Differentiation between diploid and tetraploid *Centaurea phrygia*: mating barriers, morphology and geographic distribution

Diploidní a tetraploidní *Centaurea phrygia* – reprodukční bariéry, morfologie a rozšíření

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Karyological variation, reproductive isolation, morphological differentiation and geographic distribution of the cytotypes of *Centaurea phrygia* were investigated in Central Europe. Occurrence of two dominant cytotypes, diploid (2n = 22) and tetraploid (2n = 44), was confirmed and additionally triploid, pentaploid and hexaploid ploidy levels identified using flow cytometry. Allozyme variation as well as morphological and genome size data suggest an autopolyploid origin of the tetraploids. Crossing experiments and flow cytometric screening of mixed populations revealed strong reproductive isolation of the cytotypes. Multivariate morphometric analysis revealed significant differentiation between the cytotypes in several morphological characters (pappus length, length and colour of appendages on involucral bracts, involucre width). The cytotypes have a parapatric distribution with only a small contact zone: diploids occupy the whole of the Central and North European geographic range of the species except for the major part of the Western Carpathians, whereas tetraploids are confined to the Western Carpathians and adjacent areas, both cytotypes co-occurring only in a limited area of intra-montane basins of the Western Carpathians. Based on this array of data, taxonomic treatment of the cytotypes as autonomous species is proposed. The name *Centaurea phrygia* is applied to the diploids and the name *C. erdneri* belongs to the tetraploids; nomenclature of hybrids with *C. jacea* is also resolved.

Keywords: *Centaurea* sect. *Jacea*, *Centaurea phrygia*, *Centaurea melanocalathia*, flow cytometry, geographic distribution, multivariate morphometric analysis, nomenclature, polyploidy, reproductive isolation, taxonomy

Introduction

Polyploidy is an important evolutionary phenomenon and one of the main sources of plant diversity (Soltis et al. 2003). The process of polyploidization and its consequences concerning morphological and ecological differentiation of polyploids and their ancestors, phylogenetic origin of the polyploids, genome evolution, or role of polyploidy in angiosperm diversification have attracted much attention (Otto & Whitton 2000, Ramsey & Schemske 2002, Osborn et al. 2003, Adams & Wendel 2005, Comai 2005, Soltis et al. 2009, 2010, Parisod et al. 2010). However, the taxonomic treatment of polyploids at specific or infra-specific levels is still controversial. Coexistence of two or more cytotypes within one species or a complex of closely related species is frequent and documented by dozens of studies (during the last year, e.g. Bardy et al. 2010, Cires et al. 2010, Cosendai &

Horändl 2010, Li et al. 2010, Marhold et al. 2010, Šafářová & Duchoslav 2010, Trávníček et al. 2010). If cytotypes represent more or less reproductively isolated entities and especially if there is certain morphological, ecological and/or geographical separation between them, it is reasonable to treat them as autonomous taxa of various ranks, up to species (Šmarda et al. 2007, Soltis et al. 2007, Ekrt & Štech 2008, Mráz et al. 2011). In contrast, in some cases the recognition of cytotypes as any kind of infraspecific unit is disregarded after a detailed study (Hodálová et al. 2007, Mandáková & Münzbergová 2008, Španiel et al. 2008). In general, for this morphology is a highly relevant criterion, because morphological characters can usually be easily evaluated directly in the field, which is a key condition for practical use in other botanical (e.g. vegetation science) and applied disciplines (e.g. agricultural research).

The genus *Centaurea* L. sensu lato is notorious for being taxonomically difficult. Until molecular phylogeny studies were published in the last decade it was not possible to resolve the delimitation of the genus, its internal classification and consequent nomenclatural issues (Garcia-Jacas et al. 2001, Susanna & Garcia-Jacas 2009; see Greuter et al. 2001 for the nomenclatorical consequences). In addition to the generic level, there are many difficulties in this genus at the specific and lower ranks. In many of the problematic groups polyploidy plays a considerable evolutionary role, e.g. in the *C. jacea – C. nigra* complex (Hardy et al. 2000, Vanderhoeven et al. 2002), *C. stoebe* L. (Španiel et al. 2008, Mráz et al. 2011), *C. toletana* complex (Garcia-Jacas et al. 2009) and in the closely related genus *Cyanus*, formerly part of *Centaurea* (Olšavská et al. 2011). This also holds true for the *C. phrygia* group, the subject of this study. It comprises ca 15–20 taxa in Europe (Dostál 1976), with numerous local and poorly resolved taxa, especially in the Balkans and eastern Europe (Čerepanov 1963, Prodan & Nyárády 1964, Soó 1970, Dobročaeva 1999, Ciocârlan 2000). Diploids (2n = 22) and tetraploids (2n = 44) are known. In Central Europe, up to six taxa are recognized by the majority of authors, namely *Centaurea phrygia* L., *C. pseudophrygia* C. A. Mey. [synonym *C. elatior* (Gaud.) Hayek], *C. stenolepis* A. Kern., *C. indurata* Janka, *C. melanocalathia* Czakó and *C. nigriceps* Dobrocz. (Soó 1970, Mądalski & Ciaciura 1972, Dostál 1976, 1989, Wagenitz 1987, Dostál & Červenka 1992, Ochsmann 1998, Simon 2000, Štěpánek & Koutecký 2004, Jäger & Werner 2005, Fischer et al. 2008). Depending on an author's concept, some of these taxa are treated as subspecies of *C. phrygia* L. and/or within the genus *Jacea* Mill. A recent revision (Koutecký 2007) indicates that these taxa are usually uniform in terms of chromosome number, with the only one exception, *C. phrygia* L. (hereafter referred to as "*C. phrygia*", while "*C. phrygia* agg." refers to the whole complex).

Centaurea phrygia (i.e. *C. phrygia* subsp. *phrygia* as delimited by Dostál 1976) occurs in the northern, eastern and central parts of Europe: the northern half of the European part of Russia, southern Finland, Belarus, Estonia, Latvia, Lithuania, Poland, eastern Germany, Czech Republic, Slovakia, Ukraine, Romania, Bulgaria and Albania; it is an adventive species in Denmark, Sweden and Norway (Dostál 1976, Meusel & Jäger 1992, Greuter 2006–2009). Occurrence in the Balkans deserves revision as the plants there may belong to other little known taxa of the *C. phrygia* agg. For the majority of the distribution area, only a diploid chromosome count is known. There are diploid counts with the exact localities for Russia (Poddubnaja-Arnoldi 1931, Pulkina 1988, Lavrenko et al. 1990), Belarus (Semerenko 1989), Poland (Dydak et al. 2009), Czech Republic, Slovakia and Ukraine (Koutecký 2007) and Romania (Koutecký 2008); and without citing the localities

in Flora Europaea (Dostál 1976) and floras and determination keys for Germany, Czech Republic and Slovakia (e.g. Wagenitz 1987, Dostál 1989, Dostál & Červenka 1992, Štěpánek & Koutecký 2004, Jäger & Werner 2005). In contrast, tetraploids seem to have a more narrow distribution. They are known from the Czech Republic and Slovakia: localized records are reported by Májovský & Murín (1987) and Koutecký (2007) and without localities by Dostál (1989) and Štěpánek & Koutecký (2004). Tetraploids are also reported from Bulgaria (Löve 1979, 1981), however, their taxonomic identity is unclear.

A tetraploid chromosome count is reported for another taxon of *C. phrygia* agg.: *C. melanocalathia* Czakó [= *C. phrygia* subsp. *melanocalathia* (Czakó) Dostál] by Dostál (1989). However, no populations that could be assigned to *C. melanocalathia* were identified during a previous study of the *C. phrygia* agg. and only a few individuals of "*C. melanocalathia* morphotype" (as described by Dostál 1976, 1989) occur within *C. phrygia* populations (Koutecký 2007). Inclusion of the name *C. melanocalathia* into the synonymy of *C. phrygia* L. has therefore been proposed. Notably, the individuals with "*C. melanocalathia* morphology" were always diploid (Koutecký 2007). The identity of the reported tetraploid(s) cannot be revised in any way as the chromosome count is published in a general flora (Dostál 1989) and neither the exact locality nor any voucher specimen is known. However, we doubt that the cited tetraploid chromosome count really belonged to a plant of "*C. melanocalathia* morphotype" as Dostál's New Flora of Czechoslovak Socialistic Republic (Dostál 1989) is known to contain numerous inaccuracies.

In addition to their geographic separation, cytotypes of *Centaurea phrygia*, based on our field experience, also appear to be morphologically differentiated, although a previous morphometric analysis of the *Centaurea phrygia* agg. did not separate them (Koutecký 2007). It may be reasonable to treat them as autonomous taxa that are homogeneous in terms of their chromosome number, as has been done for other Central European taxa of the *C. phrygia* agg. We therefore conducted the present detailed study, asking the following questions: (i) Are the cytotypes reproductively isolated? (ii) Are the cytotypes morphologically differentiated? (iii) What is the distribution of the cytotypes of *C. phrygia* in Central Europe? (iv) Is it reasonable to recognize the *C. phrygia* cytotypes as separate taxa? If so, what are the appropriate names for them?

Materials and methods

Field sampling

For the morphometric analyses 817 individuals from 28 populations were sampled. Part of this dataset (505 individuals from 17 populations) came from a previous study of *C. phrygia* agg. (Koutecký 2007). The new populations were added to cover the distribution areas of both cytotypes. Besides Central Europe (Czech Republic, Slovakia, Poland and western Ukraine), three population from northern Europe (Russia and Finland) were studied. Only populations not obviously influenced by hybridization with other taxa were included. *Centaurea phrygia* plants sometimes grow in lax clusters, each cluster corresponding to one genet. Therefore, only one stem from a cluster was sampled. If present, achenes were collected, either a stem with just ripe achenes (in capitula that were still enclosed by remains of withered florets) was sampled or achenes were collected from other stems of the same cluster. The chromosome numbers of all populations were esti-

mated by direct counting and/or flow cytometry. Additionally 21 populations, including several mixed populations of both cytotypes, were subjected to extensive flow cytometric screening. In total, 3271 individuals were analysed by flow cytometry. All populations and the numbers of individuals for morphometric analyses, chromosome counting and flow cytometry are listed in Appendix 1. Voucher specimens are deposited in herbarium CBFS.

Chromosome counts

Chromosome counts for 18 populations of *C. phrygia* are recorded in a previous study of the *C. phrygia* agg. (Koutecký 2007). In the present study, another five populations were analysed, including two populations from Russia (see Appendix 1). Chromosomes in the apical root meristems of seedlings that were germinated from field-collected achenes were counted. Seedlings were pre-treated with a saturated water solution of p-dichlorobenzene for 3 hours at room temperature or with 0.002M 8-hydroxyquinoline for 8 hours at 4°C, fixed in a mixture of ethanol and acetic acid (3:1) for 24 hours at 4°C and stored in 70% ethanol at 4°C. Maceration lasted about 3–5 min in a mixture of ethanol and hydrochloric acid (1:1). The apical part of a root was then cut and squashed under a cover glass in 1% lacto-propionic orcein. At least three samples (achenes originating from different individuals) per population were analyzed and at least two mitoses per plant were studied.

Flow cytometry

DNA ploidy levels were determined using a Partec PA II flow cytometer (Partec GmbH, Münster, Germany) equipped with a mercury arc lamp. Fresh leaves were analyzed (field-collected leaves from natural populations and cotyledons or first leaves of offspring from crossing experiments germinated in a growth chamber). Samples were prepared following the simplified two-step protocol (Doležel et al. 2007). About 0.25 cm^2 of intact leaf tissue was chopped with a sharp razor blade together with an appropriate volume of the internal standard (*Glycine max* 'Polanka', 2C = 2.50 pg; Doležel et al. 1994) in a Petri dish containing 0.5 ml of ice-cold Otto I buffer (0.1M citric acid, 0.5% Tween-20). The suspension was filtered through a 42-mm nylon mesh and incubated for about 1 min at room temperature. After incubation, 1 ml of the staining solution was added. The staining solution consisted of 1 ml of Otto II buffer (0.4M $Na_2HPO_4.12H_2O$), 2-mercaptoethanol (2 µl/ml) and the fluorochrome DAPI (4 µg/ml). Samples were run on the flow cytometer after about one minute of staining and the fluorescence intensity of 3000–5000 particles recorded. Only histograms with coefficients of variation for the G_0/G_1 peaks of both the analyzed *Centaurea* sample and the standard below 3.0% were considered. Pooled samples of up to 10 individuals could be used due to high-resolution histograms and absence of endopolyploidy. Nevertheless, each plant was separately reanalyzed if the occurrence of more DNA ploidy levels in the pooled sample was suspected or if the coefficients of variation of the peaks exceeded the 3% threshold. Results of the ploidy level analysis were calibrated using samples from populations for which there were direct chromosome counts.

For estimating the genome size, the same internal standard and the same method of sample preparation and staining were used, only DAPI was replaced by propidium iodide (50 µg/ml) and RNase IIa (50 µg/ml). Fresh leaves were analyzed (adult plants transplanted to the experimental garden except for the populations NSE1 and KOLI, for which about 3 month old plants grown in the experimental garden from field-collected seeds

were used). The genome size was determined using a Partec CyFlow SL flow cytometer equipped with a 532 nm (green) diode-pumped solid-state laser (100 mW output). One individual per sample was measured and the fluorescence intensity of 5000 particles was recorded. Histograms with coefficients of variation for the G_0/G_1 peaks of both the analyzed *Centaurea* sample and the standard below 3.6% were considered. Each individual was analyzed three times in three different days and the average value used as the genome size in order to minimize random instrumental error. If the variation range within the three measurements exceeded 2% of the average value, the most outlying measurement was discarded and the sample was reanalyzed.

