Katarzyna Marcysiak

Geographical differentiation of *Dryas octopetala* in Europe based on morphological features

Abstract: *Dryas octopetala* is an important arctic alpine species, but its morphological analyses are scarce. The study deals with present European biogeographical structure based on morphology and its relations to the phylogenetic structure and the climate. Characters of leaves from 23 isolated populations were measured, statistically characterized and analyzed with the use of ANOVA and the discriminant analysis. All the features differentiated the tested samples significantly. Differences between plants representing regions were greater than between the samples within regions with regard to the four characters. The most visible division ran between the samples coming from the southern and the northern parts of the range, with the sample from the Western Scandinavia occupying the central position. Correlations between characters and climactic factors were shown, and the size of the leaf was associated mainly with the minimum temperatures of spring months, while the leaf shape with the rainfall during the summer months. Characters differentiating the regions and discriminating populations strongly were mostly dependent on the influence of the climate. The results of the present study were not fully consistent with the outcomes of the biogeographic molecular analyses of the researched species. Some parallels can be drawn when comparing the present analyses with the geographic differentiation of two other arctic-alpine shrubs *Salix herbacea* and *S. reticulata*. Although morphological diversity of *Dryas octopetala* showed a geographic structure, it was most probably shaped mainly by the influence of the environmental and climatic factors.

Additional key words: arctic-alpine species, biogeography, biometrics, climatic conditions, statistical analyses

Address: K. Marcysiak, Kazimierz Wielki University, Botany Department, al. Ossolinskich 12, 85-093 Bydgoszcz, Poland, e-mail: marc@ukw.edu.pl

Introduction

Dryas octopetala is one of quite numerous arctic-alpine species (Zając and Zając 2009). In Europe, the species with this type of distribution occur in the north of the continent, in the Arctic, and in the mountains of the Alpine system. This disjunction is the result of successive contractions and expansions of plant ranges, often called plant migrations, which were forced by climate fluctuations in the Pleistocene and the early Holocene (Comes and Kadereit 1998; Hewitt 1999; Birks and Willis 2008). The migrations were accompanied by processes that contributed to the diversity and the internal variability of parts of ranges and individual populations (Jones and Gliddon 1999; Abbott and Brochmann 2003; Kapralov et al. 2006; Birks 2008; Birks and Willis 2008). Recently, popular studies of arctic-alpine species, aiming to

investigate their internal differentiation and variability at the molecular level, allow also to trace the changes of plant ranges, and conclude what were the Pleistocene refuges and migration routes of species.

Attempts to explore the diversity of arctic-alpine species with morphological markers are few. Although for many years research, including taxonomic studies, has been based on morphological characteristics, recently they have attracted less confidence, especially in the study of variation within the lower level taxa, due to the possible effects of environmental factors on this variability. In particular, there are few studies comprising large parts of plant ranges. The morphological variation of the European ranges of *Salix herbacea* and *S. reticulata* arctic-alpine dwarf shrubs was recently characterized by Marcysiak (2012 a,b), who obtained a geographical structure only partially in accordance with the postglacial history of the species and showed the relationship of features of *S. herbacea* leaves with the climate.

Still, congruency of results of molecular and morphological analyses was found in several studies of variation within species or between closely related species of gymnosperms (Douaihy et al., 2012; Boratyński et al., 2013; Jasińska et al., 2013).

Dryas octopetala, an essential component of the Pleistocene tundra, is also one of the most important species of contemporary communities occurring at high latitudes of the northern hemisphere. In Europe, we can also find it in the calcareous mountains, usually above the subalpine zone. *Dryas octopetala* reproduces both generatively and clonally, which is typical for plants of cold climates, and individuals can reach the maximum age of 500 years, but populations are predominated by young genets. Such a structure of the population enables the species to adapt to changing environmental conditions and persist in them (de Witte et al. 2012). Although seeds of *Dryas octopetala* are adapted for long-distance wind dispersal, the rates of seedling establishment are low which limits the flow of new genotypes into populations. The species also show a strong local adaptation which is manifested by the elimination of transplanted ecotypes in a foreign environment (Bennington et al. 2012). Thus the differences between isolated populations of the species may persist for a long time. However, it is widely accepted and proven that the morphology of the plant can be influenced by environmental conditions (Bazzaz et al. 1987; Mal and Lovett-Doust 2005).

