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The variability of phenol compounds based on karyological studies of taxa of the genus *Vaccinium* L., subgenus *Oxycoccus* (Hill) A. Gray

WANDA GUGNACKA-FIEDOR

Department of Taxonomy, Plant Ecology and Nature Protecion,
M. Kopernik University, Gagarina 9, 87-100 Toruń, Poland

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Abstract

52 population samples from Poland and several European countries were subjected to karyological and chemotaxonomic studies. Two diploid species (*Vaccinium macrocarpon* Ait. and *V. microcarpum* (Turcz, ex Rupr.) Schmalh.) with chromosome number 2n = 24 were found, differing essentially in the chemical composition of their phenol fractions. Besides diploid species, a tetraploid *V. oxycoccos* L., with the chromosome number 2n = 48 was distinguished. It is characterized by a high chemical variability. Its chromatograms show spots of compounds characteristic of both subspecies distinguished. No hexaploid *V. hagerupii* (Löve et Löve) Rothm. with chromosome number 2n = 72 was in the karyologically studied material. Chemical studies of a herbarium sample do not confirm the distinctness of this taxon in relation to *V. oxycoccos*. The karyological studies, which, however, need replication, have also revealed the presence of a presumed hybrid, *V. pseudonanum* Keller, with chromosome number 2n = 36. The chromatogram of that taxon is very similar to that of *V. oxycoccos* L. subsp. *microphyllus* (Lange) Löve et Löve.

INTRODUCTION

Rothmaler (1966), Hulten (1970), Webb (1972) and Meusel et al. (1978) distinguish within the genus *Vaccinium* L. subgenus *Oxycoccus* (Hill) A. Gray the following taxa: *Vaccinium macrocarpon* Ait., *V. microcarpum* (Turcz, ex Rupr.) Schmalh., *V. oxycoccos* L. and *V. hagerupii* (Löve et Löve) Rothm. Lower taxonomic units of subspecies rank have been distinguished only within the polytype species *V. oxycoccos*. These are *V. oxycoccos* L. subsp. *oxycoccos* and *V. oxycoccos* L. subsp. *microphyllus* (Lange) Löve et Löve. On the grounds of the results of studies of morphological variation (Gugnacka-Fie-

dor 1982), it is proposed to distinguish two varieties within *V. oxycoccos* L. subsp. *oxycoccos*, viz.: *V. oxycoccos* L. subsp. *oxycoccos* var. *oxycoccos* and *V. oxycoccos* L. subsp. *oxycoccos* var. *macrophyllus* W. G.

Species of the subgenus *Oxycoccus* have already been studied several times in respect to their karyology. After Löve and Löve (1958), mainly Hagerup (1940), Newcomer (1941) and Darrow et al. (1944) ascertained the basic chromosome number for all species. For *V. macrocarpon* and *V. microcarpum* it was found to be n = 12, for *V. oxycoccos* n = 24. Bolkhovskikh et al. (1969) report furthermore a high variability of the chromosome number within the species *V. oxycoccos* (2n = 24-112). A separate taxon, *V. oxycoccus* L. var. *ovalifolium* Michx, has been distinguished, regarded by Porsild (1938) as a separate species. In later works (Hulten 1970, Meusel et al. 1978) this taxon was reincluded in *V. oxycoccos*, which is in agreement with its chromosome number (2n = 48). Hagerup (1940) also first described the new species *Oxycoccus gigas* with chromosome number 2n = 72. This has been confirmed by Jörgensen et al. (1958). In the latest systematic works (Rothmaler 1966, Webb 1972, Meusel et al. 1978) this taxon has been recognized as a separate species, *V. hagerupii*. It seems surprising, however, that the chromosome number in *V. oxycoccos* reported by Jörgensen et al. (1958) is 2n = 24. It seems likely that these data refer to the species *V. microcarpum*, which in some systematic works is still reported as a subspecies of *V. oxycoccos* (Hegi 1966, Hulten 1970, Garcke 1972).

The greatest variability of the basic chromosome number has been observed during meiosis in the hexaploid taxon *V. hagerupii*. According to Hagerup (1940), the pollen tetrads in formation contain different haploid numbers (n = 18-61). Ahokas (1971), who uses improved research techniques, questions Hagerup's (1940) viewpoint, arguing that, owing to genetically controlled interchromosomal fibres, normal bivalents are formed conducive to normal meiosis, and the chromosome number variability is not above normal. The processes of mitosis and meiosis in the remaining species of the subgenus *Oxycoccus* proceeding normally.