Crossing experiments

Inflorescences of experimental plants (diploid populations CERV, KOB and LAZ, tetraploid populations LIPO1, VELF, VIS; 10 plants per population) were enclosed in nylon bags prior to anthesis. At anthesis, two capitula were gently brushed against each other once every day for 3–6 consecutive days until all the florets withered. Emasculation of flowers was not necessary due to low incidence of autogamy in *Centaurea* sect. *Jacea* (Gardou 1972, Hardy et al. 2001). Three types of crosses were performed: (i) within a cytotype (homoploid crosses), (ii) between cytotypes (heteroploid crosses), and (iii) autogamy as a control (two capitula of one individual brushed against each other). Mature achenes were harvested about 4–6 weeks after flowering and stored in a dry, dark room. Capitula infested by seed-feeding insects were not analyzed. In total, 294 pollinated capitula were examined for presence of achenes (31 and 39 from diploid and tetraploid homoploid crosses, respectively; 61 and 55 from heteroploid crosses with a diploid and tetraploid maternal plant, respectively; 36 and 72 from autogamy of diploids and tetraploids, respectively). Some of the achenes were germinated in Petri dishes containing distilled water in a growth chamber (14 h light / 10 h dark regime; temperature ca 20°C, not regulated) for the flow cytometric analysis.

The frequency of successful crosses (i.e. crosses that yielded at least one filled achene) was analyzed using a log-linear model including two predictor factors 'type of cross' (three levels: autogamy, homoploid, heteroploid) and 'maternal cytotype' (two levels: diploid, tetraploid) and a response factor 'success' (two levels: yes, no). The number of achenes in successful crosses was analyzed using a generalized linear model with a quasi-Poisson distribution and the same predictors as above.

Morphometric analysis

The same set of characters as in the previous study of *C. phrygia* agg. (Koutecký 2007) was used. However, use of some characters originally excluded from the dataset was reconsidered and several characters were excluded due to their invariability in *C. phrygia*. The final data matrix includes 13 quantitative characters, 5 ratios and 4 binary characters (Table 1). Characters such as branching of a stem, shape of leaves and dimensions of an involucre were measured on fresh plants. Plants were then dried and used to measure other morphological characters. Three quantitative characters and one ratio were recorded for achenes. It was not possible to collect all the samples after fruit ripening for logistic reasons. Therefore, achenes were not available for all individuals studied and all analyses were computed twice, with the achene characters either included (i.e. all characters were

Table 1. – List of morphological characters studied. All characters were measured on plants with a fully developed and undamaged terminal capitulum. The accuracy of the measurements is one decimal place.

Character	Description	Unit
Quantitative characters		
SN	height of the non-flowering part of the stem, i.e. height from the ground to the lowest flowering branch; short (a few cm) thin branches with reduced capitula that sometimes develop in the lower leaf axils were not considered	cm
SF	height of the flowering part of the stem, i.e. height from the lowest flowering branch to the terminal capitulum	cm
ST	total stem height (SN + SF)	cm
LL	lamina length of a middle stem leaf	cm
LW	lamina width of a middle stem leaf, including lateral teeth / lobes	cm
IL	height of the involucre of the terminal capitulum, i.e. from the base of the involucre to the top of appendages on the innermost involucral bracts	cm
IW	width of the involucre of the terminal capitulum; the distance between outer surfaces of involucral bracts is measured, recurved parts of involucral bracts were not included	cm
ML	length of the longest appendage on middle involucral bracts of the terminal capitulum, including the terminal seta on the appendage	mm
MW	maximal width of the lower widened part of the longest appendage on middle involucral bracts of the terminal capitulum; lateral teeth / fimbriae were not included	mm
0MF	number of lateral fimbriae on one side of the longest appendage on middle involucral bracts of the terminal capitulum	
AL	achene length, excluding the pappus (average of 5 achenes)	mm
AW	achene width (average of 5 achenes)	mm
AP	length of the longest setae on the pappus (average of 5 achenes)	mm
Ratios		
SFT	proportion of the height of the flowering part of the stem (SF/ST)	
LLW	length / width of the lamina of a middle stem leaf (LL/LW)	
ILW	length / width of the involucre of the terminal capitulum (IL/IW)	
MLW	length / width of the longest appendage on middle involucral bracts of the terminal capitulum (ML/MW)	
ALW	length / width of achenes (AL/AW)	
Binary characters		
LM	shape of the margin of a middle stem leaf: 0 – entire to denticulate; 1 – large teeth (> 1 mm) or a few pairs of lateral lobes present	
IV	visibility of appendages on the innermost involucral bracts of the terminal capitulum in side view: 0 – visible, exceeding appendages on middle involucral bracts, 1 – not visible, covered by appendages on middle involucral bracts	
CL	colour of the lower part of the longest appendage on middle involucral bracts (0 – brown; 1 – black)	
CA	colour of the apical part (in c. upper 1/3 of length) of the longest appendage on middle involucral bracts (0 – brown; 1 – black)	

analyzed on a reduced number of individuals, n = 452) or omitted (then all individuals/populations were analyzed, n = 817). The average values for each population were calculated because in some analyses populations were used as the operational taxonomic units (OTUs). At the population level, the achene characters were not considered if there were data for less than five individuals per population (one population, LOP, had to be excluded from the dataset).

Basic statistical measures (mean, median, maximum and minimum values, quartiles, 5 and 95 percentiles and standard deviation) were computed for each cytotype and population. The normality of the distribution of each character within cytotypes was tested using Shapiro-Wilk statistics. Values of characters SN, SF, ST, MW, SFT, LLW and ILW, which markedly deviated from a normal distribution, were log-transformed. Pearson and Spearman correlation coefficients were calculated for pairs of characters for each cytotype and for the whole data set to study relationships between the characters. Prior to principal component analyses (PCA), the data were standardized to have zero mean and unit standard deviation.

PCA based on a correlation matrix using both populations and individuals as OTUs was run to obtain a first insight into the structure of the group studied. Linear discriminant analysis (LDA; also named canonical discriminant analysis), which attempts to maximize differences between a priori defined groups, was employed to test the discriminating power of individual characters using both individuals and populations as OTUs. Forward selection procedure was used to detect the minimal subset of characters without significant loss of the discrimination power. Although LDA assumes a multivariate normal distribution of the characters, it is to a large extent robust to violations of this assumption, except for significance tests (Lepš & Šmilauer 2003). Therefore, Monte Carlo permutation tests were used instead of parametric tests. Classificatory discriminant analysis based on probabilities using populations and individuals as the OTUs was run to quantify separation of the cytotypes and the a priory probabilities of classification to a particular group were set equal. The discriminant power was determined by cross validation. Because of differences between populations and a certain hierarchical design, it was not possible to ignore population structure. Therefore, we used a population as the leave-out unit in the cross validation procedure. Individuals from a particular population were thus classified using the classification rule based on all other populations but excluding individuals from the same population. Besides the parametric method, a non-parametric k-nearest neighbour algorithm with similar design of cross validation was performed.

Geographical distribution

Distribution of the cytotypes of *C. phrygia* was estimated based on our field search and a revision of ca 3000 herbarium specimens from Czech and Slovak public herbaria (BRA, BRNM, BRNU, CB, CBFS, GM, HOMP, HR, CHOM, LIM, LIT, MJ, MP, OL, OLM, OSM, PL, PR, PRC, ROZ, SAV, SLO, SOKO, ZMT) and from private collections of some colleagues (J. Hadinec, J. W. Jongepier, P. Petřík). Additional material from B, BP, LE, UPS, W and WU was also included.

For the distribution map, the obviously adventive occurrences (e.g. railway stations) and the specimens of uncertain determination are not considered. The list of all localities is available from the corresponding author on request. From Romania and the Balkans, where *C. phrygia* also occurs, only published chromosome counts are mapped but the morphology-based data from these areas are not mapped because of unresolved taxonomy.

Software

The flow cytometric data were processed using FloMax 2.6 (Partec GmbH, Germany) and FlowJo 7.5.5 (TreeStar, Inc., Oregon, USA). Distribution maps were prepared using Quantum GIS (Quantum GIS Development Team 2010). The statistical analyses were run in STATISTICA 9.1 (Statsoft 2010), CANOCO for Windows 4.5 (ter Braak & Šmilauer 2002) and R 2.10 (R Development Core Team 2009).

Results

Chromosome counts, DNA ploidy levels and genome size

The occurrence of both diploids (2n = 2x = 22) and tetraploids (2n = 4x = 44) was confirmed by chromosome counting and/or flow cytometry, but there was only one ploidy level in most populations (Appendix 1). Typical fluorescence histograms are presented in Fig. 1 and the relative fluorescence intensities are summarized in Table 2. Geographical separation is obvious as diploids were found in most of the area studied whereas tetraploids occurred only in the Western Carpathians and adjacent Eastern Sudetes. There is a small contact zone, where both cytotypes and mixed populations occur, in Slovakia in the foothills of the Nízké Tatry Mts and margins of Liptovská kotlina basin. Three less frequent ploidy levels were identified using flow cytometric screening. We found one triploid and one pentaploid within diploid–tetraploid mixed populations (see the next section) and one hexaploid and two pentaploids in otherwise tetraploid populations (Appendix 1).

Genome sizes are listed in Table 3. The variation in genome size was small across populations, even though geographically remote populations were included. We have never observed bifurcated peaks in simultaneous analyses of individuals with extreme values (including more sensitive analyses using DAPI fluorochrome). The genome size can therefore be considered homogeneous within a cytotype. The monoploid genome sizes (Cx-value) differ only slightly between cytotypes and the ratio of the mean Cx-values of tetraploids to diploids is 0.95.

Mating barriers

Crossing experiments have demonstrated pronounced differences between the different types of crosses and a strong, though not complete, reproductive barriers between the cytotypes. Both the frequency of success and the number of seeds produced by the crosses between the different cytotypes differed. Neither marginal nor conditional effects of the maternal ploidy level were significant (Table 4). As expected, the success and the yield of heteroploid crosses were reduced compared to homoploid crosses and were comparable to autogamic crosses (Fig. 2). Flow cytometric screening of the progeny has shown that the reproductive isolation between cytotypes is very strong. The progeny of heteroploid crosses involving a diploid maternal plant comprised mainly diploids, which must have originated from autogamy. Only one triploid (1.8%), probably a true hybrid, was found. No tetraploid hybrids that might be formed via unreduced ovules of diploids were identified. From tetraploid maternal plants, 19% of the triploids among the progeny were of hybrid origin. The number of possible tetraploid hybrids (via unreduced pollen of diploids) cannot be inferred using only flow cytometry because they cannot be distinguished

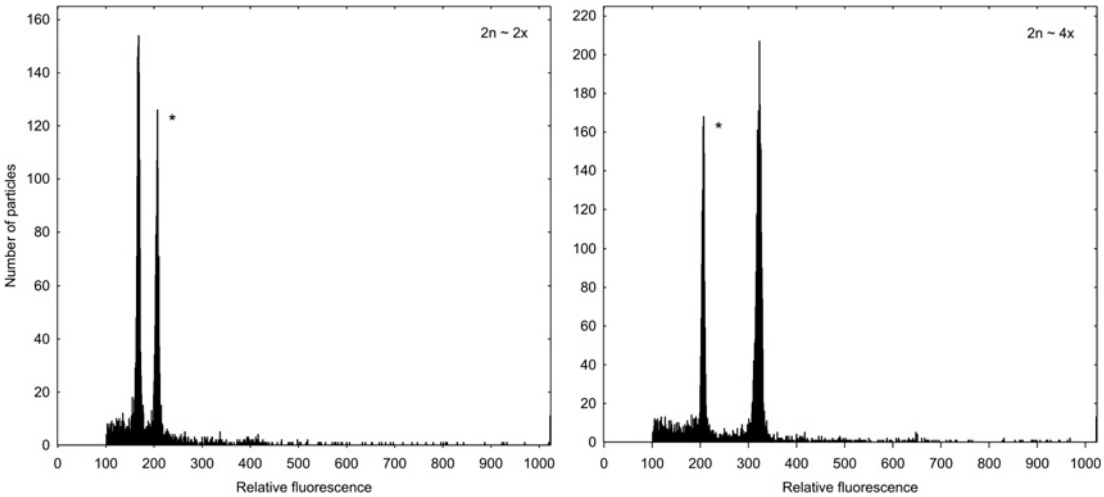

Fig. 1. – Typical histograms of the relative fluorescence (DAPI staining) of the diploid (left) and the tetraploid (right) cytotypes of *Centaurea phrygia*. The nuclei of the sample and the internal standard (*Glycine max* 'Polanka'; marked by the asterisk) were isolated, stained and analyzed simultaneously.

Table 2. – Relative genome sizes of individual ploidy levels of *Centaurea phrygia* assessed using DAPI fluorochrome (the ratio to the internal standard *Glycine max* 'Polanka', which is the given unit of relative genome size). N = number of samples, S.E. = standard error of mean.

Ploidy	N	Relative fluorescence (Mean±S.E.)	Range of relative fluorescence
2x	179	0.805±0.001	0.779–0.828
3x	11	1.175±0.005	1.152–1.198
4x	292	1.549±0.001	1.474–1.585
5x	3	1.898±0.029	1.846–1.947
6x	1	2.379	

Table 3. – Genome sizes of the diploid and the tetraploid cytotypes of *Centaurea phrygia*. N = number of samples per population analyzed, S.E. = standard error of mean. The mean 2C-value for each population and the mean 2C-value, the difference between the most extreme values (% of the mean value) and the mean Cx-value for each cytotype are given.

