierung der verschiedenen Cytotypen im Zuge pollenanalytischer Studien und auf eine karyogeographische Rekonstruktion der Fossilgeschichte des *Rumex acetosella* agg. wurde die vorliegende Untersuchung ausgeführt. Die Korrelation pollenmorphologischer Merkmale mit den Ploidiestufen 2x, 4x und 6x ist studiert worden. In einer Serie von Pollenproben aus weiten Teilen Europas wurden die folgenden Merkmale näher betrachtet: Durchmesser, das Verhältnis von tricolporaten und peri(tetra)colporaten Pollenkörnern und einige auf REM Bildern sichtbare Details der Pollenoberfläche; letztere Daten wurden in Form von Verhältniszahlen ausgedrückt, die auf Zahl und Größe der Perforationen und der Zahl der Höcker des Tectums basieren.

Der mittlere Durchmesser steigt von der 2x- über die 4x- bis zur 6x-Stufe an. Eine Variationsanalyse zeigt eine hohe Inhomogenität auf jeder der drei Ploidiestufen. Die Streuung der Mittelwerte einzelner Individuen ist so groß, daß der Pollendurchmesser als solcher nur ausnahmsweise einen diagnostischen Wert besitzt.

Diploide haben fast immer 100% tricolporate Pollenkörner, bei Tetraploiden schwankt ihr Anteil zwischen 50 und 100%, und bei Hexaploiden liegt er zwischen 0—99% mit einem Mittel in den niederen Prozentbereichen. Diese große Variabilität bei den Tetraploiden und Hexaploiden hängt vermutlich mit der regionalen, hybridogenen Struktur der Artengruppe zusammen.

Wegen der beträchtlichen Inkonstanz der Skulpturmerkmale haben diese keinen diagnostischen Wert.

Die Autoren sind der Ansicht, daß sich eine Rekonstruktion der früheren Verbreitung der Diploiden auf Grund von Untersuchungen fossilen Pollens aus Profilsäulen als praktikabel erweisen wird.

1. Introduction

Rumex acetosella is a polyploid complex with a basic chromosome number of x = 7 and four known ploidy levels, viz., 2n = 14, 28, 42 and 56, of which the first three occur in Europe and the last seems to have a more or less circumarctic distribution. The complex has at one time been subdivided on the basis of the ploidy level and of certain morphological features

into four species: *R. angiocarpus* MURB. (supposed to be diploid), *R. tenuifolius* (WALLR.) LÖVE (supposed to be tetraploid), *R. acetosella* L. s. str. (said to be hexaploid), and *R. graminifolius* LAMB. (the octoploids) by LÖVE 1941a, b and 1944. This subdivision in species has rather generally been considered to be suspect for various reasons (compare, e. g., HADAČ & HAŠEC 1948, HYLANDER in LÖVE 1960, HARRIS 1968, STERK & DEN NIJS 1971, DEN NIJS 1974, 1976, in preparation). According to DEN NIJS 1974, 1976, in the press, and SCHEFFER & DEN NIJS 1978 the distribution of the cytotypes in Western, Central, and South-Eastern Europe forms a rather fine-meshed pattern of diploid and tetraploid (relict) areas whereas in Western Europe a very widespread occurrence of hexaploids was established. The distribution of the diploid and in particular of its gymnocarpous form (which is supposed to be more primitive because the perigone lobes are not fused with the pericarp as in the angiocarpous one) is clearly of a relict nature since it is present in several glacial refugia (southern French Alps, Aosta valley, Wachau near Vienna in Austria, S.-W. Bulgaria: compare the distribution maps in the cited references). In the same relevant publications the suggestion is made that on account of their distribution pattern, among other things, the tetraploid cytotype populations arose polytopically. The direct demonstration of the relict nature of the diploids (and the tetraploids?) and of their polytopic origin is very difficult. A sufficiently thorough insight into the distributional ranges of the cytotypes in the past (i. e., into the historical karyo-geography) might prove to be an essential requirement. To this end one would, for instance, have to be able to determine the ploidy level by means of a study of fossil pollen grains. Provided the cytotypes can indeed be distinguished by their pollen grain characteristics, so that fossil pollen can be identified to the ploidy level, cogent evidence might be obtained from pollen samples from sediments. ERDTMAN & NORDBORG 1961 attempted to gain information concerning the erstwhile distributional ranges of various cytotypes of *Sanguisorba*. STEBBINS 1950, 1959 and ERDTMAN 1964 suggest that such correlations between palynological features and ploidy levels do indeed exist. Generally speaking the pollen grain diameter increases as the ploidy level is higher (SCHWARZ 1964, EHRENDORFER 1970), and the morphological characteristics (structure and sculpture of the tectum) may contribute towards the identification. OLTMANN 1972 found in *Oxalis* a manifest correlation between the basic chromosome number and the ploidy level on the one hand, and the number and the arrangement of the apertures. However, critical keys can not be drawn up in all cases: DAVIS & HEYWOOD 1963 already sounded a word of warning; BÖCHER 1960 found no size difference between the pollen grains of diploid and those of tetraploid plants of *Campanula rotundifolia* from different localities. HENRICKSON 1973, when dealing with the pollen morphology of the *Fouquieriaceae*, established that in this family the pollen size sometimes increases with the higher ploidy level, but on the other hand

hexaploids may have pollen of the smallest recorded size. He attributes this differences to flower size in connection with differences in the anthecology of the various species. In view of the fact that all forms of *R. acetosella* are anemophilous such an anthecological factor cannot possibly play a role, but one nevertheless ought to be diffident when attempting to find a correlation between pollen characteristics and ploidy levels.

