Karyological, morphological and ecological differentiation of *Sesleria caerulea* and *S. tatrae* in the Western Carpathians and adjacent regions

Karyologická, morfologická a ekologická diferenciace druhů *Sesleria caerulea* a *S. tatrae* v Západních Karpatech a v přilehlých územích

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The tetraploid (2n = 4x = 28) *Sesleria caerulea* and octoploid (2n = 8x = 56) *S. tatrae* are closely related species with very similar morphology. These species can tolerate a wide range of ecological conditions, and in areas where the two co-occur, individual plants are often hardly distinguishable and assumed to be products of hybridization. Consequently, the ecological requirements of each species and the evolutionary relationship between the two species remain unknown. The aim of this study is to determine the karyological, morphological and ecological differentiation between the two species. A total of 877 *S. caerula* and *S. tatrae* plants from 68 populations in the Western Carpathians, Alps and Sudetes mountain ranges were analysed for DNA ploidy level and subjected to morphometric examination. Moreover, phytosociological relevés for each location and Ellenberg's indicator values were used as supplementary environmental variables for interpreting the results from an ecological point of view. All individuals of *S. caerulea* were tetraploid and all those of *S. tatrae* octoploid. There were no intermediate ploidy levels, which would indicate hybrids between the two species. Morphometric analyses of 28 morphological characters revealed that eight were significantly different in these two species. Of these, density of hairs between the veins on the lemma, and length of lemma, glume and palea were the most reliable diagnostic characters. Morphological dissimilarities, however, were found only at the population level and were not always useful in identification of individual plants. Important differences between the two species were also found in ecological requirements. Detected ecological differences were mostly associated with altitude, moisture, temperature, light intensity and nutrient levels, in terms of which *Sesleria caerulea* was most tolerant. Conversely, *S. tatrae* grew under a more narrow range of conditions, occurred at high altitudes and preferred high humidity, high light intensities and nutrient-rich soils. The name *Sesleria tatrae* is typified.

K e y w o r d s: ecology, flow cytometry, morphometrics, polyploidy, *Sesleria*, typification

Introduction

The Western Carpathian mountain range is well known for its rich, unique flora and high number of endemic plant taxa (Soó 1933, Balázs 1939, Kiss 1939, Pawłowski 1970, Hendrych 1981, Kliment 1999). The floral richness is due to several physiographic features, which include diverse terrain, climatic variability and edaphic complexity (Lukniš 1972, Mirek & Piękoś-Mirkowa 1992, Petit et al. 2003, Ronikier 2011). However, despite extensive research on the Western Carpathian flora, the number of endemic species unique to this region remains unknown. One of the reasons is that the basis for the majority of the species is only material from a small number of populations that has not been critically compared with that for closely related taxa from other parts of Europe.

Species of the genus *Sesleria* (*Seslerieae*, *Pooideae*, *Poaceae*) are distributed across a large portion of Europe (west-northwards to Iceland), also occurring marginally in North Africa and western Asia (eastwards to Caucasian region) (Deyl 1946, 1980, Ujhelyi 1959a, Strgar 1981). The genus comprises up to 40 species and subspecies, mostly confined to the Balkan Peninsula (~26 taxa) or the Carpathians and Alps (~13 taxa) (Deyl 1980, Strgar 1981, Kuzmanović et al. 2013). These species play a dominant role in a number of plant communities and most of them are considered to be endemic [e.g. *S. calabrica* (Deyl) Di Pietro; Di Pietro 2010] or endangered (e.g. *S. uliginosa* Opiz; Čeřovský et al. 1999). The genus is hypothesized to have originated in the Alps, but hotspots of morphological and taxonomic diversity are found mainly in the Balkan Peninsula (Deyl 1946, Strgar 1981). Most of the *Sesleria* species are characterized by minimal morphological differentiation and large phenotypic plasticity, which often leads to taxonomical misclassification of individual plants and even, in some cases, entire populations (e.g. Strgar 1980, Kuzmanović et al. 2013). Consequently, there is little information on the evolutionary history, morphological variation, distribution and ecological preferences of individual species of *Sesleria*.

Despite this lack of life-history information, the karyological differentiation and taxonomic status of the species of *Sesleria* are well studied. The two the most widespread chromosome numbers recorded in this genus are 2n = 28 and 2n = 56; with individuals with 2n = 28 referred to as tetraploids and 2n = 56 as octoploids (Ujhelyi & Felföldy 1948, Rychlewski 1955, 1959, Ujhelyi 1959b, 1960, Di Pietro et al. 2005). Although rare, diploid (2n = 14; Deyl 1980, Lazarević et al. 2012), hexaploid (2n = 42; Strgar 1981) and dodecaploid (2n = 84; Di Pietro 2007, Lazarević et al. 2012) chromosome numbers are recorded for these species, wherein hexaploid individuals (2n = 42) are most likely the result of hybridization between tetraploid and octoploid plants (Strgar 1981).

Currently, six species *Sesleria* are recognized in the Western Carpathians (Marhold & Hindák 1998, Kliment 1999): *S. caerulea* (L.) Ard. [syn. *S. albicans* Kit. ex Schult., *S. calcaria* Opiz, *S. varia* (Jacq.) Wettst.], *S. heufleriana* Schur, *S. hungarica* Ujhelyi [syn. *S. heufleriana* subsp. *hungarica* (Ujhelyi) Deyl], *S. sadleriana* Janka, *S. tatrae* (Degen) Deyl and *S. uliginosa* (syn. *S. caerulea* sensu Wettst. 1888, *S. caerulea* sensu Deyl 1980; see Foggi et al. 2001). *Sesleria caerulea* and *S. uliginosa* are tetraploids, whereas *S. hungarica*, *S. sadleriana* and *S. tatrae* are octoploids (Ujhelyi & Felföldy 1948, Rychlewski 1955, Ujhelyi 1959b, Lysák & Doležel 1998). For a long time *Sesleria heufleriana* was exclusively reported as a tetraploid (Rychlewski 1959, Ujhelyi 1959b), but recent studies have identified rare octoploid individuals within this species (Lysák &

Doležel 1998). *Sesleria caerulea* and *S. tatrae* are the most commonly occurring species of *Sesleria* in the Western Carpathians (Deyl 1946, Lysák 1996), but their circumscription, ecological requirements and phytosociology are still ambiguous. These species are often misidentified and the main reason for the confusion and misclassification is the lack of clear morphological differentiation between the two species. Moreover, researchers hypothesize that hybridization is common where *S. caerulea* and *S. tatrae* cooccur (Deyl 1946, Bělohlávková 1980, Lysák 1996). Potential hybrids of these two species are known as "*S.* ×*tatrorum* Domin". However, this name is not valid, as only the presumed parentage *S. bielzii* × *S. calcaria* (without a description) is stated (Domin 1935). Other authors, for example Ujhelyi (1959b) or Conert (1992), consider *S.* ×*tatrorum* as a synonym of *S. sadleriana*.

To address these gaps in knowledge, this research focused on identifying karyological, morphological and ecological differentiation among the morphologically similar species *Sesleria caerulea* and *S. tatrae* in the Western Carpathians and adjacent regions (the Sudetes and Alps). The following questions are addressed in this study: (i) Are the populations of the taxa studied karyologically uniform or do they posses some degree of variation? (ii) What is the degree of morphological and ecological differentiation of the central-European populations of *Sesleria caerualea* and *S. tatrae*? (iii) Which morphological characters can be used to identify these species of *Sesleria*?

Materials and methods

Study species

Sesleria caerulea and *S. tatrae* are cespitose perennial wind-pollinated grasses with characteristic persistent sheaths surrounding the bases of numerous tillers, which could provide an effective means of vegetative (clonal) propagation.

Sesleria caerulea (2n = 4x = 28) is widely distributed across north-western and central Europe, where it grows on basiphilous substrata in the colline to alpine vegetation belt. This species' range covers continental Europe from north-eastern Spain to the Western Carpathians, northwards to northern Germany, southwards to the northern Apennines and isolated sites in Sierra Nevada, Romania and Poland; an unconfirmed and unlikely record is also documented for Albania (Ujhelyi 1938, Deyl 1946, Dixon 1982). The range of *S. caerulea* also includes island populations in Iceland, Ireland, Scotland and England. It grows in a range of natural habitats, including alpine and lowland rocky ridges and rocky faces, scree slopes, heaths, grasslands, fens and open woodlands (beech or pine forests). This species also thrives in anthropogenically influenced habitats, such as pavements, grazed calcareous or mown fens and grassland (Reisch & Poschlod 2003). Three subspecies are recognized within this species (Deyl 1980, Dixon 1982): (i) subsp. *caerulea* occurring throughout most of the species' range; (ii) subsp. *angustifolia* (Hackel et G. Beck) Deyl, recorded from Bosnia and Hercegovina into Serbia (Strgar 1973) and (iii) subsp. *islandica* Löve in Iceland. However, the taxonomic status of *S. c.* subsp. *islandica* is questionable (Dixon 1982) and *S. c.* subsp. *angustifolia*, which has an octoploid chromosome number, was recently recognized as a distinct species, *S. angustifolia* (Hackel et G. Beck) Deyl (Strgar 1973, 1980). In this study, we recognized only nominate subspecies under the name *S. caerulea*.

Sesleria tatrae (2n = 8x = 56) has a restricted distribution on a few massifs in the central part of the Western Carpathians (Slovakia, Poland): Malá Fatra Mts, Veľká Fatra Mts, Chočské vrchy Mts and Tatry (Nízke, Západné, Vysoké, Belianske) Mts. Here, it grows mainly on basiphilous substrata in the montane to alpine vegetation belts (with a vertical distribution from 850 up to 2152 m a.s.l.; Rychlewski 1955, Lysák 1996, Kliment 1999). It grows in a range of natural habitats, including areas with a rocky karst substrate, shrubland, open deciduous or mixed forests and human affected habitats (e.g. grazed calcareous grassland). There is one isolated occurrence of this species documented between 835–845 m a.s.l. in the Sudetes (Kleśnica valley in Śnieżnik Kłodzki Mts; Fabiszewski 1970). During the last century, several different taxonomic arrangements were proposed for this species. Up to the 1930s, *S. tatrae* appeared under various names, such as *S. bielzii* Schur (Domin & Podpěra 1928, Deyl 1936) or S. *caerulea* (L.) Ard. (Neilreich 1866, Sagorski & Schneider 1891). Jávorka (1924) was the first to identify this species as *S. bielzii* f. *tatrae* Degen (for more details see Results). Deyl pointed out the morphological similarity to another octoploid species, *S. sadleriana*, and described *S. tatrae* first as a species [*S. tatrae* Deyl (1938)] and later as a subspecies, *S. sadleriana* subsp. *tatrae* (Degen) Deyl, comb. inval. (Deyl 1978).

Plant material

Material was collected from 68 populations of *Sesleria caerulea* and *S. tatrae* (n_{plants} = 877; Table 1, Fig. 1) during flowering and used in karyological, morphometric and ecological analyses. Samples of *Sesleria caerulea* were collected at 31 locations distributed throughout the Western Carpathians and additional 10 locations in the Alps, including its type locality (population no. 87): montainous region near the town of Montbéliard in eastern France ["locis montosis circa Montembelgardum"] (see Foggi et al. 2001). Material of *S. tatrae* from 26 Western-Carpathian populations and a Sudetes population, which covers the entire distribution of this species, including the type locality (population no. 15): Kopské sedlo saddle in Belianske Tatry Mts (Slovakia) (see typification below), was collected.

For karyological and morphometric analyses, at each locality nine to 18 plants (depending on population size) were collected and labelled. Sampling in the Vysoké Tatry Mts, Hlinská dolina valley (mylonite bedrock population no. 13; *S. tatrae*) was an exception, as this population consisted of only seven plants. To avoid collecting clones, plants were sampled from distinct patches (at a minimum distance of 2 m apart), which represented separate, geographically distant individuals. Voucher specimens are deposited in the herbarium of the Institute of Botany, Slovak Academy of Sciences, Bratislava (SAV).

To assess phytosociological relationships, relevé plots were used to collect vegetation data at all 68 populations. The authors carried out 28 of the relevés and data on the remaining 40 came from the Slovak vegetation database (Šibíková et al. 2009, Šibík 2012). All phytosociological relevés were implemented using standard procedures of the Zürich-Montpellier School (Braun-Blanquet 1964, Westhoff & van den Maarel 1978) and stored in a TURBOVEG database (Hennekens & Schaminée 2001). This data was analysed using JUICE 7.0 software (Tichý 2002), in which the sampling scales of all relevés were expressed in terms of one uniform scale. Taxa determined only to genus, as well as all bryophytes and lichens, were not included in the analysis. Some taxa determined to subspecies or varieties, were assigned to species or broadly conceived taxa. Only clearly

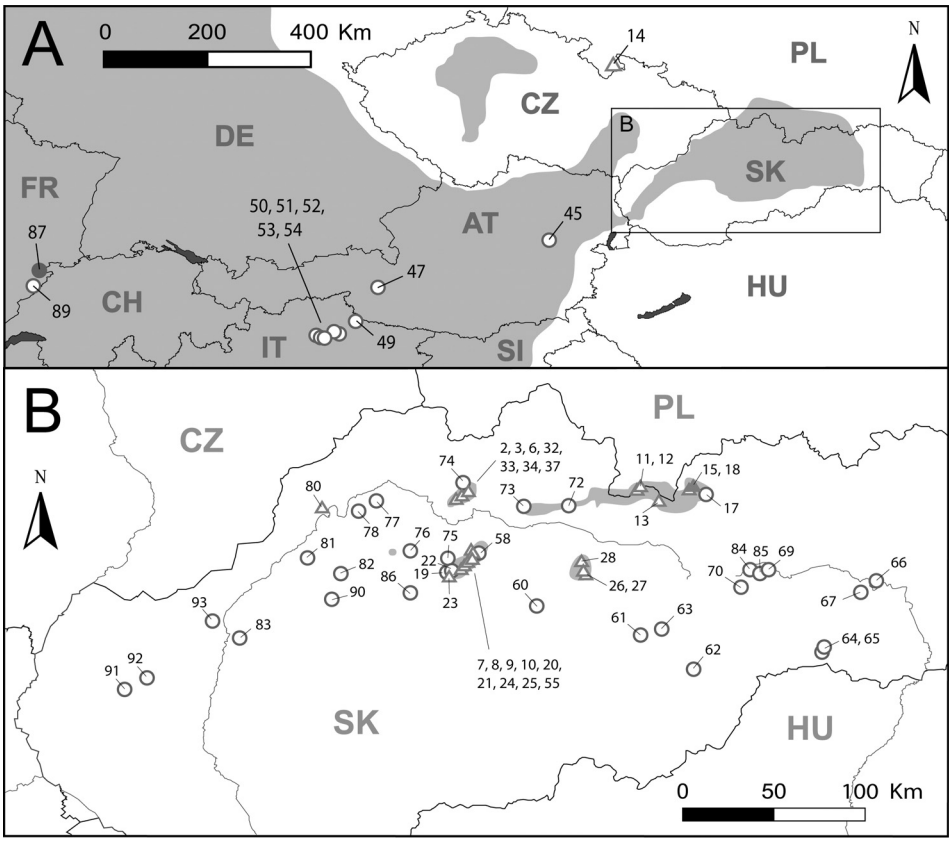

Fig. 1. – Map showing the locations of the sites sampled (Table 1) and geographic distribution of *Sesleria caerulea* (circles) and *S. tatrae* (triangles) based on data from the literature (Dixon 1982, Lysák 1996). The location of the type population of *S. caerulea* is marked by a filled circle and that of *S. tatrae* by a filled triangle. (A) overview of the study area; the area where *S. caerulea* occurs is shaded. (B) detailed view of the Western Carpathians; the area where *S. tatrae* occurs is shaded (isolated occurrence in the Sudetes is indicated in Fig. 1A).

distinguishable infraspecific taxa were used in analyses under their original designation. The nomenclature of vascular plants generally followed the checklist of Marhold & Hindák (1998) and the names of syntaxa are in accordance with Kliment et al. (2010) and Jarolímek & Šibík (2008).