Cytotype	Population	N	2C-value (pg)	Mean 2C-value ± S.E. (pg)	Variation (%)	Mean Cx-value (pg)
Diploid	KOLI	3	2.22	2.22 ± 0.01	2.2	1.11
	NSE1	3	2.24			
	VYCH	3	2.23			
	CERV	3	2.20			
Tetraploid	PRUD	3	4.27	4.22 ± 0.03	3.3	1.06
	LIPO	3	4.23			
	VELF	3	4.26			
	VIS	3	4.13			

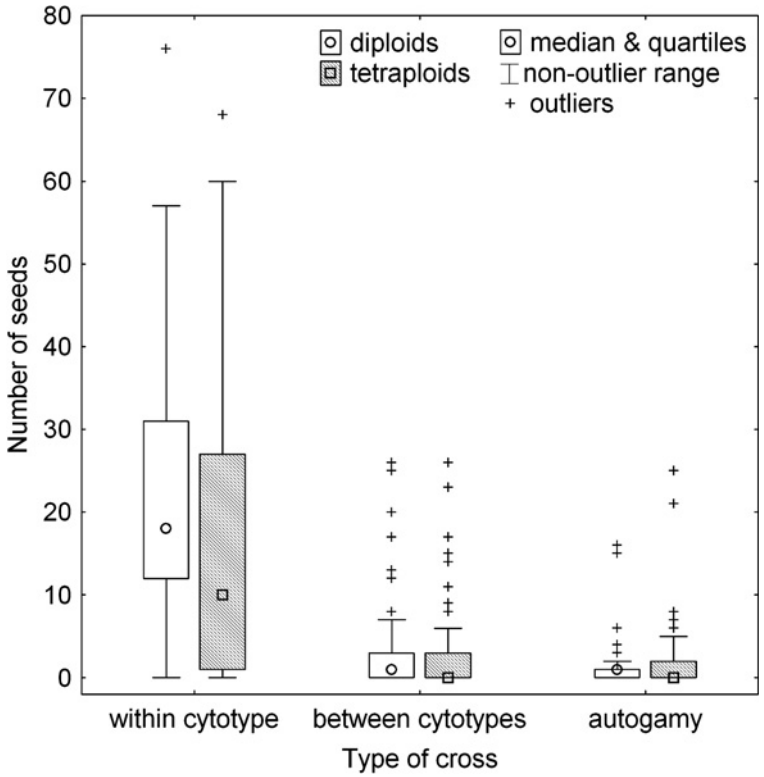

Fig. 2. – The number of seeds per capitulum for individual treatments and cytotypes used as the maternal parent in the crossing experiment with *Centaurea phrygia*. In total, 294 capitula were evaluated (31 and 39 from diploid and tetraploid homoploid crosses, 61 and 55 from heteroploid crosses with diploid and tetraploid maternal plants and 36 and 72 from autogamy of diploids and tetraploids, respectively).

from autogamic tetraploid offspring. In order to discriminate between autogamic progeny and hybrids further analyses of the progeny using allozyme markers is needed. No higher ploidy levels were found.

The results of the crossing experiment are in line with the data obtained from natural populations. Ploidy levels of the plants studied generally agreed with their morphology (see below), even in mixed populations where inter-cytotype hybridization was expected. We found only one triploid (0.1%) among the 1,002 individuals from mixed populations analyzed using flow cytometry. This triploid was probably an inter-cytotype hybrid. Moreover, we identified two tetraploids that possessed morphological characters typical of diploids and could be of hybrid origin via unreduced gametes of diploids. Surprisingly, we found one pentaploid in a diploid–tetraploid mixed population. It could have been formed either by inter-cytotype hybridization involving an unreduced gamete of a tetraploid or by crossing of an undiscovered hexaploid with a tetraploid, similar to the pentaploids recorded in one exclusively tetraploid population (see Discussion).

Table 4. – Results of a log-linear model of the frequency of successful crosses and a generalized linear model of the number of achenes produced by successful crosses. Effect – marginal effect (i.e. the effect of the variable when alone in the model) or conditional effect (i.e. the effect of the variable in addition to other variables already included in the model), df = degrees of freedom, P = significance level (based on χ^2 distribution and F-distribution, respectively).

Factor	Effect	Success			Number of achenes		
		df	Residual deviance	P	df	Residual deviance	P
Null model		5	29.70		154	2273.7	
Type of cross	marginal	2	1.63	8×10^{-7}	2	1295.9	3×10^{-11}
Maternal cytotype	marginal	1	28.66	0.308	1	2266.2	0.478
	conditional	1	0.79	0.360	1	1292.9	0.555

Morphometric analysis

There were only slight differences between the Spearman and Pearson correlation coefficients. No highly correlated characters ($r > |0.95|$) were found and all characters were used in the multivariate analyses.

PCA of populations revealed that there is morphological differentiation between the cytotypes, especially when achene characters are included (Fig. 3). Notably, there is no geographical pattern as the northern European populations (KOLI, KOV) are not separated from Central European diploid populations and the tetraploid populations from the Eastern Sudetes (KAR, PRUD) do not differ from the Western Carpathian populations. The characters that are most correlated with the separation of the cytotypes are length of the pappus (AP), colour of the apical part of the appendages (CA), length and length/width of the appendages on the middle involucral bracts (ML, MLW) and visibility of the appendages on the inner involucral bracts (IV). However, there is extensive variation within each cytotype in other characters. When individuals were used as OTUs, the groups were blurred and there was large overlap when the achene characters were considered and almost no separation when the achenes characters were omitted.

LDA of populations as OTUs confirmed morphological separation of the cytotypes. No overlap of canonical scores of the cytotypes was observed, irrespective whether achenes were included or not. Three significant characters were identified by forward selection procedure with all characters, namely (with decreasing discriminant power) pappus length (AP), visibility of appendages on inner involucral bracts (IV) and colour of the basal part of appendages on middle involucral bracts (CL). When achene characters were excluded, four significant characters were found: length of appendages on middle involucral bracts (ML), width of the involucre (IW) and characters IV and CL. At the level of individuals, there was some overlap of the groups when achene characters were included (Fig. 4) and only moderate separation when they were omitted. Characters that contributed significantly to the separation of the cytotypes in both analyses are listed in Table 5. However, the majority of the discrimination power was associated with only four characters AP, ML, IW and CA (Table 5). There is no single character that separates the cytotypes. For capitulum width (IW) and capitulum length / width ratio (ILW), there is complete overlap of the variation ranges across the cytotypes and the means and the quartiles (not shown) are only slightly shifted. The cytotypes differ in mean length of appendages on the middle involucral bracts (ML) and in

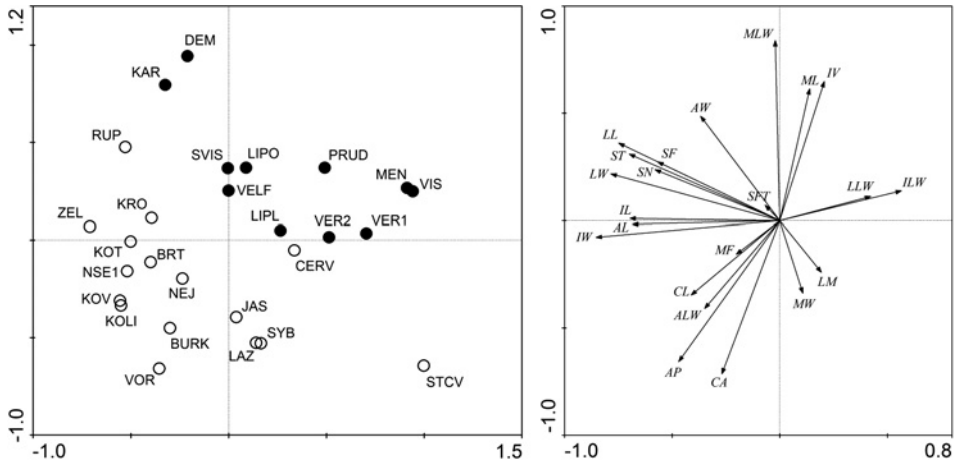

Fig. 3. – PCA of 27 populations of *Centaurea phrygia* using all 22 characters, including achenes. Empty circles – diploids, full circles – tetraploids. The first and the second ordination axis are depicted, which explain 24.5% and 16.4% of the variation, respectively. See Appendix 1 for population names.

Table 5. – Linear discriminant analyses of the cytotypes of *Centaurea phrygia* with forward selection of the characters using individuals as OTUs. Characters with a significant conditional effect (i.e. the effect of the variable in addition to other variables already included in the model) are listed. The significance was tested using a Monte Carlo permutation test (999 permutations). The analysis was run twice with achene characters either included or not. λ = eigenvalue, i.e. discriminant force of the particular character, P = significance level (conditional effect), corr. = correlation coefficient with the canonical axis (diploids have negative canonical scores, tetraploids have positive scores), marg. = characters with significant marginal effects (i.e., the effect of the variable when alone in the model) but insignificant conditional effects.

Achenes included (n = 452)				Achenes excluded (n = 817)			
Character	λ	P	corr.	Character	λ	P	corr.
AP	0.46	0.001	–0.68	ML	0.33	0.001	0.57
ML	0.17	0.001	0.62	IW	0.08	0.001	–0.20
IW	0.03	0.001	–0.26	CA	0.03	0.001	–0.29
AL	0.01	0.001	–0.16	ST	0.01	0.001	–0.17
ST	0.01	0.002	–0.18	MW	0.01	0.001	0.09
CA	0.01	0.002	–0.30	MF	0.01	0.001	0.04
MW	0.01	0.002	0.10	IV	0.01	0.006	0.21
LL	0.01	0.015	–0.14	CL	0.01	0.010	–0.20
				LLW	0.01	0.011	0.14
marg.: MLW, ILW, LW, IV, CL, LLW, SF, LL, MW				marg.: MLW, ILW, LW, SN, IL, LL, SF			

mean pappus length (AP), but the variation ranges markedly overlap even for the two best characters. The cytotypes are also partly separated by the colour of appendages, with the apical parts being more frequently black in diploids (Table 6).

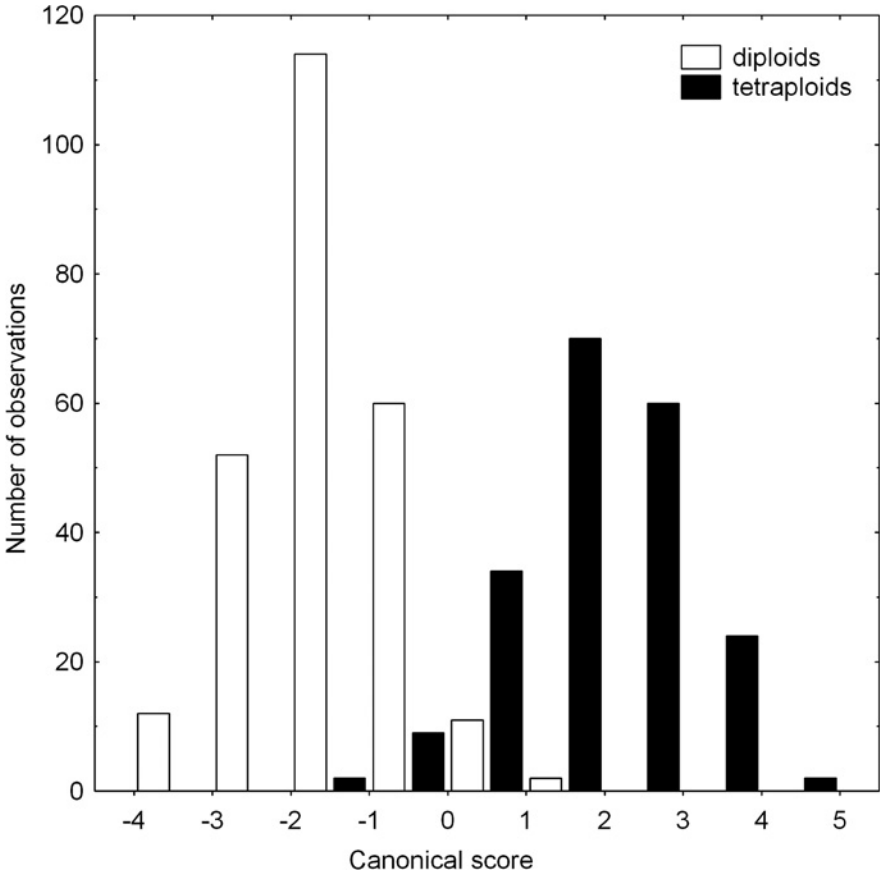

Fig. 4. – Histogram of canonical scores of linear discriminant analysis of individuals of *Centaurea phrygia* (n = 452). All characters, including achenes, were used. The canonical correlation coefficient equals 0.849 and its square, which indicates portion of variation explained by the canonical axis, equals 0.721.

Table 6. – Variation of the best discriminating characters for the cytotypes of *Centaurea phrygia*. For the binary character CA, the average value indicates the proportion of individuals having the character state 1 (i.e. distal part of appendages are black). d = diploids, t = tetraploids, N = number of individuals analyzed, SD = standard deviation, min = minimum, max = maximum, 5% and 95% indicate the respective quantiles.

