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Multivariate morphometric study of the *Cardamine pratensis* group (*Cruciferae*) in the Carpathian and Pannonian area

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Abstract: A multivariate morphometric study of the *Cardamine pratensis* group is presented, based on 84 population samples collected from the Carpathian and Pannonian area in the Czech Republic, Slovakia, Poland, Ukraine, Hungary, and Romania. Among the multivariate methods, principal component analysis, cluster analysis, and classificatory and canonical discriminant analysis were used. The analysis of chromosome numbers from all populations studied showed wide variation. The morphometric study showed that not all groups of populations characterised by their chromosome numbers and geographical criteria are morphologically, and thus taxonomically, distinguishable. Besides the morphologically well characterised species *Cardamine dentata* and *C. rivularis*, the following species were recognised in the area studied: *C. matthioli, C. majovskii* and *C. pratensis*. Within the last species, besides the typical populations, two diploid "types" are provisionally recognised: type "ucranica" and type "rivularis auct.".

Jones (1964) recognised four species of the *Cardamine pratensis* group (*Cruciferae*) – *Cardamine pratensis* L., *C. matthioli* Moretti, *C. dentata* Schult. [= *C. palustris* (Wimm. & Grab.) Peterm.] and *C. rivularis* Schur – from the Carpathian and Pannonian area. In addition, *C. majovskii* Marhold & Záborský was described from Eastern Slovakia (Marhold & Záborský 1986). However, there are many unsolved taxonomic problems in this group which result in inconsistencies in local Floras. The *Cardamine pratensis* group has been studied in Europe by Lövkvist (1956), Urbanska-Worytkiewicz & Landolt (1974), and Spasskaja (1978, 1979), but Carpathian and Pannonian populations were considered only very briefly in these works.

It was shown by the above-mentioned authors and by Marhold (1994a, b), that the pattern of morphological and chromosomal variability in this group is very complicated. Besides the diploid and eupolyploid chromosome counts up to dodecaploid level, many dysploid and aneuploid counts were recorded. Plants with dysploid numbers occur as populations, aneuploids occur only as single plants due to aneusomy, hybridisation or presence of unreduced gametes. Further, intra-individual variation of chromosome numbers is reported by some authors (Berg 1967, Vyvey & Stiepereare 1984).

The aim of the present study, which is part of a taxonomic study of the genus *Cardamine* in the Carpathian and Pannonian area, is to compare morphologically plants from the various parts of this area and to ascertain the degree to which chromosomal variability is correlated with morphological differences. The detailed taxonomic treatment, chromosome numbers and data about the distribution pattern of the taxa were presented in other parts of this study (Marhold 1994a, b).

Two species of the *Cardamine pratensis* group, *C. dentata* and *C. rivularis* were not included in the morphometric analysis. They are morphologically and karyologically isolated from the rest of the group and both are well characterised by qualitative characters (Marhold 1993, 1994b).

Material and methods

Material. For the morphometric analyses of the *Cardamine pratensis* group 84 population samples, of 30–40 plants each (rarely less than 30 plants), were collected during 1985–1990 in the Carpatho-Pannonian area. The chromosome numbers of plants from each population were counted in root tips of cultivated plants by the squash method. Most populations studied are from Slovakia, Poland, and the Ukraine, however, several population samples from the Czech Republic, Hungary, and Romania are also included (Table 1). Populations were chosen in order to represent as much as possible the morphological and karyological variability in a given area. Vouchers of all collections have been deposited in SAV.

The following groups of populations, characterised by their geographical origin and chromosome number, were treated (in cases where several aneuploid and dysploid chromosome numbers on the same polyploid level were present within the same population, only the most common number was considered):

1. *Cardamine matthioli.* Diploid populations (2n = 16) of the Pannonian area and Carpathians having a northern and eastern limit of distribution on the mountain ridges of the West and East Carpathians.
2. *Cardamine pratensis* type "rivularis auct.". [*Cardamine rivularis* auct. non Schur: Čopyk (1977: 133); Spasskaja (1973, 1978: 62); Kotov (1979: 90); Prokudin (1987: 121)]. Diploid populations (2n = 16) of the upper montane and subalpine belt of the East Carpathians.
3. *Cardamine pratensis* type "ucranica". Diploid populations (2n = 16) of the East Carpathians (Gorgany Mts, Schidni Beskydy j Nyz'ki Polonyny Mts) and the outer East Carpathian foothills (phytogeographical district of Prykarpattja).
4. *Cardamine pratensis.* Hypotetraploid populations (2n = 30) of the West and East Carpathians.
5. *Cardamine majovskii.* Tetraploid populations (2n = 32) almost coinciding in distribution with the Eastern part of the area of *C. matthioli.*
6. *Cardamine pratensis.* Hypertetraploid populations (2n = 38) of the West and East Carpathians.
7. *Cardamine pratensis.* Hypohexaploid populations (2n = 44) of the West Carpathians.
8. *Cardamine pratensis* "subsp. *major*" Tomšovic (1986). Hypohexaploid populations (2n = 44) of the flood-plain forests on the North-West border of the Pannonian area, near the Morava River.

Characters used. Only well developed plants without missing values (with exception of character HRL) were measured and scored.

Table 1. Origin of populations studied of the *Cardamine pratensis* group

Cardamine matthioli Moretti (2n = 16)

Slovakia	
MT-01	Záhorská nížina, Kátov (160 m s.m.)
MT-02	Záhorská nížina, Skalica (170 m s.m.)
MT-03	Podunajská nížina, Komárno (100 m s.m.)
MT-04	Nízke Beskydy, Brekov (140 m s.m.)
MT-05	Slovenské rudohorie, Klenovec (360 m s.m.)
MT-06	Muránska planina, Zbojská (725 m s.m.)
MT-07	Slanské vrchy, S edge of Bačkovík (280 m s.m.)
MT-08	Čergov, E edge of Malcov (450 m s.m.)
MT-09	Bukovské vrchy, Stakčín, SW of the railway station (260 m s.m.)
Hungary	
MT-10	Eupannonicum Crisicum, Sárospatak, near the River Bodrog (119 m s.m.)
MT-11	Eupannonicum Crisicum, Olaszliszka, E of the arm of the River Bodrog (113 m s.m.)
MT-12	Eupannonicum Crisicum, E of Erdöbenyei (98 m s.m.)
Ukraine	
MT-13	Schidni Beskydy j Nyz'ki Polonyny, N of Kerec'ky, near the River Boržava (263 m s.m.)
MT-14	Zakarpats'ka rivnyna, Užhorod, near the path to Onokivci (125 m s.m.)
MT-15	Zakarpats'ka rivnyna, N of Velyka Dobron', near the Serne-Močar canal (105 m s.m.)
Romania	
MT-16	Depresiunea Făgăraşului, Avrig, near the railway station (400 m s.m.)
MT-17	Depresiunea Făgăraşului, Cîrţa, near the River Olt (400 m s.m.)
MT-18	Depresiunea Sibiului, Cristian (400 m s.m.)
MT-19	Depresiunea Sibiului, Noul (450 m s.m.)