Karyological analyses

One plant per species (from population no. 75 and 8) was potted and cultivated in a greenhouse at the Institute of Botany (Slovak Academy of Sciences, Bratislava). These plants were used for chromosome counting and as reference plants for DNA ploidy level estimations: *S. caerulea*, population no. 75 (Slovakia, Veľká Fatra Mts, Gaderská dolina valley, 2n = 28) and *S. tatrae*, population no. 8 (Slovakia, Veľká Fatra Mts, Mt. Chyžky, 2n = 56) (both counted by M. Budzáková, see Table 1).

Table 1. – The plant material studied. Each record is as follows: taxon name, population number, geographic origin with corresponding altitude, date of collection, geographic coordinates (in WGS84) and numbers of individuals used both for morphometric and ploidy level analyses (N). Abbreviations of countries and months: AT – Austria, FR – France, IT – Italy, PL – Poland, SK – Slovakia; Apr – April, Aug – August, Jun – June, Jul – July. Populations marked with ^{T} refer to the type localities of *S. caerulea* and *S. tatrae*, respectively; DNA ploidy levels marked with an asterisk refer to populations for which chromosome counts were obtained.

Pop. no.	Locality description, altitude, date of collection	Geographic coordinates		N	DNA ploidy level
		Latitude	Longitude		
Sesleria caerulea					
17	SK, Belianske Tatry Mts, Mt Limbová skala, 1484 m, 21 Jul 2010	49°13'36"	20°16'38"	10	2n~ 4x~ 28
19	SK, Veľká Fatra Mts, Mt Haľamova kopa, 1204 m, 2 Aug 2010	48°53'21"	19°00'13"	10	2n~ 4x~ 28
22	SK, Veľká Fatra Mts, Mt Kráľova studňa hill, 1361 m, 4 Aug 2010	48°53'08"	19°02'46"	9	2n~ 4x~ 28
45	AT, Nördliche Kalkalpen Mts, Mt Schneeberg, 1838 m, 23 Jun 2011	47°45'31"	15°49'51"	15	2n~ 4x~ 28
47	AT, Hohe Tauern Mts, Mt Grossglockner, 2490 m, 24 Jun 2011	47°05'09"	12°44'25"	15	2n~ 4x~ 28
49	IT, Dolomiti Mts, Tre Cime di Lavaredo peaks, 2388 m, 25 Jun 2011	46°37'04"	12°19'00"	15	2n~ 4x~ 28
50	IT, Dolomiti Mts, Passo di Giau mauntain pass, 2220 m, 25 Jun 2011	46°28'44"	12°03'36"	15	2n~ 4x~ 28
51	IT, Dolomiti Mts, Passo Fedaia mauntain pass, 2090 m, 26 Jun 2011	46°27'53"	11°51'50"	15	2n~ 4x~ 28
52	IT, Dolomiti Mts, Passo Sella mauntain pass, 2280 m, 26 Jun 2011	46°30'31"	11°46'06"	15	2n~ 4x~ 28
53	IT, Dolomiti Mts, Passo Pordoi mauntain pass, 2246 m, 26 Jun 2011	46°29'26"	11°48'24"	14	2n~ 4x~ 28
54	IT, Dolomiti Mts, Passo di Varpalora mauntain pass, 2225 m, 27 Jun 2011	46°31'32"	11°59'29"	15	2n~ 4x~ 28
58	SK, Veľká Fatra Mts, Mt Čierny Kameň, 1470 m, 16 Jul 2011	48°55'53"	19°08'10"	15	2n~ 4x~ 28
60	SK, Nízke Tatry Mts, Hora hill, 550 m, 16 Apr 2012	48°48'09"	19°20'49"	15	2n~ 4x~ 28
61	SK, Muránska planina Plain, Mt Malá Stožka, 1050 m, 17 Apr 2012	48°46'33"	19°55'47"	15	2n~ 4x~ 28
62	SK, Slovenský kras Karst, Skalka hill, 530 m, 18 Apr 2012	48°37'14"	20°13'45"	13	2n~ 4x~ 28
63	SK, Muránska planina Plain, Mt Šiance, 950 m, 13 Apr 2012	48°46'13"	20°04'10"	10	2n~ 4x~ 28
64	SK, Slovenský kras Karst, Hájska dolina valley, 525 m, 18 Apr 2012	48°38'45"	20°50'42"	15	2n~ 4x~ 28
65	SK, Slovenský kras Karst, Zádielska tiesňava gorge, 580 m, 18 Apr 2012	48°37'21"	20°49'35"	15	2n~ 4x~ 28
66	SK, Slovenské rudohorie Mts, Humenec hill, 450 m, 19 Apr 2012	48°51'34"	21°09'55"	15	2n~ 4x~ 28
67	SK, Slovenské rudohorie Mts, Mt Folkmarská skala, 830 m, 19 Apr 2012	48°49'40"	21°00'46"	15	2n~ 4x~ 28
69	SK, Slovenský raj Mts, Tomášovský výhľad rock prospect, 540 m, 20 Apr 2012	48°56'30"	20°27'50"	14	2n~ 4x~ 28
70	SK, Slovenský raj Mts, Stratená village, 830 m, 20 Apr 2012	48°52'23"	20°19'45"	15	2n~ 4x~ 28
72	SK, Chočské vrchy Mts, Prosiecka jaskyňa cave, 725 m, 21 Apr 2012	49°09'38"	19°29'44"	15	2n~ 4x~ 28
73	SK, Chočské vrchy Mts, Mt Soliská, 880 m, 21 Apr 2012	49°08'56"	19°18'36"	14	2n~ 4x~ 28
74	SK, Kривánska Malá Fatra Mts, Tiesňavy gorge, 630 m, 21 Apr 2012	49°14'52"	19°02'13"	15	2n~ 4x~ 28
75	SK, Veľká Fatra Mts, Gaderská dolina valley, 545 m, 22 Apr 2012	48°56'09"	18°56'18"	15	2n~ 4x~ 28*
76	SK, Lúčanská Malá Fatra Mts, Mt Zniev, 520 m, 22 Apr 2012	48°58'06"	18°47'18"	15	2n~ 4x~ 28
77	SK, Strážovské and Súľovské vrchy Mts, Súľovské skaly Hills, 450 m, 23 Apr 2012	49°10'03"	18°34'43"	15	2n~ 4x~ 28
78	SK, Strážovské and Súľovské vrchy Mts, Manínska tiesňava gorge, 360 m, 23 Apr 2012	49°08'28"	18°30'10"	15	2n~ 4x~ 28
81	SK, Strážovské and Súľovské vrchy Mts, Mt Vápeč, 780 m, 24 Apr 2012	48°56'19"	18°19'23"	15	2n~ 4x~ 28
82	SK, Strážovské and Súľovské vrchy vrchy Mts, Mt Temešská skala, 860 m, 24 Apr 2012	48°52'09"	18°29'42"	15	2n~ 4x~ 28
83	SK, Považský Inovec Mts, Rovence hill, 413 m, 24 Apr 2012	48°39'12"	17°55'22"	15	2n~ 4x~ 28
84	SK, Slovenský raj Mts, Suchá Belá gorge, 750 m, 29 Apr 2012	48°57'07"	20°23'05"	9	2n~ 4x~ 28
85	SK, Slovenský raj Mts, Kláštorisko settlement, 800 m, 29 Apr 2012	48°56'26"	20°25'00"	15	2n~ 4x~ 28
86	SK, Kremnické vrchy Mts, Hájska skala hill, 690 m, 13 May 2012	48°46'31"	18°46'46"	9	2n~ 4x~ 28
87 ^{T}	FR, Doubs valley, Saint-Hippolyte village (near the town of Montbéliard), 630 m, 7 May 2012	47°19'59"	06°48'40"	12	2n~ 4x~ 28
89	FR, Doubs valley, Mt Mont Châtelard, 1030 m, 7 May 2012	47°05'46"	06°44'14"	15	2n~ 4x~ 28
90	SK, Strážovské and Súľovské vrchy Mts, Predný Rokoš hill, 690 m, 16 Jul 2011	48°46'55"	18°27'27"	14	2n~ 4x~ 28
91	SK, Malé Karpaty Hills, Kršlenica hill, 560 m, 4 Apr 2012	48°30'28"	17°19'12"	15	2n~ 4x~ 28
92	SK, Malé Karpaty Hills, Biela hora hill, 374 m, 4 Apr 2012	48°32'29"	17°25'31"	15	2n~ 4x~ 28
93	SK, Malé Karpaty Hills, Čachtice castle hill, 345 m, 4 Apr 2012	48°43'25"	17°45'40"	14	2n~ 4x~ 28

Pop. no.	Locality description, altitude, date of collection	Geographic coordinates		N	DNA ploidy level
		Latitude	Longitude		
Sesleria tatrae					
2	SK, Kriváńska Malá Fatra Mts, Mt Veľký Kriváň, 1575 m, 1 Jul 2010	49°11'18"	19°01'40"	9	2n~ 8x~ 56
3	SK, Kriváńska Malá Fatra Mts, Mt Steny, 1610 m, 2 Jul 2010	49°11'33"	19°03'41"	15	2n~ 8x~ 56
6	SK, Kriváńska Malá Fatra Mts, Snilovské sedlo saddle, 1471 m, 3 Jul 2010	49°11'19"	19°02'22"	9	2n~ 8x~ 56
7	SK, Veľká Fatra Mts, Mt Borišov, 1500 m, 8 Jul 2010	48°56'28"	19°05'23"	10	2n~ 8x~ 56
8	SK, Veľká Fatra Mts, Mt Chyžky (Kýšky), 1320 m, 8 Jul 2010	48°55'27"	19°06'06"	10	2n~ 8x~ 56*
9	SK, Veľká Fatra Mts, Mt Suchý vrch, 1524 m, 9 Jul 2010	48°54'31"	19°05'02"	10	2n~ 8x~ 56
10	SK, Veľká Fatra Mts, Mt Krížna, 1560 m, 10 Jul 2010	48°52'35"	19°04'43"	10	2n~ 8x~ 56
11	SK/PL, Západné Tatry Mts, Mt Stoly, 1810 m, 13 Jul 2010	49°13'23"	19°54'17"	10	2n~ 8x~ 56
12	SK/PL, Západné Tatry Mts, Mt Stoly, 1955 m, 13 Jul 2010	49°13'29"	19°54'19"	10	2n~ 8x~ 56
13	SK, Vysoké Tatry Mts, Hlinská dolina valley, 1890 m, 15 Jul 2010	49°10'32"	20°02'13"	7	2n~ 8x~ 56
14	PL, Śnieżnik Kłodzki Mts, Kleśnica valley, Pulinka rock, 845 m, 4 Jun 2010	50°14'18"	16°50'33"	10	2n~ 8x~ 56
15 [T]	SK, Belianske Tatry Mts, ridge between saddles Kopské and Vyšné Kopské, 1910 m, 20 Jul 2010	49°14'01"	20°13'07"	18	2n~ 8x~ 56
18	SK, Belianske Tatry Mts, Zadné Meďodoly valley, 1667 m, 22 Jul 2010	49°13'55"	20°12'49"	10	2n~ 8x~ 56
20	SK, Veľká Fatra Mts, ridge between Mt Krížna and Mt Frčkov, 1476 m, 3 Aug 2010	48°52'54"	19°04'54"	10	2n~ 8x~ 56
21	SK, Veľká Fatra Mts, Mt Dlhý Grúň, 1430 m, 3 Aug 2010	48°53'06"	19°05'17"	10	2n~ 8x~ 56
23	SK, Veľká Fatra Mts, Mt Smrekovica, 1414 m, 4 Aug 2010	48°52'49"	19°02'02"	10	2n~ 8x~ 56
24	SK, Veľká Fatra Mts, Mt Frčkov, 1525 m, 5 Aug 2010	48°53'36"	19°04'52"	9	2n~ 8x~ 56
25	SK, Veľká Fatra Mts, Mt Ostredok, 1545 m, 5 Aug 2010	48°54'10"	19°04'47"	10	2n~ 8x~ 56
26	SK, Nízke Tatry Mts, Králička ridge, 1657 m, 10 Aug 2010	48°55'34"	19°39'11"	10	2n~ 8x~ 56
27	SK, Nízke Tatry Mts, Kozie chrbty ridge, 1687 m, 10 Aug 2010	48°55'34"	19°38'48"	9	2n~ 8x~ 56
28	SK, Nízke Tatry Mts, Javorová priepasť chasm, 1640 m, 13 Aug 2010	48°58'48"	19°38'07"	10	2n~ 8x~ 56
32	SK, Kriváńska Malá Fatra Mts, Mt Hromové, 1478 m, 23 May 2011	49°11'26"	19°03'12"	14	2n~ 8x~ 56
33	SK, Kriváńska Malá Fatra Mts, Mt Pekelník, 1575 m, 23 May 2011	49°11'28"	19°01'07"	15	2n~ 8x~ 56
34	SK, Kriváńska Malá Fatra Mts, Mt Malý Kriváň, 1630 m, 24 May 2011	49°10'55"	18°59'31"	15	2n~ 8x~ 56
37	SK, Kriváńska Malá Fatra Mts, Mt Malý Kriváň, SE from the top, 1624 m, 24 May 2011	49°11'02"	18°59'52"	15	2n~ 8x~ 56
55	SK, Veľká Fatra Mts, Mt Ploská, 1410 m, 15 Jul 2011	48°56'12"	19°07'06"	15	2n~ 8x~ 56
80	SK, Javorníky Mts, Holíš hill, 530 m, 23 Apr 2012	49°08'57"	18°22'07"	15	2n~ 8x~ 56

Numbers of chromosomes were counted in mitotic figures in meristem cells of actively growing root tips (for details see Hodálová et al. 2008). Flow cytometry (FCM) was used to estimate nuclear DNA content and to relate it to ploidy levels. Leaf tissue collected during field sampling (a total of 877 plants) was dried using silica gel and used for FCM measurements. Ploidy was determined based on comparison with reference plants with known chromosome numbers (Doležel et al. 2007). First, samples of reference plants (see above) were analysed simultaneously with an internal DNA reference standard (*Pisum sativum* 'Ctirad', 2C DNA = 9.09 pg; Doležel et al. 1998), and the ratio of their G_0/G_1 peak positions recorded. The DNA ploidy levels of the analysed plants were then assessed by their peak position relative to the DNA reference standard peak. *Pisum sativum* 'Ctirad' was used as the internal reference standard, because its genome size does not differ markedly from those of both tetraploid and octoploid *Sesleria* and the histogram peaks of *Pisum* and *Sesleria* do not overlap.