Character	Unit	Cytotype	N	Mean	SD	(min–)5%–95%(–max)
AP	mm	d	251	1.0	0.3	(0.1–) 0.6–1.4 (–1.7)
		t	201	0.5	0.3	(0–) 0.1–1.0 (–1.6)
ML	cm	d	498	7.4	1.4	(4.5–) 5.3–9.8 (–12.4)
		t	319	9.5	1.6	(5.2–) 6.8–12.1 (–14.1)
IW	cm	d	498	1.6	0.2	(1.0–) 1.5–1.8 (–2.0)
		t	319	1.6	0.2	(1.0–) 1.3–1.9 (–2.0)
CA		d	498	0.61		
		t	319	0.32		

Table 7. – Classificatory discriminant analyses of the cytotypes of *Centaurea phrygia* using individuals as OTUs. Percentage of correctly classified individuals for both cytotypes and for the whole dataset, and the number of individuals analyzed (N) are given. The ranges on the second and the fourth line indicate the percentages of correct classifications within individual populations. The analysis was run twice, with achene characters either included (22 characters in total) or omitted (18 characters).

Achenes	Diploids	Tetraploids	Total	N
Included	94.4% 60.0%–100%	88.1% 70.0%–100%	91.6%	452
Omitted	82.1% 46.7%–100%	77.7% 56.7%–100%	80.4%	817

Morphological differentiation between the cytotypes was also confirmed by classificatory discriminant analyses. All populations when used as OTUs were correctly classified. More than 90% of the individuals were correctly classified when achene characters were included in the analysis (Table 7). Classification of diploids was slightly more successful than classification of tetraploids. The incorrect classifications were not evenly spread across the populations as while in the majority of populations the classification was 100% successful, in some populations there was a high number of misidentifications (up to 53.3%). When achene characters were omitted, the accuracy of the classification was lower (Table 7). The non-parametric k-nearest neighbour algorithm revealed a similar pattern, including differences between populations, but the overall success of the classification was lower than for the parametric method (75.7% with achenes included, 75.4% with achenes not included)

Geographic distribution

An analysis of herbarium material confirmed the geographic separation of the cytotypes, as suggested by published chromosome counts and our karyological and flow-cytometric analyses. The diploid cytotype occurs throughout the range of the species and is the only cytotype in the northern part of the range, whereas the tetraploid cytotype is confined to Central Europe (Fig. 5). There is also some geographical separation in the distribution of the cytotypes at a regional scale. Diploids occur in flat areas in the Ukraine, Poland and Germany and occasionally extend to the north of the Czech Republic and Slovakia. Diploids are the only cytotype recorded in the northern part of the Eastern Carpathians, where its occurence is connected with Ukrainian lowland localities in the north and extends in the south to Romania, where they have a more or less continuous distribution in the Eastern Carpathians (not mapped). Remarkably, diploids are nearly absent from the Western Carpathians. The tetraploid cytotype is confined to the mountain landscapes of the Western Carpathians and the adjacent Hrubý Jeseník Mts of the Sudetes, where it replaces the diploid cytotype. The tetraploid cytotype is recorded at a few other isolated localities. The cytotypes come into contact only in a relatively small area of Slovakia at the margins of Spišská kotlina and Liptovská kotlina basins and in the foothills of the Nízké Tatry (Low Tatra) Mts (Fig. 5), however, even there their distribution is rather parapatric and there are very few mixed populations.

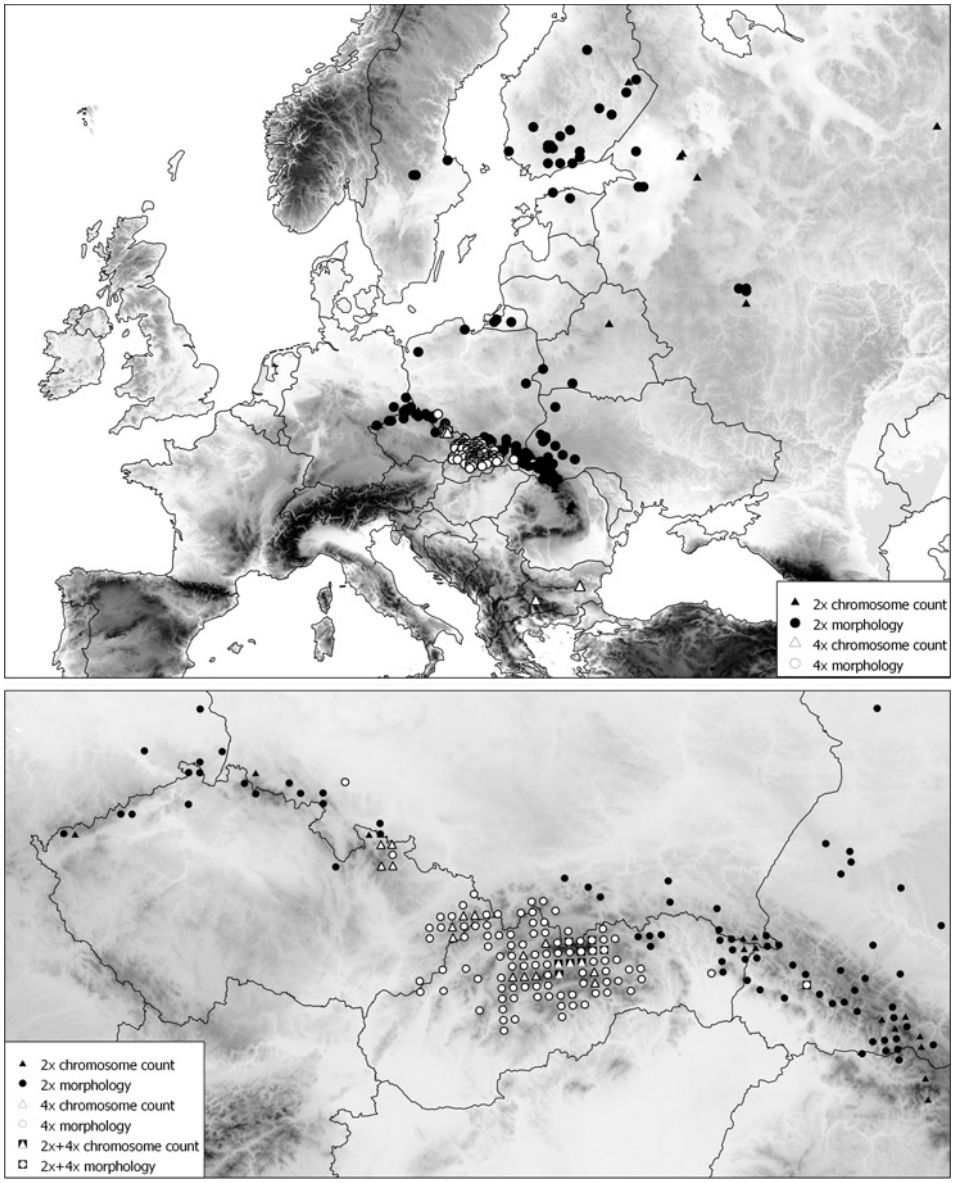

Fig. 5. – Distribution of the cytotypes of *Centaurea phrygia*. The obviously adventive occurrences (e.g. railway stations) are not mapped. For legibility, the detailed data for Central Europe are presented in a 6' × 10' grid (latitude × longitude). Note that the diploid cytotype has a more or less continuous distribution in both the Eastern Carpathians and Romania and plants similar to the tetraploid cytotype are know from Romania, Bulgaria and the former Yugoslavia; the morphology-based data from the Balkans were not mapped because of unresolved taxonomy, only published chromosome counts are mapped.

Discussion

Mating barriers and unreduced gametes

The cytotypes are reproductively well isolated. Only a few hybrids resulted from the crossing experiments and were recorded in natural mixed populations. These results are almost identical with previous detailed studies of various diploid/tetraploid pairs in *Centaurea* sect. *Jacea*, which document a low incidence of (triploid) hybrids in crossing experiments and their virtual absence in natural populations (Gardou 1972, Hardy et al. 2000, 2001, Koutecký 2007, Koutecký et al. 2011). Triploids are expected to be sterile or nearly so in *Centaurea* sect. *Jacea* (Gardou 1972, Hardy et al. 2000, 2001). Indeed, gene flow between the cytotypes of *C. phrygia* is limited.

However, based on their morphology, we have identified two tetraploid plants that are probably inter-cytotype hybrids. Fertile tetraploid hybrids are known within *Centaurea* sect. *Jacea* (Koutecký et al. 2011). Gene flow from the diploid to the tetraploid cytotype of *C. phrygia* via tetraploid hybrids is therefore possible. A tetraploid hybrid can be formed by the union of an unreduced gamete of a diploid and a reduced gamete of a tetraploid (Koutecký et al. 2011).

The higher ploidy levels recorded indicate that the tetraploid cytotype produces some unreduced gametes. One hexaploid in an otherwise tetraploid population probably originated from the fusion of a reduced and an unreduced gamete of a tetraploid. It is difficult to account for the origin of the two pentaploids recorded in a tetraploid population, because it is not possible to get a pentaploid from tetraploids in one step and there was no other taxa that could hybridize with *C. phrygia* at that locality. Most probably these pentaploids were formed as a result of a cross between a tetraploid and an undiscovered hexaploid. The one pentaploid recorded in a mixed diploid–tetraploid population may have had a similar origin, however in this case it is also possible that it was the result of the union of a reduced gamete of a diploid and an unreduced gamete of a tetraploid.

Genome size

The genome sizes were 2C = 2.22 pg for diploids and 2C = 4.22 for tetraploids. The value for the diploid cytotype is similar to the value published by Dydak et al. (2009) (2C = 2.14 pg, which differs by 3.7% from our result). Our value for the diploid cytotype is also at the upper limit of genome sizes reported for *Centaurea* sect. *Jacea* (Bancheva & Greilhuber 2006; the name *Centaurea* subgen. *Jacea* is used to denote this group in the cited work). The value for tetraploids is fully within the range reported for *Centarea* sect. *Jacea* tetraploids by Bancheva & Greilhuber (2006). Notably, the ratio of monoploid genome sizes of tetraploid to diploid *C. phrygia* (the value of 0.95) is the same as the average value reported by Bancheva & Greilhuber (2006).

Origin of tetraploids

Autopolyploidy is already documented in *Centaurea* sect. *Jacea* for tetraploid *C. jacea* L. using allozyme markers (Hardy et al. 2001). It is probable that the tetraploid cytotype of *C. phrygia* is also autotetraploid. The monoploid genome sizes (Cx-values) of the diploid and tetraploid cytotype of *C. phrygia* are similar (1.11 pg and 1.06 pg, respectively). Similarity in monoploid genome sizes is sometimes seen as an indicator of an autopolyploid

origin (Balao et al. 2009, Trávníček et al. 2010). However, it should be noted that the variation in genome size is small within *Centaurea* sect. *Jacea* (Bancheva & Greilhuber 2006), unlike in some other genera, such as the closely related *Cirsium* (Bureš et al. 2004). Little differentiation can thus be expected in *Centaurea* sect. *Jacea,* even in the case of allopolyploidy. The pattern of morphological variation also supports an autopolyploid origin, because the cytotypes of *C. phrygia* are morphologically very similar to each other. Finally, an autopolyploid origin of the tetraploid cytotype of *C. phrygia* is also supported by allozyme patterns available for a few populations (P. Koutecký et al., unpublished data). The differentiation between the cytotypes, based on the four loci studied (6-PGDH, DIA, ENP, LAP), is mainly due to rare alleles. We have not detected fixed heterozygosity in tetraploids. Indeed, various di- and triallelic heterozygotes and about 15–25% homozygotes (depending on the locus) are present among tetraploids, which suggests random segregation of alleles and tetrasomic inheritance typical of autotetraploids.

Morphological variation

The two cytotypes of *C. phrygia* can be distinguished by combination of several morphological characters, although no single character can be used to identify these cytotypes. Discriminant analysis based on probabilities was able to classify correctly more than 90% of individuals. The misclassified individuals were not evenly spread among the populations. While for the majority of populations the classification was highly successful (90–100%), there were numerous misclassifications in some populations, especially the diploid populations SYB and BURK (40% and 23.1% of wrongly classified individuals, respectively, in the analysis including achenes) and tetraploid populations VELF, VER2 and SVIS (33.3%, 30%, and 22.2%, respectively). It seems that the less successful classification of these populations is caused solely by extreme values of one or two "key" characters: the populations SYB and BURK have the longest appendages among diploids (as well as other populations from the Ukrainian Carpathians), while the populations VELF, VER2 and SVIS have the largest capitula among tetraploids; in the population SVIS there is also an unusually high number of individuals with black-coloured appendages. Nevertheless, all populations were correctly classified in the discriminant analysis based on average values for each population. The number of correctly classified individuals is similar to that recorded in a previous study (Koutecký 2007) that examined variation between widely accepted species within the *C. phrygia* agg. In conclusion, although morphological differentiation between the cytotypes of *C. phrygia* is rather weak, it is similar to that between other species of the *C. phrygia* agg., which favours treating the cytotypes of *C. phrygia* as separate taxa.