Data on the most important phenol compounds can be found in chemotaxonomic studies on species of the family *Ericaceae* and of the subfamily *Vaccinioideae* (Bate-Smith 1962, Harborne and Williams 1969, 1973, Kostecka-Mądalska and Wernikowska-Ukleja 1969, Stevens 1971). The above named authors have found simple phenols, glycosides, flavonoids and a large number of tannins. The data do not include species of the subgenus *Oxycoccus*, or refer only to the chemical composition of fruits.

The aim of the present work was to carry out chemical and karyological studies of mainly indigenous populations of cranberry, including the species *V. macrocarpon* grown in Poland, and to consider the possibility of applying the results to taxonomic purposes.

MATERIAL AND METHODS

Karyological studies were carried out on 37 population samples from natural sites of several regions of Poland (Baltic Coast, Western Pomerania, Podhale Region) and from Ireland. The fragments usually taken for study were the root growing points of germinating seeds, less frequently meristematic tissues of stems from seedlings. The material was soaked in 0.1% aqueous colchicine solution and fixed in Carnoy fixative. After chromosome staining, mainly by the aceto-carmine method and with orceine solution in 80% formic acid (Ahokas 1971), squash preparations were made. The results were based on not less than 10 counts.

Chromatograph analysis was made on 52 population and herbarium samples and on four samples of *V. oxycoccos* grown in the same habitat. Among these samples, except 37 samples studied karyologically, were population and herbarium samples from Murmańsk (USSR), Finland and Switzerland. The plant material represented all taxonomic units distinguished within the subgenus *Oxycoccus*. The material for study were dry, ground leaves, from which alcohol extracts were made. The chromatograms were run on Whatman 1 paper by descending two-way chromatography. The solvents used were 6% acetic acid and BAW (n-butanol, acetic acid and water 4 : 1 : 5). The spots of phenol compounds were observed in UV light, then stained with diazotized benzidine (Linskens 1959, Haslam 1966, Harborne 1976). The phenol compounds were identified on the grounds of standard substances, characteristic colours and Rf coefficients (Block et al. 1955, Opieńska-Blauth 1957, Linskens 1959, Leppmeier 1960, Bobbitt 1963, Haslam 1966, Harborne 1976). Statistical analysis of the material was done by calculating Marczewski and Steinhaus (1959) systematic distances (r), five chemical compounds which occurred in all samples being left out. The results of these calculations were used for drawing a dendrogram by the complete linkage method.

RESULTS

Karyological studies of indigenous population samples have confirmed the existing data on the chromosome number reported for species from the subgenus *Oxycoccus*. For *V. macrocarpon* and *V. microcarpum* that number has been found to be $2n = 24$ (Table 1). High variability

(2n = 36-72) has been found among populations of *V. oxycoccos*, this concerning 15-30% of the counts in 40.6% samples from different populations. The group of high variability samples was represented by ecotonic populations. The remaining samples of *V. oxycoccos* were characterized by a stable chromosome number 2n = 48 and came from typical

Table 1

Chromosome numbers in taxa of subgenus *Oxycoccus*

Taxon	No of sample	Peatbog	Calculated value	2n
V. macrocarpon	1	Rucianka	14	24
V. microcarpum	2	Moczadło	10	24
	3	Okonek	11	24
	4	Stręszek	10	24
V. oxycoccos	5	Z. Chocina	16	48
subsp.	6	Piecki	15	48
oxycoccos	7	Na Czerwonem	14	48
var.	8	Nawionek	13	48
oxycoccos	9	Z. Chocina	13	48
	10	Moczadło	16	48
	11	Moczadło	18	48
	12	Kiedrowice	14	48
	13	Dury	14	48
	14	Okonek	17	48
	15	Stręszek	12	(46)48(68; 72)
	16	Stręszek	10	48
	17	Zmarłe	12	(46)48(52; 70)
V. oxycoccos	18	Zmarłe	17	48(50)
subsp.	19	Piecki	10	48(58; 66)
oxycoccos	20	Okonek	18	(42)48(70; 72)
var.	21	Czarne n.L.	15	48
macrophyllus	22	Czarne n.R.	16	48
	23	Stawek	15	(44)48(64; 66)
	24	Zmarłe	10	48(72)
	25	Zmarłe	16	(44)48(64; 68)
	26	Katarzynka	9	48(72)
	27	Syconki	16	(46)48
V. oxycoccos	28	Z. Chocina	14	48
subsp.	29	Z. Chocina	15	48
microphyllus	30	Z. Chocina	18	48
	31	Zmarłe	10	(42)48(52; 72)
	32	Zmarłe	14	48
	33	Moczadło	15	(36)48
	34	Kiedrowice	16	48
	35	Dury	14	48
	36	Moczadło	16	48
V. pseudonanum	37	Kilbeggan	1	36

