Interspecific hybridization between rare and common plant congeners inferred from genome size data: assessing the threat to the Czech serpentine endemic *Cerastium alsinifolium*

Mezidruhová hybridizace mezi vzácným a hojným druhem, zjištěná na základě dat o velikosti genomu – zhodnocení ohrožení českého hadcového endemita *Cerastium alsinifolium*

Petr V í t[1,2], Kateřina W o l f o v á[1,2], Tomáš U r f u s[2,1], Přemysl T á j e k[3] & Jan S u d a[2,1*]

[1]*Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice 1, CZ-252 43 Průhonice, Czech Republic;* [2]*Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-128 01, Czech Republic, e-mail: suda@natur.cuni.cz;* [3]*Administration of the Slavkovský les Protected Landscape Area, Hlavní 504, CZ-353 01 Mariánské Lázně, Czech Republic*
**corresponding author*

Vít P., Wolfová K., Urfus T., Tájek P. & Suda J. (2014): Interspecific hybridization between rare and common plant congeners inferred from genome size data: assessing the threat to the Czech serpentine endemic *Cerastium alsinifolium*. – Preslia 86: 95–117.

Cerastium alsinifolium Tausch (*Caryophyllaceae*) is an endemic species restricted to serpentine sites in the Slavkovský les Mts (western Bohemia) in the Czech Republic. Interspecific hybridization with sympatric *C. arvense* L. has long been suspected due to the substantial and continuous morphological variation observed in the field but it has never been reliably confirmed. Although both parental species share the same number of somatic chromosomes they differ considerably in the size of their monoploid nuclear genomes (~1.5-fold), which makes it easy to identify the species. Flow cytometric investigation of more than 2200 *Cerastium* samples revealed five distinct genome size categories, corresponding to the two parental species and three types of interspecific hybrids (originating via both reduced and unreduced gametes). F1 interspecific hybrids were very common (nearly 40% of the samples analysed from the Slavkovský les Mts), which indicates the barriers to breeding between the parental species are weak. However, no backcrosses were indicated by the genome size data. In contrast to a widely held view that *C. alsinifolium* mostly occurs on open serpentine outcrops, this habitat was dominated by interspecific hybrids. The endemic species occurred mainly in moist and (semi-)shaded sites, including springs in spruce forest clearings, seeps and wet margins of forest roads. Multivariate morphometrics revealed that the shape and size of cauline leaves, development of sterile axillary shoots, bract characteristics, and lengths of calyx, petals and anthers are diagnostic for the groups investigated. While the determination of *C. arvense* usually poses few problems, distinguishing *C. alsinifolium* from interspecific hybrids on the basis of morphological characters is much more challenging; reduced pollen fertility of hybrids provides the most important clue. Our results indicate that effective conservation of this important component of the Czech flora will require more emphasis on the conservation of forest sites that host core populations of *C. alsinifolium*.

K e y w o d s: *Cerastium*, conservation, Czech Republic, endemic, flow cytometry, genome size, interspecific hybridization, multivariate morphometrics, serpentine

Introduction

Interspecific hybridization is a common and ongoing process in populations of land plants, with many important evolutionary consequences (Soltis & Soltis 2009). There are two opposing views on the role of hybridization in plant speciation. Whereas interspecific

hybridization (often connected with genome duplication) can be a source of genetic and phenotypic novelties, ultimately leading to the origin of a new species (i.e. hybrid speciation), it can also cause genetic dilution, breakdown of a species integrity and possibly species extinction. The detrimental effect of hybridization is pronounced in rare species (or species with insular-like distributions) because they usually occur at low densities and might be surrounded by larger populations of related congeners with incomplete breeding barriers. Among other factors, hybridization can contribute to the demise of rare species through the production of hybrid seeds at the expense of conspecific seeds and hybrid competition for abiotic or biotic resources (Levin et al. 1996). In addition to the demographic swamping, extensive gene flow will also influence the genetic make-up of the species by the disruption of coevolved gene complexes and affecting genetic adaptation to local environmental conditions (Givnish 2010).

One of the challenges posed by interspecific hybridization is the difficulty of recognizing it, especially when closely related species with similar phenotypes are involved. While only parental species and sterile F1 hybrids are present in some cases, complex hybrid swarms can occur in other populations, consisting of a mixture of parents, hybrids of different generations and backcrosses to parental species (Krahulcová et al. 1996, Oberprieler et al. 2010). Morphological variation in hybrid populations can span a continuum from one parent to the other, which precludes reliable determination of individual plants on the basis of phenotypic characters. Molecular markers can assist greatly in the identification of crosses and provide an insight into the dynamics of hybridization (Hegarty & Hiscock 2005). However, molecular analyses take a long time and are costly, making comprehensive population studies impractical. Cytogenetic data can also aid hybrid identification in some cases, providing the parental taxa differ in the number of somatic chromosomes or genome size (Ekrt et al. 2010, Suda et al. 2010). The field of cytotaxonomy has recently been revitalized by the advent of flow cytometry (FCM), which has made it possible to screen whole populations at large spatial and temporal scales (Kron et al. 2007, Loureiro et al. 2010, Suda & Pyšek 2010).

An illustrative example of a rare and severely threatened species in the Czech flora, whose populations have most likely been affected by interspecific hybridization, is the serpentine endemic of western Bohemia, *Cerastium alsinifolium* Tausch (Smejkal 1990, Kaplan 2012). The total area occupied by this species does not exceed 15 km^2, with all sites situated within the Protected Landscape Area Slavkovský les (Tájek et al. 2012). *Cerastium alsinifolium* ranks among the critically endangered plants in the Czech flora (Klaudisová & Čeřovský 1999, Grulich 2012, Kaplan 2012) and is listed as a priority species in Annex II of the European Commission Habitats Directive and in Appendix I of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention). It is classed as Data Deficient in the IUCN Red List of Threatened Species (IUCN 2013). Of the 74 plant taxa considered to be endemic to the Czech Republic (Kaplan 2012), *C. alsinifolium* has a prominent position because it is: (i) sexually reproducing (the majority of other endemics are apomictic microspecies), (ii) phenotypically and ecologically distinct (several other endemics are morphologically rather poorly defined taxa, often recognized at intra-specific ranks) and (iii) historically the first described (and still accepted) endemic plant, distinguished as early as 1828 (Tausch 1828).

The origin and evolutionary past of *Cerastium alsinifolium* is a matter of speculation. Although the majority of authors (e.g. Čelakovský 1873, Dostál 1989, Jalas et al. 1993) believe that *C. alsinifolium* is closely related to *C. arvense* L., Novák (1960) placed *C. alsinifolium* in the arcto-alpine *C. alpinum* agg. Currently, *C. alsinifolium* is reported from two rather contrasting types of habitat on serpentine bedrocks, namely dry open grassland on rocky outcrops and (semi)shaded springs and seeps in coniferous forests (Melichar 2005). At several sites in the Slavkovský les Mts, it cooccurs with another perennial large-flowered species, *C. arvense*, which is widely distributed in Europe and usually inhabits dry grasslands and/or semiruderal sites (Smejkal 1990). *Cerastium arvense* is relatively tolerant of soils with a high heavy metal content (Levine & Greller 2004) and in the Slavkovský les Mts it occasionally grows on outcrops of serpentine or in their immediate vicinity. Length of sepals, leaf shape and bract characteristics are considered the most important characters for the recognition of both species (Smejkal 1990, Hrouda 2002). In addition to the individuals that match the original description, there is a continuum of intermediate morphotypes in the Slavkovský les Mts, which indicate extensive interspecific hybridization (Smejkal 1990, Klaudisová & Čeřovský 1999, Rybka et al. 2004). However, the mostly quantitative species-specific morphological characters and the same number of somatic chromosomes in both species (2n = 6x = 72; Uhríková & Záborský 1980, Smejkal 1990, Měsíček & Jarolímová 1992) preclude unambiguous identification of hybrid individuals using conventional phenotype-based techniques or chromosome counting and another means of identification is needed. Despite the identical chromosome number, we found that *C. alsinifolium* and *C. arvense* differ markedly in the nuclear DNA content, which opens possibilities for a detailed examination of the structure of populations and determining the frequency of interspecific hybridization.

The main aim of this study was to assess the threat of interspecific hybridization to the survival of a rare serpentine endemic of the Czech flora, *Cerastium alsinifolium*. We utilized interspecific differences in genome size, and using DNA flow cytometry and multivariate morphometrics addressed the following questions: (i) What is the structure of the populations (proportion of hybrid individuals) at the different serpentine sites? (ii) What is the frequency of interspecific hybridization? Does data on genome size support the presence of only F1 hybrids or a more complex hybridization pattern? (iii) What are the species- and hybrid-specific phenotypic characters? Can hybrid individuals be reliably identified on the basis of morphology? (iv) Which localities host the most vigorous populations of *C. alsinifolium* and are hence of priority importance for conservation? Are the current conservation and management measures optimal for the proper protection and preservation of this endemic species or should they be revised?

Materials and methods

Field sampling

A thorough sampling of the population was done at five main localities (two open serpentine outcrops and three forest sites) in the Slavkovský les Protected Landscape Area (Electronic Appendix 1) that altogether host a substantial proportion of plants previously classified as *C. alsinifolium*. The sampling covered the entire range of microhabitats and included all the phenotypes present at these localities. A mature leaf from a total of 2086

Cerastium plants was collected for the FCM analysis. For comparative purposes, 136 individuals of *C. arvense* from 20 localities in the Czech Republic not in the Slavkovský les Mts were also included in this study (see Electronic Appendix 1 for locality details). A subset of 616 plants of known genome sizes, representing four recognized taxonomic groups (two parental species and two types of interspecific hybrids), was subsequently selected for morphometric analyses. Pressed flowering/fruiting shoots and flowers stored in 70% ethanol were collected for each individual. Herbarium vouchers are deposited at PRC (herbarium of Charles University in Prague).

Pollen viability was estimated for 174 individuals from the Slavkovský les Mts using the protocol detailed by Peterson et al. (2010). Anthers were collected before anthesis, fixed in Carnoy's fixative in the field and kept at room temperature until processed less than 2 months later. Two anthers per individual were then dissected on a microscopic slide, stained, and the slides were observed using a light microscope (Olympus BX41) at a 100× magnification. Two hundred pollen grains per sample were assessed. Because pollen viability was determined using different individuals than used in the morphometric analyses, this character was evaluated separately.