Taxa of *Centaurea* sect. *Jacea* of the same ploidy level easily hybridize. Extensive hybrid zones and introgression are documented for diploid *C. elatior* and *C. stenolepis* and for tetraploid *C. phrygia* and *C. oxylepis* (Koutecký 2007). Notably, the potential for introgression from other taxa differs between the cytotypes of *C. phrygia*. There is almost no contact between the diploid cytotype and other diploid taxa, except for a small area on the Czech–Polish border where *C. pseudophrygia* occurs close by. In contrast, the tetraploid cytotype is in wide contact with *C. oxylepis* and *C. jacea*. These species differ from *C. phrygia* in having narrower leaves, narrower capitula and absence of a pappus. We have observed that tetraploid *C. phrygia* have a shorter pappus and slightly narrower capitula than the diploids (Table 6), although we have not sampled obviously hybrid popu-

lations. Introgression at the tetraploid level could therefore promote the diversification of *C. phrygia* cytotypes.

Geographic distribution

Our data confirmed that the cytotypes of *C. phrygia* have different geographic distributions. Whereas the diploid cytotype occurs throughout the range of the species, the tetraploid cytotype is probably endemic to Central Europe. It occurs in the Western Carpathians and the Hrubý Jeseník Mts of the Eastern Sudetes. The few outlying localities for tetraploids known to us are east of the town of Świdnica in south-western Poland (probably native, growing in serpentine soil), in the foothills of the Vihorlat Mts in Slovakia (the only record is for the year 1921, possibly adventive) and in the surroundings of the town of Volovec in the Ukrainian Eastern Carpathians. Populations of a hybrid *C. oxylepis* × (tetraploid) *C. phrygia* are known from the north-east of the Czech republic, southern Poland and margins of the Western Carpathians in Slovakia. This hybrid is morphologically intermediate and due to its full fertility and back-crossing it usually prevails over parental taxa or occurs alone (Koutecký 2007). Based on our field experience, the majority of the records of *C. phrygia* and all records of *C. pseudophrygia* from this area belong to this hybrid, including the localities cited in the atlas of the distributions of plants in Poland (Zając & Zając 2001).

The diploid cytotype of *C. phrygia* is also widespread in the Eastern Carpathians in Romania (Koutecký, unpublished data). Tetraploid plants of *C. phrygia* agg. also occur in Romania (Koutecký et al., unpublished data). Based on the sparse material we have studied (both our field-collected material analyzed using FCM and herbarium material), this morphotype differs from the Western Carpathian tetraploids in having narrower leaves, smaller capitula and shorter appendages on involucral bracts. Identity of this morphotype remains obscure. Probably it could be classified as an autonomous taxon within the *C. phrygia* agg. A similar morphotype is reported from Bulgaria and the former Yugoslavia. This problem deserves further study and a thorough taxonomic revision of *Centaurea* sect. *Jacea* in Romania and the Balkans is needed.

Taxonomic classification and nomenclature

The available data are highly congruent and support treating the diploid and the tetraploid cytotypes of *C. phrygia* as autonomous taxa. The cytotypes are reproductively isolated, though the mating barrier is probably not complete. They have distinct geographical distributions with only a small contact zone and very few mixed populations. They are morphologically differentiated and can be distinguished from each other, especially when many individuals from each population are available. The morphological differences between them are rather small, but this is true of the whole *C. phrygia* agg. In conclusion, we propose treating the *C. phrygia* cytotypes as autonomous species, following the recommendations of, for example, Soltis et al. (2007).

The name *C. phrygia* L. has not been typified. Nevertheless, the name is now widely used in the sense of the diploid cytotype and usual morphological delimitation of *C. phrygia* in contemporary literature is consistent with the diploid cytotype. In fact, the majority of authors outside the Czech Republic and Slovakia work only with the diploid

cytotype due to the limited distribution of the tetraploids. Our preliminary search for the original material of the name *C. phrygia* L. (five herbarium sheets in LINN, BM, UPS and two illustrations; Koutecký & Štěpánek, unpublished data) shows that its typification in the sense of the diploid cytotype is possible, although the material is heterogeneous. Moreover, there is indirect evidence that Linnaeus personally knew of the diploid cytotype. There are three geographic areas reported in the protologue of *C. phrygia* L. (Linnaeus 1753): "Habitat in Helvetia, Austria, Finlandia". The first two are probably based on older literature and refer to other taxa of *C. phrygia* agg. that were not distinguished by Linnaeus (namely *C. nervosa* Willd. and *C. pseudophrygia* C.A.Mey.; P. Koutecký & J. Štěpánek, unpublished data). There is no reference in Linnaeus (1753) nor in his older work related to plants from Scandinavia and the record "Finlandia" is thus probably based on Linnaeus' personal knowledge; the diploid cytotype of *C. phrygia* is the only taxon of *C. phrygia* agg. that occurs in that area. We are therefore preparing typification of *C. phrygia* L. in the sense of the diploid cytotype but due to the complexity of the problem it needs to be published in a separate detailed contribution.

Two other names are typified here in the sense of the diploid *C. phrygia*. The name *C. austriaca* Willd. has been long recognized as a synonym of *C. phrygia* L. The original material from Willdenow's herbarium comprises two specimens under this name. One is certainly a specimen of diploid *C. phrygia* (collected by P. Kitaibel, locality unknown) and is here proposed as the lectotype, the other has a question mark on its original label and belongs to a later described taxon, *C. nervosa* Willd. The other name associated with diploids is *C. phrygia* subsp. *brevipennis* Čelak. Although Čelakovský (1871) probably did not distinguish between diploids and tetraploids (in the description of the distribution of this taxon there is "Ungarn", which at that time included Slovakia where tetraploids are frequent), the name can be unambiguously attributed to diploids. The geographical scope of Čelakovský's Prodromus was only Bohemia, where only diploids occur, the original diagnosis is consistent with diploids and all the original material in the herbaria PR and PRC (9 sheets altogether) studied are definitely of diploid *C. phrygia*. We propose a well-preserved specimen from Tausch's exsiccatum Plantae Selectae Florae Bohemicae as the lectotype.

Concerning the tetraploid cytotype, we have identified the name *Centaurea erdneri* J. Wagner as the correct name of the specific rank. It is the oldest name that can be unequivocally typified with tetraploids. The Hungarian botanist János Wagner described *C. ×erdneri* as a putative hybrid *C. phrygia* × *C. pseudophrygia* (Wagner 1910). Our analysis of the original material as well as that which was revised later by J. Wagner has shown that he included several taxa under this name. However, the majority of the original material is of the tetraploid cytotype of *C. phrygia* (Table 8) and the original diagnosis fits it well: *C. ×erdneri* should differ from *C. phrygia* in that only the appendages on the innermost involucral bracts are exerted from a "sheath" composed of fimbriate appendages on the outer bracts, while from *C. pseudophrygia* it should differ in having wider and shorter appendages on the outer and the middle bracts and visible appendages on the inner bracts (Wagner 1910). Moreover, the tetraploid cytotype of *C. phrygia* is morphologically somewhat "intermediate" between the "typical" (i.e. diploid) *C. phrygia* and *C. pseudophrygia* and we assume that the name was really intended to mark these "intermediate" plants. From the 20 specimens (Table 8), which definitely belong to the original material of the name *Centaurea ×erdneri* as defined by Article 9 of the International Code of Botanical

Fig. 6. – The proposed lectotype of the name *Centaurea* ×*erdneri* J. Wagner (BP 247919). The text on the label reads: "*Centaurea erdneri* Wagn. Szt. Ivan közelében. Styavnica patak völgye. ALACSONY TATRA 1907 AUG 3." The collector's name is not printed on the label, but from the handwriting and the style of the label it is clear that the collector was E. G. Nyárády. The determination was written by János Wagner according to the handwriting and was probably added later.

Table 8. – List of syntypes of the name *Centaurea ×erdneri* J.Wagner. For each specimen are presented: the code of the herbarium, the accession number, the localization on the label (recent geographic names and additional information bracketed; SK = Slovakia, RO = Romania, UA = Ukraine), collector's name and the collection date. Determination of the specimens based on our results is provided.

Herb.	No.	Localization	Collector	Date
Tetraploid cytotype of *C. phrygia*				
BP	189865	Com. Liptó: Pribylina. In finibus arvorum [SK]	S. Kupčok	12. 8. 1909
BP	247911	Liptó-Pribylina [SK]	S. Kupčok	12. 8. 1909
BP	247923	Liptó-Pribylina [SK]	S. Kupčok	12. 8. 1909
PR	32523	Liptov. Pribylina. In finibus arvorum [SK]	S. Kupčok	12. 8. 1909
PR	32524	Liptov. Pribylina. In finibus arvorum [SK]	S. Kupčok	12. 8. 1909
PR	32525	Liptov. Pribylina. In finibus arvorum [SK]	S. Kupčok	12. 8. 1909
BP	247917	Tátralomnitz közeleben Tárpaták felé [SK, Tatranská Lomnica]	E. G. Nyárády	15. 9. 1907
BP	247919	Szt. Iván közelében. Styavnica patak völgye. Alacsony-Tátra [SK, Nízke Tatry Mts, Jánská dolina valley by the town of Liptovský Ján]	E. G. Nyárády	3. 8. 1907
BP	247927	Styavnica patak völgye. Alacsony-Tátra [SK, Nízke Tatry Mts, Jánská dolina valley by the town of Liptovský Ján]	E. G. Nyárády	3. 8. 1907
BP	247922	Felkaivölgy. [?; probably SK, valley of the Velický potok brook near the village of Veľká, nowadays part of the town of Poprad]	E. G. Nyárády	4. 9. 1907
BP	247925	Kamenicza alja. Liptovyvárnal [SK, foothills of the Kamenica hill by the town of Liptovský Hrádok]	E. G. Nyárády	4. 8. 1907
BP	247928	Feher víz menten. Tátraháza [SK, along Kežmarská Biela voda river by the village of Mlynčeky]	E. G. Nyárády	7. 9. 1907
BP	247936	Podbanszka közeleben [SK, Podbanské]	E. G. Nyárády	30. 7. 1907
BP	189803	Hung. bor. Com. Abauj-Torna. In montibus supra pagum Štós [SK, Slovenské Rudohorie Mts, Štós]	L. Thaisz	16. 8. 1909
Diploid cytotype of *C. phrygia*				
BP	247921	Borszek [RO, Rodna Mts, Valea Vinului]	Pálfy	08. 1904
Centaurea stenolepis				
BP	189844	Hung. bor. Com. Abauj-Torna. In monte Szarvashegy, supra pagum Falucska [SK, Slovakian karst, Jelení vrch mt. near the village of Hačava]	L. Thaisz	15. 9. 1905
BP	247918	In silvis circa Áj (com. Abauj) [SK, Slovakian karst, Háj]	G. Lengyel	5. 8. 1905
C. phrygia agg., unresolved				
BP		Comit. Hunyad. Prope Malomvíz [RO, northern foothills of the Retezat Mts, Râu de Mori]	J. Wagner	08. 1896
PR	32539	Beregszász [UA, Berehovo]	A. Margittai	
BP	247915	Vidra [RO, Bihor Mts, Vidra]	J. Wagner	07. 1903

Nomenclature (ICBN; McNeill et al. 2006), we propose specimen BP 247919 as the lectotype (Fig. 6). It bears one well-preserved plant that is a typical tetraploid *C. phrygia* from the centre of its distribution, which was determined as *C. ×erdneri* directly by the author of the name and is consistent with the protologue of this species. Concerning nomenclature, a name originally that of a hybrid can be used even if the taxon is not a hybrid without affecting the homonymy and synonymy and author citation (articles 50 and H3.3 of the ICBN; McNeill et al. 2006).

Centaurea erdneri is most probably not the hybrid (allotetraploid) *C. phrygia* × *C. pseudophrygia*, although it was originally described as such. First, it is "intermediate" only in one character, length of appendages on involucral bracts, which determines the overall appearance of the capitula. In other characters, however, *C. erdneri* is either similar to *C. phrygia* (width, colour and shape of appendages, visibility of appendages on inner involucral bracts, size of capitula) or different from both (length of pappus, width of leaves, leaf margin). Second, *C. pseudophrygia* does not occur in the Western and Eastern Carpathians and the two "parental" taxa are almost not in contact and no hybrid zone is known. All records of *C. pseudophrygia* from Slovakia are erroneous (Koutecký 2007, 2008) including the tetraploid populations at high altitudes in the Western Carpathians provisionally assigned to *C. pseudophrygia* in a previous study (Koutecký 2007). Based on a larger dataset these populations are markedly different from this species and morphologically more similar to *C. carpatica* from Romania or the extreme *C. erdneri* (Koutecký 2008) and their status remain uncertain. Third, the available allozyme data suggest an autopolyploid origin of *C. erdneri*. We therefore consider *C. erdneri* to be a non-hybrid taxon.

The hybrid *C. jacea* × *C. phrygia* is usually designated as *C.* ×*austriacoides* Woł., Based on morphology and absence of any other taxon of *C. phrygia* agg. in the respective area the holotype of this name is definitely of this hybrid.