We used a simplified two-step nuclei isolation procedure, using Otto buffers (Doležel et al. 2007), to prepare the samples. Approximately 0.5 cm^2 of silica gel-dried *Sesleria* was chopped up together with leaf tissue of the *Pisum* standard (Suda & Trávníček 2006). This was performed with a sharp razor blade in a plastic Petri dish containing 1 ml of ice-cold Otto I buffer (0.1 M citric acid monohydrate, 0.5% Tween 20). The resulting suspension of nuclei was filtered through a 42-μm nylon mesh and stored for 10–15 min at room temperature. One ml of staining solution containing Otto II buffer (0.4 M $Na_2HPO_4 \cdot 12H_2O$) and 4',6-diamidino-2-phenylindole (DAPI, 2 μg/ml), supplemented with β-mercaptoethanol, was added to the filtered suspension of nuclei. Samples were incubated in a dark room at ambient room temperature for 5–30 minutes. Following incubation, fluorescence intensity was analysed using a Partec CyFlow ML flow cytometer (Partec GmbH, Münster, Germany), equipped with an HBO 100 W mercury arc lamp. The cytometer was adjusted so that the G_0/G_1 peak of the standard was localized on channel 200. Histograms were accumulated at a flow rate of about 25–50 particles per second for a total count of 5000 particles (stained nuclei). The resulting histograms were evaluated using Partec FloMax software (v. 2.52; Partec GmbH, Münster, Germany). For each measurement, the coefficients of variation (CV) of the standard and the analysed sample were calculated. If the CV of the G_0/G_1 peak exceeded the 5% threshold, the analysis was discarded and the sample reanalysed.

Morphometric analyses

All individuals that were processed using flow cytometry (877 plants/68 populations) were included in multivariate morphometric analyses. Twenty-eight morphological characters (nine vegetative and 19 floral) were measured (mm, cm), numbered, or scored (as 0 or 1), and two additional ratios were derived from them for further analysis (Table 2). Seven characters were not included in the morphometric analyses: four characters (SL, SLI, IL, IW) were used solely for calculating ratios, and three additional characters (SI, GA, GI) were constant or partly invariable across all the populations studied (Table 2). Consequently, 23 characters were used in the morphometric analyses.

Morphological characters studied included those traditionally used for the delimitation of *S. caerulea* and *S. tatrae* (Deyl 1938, 1946, 1980, Rychlewski 1955, Dostál 1958, 1989, Lysák 1996), as well as those found useful in our preliminary screening of Western-Carpathian populations. Although other morphometric characteristics are used for separating the species studied, they were not included in our study, because they were either invariable among populations or highly variable within populations, e.g. glume and lemma indument on veins (Deyl 1980, Dostál 1989) and length and character of ligule (Dostál 1989, Lysák 1996).

Spearman correlation coefficients (Legendre & Legendre 1998) for all pairs of morphometric characters were used to eliminate pairs of highly correlated characters that might distort further analyses.

Principal component analyses [PCA 1 – based on populations as operational taxonomic units (OTUs) and PCA 3 – based on individuals as OTUs] and a correlation matrix between the characters (Sneath & Sokal 1973) were used to clarify the overall pattern in the morphological variation among populations/individuals of *S. caerulea* and *S. tatrae*. Futhermore, to test the morphological homogeneity of one of the groups revealed by PCA 1 (those corresponding

Table 2. – List of characters measured and scored for individuals of *Sesleria caerulea* and *S. tatrae*, including eigenvectors expressing contribution of the characters to the first three principal axes (PCA 1, see Fig. 2) and total canonical structure expressing correlation of characters with first canonical axis (CDA, see Fig. 3). Only characters marked with an asterisk were used in multivariate analyses. [a] Scored on a spikelet from the centre of a spike, i.e. a spikelet with maximum number of florets per inflorescence. [b] The longest glume, lemma and palea per spikelet were measured or scored. [c] The longest awn per glume/lemma/palea was measured. [d] Glume indument = presence of hairs between the veins on the upper part of the abaxial surface. [e] Hair density = number of hairs per 0.04 mm^2 between the veins on the abaxial surface.

	Abbreviation	Character description	PCA 1			CDA
			Axis1	Axis2	Axis3	Can1
Stem	SL	Stem length, excluding inflorescence (cm)	–	–	–	–
	SLI	Stem length from the uppermost leaf to inflorescence (cm)	–	–	–	–
	SL/SLI*	Stem length (excl. inflorescence)/stem length from the uppermost leaf to inflorescence	–0.108	0.408	0.074	–0.167
Leaf	BLL*	Basal lamina length (cm)	0.124	0.127	0.070	0.119
	BLW*	Basal lamina width (cm)	–0.017	0.441	0.314	–0.060
	BLV*	Number of basal lamina veins	–0.146	0.445	0.135	–0.261
	ULL*	Uppermost lamina length (cm)	0.261	0.204	–0.185	0.540
	ULW*	Uppermost lamina width (cm)	0.277	0.160	0.230	0.431
	ULV*	Number of uppermost lamina veins	0.136	0.395	0.073	0.179
	SI	Sheaths indument	–	–	–	–
Inflorescence	IL	Inflorescence length (cm)	–	–	–	–
	IW	Inflorescence width (cm)	–	–	–	–
	IL/IW*	Inflorescence length/inflorescence width	0.121	0.078	–0.347	0.227
Spikelet[a]	FN*	Number of florets per spikelet	–0.088	0.100	0.132	–0.098
Glume[b]	GL*	Glume length, including awn (mm)	0.331	–0.030	–0.012	0.614
	GW*	Glume width (mm)	0.143	0.004	0.365	0.174
	GV*	Number of glume veins	0.142	–0.238	0.137	0.150
	GA	Number of glume teeth	–	–	–	–
	GAL*	Glume awn length[c] (mm)	0.266	0.096	–0.170	0.360
	GI	Glume indument[d] (0 absent; 1 present)	–	–	–	–
Lemma[b]	LL*	Lemma length, including awn (mm)	0.346	–0.010	0.012	0.633
	LW*	Lemma width (mm)	0.184	–0.199	0.411	0.236
	LV*	Number of lemma veins	0.115	–0.084	0.089	0.128
	LA*	Number of lemma teeth	0.083	–0.014	0.090	0.045
	LAL*	Lemma awn length (mm)	0.264	0.040	0.075	0.344
	LHD*	Lemma hair density[e]	0.283	0.071	–0.230	0.872
Palea[b]	PL*	Palea length, including awn (mm)	0.337	–0.000	0.000	0.636
	PW*	Palea width (mm)	0.230	–0.199	0.182	0.276
	PV*	Number of palea veins	0.049	–0.056	0.270	0.051
	PAL*	Palea awn length (mm)	0.232	0.148	–0.336	0.295

to *S. tatrae*), an additional PCA (PCA 2) was run based only on a subset of *S. tatrae* populations (as OTUs).

Canonical discriminant analysis (CDA, based on individuals as OTU; Klecka 1980) was performed to examine morphological separation of both groups (*S. caerulea* and *S. tatrae*) suggested by PCA 1 and determine which characters are most useful for identifying each species.

Descriptive statistics (Tukey 1977) (including mean, standard deviation, minimum, maximum, and 10 and 90 percentiles) were computed for all quantitative characters. A Tukey-Kramer multiple comparison analysis at a probability level of $P \leq 0.05$ (Tukey test for unequal sample sizes; Zar 1999, SAS Institute 2007) was used to evaluate: (i) characters in which morphologically marginal populations of *S. tatrae* differed from the remaining populations of *S. tatrae*, (ii) characters in which geographically marginal populations (population no. 14) of *S. tatrae* differed from the remaining populations of *S. tatrae* and (iii) the existence of significant differences between or within individuals of *S. caerulea* and *S. tatrae* for each morphological trait.

Analyses were done using SAS v. 9.1.3 (SAS Institute 2007). If not otherwise stated, value ranges presented below correspond to the 10 and 90 percentiles, with the minimum and maximum values in brackets.

Ecological analyses

Numerical classification of the 68 relevés was done using the program HIERCLUS in the SYN-TAX 2000 package (Podani 2001). Data transformation was not necessary, and the β-flexible clustering algorithm ($\beta = -0.25$) with similarity ratio coefficient was used. The results of numerical analyses are presented as a synoptic table (Table 3). Species with phi values greater than 0.3 ($\Phi > 30$) and Fisher's exact test of significance values (Chytrý et al. 2002) greater than 0.05 ($P < 0.05$) were designated as diagnostic. The phi coefficient was standardized to the equal relevé size of all groups, target group being of the same size as the others (Tichý & Chytrý 2006). Box and whisker plot construction for comparison of altitude in each cluster (clusters 1–4) was obtained from Statistica 8.0 (StatSoft 2014). A Tukey-Kramer multiple comparison analysis was used to determine the differences between study sites at a probability level of $P \leq 0.05$.

The main gradients in floristic composition were analysed using detrended correspondence analysis (DCA) in the CANOCO 4.5 package (ter Braak & Šmilauer 2002). Detrended correspondence analysis was used to display the relationships between communities, individual taxa and floristic gradients. Ellenberg's indicator values (Ellenberg et al. 1992) were used as supplementary environmental variables for interpretation of the ordination diagram from an ecological point of view.

We carried out a canonical correspondence analysis (CCA) of community composition, which included morphological characters of the species of *Sesleria* (Table 2) and environmental variables (altitude, slope, cover of herbaceous plant layer) as explanatory variables. The aim was to find the best predictors of species composition using the automatic forward selection procedure to identify the 10 best-fitting variables. In order to simplify the results and consequent explanations of the variation in morphology this number was selected subjectively. A partial Monte Carlo permutation test assessed the significance of each explanatory variable used in the constrained ordination model (Lepš & Šmilauer 2003). The results are depicted as an attribute diagram in which the bigger symbols of individual *Sesleria* species represent higher cover in a given community.

Table 3. – Shortened synoptic table of the relevés of the plant communities with *Sesleria caerulea* and *S. tatrae* resulting from the cluster analysis; methods used: β-flexible clustering algorithm (β = –0.25) with similarity ratio coefficient. Diagnostic taxa (Φ > 0.30) with the percentage values of their frequency, shown in bold, are sorted according to their fidelity to a particular type of vegetation (phi koeficient × 100, upper index). The species with a probability of random distribution in a particular type of vegetation lower than 0.05 based on a Fisher's exact test were excluded from the list of diagnostic species.

Cluster	1	2	3	4
No. of relevés	22	18	5	23
Sesleria tatrae	**100**$^{77.7}$	22^{---}	20^{---}	.
Carex sempervirens	**95**$^{68.4}$	39$^{1.1}$	.	17^{---}
Scabiosa lucida	**77**$^{52.9}$	28^{---}	.	30^{---}
Ligusticum mutellina	**41**$^{52.8}$	6^{---}	.	.
Campanula serrata	**41**$^{48.9}$	6^{---}	.	4^{---}
Parnassia palustris	**45**$^{45.5}$	11^{---}	.	9^{---}
Luzula luzuloides	**36**$^{44.8}$	6^{---}	.	4^{---}
Alchemilla species	**36**$^{43.7}$	11^{---}	.	.
Agrostis capillaris	**23**$^{42.5}$	.	.	.
Galium anisophyllon	**77**$^{42.1}$	33^{---}	20^{---}	35^{---}
Campanula glomerata	**27**$^{41.4}$	.	.	4^{---}
Anemone narcissiflora	**27**$^{41.4}$	.	.	4^{---}
Potentilla aurea	**41**$^{41.4}$	11^{---}	.	9^{---}
Soldanella carpatica	**45**$^{41.2}$	22$^{6.3}$	.	4^{---}
Bellidiastrum michelii	**64**$^{41.1}$	33$^{3.2}$	.	26^{---}
Thymus alpestris	**32**$^{40.4}$	6^{---}	.	4^{---}
Festuca versicolor	**41**$^{39.8}$	22$^{10.2}$	.	.
Bartsia alpina	**45**$^{39.4}$	17^{---}	.	13^{---}
Homogyne alpina	**32**$^{39.3}$	11^{---}	.	.
Phleum rhaeticum	**18**$^{37.8}$	.	.	.
Anthoxanthum alpinum	**32**$^{37.7}$	.	.	13$^{3.3}$
Primula elatior	**27**$^{36.9}$	.	.	9^{---}
Ranunculus pseudomontanus	**45**$^{35.4}$	11^{---}	.	26$^{7.7}$
Poa alpina	**41**$^{35.2}$	17^{---}	.	13^{---}
Achillea millefolium agg.	**36**$^{35.0}$	6^{---}	.	17$^{4.2}$
Vaccinium myrtillus	**32**$^{34.8}$	17$^{8.0}$	.	.
Leucanthemum margaritae	**59**$^{33.3}$	28^{---}	20^{---}	22^{---}
Phleum hirsutum	**14**$^{32.5}$	.	.	.
Festuca picturata	**14**$^{32.5}$	.	.	.
Astragalus alpinus	**14**$^{32.5}$	.	.	.
Lotus corniculatus	**64**$^{31.6}$	28^{---}	40$^{3.3}$	17^{---}
Oreogeum montanum	**18**$^{31.4}$	.	.	4^{---}
Gymnadenia conopsea agg.	**18**$^{31.4}$	.	.	4^{---}
Trifolium pratense	**23**$^{30.7}$	6^{---}	.	4^{---}
Pedicularis verticillata	**36**$^{30.2}$	22$^{8.3}$	.	9^{---}
Mercurialis perennis	5^{---}	**28**$^{37.2}$	.	4^{---}
Solidago virgaurea	9^{---}	**28**$^{37.0}$	.	.
Antennaria carpatica	.	**17**$^{36.1}$	.	.
Festuca pumila	.	**17**$^{36.1}$	.	.
Rubus saxatilis	.	**17**$^{36.1}$	.	.
Minuartia gerardii	.	**17**$^{36.1}$	.	.
Galium schultesii	.	**17**$^{36.1}$	.	.
Melica nutans	.	**17**$^{36.1}$	.	.
Epipactis helleborine	.	**17**$^{36.1}$	.	.
Carex humilis	.	11^{---}	**100**$^{76.2}$	35^{---}
Potentilla heptaphylla	.	11^{---}	**80**$^{71.0}$	13^{---}
Sanguisorba minor	.	.	**60**$^{65.6}$	9^{---}
Pilosella bauhinii	5^{---}	.	**60**$^{65.4}$	4^{---}