Characteristic features of observed chromatographic spods

Spot number	Average Rf values		Colour in UV-light before developing	Dye in visible light after developing with diazotized benzidine	Identification	Spot occurrence frequency, %				
								V. oxycoccos		
	CH_3COOH	BAW				*V. macrocarpon*	*V. microcarpum*	subsp. *oxycoccos*	subsp. *oxycoccos* var. *macrocarpon*	subsp. *microphyllus*
1	0.15	0.00	brown	red	—	100.0	100.0	100.0	100.0	100.0
2	0.21	0.52	brown	orange-red	gallocatechin	100.0	100.0	100.0	100.0	100.0
3	0.14	0.56	brown	orange-red	gallocatechin	100.0	100.0	100.0	100.0	100.0
4	0.19	0.68	brown	orange-red	gallocatechin	100.0	100.0	100.0	100.0	100.0
5	0.66	0.51	bright-blue	yellow	chlorogenic ac.	100.0	100.0	100.0	100.0	100.0
6	0.32	0.65	brown	orange-red	gallocatechin	100.0	—	100.0	100.0	88.8
7	0.35	0.48	—	red	gallocatechin	100.0	—	63.2	63.6	33.3
8	0.27	0.25	—	red	—	100.0	—	52.6	81.8	22.2
9	0.48	0.73	—	red	d-catechin	75.0	—	89.5	81.8	77.8
10	0.14	0.37	brown	orange-red	epigallocatechin	50.0	87.5	100.0	90.9	100.0
11	0.00	0.63	yellow	—	—	25.0	—	26.3	27.3	—
12	0.60	0.59	bright-blue	yellow	neochlorogenic ac.	—	100.0	100.0	90,9	100.0
13	0.44	0.59	—	copper-red	—	—	87.5	94.7	100.0	100.0
14	0.36	0.39	—	red	—	—	50.0	63.2	27.3	44.4
15	0 77	0.28	—	red	—	—	37.5	57.9	72.7	22.2
16	0.00	0.54	blue	—	—	—	37.5	52.6	63.6	55.5
17	0.84	0.43	—	yellow	arbutin	—	25.0	52.6	90.9	44.4
18	0.53	0.78	—	yellow	hydroquercetin	—	25.0	26.3	36.3	22.2
19	0.84	0.26	—	orange	—	—	12.5	52.6	63.6	22.2
20	0.29	0.55	brown	orange-red	gallocatechin	—	—	100.0	100.0	66.6
21	0.08	0.39	brown	orange-red	epigallocatechin	—	—	94.7	90.9	55.5
22	0.52	0.25	—	red	—	—	—	36.8	45.4	22.2
23	0 41	0.79	blue	—	gentisic acid	—	—	10.5	18.2	33.3
24	0.65	0.30	—	red	—	—	—	26.3	18.2	22.2
25	0.41	0.26	—	red	—	—	—	26.3	18.2	11.1
26	0.20	0.18	—	red	—	—	—	31.6	54.5	11.1
27	0.87	0.11	—	yellow	—	—	—	26.3	27.3	11.1
28	0.26	0.74	blue	—	digallie acid	—	—	10.5	18.2	11.1
29	0.86	0.55	—	yellow	—	—	—	42.1	54.5	—
30	0.79	0.77	—	yellow	hydroquinone	—	—	21.0	9.1	—
31	0.00	0.42	yellow	—	—	—	—	5.3	18.2	—
32	0.85	0.36	—	yellow	—	—	—	21.0	—	—
33	0.73	0.60	—	red	—	—	—	15.8	—	—
34	0.76	0.51	—	red	resorcinol	—	—	10.5	—	—
Number of spots in specific taxa						11	14	34	31	27

high and transitional peatbogs. No population was found which would correspond to the hexaploid taxon *V. hagerupii* with chromosome number 2n = 72. Interesting results were obtained from the material collected from the peatbog at Kilbeggan in Ireland (no. 37), in which the chromosome number was found to be 2n = 36. The sample, however, contained many immature, poorly germinating seeds, which were quickly destroyed by fungi. For that reason, the number of counts obtained from the samples does not allow the definitive establishment of its systematic rank. It seems possible that the material in question is the hybrid *V. pseudonanum* (*V. oxycoccos* x *V. microcarpum*).