We propose using the name *C.* ×*melanocalathia* Czakó for the hybrid *C. erdneri* × *C. jacea*. This name has been traditionally used to denote a putative non-hybrid taxon similar to *C. phrygia*. *Centaurea melanocalathia* was described from the foothills of Vysoké Tatry Mts (High Tatra Mts) in Slovakia (Czakó 1888a in Hungarian, Czakó 1888b in German translation) and is reported from the Carpathians, especially from the eastern part (e.g. Prodan & Nyárády 1964, Dostál 1976, Dostál 1989, Dostál & Červenka 1992, Dobročaeva 1999, Ciocârlan 2000). According to the original diagnosis, this taxon should be similar to *C. phrygia*, from which it should differ with appendages on involucral bracts that should be more overlapping, shorter and less recurved, darker coloured and having longer lateral teeth, with less hairy stems and leaves, and with a pappus ca 4–5× shorter than an achene (Czakó 1888a). However, no such non-hybrid taxon has been distinguished (Koutecký 2007). The search for the original material yielded only one herbarium sheet (BP 182623). It is consistent with the protologue and can be used as the lectotype. The distinctive shape of the appendages on the involucral bracts (relatively short and rounded in outline with long marginal teeth irregularly fused into groups) indicate it is the hybrid *C. jacea* × *C. phrygia* agg. The use of the name *C. melanocalathia* to denote some of non-hybrid populations of *C. phrygia* agg. from the Eastern Carpathians is thus erroneous and the name is also not a part of the synonymy of *C. phrygia* L., as suggested by Koutecký (2007). Most probably, the previous authors used the name *C. melanocalathia* to denote Carpathian populations of *C. phrygia* and the name *C. phrygia* to denote an assemblage consisting of unrecognized *C. erdneri* and similar undescribed populations from Romania and probably also partly of "atypical" populations of *C. phrygia.*

Indeed, it is not possible to determine whether the parent of the type specimen of *C.* ×*melanocalathia* was the diploid *C. phrygia* or tetraploid *C. erdneri*, but the latter is the most likely. There are three reasons for this: (i) a specimen of *C. erdneri* was also collected by the author of the name at the original locality of *C.* ×*melanocalathia* (BP181097: "*Centaurea phrygia* L. fl. su., Alsó Tátrafüred, in pratis et ad viam atque agrorum margines

abunde", 28. 7. 1887 leg. K. Czakó), (ii) *C. erdneri* is much more frequent than *C. phrygia* in the respective area, and (iii) hybridization between diploid and tetraploid taxa is rare in *Centaurea* sect. *Jacea*, whereas hybridization between two tetraploids is frequent (Koutecký 2007, Koutecký et al. 2011). We therefore propose using the name *C.* ×*melanocalathia* to denote the hybrid *C. erdneri* × *C. jacea* and we support this choice by an epitype, which we selected from among the plants from the tetraploid population we investigated using flow cytometry. Authorship of the name *C. melanocalathia* should also be clarified. The name is ascribed to Borbás in the protologue (Czakó 1888a) and Borbás is sometimes cited as the author of the name (e.g. in Flora Europaea; Dostál 1976). However, the diagnosis was obviously written by Czakó and is based on his own collections and according to the protologue, Czakó only consulted Borbás about the determination and used an unpublished name, which Borbás probably used to mark similar plants. In such a situation, the name should be ascribed solely to Czakó following the article 46.4 of the ICBN (McNeill et al. 2006).

Centaurea phrygia L. in Sp. pl. 2: 910. 1753.

Synonymy:
≡ *Cyanus phrygius* (L.) P. Gaertn., B. Mey. & Scherb. in Oekonom.-Techn. Fl. Wetterau 3: 173. 1801.
≡ *Jacea phrygia* (L.) Soják in Čas. Nár. Mus., Odd. Přír. 140: 132. 1972.
= *Centaurea austriaca* Willd. in Sp. pl. 3: 2283. 1803.
Lectotype (designated here): B-W 16541/1. "Centaurea austriaca calycibus recurvato plumosis foliis glabris ovalis dentatis. Centaurea phrygia Jacq. Habitat in Austria, Hungaria", sine dato legit P. Kitaibel.
≡ *C. phrygia* subsp. *austriaca* (Willd.) Gugler in Mitt. Bayer. Bot. Ges. 33: 408. 1904.
– *C. austriaca* [var.] α *genuina* W. D. J. Koch in Taschenb. Deutsch. Schweiz. Fl. 302. 1843, nom. inval. (ICBN, Art. 24.3).
– *Jacea phrygia* subsp. *austriaca* (Willd.) Dostál in Klíč k úplné květeně ČSR, ed. 2.: 740. 1958, nom. inval. (ICBN, Art. 33.4).
= *Centaurea phrygia* subsp. *brevipennis* Čelak. in Prodr. Fl. Böhmen 2: 250. 1871.
Lectotype (designated here): PRC 452349. [Plantae Selectae Florae Bohemicae] „Centaurea nigra β radiata Tausch. C. austriaca Reichenb. Auf Bergwiesen um Nixdorf", sine dato legit I. F. Tausch.

Centaurea erdneri J. Wagner in Math. Term. Közlem. 30: 422. 1910 (pro hybr.).

Lectotype (designated here): BP 247919. „Centaurea Erdneri Wagn. Szt. Ivan közelében. Styavnica patak völgye. Alacsony Tatra", 3.8.1907 legit E. G. Nyárády.
Synonymy:
≡ *Jacea erdneri* (J. Wagner) Rauschert in Feddes Rep. 83: 656. 1973 (pro hybr.).

Centaurea jacea × *C. phrygia*
Centaurea ×*austriacoides* Woł. in Spraw. Kom. Fizyogr. Akad. Umjetn. Krakow 27: 24. 1892.

Holotype: W 1926–20136. „Centaurea austriacoides Woł. C. austriaca × jacea. Przy brzegu Swicy za ujsciem Jalowego Potoku do Swicy. 760 m. Specimen unicum!" 21.8.1890 legit A. Wołoszczak.
Synonymy:
≡ *Jacea* ×*austriacoides* (Woł.) Rauschert in Feddes Rep. 83: 656. 1973.

Centaurea erdneri × *C. jacea*
Centaurea ×*melanocalathia* Borbás ex Czakó in Magyarosz. Kárpátegylet Évk. 15: 146. 1888 (pro sp.).

Lectotype (designated here): BP 182623. „Centaurea phrygia L. (austriaca W.) var. melanocalathia Borb. Alsó-Tátrafüred. Szepes m.", 10.8.1888 legit K. Czakó.

Epitype (designated here): CBFS 5941. "Centaurea ×melanocalathia Czakó. Slovakia, Vernár: meadow by the left-side tributary to the Hnilec river, ca 4 km SW of the village, 48.88834°N, 20.23777°E", 27.8.2009 legit P. Koutecký; 2n ~ 4x, the ploidy level estimated using flow cytometry.
Synonymy:
≡ *Jacea phrygia* subsp. *melanocalathia* (Czakó) Soják in Čas. Nár. Mus., Odd. Přír. 140: 132. 1972.
≡ *Jacea melanocalathia* (Czakó) Holub in Preslia 45: 145. 1973.
≡ *Centaurea phrygia* subsp. *melanocalathia* (Czakó) Dostál in Bot. J. Linn. Soc. 71: 207. 1973.

Centaurea erdneri × *C. oxylepis*

No binomial is available for a nothospecies of this hybrid formula.

Determination key

A determination key for *C. phrygia* agg. in Central Europe is presented, based on Koutecký (2007, 2008) and the present paper. The values represent 5% and 95% quantiles. Due to high variation, many individuals from a population should be studied and average values used. All measurements should be made on the terminal capitulum. "Appendages" refer to the longest appendages on the middle involucral bracts. The recurved tips of the appendages on the involucral bracts are not included in the measurement of the width of an involucre and the lateral teeth/fimbriae are not included in that of the width of an appendage.

1a Appendage margins irregularly dentate or fimbriate, the fimbriae fused in groups; basal part of appendages usually more than 2 mm wide; appendages laxly appressed to an involucre and only their tips recurved; pappus absent or short (up to 0.3 mm), often irregularly developed ..... hybrids between taxa of ***C. phrygia* agg.** and ***C. jacea*** [individual hybrids are similar to each other and a reliable determination key to them cannot be compiled]

1b Appendage margins regularly fimbriate, the fimbriae not fused; basal part of appendages less than 2.2 mm wide, linear, lanceolate, ovate, or triangular; appendages appressed to an involucre and strongly recurved apically; pappus present or absent 2

2a Appendages ovate to narrowly triangular, without a thin fimbriate acumen (upper third of an appendage is flat in the cross-section); appendages usually not covering green parts of the involucral bracts and forming a lax "sheath" around an involucre; pappus absent; involucre ovoid or cylindrical, 0.9–1.5 cm wide; leaves ovate-lanceolate to almost linear; 2n = 44 ..... ***C. oxylepis*** (Wimm. & Grab.) Hayek

2b Appendages triangular, ovate, lanceolate or linear basally, upper half attenuated into a thin fimbriate acumen (rounded in cross-section); appendages usually covering the green parts of the involucral bracts and forming a dense "sheath" around the involucre; pappus usually present; involucre cylindrical to globular; leaves lanceolate to ovate 3

3a Basal part of appendages linear, 0.4–0.9 mm wide; involucre cylindrical, markedly longer than wide, 1.4–1.8 cm long and 0.9–1.5 cm wide; low altitude plants; 2n = 22 ..... ***C. stenolepis*** A. Kern. [incl. *C. indurata* Janka, an unclear taxon that needs to be revised]

3b Basal part of appendages narrowly lanceolate, lanceolate, triangular or ovate, 0.8–2.1 mm wide; involucre cylindrical or globular; submontane to montane plants 4

4a Involucre ovoid or cylindrical, 1.3–1.7 cm long and 0.9–1.5 cm wide; appendages 5.4–11.2 mm long; basal part of appendages narrowly triangular; pappus up to 0.7 mm long, sometimes irregularly developed or absent; 2n = 44 ..... ***C. erdneri* × *C. oxylepis***

4b Involucre globular or broadly ovoid, 1.4–1.9 cm long and 1.3–1.9 cm wide, appendages 5.4–16.9 mm long; basal part of appendages triangular or lanceolate or ovate; pappus always present, regularly developed 5

5a Appendages 9.4–16.9 mm long; rounded, appendages on the inner involucral bracts covered by the fimbriate appendages on the middle involucral bracts in side view of an involucre; involucre 1.7–2.2 cm long and 1.6–2.0 cm wide 6

5b Appendages 5.4–12.1 mm long; rounded appendages on inner involucral bracts exerted over fimbriate appendages on middle involucral bracts in side view of an involucre; involucre 1.4–1.9 cm long and 1.3–1.9 cm wide 7

6a Appendages 9.4–14.5 mm long, their basal part narrowly lanceolate to lanceolate, 0.6–1.2 mm wide; pappus 0.7–1.5 mm long; stem usually branched from the middle, with numerous capitula; Alps and Bohemian Massif; meadows below timberline; 2n = 22 ..... ***C. pseudophrygia*** C. A. Mey.

6b Appendages 10.3–16.9 mm long, their basal part broadly lanceolate to ovate, 0.9–2.2 mm wide; pappus 0.7–2.2 mm long; stem usually shortly branched, with a few capitula; Carpathians; above timberline; 2n = 44 ..... montane populations of uncertain position (*C. erdneri* J. Wagner ?) occurring rarely on limestone above timberline (Veľká Fatra Mts, Nízke Tatry Mts, Západné Tatry Mts) and needing further study

7a Appendages 5.4–9.8 mm long; the basal part of appendages black; the distal part usually black or dark brown, usually only shortly recurved; pappus 0.6–1.4 mm long; 2n = 22 ..... ***C. phrygia*** L.

7b Appendages 6.8–12.1 mm long; the basal part of appendages black or brown; the distal part usually brown, often strongly recurved; pappus 0.1–1.0 mm long; 2n = 44 ..... ***C. erdneri*** J. Wagner

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Souhrn

Komplex *Centaurea phrygia* agg. zahrnuje ve střední Evropě podle většiny současných autorů 6 druhů, které jsou homogenní pokud jde o chromosomové počty. Jedinou výjimkou je druh *Centaurea phrygia* L., v rámci kterého je znám diploidní (2n = 22) i tetraploidní cytotyp (2n = 44). Naše studie byla zaměřena na reprodukční izolaci a rozdíly v morfologii a rozšíření obou cytotypů. Byl potvrzen výskyt obou. Zároveň byly s využitím průtokové cytometrie nalezeny i ojedinělé rostliny dalších ploidních úrovní (triploidní, pentaploidní a hexaploidní), které se ale vždy vyskytují v rámci populací dominantních cytotypů. Velikost genomu byla stanovena 2C = 2.22 pg pro diploidy a 2C = 4.22 pro tetraploidy. Minimální rozdíl v monoploidní velikosti genomu i variabilita 4 studovaných isozymových systémů naznačují, že tetraploidi jsou asi autopolyploidního původu. Hybridizační experimenty i studium přírodních smíšených populací pomocí průtokové cytometrie potvrdily silnou reprodukční izolaci cytotypů. Cytotypy se od sebe liší v několika morfologických znacích (délka chmýru nažek, délka a barva přívěsků zákrovních listenů, šířka zákrovu, zakrytí přívěsků vnitřních zákrovních listenů přívěsky středních zákrovních listenů) a mohou být většinou úspěšně odlišeny, zejména pokud je k dispozici více jedinců z populace. Zatímco diploidní cytotyp se vyskytuje ve většině severo-středoevropského areálu druhu s výjimkou Západních Karpat, tetraploidní cytotyp je vázán pouze na Západní Karpaty, Hrubý Jeseník a několik izolovaných lokalit v sousedství. Oba cytotypy rostou na společných lokalitách pouze v malém území na okrajích podtatranských kotlin a na úpatí Nízkých Tater. Všechna tato data jednoznačně ukazují, že je oprávněné klasifikovat tyto cytotypy jako samostatné druhy. Diploidům potom přísluší jméno *C. phrygia* L., zatímco pro tetraploidy je možné použít jméno *C. erdneri* J. Wagner. Výsledky morfometrických analýz i revize herbářového materiálu také ukazují, že není možné rozlišit další taxon udávaný většinou flór v úrovni druhu nebo poddruhu (*C. melanocalathia*, *C. phrygia* subsp. *melanocalathia*), podle originálního herbářového materiálu se toto jméno vztahuje na kříženec mezi *C. jacea* a *C. erdneri.*

References

Adams K. L. & Wendel J. F. (2005): Polyploidy and genome evolution in plants. – Curr. Opp. Plant Biol. 8: 135–141.