Cardamine pratensis L. the type "ucranica" (2n = 16)

Ukraine	
UC-20	Prykarpattja, between Dovholuka and Ulyčne (350 m s.m.)
UC-21	Prykarpattja, S of Ševčenkovo (480 m s.m.)
UC-22	Prykarpattja, Bolechov, Hošiv (370 m s.m.)
UC-23	Schidni Beskydy j Nyz'ki Polonyny, Vyškiv (900 m s.m.)
UC-24	Gorgany, Jasen' (566 m s.m.)

Cardamine pratensis L. the type "rivularis auct." (2n = 16)

Ukraine	
RI-25	Svydovec', Polonyna Drahobrat (1500 m s.m.)
RI-26	Čornohora, Mt Hoverla, NE slope (1450 m s.m.)
RI-27	Čornohora, between Mt Breskul and Mt Hoverla (1700 m s.m.)
RI-28	Čornohora, the source of Breskul'skyj stream (1600 m s.m.)
RI-29	Čornohora, Mt Požyževs'ka, SW slope (1600 m s.m.)
RI-30	Čornohora, Mt Turkul, N slope (1600 m s.m.)
RI-31	Čornohora, between Mt Dančer and Mt Turkul, NE slope (1450 m s.m.)
RI-32	Čornohora, near the source of Arendarčyk stream (1550–1600 m s.m.)

Table 1 (continued)

Cardamine majovskii MARHOLD & ZÁBORSKÝ (2n = 32)

Slovakia

MJ-33	Východoslovenská nížina, Veľký Horeš (100 m s.m.)
MJ-34	Východoslovenská nížina, E of Borša (100 m s.m.)
MJ-35	Východoslovenská nížina, E of Plešany (100 m s.m.)
MJ-36	Východoslovenská nížina, Zatín, near the Tica ox bow lake (99 m s.m.)
MJ-37	Východoslovenská nížina, Leles, near the Tica ox bow lake (type locality) (102 m s.m.)
MJ-38	Východoslovenská nížina, Leles, near the bridge over the River Latorica (102 m s.m.)
MJ-39	Východoslovenská nížina, Rad, Hrušov, near the ox bow lake (100 m s.m.)
MJ-40	Východoslovenská nížina, Ptrukša, in the Mokraď forest (100 m s.m.)
MJ-41	Východoslovenská nížina, NE of Laškovce (115 m s.m.)
MJ-42	Východoslovenská nížina, Nacina Ves, near the railway station (120 m s.m.)
MJ-43	Východoslovenská nížina, Kuzmice, near the path from Čelovce to Kuzmice (170 m s.m.)
MJ-44	Nízke Beskydy, Rokytovce (350 m s.m.)

Hungary

MJ-45	Eupannonicum Samicum, Nagytanya, near the Lonyai-csatorna canal (105 m s.m.)
MJ-46	Eupannonicum Nyírségense, Napkor, E of the railway station (140 m s.m.)
MJ-47	Eupannonicum Samicum, Buj, near the Lonyai-csatorna canal (98 m s.m.)

Ukraine

MJ-48	Vulkanični Karpaty, W of Svaljava, near Karpaty sanatorium (220 m s.m.)
MJ-49	Vulkanični Karpaty, Kam"janycja, near Nevyc'kyj Castle (140 m s.m.)
MJ-50	Zakarpats'ke peredhir"ja, Nankove (170 m s.m.)
MJ-51	Zakarpats'ka rivnyna, Velyki Komjaty (129 m s.m.)
MJ-52	Zakarpats'ka rivnyna, E of Čop (110 m s.m.)
MJ-53	Gorgany, Nehrovec', nature reserve (602 m s.m.)

Romania

MJ-54	Depresiunea Sibiului, Tălmaciu, in the valley of the River Sadu (400 m s.m.)
MJ-55	Depresiunea Sibiului, Sibiu, Pădurea Dumbrava forest (420 m s.m.)

Cardamine pratensis L. (2n = 30)

Slovakia

P30-56	Slovenský raj, Dobšinská ľadová jaskyňa, near the railway station (855 m s.m.)
P30-57	Nízke Tatry, W edge of Pohorelá (690 m s.m.)
P30-58	Nízke Tatry, S edge of Vernár (780 m s.m.)
P30-59	Nízke Tatry, the valley of the River Čierny Váh, near the Veľký Brunov forester's house (1120 m s.m.)
P30-60	Nízke Tatry, Telgárt, near the Telgárt-penzión railway station (890 m s.m.)
P30-61	Liptovská kotlina, E of Važec (790 m s.m.)
P30-62	Spišské kotliny, SE edge of Spišská Teplica (700 m s.m.)
P30-63	Spišské kotliny, Huncovce, near the railway station (640 m s.m.)
P30-64	Spišské kotliny, Strážky, near the railway station (620 m s.m.)
P30-65	Šarišská vrchovina, NW edge of Kamenica nad Torysou (470 m s.m.)

Table 1 (continued)

Ukraine	
P30-66	Schidni Beskydy j Nyz'ki Polonyny, NE edge of Turka (650 m s.m.)
P30-67	Prykarpattja, Pidhorodci (430 m s.m.)
Cardamine pratensis L. (2n = 38)	
Slovakia	
P38-68	Nízke Beskydy, Čertižné, Mokré lúky nature reserve (420 m s.m.)
P38-69	Bukovské vrchy, SW edge of Nová Sedlica (410 m s.m.)
Ukraine	
P38-70	Prykarpattja, Ždanivka (640 m s.m.)
P38-71	Prykarpattja, Busovys'ko (380 m s.m.)
Cardamine pratensis L. (2n = 44)	
Slovakia	
P44-72	Poľana, Mt Predná Poľana, near the chalet (1000 m s.m.)
P44-73	Nízke Tatry, SE edge of Vyšná Boca (950 m s.m.)
P44-74	Nízke Tatry, Moštenica, the valley of the Kyslá stream (470 m s.m.)
P44-75	Západné Tatry, Zverovka (1210 m s.m.)
P44-76	Nízke Beskydy, E edge of Nižná Polianka (402 m s.m.)
Poland	
P44-77	Kotlina Orawsko-Nowotarska, Długopole (620 m s.m.)
P44-78	Beskid Wyspowy, N of Kasina Wielka (600 m s.m.)
P44-79	Gorce, Rdzawka II, Mt Kulakowy Wierch (800 m s.m.)
Cardamine pratensis L. "subsp. *major* Tomšovic" (2n = 44)	
Slovakia	
PM44-80	Záhorská nížina, on the levee of the River Morava between Skalica and Hodonín (164 m s.m.)
PM44-81	Záhorská nížina, Holíč, the Holíčsky les forest (160 m s.m.)
PM44-82	Záhorská nížina, Brodské, near the River Morava (155 m s.m.)
Czech Republic	
PM44-83	Dolnomoravský úval, Uherský Ostroh, in the Singulární les forest (type locality) (170 m s.m.)
PM44-84	Dyjsko-svratecký úval, Tvrdonice, near the River Morava (157 m s.m.)