Cluster	1	2	3	4
No. of relevés	22	18	5	23
Hippocrepis comosa	.	6^{---}	$\mathbf{60}^{\mathbf{61.5}}$	9^{---}
Allium senescens subsp. *montanum*	5^{---}	.	$\mathbf{60}^{\mathbf{59.3}}$	13^{---}
Bromus monocladus	.	.	$\mathbf{40}^{\mathbf{57.7}}$	.
Tithymalus cyparissias	.	22^{---}	$\mathbf{80}^{\mathbf{55.7}}$	35^{---}
Tephroseris integrifolia	.	.	$\mathbf{40}^{\mathbf{53.2}}$	4^{---}
Salvia pratensis	.	.	$\mathbf{40}^{\mathbf{53.2}}$	4^{---}
Brachypodium pinnatum	.	6^{---}	$\mathbf{40}^{\mathbf{52.0}}$	.
Pilosella officinarum	.	.	$\mathbf{40}^{\mathbf{49.1}}$	9^{---}
Sedum album	.	.	$\mathbf{40}^{\mathbf{49.1}}$	9^{---}
Epipactis atrorubens	.	6^{---}	$\mathbf{40}^{\mathbf{48.1}}$	4^{---}
Teucrium chamaedrys	.	17^{---}	$\mathbf{60}^{\mathbf{47.5}}$	22^{---}
Pinus sylvestris	.	11^{---}	$\mathbf{40}^{\mathbf{47.1}}$	.
Festuca pallens	.	6^{---}	$\mathbf{60}^{\mathbf{46.5}}$	$35^{12.9}$
Scabiosa ochroleuca	.	6^{---}	$\mathbf{40}^{\mathbf{44.6}}$	9^{---}
Erysimum odoratum	.	6^{---}	$\mathbf{40}^{\mathbf{44.6}}$	9^{---}
Acinos alpinus	14^{---}	17^{---}	$\mathbf{60}^{\mathbf{43.1}}$	17^{---}
Genista pilosa	.	.	$\mathbf{40}^{\mathbf{42.2}}$	$17^{5.0}$
Pimpinella saxifraga	.	6^{---}	$\mathbf{40}^{\mathbf{41.4}}$	13^{---}
Minuartia langii	5^{---}	6^{---}	$\mathbf{40}^{\mathbf{41.3}}$	9^{---}
Jacea pratensis	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Aster amelloides	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Ononis spinosa	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Orthantha lutea	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Erophila verna	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Erigeron acris	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Salvia verticillata	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Larix decidua	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Campanula trachelium	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Medicago falcata	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Veronica austriaca	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Viola rupestris	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Cerastium pumilum	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Berberis vulgaris	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Veronica vindobonensis	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Monotropa hypopitys	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Dorycnium pentaphyllum agg.	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Crataegus monogyna	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Orchis militaris	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Corylus avellana	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Koeleria macrantha	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Polygala major	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Rosa canina	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Cirsium pannonicum	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Fragaria viridis	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Silene nemoralis	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Primula veris	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Cornus mas	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Seseli annum	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Ranunculus bulbosus	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Taraxacum erythrocarpum	.	.	$\mathbf{20}^{\mathbf{39.7}}$	.
Leontodon incanus	.	6^{---}	$\mathbf{40}^{\mathbf{38.5}}$	$17^{2.6}$
Origanum vulgare	.	17^{---}	$\mathbf{40}^{\mathbf{36.9}}$	9^{---}
Inula ensifolia	.	.	$\mathbf{40}^{\mathbf{36.5}}$	$26^{14.9}$
Sorbus aria	.	17^{---}	$\mathbf{40}^{\mathbf{34.4}}$	13^{---}
Platanthera bifolia	.	.	$\mathbf{20}^{\mathbf{33.6}}$	4^{---}

Cluster	1	2	3	4
No. of relevés	22	18	5	23
Ligustrum vulgare	.	.	**20**$^{\mathbf{33.6}}$	4^{---}
Carex caryophyllea	.	.	**20**$^{\mathbf{33.6}}$	4^{---}
Festuca rupicola	.	.	**20**$^{\mathbf{33.6}}$	4^{---}
Carpinus betulus	.	.	**20**$^{\mathbf{33.6}}$	4^{---}
Galium verum	.	.	**20**$^{\mathbf{33.6}}$	4^{---}
Luzula campestris	5^{---}	.	**20**$^{\mathbf{33.4}}$	.
Trommsdorffia maculata	5^{---}	.	**20**$^{\mathbf{33.4}}$	.
Jovibarba globifera	9^{---}	28^{---}	**60**$^{\mathbf{33.3}}$	35$^{2.3}$
Vincetoxicum hirundinaria	.	33^{---}	**60**$^{\mathbf{33.0}}$	39$^{7.4}$
Echium vulgare	.	6^{---}	**20**$^{\mathbf{32.1}}$	.
Senecio umbrosus	.	6^{---}	**20**$^{\mathbf{32.1}}$	.
Pulsatilla slavica	.	17^{---}	**40**$^{\mathbf{31.9}}$	17^{---}
Sesleria caerulea	.	78$^{16.1}$	80$^{18.8}$	**100**$^{\mathbf{42.9}}$
Carex ornithopoda	.	.	.	**13**$^{\mathbf{31.8}}$
Other taxa with higher values of fidelity or frequency:				
Galium glaucum	.	6^{---}	.	17$^{28.9}$
Helianthemum nummularium	.	6^{---}	.	17$^{28.9}$
Rhodax alpestris	.	11$^{6.1}$	.	22$^{28.4}$
Seseli osseum	.	.	40$^{29.3}$	39$^{28.0}$
Horminum pyrenaicum	.	.	.	9$^{25.8}$
Cotoneaster tomentosus	.	.	.	9$^{25.8}$
Festuca intercedens	.	.	.	9$^{25.8}$
Soldanella minima	.	.	.	9$^{25.8}$
Colymbada scabiosa	.	.	.	9$^{25.8}$
Festuca varia	.	.	.	9$^{25.8}$
Festuca alpina	.	.	.	9$^{25.8}$
Chamaecytisus hirsutus	.	.	.	9$^{25.8}$
Polygala chamaebuxus	.	.	.	9$^{25.8}$
Saxifraga caesia	.	.	.	9$^{25.8}$
Pulsatilla subslavica	.	.	.	9$^{25.8}$
Tilia cordata	.	.	.	9$^{25.8}$
Pulsatilla grandis	.	.	.	9$^{25.8}$
Ranunculus thora	.	.	.	9$^{25.8}$
Campanula rotundifolia agg.	.	.	.	9$^{25.8}$
Primula auricula	5^{---}	11$^{3.5}$	.	22$^{24.6}$
Hieracium bupleuroides	5^{---}	6^{---}	.	17$^{24.0}$
Gentiana verna	.	6$^{2.5}$	.	13$^{23.0}$
Coronilla vaginalis	.	6$^{2.5}$	.	13$^{23.0}$
Pinus sylvestris	.	6$^{2.5}$	.	13$^{23.0}$
Teucrium montanum	.	6^{---}	20$^{12.2}$	26$^{22.7}$
Anthericum ramosum	.	17^{---}	40$^{21.7}$	39$^{20.5}$
Thymus pulcherrimus agg.	27$^{14.8}$	17^{---}	.	26$^{13.0}$
Elyna myosuroides	.	17$^{20.3}$	.	13$^{12.4}$
Festuca tatrae	27$^{25.6}$	6^{---}	.	17$^{8.4}$
Carex digitata	9^{---}	28$^{24.0}$	.	17$^{6.5}$
Carduus glaucinus	18^{---}	22^{---}	20^{---}	26$^{6.3}$
Pimpinella major	23$^{14.1}$	17$^{4.1}$	.	17$^{5.3}$
Vicia oreophila	18$^{26.4}$	.	.	9$^{4.6}$
Polygala amara subsp. *brachyptera*	23$^{5.7}$	11^{---}	20$^{1.6}$	22$^{4.2}$
Ranunculus breyninus	32$^{19.0}$	22$^{4.8}$	.	22$^{4.1}$
Anthyllis vulneraria subsp. *alpestris*	32^{---}	28^{---}	60$^{22.6}$	43$^{3.2}$
Potentilla crantzii	18$^{21.3}$	6^{---}	.	9$^{1.2}$
Androsace chamaejasme	14$^{25.5}$	.	.	4^{---}
Carex atrata	14$^{25.5}$	.	.	4^{---}

Cluster	1	2	3	4
No. of relevés	22	18	5	23
Knautia arvensis	$14^{25.5}$	.	.	4^{---}
Salix reticulata	$14^{25.5}$	.	.	4^{---}
Helianthemum grandiflorum agg.	$50^{23.0}$	6^{---}	$40^{10.6}$	30^{---}
Saxifraga paniculata	$27^{6.1}$	22^{---}	20^{---}	22^{---}
Hieracium bifidum	$32^{29.7}$	11^{---}	.	13^{---}
Carlina acaulis	$41^{23.8}$	11^{---}	20^{---}	22^{---}
Campanula persicifolia	.	$17^{29.5}$	.	4^{---}
Geranium sanguineum	.	$17^{29.5}$	.	4^{---}
Bistorta vivipara	$41^{22.9}$	$33^{12.6}$	.	22^{---}
Dryas octopetala	$14^{4.6}$	$22^{20.3}$	.	9^{---}
Phyteuma orbiculare	$68^{28.4}$	28^{---}	40^{---}	39^{---}
Plantago lanceolata	5^{---}	.	$20^{28.5}$	4^{---}
Dianthus nitidus	$14^{14.1}$	$11^{8.5}$	.	4^{---}
Linum extraaxillare	$14^{14.1}$	$11^{8.5}$	.	4^{---}
Thesium linophyllon	.	6^{---}	$20^{27.5}$	4^{---}
Betonica officinalis	.	6^{---}	$20^{27.5}$	4^{---}
Aster alpinus	.	6^{---}	$20^{27.5}$	4^{---}
Acinos arvensis	.	6^{---}	$20^{27.5}$	4^{---}
Stachys recta	.	6^{---}	$20^{27.5}$	4^{---}
Cornus mas	.	6^{---}	$20^{27.5}$	4^{---}
Viola hirta	.	6^{---}	$20^{27.5}$	4^{---}
Carlina vulgaris agg.	.	6^{---}	$20^{27.5}$	4^{---}
Globularia punctata	.	6^{---}	$20^{27.5}$	4^{---}
Sedum sexangulare	.	6^{---}	$20^{27.5}$	4^{---}
Ajuga genevensis	.	6^{---}	$20^{27.5}$	4^{---}
Quercus petraea	.	6^{---}	$20^{27.5}$	4^{---}
Saxifraga aizoides	$9^{3.4}$	$17^{20.0}$	.	4^{---}
Polygonatum odoratum	.	$22^{16.4}$	$20^{12.6}$	9^{---}
Briza media	$23^{17.0}$	.	$20^{12.3}$	9^{---}
Selaginella selaginoides	$27^{24.1}$	$17^{6.0}$	.	9^{---}
Euphrasia salisburgensis	$32^{28.9}$	$17^{3.9}$	.	9^{---}
Gentianella fatrae	$23^{25.9}$	$11^{3.1}$	.	4^{---}
Cardaminopsis arenosa	$23^{11.3}$	11^{---}	$20^{6.9}$	9^{---}
Campanula rapunculoides	.	$17^{12.2}$	$20^{18.5}$	4^{---}
Plantago media	$18^{14.1}$	.	$20^{17.5}$	4^{---}
Fagus sylvatica	5^{---}	$17^{9.6}$	$20^{15.6}$	4^{---}
Knautia kitaibelii	$23^{16.3}$	6^{---}	$20^{11.7}$	4^{---}
Vaccinium vitis-idaea	$23^{25.0}$	$17^{13.2}$	.	.
Fagus sylvatica	5^{---}	$17^{12.1}$	$20^{18.4}$	.
Leontodon hispidus	$32^{20.4}$	17^{---}	$20^{2.7}$	4^{---}
Mycelis muralis	.	$28^{28.2}$	$20^{14.3}$	.