Balao F., Casimiro-Soriguer R., Talavera M., Herrera J. & Talavera S. (2009): Distribution and diversity of cytotypes in *Dianthus broteri* as evidenced by genome size variations. – Ann. Bot. 104: 965–973.

Bancheva S. & Greilhuber J. (2006): Genome size in Bulgarian *Centaurea* s.l. (*Asteraceae*). – Plant Syst. Evol. 257: 95–117.

Bardy K. E., Albach D. C., Schneeweiss G. M., Fischer P. A. & Schönswetter P. (2010): Disentangling phylogeography, polyploid evolution and taxonomy of a woodland herb (*Veronica chamaedrys* group, *Plantaginaceae* s.l.) in southeastern Europe. – Mol. Phylogen. Evol. 57: 771–786.

Bureš P., Wang Y.-F., Horová L. & Suda J. (2004): Genome size variation in Central European species of *Cirsium* (*Compositae*) and their natural hybrids. – Ann. Bot. 94: 353–363.

Čelakovský L. (1871): Prodromus der Flora von Böhmen. II. – Arch. Naturwiss. Landesdurchforch. Böhm., sect. 3a, fasc. 2: 113–388.

Čerepanov S. K. (1963): *Centaurea* podrod *Jacea* [Subgenus *Jacea*]. – In: Bobrov E. G. & Čerepanov S. K. (eds), Flora SSSR [Flora of the USSR] 28: 440–463, Botanicheskij Institut B. L. Komarova Akademii Nauk SSSR, Leningrad.

Ciocârlan V. (2000): Flora ilustrată a României [Illustrated flora of Romania]. – Editura Ceres, Bucureşti.

Cires E., Cuesta C., Revilla M. A. & Prieto J. A. F. (2010): Intraspecific genome size variation and morphological differentiation of *Ranunculus parnassifolius* (*Ranunculaceae*), an Alpine–Pyrenean–Cantabrian polyploid group. – Biol. J. Linn. Soc. 101: 251–271.

Comai L. (2005): The advantages and disadvantages of being polyploid. – Nat. Rev. Genet. 6: 836–846.

Cosendai C. & Horändl E. (2010): Cytotype stability, facultative apomixis and geographical parthenogenesis in *Ranunculus kuepferi* (*Ranunculaceae*). – Ann. Bot. 105: 457–470.

Czakó K. (1888a): Az alsó-tátrafüredi lápos vidék nyári flórája [The summer flora of bogs by Dolný Smokovec]. – Magyarorsz. Kárpátegylet Évk., Igló [Spišská Nová Ves] 15: 132–160.

Czakó K. (1888b): Die Sommerflora des Unterschmecker Moorbodens. – Jahrbuch des ungarisches Karpathen-Vereines, Zipser Neuendorf [Spišská Nová Ves] 15: 194–224.

Dobročaeva D. N. (1999): *Centaurea* L. – In: Prokudin J. N. (ed.), Opredelitel vyšših rastenij Ukrainy [Key to higher plants of Ukraine], Ed. 2, p. 355–364, Fitosociocentr, Kyjiv.

Doležel J., Doleželová M. & Novák F. J. (1994): Flow cytometric estimation of nuclear DNA amount in diploid bananas (*Musa acuminata* and *M. balbisiana*). – Biol. Plant. 36: 351–357.

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

Dostál J. (1976): *Centaurea* L. – In: Tutin T. G., Heywood V. H., Burges N. A., Valentine D. H., Walters S. M. & Webb D. A. (eds), Flora Europaea 4: 254–301, Cambridge Univ. Press, Cambridge.

Dostál J. (1989): Nová Květena Československé socialistické republiky [New Flora of the Czechoslovak Socialistic Republic]. – Academia, Praha.

Dostál J. & Červenka M. (1992): Veľký klúč na určovanie vyšších rastlín [Big key to the determination of the higher plants]. – Slovenské pedagogické nakladateľstvo, Bratislava.

Dydak M., Kolano B., Nowak T., Siwinska D. & Maluszynska J. (2009): Cytogenetic studies of three European species of *Centaurea* L. (*Asteraceae*). – Hereditas 146: 152–161.

Ekrt L. & Štech M. (2008): A morphometric study and revision of the *Asplenium trichomanes* group in the Czech Republic. – Preslia 80: 325–347.

Fischer M. A., Adler W. & Oswald K. (2008): Exkursionsflora für Österreich, Liechtenstein und Südtirol, Ed. 3. – Land Oberösterreich & Biologiezentrum der OÖ Landesmuseen, Linz.

Garcia-Jacas N., Soltis P. S., Font M., Soltis D. E., Vilatersana R. & Susanna A. (2009): The polyploid series of *Centaurea toletana*: glacial migrations and introgression revealed by nrDNA and cpDNA sequence analyzes. – Mol. Phylogen. Evol. 52: 377–394.

Garcia-Jacas N., Susanna A., Garnatje T. & Vilatersana R. (2001): Generic delimitation and phylogeny of the subtribe *Centaureinae* (*Asteraceae*): a combined nuclear and chloroplast DNA analysis. – Ann. Bot. 87: 503–515.

Gardou C. (1972): Recherches biosystématiques sur la section *Jacea* Cass. et quelques sections voisines du genre *Centaurea* L. en France et dans les régions limitrophes. – Feddes Repert. 83: 311–472.

Greuter W. (2006–2009): *Compositae* (pro major parte). – In: Greuter W. & von Raab-Straube E. (eds), *Compositae*. Euro+Med Plantbase – the information resource for Euro-Mediterranean plant diversity, URL: [http://ww2.bgbm.org/EuroPlusMed, accessed 6 October 2010].

Greuter W., Wagenitz G., Aghababian M. & Hellwig F. H. (2001): Proposal to conserve the name *Centaurea* (*Compositae*) with a conserved type. – Taxon 50: 1201–1205.

Hardy O. J., de Loose M., Vekemans X. & Meerts P. (2001): Allozyme segregation and inter-cytotype mating barriers in the polyploid complex *Centaurea jacea*. – Heredity 87: 136–145.

Hardy O. J., Vanderhoeven S., de Loose M. & Meerts P. (2000): Ecological, morphological and allozymic differentiation between diploid and tetraploid knapweeds (*Centaurea jacea*) from a contact zone in the Belgian Ardennes. – New Phytol. 146: 291–290.

Hodálová I., Grulich V., Horová L., Valachovič M. & Marhold K. (2007): Occurrence of tetraploid and octoploid cytotypes in *Senecio jacobaea* ssp. *jacobaea* (*Asteraceae*) in Pannonia and the Carpathians. – Bot. J. Linn. Soc. 153: 231–242.

Jäger E. J. & Werner K. (2005): Rothmaler, Exkursionsflora von Deustschland. 4. Gefäßpflanzen: Kritischer Band, Ed. 10. – Spektrum Akad. Verlag, Berlin.

Koutecký P. (2007): Morphological and ploidy level variation of *Centaurea phrygia* agg. (*Asteraceae*) in the Czech Republic, Slovakia and Ukraine. – Folia Geobot. 42: 77–102.

Koutecký P. (2008): Taxonomická studie středoevropských zástupců *Centaurea* sect. *Jacea* [Taxonomic study of Central European taxa of *Centaurea* sect. *Jacea*]. – Ph.D. Thesis, University of South Bohemia, České Budějovice.

Koutecký P., Baďurová T., Štech M., Košnar J. & Karásek J. (2011): Hybridization between diploid *Centaurea pseudophrygia* and tetraploid *C. jacea* (*Asteraceae*): the role of mixed pollination, unreduced gametes, and mentor effects. – Biol. J. Linn. Soc. 104: 93–106.

Lavrenko A. N., Serditov N. P. & Ulle Z. G. (1990): Čisla chromosom nekotorych vidov cvetkovych rastenij evropejskogo severo-vostoka SSSR [Chromosome numbers in some species of flowering plants from the north-eastern regions of the European part of the USSR]. – Bot. Zhurn. 75: 1319–1321.

Lepš J. & Šmilauer P. (2003): Multivariate analysis of ecological data using CANOCO. – Cambridge Univ. Press, Cambridge.

Li D., Liu Y., Zhong C. & Huang H. (2010): Morphological and cytotype variation of wild kiwifruit (*Actinidia chinensis* complex) along an altitudinal and longitudinal gradient in central-west China. – Bot. J. Linn. Soc. 164: 72–83.

Linnaeus C. (1753). Species plantarum, Ed. 1. – Holmia, Stockholm.

Löve Á. (ed.) (1979): IOPB chromosome number reports LXIV. – Taxon 28: 391–408.

Löve Á. (ed.) (1981): IOPB chromosome number reports LXXII. – Taxon 30: 694–708.

Mądalski J. & Ciaciura M. (1972): *Centaurea* L. – In: Pawłowski B. & Jasiewicz A. (eds), Flora Polska, Rośliny naczyniowe Polski i ziem ościennych [Flora of Poland, vascular plants of Poland and adjacent countries] 13: 35–91, Państw. Wydaw. Nauk., Warszawa & Kraków.

Májovský J. & Murín A. (eds) (1987): Karyotaxonomický prehľad flóry Slovenska [Karyotaxonomic survey of the flora of Slovakia]. – Veda, Bratislava.

Mandáková T. & Münzbergová Z. (2008): Morphometric and genetic differentiation of diploid and hexaploid populations of *Aster amellus* agg. in a contact zone. – Plant Syst. Evol. 274: 155–170.

Marhold K., Kudoh H., Pak J. H., Watanabe K., Španiel S. & Lihová J. (2010): Cytotype diversity and genome size variation in eastern Asian polyploid *Cardamine* (*Brassicaceae*) species. – Ann. Bot. 105: 249–264.

McNeill J., Barrie F. R., Burdet H. M., Demoulin V., Hawksworth D. L., Marhold K., Nicolson D. H., Prado J., Silva P. C., Skog J. E., Wiersema J. H. & Turland N. J. (eds) (2006): International Code of Botanical Nomenclature (Vienna Code) adopted by the Seventeenth International Botanical Congress Vienna, Austria, July 2005. – Regnum Veg. 146, A. R. Gantner Verlag, Ruggell.

Meusel H. & Jäger E. J. (1992): Vergleichende Chorologie der zentraleuropäischen Flora. Band 3. – Gustav Fischer Verlag, Jena.

Mráz P., Bourchier R. S., Treier U. A., Schaffner U. & Müller-Schärer H. (2011): Polyploidy in phenotypic space and invasion context: a morphometric study of *Centaurea stoebe* s.l. – Int. J. Plant Sci. 172: 386–402.

Ochsmann J. (1998): *Centaurea* L. – In: Wisskirchen R., Haeupler H. & Albers F. (eds), Standardliste Farn- und Blütenpflanzen Deutschlands: mit Chromosomenatlas von Focke Albers, p. 133–136, Eugen Ulmer Verlag, Stuttgart.

Olšavská K., Perný M., Kučera J. & Hodálová I. (2011): Biosystematic study of the *Cyanus triumfetti* group in Central Europe. – Preslia 83: 59–98.

Osborn T. C., Pires J. C., Birchler J. A., Auger D. L., Chen Z. J., Lee H.-S., Comai L., Madlung A., Doerge R. W., Colot V. & Martienssen R. A. (2003): Understanding mechanisms of novel gene expression in polyploids. – Trends Genet. 19: 141–147.

Otto S. P. & Whitton J. (2000): Polyploid incidence and evolution. – Ann. Rev. Genet. 34: 401–437.

Parisod C., Holderegger R. & Brochmann C. (2010): Evolutionary consequences of autopolyploidy. – New Phytol. 186: 5–17.

Poddubnaja-Arnoldi W. (1931): Ein Versuch der Anwendung der embryologishen Methode bei der Lösung einiger systematischer Fragen. I. Vergleichend embryologishen zytologische Untersuchungen über die Gruppe Cynareae, Fam. *Compositae*. – Beihefte Bot. Centralblatt 48: 141–237, 545–550.

Prodan I. & Nyárády E. I. (1964): *Centaurea* L. – In: Savulescu T. (ed.), Flora Reipublicae Socialisticae Romania [Flora of the Socialistic Republic of Romania] 9: 758–951, Editio Academiae Reipublicae Socialisticae Romania, Bucureşti.

Pulkina S. V. (1988): Čisla chromosom nekotorych vidov semejstva *Asteraceae* [Chromosome numbers in some species of the family *Asteraceae*]. – Bot. Zhurn. 73: 607–608.

Quantum GIS Development Team (2010): Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. – URL: [http://qgis.osgeo.org].

R Development Core Team (2009): R: A language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, URL: [http://www.R-project.org].

Ramsey J. & Schemske D. W. (2002): Neopolyploidy in flowering plants. – Ann. Rev. Ecol. Syst. 33: 589–639.

Šafářová L. & Duchoslav M. (2010): Cytotype distribution in mixed populations of polyploid *Allium oleraceum* measured at a microgeographic scale. – Preslia 82: 107–126.