The application of SEM techniques in the last decade has opened up new perspectives in the study of morphological pollen characters (see, e. g., SMIT & WIJMSTRA 1970, SMIT 1973, GUGGENHEIM 1975, FERGUSON & MULLER 1976). For the present study a forthcoming paper by HOOGHIEMSTRA & SMIT (in preparation) is of direct importance since it shows that a combination of features discernable in a light microscope and on SEM photographs (e. g., sculpture indices based on magnifications of about $\times$ 20,000) enabled them to distinguish the rather similar pollen of *Oxyria digyna*, *Rumex acetosa*, and *R. acetosella* from one another. When the present study was undertaken it was hoped that such a combination of microscopical methods would also yield useful information to separate the various ploidy levels of the *R. acetosella* aggregate, so that a reliable key for the identification of their pollen could be drawn up by means of which the erstwhile occurrence of a certain cytotype in a sample of fossil pollen could be established by the study of, say, 10 grains in a satisfactory state of preservation.

2. Material and Methods

2.1. Samples studied

The 25 samples studied represent a selection from the collections brought together for the karyo-geographical analysis of the aggregate constituting a major part of a biosystematic inquiry by the senior author. Fig. 1 shows the localities of the selected samples, and in Table 1 they are arranged, per ploidy level, in a sequence according to their geographical origin from W. to S.E.-Europe. The numbering of the samples (and the recorded chromosome numbers) corresponds with those used and reported in previously published papers as indicated in the map legend.

The samples consisted of pollen extracted from plants collected in the field (usually from herbarium specimens) with the exception of the numbers between brackets which are samples obtained from living plants raised in the experimental garden from seeds collected in the field.

2.2. Light-microscopical observations of pollen diameter and structure

Each sample number represents 1 to 5 (usually 2) male plants selected for the present study. Of each plant the pollen of at least 10 individual flowers was collected. The following procedure was employed:

(1) boiling for 5 min. in 10% KOH which is stirred all the time;
(2) straining of the mixture, and transfer to distilled water;

(3) centrifugation, twice;
(4) rinsing in glacial acetic acid and another centrifugation;
(5) acetolysis in an acetic acid anhydride-H_2SO_4 mixture (9 : 1) for 5 min. at 100° C;
(6) rinsing, twice, in distilled water followed by centrifugation;

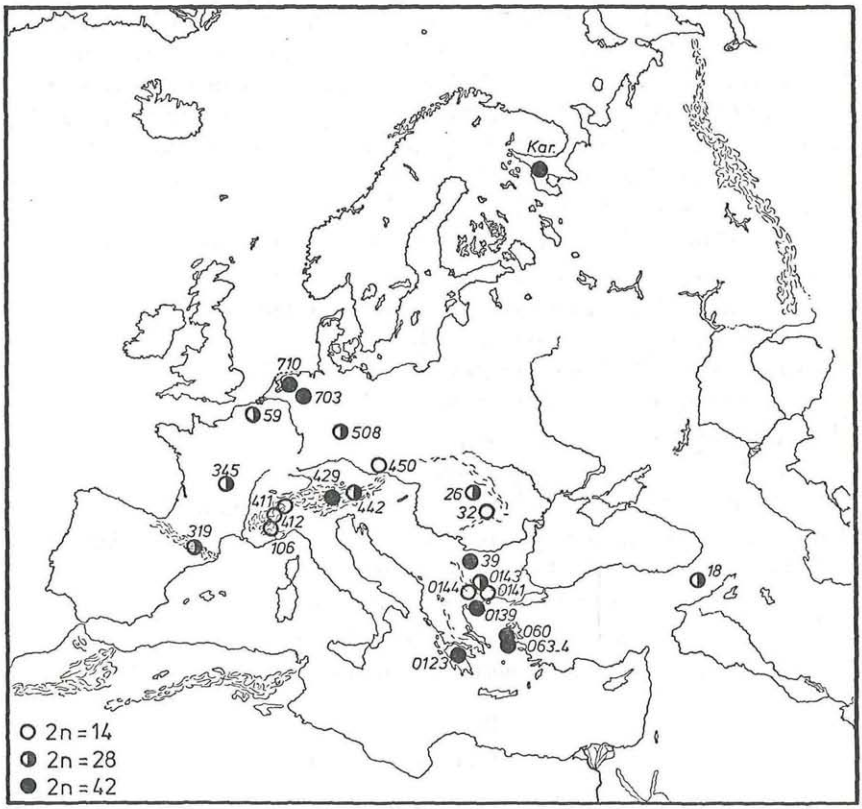

Fig. 1. Situation of the sampled populations of the *Rumex acetosella* agg. and the corresponding chromosome numbers after DEN NIJS 1970, 1974, 1976, SCHEFFER 1978, and unpublished data

(7—10) rinsing followed by centrifugation in 96% ethanol, absolute alcohol, xylene, and cyclohexane, successively;
(11) mounting in silicone oil without coverglass supports.

N.B. The treatments 7 up to 10 were applied in order to obtain completely water-free slides.

The preparations were examined under a Zeiss RA research microscope at a magnification of × 780. Per slide the diameter of 50 pollen grains was measured: of the somewhat dorsoventrally compressed tricolporate grains

the diameter of the equatorial plane, and of the subglobose pericolporate ones in any direction. For the calculation of the ratios tri- vs. pericolporate grains mostly about 300 grains were counted.