LP length of petals, mm.
WP width of petals, mm.
LS length of sepals, mm.
LFL length of filaments of longer stamens, mm.
LFS length of filaments of shorter stamens, mm.
NL number of cauline leaves.
NS number of segments on the third cauline leaf (in the case when only two leaves occurred, the second leaf was scored).
PG average diameter of 30 pollen grains from flowers in the main inflorescence.
NI number of lateral inflorescences (longer than 1 cm).

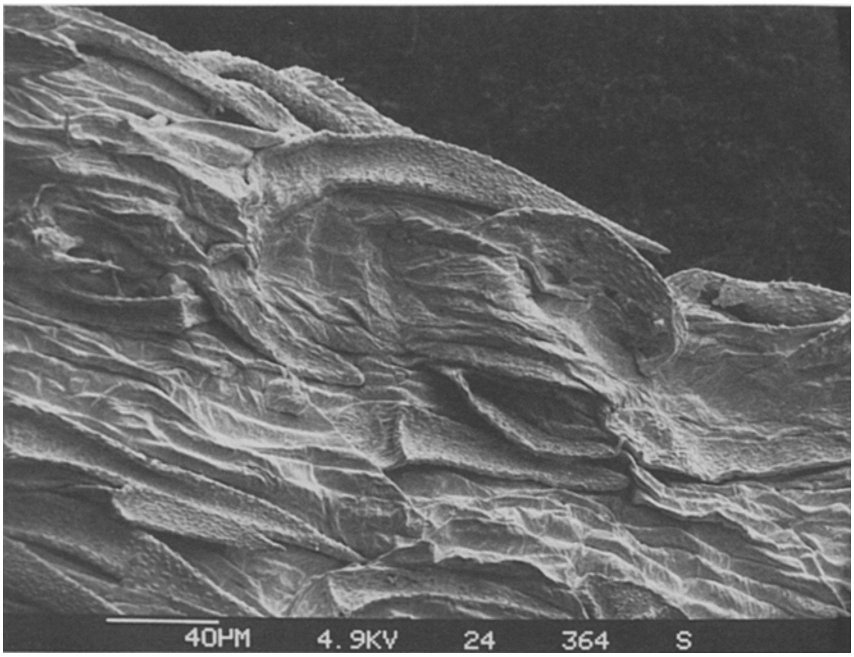

Fig. 1. Appressed hairs on the rachis of the rosette leaves of *Cardamine matthioli*, population MT-13

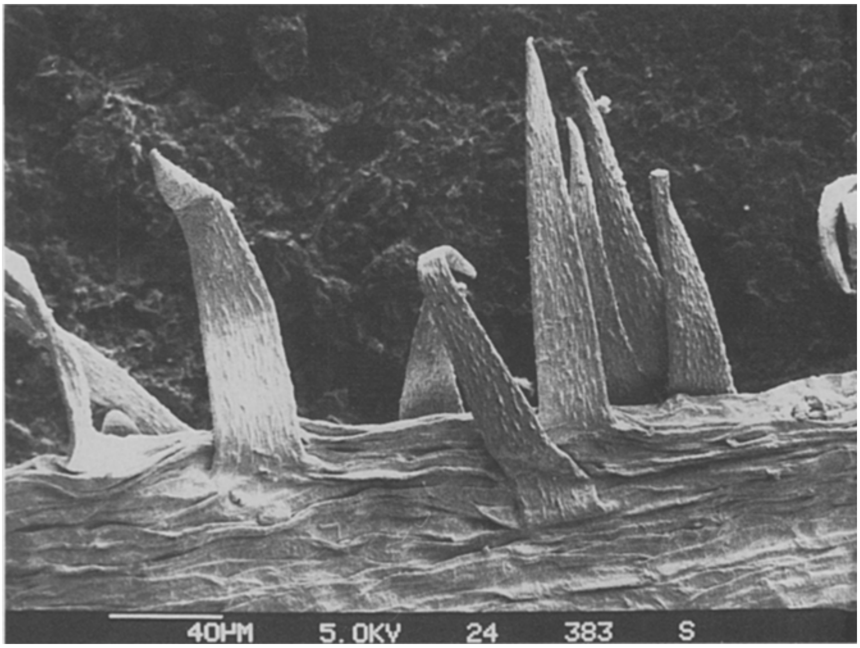

Fig. 2. Patent hairs on the rachis of the rosette leaves of type "rivularis auct." of *Cardamine pratensis*, population RI-30

PS position of lower segments on the mid-cauline leaves: 1 at least some segments slightly deflexed, 2 segments spreading under acute angles.

HRL hairs on the rachis of the rosette leaves: 1 appressed (Fig. 1), 2 hairs absent, 3 patent (90°) (Fig. 2). Hairs of both types never occur in the same population.

FC colour of petals: 1 white, 2 white with pale reddish-violet veins, 3 reddish-violet or purple.

Data analyses. (1) Exploratory data analysis (EDA) (TUKEY 1977, SAS INSTITUTE 1990a) was carried out on the data matrices of all eight groups of populations. Within each group the mean, minimum and maximum values, coefficient of variation, measures of skewness and kurtosis, the Shapiro-Wilk (W) statistic with associated probability (Prob < W) for the test of normality, were computed for each character. The EDA showed that all variables more or less deviate from the normal distribution. To meet assumptions of some further analyses, which require variables to be normally distributed, variables were transformed using the arcsin, logarithmic or square root transformation. The probabilities Prob < W (W statistics, respectively) were slightly improved by arcsin transformation of the characters PG and WP, and by square root transformation of the characters NL, NS, LP, LS, and NI. In analyses using individual plants as operational taxonomic units (OTUs), transformed values of the above-mentioned characters were used unless otherwise stated.

Principal component analyses (PCA) (SNEATH & SOKAL 1973, KRZANOWSKI 1990) of correlation matrices were used as an ordination method in order to study morphological homogeneity of the population samples and to find outliers, i.e., plants with extreme values of some characters. From these analyses characters NI, PS, HRL, and FC were excluded because they are invariable or nearly invariable in many populations. As a result of PCA, up to 4 plants were removed from some population samples for the purpose of further analyses, on the basis of average values of characters from individual populations. The aim of this step was to prevent serious distortion of the population character average values by several individual plants. These plants, however, were not excluded from further analyses based on the individual plants, as they are part of the natural variation.

(2) In order to obtain further insight into variables two correlation coefficients, Pearson and Spearman, were calculated on the pooled data matrix of all material and on the data matrices of the individual groups. The second correlation coefficient is a rank coefficient and was used because the variables are not normally distributed.

