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Phenotypic differentiation of *Rhododendron ferrugineum* populations in European mountains

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Abstract: *Rhododendron ferrugineum* occurs in the mountains of the Central Europe with large disjunction in geographic range between Pyrenees and Alps and between Alps and Sudetes. We expect that these discontinuities in the species occurrence should involved their phenotypic differentiation, similar as described on the studies of molecular markers.

The aim of the study was verification of phenotypic differences between the species populations from the Pyrenees, Alps and Sudetes.

We examined characters of leaves and capsules from 13 populations, each represented by 25–30 individuals, using ANOVA, discrimination analysis, principal components analysis and agglomeration on the closest Euclidean distances.

Every from examined characters of leaves and capsules except of apex angle of a capsule valve and capsule valve shape differentiated between populations at P≤0.001. Despite, range of character variation differentiating even at the highest level overlapped between populations. The analysis of discrimination, principal component analysis and agglomeration gave similar results indicating differences between populations from Pyrenees, Alps and Sudetes. However, the differences between individuals did not allow distinguish regions.

The morphological characteristics of the leaves and capsules allowed to distinguish among populations of *R. ferrugineum* from the Pyrenees, Alps and Sudetes, and the pattern of their phenotypic differentiation was similar to that described based on genetic markers. The relict population from the Sudetes is phenotypically more similar to the populations from the Pyrenees and the Western Alps than to the spatially closest populations from the Eastern Alps.

Keywords: Alps, biogeography, discrimination analysis, Ericaceae, plant variation, Pyrenees, Sudetes

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Introduction

Phenotypic characteristics remain important in phylogenetic studies, although molecular markers dominated in the last decades (e.g., Jenner, 2004). In plants, leaf characteristics are the most easily attainable and consequently, frequently used features in studies on taxonomy and hybridisation and for the recognition of geographic patterns of variation (e.g., Scotland et al., 2003; Boratyńska et al., 2014, 2015a, b, 2021a, b; Jasińska et al., 2017a, b; 2021), although leaves are among the most variable plant organs. Their dimensions and shape characteristics in the same tree/shrub depend on the position on the long- versus short-shoot and the central versus basal and/or apical position on the shoot, as well as exposure to sun radiation (Jentys-Szaferowa, 1970; Wójcicki, 1997; Jasińska et al., 2015, 2021). In the case of *Rhododendron*, the local environment can influence the variation among individuals (Koksheeva et al., 2017; Sosnovsky et al., 2017, 2021). The influences of different position in the crown and level of radiation can be omitted or at least strongly reduced when leaves are sampled from the central part of shoots exposed to sunlight, from individuals evenly dispersed within the population.

Rhododendron ferrugineum occurs in the Pyrenees, Jura and the Alps, in the northern Apennines, the Dynaric Alps and the Sudetes (Fig. 1). It is frequently a main component of the subalpine vegetation belt (Ozenda, 1988; Villar et al., 2001; Căprar et al., 2014; Gómez et al., 2020), where it forms dense thickets thanks to its clonal expansion, which allows the spreading out of an individual by several metres (Escaravage et al., 1998; Pornon & Escaravage, 1999; Pornon et al., 2000). In the Pyrenees, the species forms plant communities at elevations between 1,360 and 2,470 m, sometimes up to 2,780 – 2,800 m (Gómez, 1993), but altitudinal maximum of *R. ferrugineum* was found there at 3,200 m (Gómez et al., 2020). In the Alps, it occurs between 1,500 and 2,600 m (Ozenda, 1988; Ozenda & Borrel, 2003), but sometimes down to 800–1,000 m and up to 2,800–3,000 m (Polatschek, 1999). In the Sudetes, it is known from only one relict locality at 1,175–1,190 m (Malicki et al., 2019).

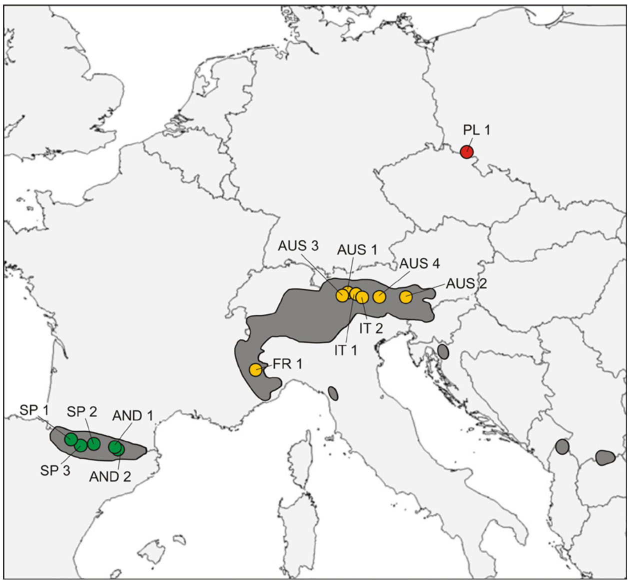

Fig. 1. Geographical positions of examined populations of *Rhododendron ferrugineum* against the species range (after Meusel et al., 1978, supplemented with data of Căprar et al., 2014; Suchan et al., 2019). Colours of dots indicate the population position in the Pyrenees (green) Alps (yellow) and Sudetes (red)