In the karyologically studied material, extended by samples from other European countries, including a herbarium sample of *V. hagerupii* 34 phenol and phenol-derivative compounds have been found. The mean number of these compounds varies in most taxa, and tends to increase with the polyploid sequence. The differences in their numbers are particularly wide between the diploid and the polyploid species (Table 2). Among the compounds observed, the following were identified: gallocatechin, epigallocatechin, two chlorogenic acids, gentisic acid, m-digallic acid, d-catechin, arbutin, 2-hydroquercetin, hydroquinone and resorcinol, and 16 unknown substances were distiguished. In order to illustrate the chemical variability of cranberries, chromatograms are presented, on which are marked all distinguishable spots in the samples of individual taxonomic units (Figs. 1-3).

In *V. macrocarpon* samples (Fig. 1A) eleven spots of compounds have been distinguished, eight of which reach 100% frequency. Neochlorogenic acid, always present in all other species, is absent here. The fraction from *V. microcarpum* leaves (Fig. 1B) contains 14 phenol compounds. Here belong substances common to both diploid species and a number of compounds absent from the chromatograms of *V. macrocarpon*. Those compounds are of considerable taxonomic importance and reach a high frequency in the following polyploid species. The phenol components of *V. oxycoccos* leaves are in three chromatograms, which illustrate their variability in lower taxonomic units of the species. The most diversified and richest in the compounds in question is the typical subspecies. The collective chromatogram of this taxon contains 34 spots. The remaining *V. oxycoccos* units show smaller numbers of phenol compounds in their chromatograms (Figs. 2A, B and 3A). The chromatogram of *V. hagerupii* shows no significant differences as compared to the former ones (Fig. 3B). All the substances distinguished in it occur with various frequencies also in the chromatograms of *V. oxycoccos*.

The results of sample grouping are presented in a dendrogram (Fig. 4). The samples of *V. microcarpum* are clearly distinct from the rest, as most of them reach low values of systematic distance, which is evi-

dence of a high degree of similarity of the chemical characteristics compared. Similarly distinct are samples of *V. macrocarpon*. Among the samples of *V. oxycoccos* there are several groups, not always coinciding with their division into lower taxonomic units adopted as a result of morphological studies. It follows from the dendrogram that *V. oxycoccos* shows more resemblance to *V. macrocarpon* (r = 0.93) than to *V. microcarpum* (r = 1.00), where no common elements for the two groups have been found. *V. hagerupii* shows the greatest similarity to *V. oxycoccos* subsp. *oxycoccos* var. *macrophyllus* (sample 37). In all

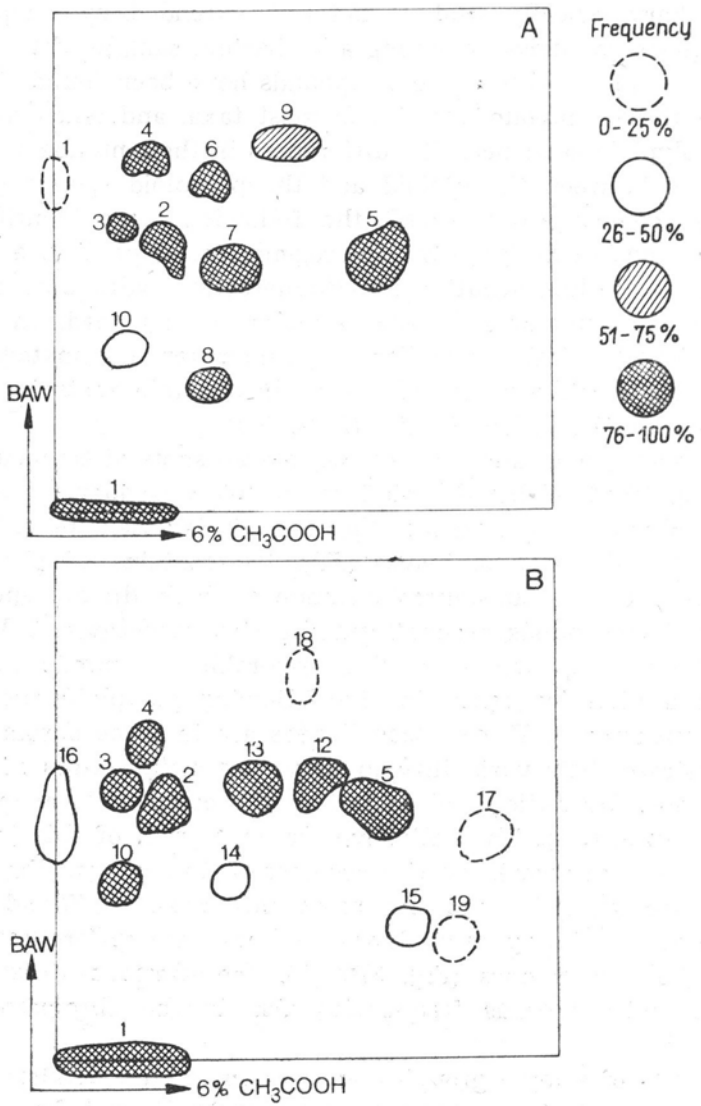

Fig. 1. Chromatograms of phenol compounds present in all samples of *V. macrocarpon* (A) and *V. microcarpum* (B)