Results

Karyological analyses

FCM detected two DNA ploidy levels. All individuals morphologically corresponding to *S. caerulea* were tetraploid with 2n ~4x ~28, while those morphologically corresponding to *S. tatrae* were octoploid with 2n ~8x ~56 (Table 1). Population no. 80 from Holíš hill in the Javorníky Mts, previously designated as *S. calcarea* (Fajmonová 1970), was octoploid

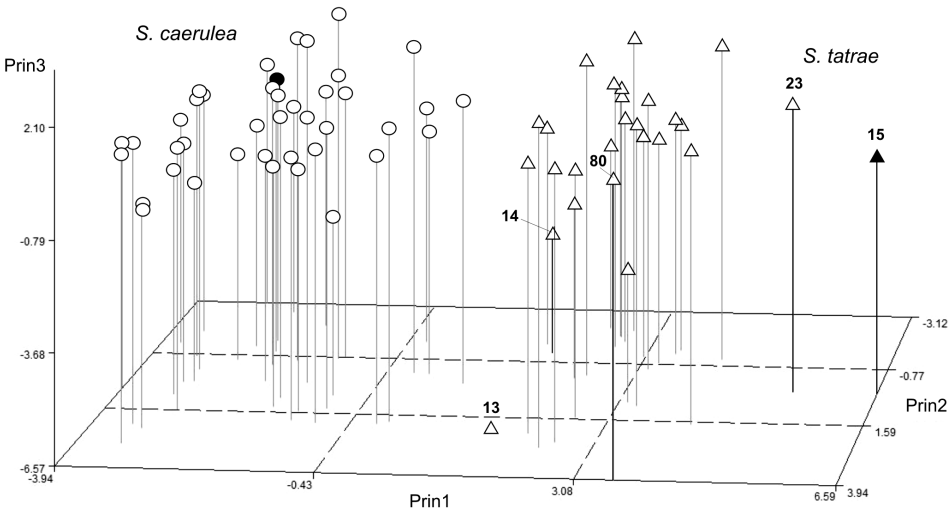

Fig. 2. – Principal component analysis (PCA 1) of 68 populations of *Sesleria caerulea* (circles) and *S. tatrae* (triangles) based on 23 morphological characters. The type population of *S. caerulea* is marked by a black circle and that of *S. tatrae* by a black triangle. Numbers of the populations of *S. tatrae* selected are as listed in Table 1. The first three axes explained 51.8% of the total variation.

in this analysis. Chromosome counts revealed 2n = 28 for *S. caerulea* and 2n = 56 for *S. tatrae* (populations no. 75 and 8, see Table 1), thus confirming the chromosome numbers previously reported for these species (Ujhelyi & Felföldy 1948, Bielecki 1955, Rychlewski 1955, Ujhelyi 1959b, Lysák & Doležel 1998).

Morphometric analyses

Spearman correlation coefficients did not reveal the presence of any highly correlated pairs of characters (exceeding 0.9); the highest value obtained was 0.621 (between the characters BLW and BLV), so all characters were used in subsequent analyses.

Principal component analysis (PCA 1, based on populations as OTUs) resulted in two main groups (Fig. 2): (i) one forming a relatively compact cluster of populations morphologically corresponding to *S. caerulea*, including the type population (no. 87) and (ii) one forming a more heterogenous cluster of populations that morphologically corresponds to *S. tatrae*, with some shift in morphology of its type population (no. 15) and population no. 23 along the first axis, and population no. 13 (from mylonite zone of Hlinská dolina valley) along the third axis. The geographically isolated population from Śnieżnik Kłodzki Mts in Poland (no. 14) occurred in the middle of a cluster of *S. tatrae*. The low-lying octoploid population from Javorníky Mts (no. 80) grouped at the margin of the *S. tatrae* cluster. The greatest influence on division of both groups by species in PCA 1 was along the first axis (31.3% of the variance), based principally on floral characters: glume, lemma and palea length, plus lemma hair density (Table 2). The second and third axes accounted for only 11.1 and 8.1% of the variation observed, respectively, and did not have a major effect on the division of the populations (Fig. 2).

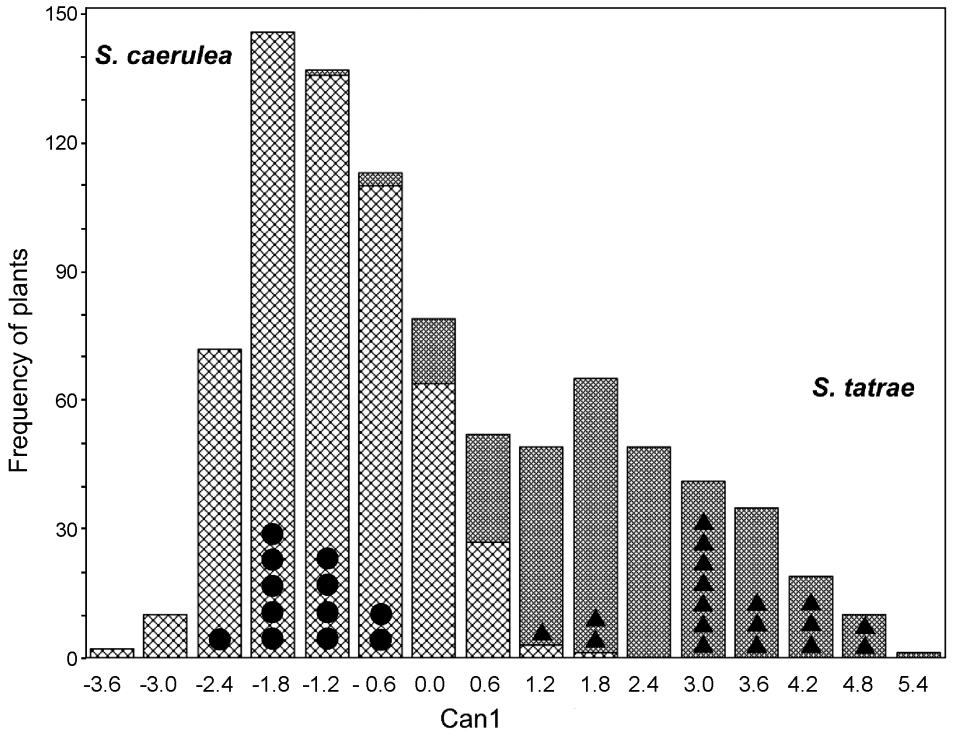

Fig. 3. – Canonical discriminant analysis (CDA) of 877 individuals based on 23 morphological characters with two groups pre-defined based on the results of PCA 1, representing *Sesleria caerulea* and *S. tatrae*; black circles show the position of individual plants from the type population of *S. caerulea* and black triangles of the type population of *S. tatrae*.

To better show intra-population homo-/or heterogeneity of morphological characters within *S. tatrae*, PCA 2 (based on populations as OTUs) was performed (Electronic Appendix 1). This analysis did not show any further grouping or separation within this species, with all five above-mentioned morphologically marginal populations (no. 13, 14, 15, 23 and 80) clustering more or less together. The first three axes explained 43.2% of the total variation.

Plotting the first three principal coordinates of PCA 3 based on individuals of both *Sesleria* species as OTUs showed one large cloud of all the objects analysed (Electronic Appendix 2). Despite some grouping of ploidy levels (with tetraploids on the left side of the diagram and octoploids on the right), there was considerable overlap between clusters and no clear delineation between ploidy levels or *S. caerulea* and *S. tatrae* individuals, respectively. Plants from type localities of both species (population no. 87 and 15) were deeply nested in the main cloud of variation. As found in PCA 1, variable loadings showed that the first component (explained around 16.8%) was most highly influenced by floral characters, namely length of glume, lemma and palea, and lemma hair density. The second and third axes account for 8.9 and 6.3% of the variation, respectively, and did not influence division of species.

Canonical discriminant analysis (CDA, Fig. 3), based on individual plants as OTUs, was run to reveal the differentiation between the groups (i. e. tetra- and octoploids) identified by PCA 1. In this analysis, there was a clear separation of *S. caerulea* and *S. tatrae*, with only a slight overlap of individuals. Plants from the type localities of *S. caerulea* and *S. tatrae* (no. 87 and 15) were clearly associated with their maternal species.

There were three Tukey-Kramer tests of the morphologically marginal populations nos. 13, 15 and 80 (placed by PCA 1 in a peripheral position of the non-compact cluster of *S. tatrae*, Fig. 2) versus the remaining populations of *S. tatrae*. The first revealed that population no. 13, from mylonite zone, significantly differs from the remaining populations of *S. tatrae* in five characters [given in square brackets are the value ranges corresponding to the (minimum–) average (–maximum) for marginal populations vs remaining *S. tatrae* populations]: inflorescence length/width [(1.55–) 1.97 (–2.22) vs (1.25–) 2.38 (–5.5)], glume width [(1.22–) 1.62 (–2.08) vs (1.0–) 2.18 (–3.62) mm], lemma width [(1.45–) 1.84 (–2.14) vs (1.00–) 2.57 (–4.00) mm], palea width [(1.10–) 1.29 (–1.67) vs (0.50–) 1.68 (–3.00) mm] and number of palea veins [2 vs (1–) 1.99 (–4)]. The second test revealed that plants from the type population of *S. tatrae* (no. 15) have significantly longer uppermost laminas [(1.25–) 1.93 (–3.20) vs (0.22–) 1.40 (–4.75) cm], lemmas (including awn) [(3.90–) 5.91 (–7.00) vs (3.30–) 5.35 (–6.90) mm] and awns of lemmas [(0.60–) 1.19 (–2.20) vs (0–) 0.93(–2.10) mm] than plants from the remaining populations of *S. tatrae*. Interestingly, the last two mentioned characters (LL and LAL) are also the best discriminators between *S. caerulea* and *S. tatrae* (Table 2, see results of PCA 1 and CDA). The third test revealed that population no. 80 significantly differs from the remaining populations in three leaf characters: number of basal lamina veins [(15–) 19.36 (–24) vs (4–) 14.8 (–28)], uppermost lamina length [(0.65–) 1.91 (–4.75) vs (0.22–) 1.41 (–3.75) cm] and number of uppermost lamina veins [(12–) 16.07(–24) vs (5–) 12.65 (–22)].

The fourth Tukey-Kramer test was used to determine if the morphological characters of individuals in the geographically marginal Polish population (no. 14) differ from the remaining populations of *S. tatrae* occurring in the Western Carpathians. This test showed that there is only one character in which these two populations of *S. tatrae* significantly differ, namely the width of the lemma [(1.00–) 1.97 (–2.81) in pop. 14 vs (1.20–) 2.56 (–4.00) mm in the Western Carpathians populations].

Descriptive statistics of characters measured and scored in *S. caerulea* and *S. tatrae* are listed in Table 4. Comparison of inter-species variation by the fifth Tukey-Kramer test revealed significant differences (P = 0.05) between *S. caerulea* and *S. tatrae* in nearly all characters. Of the 23 characters assessed, only seven were not significantly variable (BLW, SI, IL, GA, GI, LA and PV; Table 4).

Table 4. – Mean ± standard deviation (upper row), and minimum, 10 and 90 percentiles, and maximum (lower row) of quantitative characters, and character state frequencies of qualitative characters of *Sesleria caerulea* and *S. tatrae*. Values that were not significantly different between species (P = 0.05) are marked with an asterisk. For detailed explanations of the characters see Table 2.

Character	*S. caerulea* (N = 572)	*S. tatrae* (N = 305)
SL: Stem length, excluding inflorescence (cm)	25.9±9.4	39.7±14.2
	(6.0–) 17.0–38.1 (–73.5)	(11.4–) 19.8–57.3 (–79.0)
SLI: Stem length from the uppermost leaf to inflorescence (cm)	15.60±8.63	26.36±12.00
	(3.00–) 6.75–26.70 (–60.2)	(3.00–) 9.39–41.16 (–56.10)
SL/SL1	1.87±0.55	1.7±0.6
	(1.09–) 1.31–2.62 (–5.02)	(1.05–) 1.28–2.48 (–4.47)

Character	*S. caerulea* (N = 572)	*S. tatrae* (N = 305)
BLL: Basal lamina length (cm)	16.8±6.8 (0.79–) 8.80–25.90 (–58.00)	18.5±7.4 (4.1–) 9.6–27.1 (–77.5)
BLW: Basal lamina width (cm)	0.36±0.08* (0.14–) 0.25–0.45 (–0.55)	0.35±0.11* (0.10–) 0.21–0.50 (–0.65)
BLV: Number of basal lamina veins	16.61±3.60 (8–) 12–21 (–30)	14.95±3.70 (4–) 10.7–20.0 (–28)
ULL: Uppermost lamina length (cm)	0.90±0.42 (0.11–) 0.49–1.45 (–3.30)	1.43±0.59 (0.22–) 0.80–2.17 (–4.75)
ULW: Uppermost lamina width (cm)	0.24±0.06 (0.04–) 0.16–0.31 (–0.42)	0.29±0.07 (0.13–) 0.20–0.38 (–0.48)
ULV: Number of uppermost lamina veins	11.70±3.23 (4–) 8–16 (–24)	12.77±3.23 (5–) 8–17 (–24)
SI: Sheaths indument	absent: 99.3%* present: 0.7%	absent: 99.4%* present: 0.6%
IL: Inflorescence length (cm)	1.91±0.38* (0.28–) 1.50–2.40 (–3.70)	1.94±0.34* (1.0–) 1.5–2.4 (–2.9)
IW: Inflorescence width (cm)	0.74±0.14 (0.4–) 0.6–0.9 (–1.2)	0.84±0.15 (0.50–) 0.65–1.00 (–1.20)
IL/IW: Inflorescence length/inflorescence width	2.66±0.78 (0.47–) 1.88–3.64 (–6.25)	2.37±0.56 (1.25–) 1.75–3.17 (–5.50)
FN: Number of florets per spikelet	2.12±0.37 (1–) 2–3 (–4)	2.05±0.33 (1–) 2 (–4)
GL: Glume length, including awn (mm)	4.56±0.66 (2.8–) 3.8–5.5 (–6.9)	5.45±0.74 (3.6–) 4.5–6.5 (–7.59)
GW: Glume width (mm)	2.03±0.41 (0.2–) 1.6–2.6 (–3.6)	2.17±0.46 (1.00–) 1.60–2.80 (–3.62)
GV: Number of glume veins	1.14±0.55 1 (–5)	1.28±0.72 1–3 (–5)
GA: Number of glume awns	1.01±0.17* (0–) 1 (–3)	1.01±0.16* (0–) 1 (–3)
GAL: Glume awn length (mm)	0.67±0.32 (0–) 0.3–1.1 (–2.4)	0.91±0.37 (0.20–) 0.50–1.39 (–2.50)
GI: Glume indument	absent: 100%* present: 0%	absent: 100%* present: 0%
LL: Lemma length, including awn (mm)	4.61±0.54 (3.0–) 4.0–5.3 (–6.5)	5.38±0.60 (3.3–) 4.6–6.1 (–7.0)
LW: Lemma width (mm)	2.34±0.43 (1.1–) 1.8–2.9 (–4.1)	2.55±0.53 (1–) 1.8–3.2 (–4)
LV: Number of lemma veins	3.49±1.12 (1–) 3–5 (–7)	3.73±1.13 (1–) 3–5 (–7)
LA_ Number of lemma awns	2.89±0.75* (1–) 2–3 (–5)	2.96±0.69* (0–) 3 (–5)
LAL: Lemma awn length (mm)	0.74±0.28 (0–) 0.4–1.1 (–1.84)	0.94±0.36 (0–) 0.5–1.3 (–2.2)
LHD: Lemma hair density (per 0.04 mm^2)	0.89±1.59 0–3 (–8)	6.58±3.61 (0–) 2–11.3 (–16)
PL: Palea length, including awn (mm)	3.81±0.48 (2.0–) 3.2–4.4 (–5.5)	4.52±0.58 (2.40–) 3.80–5.22 (–6.00)
PW: Palea width (mm)	1.39±0.32 (0.5–) 1.0–1.8 (–2.8)	1.67±0.44 (0.50–) 1.20–2.29 (–3.00)
PV: Number of palea veins	1.99±0.12* (1–) 2 (–3)	1.99± 0.17* (1–) 2 (–4)
PAL: Palea awn length (mm)	0.13±0.14 0–0.27 (–1.6)	0.22±0.21 (0–) 0.1–0.4 (–2)