2.3. SEM method: Sculpture of the tectum

After acetolysis according to ERDTMAN 1960, the pollen is suspended in alcohol. A few drops of this suspension are placed on a SEM stub holder covered with a thin layer of a mixture of rubber cement and carbon powder. After drying of the pollen, a thin layer of gold is applied by evaporation in a SEM Coating Unit E 5000, Polaron Equipment Ltd. (2 min./1,2 kV./ 40 mA resulting in a goldlayer of about 20 to 50 nm.). The SEM used was a Cambridge Mark II Stereoscan. The best results were obtained with an accelerating voltage of 10 kV.

The following characteristics were scored from the SEM-micrographs:

— perforations and deep depressions in the tectum;
— surface area of the perforations per unit of surface area;
— the number of processes on the tectum.

From these data the following three ratios were obtained:

$$R_1 = \frac{\text{surface area of tectum part measured}}{\text{total surface area of perforations}}$$

$$R_2 = \frac{\text{surface area of tectum part measured}}{\text{number of processes} \times \text{surface area of one processus}}$$

(This surface area of a single process is put at 1 at a magnification of $\times$ 20,000, so that R_2 has also no dimension.)

$$R_3 = \frac{\text{number of processes per unit of surface area}}{\text{number of perforations per unit of surface area}}$$

The statistical procedures are according to SOKAL & ROHLF 1969.

3. Results

3.1. Collected data of size differences

In Table 1 the means and the standard deviations of the pollen diameters per plant are tabulated. This survey shows that the pollen dimensions gradually increase. The s. d. of the diameter per individual plant is rather variable. Fig. 2 shows the relative frequency distribution of the size for each ploidy level. All records per ploidy level were summed. The increase in mean diameter is unmistakable ($\bar{x}$ = 15.8—17.7—19.1 µm.) but owing to the appreciable variation, the overlapping is considerable. Table 2 shows the results of the analysis of variance as applied to the recorded data arranged groupwise according to the ploidy levels.

The conclusion to be drawn from this analysis is twofold, viz., (1) at every ploidy level there are highly significant differences between the mean pollen sizes of individual plants; they are in fact very heterogeneous assemblies: the means per ploidy level calculated for the sake of a surveyable

Tabel 1

Survey of samples studied, their origin, and results of analyses

Degree of ploidy	Country	Population, origin	Plant no.	Diam. $\bar{x} \pm s$	aperture type n	aperture type % tri-colporate grains	SEM yes	SEM no
2 x	France	Alps, Tinée	(106-9)	16.6 ± 1.2	300	100		
			(106-13)	14.7 ± 0.9	300	99		
2 x	Italy	Alps, Aosta	(412-5)	14.7 ± 1.2	322	99	yes	
2 x	"	" "	(411-14)	-	-	-	yes	
2 x	Austria	Wachau, Dürnstein	(450 coll.)	15.0 ± 0.8	300	96	yes	
2 x	Roumania	the Carpathians	32-1	16.7 ± 1.7	279	86	yes	
			32-2	16.8 ± 1.4	300	100	yes	
			32-3	16.3 ± 0.7	253	100	yes	
2 x	Greece	Macedonia, Pirgi	(0141-2) # 1	14.7 ± 1.3	300	97	yes	
			# 2	-	-	-	yes	
2 x		Macedonia, Serrai	(0144-1) # 1	15.0 ± 0.7	300	100	yes	
			# 2	-	-	-	yes	
2x	Exper. garden F1	S.E. France	R422	16.2 ± 1.2	309	100	yes	
2 x	Exper. garden F2	S.E. France	R467	16.8 ± 1.3	100	98		
4 x	Belgium	Flanders, Wachtebeke	59-32	17.0 ± 0.9	300	100	yes	
			59-53	17.4 ± 0.9	300	95	yes	
4 x	France	Haute Loire, Pinols	345-22	18.9 ± 1.1	304	56	yes	
			345-23	17.7 ± 0.8	305	76	yes	
			345-25	18.0 ± 1.4	207	61	yes	
			345-26	18.5 ± 1.4	250	70	yes	
			(345-34)		300	97	yes	
			(345-29)		300	73		
4 x	France	Pyrenees, Col Quillane	(319 coll.)	15.5 ± 1.1	300	99	yes	
			(319 ind.)					
4 x	Austria	Salzburg, Sauerfeld	(442) # 1	-	-	-	yes	
			# 2	-	-	-	yes	
4 x	Roumania	-	26-1	17.9 ± 1.1	275	91		
			26-2	18.6 ± 1.0	303	99	yes	
4 x	Greece	Macedonia, Mokros	(0142-3)	15.2 ± 1.0	300	83		
			(0143-4)	17.8 ± 1.1	300	72		
4 x	Turkey	E.Anatolia, Erzurum	18-65	21.9 ± 1.3	17	82		
			18-72	19.7 ± 1.9	20	60		
4 x	German F.R., Bavaria	Neumarkt i.O.	(508) # 1	-	270	98		
			# 2	-	314	95		
			# 3	-	207	97		
6 x	Austria	Alps, Obergurgl	(429b-2)	-	300	1		
6 x	USSR	Karelia, Kizhi	Kar. 1	18.5 ± 1.0	274	68	yes	
			Kar. 2	19.6 ± 1.4	203	61		
6 x	Bulgaria	(N.E.) Granitovo	(39 coll.) # 1	17.5 ± 0.9	241	99	yes	
			# 2	19.2 ± 1.2	320	93	yes	
			# 3	17.7 ± 1.0	258	44	yes	
6 x	Greece	Chalkidike, Arnea	(0139-1) # 1	20.4 ± 1.2	271	34	yes	
			# 2	19.9 ± 1.2	275	58	yes	
			# 3	19.6 ± 0.9	277	13	yes	
			# 4	20.1 ± 1.3	254	78	yes	
			# 5	21.4 ± 2.7	165	70		
6 x	Greece	Peleponnesos, Langada	0123-4	17.5 ± 1.4	300	62		
			0123-6	17.8 ± 0.8	300	76	yes	
			0123-8	18.4 ± 2.2	300	57		
6 x	Greece	Aegeis, Mykonos	060	19.5 ± 1.5	300	10		
			063-4	19.1 ± 1.4	300	2	yes	
6 x	Exper. garden F1	Belgium and France	R427-7	-	300	16		
			R427-26	-	300	30	yes	
6 x	German F.R.	Nordrhein-Westfalen, Ahaus	(703) # 1	-	265	16		
			# 2	-	312	4		
	*) USSR	Siberia	Sib. 1	20.6 ± 1.7	228	3	yes	
			Sib. 2	19.8 ± 1.5	282	11	yes	

*) On the ground of distribution and characters assessed here supposed to be hexaploid.