V. oxycoccos samples from the plantation, a reduction in the number of phenol components is found (Fig. 5), the changes remaining within the observed variability of the species.

DISCUSSION

The species of the genus *Vaccinium,* subgenus *Oxycoccus* form a polyploid sequence. *V. macrocarpon* and *V. microcarpum* are diploids with chromosome number 2n = 24. Both diploid species are characteri-

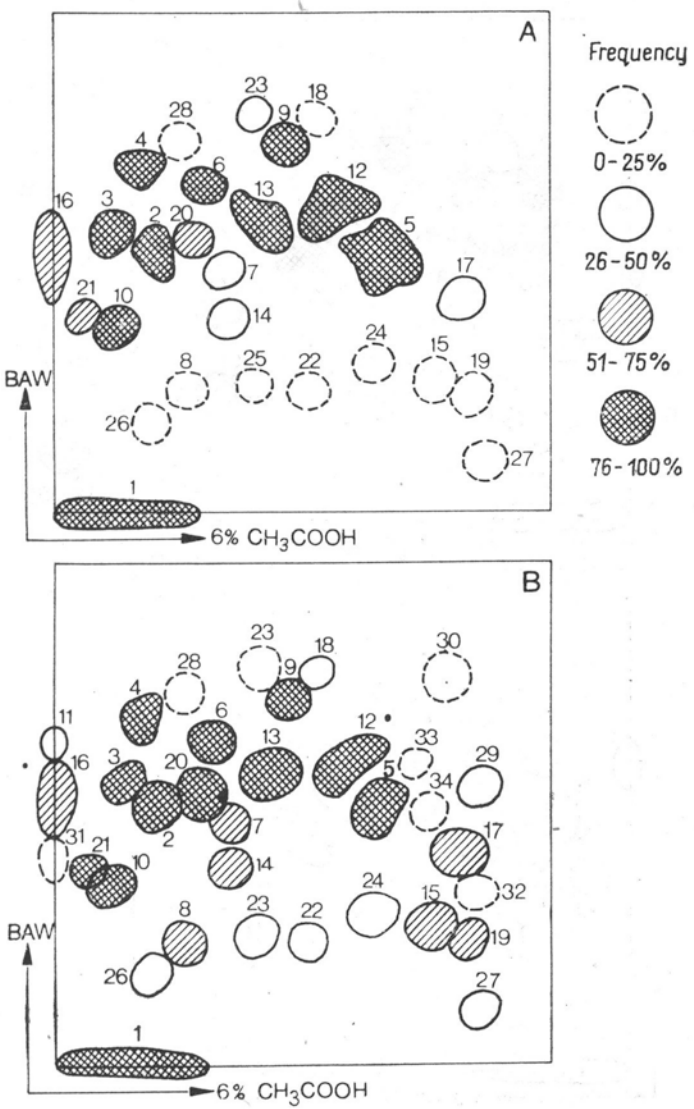

Fig. 2. Chromatograms of phenol compounds present in all samples of *V. oxycoccos* subsp. *microphyllus* (A) and *V. oxycoccos* subsp. *oxycoccos* (B)

zed by little morphological variation, a stable chromosome number and a specific composition of phenol compounds, characterictic for each of them only. In the literature referring to the species under study, there are differences in treating the taxon *V. microcarpum*. Some authors (H e g i 1966, H u l t e n 1970, G a r c k e 1972) regard *V. microcarpum* as a subspecies of *V. oxycoccos*. In the light of the results obtained in the present work, this view seems to lack adequate evidence. Considering the pronounced differences in morphology, karyology and chemistry, *V. microcarpum* fully deserves the rank of species.