Flow cytometry

Genome sizes (2C-values; Greilhuber et al. 2005) were estimated using propidium iodide flow cytometry following the simplified two-step protocol using Otto buffers (Doležel et al. 2007). Briefly, ~0.5 cm^2 of intact leaf tissue per analysed plant and an appropriate volume of the internal reference standard (*Glycine max* 'Polanka', 2C = 2.50 pg; Doležel et al. 2007) were chopped up using a sharp razor blade in a Petri dish containing 0.5 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween-20; Otto 1990). The suspension was filtered through a 42-μm nylon mesh and incubated for ~30 min at room temperature. The staining solution consisted of 1 ml of Otto II buffer (0.4 M $Na_2HPO_4.12\ H_2O$) supplemented with β-mercaptoethanol (final concentration of 2 μl/ml), propidium iodide and RNase II type IIA (both at a final concentrations of 50 μg/ml). After a short incubation, the samples were analysed using a Partec CyFlow instrument (Partec GmbH., Münster, Germany) equipped with a green (532 nm, 100 mW output) diode-pumped solid state laser. Fluorescence intensity of 5000 particles was recorded. Histograms were evaluated using Partec Flomax (ver. 2.4b). Only analyses with coefficients of variation for the G0/G1 *Cerastium* peaks below 4.0% were considered. Bulk samples of up to five individuals were processed during a large-scale population screening; mixed samples (i.e. consisting of plants with different fluorescence intensities) were reanalysed separately. One plant per fluorescence category from each locality studied in the Slavkovský les Mts was then selected and its absolute genome size estimated based on three replicates on different days. Differences in genome size values were tested using a general linear model (GLM, due to unbalanced data design) in the SAS 9.2 package (SAS Institute, Cary, NC, USA).

Multivariate morphometrics

Seventy quantitative features (incl. ratios) of vegetative and generative characters (for details see Table 1 and Electronic Appendix 2) were measured and calculated for 616 individuals, including 206 individuals of *Cerastium alsinifolium*, 191 individuals of *C. arvense* (95 and 96 from the Slavkovský les Mts and beyond, respectively), and 219

Table 1. – List of the morphological characters analysed and their contributions to the first canonical axis in canonical discriminant analyses of (i) *Cerastium arvense* vs. *C. alsinifolium* + interspecific hybrids (616 samples, 69 characters), and (ii) *C. alsinifolium* vs. interspecific hybrids with *C. arvense* (425 samples, 69 characters). Canonical correlates with the highest absolute loadings that were selected for the determination key are highlighted in bold type. Numbers in parentheses represent ranks of the strength of the correlation of each variable with the canonical axis. Character v41 was excluded from discriminant analyses because of its very strong correlation with v40.

No.	Character description	Unit	*C. arvense* vs others	*C. alsinifolium* vs hybrids
Leaf and bract characters				
v1	Stem length (excluding inflorescence)	mm	0.5399 (21)	–0.0404 (69)
v2	Number of sterile shoots in leaf axils along the entire length of the stem	number	**0.7312 (8)**	0.1406 (55)
v3	Length of the uppermost leaf	mm	0.6875 (12)	0.1220 (58)
v4	Width of the uppermost leaf	mm	–0.2769 (46)	–0.4246 (28)
v5	Length of hairs on the margin of the uppermost leaf	mm	–0.3584 (34)	0.0219 (67)
v6	Number of hairs on the margin of the uppermost leaf (per 10 mm)	number	0.1642 (58)	0.3461 (34)
v7	Angle of the tip of the uppermost leaf	degree	–0.5603 (19)	**–0.6278 (9)**
v8	Length of the second uppermost leaf	mm	**0.7794 (2)**	0.2827 (41)
v9	Width of the second uppermost leaf	mm	–0.2796 (44)	–0.3259 (35)
v10	Length of hairs on the margin of the second uppermost leaf	mm	–0.1811 (53)	0.0904 (60)
v11	Number of hairs on the margin of the second uppermost leaf (per 10 mm)	number	0.2793 (45)	0.5260 (20)
v12	Angle of the tip of the second uppermost leaf	degree	–0.6230 (16)	**–0.6423 (7)**
v13	Length of the third uppermost leaf	mm	**0.8003 (1)**	0.4071 (31)
v14	Width of the third uppermost leaf	mm	–0.2542 (47)	–0.2507 (43)
v15	Length of hairs on the margin of the third uppermost leaf	mm	0.1411 (60)	0.2232 (45)
v16	Number of hairs on the margin of the third uppermost leaf (per 10 mm)	number	0.4659 (26)	0.5271 (19)
v17	Angle of the tip of the third uppermost leaf	degree	–0.6207 (17)	–0.5699 (12)
v18	Length of the lowermost bract	mm	0.2153 (51)	–0.1995 (49)
v19	Width of the lowermost bract	mm	–0.0511 (66)	–0.2880 (40)
v20	Length of the scarious margin at the tip of the lowermost bract	mm	0.4114 (31)	0.4881 (22)
v21	Width of the scarious margin on the side of the lowermost bract	mm	0.4827 (25)	0.5553 (15)
v22	Length of the scarious margin of the lowermost bract	mm	0.6887 (11)	**0.6847 (2)**
v23	Length of hairs on the margin of the lowermost bract	mm	–0.0950 (62)	0.1499 (54)
v24	Number of hairs on the margin of the lowermost bract (per 10 mm)	number	0.0755 (64)	0.3895 (33)
v25	Angle of the tip of the lowermost bract	degree	–0.2847 (42)	–0.4159 (29)
v26	Length of the second lowermost bract	mm	0.3402 (37)	–0.0454 (64)
v27	Width of the second lowermost bract	mm	0.1406 (61)	–0.0376 (66)
v28	Length of the scarious margin at the tip of the second lowermost bract	mm	0.3759 (33)	0.4153 (30)
v29	Width of the scarious margin on the side of the second lowermost bract	mm	0.4529 (29)	0.5344 (18)
v30	Length of the scarious margin of the second lowermost bract	mm	0.6342 (15)	**0.6752 (4)**
v31	Length of hairs on the margin of the second lowermost bract	mm	–0.1777 (56)	–0.0713 (62)
v32	Number of hairs on the margin of the second lowermost bract (per 10 mm)	number	–0.0271 (68)	0.0779 (61)
v33	Angle of the tip of the second lowermost bract	degree	–0.1660 (57)	–0.1712 (53)
Flower characters				
v34	Calyx length (excluding the scarious margin)	mm	0.6996 (9)	0.4636 (24)
v35	Calyx width (excluding the scarious margin)	mm	0.4627 (27)	0.2533 (42)
v36	Length of the scarious margin at the tip of the calyx	mm	0.3280 (39)	0.2129 (47)

v37 Length of the scarious margin on the side of the calyx	mm	0.4183 (30)	0.2228 (46)
v38 Petal length	mm	**0.7718 (4)**	–0.0558 (63)
v39 Petal length to the notch	mm	0.6851 (13)	–0.1356 (57)
v40 Petal width	mm	0.6982 (10)	0.2298 (44)
v41 Width of the lobe of a petal	mm	–	–
v42 Filament length	mm	0.5434 (20)	–0.2944 (39)
v43 Length of anther	mm	**0.7736 (3)**	–0.0419 (65)
v44 Width of anther	mm	0.4846 (24)	–0.4365 (27)
Fruit and seed characters			
v45 Capsule length on the convex side	mm	0.2797 (43)	–0.6093 (10)
v46 Capsule length on the concave side	mm	0.1797 (54)	**–0.6682 (5)**
v47 Capsule width	mm	0.3533 (35)	–0.4601 (26)
v48 Tooth length of a dehiscent capsule	mm	0.5281 (23)	0.5766 (11)
v49 Tooth width of a dehiscent capsule	mm	0.5843 (18)	0.1916 (50)
v50 Calyx length at fruiting stage	mm	**0.7391 (6)**	**0.6568 (6)**
v51 Seed length	mm	0.0466 (67)	0.3107 (37)
v52 Seed width	mm	0.2319 (50)	0.1403 (56)
Ratios			
v53 Length / width of the uppermost leaf		–0.6661 (14)	–0.5633 (13)
v54 Length / width of the second uppermost leaf		**–0.7345 (7)**	–0.5352 (17)
v55 Length / width of the third uppermost leaf		**–0.7685 (5)**	–0.5530 (16)
v56 Length / width of the lowermost bract		–0.3271 (40)	–0.1803 (51)
v57 Width of the scarious margin on the side / total width of the lowermost bract		0.3980 (32)	0.5622 (14)
v58 Length of the scarious margin / total length of the lowermost bract		0.5298 (22)	**0.6757 (3)**
v59 Length / width of the second lowermost bract		–0.2382 (49)	–0.0047 (68)
v60 Width of the scarious margin on the side / total width of the second lowermost bract		0.3397 (38)	0.4621 (25)
v61 Length of the scarious margin / total length of the second lowermost bract		0.4626 (28)	**0.6363 (8)**
v62 Length / width of the calyx		–0.1782 (55)	–0.0949 (59)
v63 Petal length to notch / total petal length		–0.1521 (59)	–0.1746 (52)
v64 Length / width of the petal		0.0101 (69)	0.2947 (38)
v65 Calyx length / petal length		0.0721 (65)	–0.4843 (23)
v66 Length / width of the anther		–0.1917 (52)	–0.4972 (21)
v67 Capsule length on the convex / concave side		–0.3439 (36)	–0.3114 (36)
v68 Length / width of the capsule		–0.0907 (63)	0.3978 (32)
v69 Capsule length / calyx length at fruiting stage		–0.3068 (41)	**–0.7758 (1)**
v70 Length / width of the seed		0.2396 (48)	–0.2039 (48)

individuals of interspecific crosses. Missing character values were replaced by population means provided that measurements for at least 90% of the individuals from a particular population were available. The set of morphological characters analysed was selected on the basis of published determination keys, flora handbooks (Smejkal 1967, Hegi & Weber 1975, Smejkal 1990, Hrouda 2002) and our own field observations. Morphometric data were analysed using CANDISC (canonical discriminant analysis), CORR (correlation analysis), DISCRIM (classification discriminant analysis), STEPDISC (stepwise discriminant analysis with forward or stepwise selection of characters), PRINCOMP (principal component analysis) and UNIVARIATE (basic statistics) procedures in SAS

9.2 following Rosenbaumová et al. (2004). Individual plants were used as operational taxonomic units (OTUs). First insights into phenetic relationships among OTUs were gained using principal component analysis (PCA) while discriminant analyses were employed to select a set of characters that allowed for the best separation of a priori defined groups of OTUs (i.e. species and hybrids characterized by their genome sizes) and to determine the proportion of correctly classified individuals. Because the data distributions within groups were not multivariately normal (Wilks-Shapiro test), the non-parametric k-nearest neighbour discriminant function and non-parametric correlation coefficients were employed. Discriminant power was determined by cross-validation (Klecka 1980). Various modifications of discriminant analyses were performed, including all four recognized taxonomic groups (parental species, hybrids originating from unreduced gametes and those originating via reduced plus unreduced gametes), three groups (with all hybrids merged into a single category), separate analysis of the two groups of hybrids, *C. arvense* vs. *C. alsinifolium* + hybrids, *C. alsinifolium* vs. *C. arvense*, C. *alsinifolium* vs. hybrids, etc. Flowering and fruiting plants were analysed together and separately. The determination key was largely constructed on the basis of results of discriminant analyses (characters most tightly correlated with canonical axes); characters that can be easily measured/observed in the field were preferentially selected.