The Pyrenean and Alpine-Apennine-Jurassic areas of the species occurrence centres are distant of about 400 km. This geographic range disjunction was indicated as a reason for the species´ genetic main split into Pyrenean and Alpine populations, however, the spatial analysis of amplified fragment length polymorphism (AFLP) did not reveal correlation between genetic and geographic distances (Wolf et al., 2004). The analysis of nuclear microsatellite markers detected two separate genetic lineages in the Alps and Pyrenees (Charrier et al., 2014). The most recent AFLP analysis of Pyrenean, Alpine and Sudetan populations of *R. ferrugineum* detected a close genetic relationships between Pyrenean and West-Alpine populations, allowed to distinguish the geographic structure of the species in the Alps and precised the position of population from the Sudetes among all studied. Interestingly, the latter population appeared more close to the geographically distant Pyrenean populations than to the nearest Alpine ones (Suchan et al., 2019).

We hypothesise that the differentiation of *R. ferrugineum*, described using molecular markers (Charrier et al., 2014; Wolf et al., 2004; Bruni et al., 2012; Suchan et al., 2019), should also be visible in the morphological characteristics, and differences among their populations from the Pyrenees, Alps and Sudetes should be detected. In this context, the main aim of the study was the verification of the mentioned above assumption using biometrical methods applied to leaves and fruits. Considering the fact that phenotype is environmentally influenced: we also verified possible influence of the climate conditions to the variation of particular characteristics.

Materials and methods

Plant material

Rhododendron ferrugineum L. is one of the three high-mountain representatives of the genus in Central European mountains. It is a densely ramified, upright, evergreen, glabrous, monoecious shrub, about

30–80 (120) cm in height. The main roots are thick, lignified, and at most 20% of them are at a depth to 25–50 cm (Kutschera et al., 1997). The leaves are elliptic to oblong-elliptic, ±entire, revolute, shining dark green above and with peltate scales (lepidote) beneath, yellow when young and then ferruginous. Inflorescence upright, corymbose, with 6–14 flowers set on 7–13-mm-long pedicels. Flowers actinomorphic, sympetalous; calyx with sepals connate at base; corolla 10–15 mm, bell-shaped, with 5 petals pale-pink to purple, lobes ± as long as tube; fruit is a five-valve dehiscent, lepidote capsule 4–8 (15)-mm long (Popova, 1972; Gómez, 1993). This species is frequently mass flowering (Delmas et al., 2014), entomophilous, pollinated mainly by honeybees and bumblebees, but also self-compatible (Escaravage et al., 1997; Escaravage & Wagner, 2004). The dispersal of diaspores is baleochorous and anemochorous. Maximum age, counted from the annual rings root collar, has been determined as 132 years (Landolt et al., 2010).

We sampled 13 natural populations of *R. ferrugineum*, 5 from the Pyrenees, 7 from the Alps and 1 from the Sudetes (Table 1). The sampled populations represented three main clusters distinguished using different molecular markers, namely the Pyrenean, Alpine and Sudetan ones (Bruni et al., 2016; Charrier et al., 2014; Suchan et al., 2019).

Each population was represented by 26–30 individuals, separated by a distance of about 20 m; the small population form the Sudetes was represented by 20 individuals more grouped and the west-Alpine one by 11 individuals. From each individual, we took ten 2-year-old leaves, from the central parts of upright, well insolated shoots. One fully developed fruit was sampled from the same individuals in nine populations, four in the Pyrenees, four in the Alps and one in the Sudetes. Each population was represented by 30 fruits, except for the Sudetes, where only 12 fruits were gathered. In total, 3,465 leaves from 354 individuals and 252 fruits were analysed (Table 1). The samples were dried, and the leaves were pressed in a herbarium press. The material was conserved in dry state prior to measurements.

Measurement procedures

Measurements were performed on the images of the scanned material, using digiShape software (Moraczewski, 2005). Leaf characteristics are frequently used to distinguish *Rhododendron* taxa in Europe (Popova, 1972; Polatschek, 1999). In our study, we used 14 leaf characteristics (Table 2, Supplementary Material Fig. S1A), based on leaf variation of *Rhododendron* species from the subsection *Rhododendron* (Sosnovsky et al., 2017, 2021) and other species as *Salix herbacea* L., *Betula nana* L. and *S. reticulata* L. (Marcysiak, 2012a, b), *Alnus incana* (L.) Moench (Krauze-Michalska & Boratyńska, 2013) and *A. viridis* (Chaix) DC. (Wójcicki, 1997).

The fruit (capsule) characteristics are useful in distinguishing among *Rhododendron* species (Davidian, 1982; Cox & Cox, 1997; Cullen, 2005). We analysed twelve characteristics, mostly concerning capsule valves (Table 2, Supplementary Material Fig. S1B).