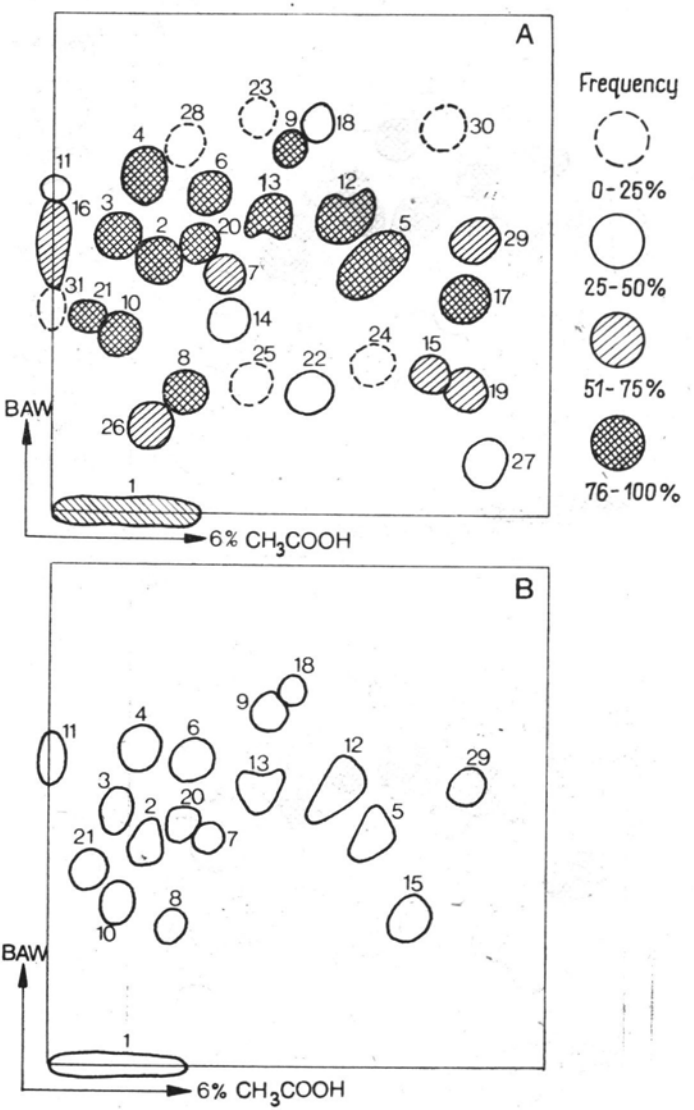

Fig. 3. Chromatograms of phenol compounds present in all samples of *V. oxycoccos* subsp. *oxycoccos* var. *macrophyllus* (A) and in sample of *V. hagerupii* (B)