Results

Variation in genome size

Flow cytometric analysis yielded high-resolution histograms with distinct peaks of both the plant sample(s) and internal standard, and with little background fluorescence signals (Fig. 1). The average coefficients of variation of the G0/G1 peaks of *Cerastium* samples and the internal reference standard were 2.65% (range 1.27–3.89%) and 3.02% (range 1.55–3.84%), respectively. Estimates of genome sizes of 2222 *Cerastium* plants resulted in five distinct categories, corresponding to the two parental species and three types of interspecific hybrids (Fig. 2). Individuals morphologically matching *C. arvense* from the Slavkovský les Mts did not differ in genome size from those collected elsewhere in the Czech Republic (GLM, $F = 0.79$, $P = 0.376$). Nuclear DNA contents of the serpentine endemic *C. alsinifolium* (mean 2C-value = 4.25 pg; Figs 1, 2) was about 1.5-times greater than that of *C. arvense* (mean 2C = 2.83 pg), enabling not only the reliable recognition of both parental species but also that of their interspecific crosses, including potential back-crosses. A considerable percentage of the samples (37.4%; Table 2) from the Slavkovský les Mts had intermediate genome sizes between the putative parental species, suggesting they were of hybrid origin. In addition, a few individuals (1.3%; Table 2) had genomes markedly larger than *C. alsinifolium*, with a mean value of 4.98 pg/2C. The most parsimonious explanation for these genome values is interspecific hybridization by means of an unreduced gamete of *C. arvense*. The highest 2C-value (5.43 pg) was recorded for one individual from population Vlček (Table 2), which most likely originated via a syngamy of a 2n gamete of *C. alsinifolium* and a reduced gamete of *C. arvense* (all crosses involving unreduced gametes are further referred to as "polyploid hybrids"). Clear discontinuities between the five genome size categories (Fig. 2) indicate the lack of back-crosses.

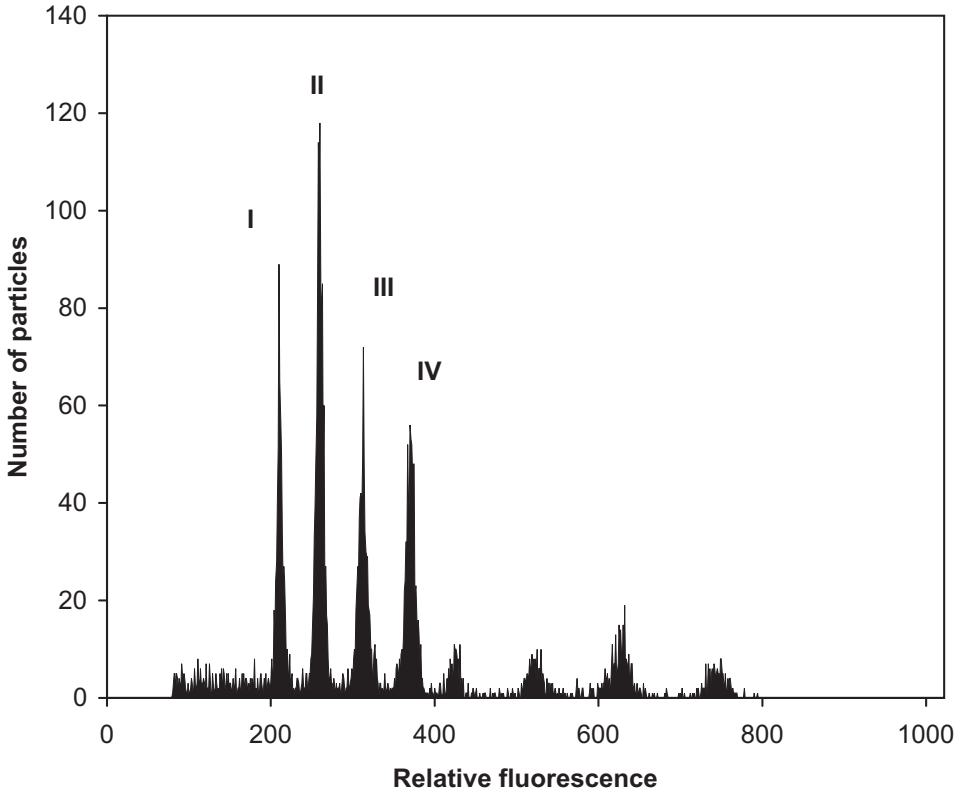

Fig. 1. – Simultaneous flow-cytometric analysis of *Cerastium* plants of four different holoploid genome sizes. Nuclei of all samples were isolated, stained with propidium iodide and analysed simultaneously. Peak designations: I – *C. arvense*; II – F1 hybrid (originating via unreduced gametes); III – *C. alsinifolium*; IV – polyploid hybrid (originating from an unreduced gamete of *C. arvense* and a reduced gamete of *C. alsinifolium*).

Ecological preferences

Representative samples from five serpentine localities revealed dramatic differences in the frequency of *C. alsinifolium* and interspecific hybrids in the two main types of habitat (Table 2). Whereas moist places in coniferous forests on serpentine bedrocks (spring areas in forest clearings, seeps, wet margins of forest roads, etc.) are dominated by *C. alsinifolium* (73.2–91.7% of the individuals sampled), open and dry serpentine outcrops are largely inhabited by interspecific hybrids and the endemic species only constitutes a minority of the individuals (10.6–15.7% of samples analysed). On rocky outcrops, *C. alsinifolium* clearly occurs mainly in sheltered and the most humid microhabitats, such as rock crevices covered with moss. In contrast, both hybrid plants and *C. arvense* are more heliophilous, competition- and drought-tolerant and usually occur in open short grassland.

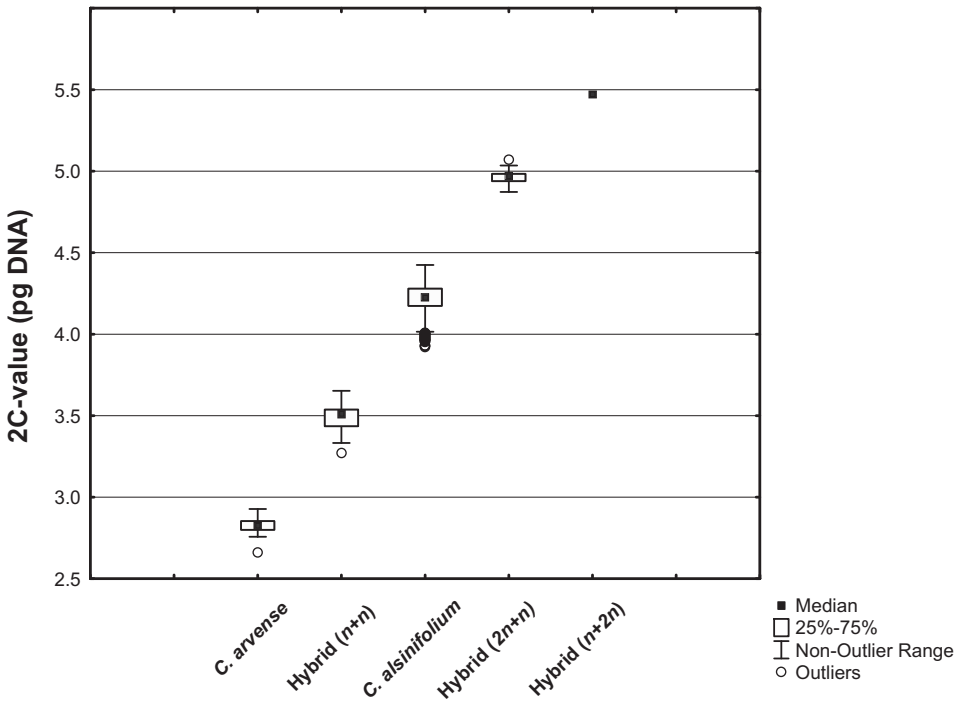

Fig. 2. – Box-and-whisker plots showing the holoploid genome sizes (2C-values) estimated for samples of large-flowered *Cerastium*, mainly from the Slavkovský les Mts. See Table 2 for number of samples in individual categories.

Table 2. – Numbers and percentages (in parentheses) of *Cerastium* samples corresponding to two parental species and three types of interspecific hybrids at five thoroughly investigated localities in the Slavkovský les Mts in western Bohemia. Habitats in coniferous forests represent moist places on serpentine bedrock.

Locality	Habitat	*C. arvense*	F1 hybrid	*C. alsinifolium*	Polyploid hybrid (2n *C. arvense* + n *C. alsinifolium*)	Polyploid hybrid (n *C. arvense* + 2n *C. alsinifolium*)
Dominova skalka	open serpentine outcrops	100 (19.4%)	325 (63.0%)	81 (15.7%)	10 (1.9%)	0
Křížky	open serpentine outcrops	94 (20.8%)	310 (68.6%)	48 (10.6%)	0	0
Pluhův bor	coniferous forest	12 (4.5%)	6 (2.3%)	244 (91.7%)	4 (1.5%)	0
Planý vrch	coniferous forest	0	109 (24.9%)	320 (73.3%)	8 (1.8%)	0
Vlček	coniferous forest	1 (0.2%)	30 (7.2%)	378 (91.2%)	5 (1.2%)	1 (0.2%)

Phenotypic variation

Correlation analysis using Spearman coefficients revealed one tightly correlated pair of characters of petals (total petal width and width of the petal lobe), therefore the latter character (v41) was excluded from the discriminant analyses.