Dryas octopetala has already been the subject of various studies (McGraw and Antonovics 1983; Rieley et al. 1995; Vasari 1999; Šibik et al. 2004). Some of them have focused on the genetic variability of the species, the possibility of crossing with *D. integrifolia*, its postglacial migration and possible refugia (Philipp and Siegismund 2003; Vik et al. 2010). Skrede et al. (2006), in her research on molecular differentiation of *Dryas octopetala sensu lato* within the whole circumpolar range found that the populations of the species

Table 1. Collection data of *Dryas octopetala* samples

| Acronym	Location	Region	No. of individuals	No. of leaves	alt.	N	E
ES	Norway, Finnmark, Nordkapp	Eastern Scandinavia	30	300	100	71.0893	25.7840
WS	Norway, Hardangevidda	Western Scandinavia	30	298	1300	60.5593	08.0792
EC1	Romania, Piatra Craiului, Şaua Grindului	Eastern Carpathians	33	310	2206	45.525	25.2086
EC2	Romania, Muntii Rodnei		33	320	1910	47.5383	24.8587
SC1	Romania, Parâng Mts.	Southern Carpathians	31	282	2390	45.3540	23.5268
SC2	Romania, Muntii Retezat		15	111	2210	45.3567	22.9172
NC1	Polska, Małe Pieniny	Western Carpathians	16	160	715	49.4017	20.5712
NC2	Slovakia, Belanské Tatry		20	157	1615	49.2503	20.2032
NC3	Slovakia, Nizke Tatry		29	241	1750	48.9808	19.6297
NC4	Poland, Tatra Mts., Wielka Kopa Królowa		26	260	1530	49.2645	20.0030
NC5	Poland, Tatra Mts., Sarnia Skała		30	300	1377	49.2762	19.9578
NC6	Poland, Tatra Mts., Czerwone Wierchy		30	300	1750	49.2533	19.9018
Alp1	Italy, Dolomites, Monte Cristallo	Alps	27	270	2167	46.5691	12.1839
Alp2	Austria, Grubigstein, top, meadow		29	290	2207	47.3831	10.8411
Alp3	Austria, Grubigstein, steep slope, on loose rocks		30	300	2166	47.3832	10.8439
StPla	Bulgaria, Centralna Stara Planina mountains, Kozia Stena reserve	Balkans	31	307	1660	42.7843	24.5350
Ril	Bulgaria, Rila mountains, Sedemte Ezera valley		32	290	2425	42.1898	23.3238
SPla	Macedonia, Šar-Planina mountains		30	300	2010	42.0243	20.8578
SuPla	Serbia, Suva Planina		25	250	1680	43.1815	22.1587
Din1	Montenegro, Dinaric Mountains, Durmitor		25	250	2120	43.1215	19.0325
Din2	Montenegro, Dinaric Mountains, Komovi		27	270	1900	42.6883	19.6745
Ap	Italy, Apuan Alps	Appenines	27	270	1600	44.10	10.1333
Pyr	Andorra, Pico de Casamanya	Pyrenees	30	300	2670	42.5812	01.5713
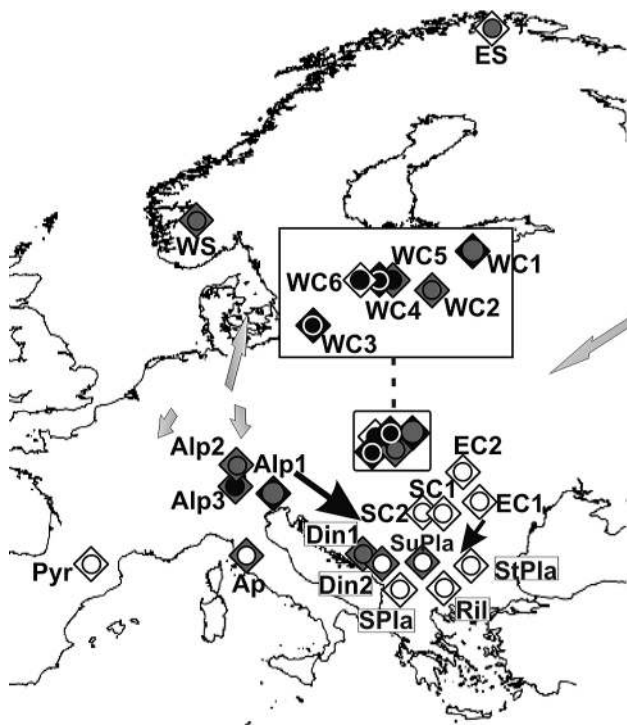
Fig. 1. Location and selected characteristics of *Dryas octopetala* samples, compared to the previously proposed migration routes of the species; squares - mean *bl/blw* values: 1.65<white<1.82, 1.82<grey<2.00, 2.00<black<2.36; circles - mean *blw/aw* values: 2.57<black<3.00, 3.00<grey<4.08, 4.08<white<5.13; arrows - migration routes based on molecular studies by: light grey - Skrede et al. 2006, black - Ronikier unpubl. preliminary analysis; characters acronyms as in Table 2

in the Alps and Western Scandinavia originated from the glacier foreland on the European lowland, while the populations in the Carpathians and Eastern Scandinavia came from the Northern Siberia. Recently Ronikier and Cieślak (2012) described three distinct AFLP lineages of the species across Europe and concluded that present Balkan range emerge from the last glacial migration from the Carpathians and the Alps.