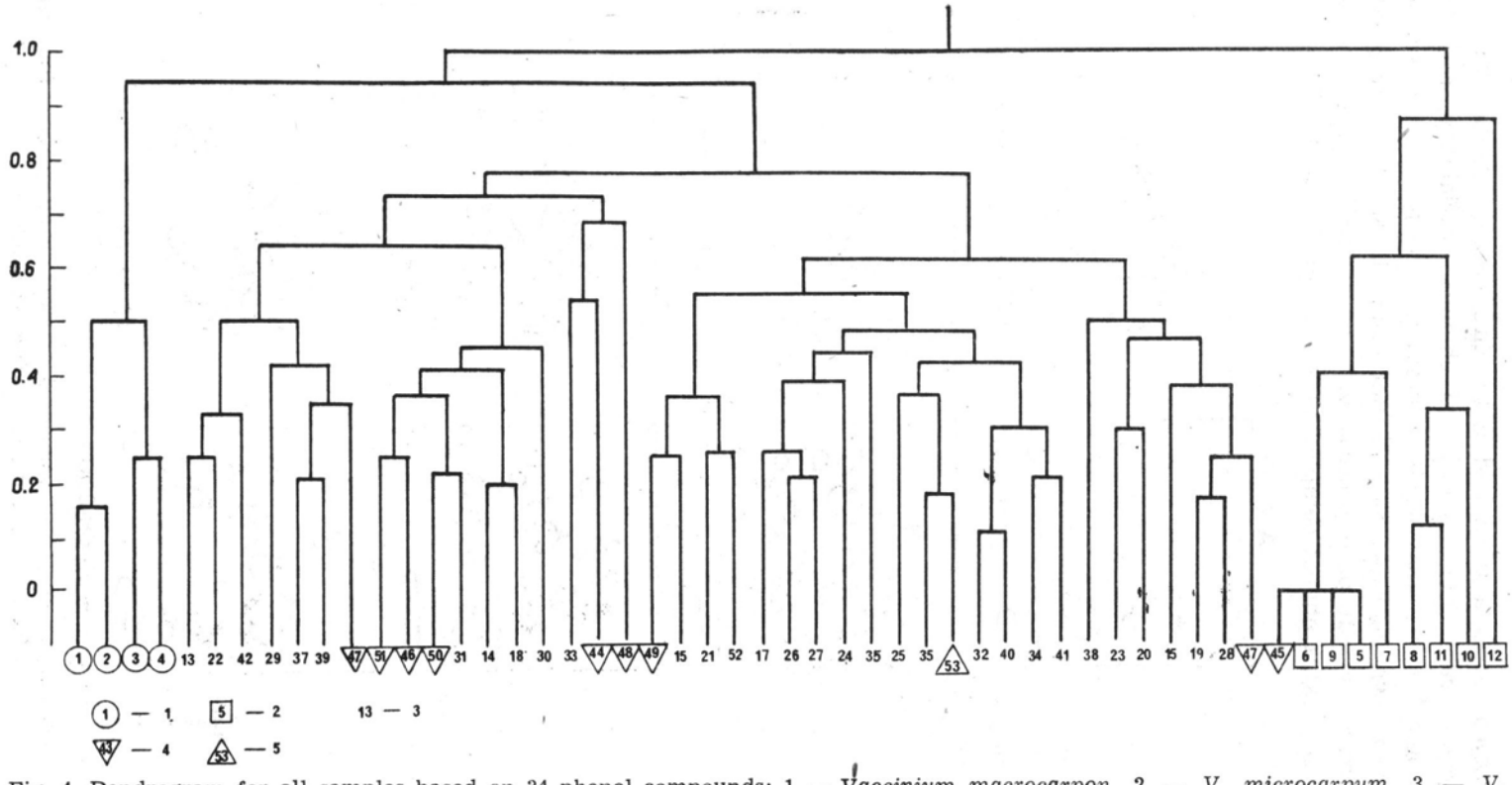

Fig. 4. Dendrogram for all samples based on 34 phenol compounds: 1 — *Vaccinium macrocarpon*, 2 — *V. microcarpum*, 3 — *V. oxycoccos* subsp. *oxycoccos*, 4 — *V. oxycoccos* subsp. *microphyllus*, 5 — *V. hagerupii*

V. oxycoccos is a tetraploid with chromosome number 2n = 48. It shows considerable karyological and chemical variability, reflected in morphological variability. It is a polytype species, whose subspecies show their own characteristic phenol compounds. The only exception is *V. oxycoccos* subsp. *oxycoccos* var. *macrophyllus*, which does not contain any extra phenol compounds, characteristic of itself.