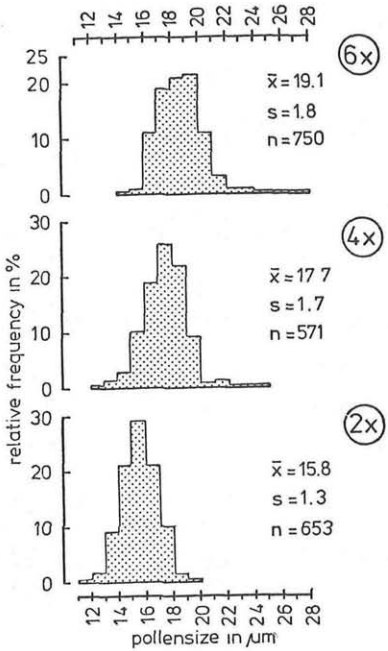

Fig. 2. Relative frequencies of pollen size classes per ploidy level. All dimensions in μm

presentation and shown in Fig. 2 do not give a very good idea of the actual variation within the ploidy levels; and
(2) the variance between 4x individuals is much greater than that within the 2x and the 6x groups.

In order to get an impression of the possible use of (among other data) the diameter as a distinguishing feature, the extreme values between which a pollen size may belong at a significant level of $\alpha = 0.05$ to one of the assemblies 2x, 4x, or 6x, are calculated by using the Student t-test. Since

Table 2

Results of the analysis of variance

Group	Source of Var.	df.	s. s.	m. s.	F
2x	Between plants	10	712.58	42.33	32.01 ***)
	Within plants	539	1135.85	1.32	
4x	Between plants	12	886.37	143.95	103.45 ***)
	Within plants	637	2613.75	1.39	
6x	Between plants	16	1712.91	66.28	32.23 ***)
	Within plants	833	2772.42	2.05	

***) significant at $\alpha = 0.001$.

the variation pattern within each ploidy level is insufficiently known (does perhaps an ecogeographical differentiation play a role?) at each ploidy level for the lower limit the smallest, and for the upper limit the largest individual mean was used. The results are shown in Fig. 3, which once more demonstrates that in spite of the clear differences in general means, the diameter cannot be employed as a reliable character owing to the great variation between individual plants. Only the very small (<13,5 μm.) and the very large (>24 μm.) grains can with a 95% probability be referred to the 2x and 6x categories, respectively.

3.2. Collected data of structural features

Fig. 5 shows some examples of tri- and of pericolporate pollen in lightmicroscopical and SEM photomicrographs. The percentages of tricol-

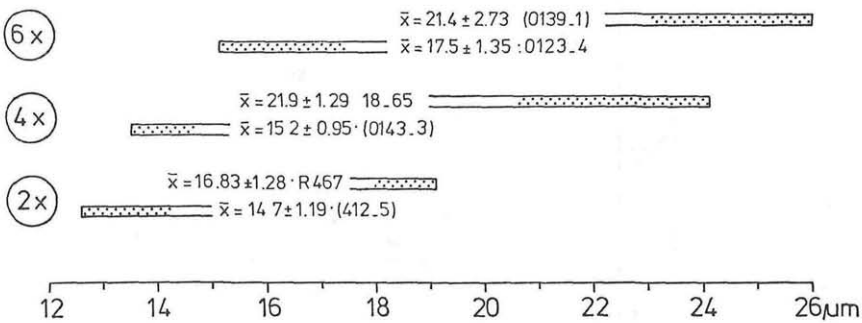

Fig. 3. The significance ranges ($\alpha = 0.05$) for individual pollen grains under the hypothesis that they can be referred to one of the ploidy levels

porate pollen and the number of grains examined are tabulated in Table 1. Stephanocolporate grains occur very sporadically (only twice), so that the complementary percentages to all intents and purposes represent peri-(4)colporate pollen. The following conclusions can be drawn:

- without exception the diploids always have a very high percentage of tricolporate pollen;
- at the tetraploid level this percentage varies from 56% to 100%, the heterogeneity of population sample no. 345 being especially striking: 56—97%;
- at the hexaploid level the range of variation is even wider: between 1% and 99%. Also in this group a great heterogeneity may be present in a single population (in no. 39 from Bulgaria and in no. 0139-1 from Greece plants vary from 13% to 78%).

Fig. 4 shows the relation between chromosome number, size, and percentage of tricolporate pollen, as recorded per individual plant.

3.3. Collected data of SEM images

On Fig. 6 and 7 a number of representative examples of photomicrographs are reproduced. These photo-micrographs and the corresponding Ratios indicate that there are no clear discontinuities between the ploidy levels and that the variance within a single individual is already appreciable (Fig. 7: 13—16).

In Table 3 the F-values of the analysis of variance are presented per ploidy level and per Ratio, and in addition the added variance component among these groups is given.