Ecological analyses

N u m e r i c a l c l a s s i f i c a t i o n. The results of the hierarchical classification (Fig. 4) indicate differences in local ecological conditions, which reflect differences in the species composition of the populations studied. Cluster 1 includes populations and communities from high and a relatively narrow range of altitudes (Fig. 5). At the level of syntaxa, we can label these communities as a part of the alliance *Seslerion tatrae*. Vegetation units included in this alliance characteristically occur in habitats consisting of calcareous alpine swards on deep soils with prolonged snow cover in the Western Carpathians. *Sesleria tatrae* and *Carex sempervirens* (mainly *C. s.* subsp. *laxiflora*, syn. *C. s.* subsp. *tatrorum*) dominate these communities. Cluster 2 includes relevés mainly dominated by *Sesleria caerulea*, which occur in a more heteregenous group of ecological conditions, with relatively deep and more humid soils. Relevés in this cluster come from both the Western Carpathians and Alps and phytosociologically belong to three alliances occurring at montane to alpine altitudes: *Caricion firmae* (calcareous alpine cushion-like open sedge and tussocky swards on northern windswept slopes), *Oxytropido-Elynion* (alpine xero-cryophilous wind-exposed swards and carpets on base-rich soils) and *Astero alpini-Seslerion calcariae* (calcareous montane and alpine communities predominantly on shallow soils on sunny slopes with a short period of snow cover in the Western Carpathians). The transitional character of some populations, particularly those from low altitudes, illustrates a shift between several communities and the potential for natural altitudinal vicariants, such as *Diantho lumnitzeri-Seslerion* (for those from the *Astero alpini-Seslerion calcariae*) and *Cystopteridion* (which at low altitudes change into plant communities of the alliance *Caricion firmae*, which are typical of high altitudes; see Šibík et al. 2004,

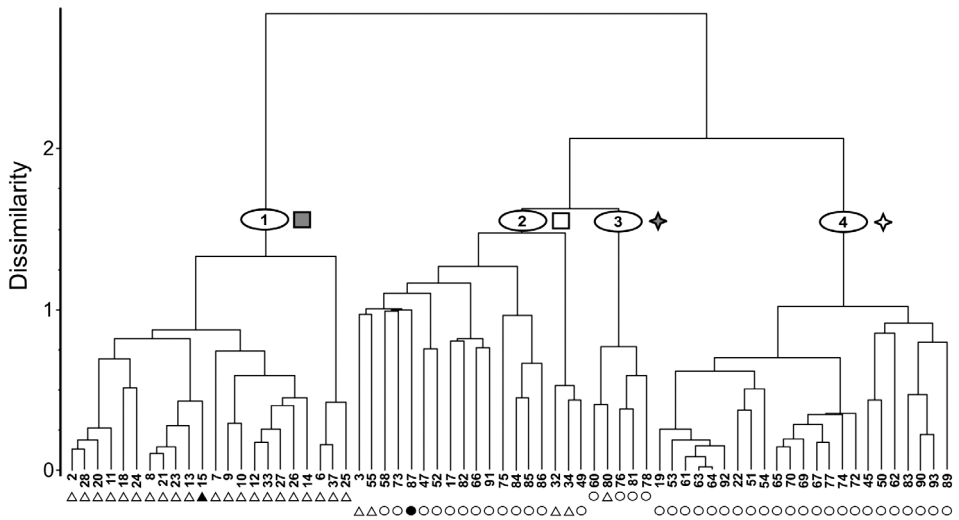

Fig. 4. – Dendrogram of the numerical classification of the populations with *Sesleria caerulea* (circles) and *S. tatrae* (triangles) studied. Clusters 1–4 represent floristically and ecologically different plant communities. The type population of *S. caerulea* is marked by a black circle and that of *S. tatrae* by a black triangle. Numbers of the populations are as listed in Table 1.

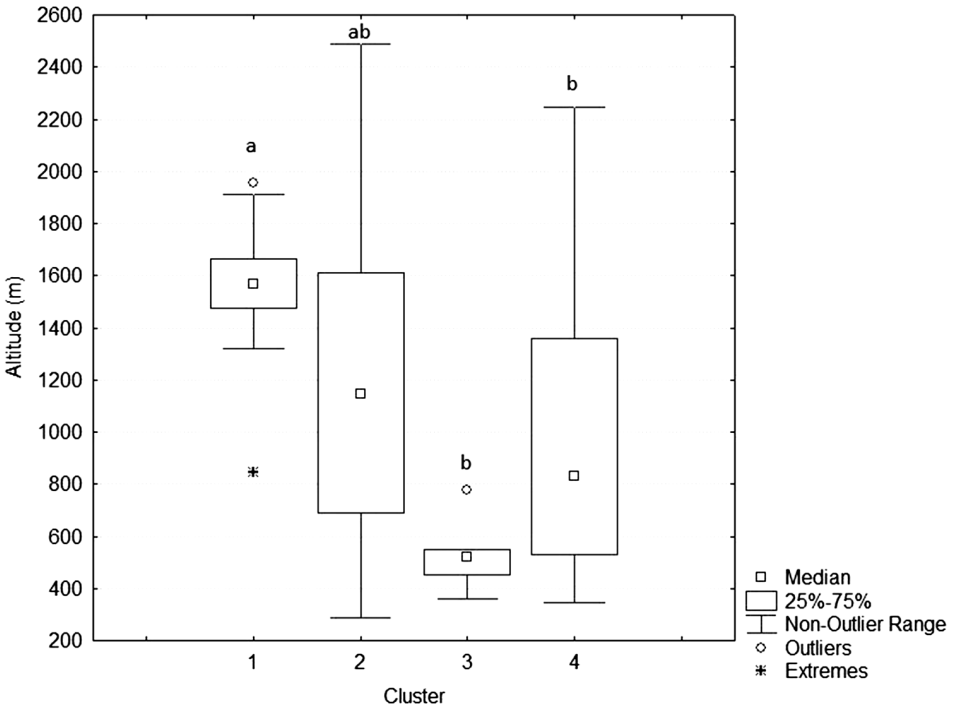

Fig. 5. – Box and whisker plots of the altitudinal ranges of communities within each cluster. Labels a–b indicate homogenous groups according to post-hoc comparisons following one-way ANOVA (Tukey-Kramer multiple comparison analysis at a probability level of $P \leq 0.05$). Numbers of clusters are as indicated in Fig. 4.

Kliment et al. 2005, Petrík et al. 2006). *Sesleria caerulea* dominates at low altitudes, whereas at these altitudes the abundance and occurrence of *S. tatrae* is low (Table 3). There is a higher occurrence and abundance of *Sesleria caerulea* in clusters 3 and 4. These results indicate that plant communities with *S. caerulea* are more diverse than those with *S. tatrae*. Plant communities occurring at low altitudes (Fig. 5) dominated by *Carex humilis* group together in cluster 3. These populations represent communities from the alliances *Festucion valesiacae* (subxerothermophilous grasslands at low altitudes) and *Pulsatillo slavicae-Pinion* (relic pine forests). The octoploid plants in population no. 80 (pine forest in the Javorníky Mts) occur in an atypical locality for *S. tatrae*, given at a very low altitude. Finally, cluster 4 comprises plant communities from various altitudes (Fig. 5); the link between associated plots is that *S. caerulea* is very abundant in all of them and there are some thermophilous or heliophilous species (or species confined to southern slopes, see Table 3) growing on shallow, skeletal soils. The main difference between these communities and those grouped in cluster 2 is related to specific soil characteristics, including soil thickness and structure and consequently soil reaction. Syntaxonomically, we can place the phytocoenoses grouped in this cluster into the alliances *Astero-Seslerion*, *Caricion firmae*, *Seslerion albicantis* (in the Alps), *Diantho lumnitzeri-Seslerion* and *Festucion valesiacae*.

O r d i n a t i o n. The results of the DCA analysis of the 68 relevés are shown in Fig. 6. In the analysis of supplementary data, Ellenberg's indicator values defined correlations between habitat attributes. The main factors dividing plant communities are temperature, moisture, light, nutrient availability and soil reaction. Axis 1 is highly correlated with nutrient availability, which demonstrates its importance in determining community composition. Communities dominated by *Sesleria tatrae* occur in cluster 1, which is best explained in terms of soil nutrient availability (highest values on left side of Fig. 6). Other groups identified as significant clusters (cluster 2, 3 and 4) are more or less scattered in the diagram. In general, most of the *S. caerulea* populations are located on the upper right side, while populations with *S. tatrae* are grouped on the lower left side of the diagram. The second axis is associated with temperature and moisture conditions. Communities

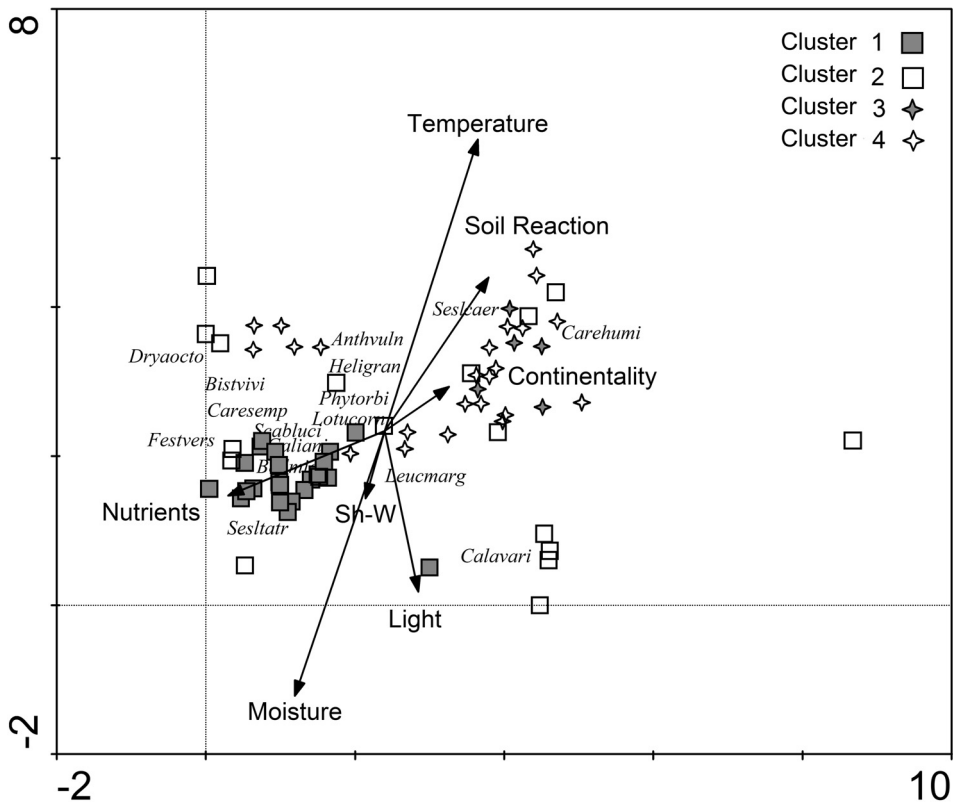

Fig. 6. – Detrended correspondence analysis (DCA) of 68 relevés with *Sesleria caerulea* and *S. tatrae*. Communities are those recorded at the localities and habitats of the populations from which samples were collected for the karyological and morphometric analyses. Ellenberg's indicator values were used as supplementary environmental variables. Length of gradients: 8.673 (1. axis), 4.777 (2. axis); eigenvalues: 0.818 (1. axis), 0.524 (2. axis). Anthvuln – *Anthyllis vulneraria* subsp. *alpestris*, Bellmich – *Bellidiastrum michelii*, Bistvivi – *Bistorta vivipara*, Calavari – *Calamagrostis varia*, Carehumi – *Carex humilis*, Caresemp – *Carex sempervirens*, Dryaocto – *Dryas octopetala*, Festvers – *Festuca versicolor*, Galianis – *Galium anisophyllon*, Heligran – *Helianthemum grandiflorum* agg., Leucmarg – *Leucanthemum margaritae*, Lotucorn – *Lotus corniculatus*, Phytorbi – *Phyteuma orbiculare*, Scabluci – *Scabiosa lucida*, Seslcaer – *Sesleria caerulea*, Sesltatr – *Sesleria tatrae*.

growing at moist sites group together at the bottom of the diagram, whereas those growing where conditions are hotter and drier group together in the upper part of the diagram. These clusters include those with *S. tatrae* and *S. caerulea*, respectively. High values of pH correlate with the occurrence of *S. caerulea*. This species seems to prefer an alkaline pH, whereas *Sesleria tatrae*, although preferring alkaline conditions, appears to be more tolerant of variations in pH and soil moisture.