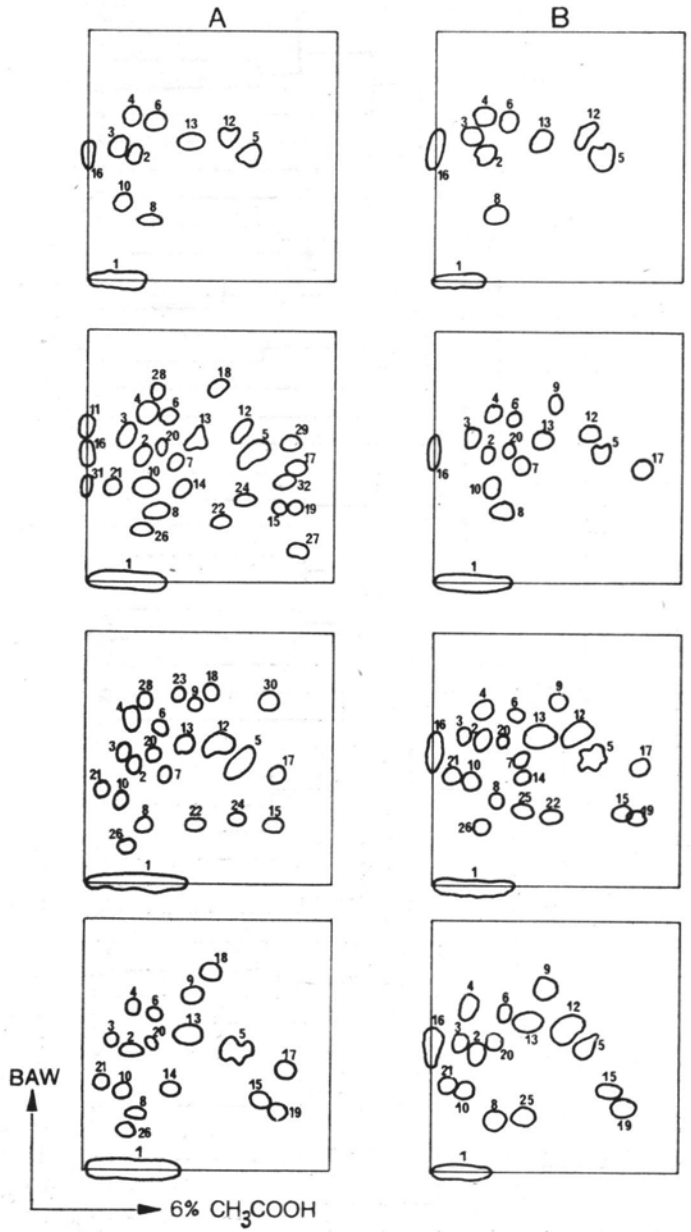

Fig. 5. Chromatograms of phenol compounds present in all samples from natural habitat (A) and from cultivated plants (B)

The polyploid sequence is terminated by *V. hagerupii* with chromosome number 2n = 72. In the literature referring to that taxon, the most controversial problem is its systematic position. The results referring to that species in the present work were based on only one collective, karyologically tested, herbarium sample from Finnish materials. Chemical analyses showed no qualitative differences in relation to other species of the subgenus *Oxycoccus*. Studies on the morphological variability have demonstrated that *V. hagerupii* is a taxon sympatric in relation to *V. oxycoccos*. It must be admitted that the different chromosome number, normal sexual reproduction and the development of additional isolatory barriers, demonstrated by the experiments of Ahokas (1971), justify the distinction of the taxon in question in the rank of a separate species. However, to solve the problem definitively, further extended studies and observations are needed.

Similar complementary studies are necessary for *V. pseudonanum*. The present results of karyological studies of one population sample are not enough to recognize the sample as an interspecific hybrid. The chromosome number 2n = 36 also comes within the variability limits occurring in *V. oxycoccos*. On the grounds of the results of chromatographic studies it can also be admitted that the sample from Ireland is *V. oxycoccos* subsp. *microphyllus*. For a conclusive confirmation of the taxonomic status of that sample more studies on larger material are necessary.

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Zmienność związków fenolowych na tle badań kariologicznych taksonów rodzaju Vaccinium L., podrodzaju Oxycoccus (Hill) A. Gray

Streszczenie

Badaniami kariologicznymi i chemotaksonomicznymi objęto 52 próby populacyjne z obszaru Polski i kilku innych krajów europejskich. Wśród badanych kariologicznie 37 prób z obszaru Polski i Irlandii stwierdzono występowanie dwóch gatunków diploidalnych o liczbie chromosomów n=12 (*Vaccinium macrocarpon* Ait. i *V. microcarpum* (Turcz. ex Rupr.) Schmalh.), jednego gatunku tetraploidalnego o liczbie chromosomów n=24 (*V. oxycoccos* L.) i jednego taksonu mieszańcowego o liczbie chromosomów n=18 (*V. pseudonanum* Keller). W populacjach

V. oxycoccos wyróżniono grupę prób o ustabilizowanej liczbie chromosomów 2n=48, która obejmuje próby z typowych torfowisk wysokich i przejściowych. Druga grupa reprezentuje populacje ekotonowe o różnych liczbach chromosomów (2n=42-72), rosnące na skraju torfowisk, na siedliskach przeważnie bardzo zmienionych pod względem edaficznym. Nie stwierdzono na obszarze Polski obecności heksaploidalnego gatunku *V. hagerupii* (Löve et Löve) Rothm. o liczbie chromosomów 2n=72.

Badania chromatograficzne ekstraktów z liści żurawin wykazały obecność 34 substancji fenolowych. Średnia ilość tych związków jest różna dla poszczególnych taksonów i przejawia tendencję wzrastającą wraz z szeregiem poliploidalnym. Szczególnie duże różnice w ich ilości występują między gatunkami diploidalnymi i poliploidalnymi. Najbardziej różnią się pod względem chemicznym gatunki *V. macrocarpon* i *V. microcarpum*. Chromatogramy ich są dobrze odróżnialne i posiadają kilka charakterystycznych tylko dla siebie związków. *V. oxycoccos* jest gatunkiem najbardziej jednorodnym pod względem składu chemicznego. W jego niższych jednostkach aż 79,41% stanowią związki wspólne dla całego taksonu. Badania chemiczne nie potwierdziły odrębności gatunku *V. hagerupii*. Wyniki przedstawionych badań nad substancjami fenolowymi potwierdzają w zasadzie wcześniejsze dane, uzyskane dla przedstawicieli rodziny *Ericaceae*. Ze związków fenolowych, charakterystycznych dla taksonów podrodziny *Vaccinioideae* nie stwierdzono jednak obecności kempferolu.