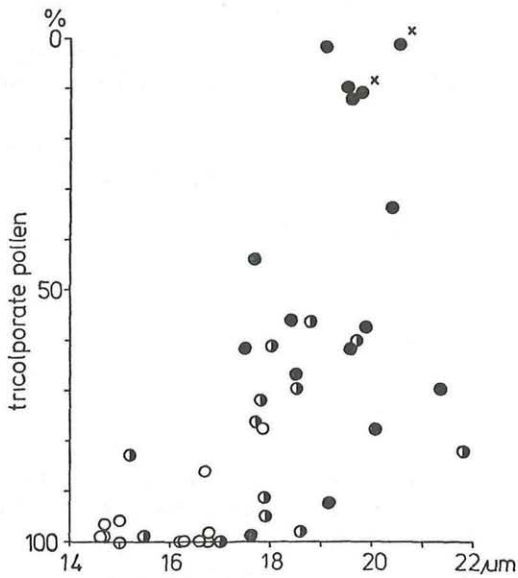

Fig. 4. Relation between diameter, percentage of tricolporate pollen and ploidy level. The significance limits of Fig. 2 also drawn in here. Symbols as in Fig. 1. — x indicates plants from Siberia whose chromosome number have not been counted

Table 3

F-values of and the added variance component among the ploidy levels for each of the ratios R_1, R_2 and R_3

Group	R_1		R_2		R_3	
	F	A.V.C.%	F	A.V.C.%	F	A.V.C.%
2x	4.57 *)	42.8	6.21 *)	52.2	3.70 *)	36.2
4x	2.43 *)	22.3	3.06 *)	29.3	0.96	0
6x	3.08 *)	29.5	6.23 *)	51.2	3.74 *)	35.5

A.V.C. = added variance component.
*) significant at $\alpha = 0.05$.

The following conclusions may be drawn:

— with the exception of R_3 in the 4x assembly all groups are significantly heterogeneous albeit to a lesser extent than in the case of the diameter variance, and

— as far as the recorded structural features are concerned, the tetraploid assembly as such is the least variable, in contrast to the diameter.

The range of variance of the Ratios within one origin is so excessive that it was not deemed to serve a useful purpose to calculate the reliability intervals as was done in the case of the diameter. Although the analysis of variance indicates that the ploidy levels are heterogeneous $\bar{x}$ and s. d. have nevertheless been calculated in order to present the results as concisely as possible in Table 4.

These Ratios can obviously not be used for a key by means of which, from data obtained from about 10 pollen grains, a reasonably reliable identification of the ploidy level can be made.

Table 4

Sculptural features ratios R_1, R_2, and R_3; means and standard deviations per ploidy level

Cyto-type	n	R_1 $\bar{x}$	R_1 s	R_2 $\bar{x}$	R_2 s	R_3 $\bar{x}$	R_3 s
2x	40	36.11	14.30	50.71	9.02	2.70	0.63
4x	51	24.50	13.75	39.62	8.59	3.26	0.84
6x	61	32.19	15.59	50.18	18.55	2.48	0.81

4. Discussion

4.1. Discussion of diameter and structure of the pollen

Diameter: Extensive data had earlier been published by Löve 1940, 1944, Hadač & Hašec 1948, and Harris 1969.

Table 5 is a survey of the pollensize records per ploidy level as given in the relevant literature and as found by us.

In the first place the discrepancies between previously recorded sizes and the present data needs some explanation. Reitsma 1969 showed in a comparative study of the reactions of pollen grains upon various methods of storage and preparation that the procedure followed in our present investigation (i. e., a treatment of usually air-dried pollen with KOH followed by acetolysis) causes a shrinkage of the grains of up to about 10%. Löve 1944 and Harris 1969 state that the grains they studied were mounted in glycerol; it is to be expected that they boiled the grains in water. On the basis of his own experiments and of data from the literature Reitsma 1969 found evidence of a swelling of up to 10% after this treatment. Van der Leeuw 1968, mentioning as method boiling up the dried pollen in water

and inclusion in glycerol, found mean diameters of 20.6—23.6 μm. in tetraploid populations.

The opposite effects of the method employed by LÖVE 1944 and HARRIS 1969 and ours (together causing a difference of up to 20% in the size measurements) most probably account for the size differences recorded.

The statistically significant increase in size of polyploid series has once more been confirmed, but application of this characteristic to the *R. acetosella* agg. has by itself no diagnostic value. The reliability intervals reveal that the individual variation is too great to decide on the basis of pollen of a single individual alone what the chromosome number of that plant is. HARRIS 1969 found in a series of 6x populations also significant differences (at $\alpha = 0.01$) between the extremes of this series, and we could confirm this at the 2x and 4x levels. Only in extreme cases (very large or very small grains) does the pollen diameter of a plant (or a population sample, respectively) provide a reliable criterion for the recognition of the cytotype (as the 6x or 2x level, respectively). Generally speaking herbarium material is inadequate for that purpose.