Statistical analysis

The data distribution inside the populations was verified using Shapiro-Wilk's test, and homoscedasticity of the data variances was tested using the Brown-Forsythe test prior to multivariate comparisons. The Pearson's correlation coefficient was determined between pairs of characteristics to detect possible redundant variables (Zar, 1999; Sokal & Rohlf, 2003). The data were standardised using the STATISTICA 12 software (StatSoft PL) to avoid a possible

Table 1. *Rhododendron ferrugineum* populations biometrically analysed; cluster indicates affiliation to genetic differentiation (after Bruni et al., 2012; Charrier et al., 2014; Suchan et al., 2019): P – Pyrenees, A – Alps, SU – Sudetes (see Fig. 1)

Acronym	Locality	Cluster	Latitude [°]	Longitude [°]	Altitude [m]	No of individuals	
						Leaves	Capsules
AND 1	Pyrenees, Andorra, Puig Alt de la Capa	P	42.583	1.450	1700	30	30
AND 2	Pyrenees, Andorra, Estany d'Engolasters	P	42.517	1.550	1650	30	30
SP 1	Pyrenees, Spain, Puerto de Formigal	P	42.783	−0.350	1850	29	30
SP 2	Pyrenees, Spain, above Lago de Bardamina	P	42.667	0.550	2300	30	–
SP 3	Pyrenees, Spain, Ordesa	P	42.633	−0.017	1900	30	30
IT 1	Alps, Italy, Penser Joch	A	46.850	11.383	1900	26	30
IT 2	Alps, Italy, Königsangerspitze	A	46.706	11.577	2260	28	–
AUS 1	Alps, Austria, Obergurgl – Hochgurgl	A	46.880	11.041	2040	30	30
AUS 2	Alps, Austria, Spittal – Goldeck	A	46.757	13.462	2070	30	30
AUS 3	Alps, Austria, Vent – Kreuz Kögl	A	46.842	10.914	2100	30	30
AUS 4	Alps, Austria, Thurnteler Kögl	A	46.773	12.389	2340	30	–
FR 1	Alps, France, Col de Vars	A	44.546	6.689	2250	11	–
PL 1	Sudetes, Poland, Karkonosze	SU	50.740	15.78	1170	20	12

Table 2. Means (M) and standard deviations (SD) of characters of *Rhododendron ferrugineum* leaves and capsules for all data, the geographical regions and ANOVA results

Character	Code	M±SD	Region			ANOVA	
			Pyrenees	Alps	Sudetes	F	P
Perimeter of a leaf blade [mm]	LP	53.42±8.91	56.28±8.00	50.50±8.64	58.47±8.99	23.13	0.000
Length of a leaf blade [mm]	LL	22.84±4.05	24.28±3.64	21.45±3.91	24.62±4.00	24.38	0.000
Length of a petiole [mm]	PeL	4.04±0.75	4.20±0.68	3.89±0.79	4.26±0.72	10.41	0.000
Width of a leaf blade at ½ of its length [mm]	W	8.25±1.28	8.27±1.14	8.05±1.26	9.84±1.41	15.93	0.000
Width of a leaf blade at ¼ of its length [mm]	W¼	6.43±0.89	6.39±0.81	6.37±0.88	7.29±1.12	11.28	0.000
Width of a leaf blade ¾ of its length [mm]	W¾	6.54±1.07	6.77±0.96	6.18±0.94	8.08±1.14	17.13	0.000
Apex angle of a leaf blade [°]	AA	92.01±10.62	86.13±8.63	97.54±9.20	85.66±9.13	29.76	0.000
Base angle of a leaf blade [°]	BA	95.42±11.86	93.68±10.22	96.40±12.69	99.42±13.96	16.07	0.000
Ratio of perimeter/length of a leaf blade	LP/LL	2.35±0.05	2.32±0.03	2.36±0.04	2.38±0.05	22.35	0.000
Ratio of leaf blade length/petiole length	LL/PeL	5.87±1.01	6.04±1.02	5.73±1.00	5.93±0.85	11.73	0.000
Ratio of length/width at ½	LL/W	2.79±0.34	2.96±0.31	2.68±0.31	2.52±0.24	25.93	0.000
Ratio of width at ¼/width at ½	W¼/W	0.78±0.04	0.78±0.03	0.79±0.04	0.74±0.03	19.09	0.000
Ratio of width at ¼/width at ¾	W¼/W¾	0.99±0.09	0.95±0.07	1.04±0.08	0.91±0.07	17.44	0.000
Ratio of leaf apex angle/leaf base angle	AA/BA	0.98±0.11	0.93±0.09	1.03±0.10	0.88±0.12	14.02	0.000
Perimeter of a capsule valve [mm]	CP	14.22±1.83	14.84±1.73	13.85±1.58	11.87±2.40	16.46	0.000
Length of a capsule valve [mm]	CL	6.03±0.83	6.33±0.79	5.84±0.70	5.03±1.09	15.89	0.000
Length of a stalk [mm]	SL	13.36±2.66	13.11±2.54	13.32±2.56	16.32±3.25	13.73	0.000
Width of a capsule valve at ½ of its length [mm]	CW	1.67±0.20	1.66±0.18	1.69±0.20	1.54±0.28	4.18	0.000
Width of a capsule valve at ¼ of its length [mm]	CW¼	1.39±0.16	1.37±0.15	1.43±0.16	1.23±0.20	4.93	0.000
Width of a capsule valve at ¾ of its length [mm]	CW¾	1.61±0.22	1.62±0.20	1.62±0.21	1.44±0.32	8.03	0.000
Apex angle of a capsule valve [°]	CA	98.19±11.84	96.33±11.14	99.61±12.09	102.08±13.88	1.64	0.116
Ratio of perimeter/length of a capsule valve	CP/CL	2.36±0.05	2.35±0.05	2.37±0.05	2.37±0.06	6.23	0.000
Ratio of capsule valve length/ stalk length	CL/SL	0.47±0.10	0.50±0.11	0.45±0.08	0.31±0.06	19.71	0.000
Ratio of length/width at ½ capsule valve	CL/CW	3.64±0.47	3.84±0.45	3.49±0.42	3.26±0.36	11.11	0.000
Ratio of width at ¼/width at ½	CW¼/CW	0.84±0.07	0.83±0.06	0.85±0.07	0.80±0.10	4.69	0.000
Ratio of width at ¼/width at ¾	CW¼/CW¾	0.87±0.10	0.86±0.09	0.89±0.10	0.87±0.12	2.25	0.025