Morphological characters of *Dryas octopetala* have rarely been used in research. Several features of the leaves were used by Philipp and Siegismund (2003), who analyzed the hybrid zone between *D. octopetala* and *D. integrifolia* in Greenland. The parallel analysis of isoenzymes carried out by the authors gave results not fully complying with the grouping based on morphology.

In the present paper, I characterize the morphological variation and differentiation between populations of *Dryas octopetala*, coming from different parts of the range in Europe. I expected that the morphological differences may occur between isolated mountain ranges and populations within them, and their clustering may reflect the existing biogeographic divisions. I want to verify whether the biogeographical structure based on the leaf morphology is similar to

that obtained in the molecular studies by Skrede et al. (2006) and Ronikier and Cieślak (2012). I also try to check if *Dryas* morphology depended on the climate, and if some parallels between morphological differentiation of *Dryas octopetala* and other arctic-alpine species, investigated by Marcysiak (2012 a, b), could be observed.

Materials and methods

Collection of material and measuring

The research material consisted of leaves of *Dryas octopetala* from Eastern and Western Scandinavia, the Western, Eastern and Southern Carpathians, the Central Alps, the Appenines, the Pyrenees and the Balkans (Fig. 1, Table 1). From each of the 23 population, depending on its size, the leaves were collected from (15) 20–30 distinguishing individuals, spaced out at least 3 m, to avoid the collection of one genet. From each individual, from the central part of the stem of at least 5 different shoots, about 10 leaves were collected, without any visible infection or damage. After collection, the leaves were dried and herbarized. The herbarium is deposited at the Department of Botany of Kazimierz Wielki University in Bydgoszcz.

The herbarized material was measured. As a small portion of the leaves was damaged during transport and drying, ultimately, there were 6136 leaves of 637 individuals measured in total. (Table 1). The leaves of each individual were scanned and measured automatically with the use of digiShape software (CortexNova); additionally, the teeth were counted. The measured characteristics were used as the basis for calculating the synthetic ones, which describe the leaf shape. Typical features used in the morphological analyses of leaves were chosen (Philipp and Siegismund 2003; Boratyński et al. 2008; Marcysiak 2012 c). In general, the leaves were characterized on the basis of nine measured/counted characteristics and 5 proportions (Fig. 2, Table 2).

Analyses

Before starting the analyses, the normality of distribution of characters was examined with K-S and Lilliefors test.

Descriptive statistics were provided for the entire data set, for each region and sample. The variability of characters in each sample, region and the entire set of data was tested using the variation coefficient ($Cv=M/SD\times100$). For comparisons of the variability between samples and regions, arithmetic means of variation coefficients of characters in populations were used (Soulé and Zegers 1996). In the entire data set, mutual correlations of characters were examined using Pearson's correlation coefficient.

Table 2. Characters of *Dryas octopetala* leaves, their descriptive statistics, correlations and importance in the differentiation of populations and regions

Character	Acronym	Aryth-metic mean	Min	Max	Cv	Characters correlated at $p>0.01$ with		Characters diversifying regions*	Discrimination power testing among populations partial Wilk's λ $p<0.001$
						tem-pera-ture	preci-pita-tion		
Perimeter of a leaf blade [mm]	per	39.59	13.59	110.01	30.85				0.777
Area of a leaf blade [mm]	ar	67.10	8.70	381.44	56.21	×			0.848
Length of a leaf blade [mm]	bl	12.51	4.10	34.52	29.67	×			0.850
Maximum width of a leaf blade [mm]	blw	3.34	1.11	8.00	27.61				0.830
Width of a leaf base [mm]	bw	4.57	0.89	11.35	30.61				0.913
Maximal width of an apical tooth [mm]	aw	1.75	0.35	5.88	36.42	×		×	0.668
Length of a biggest tooth [mm]	tl	2.24	0.81	5.30	26.11	×			0.722
Length of a petiole [mm]	pl	16.02	4.19	47.46	33.84				0.880
Number of teeth on a leaf blade margin	tn	14.20	6.00	27.00	17.06		×	×	0.769
Ratio of area/perimeter (char. 2/char. 1)	ar/per	1.59	0.58	3.76	25.73	×			0.829
Ratio of leaf length/max. width × 2 (char. 3/(char.4 × 2))	bl/blw	1.89	0.97	4.43	18.99				0.832
Ratio of max. width × 2/ width of leaf base (char. 4× 2/char.5)	blw/bw	1.51	0.44	4.29	22.42		×		0.871
Ratio of max. width × 2/ width of apical tooth (char. 4× 2/char.6)	blw/aw	4.20	1.26	25.36	39.15	×	×	×	0.776
Ratio of petiole length/sum of blade and petiole length (character 11/ (char.3 + char.11))	pl/pbl	0.56	0.29	0.72	10.46			×	0.889