Following the CCA analyses (Figs 7 and 8, Table 5), the most significant explanatory variables were identified using a Monte Carlo permutation test. This analysis revealed that only one character, ratio of stem length (excl. inflorescence) to stem length from the uppermost leaf to the inflorescence (SL/SLI), is positively correlated with occurrence of *S. caerulea*, which suggests that the distance between uppermost leaf and inflorescence is shorter in *S. caerulea* than in *S. tatrae*. The relative importance of the five other significant morphological characters increased with presence of *S. tatrae* (Figs 7, 8). One character (number of uppermost lamina veins, ULV) appeared to be important in the separation of

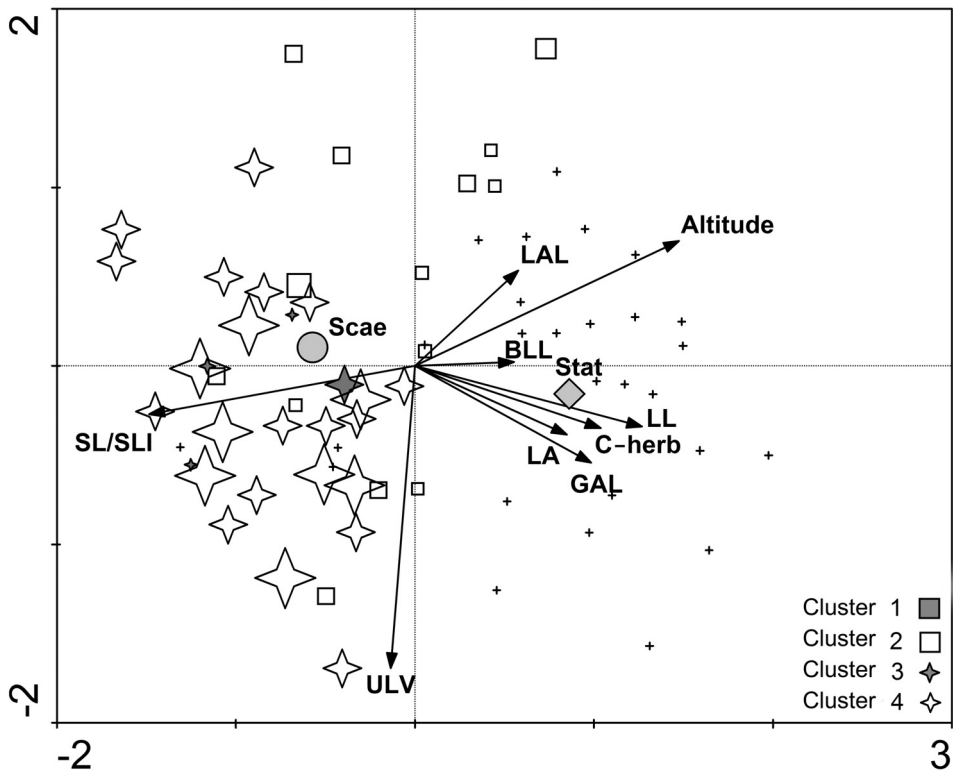

Fig. 7. – Canonical correspondence analysis (CCA) of 68 relevés. Percentage cover of *Sesleria caerulea* in each community was divided by cluster analysis values and used as attribute data. For explanations of the characters measured and scored for the two species see Table 2, Scae – tetraploid populations corresponding to *Sesleria caerulea*, Stat – octoploid populations corresponding to *S. tatrae*, C-herb – percentage cover of herbaceous plant layer, plus – species did not occur in this relevé.

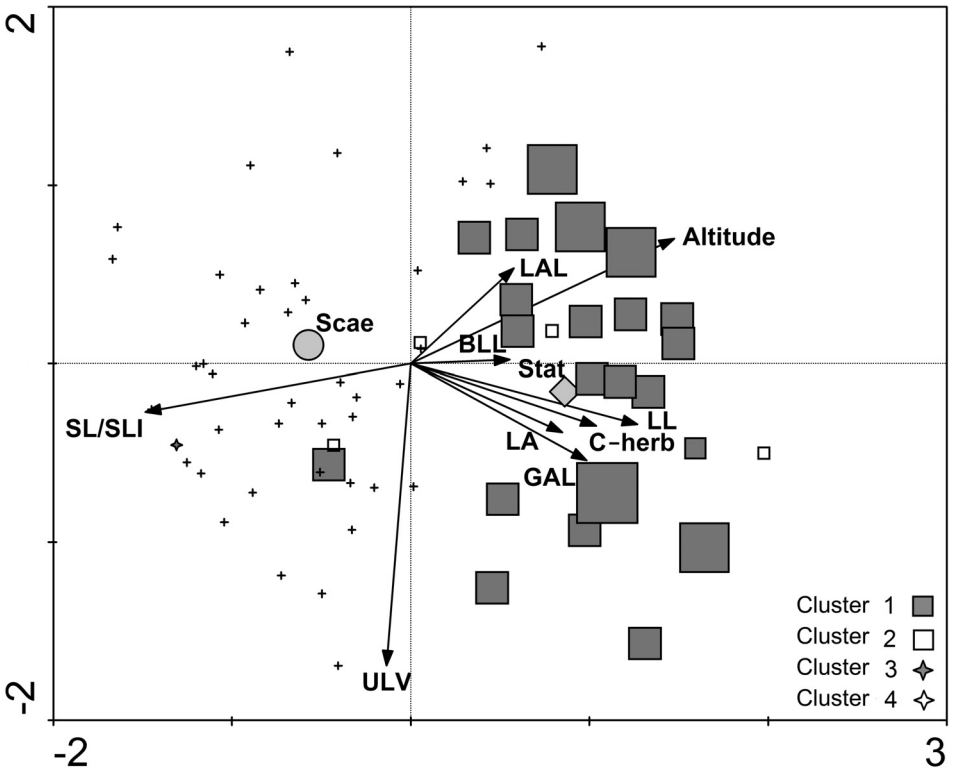

Fig. 8. – Canonical correspondence analysis (CCA) of 68 relevés. Percentage cover of *Sesleria tatrae* in each community was divided by cluster analysis values and used as attribute data. For explanations see Fig. 7.

communities along the axes, but this character is correlated with communities dominated by both *S. caerulea* and *S. tatrae*. Of the environmental characteristics assessed, two were significant, percentage cover of the herbaceous plant layer and altitude. Both of which are positively associated with the occurrence of *S. tatrae*. Herbaceous plant cover was higher in communities with *S. tatrae* [(45–) 63–100%], which grows in denser plant communities than *S. caerulea* [(15–) 50–98 (–100)%]. Altitude separated the tetraploid and octoploid plants. Tetraploid plants (*S. caerulea*) occurred in the Western Carpathians in the colline to submontane vegetation belts [(345–) 413–970 (–1484) m a.s.l.], whereas octoploid plants (*S. tatrae*) occurred predominantly in the subalpine belt [(530–) 1412–1866 (–1955) m a.s.l. – given values correspond to 10 and 90 percentiles, with minimum and maximum in round brackets; Table 1, Fig. 5]. Only two of the octoploid populations occurred at an altitude below 1320 m a.s.l.: (i) the population in the Javorníky Mts (Slovakia, no. 80), growing at an altitude of only 530 m, and (ii) the population in the Śnieżnik Kłodzki Mts (Poland, no. 14), growing at an altitude of 845 m.

Table 5. – Inter-set correlations of environmental variables with CCA ordination axes for Figs 7 and 8. For explanations of characters measured and scored on both species of *Sesleria* see Table 2.

	Axis 1	Axis 2	Axis 3	Axis 4
Sesleria tatrae	0.657	–0.141	0.078	–0.490
Sesleria caerulea	–0.657	0.141	–0.078	0.490
Altitude	0.558	0.258	0.422	0.125
Cover of herb layer	0.392	–0.128	0.057	0.318
BLL	0.209	0.008	–0.610	–0.329
ULV	–0.051	–0.622	–0.064	–0.302
SL/SL1	–0.561	–0.099	0.107	–0.107
GAL	0.373	–0.199	0.254	–0.545
LL	0.480	–0.125	0.143	–0.531
LAL	0.218	0.197	0.213	–0.439
LA	0.321	–0.141	0.154	0.304

Discussion

Karyological uniformity of the populations studied

All studied populations possessed uniform ploidy level. Plants morphologically corresponding to *S. caerulea* (572 individuals) were tetraploid, with 2n ~28, and those morphologically corresponding to *S. tatrae* (305 individuals) octoploid, with 2n ~56. We did not find two cytotypes within one species or population, as found previously for other species of *Sesleria* (Lysák & Doležel 1998, Kuzmanović et al. 2013). This indicates that polyploidization does not occur or is very infrequent within populations of *S. caerulea* and *S. tatrae*, respectively.

An important result of this study is the lack of hexaploid individuals, which would indicate hybridization between these species. This is surprising, because some of the areas sampled are reported to contain intermediate/hybrid individuals and populations, e.g. on mountain ridges of the Krivánska Malá Fatra Mts (populations no. 2, 3, 6, 32–34, 37) and Veľká Fatra Mts (populations no. 7–10, 19–25, 55, 58) (Deyl 1938: 24, 1946: 154, Bělohlávková 1980: 25–27, Lysák 1996: 86–87, 107–108). In all the populations sampled, tetraploid and octoploid individuals grew more or less separately and did not form karyologicaly mixed populations (Table 1). The same situation is recorded also on the Balkan Peninsula for tetra- and octoploids of *S. filifolia* and *S. rigida* s.l., where there are neither odd-ploidy level cytotypes nor mixed-ploidy populations (Kuzmanović et al. 2013). Despite the lack of hexaploid individuals in areas where *S. caerulea* and *S. tatrae* grow in sympatry, we cannot exclude the possibility that these two species hybridize. The formation of hybrid plants may be prevented by a postzygotic reproductive barrier, or hexaploid individuals are very rare and detectable only by a much more detailed study, or hybrid individuals could theoretically arise by non-reduced (4x) gametes of *S. caerulea* fusing with reduced (4x) gametes of *S. tatrae* to form octoploid hybrid individuals. Although, hybridization between *S. caerulea* and *S. tatrae* cannot be completely excluded, this study revealed that it is unlikely to be as common as previously assumed.

Morphological variation of Sesleria caerulea and S. tatrae

Our analyses show (Table 4, Electronic Appendix 2) that *S. caerulea* and *S. tatrae* are morphologically very similar, and significant morphological differences can be found only at the population level (Fig. 2). The two species differ mainly in quantitative characters that relate to their ploidy level, with larger values for characters generally found in the octoploid taxon, *S. tatrae*. This is a common trait of many other species of plants (Oliveira et al. 2004, De Las Mercedes Sosa et al. 2012). In the genus *Sesleria*, a similar correlation between ploidy level and size of some characters (anatomical characters of the leaves) is recorded for populations of *S. filifolia* (Kuzmanović et al. 2013).

The most reliable character for distinguishing the taxa studied is the density of hairs between the veins on the abaxial surface of a lemma (Fig. 9), which in *Sesleria tatrae* is often densely hairy, although plants with an almost complete lack of hairs between the lemma veins were recorded (2.8% of those measured). On the other hand, the lemmas of *S. caerulea* are clearly glabrous or only sparsely hairy (see Table 2, Electronic Appendix 3 and 4, and identification key below). Length of lemma, glume and palea can also be used for distinguishing between these species, however, the values for these characters should only be considered after determining the average for a population (see Table 2 and 4, and identification key below). Deyl (1938: 42, 1980: 174) and Dostál (1958: 874, 1989: 1350) previously also used some of these four characters to identify these two species. In addition to these characters, some authors have identified other characters, such as the width of an inflorescence (e.g. Deyl 1938: l.c., 1980: l.c.), length of a lemma awn (Deyl 1938: l.c.) and palea indument (e.g. Deyl 1938: l.c., 1946: 80, Dostál 1958: l.c.), as useful for delineating the species. Although there are interspecific differences in the width of an inflorescence and length of lemma awn, these characters are not pronounced and thus not suitable for distinguishing between the two species (Table 4). We did not assess palea indument in this study, but preliminary field observations indicated that these species could be distinguished using this character. Deyl (1980: l.c.) states that *S. caerulea* and *S. tatrae* differ also in the width of basal lamina (Electronic Appendices 3 and 4), however, this character in the two species did not differ significantly in this study (Table 4).

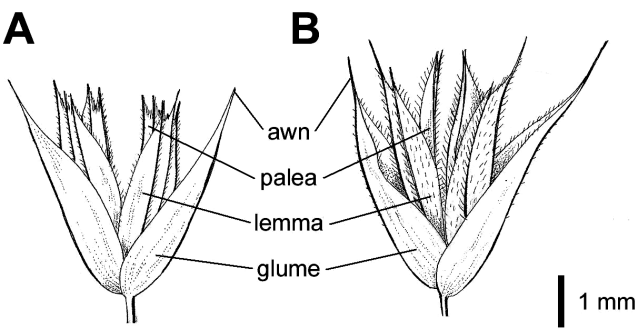

Fig. 9. – Floret of (A) *Sesleria caerulea* and (B) *S. tatrae*: (a) glume, (b) lemma, (c) palea, (d) awn.

Our research has shown that characters identified as diagnostic vary widely within both of the species studied. Several of the trait values presented here lie outside the range of values most commonly cited in the literature (see Electronic Appendices 2 and 3). In studies by Deyl (1980) and Dostál (1989), nominate subspecies of S. *caerulea* had leaves with 17–19 veins. In our data set, however, leaves have (8–) 12–21 (–30) veins, and for 70% of the plants studied, the number of veins did not come within the range of variation cited in these studies. Furthermore, these studies (Deyl 1980, Dostál 1989) identified 17 or more veins as a common character of *S. tatrae*, but our study identified 15 veins as the most common value (and 77.4% of plants had ≤ 17 veins). Some of the *Sesleria* characters, such as leaf width or number of leaf veins, depend to a large extent on the habitat (Deyl 1980, Dostál 1989). The range of variation in morphological characters of *S. caerulea* and *S. tatrae* recorded in this study was often so large that differentiation of the species was blurred in many cases and identification was questionable. For example, the length of the uppermost stem leaf is up to 10 mm in *S. caerulea*, whereas in the related species *S. uliginosa* it is between 10 and 20 mm (Deyl 1980: 176). In our measurements, the length of the uppermost leaf of *S. caerulea* was (1.1–) 4.9–14.5 (–33) mm, and up to 29.9% of the plants of *S. caerulea* studied would be misidentifed as *S. uliginosa* using the reported values.