influence of their different types on the results of the multivariate analyses.

Minimum and maximum values of particular characteristics were found, and the arithmetical means (M), standard deviations (SD) and variation coefficients (V) were calculated and analysed for each population and cluster. The post hoc T test with Bonferroni corrections was used for multiple comparisons between pairs of populations. Analysis of discrimination (DA), principal components analysis (PCA) and agglomeration on the closest Euclidean distances according to the Ward's method were used to detect groupings of populations (Sokal & Rohlf 2003). In DA, we used proportions and characteristics that were not used in the proportion calculation, namely for leaf characteristics: apex angle of leaf blade (AA), base angle of blade (BA), ratio of perimeter to length of a leaf blade (LP/LL), ratio of leaf blade length to petiole length (LL/PeL), ratio of leaf blade length to leaf width measured at ½ of its length (LL/W), ratio of blade width at ¼ to its width at ½ (W¼/W) and ratio of blade width at ¼ to its width at ¾ (W¼/W¾), and for capsule characteristics: length of capsule stalk (SL), ratio of perimeter to length of a capsule valve (CP/CL), ratio of length to width of capsule valve measured at ½ of its length (CL/CW), ratio of width at ¼ of capsule valve to its width at ½ (CW¼/CW) and ratio of width at ¼ of capsule valve to its width at ¾ (CW¼/CW¾). Analyses of leaves and capsules were conducted separately. For mathematical calculations, we used STATISTICA 12.0 for Windows.

The relation of particular leaf and capsule characteristics to the 19 bioclimatic factors and Ellenberg's Quotient (EQ = 1000 * mean temperature of July/ average yearly precipitation) of sampled localities were verified with implementation of regression analysis, using of JMP 13 software (SAS Institute Inc.). The climatic data (Supplementary Material, Table S5) were retrieved from the WorldClim database (Hijmans et al., 2005).

Results

Variation and correlation of characteristics

The frequency distributions of values of the leaf and capsule characteristics were unimodal and normal or at least close to normal, and variances were homoscedastic in the majority of populations examined. These circumstances allowed the use of multi-characteristic analyses.

Within leaf characteristics, the most variable one, irrespective of the population, was petiole length (PeL), with values of V around 25%, whereas the least variable characteristic was leaf apex angle (AA), with V values from 9 to 17%, depending on the population (Supplementary Material, Table S1). The dimensional characteristics of leaves correlated positively to each other at a statistically significant level ($P<0.01$). Apex and base leaf angles were negatively correlated with all other measured leaf characteristics (Supplementary Material, Table S2). From the pairs of the characteristics correlating at $r \geq |0.95|$, the perimeter of leaf blade (LP) and width of a leaf blade measured at ½ of its length (W) were not included in the multivariable analyses.

The capsule characteristics were generally less variable than those of leaves; however, stalk length (SL), the most variable one, had V values between 12 and 24% in some populations (Supplementary Material, Table S3). The variations of the other characteristics did not exceed 14–15%.

The measured capsule characteristics correlated positively to each other at a significant level ($P<0.01$), except for capsule valve angle (CA), which was negatively correlated with most of the other characteristics. We excluded the perimeter of capsule valve (CP) correlating to the length of capsule valve (CL) at $r = 0.99$ from further multivariable analyses (Supplementary Material, Table S4).

The apex angle of the leaf blade (AA) revealed reverse significant ($P \leq 0.01$) dependence on the average yearly temperature, while perimeter and length of leaf blade (LP and LL, respectively) had direct significant relation ($P \leq 0.05$) level. (Supplementary Material, Fig. S4 A–C). Similar relations demonstrated LL and LP to the Ellenberg's Quotient (Supplementary Material, Fig. S4 D–E).