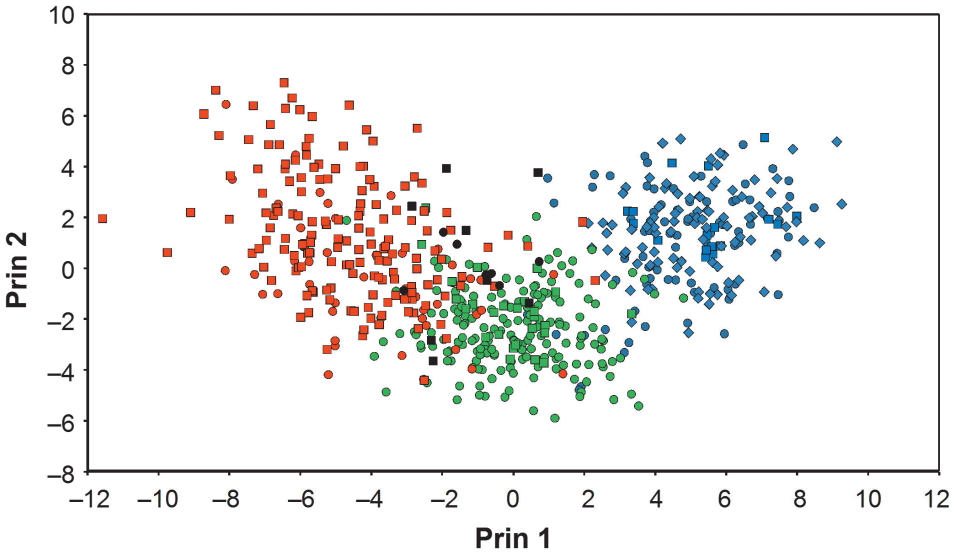

Fig. 3. – Principal component analysis of 616 *Cerastium* samples based on 70 characters (see Table 1 for character description). *C. alsinifolium* – red, *C. arvense* – blue, F1 hybrids – green, polyploid hybrids – black. Samples from open rocky outcrops and (semi)shaded forest habitats are depicted by circles and squares, respectively. Diamonds denote samples of *C. arvense* from outside the Slavkovský les Mts. The first and second PCA axes explain 26.3% and 9.8% of the total variation, respectively.

Principal component analysis of the entire data set revealed that the samples of *C. arvense* from the Slavkovský les Mts were very similar to those collected elsewhere, as were samples of *C. alsinifolium*/hybrids from different habitats within the serpentine area investigated (Fig. 3). Slight differences between plants from open rocky and (semi)shaded forest sites were mainly in stem length. Three distinct, though partially overlapping groups of characters were revealed by the PCA plot (Fig. 3). Interspecific hybrids occupied an intermediate position between their putative parents, but generally were somewhat closer to *C. alsinifolium*. Discriminant analysis of the same taxonomic groups (*C. alsinifolium*, *C. arvense* and interspecific hybrids) yielded a very similar picture (with better separated groups of OTUs; Electronic Appendix 3).

In order to follow the structure of dichotomous determination keys, we performed separate discriminant analyses on two groups of characters. Major morphological differences (characters most tightly correlated with the canonical axis) between flowering individuals of *C. arvense* and a group of *C. alsinifolium* + hybrids were revealed in the number of short sterile shoots emerging in axils of cauline leaves (character v2; see Table 1), lengths and shapes (length/width ratios) of the second and third uppermost leaves (v8, v13, v54, v55), length of the scarious margin of the lowermost bract (v22), petal length (v38) and anther length (v43) (Table 1, Fig. 4A). While the involvement of all measured and scored characters resulted in a misclassification of 14 out of 616 individuals (= 2.3%), the discrimination power was very similar when only the eight above-mentioned characters with the highest absolute canonical loadings were used (20 misclassified individuals out of 616; 3.2%). Fruiting individuals can most reliably be determined using differences in calyx length (v50), in addition to leaf and bract characters (results not shown). The taxonomic

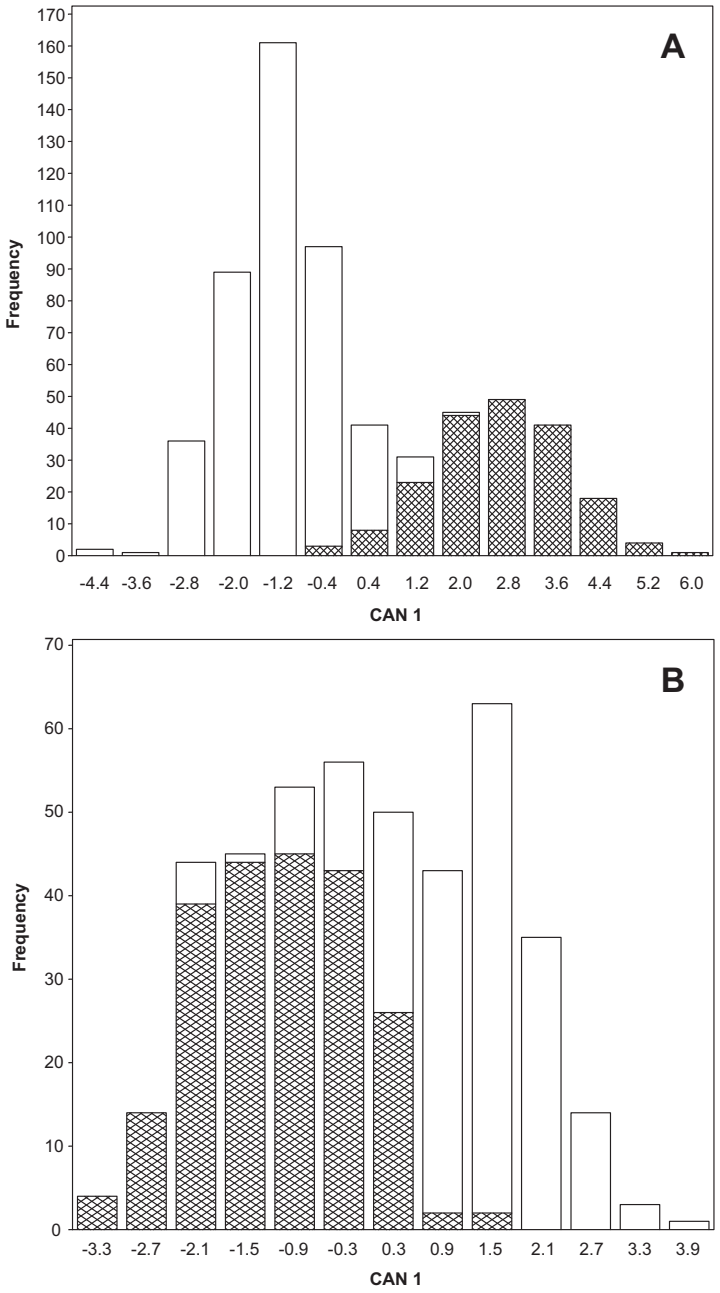

Fig. 4. – (A) Canonical discriminant analysis of a group of *Cerastium alsinifolium* + interspecific hybrids (open columns; 425 individuals) and *C. arvense* (hatched columns; 191 individuals). Eight taxonomically-important characters (v2, v8, v13, v22, v38, v43, v54, v55; see Table 1) were used for discrimination. The analysis resulted in 3.2% of the samples being misclassified. (B) Canonical discriminant analysis of *C. alsinifolium* (open columns; 206 individuals) and its hybrid with *C. arvense* (hatched columns: 219 individuals). Seven taxonomically-important characters (v22, v30, v53, v54, v55, v58, v61; see Table 1) were used for discrimination. The analysis resulted in 11.8% of the samples being misclassified.

importance of the selected characters was also confirmed in a separate analysis of parental species with all hybrids excluded; in this modified analysis, calyx length at the flowering stage (v34) was included among the diagnostic characters (results not shown).

Correct identification of *C. alsinifolium* and interspecific hybrids based on morphological characters is more challenging. Characters with the highest discrimination power and suitability in determination keys include leaf shape (= length/width of leaves; v53, v54, v55) and the size and shape of scarious margins of bracts (both absolute length and its proportion with respect to total bract length; v22, v30, v58, v61) (Table 1, Fig. 4B). There are some differences in the shape of the leaf apex (acute vs. subobtuse). The percentage of correctly classified flowering individuals reached 92.1% and 88.2% using all characters and the seven selected characters with the highest absolute canonical loadings, respectively. Calyx length (v50) and the ratio of it and capsule length (v69) are additional taxonomically-important characters that can help in the identification of fruiting individuals. Polyploid hybrids differed from their counterparts originating via crosses in which both gametes are unreduced mainly in terms of calyx (v36, v50, v69) and capsule (v48, v49) characters (Table 1) but the discrimination was unreliable and the success comparatively low due both to tiny differences and unbalanced data design.

Striking differences between parental species and their hybrids were found in pollen fertility. While the mean percentage of viable pollen grains was 95.1% (range 73.1–100%) and 97.3% (range 88.3–100%) for *C. arvense* and *C. alsinifolium*, respectively, the mean value dropped down to 25.1% (range 0–56.9%) for interspecific crosses.

Discussion

Our study provides a detailed investigation of a phytogeographically and evolutionarily important component of the Czech flora, *Cerastium alsinifolium.* Although this species belongs among the most well-known endemic plants of the Czech Republic (Novák 1960, Smejkal 1990, Klaudisová & Čeřovský 1999, Rybka et al. 2004, Kaplan 2012), reliable data on its morphology, ecology and fine-scale distribution were missing because it is difficult to identify, in particular to distinguish it from putative hybrids with another large-flowered and sympatric species, *C. arvense*. Unlike previous studies, we used genome size as an independent marker for taxonomic decision-making and assessed phenotypic variation and ecological preferences of individuals characterized by their distinct nuclear DNA amounts.

Taxonomic significance of genome size data

The last decade has seen a rise in the number of studies that acknowledge the taxonomic value of genome size data (Kron et al. 2007, Loureiro et al. 2010). The amount of nuclear DNA can vary considerably among different congeneric species irrespective of the number of chromosomes (Bennett & Leitch 2011) but is usually stable within the same species/evolutionary unit (Greilhuber 2005). Consequently, genome size can be employed as a useful character to delimit species boundaries, identify improperly developed individuals and/or resolve complex low-level taxonomies even in groups with identical chromosome numbers (Suda et al. 2007). Provided that interspecific differences are sufficiently large (dozens of percent), genome size also offers the opportunity to detect interspecific hybrids

and/or backcrosses. This promise has been recently fulfilled in several taxonomically challenging plant genera, including *Amaranthus* (Jeschke et al. 2003), *Dryopteris* (Ekrt et al. 2010) and *Elytrigia* (Mahelka et al. 2005).