*results of nested variance analysis; variance greater between regions than between samples within regions at $p<0.05$

Differentiation between samples and regions was examined with the help of univariate analysis of variance (ANOVA / ANCOVA). To determine which features differentiated between populations, the post-hoc procedure with the use of the RIR Tukey test for unequal frequencies were conducted. The results were illustrated by means of box-and-whiskers plots. The significance of the variation between regions and between samples within regions was determined with the nested variance analysis (Sokal and Rohlf 2003).

Multivariate relationships between populations and discrimination power of chosen characters were tested using stepwise discrimination analysis. Only shape characters were selected for this analysis, as mutual correlations between them were weaker and less frequent, compared to the size characters. The position of the populations along the first two discriminant variables were examined and presented on the scatterplot (Sokal and Rohlf 2003; Tabachnik and Fidell 2007).

The connections of *Dryas octopetala* features to the altitude and the bioclimatic factors were checked using Spearman's r coefficient for non-parametric distributions. The data concerning the bioclimatic factors were obtained from publicly available databases WorldClim; 'Data for current conditions (1950–2000)' and highest resolution 30 arc-seconds were used (Hijmans et al. 2005).

Calculations and analyzes were performed using software STATISTICA (StatSoft), with the exception of the nested variance analyses that was performed by means of the JMP software.

Results

The distribution of values of analyzed characters were unimodal or very close to unimodal.

The mean coefficient of variation of characters was almost 29%, and ranged from about 10% for *pl/pbl* to over 56% of leaf area (*ar*) (Table 2). The mean coefficient of individual samples ranged from less than 15% for WC1 to over 21% for the SuPla. In general, higher values of Cv were observed in samples from the Balkans, the Low and the High Tatras in the Western Carpathians and in Eastern Scandinavia.

Most features of *Dryas octopetala* showed significant correlations at $p<0.01$. The highest values of correlation coefficients were found between the measured features; the synthetic features were tied together less frequently, and the correlations were weaker (not shown).

All the features differentiated significantly the tested samples at $p <0.01$, whereas, Tukey's test showed similarities between some of the samples in terms of certain characters. As far as size characters were concerned, two population groups were appar-
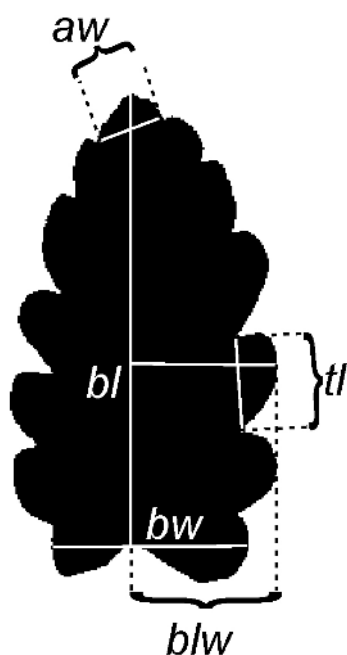
Fig. 2. Way of measuring the characteristics of Dryas octopetala leaf: bl - length of a leaf blade; blw - maximum width of a leaf blade; bw - width of a leaf base; aw - maximal width of an apical tooth; tl - length of a biggest tooth

ently distinct: one of the smallest leaves – Alp3, ES SC1-2, and one of the largest leaves: Ap, NC2, Din2, SuPla and StPla, while other samples achieved intermediate values. In case of *aw* character, the samples grouped in differently: the leaves from the Western Carpathians (NC2-6) had the widest apex (*aw*), while those from the Southern and Eastern Carpathians, the Pyrenees, the Rila Mountains, the Šar-Plani-