Structure: An increase of the number of the colpores at higher ploidy levels is a well known phenomenon (e. g., OLTMANN 1972). A clear

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Fig. 6. Examples of SEM Photo-micrographs studied at ×20,000, reproduced here at about ×12,000. Ploidy level 2x and 4x

	2n =	pop. no.	R_1	R_2	R_3
1	14	32.1	44.44	43.13	3.40
2	14	0142	19.24	47.34	2.56
3	14	411	32.53	43.88	2.52
4	14	R 422	21.47	38.22	2.27
5	28	345.26	13.87	43.89	3.40
6	28	442	19.41	38.04	3.13
7	28	319	33.09	43.68	3.57
8	28	26.2	19.71	35.14	4.64

Fig. 7. Examples of SEM photo-micrographs studied at ×20,000, reproduced here at about ×12,000. Somatic chromosome number of all specimens: 2n = 42

	pop. no.	R_1	R_2	R_3
9	940.2	35.00	46.05	2.92
10	Sib.2	16.97	44.01	2.51
11	R 571.5	80.10	47.03	3.50
12	Khizi	19.44	45.46	3.78
13	942.1.1	36.38	43.25	3.33
14	942.1.1	16.89	34.17	2.81
15	942.1.1	44.00	60.05	1.96
16	942.1.1	31.71	41.38	2.65

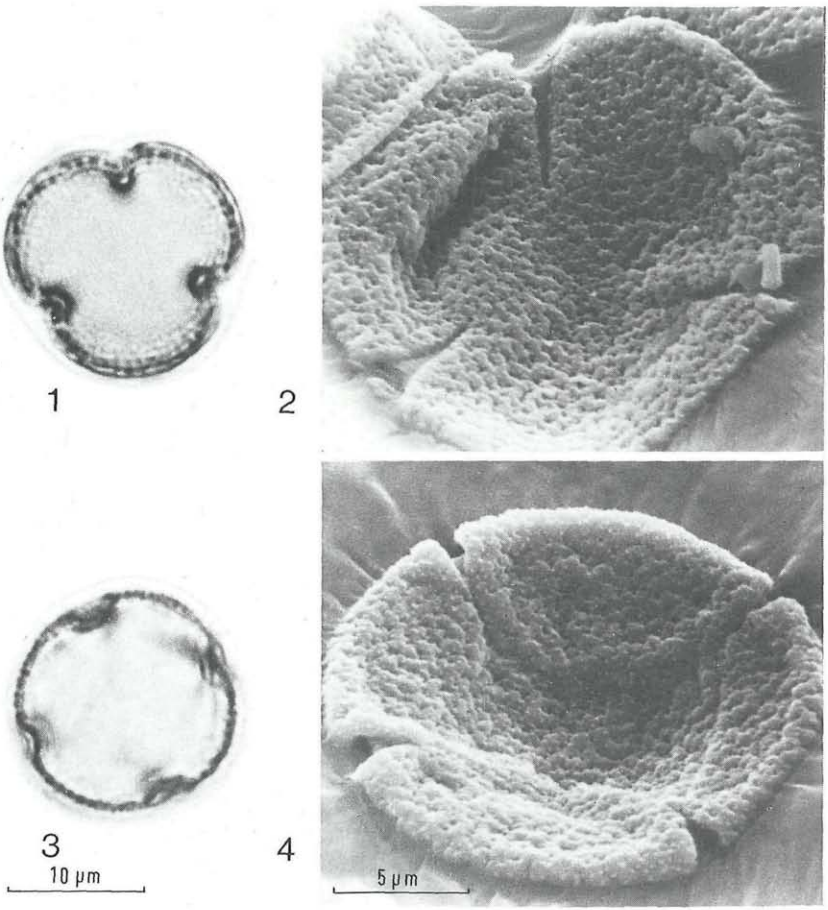

Fig. 5. Examples of tri- and peri-colporate pollen (light microscopy and SEM). — 1 # Population no. 32. 1, Roumania: 2n = 14. — 2 # Pop. no. 442, Austria: 2n = 28. — 3 # Pop. no. 32.1, Roumania: 2n = 14. — 4 # Pop. no. R 427.26, France: 2n = 42