(3) To get an overall view on the phenetic relations among populations and groups of populations, cluster analysis (EVERITT 1986) and canonical discriminant analysis (KLECKA 1980, KRZANOWSKI 1990) were made, using populations characterised by average values of characters as OTUs, and the above-mentioned groups of populations as groups. The clustering algorithm chosen was the average clustering method (UPGMA), using squared euclidean distance as a dissimilarity coefficient. Characters were standardised, so that they all had zero means and unit standard deviation, before analysis.

(4) For a better insight into the phenetic relations among individual plants and groups of plants, characterised by their geographical distribution and chromosome numbers, canonical and classificatory discriminant analyses (KLECKA 1980, KRZANOWSKI 1990) were made. These analyses used individual plants as OTUs, and the above-mentioned groups of populations as groups.

The discriminant analyses (DA) generally require multivariate normality of the distribution of variables and the equality of within group covariance matrices. However, as is pointed out by many authors (SNEATH & SOKAL 1973, THORPE 1976, KLECKA 1980) DA are to a large extent robust to violations of these assumptions. Such a violation of the assumptions could especially influence the results of the tests of significance and accuracy of prediction in the classification procedures.

In the classificatory discriminant analyses, discriminant function was determined by the crossvalidation (i.e., the discriminant function was based on n-1 out of n observations and than it was applied to classify the one observation left out). Because the distribution within the groups was not multivariate normal, a nonparametric k-nearest-neighbour method, using raw, untransformed data was performed.

As is shown by Thorpe (1976), in the case of more complicated taxonomic structure, ordination analysis could portray differentiation among groups by the first few vectors, but does not adequately portray differentiation within these primary clusters. Subsequent vectors could only be the compromise between somewhat different variation within primary clusters. This fact was the main reason why canonical and classificatory discriminant analyses were made separately within each primary cluster, resulting from the cluster analyses in the third step. Only classificatory discriminant analysis was carried out on the pooled data.

All numerical analyses were made on the mainframe computer of the University of Oxford. The clustering analyses were made using CLUSTER and PLINK procedures of CLUSTAN package (Wishart 1987). Other analyses were computed using procedures CANDISC, CORR, DISCRIM, PRINCOMP, and UNIVARIATE, available in the SAS package (SAS Institute 1990a, b).

Results

Exploratory data analysis (Tables 2, 3). In spite of the differences in the average values in several characters among groups of populations, a serious overlap in their extreme values could be seen. As a result of invariability in the groups no. 3, 4, 5, 6, and 8 character FC must be removed from all discriminant anlyses in which these groups are included because of producing singularities.

There are only slight differences among the results of Pearson and Spearman correlation coefficients among characters in the pooled data matrix and in the data matrices of individual groups of populations. No characters are either very highly correlated (exceeding value 0.95) or highly correlated (exceeding value 0.9), thus all characters are used in further analyses. Correlations exceeding the arbitrary level of 0.6 occur among floral characters, including the diameter of pollen grains (WP, LP, LS, LFL, LFS, and PG) and among the number of cauline leaves, number of cauline leaf segments and number of lateral inflorescences (NL, NS, and NI).

Cluster analysis (Fig. 3). On the dendogram two main clusters could be seen. In the first main cluster all populations of *C. matthioli* and, with two exceptions (populations no. 44 and 53), all populations of *C. majovskii* as well as the type "ucranica" of *C. pratensis* are together. The latter two form separate clusters, while *C. matthioli* is clustered together with four relatively small flowered populations of *C. majovskii*. In the second main cluster, a separate cluster is formed by the hypohexaploid populations of *C. pratensis*, including the populations of *C. pratensis* "subsp. *major*", while populations of *C. pratensis* with 2n = 30 and 2n = 38 are clustered together with the type "rivularis auct." of *C. pratensis,* which here forms a separate cluster.

Discriminant analyses. On the ordination diagram of the canonical DA, based on populations as OTUs (Fig. 4), the groupings of populations of *Cardamine matthioli, C. majovskii, C. pratensis* with 2n = 30, and the types "ucranica" and "rivularis auct." of *C. pratensis* are clearly remote from each other and from the other populations. However, populations of *Cardamine pratensis* with 2n = 38, 44, and

Table 2. Results of exploratory data analysis of the groups of populations of *Cardamine pratensis* group. *np* number of studied plants *1 Cardamine matthioli* (np = 716), *2 C. majovskii* (np = 871), *3 Cardamine pratensis* "subsp. *major*" (np = 178), *4 C. pratensis* (2*n* = 44) (excl. "subsp. *major*") (np = 273), *5 C. pratensis* (2*n* = 38) (np = 155), *6 C. pratensis* (2*n* = 30) (np = 422), *7* the type "ucranica" of *C. pratensis* (np = 174), *8* the type "rivularis auct." of *C. pratensis* (np = 316). [1] In the case of the character PG 1% and 99% percentiles are presented. For character abbreviations see material and methods

Character	Group	Mean	Standard deviation	Minimum (5% percentile)	Maximum (95% percentile)[1]	Cv
PG	1	26.035	0.886	21.015 (24.027)	31.053 (27.835)	3.404
	2	30.193	0.848	27.427 (28.372)	32.994 (32.312)	2.808
	3	33.318	1.022	29.743 (30.459)	36.398 (35.719)	3.068
	4	32.023	0.994	29.215 (29.804)	36.251 (35.116)	3.103
	5	31.862	0.873	28.807 (29.941)	34.492 (34.352)	2.740
	6	29.218	0.834	26.831 (27.290)	32.238 (31.499)	2.856
	7	26.128	0.631	24.163 (24.308)	27.407 (27.391)	2.416
	8	26.943	0.800	23.046 (24.758)	29.205 (28.844)	2.968
NL	1	7.015	2.539	2 (3)	18 (12)	36.198
	2	5.905	2.113	2 (3)	14 (10)	35.779
	3	4.584	1.421	2 (3)	9 (7)	30.990
	4	3.652	1.111	2 (2)	8 (6)	30.431
	5	3.639	1.056	1 (2)	7 (5)	29.024
	6	4.545	1.580	2 (3)	13 (7)	34.758
	7	5.983	1.891	2 (3)	11 (10)	31.616
	8	4.994	1.405	3 (3)	10 (8)	28.139
NS	1	15.581	4.348	4 (8)	29 (22)	27.907
	2	13.393	4.020	2 (7)	31 (21)	30.017
	3	8.404	2.399	3 (5)	15 (13)	28.547
	4	8.524	2.532	2 (5)	19 (13)	29.708
	5	8.871	2.382	5 (5)	19 (13)	26.846
	6	9.865	2.814	2 (6)	21 (15)	28.523
	7	12.489	2.824	5 (9)	22 (17)	22.611
	8	11.063	2.812	5 (7)	19 (17)	25.417
NI	1	3.810	3.154	0 (0)	18 (10)	82.778
	2	2.463	2.334	0 (0)	12 (7)	94.766
	3	1.890	1.713	0 (0)	8 (5)	94.705
	4	0.857	1.395	0 (0)	8 (4)	162.767
	5	0.684	1.138	0 (0)	6 (3)	166.436
	6	1.190	1.685	0 (0)	9 (5)	141.619
	7	2.000	2.110	0 (0)	9 (7)	105.485
	8	0.320	0.874	0 (0)	6 (2)	273.505
LP	1	8.750	1.519	4.9 (6.6)	15.3 (11.6)	17.361
	2	11.687	1.820	6.4 (8.5)	16.8 (14.6)	15.576
	3	12.430	1.433	9.4 (9.9)	17.0 (14.9)	11.530
	4	11.436	1.438	8.2 (9.2)	16.3 (14.1)	12.571
	5	10.158	1.241	6.6 (8.3)	13.7 (12.5)	12.216
	6	9.598	1.238	6.1 (7.5)	13.5 (11.6)	12.901
	7	9.887	1.006	7.3 (8.3)	12.7 (11.5)	10.176
	8	8.599	1.020	5.9 (7.1)	11.6 (10.2)	11.849