na Mountains and Eastern Scandinavia (SC1-2, EC1-2, Pyr, Ril, SPla, ES), the narrowest (App. 1). The shape characters differed populations somewhat less distinctly. The groups with the biggest and smallest character values, as in the case of size characters, can be found for the character *ar/per*. The leaves from the Western Carpathians (except NC6) and the Dolomites (Alp1) were more elongated (greater value of *bl/blw*) than in the rest of samples. The proportion of the petiole length in the whole leaf length (*pl/pbl*) reached the highest values in WS and the lowest in Ap and did not seem to show any regularity. Character *blw/bw* describes the shape of the lower half of the leaf: its lower values (tending to 1) mean almost linear leaf, and leaves of this shape were found in the Tatras and Alps (NC4-5, Alps1-3). Values of the ratio of the maximum leaf width to the width of leaf apex (*blw/aw*) divided samples into two groups: the northern group, comprising samples from the Scandinavia (ES, WS), Western Carpathians (NC1-6) and Alps (Alp1-3), and the southern group with the rest of the samples. In the northern group, leaves generally had wider apical tooth (*aw*) and the difference between the leaf width and *aw* was smaller (lower values of *blw/aw*). The wide apex tapered quite sharply and as a result, the very top of the leaf was more acute compared to the leaves of the southern groups. The leafs of the southern group usually had narrow and small apices somewhat hidden between the teeth below, so the top of the leaf was usually rounded. The division into northern and southern group is also evident in character *tn*, though not so distinctly, and the leaves from the north had fewer teeth than those from the south (App. 2).

Table 3. Results of the nested variance analysis of the *Dryas octopetala* leaf characters

Character	Component	%	SS	MS	df	F	Prob > F
aw	between regions	21.371	497.224	49.7224	10	6.0356	0.0034*
	between populations in regions	6.890	82.6862	7.51692	11	9.1518	<.0001*
	within population	16.240	498.826	0.85856	581	3.8304	<.0001*
	Residual	55.499					
	Total	100					
tn	between regions	16.543	7197.99	719.799	10	3.0362	0.0417*
	between populations in regions	13.679	2372.59	215.69	11	13.6033	<.0001*
	within population	23.612	9684.66	16.6689	581	5.9471	<.0001*
	Residual	46.166					
	Total	100					
blw/aw	between regions	27.288	3924.35	392.435	10	10.8809	0.0003*
	between populations in regions	4.476	362.251	32.9319	11	8.9712	<.0001*
	within population	8.249	2206.64	3.79801	581	2.3302	<.0001*
	Residual	59.987					
	Total	100					
pl/pbl	between regions	13.135	2.60577	0.26058	10	6.4049	0.0029*
	between populations in regions	3.243	0.41328	0.03757	11	3.6171	<.0001*
	within population	26.473	6.33874	0.01091	581	5.4807	<.0001*
	Residual	57.149					
	Total	100					

Table 4. Spearman's rank correlation coefficient between bioclimatic factors and characters of *Dryas octopetala*; only factors and characters expressing significant correlations at p<0.01 (numbers in bold) are shown

	ar	bl	bw	tl	aw	blw/bw	blw/aw	tn	ar/per
tmin3	0.38	0.46	**0.53**	0.42	0.30	−0.13	0.18	0.14	**0.55**
tmin4	**0.59**	**0.58**	**0.59**	0.48	0.40	0.02	0.13	0.18	**0.67**
tmin5	**0.60**	**0.58**	**0.61**	0.48	0.39	0.04	0.16	0.19	**0.69**
tmin6	**0.59**	**0.56**	**0.57**	0.44	0.35	0.08	0.18	0.23	**0.66**
tmin7	0.46	0.42	0.50	0.36	0.22	0.11	0.25	0.23	**0.56**
tmin8	0.43	0.37	0.48	0.28	0.14	0.09	0.32	0.26	**0.53**
tmax6	0.48	0.51	0.46	0.34	0.20	0.01	0.27	0.31	**0.53**
tmax8	0.34	0.34	0.34	0.17	−0.11	0.28	**0.54**	**0.53**	0.37
prec6	−0.06	−0.07	−0.06	0.00	0.34	−0.33	**−0.56**	**−0.54**	0.00
prec7	−0.08	−0.02	−0.04	0.09	0.48	**−0.54**	**−0.70**	**−0.66**	0.01
prec8	−0.03	0.02	0.02	0.15	**0.55**	**−0.54**	**−0.74**	**−0.70**	0.05
prec9	0.10	0.12	0.20	0.28	**0.57**	−0.43	**−0.59**	**−0.53**	0.16
MTWeQ	0.35	0.48	0.36	**0.53**	**0.60**	−0.34	−0.37	−0.30	0.49
MTDQ	0.03	0.01	0.13	−0.12	−0.41	0.38	**0.66**	0.51	0.08
AP	0.03	0.02	0.12	0.12	0.47	−0.33	**−0.54**	−0.50	0.11
PWeQ	−0.08	−0.06	−0.05	0.02	0.43	−0.40	**−0.66**	**−0.65**	0.00
PWaQ	−0.07	−0.03	−0.05	0.07	0.48	−0.48	**−0.71**	**−0.67**	0.01