Multivariate population differentiation

All leaf and capsule characteristics differed significantly ($P<0.01$) among populations in the one-way variation analysis (Table 2). Multiple comparison post hoc T test with Bonferroni corrections detected these differences at lower level. The populations which revealed the highest number of significant differences in the leaf characteristics were the Alpine AUS4 and Sudetan PL1 (Supplementary Material, Table S6). The latter population was also different from the other in the capsule characteristics (Supplementary Material, Table S7).

Every leaf characteristic used in DA discriminated significantly among populations ($P<0.001$). The highest discrimination power among populations had apex angle of a leaf blade (AA), ratio of leaf width at ¼ and width at ¾ (W¼/W¾) and ratio of

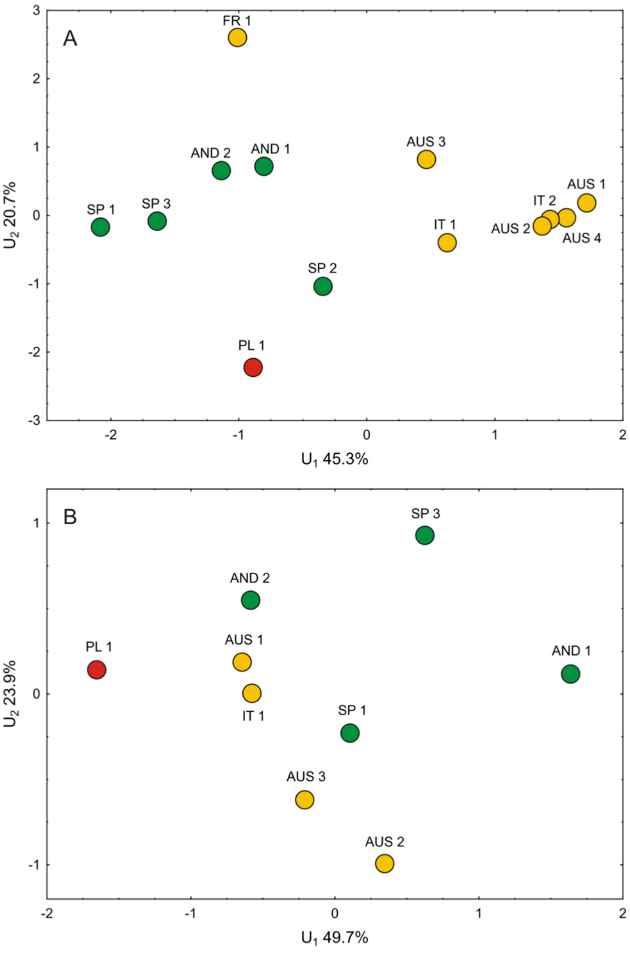

Fig. 2. Results of discrimination analysis among populations of *Rhododendron ferrugineum* based on leaf characters – A, and capsule characters – B; population acronyms as in Table 1; colours of dots indicate the populations from Pyrenees (green), Alps (yellow) and Sudetes (red) as on Fig. 1

leaf width at ¼ and width at ½ (W¼/W), with partial Wilk's λ values of 0.66, 0.69 and 0.74, respectively. The dispersion of populations in the space between the two first discrimination variables, responsible for 66% of the total variation, indicated the separation between populations from the Alps, Pyrenees and Sudetes (Fig. 2A). Interestingly, the population from the latter mountains appeared most separated by discrimination variable U$_2$, determined mostly by LL/W, W¼/W and W¼/W¾, whereas U$_1$, determined by AA, LL/W and LP/LL, did not distinguish it. Additionally, the population from the Sudetes was allied to population FR1 from Maritime Alps and Pyrenean populations (Fig. 2A). Despite the multivariate differences between populations, the individuals from the Pyrenees and Alps were partly intermingling among the 95% confidential intervals for each group, whereas those from the Sudetes were marginal considering the discrimination variable U$_2$ and intermingled mostly with the Pyrenean group (Supplementary Material, Fig. S2A).