Table 2 (continued)

Character Group	Mean	Standard deviation	Minimum (5% percentile)	Maximum (95% percentile)[1]	Cv
WP 1	4.832	1.098	2.6 (3.5)	9.2 (7.3)	22.721
2	6.936	1.588	3.3 (4.3)	12.3 (9.5)	22.900
3	7.391	1.040	5.0 (5.4)	10.1 (9.4)	14.074
4	6.563	0.955	4.2 (5.0)	9.2 (8.2)	14.546
5	5.530	0.901	2.8 (4.0)	8.5 (6.9)	16.291
6	5.643	1.027	3.1 (4.0)	9.9 (7.5)	18.198
7	5.655	0.749	4.0 (4.3)	7.5 (6.9)	13.237
8	5.025	0.707	3.1 (4.0)	7.1 (6.4)	14.063
LS 1	3.481	0.437	2.3 (2.8)	6.4 (4.3)	12.565
2	4.279	0.643	2.4 (3.3)	7.1 (5.4)	15.033
3	4.262	0.473	3.3 (3.5)	5.6 (5.0)	11.089
4	4.229	0.519	2.9 (3.5)	5.6 (5.2)	12.263
5	3.436	0.354	2.6 (2.9)	4.3 (4.2)	10.294
6	3.629	0.411	2.4 (2.9)	4.7 (4.3)	11.339
7	3.539	0.370	2.6 (2.9)	4.5 (4.2)	10.448
8	3.461	0.379	2.3 (2.9)	5.2 (4.2)	10.962
LFS 1	3.075	0.584	1.2 (2.1)	4.9 (4.2)	18.988
2	3.797	0.660	1.7 (2.8)	6.4 (5.0)	17.382
3	4.171	0.780	2.3 (2.9)	6.1 (5.6)	18.710
4	3.624	0.545	1.4 (2.8)	5.0 (4.5)	15.039
5	3.302	0.445	2.1 (2.4)	4.5 (4.0)	13.488
6	3.170	0.559	1.6 (2.3)	4.9 (4.0)	17.631
7	2.880	0.388	1.7 (2.3)	4.0 (3.5)	13.463
8	2.862	0.434	1.0 (2.1)	4.2 (3.5)	15.151
LFL 1	4.926	0.729	2.8 (3.8)	7.1 (6.2)	14.792
2	6.057	0.787	3.3 (4.7)	8.8 (7.3)	12.998
3	6.966	0.744	4.7 (5.9)	8.8 (8.3)	10.687
4	6.186	0.662	2.9 (5.2)	8.2 (7.3)	10.697
5	5.708	0.542	4.0 (4.7)	7.1 (6.6)	9.494
6	4.994	0.690	3.3 (4.0)	7.3 (6.2)	13.809
7	4.394	0.398	3.3 (3.8)	5.6 (5.0)	9.047
8	5.013	0.553	3.3 (4.2)	6.8 (6.1)	11.022

C. pratensis "subsp. *major*" are fused into one grouping. The first three axes account for 97.3% of the ability of characters to distinguish among groups (first axis: 71.5%, second: 20.6%, and third: 5.2%). The characters PG, LFL, LP(CA1); PS, HRL, NI (CA2); LP, WP, PS (CA3) have the strongest correlation with the first three canonical axes.

The results of discriminant analyses based on the individual plants, give a more detailed view of the variability of populations and groups of populations. In the classificatory DA, based on the pooled data of the individual plants as OTUs (Table 4), the best separation, with more than 92% of plants correctly classified, is

Table 3. Frequency of the states of characters HRL, FC and binary character PS in the groups of populations of *Cardamine pratensis*. For group numbers see Table 2 and for character abbreviations material and methods

Frequency in the group		State of character 1	2	3
HRL	1	229	417	–
	2	234	637	–
	3	–	45	133
	4	–	10	263
	5	–	23	132
	6	–	90	332
	7	–	118	56
	8	–	3	313
FC	1	138	482	96
	2	52	440	379
	3	–	–	178
	4	–	–	273
	5	–	–	155
	6	2	4	416
	7	163	11	–
	8	–	–	316
PS	1	622	94	
	2	634	237	
	3	17	161	
	4	33	240	
	5	45	110	
	6	13	409	
	7	3	171	
	8	30	286	

shown by *Cardamine matthioli*, *C. majovskii* and the types "rivularis auct." and "ucranica" of *C. pratensis*.

In the discriminant analyses based on the individual plants from the populations from the first cluster of the CA the groups of *Cardamine matthioli, C. majovskii* and the type "ucranica" of *C. pratensis* (populations no. 44 and 53 are treated as members of the first cluster because of their chromosome number) are clearly distinct on the ordination graph (Fig. 5). According to the values of correlations of the characters with the canonical axes (total canonical structure) the characters PG, LFL, LP, LS, and WP are most important for the separation of *C. majovskii* from the other two taxa (1st canonical axis) and PS, FC, and HRL for the separation of *C. matthioli* and the type "ucranica" of *C. pratensis* (2nd canonical axis). The first canonical axis accounts for 84.5% and the second one for 15.5% of the ability of characters to distinguish among groups. The results of the nonparametric classificatory DA (Table 5) confirm the division. The percentage of correct classification is very high. Even if the character PG is not taken into con-