in months: tmin - minimum temperature, tmax - maximum temperature, prec - precipitation; MTWeQ - Mean Temperature of Wettest Quarter; MTDQ - Mean Temperature of Driest Quarter; AP - Annual Precipitation; PWeQ - Precipitation of Wettest Quarter; PWaQ - Precipitation of Warmest Quarter

The nested variance analysis showed that differences between plants representing regions were greater than between the samples within regions with regard to only four characters. The variance within populations was generally high, but the residual variation reached the highest level, indicating the great differences between leaves in individuals and populations (Table 3).

All the characters significantly discriminated the analyzed samples, although their discriminatory power was not big, the biggest for characters *aw*, *tl* and *tn* (Table 2). The first two discriminatory variables explained nearly 75% of the total variance. The
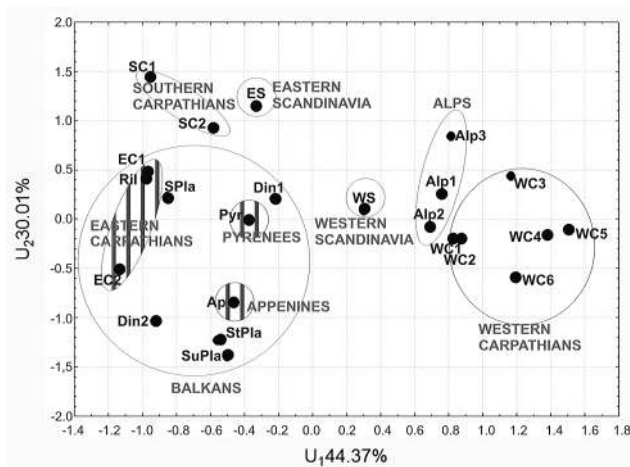

Fig. 3. Results of discrimination analysis for *Dryas octopetala* on the plot of two first discrimination variables; black points - samples means; black letters - samples acronyms as in Table 1; areas surrounded by grey lines - samples belonging to one region, striped areas - overlapping regions; grey letters - regions name

grouping split the samples into two parts, the first comprising samples from the Alps and the Western Carpathians and the second, containing all samples from the southern parts of the species range and Eastern Scandinavia. The sample from the Western Scandinavia occupied the central position. Variable U_1 was correlated with features *blw/aw*, *aw* and *tn* most strongly (Fig. 3).

The correlations of *Dryas octopetala* characters with the climactic factors were shown by some characters of size and shape, and the size of the leaf (*ar, bl* and *bw*) was associated mainly with the minimum temperatures of the spring months (positive correlation), while the leaf shape (*ar, bl* and *bw*) with the rainfall during the summer months (negative correlation). As a result, leaves from the more rainy regions had fewer teeth and were more linear, but with an acute apex. The exception was character *ar/per*, dependent on the temperature of the spring and the summer, which means that leaves were more indented in the regions with lower temperatures. All correlations were moderate (Table 4). The correlations with altitude were found only for the Balkan samples, significant at p<0.05 for: *ar, bw, tl, pl* and *ar/per*.

Discussion

Biogeographic structure of *Dryas octopetala* based on leaf morphology

On the basis of the results of this study one can conclude that *Dryas octopetala* populations differed in terms of leaf morphology and this diversity showed a