Considerable karyological variation is recorded in the genus *Cerastium*. Disregarding one uncertain diploid count, all *Cerastium* species are polyploid, with chromosome numbers ranging from 2n = 4x = 36 to 2n = 16x = 144 (Hegi & Weber 1975, Goldblatt & Johnson 1979 onwards, Jalas et al. 1993, Scheen et al. 2004). Despite substantial variation in ploidy levels, aneuploidy seems to be very rare, at least among European species. Flow cytometric measurements (Boşcaiu et al. 1999, Niketić et al. 2013) reveal an approximately three-fold variation in the size of the monoploid genome in different species of *Cerastium*. A combination of chromosome numbers and nuclear DNA values led to the recognition of three different cytogenetic groups within the monophyletic section *Cerastium*, which correspond to species aggregates (Niketić et al. 2013). While the *C. arvense* agg. has the lowest genome sizes, the *C. alpinum* agg. has medium to high genome size.

Our genome size estimates for more than 2000 large-flowered *Cerastium* plants inhabiting serpentine sites in the Slavkovský les Mts revealed clear discontinuities in the amount of nuclear DNA, resulting in three main genome size categories (in addition there were a few individuals with outlying values; see Fig. 2). Disregarding the outliers, the smallest C-value category morphologically matched *C. arvense* while the medium and large categories were referred to as interspecific hybrids and the endemic *C. alsinifolium*, respectively. The estimated values for the *C. arvense* genome in our study (mean 2C = 2.83 pg) differs slightly from those previously published (2C = 2.6 pg; Boşcaiu et al. 1999), which may be due to the use of different reference standards or, perhaps more probably, of different DNA-selective fluorochromes. A more recent estimate for *C. arvense* subsp. *rigidum* (2C = 2.76 pg; Niketić et al. 2013), a taxon that has often been synonymized with the nominate subspecies, fits our data very well.

Although our FCM results were not complemented by chromosome counts, we are convinced that this limitation does not affect our interpretations and undermine the usefulness of genome size data for taxonomic purposes. According to the literature, *Cerastium alsinifolium* is octoploid (2n = 8x = 72; Měsíček & Jarolímová 1992) while tetra- (2n = 4x = 36) and octoploid (2n = 8x = 72) counts are known for the nominate subspecies of *C. arvense* (Goldblatt & Johnson 1979 onwards). Considering the genome sizes that were determined for karyologically-counted samples of *C. arvense* (Boşcaiu et al. 1999, Niketić et al. 2013) we assume that in our study we were dealing with the octoploid cytotype and this chromosome number was also shared by the majority of interspecific hybrids.

Considerable differences in the monoploid genome sizes of *Cerastium alsinifolium* and *C. arvense* (~50%) do not support their close phylogenetic relationships (e.g. placement in the same species aggregate) as suggested in some earlier works (Čelakovský 1873, Dostál 1989). Although molecular markers are necessary to elucidate the phylogenetic position of this Czech endemic, available pieces of evidence based on DNA content favour the hypothesis of Novák (1960) that *C. alsinifolium* is a descendant of some taxon from the *C. alpinum* agg. Some ancestral populations of the latter species might have reached serpentine sites in western Bohemia during climatically-driven migrations in the Quaternary and, in response to specific soil conditions, evolved there into a new taxon. After climatic amelioration and subsequent forest expansion in the Early Holocene, ancestral populations could have survived only at serpentine sites and gradually evolved into a new species.

Our FCM measurements of octoploid *C. alpinum* from Scandinavia (7 accessions), the Alps (9 accessions) and Western Carpathians (7 accessions), with mean genome size of 4.11 pg/2C, accurately matched the values for *C. alsinifolium* (mean 2C = 4.25 pg). Importantly, *C. alpinum* often inhabits serpentine sites in Scandinavia and serpentine tolerance evolved repeatedly during the postglacial colonization of Northern Europe (Berglund et al. 2003).

Ecological requirements

One of the most important findings of our study is a fundamental reshaping of traditional views on habitat preferences and spatial distribution of the pure *Cerastium alsinifolium* in the Slavkovský les Mts. Previous studies assumed this endemic favours open serpentine outcrops with shallow soil covered by heliophilous plant communities from where it occasionally colonizes open pine forest, heathlands and dry grasslands (Novák 1960, Smejkal 1990). Only rarely is its occurrence in wet meadows or spring areas in forest margins mentioned. However, according to our data, dry and open sites are dominated by interspecific hybrids with *C. arvense*, whereas the genuine *C. alsinifolium* mostly inhabits moist and semi-shaded sites in spruce forests, including springs in forest clearings, seeps, wet margins of forest roads, etc. The character of these localities suggests that *C. alsinifolium* is a poor competitor. This species attains its highest densities in slightly elevated sites covered by mosses with little surrounding vegetation and can be particularly common on remnants of rotten spruce trunks and along margins of forest roads, occasionally disturbed by vehicles. In forest habitats, samples corresponding to *C. alsinifolium* outnumbered by approx. 5.8-fold their hybrid counterparts, whereas the opposite was true in open rocky outcrops where the ratio of *C. alsinifolium* to hybrids was about 1: 5. Different percentages of interspecific hybrids mirror frequencies of *C. arvense* in particular habitat types. This heliophilous species is relatively common nearby serpentine outcrops (~20% of all samples from this habitat) but only rarely grows in moist sites in forests (1.2% of all samples from forests). According to our field observations, ecological requirements of hybrids seem to be intermediate between those of their parents (moist and partially shaded habitats on serpentine soils for *C. alsinifolium* vs. open and dry habitats on non-serpentine soils for *C. arvense*). However, a deeper insight into abiotic conditions of sites, associated vegetation types and the width of ecological niches of large-flowered *Cerastium* species (and their hybrids) in the Slavkovský les Mts requires further detailed study.

Frequency of interspecific hybridization

Reproductive barriers between species of *Cerastium* are often weak and both homoploid and heteroploid crosses are reported, especially in large-flowered perennial species growing in sympatry (Smejkal 1990, Brysting 2000, Hagen et al. 2002). For example, Brysting (2000) records plants with 90 chromosomes that originated from the hybridization of octoploid *C. alpinum* (2n = 72) and dodecaploid *C. nigrescens* (2n = 108). In sympatric populations of both species, up to 8.4% of the individuals were presumably of hybrid origin, and some back-crosses were also suspected (Hagen et al. 2002). In addition, natural hybrids between *C. arvense* and *C. alpinum* are reported on the basis of morphological characters (e.g. Richter & Gürke 1899, Hegi & Weber 1975) and the artificial crossing of both species results in vigorous hybrids (Khalaf & Stace 2000).

Although ecological optima of both *Cerastium alsinifolium* and *C. arvense* differ considerably, both species occasionally come into contact and then hybridize freely. Open serpentine outcrops provide more or less intermediate conditions with respect to the ecological requirements of parental species and are largely inhabited by interspecific crosses (645 out of the 968 samples analysed; = 66.6%). The history of hybridization is difficult to ascertain but we can speculate that *C. alsinifolium* might have been more common in suitable microhabitats on serpentine outcrops in the past. Its contact with *C. arvense* resulted in interspecific crosses that were better adapted to the ecological conditions at open serpentine sites and possibly outcompeted the endemic species (currently, *C. alsinifolium* is the least common plant on serpentine outcrops and, on average, accounts only for 13.3% of the plants studied). Hybridization might have been accelerated by natural or anthropogenic disturbances. An illustrative example is provided by the serpentine outcrop Křížky, which was used as a shooting range in the 1970s and 1980s, and this activity resulted in an increase in abundance of large-flowered *Cerastium* plants (J. Schlossar, pers. comm.). Although these were referred to as *C. alsinifolium*, they were most likely hybrids with *C. arvense.* Similarly, another such hybrid in the genus *Cerastium* seems to be *C. arvense* × *C. tomentosum*, which grows in abundance along the roads in Scandinavia (Nilsson 1977).

Spatial isolation due to ecological segregation seems to be the main prezygotic breeding barrier between *Cerastium alsinifolium* and *C. arvense*. When this barrier is overcome, extensive hybridization occurs. The overall frequency of interspecific crosses (mean 38.7%, range 3.8–68.6%) in the *Cerastium* populations studied is unusually high for any hybridizing group of vascular plants. In other plants, F1 hybrids mostly occur as single individuals within populations of parental species even in genera with frequently hybridizing species (e.g. 0–19.4% with a mean of 8.0% in the genus *Cirsium*; Bureš et al. 2010). Although backcrosses can be expected in populations harbouring substantial percentages of F1 hybrids, our FCM analyses (i.e. the uniformity in the sizes of the genomes of the hybrids and clear discontinuities between values of both parental species) refuted this assumption. The pattern of hybridization in *Cerastium alsinifolium – C. arvense* populations differs from that in other hybrid-prone genera, including *Prunus* (Wójcicki & Marhold 1993), *Senecio* (Oberprieler et al. 2010) and *Viola* (Krahulcová et al. 1996), in which complex hybrid swarms are often formed and the genetic integrity of the hybridizing species possibly threatened. Hybrid individuals of *Cerastium* have a considerably reduced pollen fertility (25.1% of stainable pollen grains on average) and their capsules contain high percentages of undeveloped seeds (our own observations), which possibly decrease the chances of further hybridization. Unfortunately, no data are currently available either on the germinability or origin of the seeds of F1 hybrids. Whether backcrosses occasionally occur at the seed stage but are later outcompeted or whether seeds produced by F1 hybrids are uniform (originating by self-pollination or cross-pollination with other hybrid individual) remains to be established. Another moot question is the direction of hybridization (i.e. the recognition of maternal and paternal parent) although our preliminary results (sequences of *psbJ-petA* inter-genic spacer and *trnG2-trnG* intron) indicate bidirectionality.

In addition to reduced gametes, unreduced gametes also participate in the origin of some hybrids (~3.5%), giving rise to presumably dodecaploid individuals. Interestingly, unreduced gametes of both parental species can enter into hybridization although those of

C. arvense were involved much more often (detected in 27 polyploid hybrids) than the 2n gametes of *C. alsinifolium* (detected only in one polyploid hybrid). Interspecific hybrids originating via unreduced gametes are known for example in *Elytrigia* (Mahelka et al. 2005) and *Pilosella* (Krahulcová et al. 2011).

We deliberately do not provide a name for the interspecific hybrid as the nomenclature of the group requires a separate study. The original herbarium material of I. F. Tausch consists of a mixture of *Cerastium alsinifolium* and interspecific hybrids. Considerable nomenclatural chaos resulted recently when Toman (2003) uncritically introduced several new names (e.g. *C. caesarosylvaticum*) for plants occurring in the Slavkovský les Mts.