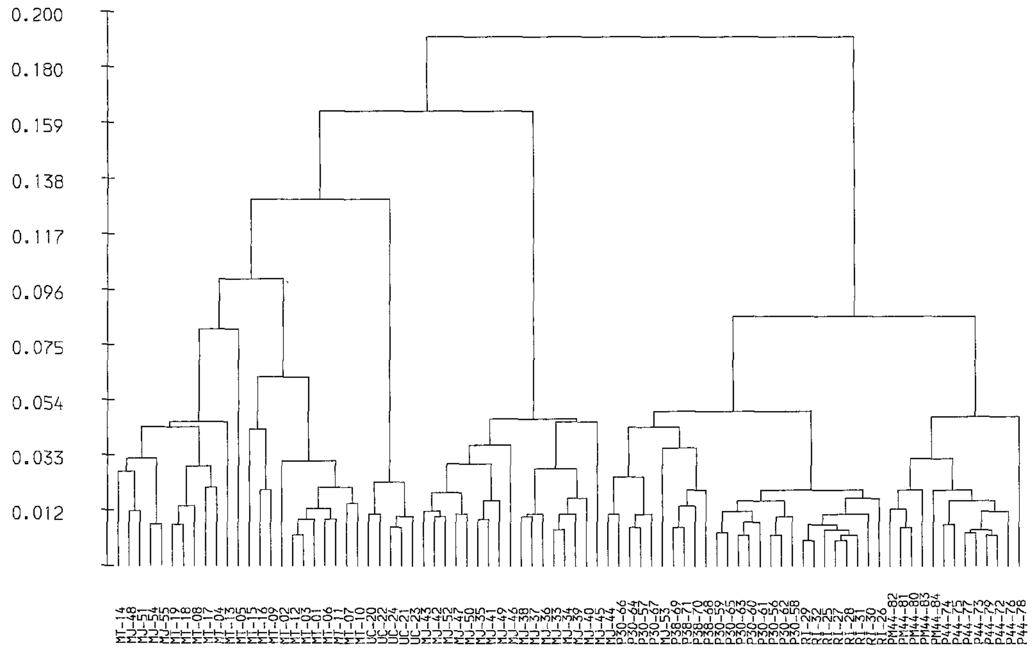

Fig. 3. Cluster analysis of the *Cardamine pratensis* group

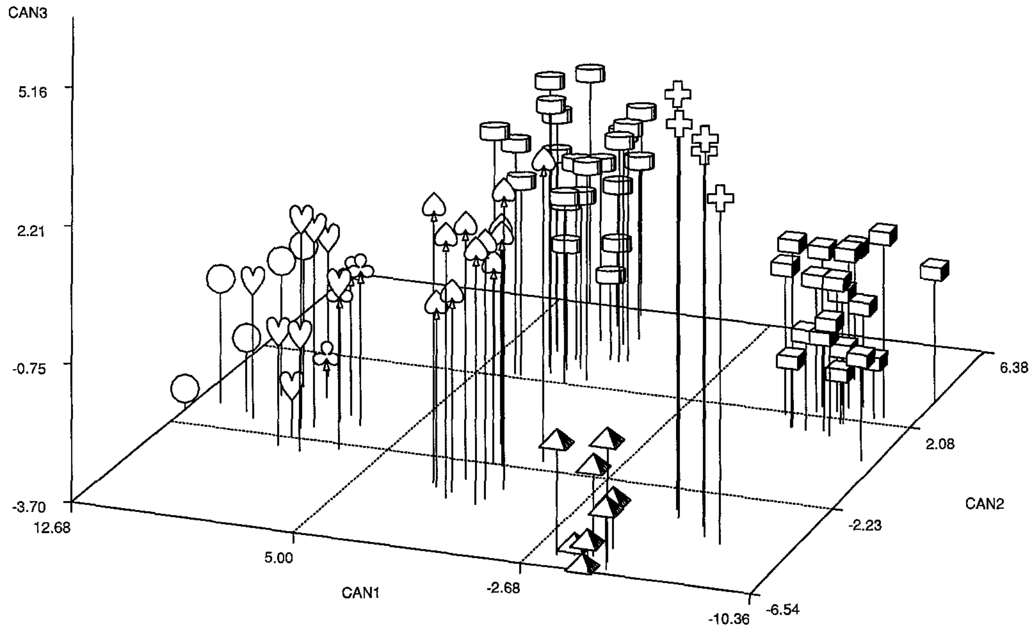

Fig. 4. Canonical discriminant analysis of the *Cardamine pratensis* group, based on populations as OTUs. Cube *Cardamine matthioli*, cylinder *C. majovskii*, cross the type “ucranica” of *C. pratensis*, pyramid the type “rivularis auct.” of *C. pratensis*, club *C. pratensis* with 2n = 30, spade 2n = 38, heart 2n = 44 (with the exception of *C. pratensis* “subsp. *major*”), balloon *C. pratensis* “subsp. *major*”

Table 4. Results of the nonparametric classificatory discriminant analysis based on the plants of the groups *Cardamine matthioli* (1), *C. majovskii* (2), *C. pratensis* (2n = 30 (3), 2n = 38 (4), 2n = 44 (5), *C. pratensis* "subsp. major" (6), and the types "ucranica" (7) and "rivularis auct." of *C. pratensis* (8)

Actual group	Predicted group membership (number of observations and percent classified into groups)							
	1	2	3	4	5	6	7	8
1	**665/92.88**	4/0.56	0.00	0/0.00	0/0.00	0/0.00	45/6.28	2/0.28
2	8/0.92	**803/92.19**	41/4.71	15/1.72	2/0.23	2/0.23	0/0.00	0/0.00
3	1/0.24	19/4.50	**353/83.65**	10/2.37	7/1.66	1/0.24	4/0.95	27/6.40
4	0/0.00	5/3.23	3/1.94	**135/87.10**	10/6.45	2/1.29	0/0.00	0/0.00
5	0/0.00	3/1.10	8/2.93	56/20.51	**183/67.03**	23/8.42	0/0.00	0/0.00
6	0/0.00	3/1.69	1/0.56	13/7.30	29/16.29	**132/74.16**	0/0.00	0/0.00
7	0/0.00	0/0.00	1/0.57	0/0.00	0/0.00	0/0.00	**161/92.53**	12/6.90
8	1/0.32	0/0.00	11/3.48	0/0.00	0/0.00	0/0.00	4/1.27	**300/94.94**