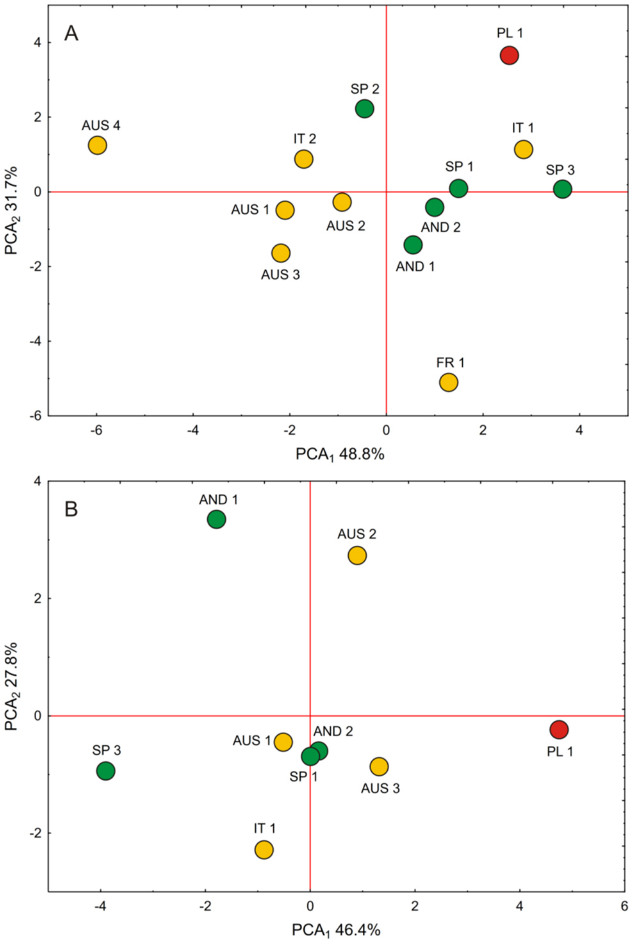

Fig. 3. Results of PCA among populations of *Rhododendron ferrugineum* based on leaf characters - A; and capsule characters – B; population acronyms as in Table 1; colours of dots indicate the populations from Pyrenees (green), Alps (yellow) and Sudetes (red) as on Fig. 1

Capsule characteristics also significantly discriminated among populations (P<0.001). The strongest discrimination power was found for stalk length (SL) and ratio of capsule valve length and width at ½ (CL/CW), with partial Wilk's λ values of 0.68 and 0.74, respectively. The populations' dispersion in the space between the two first DA variables U$_1$ and U$_2$, responsible for more than 73% of the total variation, indicated the separation of the population from the Sudetes and differences between the Alpine and Pyrenean populations (Fig. 2B). However, the individuals intermingled the confidence intervals among the Pyrenees, Alps and Sudetes (Supplementary Material, Fig. S2B).

The PCA on the leaf characteristics confirmed the separation between populations from the Pyrenees, Alps and Sudetes (Fig. 3A). The dispersion of populations between the two first principal components, which covered more than 80% of the total variation, was mainly determined by leaf blade perimeter (LP), length (LL), and apex angle of a leaf blade (AA). All Pyrenean populations were separated from the Alpine ones, except for the populations from the Maritime Alps (FRA1) and southern Tyrol (IT1). The position of the latter two populations was mostly determined by the first factor of the PCA. The population from the Sudetes remained also separate, surprisingly revealing a close connection to the Maritime Alps. Despite the differences among populations, the individuals intermingled the 95% confidence intervals for each region (Supplementary Material, Fig. S3A). The individuals from the Sudetes were marginal, and overlapped the largest area from the three compared regions, indicating a high level of phenotypic diversity.

The PCA on the capsule characteristics confirmed the results found for the leaves. The dispersion of populations in the space between the two first components, covering more than 74% of the total variation, was mostly determined by CP, CL and CL/CW. The dispersion of the population indicated the differences among populations from the Sudetes, Pyrenees and Alps. The populations on the space between the first and second PCA components were dispersed and intermingled among regions, but with a clear distance of the Sudetan one (Fig. 3B). The individuals from the three regions intermingled on the dispersion diagram, with a marginal position of those from the Sudetes, which were the most variable (Supplementary Material, Fig. S3B).

Agglomeration on the leaf characteristics well separated the Pyrenean from the Alpine populations, with the population from the Sudetes being closest to the Pyrenean SP2 (Fig. 4A). Separation based on capsule characteristics was at the lower level, with the population from the Sudetes being related to the Alpine population AUS1 (Fig. 4B).