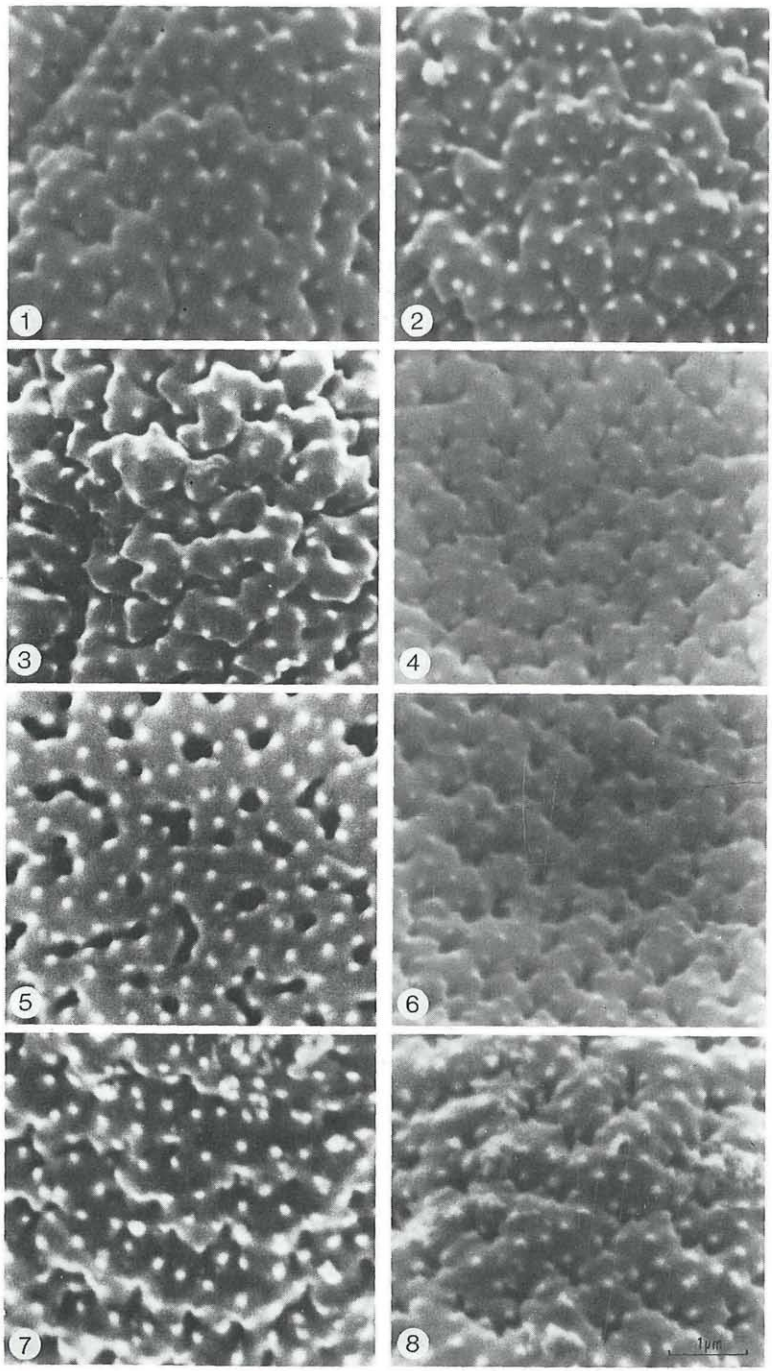

Fig. 6. Explanation of figure on p. 318

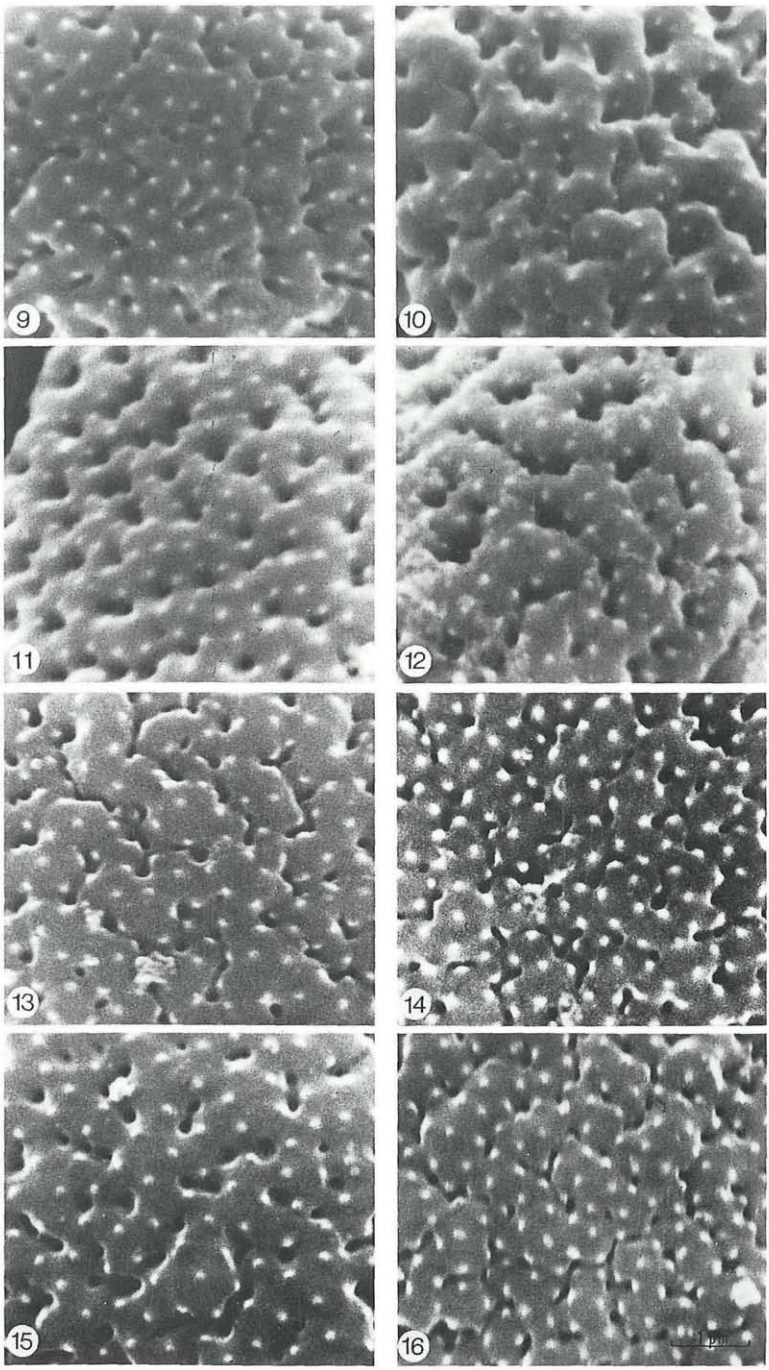

Fig. 7. Explanation of figure on p. 318

Table 5

Pollen diameters: a comparison of previously reported data with those of present study. All dimensions in μm

	Löve (1940, 1944)				Hadač & Hašec (1948)	Harris (1969)				Present study			
	no. pops	no. plts	n pollen	x̄ *)	x̄	no. pops	no. plts	n pollen	x̄ *)	no. pops	no. plts	n pollen	x̄
2x	—	—	243	19.8±0.10	20.5	1	4	80	19.9±0.9	8	11	653	15.8±1.3
4x	5	5	1047	22.2±0.07	22.0	4	25	125	22.0±0.4	6	16	571	17.7±1.7
6x	4	4	1312	23.9±0.05	24.0	40	189	945	25.3±0.1	7	18	750	19.1±1.8
	"the differences are so distinct that they may be of great help in determining the systematic position of herbarium material."					Statistically significant 2x < 4x < 6x				Statistically significant 2x < 4x < 6x			

*) The relevant publication does make it clear what the component ± behind the mean signifies. A re-calculation of the figures showed that is it neither the s. d. nor the standard error of the mean.

correlation between the level and that number has not been established, however. The appreciable variation of the percentage of pericolporate grains in *R. acetosella* at both the 4x and the 6x level and also within the progeny of a single plant was somewhat surprising. A possible explanation may be found in the geographic and genetical background of these cytotypes.