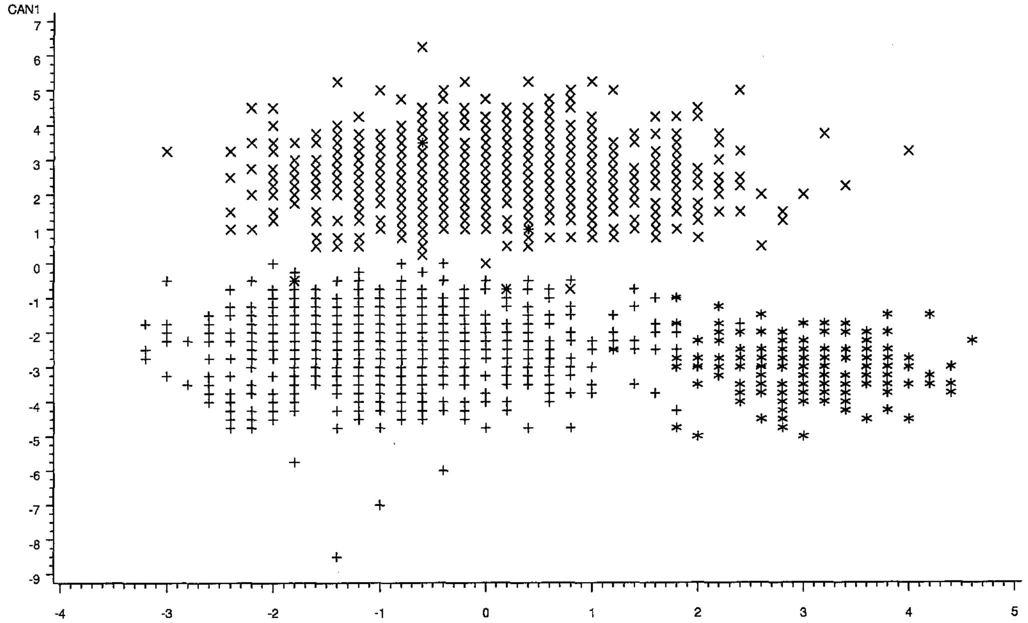

Fig. 5. Canonical discriminant analysis of the individual plants of *Cardamine matthioli* (+), *C. majovskii* (×) and the type "ucranica" of *C. pratensis* (*)

Table 5. Results of the nonparametric classificatory discriminant analysis based on the plants of the groups *Cardamine majovskii* (1), *C. matthioli* (2) and the type "ucranica" of *C. pratensis* (3). [1] Two plants (0.23%) are classified with equal probability into *C. majovskii* and the type "ucranica" of *C. pratensis*. *PG* Pollen grains, *a* character PG included, *b* without character PG

Actual group	Predicted group membership (number of observations and percent classified into groups		
	1	2	3
1 a	**860/98.74**	10/1.15	1/0.11
b	**743/85.30**[1]	104/11.94	22/2.53
2 a	3/0.42	**698/97.49**	15/2.09
b	74/10.34	**620/86.59**	22/3.07
3 a	0/0.00	0/0.00	**174/100.00**
b	0/0.00	0/0.00	**174/100.00**

sideration (it is not possible in every case to measure pollen grains), more than 85% of plants representing *Cardamine matthioli* and *C. majovskii* can be correctly classified.

In the canonical discriminant analysis based on the individual plants from the second cluster of the CA the plants do not form distinct groups (Fig. 6). (The character HRL was excluded from these analyses because it is clearly of no use for distinguishing the above-mentioned groups.) The plants of the type "rivularis auct." of *C. pratensis* form a grouping which slightly overlaps with plants of *C. praten-*

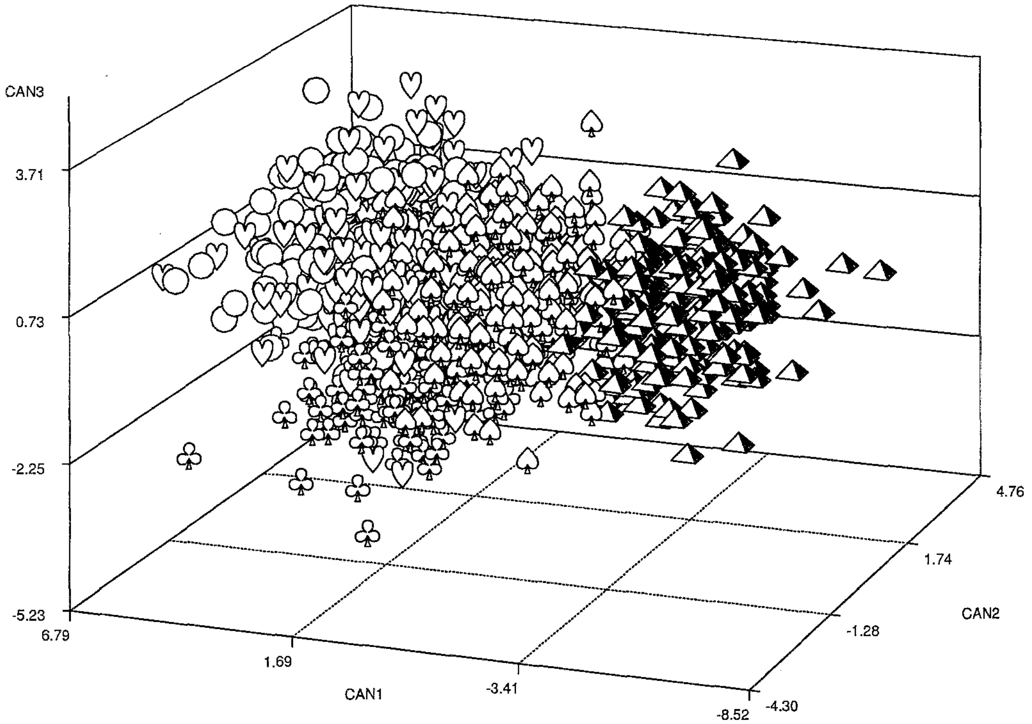

Fig. 6. Canonical discriminant analysis of the individual plants of *Cardamine pratensis* with 2n = 30 (spade), 2n = 38 (club), 2n = 44 (with the exception of *C. pratensis* “subsp. *major*”) (heart), the type “rivularis auct.” of *C. pratensis* (pyramid) and *C. pratensis* “-subsp. *major*” (balloon)

Table 6. Results of the nonparametric classificatory discriminant analysis based on the plants of the groups *Cardamine pratensis* 2n = 30 (1), 2n = 38 (2), 2n = 44 (3), *C. pratensis* “subsp. *major*” (4), and the type “rivularis auct.” of *C. pratensis* (5). *PG* Pollen grains, *a* character PG included, *b* without character PG

Actual group	Predicted group membership (number of observations and percent classified into groups)				
	1	2	3	4	5
1 a	**368/87.20**	16/3.79	11/2.61	1/0.24	26/6.16
b	**260/61.61**	37/8.77	28/6.64	16/3.79	81/19.19
2 a	2/1.29	**138/89.03**	14/9.03	1/0.65	0/0.00
b	8/5.16	**126/81.29**	11/7.10	2/1.29	8/5.16
3 a	8/2.93	51/18.68	**185/67.77**	29/10.62	0/0.00
b	26/9.52	38/13.92	**165/60.44**	35/12.82	9/3.30
4 a	1/0.56	15/8.43	32/17.98	**130/73.03**	0/0.00
b	8/4.49	11/6.18	42/23.60	**115/64.61**	2/1.12
5 a	12/3.80	0./0.00	0/0.00	0/0.00	**304/96.20**
b	30/9.49	29/9.18	5/1.58	0/0.00	**252/79.75**

sis with 2n = 30, while plants of *C. pratensis* with 2n = 30, 38, 44 and *C. pratensis* "subsp. *major*" are fused together. The first canonical axis accounts for the greatest part (88.4%) of the whole ability of characters to distinguish among groups, while the remaining two axes account only for 5.2% (2nd axis) and 4.5% (3rd axis), respectively. The characters PG, LP, and LFL have the highest values of correlation with the 1st canonical axis (total canonical structure), LFL with the 2nd canonical axis and LS with the 3rd. However, the character PG is much more powerful than the other ones. These phenetic relations are confirmed by the results of the nonparametric classificatory discriminant analysis (Table 6). With the exception of the type "rivularis auct." of *C. pratensis* the percentage of correctly classified plants is relatively low and, if pollen grains are not measured, the percentage of correctly classified plants sharply decreases.