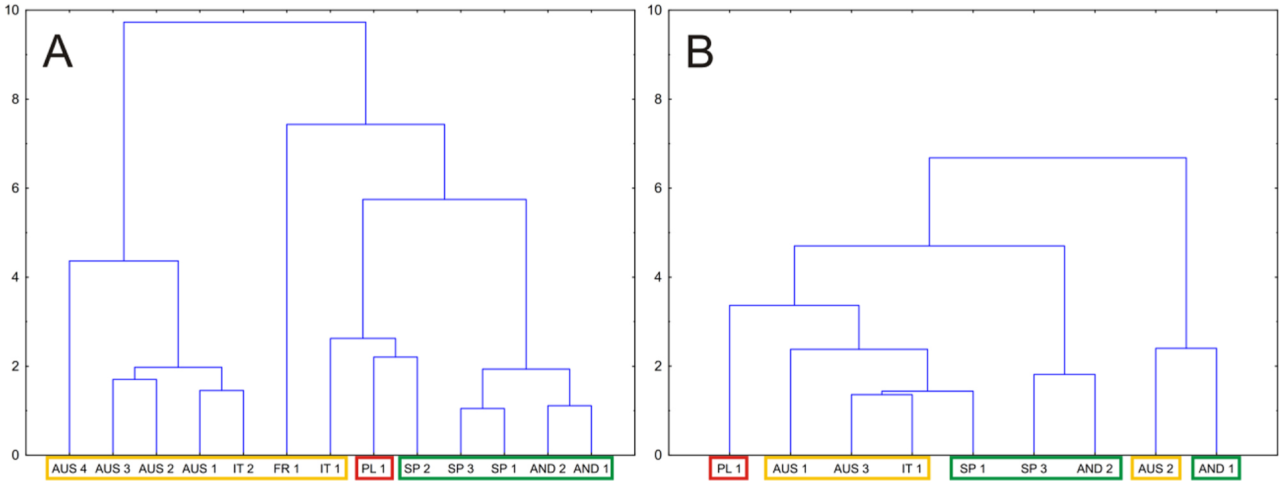

Fig. 4. Results of agglomeration (Ward's method) of populations of *Rhododendron ferrugineum* based on leaf characters - A; and capsule characters – B; population acronyms as in Table 1; colours of frames indicate the populations from Pyrenees (green), Alps (yellow) and Sudetes (red) as on Fig. 1

Discussion

Influence of characteristics on population discrimination

Consistent leaf sampling in this study reduced the level of leaf variation, even for the most variable characteristics such as petiole length, perimeter and length of the leaf blade. Interestingly, each leaf and capsule characteristic of *R. ferrugineum* differed significantly among populations. It can be interpreted that the leaf characteristic were, to a great degree, specific for populations; however, the differences among the particular characteristics were relatively small, and their ranges of variation overlapped among populations. Comparing to the Alpine, the populations from Pyrenees and Sudetes had generally larger leaves, with narrower apex angle (Table 2).

The climate factors did not differ substantially between places of population origin (Supplementary Material, Table S5), except of yearly mean temperatures (BIO1). Consequently, we found only three measured characteristics dependent on this factor, indicating positive relation of the leaf blade dimensions and negative relation of the angle of leaf apex to the higher temperatures. Nevertheless, the positive reaction of leaf blade length and perimeter to the yearly temperature revealed also geographic segregation among populations from Pyrenees, Alps and Sudetes (Supplementary Material, Fig. S4).

Our study presents, for the first time, the biometrical characteristics of populations of *R. ferrugineum* from quite entire geographic range of the species based on a large set of leaf and capsule characteristics. So far, only a few basic characteristics, namely leaf length (LL), leaf width (W) and length of leaf petiole (PeL), as well as capsule length (CL) and stalk length (SL), have been considered in species descriptions, basing on the herbarium materials. Our data based on a large number of leaves collected from the central parts of the yearly increments of the 2-year-old shoots exposed to sunlight, which reduced their variation. The capsules were sampled only from the plants exposed to direct radiation, which presumably reduced variation. The large numbers of both leaves and capsules examined resulted in reliable results. The data obtained in our study were generally comparable to those reported in the literature, despite the standardised sampling procedures (Table 3).

Several characteristics used in our study have not been reported before. For example, data on leaf (LP, W¼, W¾, AA, BA) and capsule (CP, CW, CW¼, CW¾, CA) characteristics calculated as their proportions (Table 2) have not been published for *R.*

Table 3. Average values and range of variation of characters of leaf (LL, PeL and W) and capsule (CL and SL) of *Rhododendron ferrugineum* received in the study (bolded), compared to literature data; character codes of leaf and capsule as in Table 2

LL [mm]	PeL [mm]	W [mm]	CL [mm]	SL [mm]	Source of data
22.9 (9.9–42.1)	**4.0 (0.8–8.8)**	**8.3 (4.3–15.2)**	**6.1 (3.6–8.4)**	**13.4 (6.3–21.6)**	our results
15–35 (55)	5	5–10			Braun-Blanquet 1926
20–40				8	Popova 1972
16–43	2–8	6–16	4–7	6–17	Davidian 1982
(15) 20–40 (45)	3–7		4–8 (11)	7–13	Gómez 1993

ferrugineum. All these data could be useful in further investigations and in possible comparisons of fossil material of the genus *Rhododendron*.

Environmental influence to variability

The climate conditions of the sampled localities of *R. ferrugineum* including the Sudetes, appeared relatively similar. Even the climatic conditions of the latter population, despite its location below the forest line, did not differ significantly from the conditions of the Alpine and Pyrenean populations (Supplementary Material, Table S5). In this context, the general lack of influence of the climatic factors to the leaf characteristics was not surprising. We detected only slightly significant direct correlation ($P \leq 0.05$) of leaf length and perimeter to mean yearly temperature and Ellenberg's Quotient, and reverse correlation of the apical leaf blade angle to mean yearly temperature. This finding in some degree supported the pattern of relation of the leaf characteristics to the climate and orographic conditions described for *R. myrtifolium* in the East Carpathians in Ukraine. The leaves of that closely related to *R. ferrugineum* species were smaller and more xeromorphic in higher elevations and in the harsher climatic conditions (Sosnovsky et al., 2021).

Geographic differentiation

Our morphometric analyses provided some interesting results concerning the geographic distinctiveness at intra-specific level, namely among the three groups of populations representing the Pyrenees, Alps and Sudetes.