geographic structure. Grouping of the samples and regions in the present work confirmed the biogeographic and phylogeographic patterns reported for other arctic-alpine species. The clustering of the Carpathian samples was congruent with the biogeographic division of the massif to the western, southern and eastern parts (Ronikier 2011). A discrepancy between the samples from western and eastern Scandinavia, found in the present analyses, has been observed long also for other species and interpreted as the result of their migration from different directions (Nordal and Jonsell 1988; Hewitt 2000). However, in some cases the sample grouping was not always consistent with the region affiliation. Especially, the regions from the southern Europe were overlapping on the plane of the discriminant analysis (Fig. 3). The great differentiation of the Balkan samples can be explained by the dependence of the characters values on altitude: three of the Balkan samples, collected in lower elevation: StPla, SuPla, Din2 had bigger leaves, and so the samples clustered together, despite the location in different parts of the Balkans: eastern, central and western, respectively. The other three samples: Ril, Spla and Din2, were collected in the same Balkan parts, but at higher elevation, and their leaves were smaller (Table 1, Fig. 3, App.1). These situation is responsible for the high variation of the Balkan samples. At the same time, the characters of the Balkan samples showed correlation with altitude. It is possible that this relation is also true for the rest of the species range and was not evident in the present study only because the elevation of particular samples were similar. The diminishing of vegetative parts of plants with the growing altitude in the mountains were reported previously (Fabbro and Körner 2004).

Morphological analyses showed resemblances of all regions and the samples from the southern part of Europe, what might be explained by the dependence of *Dryas octopetala* characters on climate, proved in the present study. Still, the closeness of the Eastern Scandinavian sample to this group is surprising and hard to comment on.

The results of the present study were not fully consistent with the results of the biogeographic molecular analyses of the researched species (Fig. 1). Skrede et al. (2006) indicated a very strong resemblance of the Scandinavian population of *Dryas* to the western-Alpine population: these populations, together with those of the Pyrenees and Abruzzo, formed a group derived from a common source in the Central European tundra. The division into northern and southern part of the range, demonstrated in the present study, is therefore not justified. Ronikier and Cieślak (2012), in his preliminary analysis, described the relationship – being founded on molecular basis – between populations of the Southern and the Eastern Carpathians and the eastern part of the Balkans,

which can be confirmed by the present analyses, as well as between the Alps and the Northern and the Western Balkans, which could not be demonstrated (Fig. 1). The present diversification of morphological characters of the Balkan samples did not reflect the east-west gradient described by the author.

Some parallels can be drawn when comparing the present analyses with the geographic differentiation of two other arctic-alpine shrubs *Salix herbacea* and *S. reticulata*. For all species, the closeness of the Alpine and the Western Carpathian samples was found, although in the case of *S. herbacea*, also samples from other parts of the Carpathians belonged to this group (Marcysiak 2012 a). Multidimensional grouping of the latter species showed also closeness of the samples from the Pyrenees and to both part of Scandinavia, and in the present results some similarities between the Pyrenean and the Eastern Scandinavian samples can be found. In the case od *S. reticulata*, the sample from Western Scandinavia joined the Western Carpathian-Alpine group, and means of samples from the Eastern Carpathians and Eastern Scandinavia were located on the opposite side of the graph, although not close to each other (Marcysiak 2012b). The distribution of the *Dryas* samples on the scatterplot (Fig. 3) is quite comparable, and common preferences to the limestone substrate are the proposed explanation for these similarities.

The dependence of *Dryas octopetala* leaf characters on the climate

Analyzing the correlation of *Dryas octopetala* characters with the climatic factors one should remember that these data are generalized for larger areas. Nonetheless, positive relations of some features of the leaf size with spring months minimum temperature can be regarded as typical: Welker et al. (1997) described the increase in leaf biomass in response to warmer conditions; he also pointed to the importance of higher temperatures in spring and autumn on *Dryas* phenology. It is more difficult to interpret the negative correlation between the rainfall in the summer months and the number of teeth (*tn*), as well as the characters describing the narrowing at the base of the leaf (*blw/bw*) and at the top (*blw/aw*). The large width of the peak tooth (*aw*) clearly distinguishes the Western Carpathian samples. A negative correlation between the number of teeth and the rainfall in June and July was already reported for another arctic-alpine subshrub, *Salix herbacea* (Marcysiak 2012 b). Toothed leaves are usually associated with the cold climate, which has been confirmed in many studies (Greenwood 2005; Royer and Wilf 2006; Royer et al., 2008). In the case of this study, this relationship may be confirmed by the positive correlation between the

temperature and the leaf area-perimeter ratio (*ar/per*), indirectly informing about the indentation depth. However, the greater number of teeth occurred in populations from the southern part of the range, so the correlation 'the number of teeth – precipitation' presumably informed about the relationship between the number of teeth and a the geographic location. The ratio of maximum width to the width of the peak tooth (*blw/aw*), which had the highest number of correlations with the climate, at the same time clearly differentiated parts of the range (Fig. 3).