Phenotypic variation and species-specific characters

The number of quantitative characters used to distinguish *Cerastium alsinifolium* and *C. arvense* (Smejkal 1990, Hrouda 2002) predisposes this group to morphometric analysis. Previously, multivariate morphometrics proved successful in the assessment of phenotypic variation and identification of species-specific characters in several taxonomically challenging *Cerastium* complexes, including *C. alpinum–C. arcticum* (Brysting & Elven 2000, Grundt et al. 2000) and *C. pumilum–C. glutinosum* (Letz et al. 2012).

According to published determination keys (Smejkal 1990, Hrouda 2002), *Cerastium alsinifolium* and *C. arvense* should mainly differ in calyx length, leaf shape, bract characteristics, development of axillary shoots and plant colour; no diagnostic characters have ever been provided for hybrids. Our morphometric analysis of 616 individuals largely confirmed the taxonomic value of the above characters. The most distinct taxon is *C. arvense*, whose determination usually poses few problems. In accordance with previous studies, leaves of this species are comparatively long and narrow (mostly at least 3.6-times longer than wide), acute at the apex, the lowermost bracts have distinct scarious margins (at least 1.7 mm long; Fig. 5C) and there are several (mostly 2–10) short sterile shoots in axils of cauline leaves (see Appendix 1 for values of the diagnostic characters discussed). The calyx of *C. arvense* is rather large as are the anthers and petals (the last character was not considered taxonomically-important by previous works). Greyish-green colour of vegetative parts can also help in the identification of *C. arvense* in the field.

In contrast, distinguishing *Cerastium alsinifolium* from interspecific hybrids with *C. arvense* on the basis of morphological characters is a more challenging task. As expected, hybrids have far fewer properly developed pollen grains (on average 25.1% vs. 97.3% in *C. alsinifolium*) although this character is only of limited value in the field. The taxonomically important morphological characters are mostly for vegetative parts and the values for interspecific hybrids are usually intermediate between those of their parents. Specifically, *C. alsinifolium* has only an indistinct scarious margin to lowermost bracts, which is mostly (in 3/4 of the individuals analysed) confined to the upper third of the bract and only rarely (in 7% of plants) reaches its bottom half (Fig. 5A). While the median value of scarious margin length/bract length was only 0.18 in *C. alsinifolium*, it was 0.53 in hybrids (Appendix 1). In plants of *C. alsinifolium* from (semi)shaded forest sites the scarious margin is often lacking. Second lowermost bracts can also be used in the identification of species although the differences are less pronounced and the scarious margin is generally more developed. Among leaves, the third uppermost leaf pair has the highest discriminating power, although there are useful characters on the upper and median cauline leaf pair

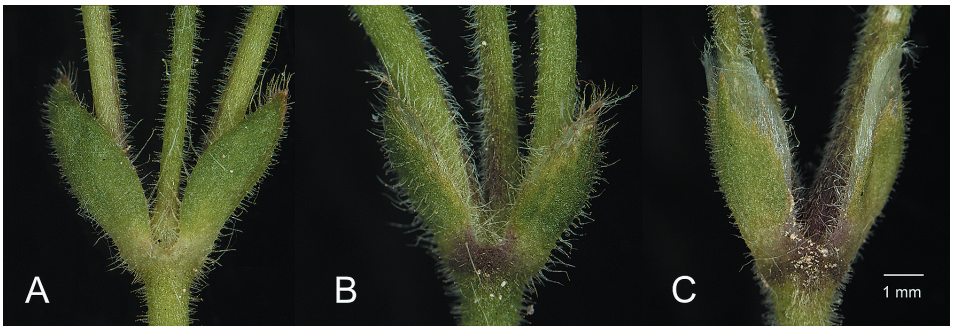

Fig. 5. – Lowermost bracts of *Cerastium alsinifolium* (A; locality Pluhův bor), *C. alsinifolium* × *C. arvense* (B; locality Pluhův bor), and *C. arvense* (C; locality Smečno). Note differences in the shape and size of scarious margins.

(most likely also on bottom pairs but these were not analysed in this study). Leaves of *C. alsinifolium* are usually less than 3.7-times longer than broad, comparatively short (4–10 mm and 5–12 mm for third and second uppermost cauline leaf pairs, respectively) and their apices are more obtuse than those of corresponding leaves of interspecific hybrids. In general, the shape and colour of cauline leaves of *C. alsinifolium* resemble those of small-flowered *C. holosteoides*. In contrast to what is cited in determination keys (Smejkal 1990, Hrouda 2002), the leaves of *C. alsinifolium* are dark (not light) green (Fig. 6). Axillary shoots in *C. alsinifolium* are lacking in about one third (35%) of individuals while there was only one in another third (29%) of the plants analysed; more than three axillary shoots occurred in 3.4% of the individuals. Hybrids were similar to *C. alsinifolium* in terms of the number of axillary shoots (24% of individuals had no axillary shoots, 31% one shoot, 38% two or three shoots and 7% four or five shoots). The floral parts measured were very similar in *C. alsinifolium* and interspecific hybrids. A clue in fruiting plants is provided by the capsules, which in *C. alsinifolium* are more exserted from a comparatively shorter calyx.

The native large-flowered *Cerastium* plants occurring in the Slavkovský les Mts can be identified using the determination key below. Values of quantitative characters are expressed as (minimum–) 5 percentile – 95 percentile (–maximum). Electronic Appendix 4 contains a modified key with added data on pollen fertility. In addition, linear discriminant functions are provided in Electronic Appendix 5.

1a Median and upper cauline leaves (2.5–) 3.6–10.2 (–14.7)-times longer than broad, (6.6–) 9.6–22.2 (–29.6) mm long, sterile shoots in leaf axils usually well-developed, (0–) 2–10 (–14) in number, scarious margin of the lowermost bract (1.1–) 1.7–4.2 (–4.9) mm long, calyx (excluding the scarious margin) (3.7–) 4.2–5.9 (–6.9) mm long, petals (7.0–) 7.9–11.6 (–12.6) mm long, anthers (0.6–) 0.9–1.3 (–1.4) mm long, plants of open habitats ..... ***C. arvense* L.**

1b Median and upper cauline leaves (1.2–) 1.7–4.7 (–7.1)-times longer than broad, (2.7–) 5.0–13.7 (–19.4) mm long, sterile shoots in leaf axils lacking or only a few [1–3 (–5)] in number, scarious margin of the lowermost bract (0–) 0.1–3.0 (–4.4) mm long, calyx (excluding the scarious margin) (2.6–) 3.4–5.2 (–5.7) mm long, petals (5.4–) 6.2–9.3 (–11.3) mm long, anthers (0.4–) 0.6–1.0 (–1.2) mm long, plants of open or (semi-)shaded habitats **2**

2a Median and upper cauline leaves (1.2–) 1.6–3.7 (–5.3)-times longer than broad, often sub-obtuse (angle at apex usually 40–100°), scarious margin of the lowermost bract indistinct, 0–1.8 (–2.7) mm long, mostly

confined to the apical third of the bract (rarely reaches its bottom half), scarious margin of the second lowermost bract (0–) 0.2–2.2 (–2.8) mm long, usually confined to the apical half, calyx (2.6–) 3.0–4.6 (–5.1) mm long, capsule (1.1–) 1.6–3.2 (–3.6)-times longer than calyx, plants of moist and at least partially shaded habitats, usually in spring areas in spruce forests ..... ***C. alsinifolium* Tausch** (Fig. 6)

2b Median and upper cauline leaves (1.4–) 2.1–5.0 (–6.4)-times longer than broad, acute (angle at apex usually 25–65°), scarious margin of the lowermost bract usually distinct, (0–) 0.7–3.3 (–4.4) mm long, mostly reaching beyond the apical third of the bract (often up to bottom half), scarious margin of the second lowermost bract (0.2–) 0.9–2.9 (–3.6) mm long, usually reaching beyond the apical half, calyx (3.1–) 3.4–5.2 (–5.7) mm long, capsule 1.1–2.2 (–2.8)-times longer than calyx, plants of more open habitats, usually on serpentine outcrops ..... ***C. alsinifolium* × *C. arvense*** (Fig. 6)

Implications for conservation

The results of this study have direct and far-reaching practical implications for the protection and conservation of *Cerastium alsinifolium* in the Slavkovský les Mts. Until now, conservation measures have been selectively targeted at open serpentine outcrops, which were believed to be the main reservoir of the endemic's gene pool. Assuming a heliophilous nature of *C. alsinifolium*, the sites were regularly subjected to controlled grazing, removal of shrubs and emerging trees and/or mild disturbance. Although the presence of plants tentatively determined as *C. alsinifolium* in moist forest sites has also been known, these habitats were largely neglected by conservationists (Melichar 2005).

Paradoxically, open serpentine outcrops are mostly inhabited by *Cerastium alsinifolium* × *C. arvense* hybrids, whereas *C. alsinifolium* is relatively uncommon and is confined to suitable (moist and sheltered) microhabitats. The management practices adopted could have facilitated the establishment of hybrid seedlings. However, it is unlikely that any change in conservation measures would cause an increase in the abundance of the endemic species. These open serpentine sites (Dominova skalka, Křížky) should be viewed as natural laboratories where the contact between the two ecologically and morphologically differentiated species results in extensive hybridization. Generally, hybrids are rarer and more spatially restricted than pure *C. alsinifolium* and deserve appropriate protection. As a curiosity, most published pictures of *C. alsinifolium* actually show the hybrid, which is usually more showy than the endemic species.

Our finding that *Cerastium alsinifolium* favours moist spruce forests on serpentine bedrocks will require the development of different conservation strategies with more emphasis on forest sites. Because interspecific hybridization clearly presents a major threat to the genetic integrity of *C. alsinifolium*, spread of *C. arvense* in serpentine forest sites (along forest paths and roads, in clearings, etc.) should be controlled. The fact that only F1 crosses were encountered among mature plants *in situ* slightly diminishes the threat posed by hybridization and indicates that it is likely that the genetic integrity of the endemic species will be maintained. Due to its weak competitive ability, *C. alsinifolium* is likely to benefit from occasional disturbance. An appropriate management would seem to be an occasional passage of vehicles along forest roads. According to current knowledge, nature reserves Vlček and Pluhův bor host the most abundant and cytologically pure populations of the Czech endemic *C. alsinifolium* and these forests on serpentine bedrocks should therefore receive the highest conservation priority.