Both higher ploidy levels must be supposed to have originated polytopically (and polyphyletically), which is a common situation at the higher levels of polyploid complexes (GOTTSCHALK 1976) and also highly probable in the *R. acetosella* agg. (DEN NIJS 1974, 1976, in the press). Such a multiple advent of 4x and 6x cytotypes may be a major cause of variation.

However, the incidence of rather large-scale hybridisations between higher ploidy levels may be even more important. DEN NIJS (l. c.) found in Central France, Bohemia and Anatolia, among other places, hybrid swarms and the probable products of backcrosses. Such situations easily lead to a increasing genetical heterogeneity and a blurring of the differences between the ploidy levels at least in areas where such hybrid swarms occur. If one takes in account, when interpreting Table 1, the sympatric occurrence of different cytotypes and the presence of interlevel hybrids in the natural vegetations (see DEN NIJS l. c.), it appears that the populations exhibiting the greatest variation in the degree of colporateness (both at the 4x and the 6x levels) originated from precisely those regions of Europe: compare, e. g., the populations from Pinols, Haute Loire (345), Mokros, Macedonia (0143), Granitovo, Bulgaria (39), and Arnea, Chalkidike, Greece (0139).

Populations from karyologically more homogeneous or isolated areas are less variable: tetraploids appear to have very high percentages of tricolporate pollen only: Wachtebeke, E. Flanders (59), Col de Quillane, Pyrenees (319), Neumarkt i. O., GFR (508), and hexaploids are almost exclusively pericolporate: Mykonos, Aegean Archipelago (063—063), Ahaus, GFR (703), Siberia (Sib. 1 and 2).

These data and explanations agree with the results of crossing experiments between the ploidy levels (DEN NIJS in preparation), which indicate the incidence of back-crosses in nature towards both parental ploidy levels. Owing to this complication it is impossible to key out the three ploidy levels when only about 10 fossil pollen grains in samples of fossil pollen are available. Fig. 4 shows quite clearly that only the 2x cytotype can be recognised with a fair degree of certainty by the combination of the small pollen size and the always high percentage of tricolporateness. This would enable us to reconstruct the historical phytogeography of the 2x taxon, so that it may be clear, among other things, since when the diploids have been restricted in their distribution to relict (refugial) areas (DEN NIJS 1976, in the press; SCHEFFER & DEN NIJS 1978). In this connection the occurrence of diploid representatives of the *R. acetosella* agg. in N.-E. Siberia (ZHUKOVA 1968, DEN NIJS 1974) may be a pointer.

4.2. Discussion of the sculpture Ratios

The photographs of Fig. 6 and 7 and Table 4 indicate that the sculptural features of the tectum used do not provide an adequate criterion for the recognition of the ploidy level. The variance of these Ratios is excessive both within and between the ploidy levels, as is evident from the unduly high s. d. of the means. Contrary to our expectation based on earlier records (SMIT & WIJMSTRA 1970; HOOGHIEMSTRA & SMIT in preparation and pers. com.) the tectal perforations and processes are not sufficiently constant in size and in number to provide useful evidence. This is the more important because the key we aimed at would, for practicle reasons, have to be reliable when only about 10 pollen grains are available.

The following considerations about the effects of a number of unchangable variables may give some explanation of the variability:

(1) The relative age of the pollen: both not fully matured and mature grains may become incorporated in the sample. *R. acetosella* produces botrytic inflorescences with an in time staggered anthesis. The increase of the grains during the last stages of development will have an effect especially on the value of R_1 (an increase). For the following survey young and mature anthers from one individual were separately processed:

pop. no.		R_1	R_2	R_3	
442	young	27.1± 8.0	35.8±16.2	3.5±1.2	n = 6
	mature	39.7±16.8	43.6± 4.7	3.6±0.9	n = 8

The very great s. d.'s again point to the relativity of all collected data.

(2) The relative position of the flower in the inflorescence may have some bearing upon the pollen development. RAHN 1974 found in the spikes of species of *Plantago* the largest grains in the lowermost part, where flowering starts. During that early phase the supply of nutrient is apparently largest. A similar effect was recorded by STERK 1968 in *Spergularia* species: a progressive lower number of stamens is formed in later initiated flowers.

(3) GUGGENHEIM 1975 found that in species of *Tilia* significant differences in sculptural characteristics of the pollen exists between populations, and he concluded that the influence of the local habitat should not be under-estimated. In other words, such characteristics exhibit a great deal of phenotypic plasticity.

(4) A genetic heterogeneity as discussed in the preceding paragraph will have its repercussions. Conceivably certain isolated population groups can be distinguished, but this is not relevant the set purpose.

(5) The interpretation of the photo-micrographs, especially the distinction of the tectum perforations, is not always clearcut, which introduces another statistical bias.

4.3. Conclusions

The presence of a practically 100% tricolporate pollen type in conjunction with indications from the grain diameter yield a reasonably possibilty to recognise diploid pollen as such. The 4x pollen is not clearly identifiable, and only the presence of an almost consistently pericolporate and large pollen type is sufficiently indicative of the 6x cytotype. In view of the known karyo-geographic situation of the 2x taxa at present it is recommendable to apply this method in an attempt to unrevel the historical karyo-geography of these diploids.

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