Conclusion

Geographic differentiation of *Dryas octopetala* leaf morphology reflect the biogeographic divisions of arctic-alpine species. It seems that it is the influence of the environmental and climatic factors, similar or identical for the populations located in one region, decided about the grouping of samples, because the characters differentiating the regions and strongly discriminating populations were mostly dependent on the influence of the climate (Table 2).

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Appendix 1. Box-whiskers plot of *Dryas octopetala* measured characters, in samples; point - mean, box - mean ± standard error, whiskers - mean ± 1.96 standard error; samples acronyms as in Table 1
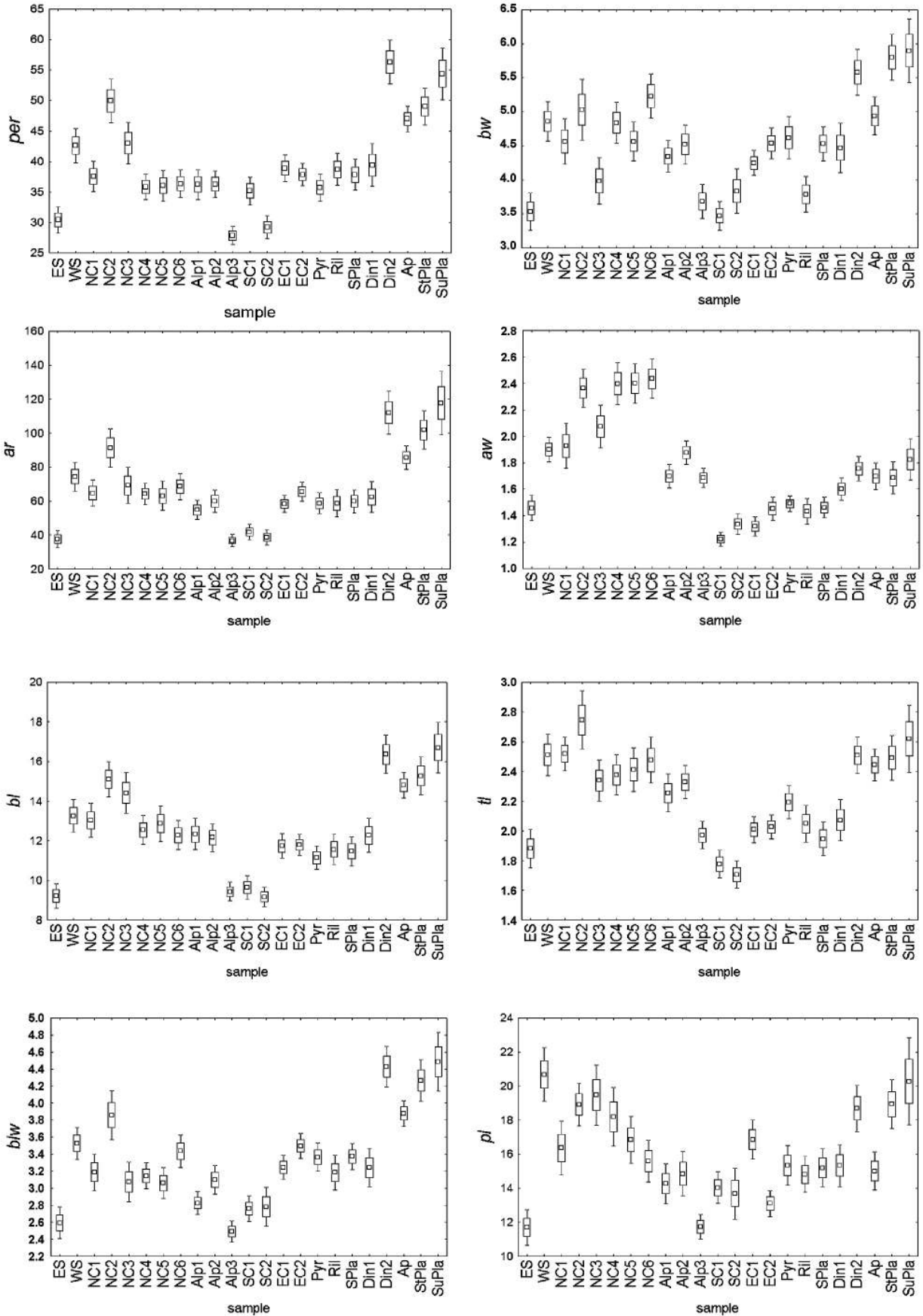
Appendix 2. Box-whiskers plot of Dryas octopetala calculated characters and teeth number (tn), in samples; point - mean, box - mean ± standard error, whiskers - mean ± 1.96 standard error; samples acronyms as in Table 1