See http://www.preslia.cz for Electronic Appendix 1–5

Fig. 6. – Pictures of *Cerastium alsinifolium* (locality Vlček; top) and its interspecific hybrid with *C. arvensis* (locality Dominova skalka; bottom).

Acknowledgements

We thank J. Kalůsková and P. Trávníček for assistance with some of the flow cytometric analyses, R. Sudová for her help in the field and Josef Brůna for preparing the map. The research was supported by the Grant Agency of the Academy of Sciences of the Czech Republic (project no. KJB601110709) and Grant Agency of Charles University (project no. 29507/2007). Additional support was supplied by long-term research development project no. RVO 67985939 (Academy of Sciences of the Czech Republic), institutional resources of the Ministry of Education, Youth and Sports of the Czech Republic for the support of science and research, and project no. 14-36079G, Centre of Excellence PLADIAS (Czech Science Foundation).

Souhrn

Článek se zabývá mezidruhovou hybridizací endemického rožce kuřičkolistého (*Cerastium alsinifolium*) a široce rozšířeného rožce rolního (*C. arvense*) na hadcových tělesech Slavkovského lesa. Hybridizace byla studována pomocí průtokové cytometrie a mnohorozměrných morfometrických analýz (celkem bylo cytometricky zpracováno 2222 jedinců a morfometricky 616 jedinců). Navzdory stejnému počtu somatických chromozómů se oba druhy výrazně (zhruba 1,5násobně) liší velikostí jaderného genomu, což umožňuje jejich spolehlivé určení. Jejich mezidruhový kříženec vykazuje intermediární hodnoty obsahu jaderné DNA. Vzácně (28 jedinců) byly též nalezeny rostliny s nápadně většími genomy; nejpravděpodobněji se jedná o křížence vzniklé splynutím neredukované a redukované gamety rodičovských druhů. Celkově je mezidruhová hybridizace ve Slavkovském lese velice častá a na křížence připadá 38.7 % všech studovaných jedinců. Ekologické preference *C. alsinifolium* se ukázaly být velmi odlišné od informací udávaných v literatuře. Jeho ekologické optimum leží na prameništích ve smrkových lesích na hadcových podkladech (PR Planý vrch, NPR Pluhův bor, PP Vlček), zatímco na otevřených hadcových výchozech (PP Dominova skalka, NPR Křížky) převažují kříženci a vlastní *C. alsinifolium* se vzácně vyskytuje pouze ve stinných a vlhčích štěrbinách skalek. Morfometrické analýzy odhalily, že studované druhy a jejich kříženec se nejvýrazněji liší ve tvaru a délce lodyžních listů, počtu sterilních větévek vyrůstajících v úžlabí listů, tvaru a velikosti blanitého okraje listenů (zejména nejspodnějšího páru), délce kalicha, koruny a nitky. Zatímco determinace *C. arvense* většinou bývá poměrně snadná, rozlišení druhu *C. alsinifolium* a mezidruhového křížence na základě morfologických charakteristik je mnohem obtížnější; jako nejspolehlivější znak se ukázala fertilita pylu, která je u kříženců výrazně snížena. Zjištěné výsledky mají praktický význam pro druhovou ochranu významného představitele naší endemické flóry, přičemž větší pozornost bude potřeba věnovat populacím velkokvětých rožců rostoucích na lesních stanovištích, které dosud stály spíše stranou ochranářského zájmu.

References

Bennett M. D. & Leitch I. J. (2011): Nuclear DNA amounts in angiosperms: targets, trends and tomorrow. – Ann. Bot. 107: 467–590.

Berglund A. B. N., Dahlgren S. & Westerbergh A. (2003): Evidence for parallel evolution and site-specific selection of serpentine tolerance in *Cerastium alpinum* during the colonization of Scandinavia. – New Phytol. 161: 199–209.

Boşcaiu M., Vicente O. & Ehrendorfer F. (1999): Chromosome numbers, karyotypes and nuclear DNA contents for perennial polyploid groups of *Cerastium* (*Caryophyllaceae*). – Plant Syst. Evol. 218: 13–21.

Brysting A. K. (2000): Chromosome number variation in the polyploid *Cerastium alpinum* – *C. arcticum* complex. – Nord. J. Bot. 20: 149–156.

Brysting A. K. & Elven R. (2000): *Cerastium alpinum* – *C. arcticum* complex (*Caryophyllaceae*): numerical analyses of morphological variation and a taxonomic revision of *C. arcticum* Lange s.l. – Taxon 49: 189–216.

Bureš P., Šmarda P., Rotreklová O., Oberreiter M., Burešová M., Konečný J., Knoll A., Fajmon K. & Šmerda J. (2010): Pollen viability and natural hybridization of Central European species of *Cirsium*. – Preslia 82: 391–422.

Čelakovský L. (1873): Prodromus květeny české [Prodromus of the Czech flora]. Vol. 2: 496–499. Arch. Přírod. Výzk. Čech, Praha.

Doležel J., Greilhuber J. & Suda J. (2007): Estimation of nuclear DNA content in plants using flow cytometry. – Nature Protocols 2: 2233–2244.

Dostál J. (1989): Nová květena ČSSR [New flora of the Czechoslovak Socialist Republic]. Vol. 1. – Academia, Praha.

Ekrt L., Holubová R., Trávníček P. & Suda J. (2010): Species boundaries and frequency of hybridization in the *Dryopteris carthusiana* (*Dryopteridaceae*) complex: a taxonomic puzzle resolved using genome size data. – Am. J. Bot. 97: 1208–1219.

Givnish T. J. (2010): Ecology of plant speciation. – Taxon 59: 1326–1366.
Goldblatt P. & Johnson D. E. (1979 onwards): Index to plant chromosome numbers. – Missouri Botanical Garden, St. Louis.
Greilhuber (2005): Intraspecific variation in genome size in angiosperms: identifying its existence. – Ann. Bot. 95: 91–98.
Greilhuber J., Doležel J., Lysák M. A. & Bennett M. D. (2005): The origin, evolution and proposed stabilization of the terms "genome size" and "C-value" to describe nuclear DNA contents. – Ann. Bot. 95: 255–260.
Grulich V. (2012): Red List of vascular plants of the Czech Republic: 3rd edition. – Preslia 84: 631–645.
Grundt H. H., Borgen L. & Elven R. (2000): Intraspecific variation in *Cerastium alpinum* s. lat. (*Caryophyllaceae*) in Central Norway. – Nord. J. Bot. 20: 641–653.
Hagen A. R., Saether T., Borgen L., Elven E., Stabbetorp O. E. & Brochmann C. (2002): The artic-alpine polyploids *Cerastium alpinum* and *C. nigrescens* (*Caryophyllaceae*) in a sympatric situation: breakdown of species integrity? – Plant Syst. Evol. 230: 203–219.
Hegarty M. J. & Hiscock S. J. (2005): Hybrid speciation in plants: new insights from molecular studies. – New Phytol. 165: 411–423.
Hegi G. & Weber H. (1975): Illustrierte Flora von Mittel-Europa, ed. 2. Vol. 3/2. – Paul Parey, Berlin & Hamburg.
Hrouda L. (2002): *Cerastium* L. – rožec [chickweed]. – In: Kubát K., Hrouda L., Chrtek J. jun., Kaplan Z., Kirschner J. & Štěpánek J. (eds), Klíč ke květeně České Republiky [Key to the flora of the Czech Republic], p. 383–386, Academia, Praha.
IUCN (International Union for Conservation of Nature) (2013): IUCN Red List of threatened species. Version 2013.1. – IUCN, Species Survival Commission, Gland, Switzerland, URL: http://www.iucnredlist.org (accessed August 2013).
Jalas J., Wyse Jackson M. B., Sell P. D. & Whitehead F. H. (1993): *Cerastium* L. – In: Tutin T. G., Burges N. A., Chater O. A., Edmonson J. R., Heywood V. H., Moore D. M., Valentine D. H., Walters S. M. & Webb D. A. (eds), Flora Europaea, Ed. 2., 1: 164–174, Cambridge University Press, Cambridge.
Jeschke M. R., Tranel P. J. & Rayburn A. L. (2003): DNA content analysis of smooth pigweed (*Amaranthus hybridus*) and tall waterhemp (*A. tuberculatus*): implications for hybrid detection. – Weed Sci. 51: 1–3.
Kaplan Z. (2012): Flora and phytogeography of the Czech Republic. – Preslia 84: 505–573.
Khalaf M. K. & Stace A. (2000): Breeding systems and relationships of the *Cerastium tomentosum* group. – Preslia 72: 323–344.
Klaudisová A. & Čeřovský J. (1999): *Cerastium alsinifolium* Tausch. – In: Čeřovský J., Feráková V., Holub J., Maglocký Š. & Procházka F. (eds), Červená kniha ohrožených a vzácných druhů rostlin a živočichů ČR a SR. Vol. 5. Vyšší rostliny [Red Data Book of endangered and rare plant and animal species of Czech Republic and Slovak Republic, Vol. 5. Vascular plants], p. 91, Príroda, Bratislava.
Klecka W. R. (1980): Discriminant analysis. – Sage University papers, series: Quantitative applications in the social sciences, no. 19, Sage Publications, Beverly Hills and London.
Krahulcová A., Krahulec F. & Kirschner J. (1996): Introgressive hybridization between a native and an introduced species: *Viola lutea* subsp. *sudetica* versus *V. tricolor*. – Folia Geobot. 31: 219–244.
Krahulcová A., Krahulec F. & Rosenbaumová R. (2011): Expressivity of apomixis in 2n + n hybrids from an apomictic and a sexual parent: insights into variation detected in *Pilosella* (*Asteraceae*: *Lactuceae*). – Sex. Plant Reprod. 24: 63–74.
Kron P., Suda J. & Husband B. C. (2007): Applications of flow cytometry to evolutionary and population biology. – Annu. Rev. Ecol. Evol. Syst. 38: 847–876.
Letz D. R., Dančák M., Danihelka J. & Šarhanová P. (2012): Taxonomy and distribution of *Cerastium pumilum* and *C. glutinosum* in Central Europe. – Preslia 84: 33–69.
Levin D. A., Francesco-Ortega J. & Jansen R. K. (1996): Hybridization and the extinction of rare plant species. – Conserv. Biol. 10: 10–16.
Levine M. E. & Greller A. M. (2004): Ecological and floristic analyses of vascular plants along a gradient on disturbed serpentinite on opposing slopes in Staten Island, NY. – J. Torrey Bot. Soc. 131: 69–92.
Loureiro J., Trávníček P., Rauchová J., Urfus T., Vít P., Štech M., Castro S. & Suda J. (2010): The use of flow cytometry in the biosystematics, ecology and population biology of homoploid plants. – Preslia 82: 3–21.
Mahelka V., Suda J., Jarolímová V., Trávníček P. & Krahulec F. (2005): Genome size discriminates between closely related taxa *Elytrigia repens* and *E. intermedia* (*Poaceae*: *Triticae*) and their hybrid. – Folia Geobot. 40: 367–384.
Melichar V. (2005): Metodika monitoringu evropsky významného druhu rožec kuřičkolistý (*Cerastium alsinifolium*) [Guide for monitoring *Cerastium alsinifolium*, a priority European species]. – Ms. [Depon. in: AOPK, Praha].