The differences between Pyrenean and Alpine populations, together with non-uniform genetic structure of *R. ferrugineum* in these mountain chains were described on the basis of nuclear microsatellite markers (Charrier et al., 2014: Fig. 2). In our study, the most the Pyrenean populations of the species differed from the Alpine ones except of FR1 and IT1 from the West Alps, which appeared intermediate. For species growing in both the Alps and the Pyrenees, genetic differences were often shown between populations from these massifs. The distinctness between Pyrenean and Alpine populations of *Pinus uncinata* Ramond ex DC. was detected using chloroplast microsatellite markers (Dzialuk et al., 2009, 2016). This pattern of genetic split was found in several other subalpine/alpine species, as for example in *Cicerbita alpina* (L.) Wallr. (Michl et al., 2010), *Ranunculus glacialis* L. (Ronikier et al., 2012), *Ranunculus platanifolius* L. (Stachurska-Swakoń et al., 2013), *Soldanella alpina* L. (Kropf et al., 2012). Interestingly, the phenotypic differences between Alpine and Pyrenean populations were also detected for *Pinus uncinata* (Boratyńska et al., 2015a).

Populations of *R. ferrugineum* in the Pyrenees and in the Alps revealed high level of genetic differentiation (Charrier et al., 2014; Suchan et al., 2019). In our study, populations from the Pyrenees and the Alps formed a dispersed group in DA and PCA for both leaf and capsule characteristics, supporting the high level of genetic differentiation.

The phylogeographic structure of *R. ferrugineum* in the Alps underlines separate position of populations from the Maritime, the North-Western and the Eastern Alps (Suchan et al., 2019: Fig. 1). The genetic differences between populations of *R. ferrugineum* from Eastern and Western Alps well fit to the more general pattern of genetic differentiation described for *Abies alba* Mill., *Larix decidua* Mill. *Pinus cembra* L. and *Pinus mugo* Turra using single nucleotide polymorphisms (SNPs) (Mosca et al., 2012). Some other subalpine and alpine species revealed similar differentiation between the Eastern- and Western-Alpine populations, resulting from a distinct glacial history of populations in these areas (Thiel-Egenter et al., 2011; Stachurska-Swakoń et al., 2013). The phenotypic differentiation of *R. ferrugineum* in the Alps resembles the genetic pattern of their variation, however, to describe it more clearly some additional samples should be studied biometrically.

The northernmost and isolated population of *R. ferrugineum* in the Sudetes in our examination appeared closer in terms of the leaf and the capsule characteristics to the Pyrenean and West-Alpine populations than to the nearest East-Alpine ones. Interestingly, it was similar to the pattern of differentiation described for the AFLP (Suchan et al., 2019: Fig. 2). Such geographic differentiation supports the hypothesis of a possible older origin of the Sudetan population than from direct post-glacial colonization discussed by Suchan et al. (2019).

The similarities between northern pre-Alpine and Pyrenean populations of several other subalpine plants have been reported, as for *Ranunculus glacialis* L. (Ronikier et al., 2012), complex of *Pinus mugo* Turra – *P. uncinata* Ramond ex DC. – *P. uliginosa* G.E.Neumann (Boratyńska et al., 2014, 2015a; Dzialuk et al., 2016; Sobierajska et al., 2020; Boratyńska et al., 2021a) and *Polygaloides chamaebuxus* (L.) O.Schwarz (Windmaißer et al., 2016).

We highlight that the pattern of phenotypic differentiation detected in *R. ferrugineum* (our Figs 2–4) appeared similar to that found using microsatellite markers (Charrier et al., 2014: Fig 2) and AFLP (Suchan et al., 2019: Fig. 1). It was expected, as the results received using morphometric methods generally confirmed the results of genetic examinations, such as for coniferous *Pinus mugo* complex (e.g., Boratyńska et al., 2014, 2015a), for Mediterranean *Juniperus* species (Boratyński et al., 2013, 2014), *Juniperus excelsa* (Douaihy et al., 2012), *Cupressus* species

(Sękiewicz et al., 2016), *Abies* species (Boratyńska et al., 2015b; Litkowiec et al., 2021) and for broadleaved trees (Ballian et al., 2012; Poljak et al., 2015; Jasińska et al., 2015, 2021). However, it should be considered that the geographic differentiation of the species detected using molecular markers, which are not subject to environmental influences, are more reliable.

Presently observed genetic and phenotypic differentiation of *R. ferrugineum* results probably from the Pleistocene history of the species. In this context, similarity of its' population from the Sudetes to Pyrenean ones could be also explained by possible colonization route of the alpine plants during interglacials along the northern pre-Alpine regions (Birks, 2008). However, this topic needs the additional special study with implementation of the molecular markers.

Conclusions

The morphological characteristics of the leaves and capsules allowed to distinguish among populations of *R. ferrugineum* from the Pyrenees, Alps and Sudetes, and the pattern of their phenotypic differentiation was similar to that described based on genetic markers. The relict population from the Sudetes is phenotypically more similar to the populations from the Pyrenees and the Western Alps than to the spatially closest populations from the Eastern Alps.

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