Discussion and conclusions

The karyological study (Marhold 1994a) has shown wide variability in chromosome numbers of the *Cardamine pratensis* group in the Carpathian and Pannonian area. Beside the two morphologically well defined taxa – *Cardamine rivularis* and *C. dentata* (see the key below), eight groups of populations could be recognised on the base of karyological and geographical criteria. However, the present morphometric study shows that not all these groups are morphologically and thus, in the opinion of the present author, taxonomically distinguishable.

Cardamine matthioli and *C. majovskii* are rather well characterised against other studied groups of populations by the position of hairs on the rachis of the rosette leaves (HRL), the position of lower segments on the mid-cauline leaves (PS) and by the colour of petals (FC). They differ from each other in the size of the flower parts (LP, WP, LS, LFL) and in the diameter of the pollen grains (PG). When all these characters are used for identification of the two taxa the probability of a correct result is more than 97%. If the diameter of pollen grains is not known this probability is still more than 85%. However, one must be cautious in the case of extreme ecological conditions [very rich soils in flood-plain forests (as in the locality MT-15) or poor soils (as in the locality MJ-55)] which could influence the size of flower parts. In such cases the measurement of pollen grains is essential. These morphological differences favour the idea that *C. majovskii* is an autotetraploid species which arose directly from *C. matthioli*.

The type "ucranica" of *C. pratensis* forms a rather isolated group of populations which is provisionally included in *Cardamine pratensis*, until a definite taxonomic decision about its status can be made. It differs from other groups of populations in the colour of petals (FC) being always pure white, in the position of hairs on the rachis of the rosette leaves (HRL) and partly also in the position of lower segments on the mid-cauline leaves (PS). The percentage of correct identification of this group is at least more than 92%. However, there are populations of the *Cardamine pratensis* group widespread in the Ukraine to the East of the Carpathians which are, with the exception of the chromosome number (probably hexaploid) and the diameter of pollen grains, almost identical with the type "ucranica" of *C. pratensis*. Thus the final taxonomic decision should be made only after detailed evaluation of these populations.

The type "rivularis auct." of *C. pratensis* represents populations which differ from other populations of the *C. pratensis* group in chromosome number and geographical distribution (upper montane and subalpine belt of the East Carpathians). Morphologically, however, they differ only poorly from the populations of *C. pratensis* with 2n = 30, 38, and 44. The only sharp difference among these plants is the diameter of pollen grains. When the size of the pollen grains is unknown, the probability of correct classification of this type is much lower (in such a case 19.2% of plants of *C. pratensis* with 2n = 30 in classificatory DA were wrongly classified as *C. pratensis* the type "rivularis auct."). The populations of the type "rivularis auct." of *C. pratensis* were in the past wrongly identified either as *C. pratensis* proles *crassifolia* (Pourr.) O. E. Schulz or as *C. rivularis* Schur. *Cardamine crassifiolia* Pourr. is an endemic species occurring only in the Pyrenees and differs from the type "rivularis auct." of *C. pratensis* in having a stoloniferous rhizome. *Cardamine rivularis* is, according to the present author, the species which only occurs in the South Carpathians and some Bulgarian mountains. It differs from the type "rivularis auct." of *C. pratensis* and from the populations from the Alps, Apennines, Massif Central, and Vosges, previously treated as *C. rivularis*, in having purplish anthers before dehiscence, the terminal leaflet of the basal leaves approximately having the same size as the lateral ones and hairs being appressed on the rachis of basal leaves. However, the relations between the type "rivularis auct." of *C. pratensis* and diploid populations of *C. rivularis* auct. non Schur from the Alps and diploid populations of *C. pratensis* (s. str.) reported by Urbanska-Worytkiewicz & Landolt (1974) from the north of the Alps, from the surroundings of Lake Constance (Bodensee) are still not clear. Thus the final decision concerning the taxonomic position of this Carpathian high mountain type should be made after careful comparison with these populations.

Among the groups of populations of *Cardamine pratensis* with the chromosome numbers 2n = 30, 38, and 44 and *Cardamine pratensis* "subsp. *major*" the discriminant and, to some extent, cluster analysis have shown the possibility of the morphological recognition of the populations with 2n = 30 from the populations with higher chromosome numbers. This, however, was not confirmed on the level of individual plants. All discriminant analyses based on the individual plants support the idea that all these populations should be treated as one taxon. The subspecies *C. pratensis* subsp. *major,* described by Tomšovic (1986), which differs from other populations of *C. pratensis* in its ecology (it grows in the flood-plain forests) is not reliably recognisable morphologically, especially from the other hypohexaploid populations.

The above-mentioned species (including *C. dentata* and *C. rivularis*) can be identified using the following key:

1a	All cauline leaves pinnate, leaflets distinctly stalked	*C. dentata*
1b	At least upper cauline leaves pinnatisect	2
2a	At least some basal leaves hairy	3
2b	All basal leaves glabrous or shrivelled at anthesis	5
3a	Hairs on rachis of basal leaves patent (90°)	*C. pratensis*

3b Hairs on rachis of basal leaves appressed 4

4a Corolla purple; anthers purplish before dehiscence .... *C. rivularis*

4b Corolla white or pale reddish violet; anthers yellow before dehiscence 7
5a (2b) Lower segments of mid-cauline leaves slightly ascending 6
5b Lower segments of mid-cauline leaves slightly deflexed 7

6a Terminal leaflet of basal leaves of approximately the same size as lateral ones; anthers purplish before dehiscence .... *C. rivularis*
6b Terminal leaflet of basal leaves distinctly larger than lateral ones; anthers yellow before dehiscence .... *C. pratensis*

7a (4b) Petals 5.0–9.0(–12.0) × 2.5–5.5(–6.0) mm, average size of diameter of pollen grains 24.0–27.8 µm .... *C. matthioli*
7b Petals 8.5–16.5 × (5.0–)5.5–12.0 mm, average size of diameter of pollen grains 28.3–32.3 µm .... *C. majovskii*

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