Měsíček J. & Jarolímová V. (1992): List of chromosome numbers of the Czech vascular plants. – Academia, Praha.

Niketić M., Siljak-Yakovlev S., Frajman B., Lazarević M., Stevanović B., Tomović G. & Stevanović V. (2013): Towards resolving the systematics of *Cerastium* subsection *Cerastium* (*Caryophyllaceae*): a cytogenetic approach. – Bot. J. Linn. Soc. 182: 205–224.

Nilsson A. (1977): Spontana hybrider mellan fältarv och silverarv (*Cerastium arvense* x *tomentosum*) [Spontaneous hybrids between *Cerastium arvense* and *C. tomentosum*]. – Svensk Bot. Tidskr. 71: 263–272.

Novák F. A. (1960): Fylogenese serpentinových typů [Phylogeny of serpentine morphotypes]. – Preslia 32: 1–8.

Oberprieler C., Barth A., Schwartz S. & Heimann J. (2010): Morphological and phytochemical variation, genetic structure and phenology in an introgressive hybrid swarm of *Senecio hercynicus* and *S. ovatus* (*Compositae*, *Senecioneae*). – Plant Syst. Evol. 286: 153–166.

Otto F. (1990): DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. – In: Crissman H. A. & Darzynkiewicz Z. (eds), Methods in cell biology 33: 105–110, Academic Press, New York.

Peterson R., Slovin J. P. & Chen C. (2010): A simplified method for differential staining of aborted and non-aborted pollen grains. – Int. J. Plant Biol. 1: 66–69.

Richter K. & Gürke M. (1899): Plantae Europaeae 2. – Wilhelm Engelmann, Leipzig.

Rosenbaumová R., Plačková I. & Suda J. (2004): Variation in *Lamium* subg. *Galeobdolon* (Lamiaceae): insights from ploidy levels, morphology and isozymes. – Plant Syst. Evol. 244: 219–244.

Rybka V., Rybková R. & Pohlová R. (2004): Rostliny ve svitu evropských hvězd [Plant species of the system of Natura 2000 in the Czech Republic]. – Sagittaria, Olomouc & Praha.

Scheen A. C., Brochmann C., Brysting A. K., Elven R., Morris A., Soltis D. E., Soltis P. S. & Albert V. A. (2004): Northern hemisphere biogeography of *Cerastium* (*Caryophyllaceae*): insights from phylogenetic analysis of non-coding plastid nucleotide sequences. – Am. J. Bot. 91: 943–952.

Smejkal M. (1967): Určovací klíč a přehled československých druhů a poddruhů rodu *Cerastium* L. [Determination key to and a synopsis of species and subspecies of *Cerastium* in Czechoslovakia]. – Zpr. Čs. Bot. Společ. 3: 137–146.

Smejkal M. (1990): *Cerastium* L. – rožec [chickweed]. – In: Hejný S. & Slavík B. (eds), Květena České republiky [Flora of the Czech Republic] 2: 136–151, Academia, Praha.

Soltis P. S. & Soltis D. E. (2009): The role of hybridization in plant speciation. – Annu. Rev. Plant Biol. 60: 561–588.

Suda J., Krahulcová A., Trávníček P., Rosenbaumová R., Peckert T. & Krahulec F. (2007): Genome size variation and species relationships in *Hieracium* subgen. *Pilosella* (*Asteraceae*) as inferred by flow cytometry. – Ann. Bot. 100: 1323–1335.

Suda J. & Pyšek P. (2010): Flow cytometry in botanical research: introduction. – Preslia 82: 1–2.

Suda J., Trávníček P., Mandák B. & Berchová-Bímová K. (2010): Genome size as a marker for identifying the invasive alien taxa in *Fallopia* section *Reynoutria*. – Preslia 82: 97–106.

Tájek P., Klaudisová A. & Vít P. (2012): Vývoj populace křížence rožce kuřičkolistého (*Cerastium alsinifolium*) a rožce rolního (*C. arvense*) v NPP Křížky v letech 1984–2012 [Population dynamics of an interspecific hybrid between *Cerastium alsinifolium* and *C. arvense* in NPP Křížky during 1984–2012]. – Sborn. Muz. Karlovar. Kraje 20: 173–194.

Tausch I. F. (1828): Diagnoses plantarum novarum itemque minus cognitarum. – Sylloge plantarum novarum itemque minus cognitarum a prestantissimis botanicis adhuc viventibus collecta et a Societate regia botanica ratisbonensis edita 2: 240–256.

Toman M. (2003): Charakteristische ausdauernde Arten der Gattung *Cerastium* im südöstlichen Teil von Slavkovský les (Kaiserwald, Tschechische Republik) und die Problematik der Art *Cerastium alsinifolium*. – Čas. Nár. Muz, ser. nat.-hist., 172: 81–108.

Uhríková A. & Záborský J. (1980): *Cerastium arvense*. – In: Löwe Á. (ed.), Chromosome number reports LXIX. Taxon 29: 729.

Wójcicki J. J. & Marhold K. (1993): Variability, hybridization and distribution of *Prunus fruticosa* (*Rosaceae*) in the Czech Republic and Slovakia. – Polish Bot. Stud. 5: 9–24.

Received 6 September 2013
Revision received 19 November 2013
Accepted 20 November 2013

Appendix 1. – Values of selected taxonomically-important morphological characters for *Cerastium arvense*, interspecific hybrids and *C. alsinifolium*. Group abbreviation: H – interspecific hybrid, L – *C. alsinifolium*, R – *C. arvense*. Lengths are given in millimetres.

	Number of sterile shoots in leaf axils (v2)			Length of the uppermost leaf (v3)			Length / width of the uppermost leaf (v53)			Angle of the tip of the uppermost leaf (v7)			Length of the second uppermost leaf (v8)			Length / width of the second uppermost leaf (v54)		
Group	R	H	L	R	H	L	R	H	L	R	H	L	R	H	L	R	H	L
min	0	0	0	6.9	4.2	4.0	2.5	1.6	1.2	15	15	20	6.6	4.0	3.2	2.8	1.6	1.2
5%	2	0	0	9.7	6.1	5.4	3.6	2.2	1.6	20	20	35	10.2	5.7	4.7	3.5	2.1	1.6
25%	3	1	0	12.9	8.0	7.5	4.6	3.0	2.1	20	30	50	12.5	7.6	6.4	4.6	2.7	2.0
50%	4	1	1	15.4	9.8	9.2	5.6	3.5	2.5	30	40	60	15.5	9.2	8.2	5.6	3.3	2.4
75%	6	2	2	18.1	11.8	10.8	6.7	4.2	3.0	30	50	70	18.1	11.3	9.8	7.5	3.8	2.8
95%	10	4	3	21.3	15.5	14.5	9.7	5.2	3.8	40	60	100	22.0	13.8	12.0	10.1	4.9	3.7
max	14	5	5	27.0	18.9	19.4	12.0	6.1	5.3	50	75	125	29.6	16.4	16.5	11.8	6.8	5.3

	Angle of the tip of the second uppermost leaf (v12)			Length of the third uppermost leaf (v13)			Length / width of the third uppermost leaf (v55)			Angle of the tip of the third uppermost leaf (v17)			Length of the scarious margin of the lowermost bract (v22)			Length of the scarious margin / total length of the lowermost bract (v58)		
Group	R	H	L	R	H	L	R	H	L	R	H	L	R	H	L	R	H	L
min	15	20	20	6.9	3.3	2.7	2.7	1.4	1.2	20	25	20	1.1	0.0	0.0	21	0	0
5%	20	25	40	9.0	4.8	3.9	3.5	1.9	1.5	20	30	40	1.7	0.7	0.0	35	14	0
25%	25	35	53	11.8	6.5	5.5	4.7	2.6	2.0	30	40	60	2.4	1.3	0.3	51	34	7
50%	30	40	65	14.5	8.4	6.6	6.0	3.2	2.3	30	45	65	2.9	1.9	0.6	65	53	18
75%	35	50	70	18.0	10.3	7.8	7.6	3.9	2.7	40	60	75	3.4	2.5	1.1	73	66	31
95%	40	70	95	23.2	13.1	10.0	10.6	4.8	3.5	50	70	100	4.2	3.3	1.8	82	79	54
max	60	90	110	29.5	17.3	13.3	14.7	6.5	4.9	80	110	110	4.9	4.4	2.7	95	100	72

	Length of the scarious margin of the second lowermost bract (v30)			Length of the scarious margin / total length of the second lowermost bract (v61)			Petal length (v38)			Anther length (v43)			Calyx length at fruiting stage (v50)			Capsule length / calyx length at fruiting stage (v69)		
Group	R	H	L	R	H	L	R	H	L	R	H	L	R	H	L	R	H	L
min	0.8	0.2	0.0	31	7	0	7.0	5.4	5.6	0.6	0.4	0.5	3.7	3.1	2.6	1.1	1.1	1.1
5%	1.6	0.9	0.2	47	32	4	7.9	6.1	6.4	0.9	0.6	0.6	4.2	3.4	3.0	1.3	1.1	1.6
25%	2.2	1.5	0.4	63	52	15	9.3	7.0	7.3	1.0	0.7	0.7	4.6	3.9	3.3	1.6	1.4	2.1
50%	2.6	1.9	0.9	72	65	32	10.1	7.7	7.9	1.1	0.8	0.8	5.0	4.2	3.6	1.8	1.7	2.3
75%	2.9	2.3	1.3	80	74	49	11.0	8.2	8.3	1.2	0.9	0.9	5.3	4.6	4.0	2.0	2.0	2.7
95%	3.6	2.9	2.2	89	91	70	11.6	9.5	9.1	1.3	1.0	1.0	5.9	5.2	4.6	2.2	2.2	3.2
max	4.8	3.6	2.8	100	100	100	12.6	11.3	10.3	1.4	1.1	1.2	6.9	5.7	5.1	2.6	2.8	3.6