Based on our results, *S. tatrae* is more similar in its morphology to *S. sadleriana* than previously documented. Length of the uppermost leaves is one of the morphological characters commonly used for distinguishing between *S. sadleriana* and *S. tatrae*. Deyl (1980) and Dostál (1989) report a length of 0.8–1.5 cm for *S. sadleriana* and 2–4 cm for *S. tatrae*. In contrast, our study revealed a leaf length of (0.22–) 0.80–2.17 (–4.75) cm for *S. tatrae*, which falls outside the previously documented range for this species. Of all individual plants of *S. tatrae* measured, only a handful (~15%) of them exceeded 2 cm in length, while more than half (56.6%) of measured plants corresponded with values traditionally reported for *S. sadleriana* (Deyl 1980, Dostál 1989).

Ecological requirements of Sesleria caerulea and S. tatrae

This study revealed important differences in the ecological requirements of the two species studied. The main ecological factors that differentiate the habitats of *S. caerulea* and *S. tatrae* are mostly associated with altitude, moisture, temperature, light availability and nutrient levels (Figs 4–8). Results of the numerical classification and indirect analyses also revealed that communities with *S. caerulea* are more diverse than those with *S. tatrae*. Greater community diversity is most likely associated with the ecological breadth of *S. caerulea*, which commonly occurs at sites that differ from each other in terms of habitat quality and land use (Reisch 2002, Reisch & Poschlod 2003, Budzáková & Šibík 2012). This species is phenotypically plastic and able to grow in a wide range of habitat conditions, which complicates our understanding of species' habitat requirements and makes species identification more challenging.

As documented in this study, *S. caerulea* mostly occurs at low altitudes (colline and submontane belts), whereas *S. tatrae* occurs more commonly over a narrow range of altitudes in the subalpine belt (Table 1, Fig. 5). These results agree with those of Rychlewski (1955), who states that *S. tatrae* grows abundantly at altitudes between 1400 and 1500 m a.s.l. in the Polish High Tatras. A similar vertical differentiation of tetra- and octoploid

plants is recorded for *S. filifolia* on the Balkan Peninsula, with the higher polyploids growing at high altitudes (Kuzmanović et al. 2013). *Sesleria caerulea* occurs at a wide range of altitudes. The altitudinal threshold of this species is high and it reaches its highest altitudes in the Alps (Table 1). Therefore, we can infer that altitude is not the primary determinant of its ecological distribution, which is most likely driven by characteristic conditions of the microsites it occupies.

The population of *S. tatrae* in the Sudetes (pop. 14, Śnieżnik Kłodzki Mts, 845 m a.s.l.) is an interesting exception in terms of its typical geographic location and altitude. This population is in a very isolated geographical position in the species range and at unusually low altitude. Researchers have developed a few hypotheses to explain the atypical occurrence of this population. One hypothesis suggests that this population is autochthonous and has been isolated from the Carpathians populations for a long time, while another hypothesis proposes a more recent and allochthonous occurrence (Fabiszewski 1970). Finally, it is not possible to rule out an autopolyploid origin from Sudetes *S. caerulea* tetraploids. Currently, however, the available data is insufficient to distinguish between these hypotheses.

Microclimate conditions also determine each species' presence or absence at a given location. Our investigation found that *S. caerulea* colonizes steeper, drier slopes and is more resistant to drought. The ability of this species to tolerate both dry as well as wet conditions is recorded (Dixon 1986, 1996). It is hypothesized that *S. caerulea* is typically a hygrophyte that has recently adapted to grow in xerophilic conditions in the subalpine and alpine zones (Pignatti & Pignatti 1975). Conversely, *S. tatrae* dominates on gentler slopes with more moist soils and, in comparison with *S. caerulea*, is more restricted to humid conditions.

Temperature and light availability are also important determinants of the two species' occurrence. In this study, *S. caerulea* was recorded in habitats with high temperatures. Similarly, Dixon (1982) identified *S. caerulea* as a highly eurythermal species that can tolerate a wide range of temperatures. *Sesleria tatrae*, on the other hand, was mainly recorded in open areas at high altitudes, where characteristically air temperatures are low and light intensities high. Both species studied also appear to thrive under different soil nutrient conditions. *Sesleria caerulea* usually dominates on steeper slopes with shallow leached soils. It is likely that *S. tatrae* requires a greater level of nutrient availability, as this species commonly occurs in areas with moderate slopes and deeper soils, which presumably have a higher nutrient content.

In the Western Carpathians, both *S. caerulea* and *S. tatrae* occur predominantly on basiphilous substrates, such as limestone and dolomite (Deyl 1936, Dixon 1982, Lysák & Doležel 1998). Dixon (1982) gives pH values for *S. caerulea* of between 5.5 and 8.0. Our study using Ellenberg's indicator values confirmed that this species prefers habitats on more alkaline soils with high pH values. Deyl (1936) previously established the soil conditions preferred by *Sesleria tatrae*, which occurs on deeper soils with a wide range of pH values (between 5.2 and 8.5) but have the greatest vitality when growing in neutral to slightly alkaline soils. The ability of *S. tatrae* to grow in more acid soils than *S. caerulea* is supported by our study. *S. tatrae* was also recorded growing on mylonite bedrocks, which are poorer in mineral content in comparison with limestone and dolomite, but the soils are generally base-rich. Deyl (1936) assumes that this species is not restricted to a specific soil pH but rather depends on soil substrate, which is usually limestone or dolomite.

Systematic position of the octoploid Sesleria population at Holíš hill

One of the interesting discoveries of this study was the occurrence of octoploid plants at Holíš hill (pop. 80, Javorníky Mts, 530 m a.s.l.). In view of (i) the atypical nature of the habitat of the population at Holíš hill compared to that of other sites with *S. tatrae*, (ii) the occurrence of octoploid plants growing in similar conditions on Vršatec hill at a distance of only 18 km, which were indentified by Lysák et al. (1997) as *S. sadleriana* and (iii) the morphological similarity of *S. tatrae* and *S. sadleriana* mentioned above (several authors have also suggested the possibility that *S. sadleriana* and *S. tatrae* originated from a common ancestor; e.g. Deyl 1938: 26, 1946: 154, Lysák & Doležel 1998: 130) a few hypotheses of the systematic position of *Sesleria* plants at Holíš hill come into consideration. These plants could represent: (i) true *S. tatrae* (and thus the lowermost occurrence of a population of this species, with a unique ecology and phytocenology), (ii) *S. sadleriana*, (iii) the hybrid *S. sadleriana* × *S. tatrae*, or potentially (iv) hybridogenous population originating from the fusion of non-reduced gamets of *S. caerulea* × reduced-gamete of *S. tatrae* or *S. sadleriana*. This population needs further study in order to clarify the relationship of these plants.

The history of discovery and typification of Sesleria tatrae

The discovery of *S. tatrae* dates back to September 1905 when the Hungarian botanist Árpád Degen visited the Belianske Tatry Mts, which were in Hungary at that time (Degen 1906). During his trips, Degen collected *Sesleria* specimens from cliff "Vaskapu" (= Mt. Skalné vráta) at the end of the "Drechslerhäuschen" valley (= Dolina Siedmych prameňov valley) (BP 729909) and at the "Kopa pass" (= Kopské sedlo saddle) (BP 729910) on 9 and 13 September 1905, respectively. Although Degen labelled both specimens as *S. bielzii*, he found these plants, as well as those previously collected by other botanists (Fritze, Wetschky) in the Tatra Mts, remarkable. Degen's particular interest is expressed in his handwritten notes attached to specimen BP 729908, which was collected by Wetschky in the "Drechslerhäuschen" valley in 1872. Degen asked Eduard Hackel, a world expert on grasses at that time, to investigate the freshly collected material. As documented in the notes on the specimens, Hackel identified Degen's first specimen (BP 729909) as *S. caerulea* and the second sample (BP 729910) as intermediate between *S. caerulea* (s.l.) and *S. bielzii*. Next year (1906), many of the conspicuous "intermediate" *Sesleria* plants growing at Kopa pass were collected for the exsiccata of "Gramina Hungarica", which was controlled and edited by Degen himself (Priszter 1978). The ominous taxon, named by Degen as *S. bielzii* f. *tatrae*, was eventually distributed in multiple specimens within the 7th pack of this exsiccata series (Nr. 313) in 1911 (Kümmerle 1912, Priszter 1978). Important to our study, the printed labels on *S. bielzii* f. *tatrae* are accompanied by a Latin diagnosis.

Up to the present day, a widely held, albeit incorrect, belief of botanists (e.g. Deyl 1938: 41) was that the aforementioned basionym of *S. tatrae* (i.e. *S. bielzii* f. *tatrae*) was first validly published by Jávorka (1924: 84). According to the Code (McNeill et al. 2012), however, the name *S. bielzii* f. *tatrae* was effectively (Art. 30.7) and validly (Art. 39.1) published on the printed label of Degen's "Gramina Hungarica" (Nr. 313), and all the information on the label, therefore, automatically constitutes the protologue of this name (published in 1911). Additionally, since the relevant data (collector, collecting date, locality)

of this sample are on the label (i.e. the details are cited in the protologue), all specimens from this gathering are syntypes and have equal status for typification.

During our studies, we have ascertained that, despite a few attempts, the name *S. bielzii* f. *tatrae* has not yet been correctly typified. In online plant databases (ITHAKA 2014, Herbarium WU 2014), we found a specimen (GZU 000282700) collected by Klášterský and Deyl in the Malá Fatra Mts in 1935 that bears a label reading "typus". Although we are not aware that this typification was effectively published, the specimen should be considered as neotype, which has no standing in the presence of the original material (see below). We also found four duplicates of Degen's "Gramina Hungarica" (Nr. 313) in W, which were designated as "typus" by L. Pignotti in October 2011. Nevertheless, this typification has not been effectively published (Pignotti, in litt. 2013). Consequently, a lectotype designation from among the existing syntypes is required and is given below.

Sesleria tatrae (Degen) Deyl, Acta Musei Nationalis Pragae 1 B No. 3: 41, 1938.

B a s i o n y m: *Sesleria bielzii* f. *tatrae* Degen. **Lectotype (designated here)**: [SLOVAKIA], Alpes Belaënses, in saxosis herbosis jugi Kopa [Belianske Tatry Mts, Kopské sedlo saddle, stony places in grassland vegetation], alt. c. 1700 m. s. m., sol. calc., 23 June 1906, I. Győrffy ["J. Györffy"] (W 1916-0038514, exsiccate specimen of "Gramina Hungarica" no. 313). The lectotype is accessible in the online plant database of Herbarium WU (2014) and shown here in Electronic Appendix 5.

I s o l e c t o t y p e s: BP 236091, BP 236092, BP 239136, W 1916-0032711, W 1916-0032712, W 1958-0006252, and presumed duplicates deposited in other herbaria, which were not studied by us. Further original material: Poland, Tatry Zachodnie Mts, Mt. Giewont, 8 Sep 1865, R. Fritze (BP 593347, BP 593348, BP 593370); Slovakia, Belianske Tatry Mts, Drechslerhäuschen valley [Dolina Siedmych prameňov valley], 10 Aug 1872, M. Wetschky (BP 729907, BP 729908); Slovakia, Belianske Tatry Mts, Kopa pass [Kopské sedlo saddle], 13 Sep 1905, A. Degen (BP 729910).

Key to the species of *Sesleria caerulea* and *S. tatrae* (Fig. 9)

1a Lemma glabrous or sparsely ciliate between veins [with 0–3 (–8) hairs per 0.04 mm^2], (3.0–) 4.0–5.3 (–6.5) mm long (including awn); glume (2.8–) 3.8–5.5 (–6.9) mm long (including awn); 2n = 28 ..... ***S. caerulea***

1b Lemma usually densely ciliate between veins [with (0–) 2–11 (–16) hairs per 0.04 mm^2], (3.3–) 4.6–6.1 (–7.0) mm long (including awn); glume (3.60–) 4.50–6.50 (–7.59) mm long (including awn); 2n = 56 ..... ***S. tatrae***

See www.preslia.cz for Electronic Appendices 1–5

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Souhrn

Tetraploidní (2n = 4x = 28) *Sesleria caerulea* a oktoploidní (2n = 8x = 56) *S. tatrae* jsou blízce příbuzné taxony, které nalézáme na mnoha stanovištích s různými ekologickými podmínkami. Někteří jedinci jsou často stěží rozeznatelní a předpokládá se, že atypické rostliny, které jsou nalézané v některých regionech, jsou výsledkem

křížení těchto druhů. V důsledku toho jsou i ekologické nároky obou druhů a jejich fytocenologické vazby pořád nejednoznačně známé. Cílem předložené studie je prozkoumat karyologickou, morfologickou a ekologickou diferenciaci těchto druhů. Celkem 877 rostlin z 68 populací ze Západních Karpat (Slovensko a hraniční pásmo s Polskem), Alp (Rakousko, Itálie, Francie) a Sudet (Polsko) bylo podrobeno karyologické a morfometrické analýze. Kromě toho byly v každé lokalitě pořízeny fytocenologické snímky a Ellenbergovy indikační hodnoty byly použity jako doplňkové proměnné prostředí. Všechny analyzované rostliny *S. caerulea* se ukázaly být tetraploidní, rovněž všechny rostliny *S. tatrae* oktoploidní. Žádná přechodná ploidie mezi rostlinami, která by poukazovala na hybridizaci druhů nebyla zaznamenána. Morfometrické analýzy dokázaly, že 8 z 28 studovaných morfologických znaků prokázalo signifikantní rozdíly mezi oběma druhy. Odění pluchy mezi žilkami, délka pluchy, délka plušky a délka plevy jsou znaky, které lze spolehlivě použít jako diagnostické znaky druhů. Nicméně, oba druhy jsou velmi podobné a morfologické rozdíly lze nalézt pouze na úrovni populace. Důležité rozdíly mezi studovanými taxony byly nalezeny také v ekologických nárocích druhů. Zjištěné ekologické rozdíly souvisely především s nadmořskou výškou, vlhkostí, teplotou, osvětlením stanoviště a množstvím živin. V rámci obou studovaných taxonů, *Sesleria caerulea* vykazovala vyšší úroveň ekologické variace. Obecně upřednostňuje strmější slunnější a sušší stanoviště v nižších nadmořských výškách. *Sesleria tatrae* se na rozdíl od předešlého druhu vyskytuje spíše ve vyšších polohách a dominuje ve vlhčích stanovištích se zvýšenou světelnou expozicí a na hlubších půdách bohatých na živiny.

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