Semerenko L. B. (1989): Čisla chromosom nekotorych predstavitelej semejstv *Asteraceae*, *Fabaceae*, *Orchidaceae*, *Poaceae* flory Berezinskogo zapovednika (BSSR) [Chromosome numbers in some members of the families *Asteraceae*, *Fabaceae*, *Orchidaceae*, *Poaceae* from the Berezinsky biosphere reservation (Belorussia)]. – Bot. Zhurn. 74: 1671–1673.

Simon T. (2000): A magyarországi edényes flóra határozója [Vascular flora of Hungary]. – Nemzeti Tankönyvkiadó, Budapest.

Šmarda P., Šmerda J., Knoll A., Bureš P. & Danihelka J. (2007): Revision of central European taxa of *Festuca* ser. *Psammophilae* Pawlus: morphometrical, karyological and AFLP analysis. – Plant Syst. Evol. 266: 197–232.

Soltis D. E., Albert V. A., Leebens-Mack J., Bell C. D., Paterson A. H., Zheng C., Sankoff D., de Pamphelis C. W., Kerr Wall P. & Soltis P. S. (2009): Polyploidy and angiosperm diversification. – Amer. J. Bot. 96: 336–348.

Soltis D. E., Buggs R. J. A., Doyle J. J. & Soltis P. S. (2010): What we still don't know about polyploidy. – Taxon 59: 1387–1403.

Soltis D. E., Soltis P. S., Schemske D. W., Hancock J. F., Thompson J. N., Husband B. C. & Judd W. S. (2007): Autopolyploidy in angiosperms: have we grossly underestimated the number of species? – Taxon 56: 13–30.

Soltis D. E., Soltis P. S. & Tate J. A. (2003): Advances in the study of polyploidy since Plant speciation. – New Phytol. 161: 173–191.

Soó R. (1970): A magyar flóra és vegetáció rendszertani-növényföldrajzi kézikönyve [Taxonomic-phytogeographic overview of Hungarian flora and vegetation], Vol. 4. – Akadémiai Kaidó, Budapest.

Španiel S., Marhold K., Hodálová I. & Lihová J. (2008): Diploid and tetraploid cytotypes of *Centaurea stoebe* (*Asteraceae*) in Central Europe: morphological differentiation and cytotype distribution patterns. – Folia Geobot. 43: 131–158.

StatSoft (2010): STATISTICA (data analysis software system), version 9.1. – StatSoft Inc., URL: [http://www.statsoft.com].

Štěpánek J. & Koutecký P. (2004): *Centaurea* L. – In: Slavík B. & Štěpánková J. (eds), Květena České republiky [Flora of the Czech Republic] 7: 426–449, Academia, Praha.

Susanna A. & Garcia-Jacas N. (2009): *Cardueae* (*Carduoideae*). – In: Funk V. A., Susanna A., Stuessy T. F. & Bayer R. J. (eds), Systematics, evolution, and biogeography of *Compositae*, p. 293–313, International Association for Plant Taxonomy, Wien.

ter Braak C. J. F. & Šmilauer P. (2002): CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). – Microcomputer Power, Ithaca, NY.

Trávníček P., Eliášová A. & Suda J. (2010): The distribution of cytotypes of *Vicia cracca* in Central Europe: the changes that have occurred over the last four decades. – Preslia 82: 149–163.

Vanderhoeven S., Hardy O. J., Vekemans X., Lefèbvre C., de Loose M., Lambinon J. & Meerts P. (2002): A morphometric study of populations of the *Centaurea jacea* complex (*Asteraceae*) in Belgium. – Plant Biol. 4: 403–412.

Wagenitz G. (1987): Hegi, Illustrierte Flora von Mitteleuropa. Vol. 6/4 (*Compositae* II: *Matricaria–Hieracium*), Ed. 2. – Verlag Paul Parey, Berlin & Hamburg.

Wagner J. (1910): A magyarországi Centaureák ismertetése (*Centaureae* Hungariae) [A review of *Centaurea* in Hungary]. – Math. Term. Közlem. 30, suppl.: 271–451.

Zając A. & Zając M. (eds) (2001): Atlas rozmieszczenia roślin naczyniowych w Polsce [Distribution atlas of vascular plants in Poland]. – Inst. Bot. Uniw. Jagiel., Kraków.

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Appendix 1. – List of *Centaurea phrygia* populations studied. Abbreviations of countries: CZ – Czech Republic, FI – Finland, PL – Poland, RU – Russia, SK – Slovakia, UA – Ukraine. Morf – the number of individuals included in the morphometric analyses, ach – the number individuals with achenes; Karyo/FCM – the number of individuals analyzed by chromosome counting and flow cytometry, respectively; 2n – chromosome counts or DNA ploidy levels estimated using flow cytometry. Morphometric data and chromosome counts (but not flow cytometric results) for the populations marked with an asterisk are taken from a previous study of *C. phrygia* agg. (Koutecký 2007).

Population	Localization	Altitude	Latitude	Longitude	Morf all / ach	Karyo/ FCM	2n
BORS	SK, Bukovské vrchy Mts, Ruský Potok: abandoned meadow near the lower border of „Borsučiny“ nature reserve, ca 2 km NE of the church in the village	820	49°02'36"N	22°25'42"E	–	– / 60	2x
BRT1*	SK, Nízke Tatry Mts, Liptovský Hrádok: meadows in Brtkovica valley, ca 3.5 km SSW of the railway station (subpopulation 1)	1000	49°00'18"N	19°42'45"E	27 / 14	7 / –	22, 33
BRT2	SK, Nízke Tatry Mts, Liptovský Hrádok: meadows in Brtkovica valley, ca 3.5 km SSW of the railway station (subpopulation 2; replacement for BRT1 that was destroyed recently)	1000	49°00'21"N	19°42'44"E	–	– / 105	2x, 3x, 4x
BURK*	UA, Ivano-Frankivskaya oblast', Burkut: SSE of the town of Verchovyna, meadow N of the village	920	47°56'47"N	24°41'30"E	30 / 13	3 / 1	22
CAR	CZ, Moravskoslezské Beskydy Mts, Karolinka: meadows ca 150 m SW of the Čarták saddle, ca 4.1 km N of the railway station in the village	810	49°23'13"N	18°14'30"E	–	– / 100	4x
CERV*	CZ, Silesia, Černá Voda: meadow by the Černý potok brook near the „Rokliny“ hamlet, ca 1.4 km NE of the church in the village	300	50°19'17"N	17°10'07"E	30 / 9	3 / 108	22
DEM*	SK, Nízke Tatry Mts, Demänová: Repiská meadows in Demänovská dolina valley, ca 8 km S of the village	900	48°59'31"N	19°34'45"E	30 / 27	3 / 5	44
HOU	CZ, Hrubý Jeseník Mts, Horní Údolí: meadows at the SE end of the village	640	50°12'56"N	17°21'15"E	–	– / 105	4x
HUB	SK, Veľká Fatra Mts, Hubová: meadows in the mouth of Bystrá dolina valley, ca 2.9 km E of the church in the village	470	49°06'53"N	19°13'26"E	–	– / 96	4x
HUT	CZ, Moravskoslezské Beskydy Mts, Hutisko-Solanec: meadows ca 3.9 km SE of the church in the village, near „Za Hutí“ hamlet	630	49°24'11"N	18°15'07"E	–	– / 113	4x
JAN	SK, Nízke Tatry Mts, Liptovský Ján: Jánska dolina valley, meadows „Pred Bystrou“, ca 8 km S of the centre of the village	880	48°58'32"N	19°40'53"E	–	– / 105	2x, 4x, 5x

Population	Localization	Altitude	Latitude	Longitude	Morf all / ach	Karyo/ FCM	2n
JAS*	UA, Svidovets Mts, Jasinya: meadow in the low end of the Svidovets brook valley, ca 5.5 km SW of the centre of the village	650	48°13'58"N	24°18'57"E	30 / 24	3 / –	22
KAR	CZ, Hrubý Jeseník Mts, Karlov pod Pradědem: meadows S of the village	690	50°01'03"N	17°18'15"E	30 / 20	3 / –	44
KOB	CZ, Silesia, Kobylá nad Vidnavkou: abandoned meadow ca 100 m N of the railway station, E of the village	290	50°20'21"N	17°07'32"E	–	– / 108	2x
KOLI	FI, North Karelia region: grassy patches at the parking place at the entrance to Koli national park, ca 27 km SSW of the town of Lieksa	280	63°05'41"N	29°48'14"E	24 / 23	– / 10	2x
KOT	PL, Góry Izerskie Mts, Gierczyn: ca 1.5 km S SW of the church of the village, meadows at „Kotlina“ hamlet	530	50°55'31"N	15°22'41"E	29 / 22	3 / –	22
KOV	RU, region Leningradskaya oblast', Kovkenitsy: meadows E of the village	10	60°38'56"N	33°13'56"E	30 / 6	3 / –	22
KRAS	CZ, Moravskoslezské Beskydy Mts, Krásná: meadow at the N end of the village, ca 0.9 km SWW of the summit of Obora hill (709)	500	49°34'57"N	18°28'48"E	–	– / 431	4x, 5x
KRL	SK, Nízke Tatry Mts, Kráľova Lehota: meadows S of the confluence of Biely Váh and Čierny Váh Rivers, ca 1.2 km SEE of the church in the village	670	49°00'58"N	19°48'29"E	–	– / 102	2x, 3x, 4x
KRO	PL, Góry Izerskie Mts, Krobica: meadows ca 1 km E of the village	430	50°55'49"N	15°22'06"E	30 / 21	3 / –	22
LAZ*	UA, Chornohora Mts, Lazeshchyna: in the valley of the Lazeshchanka brook, ca 9 km SSE of the centre of the village	940	48°11'59"N	24°27'39"E	30 / 8	3 / 2	22
LIPL	SK, Nízke Tatry Mts, Liptovská Lúžna: abandoned meadows along Lužnianka brook, ca 4.5 km E of the centre of the village	1030	48°56'22"N	19°22'52"E	22 / 20	– / 112	4x, 6x
LIPO1*	SK, Liptovská kotlina basin, Liptovská Porúbka: an old garden and margin of a meadow at the S edge of the village (subpopulation 1)	690	49°01'23"N	19°43'24"E	30 / 18	3 / 20	44, 4x

Population	Localization	Altitude	Latitude	Longitude	Morf all / ach	Karyo/ FCM	2n
LIPO2	SK, Liptovská kotlina basin, Liptovská Porúbka: meadows by the S edge of the village (subpopulation 2; ca 250 m far from LIPO1; the opposite side of a meadow with a large tetraploid population; in subpopulation 2 there were a few diploid individuals)	710	49°01'21"N	19°43'09"E	–	– / 79	2x, 4x
LOP	RU, region Leningradskaya oblast', Lodeynoye Pole: margin of the forest road, ca 5 km NWW of the centre of the town	15	60°44'47"N	33°28'15"E	30 / 3	3 / –	22
MEN	SK, Podtatranská kotlina basin, Mengusovce: meadow ca 0.5 km SSE of the village	800	49°04'11"N	20°08'28"E	30 / 25	– / 106	4x
NEJ*	CZ, Krušné hory Mts, Nejdek: meadow ca 1.6 km SSE of Javorník hill, ca 2 km W of the railway station in the town	640	50°19'02"N	12°40'17"E	30 / 19	3 / 100	22
NSE1	SK, Bukovské vrchy Mts, Nová Sedlica: meadows at Packova Kýčera hill (871.6), ca 3 km NNE of the village	820	49°04'09"N	22°32'26"E	30 / 17	3 / 20	22
NSE2	SK, Bukovské vrchy Mts, Nová Sedlica: margin of the forest road near Vrch hrbu hill (585.9), ca 2.5 km SSE of the centre of the village	690	49°01'34"N	22°31'36"E	–	– / 20	2x
POD	SK, Nízké Tatry Mts, Podsuchá: meadows ca 0.4 km of the main road crossing at the E end of the village	590	48°59'29"N	19°17'14"E	–	– / 100	4x
PRUD	CZ, Hrubý Jeseník Mts, Pustá Rudná: meadows ca 0.25 km NE of the centre of the village	720	50°04'47"N	17°23'55'E	27 / 25	– / 105	4x
REJ	CZ, Hrubý Jeseník Mts, Rejvíz, part Starý Rejvíz: meadows at the N edge of the village	750	50°13'35"N	17°19'06"E	–	– / 30	4x
RUP	SK, Bukovské vrchy Mts, Ruský Potok: Príslopec saddle between Velký Bukovec and Malý Bukovec hills, ca 2.2 km N of the church in the village	670	49°02'53"N	22°25'00"E	30 / 25	– / 108	2x
STCV*	CZ, Silesia, Stará Červená Voda: meadow by the E edge of the village, ca 0.7 km SSW of the church in the village	310	50°19'27"N	17°11'58"E	30 / 6	3 / –	22
STIS	SK, Bukovské vrchy Mts, Nová Sedlica: dry margin of the Stinská slatina peat bog and adjacent forest road, ca 2.9 km SSE of the centre of the village	670	49°01'14"N	22°31'46"E	–	– / 50	2x

Population	Localization	Altitude	Latitude	Longitude	Morf all / ach	Karyo/ FCM	2n
STP	SK, Nízké Tatry Mts, Liptovský Ján: Stanišovská po'lana meadows in Stanišovská dolina valley, ca 4.8 km SSE of the centre of the village	880	49°00'21"N	19°41'24"E	–	– / 156	2x, 4x
STR	SK, Podtatranská kotlina basin, Štrba: meadows ca 3.3 km NWW of the church in the village	900	49°03'52"N	20°02'17"E	–	– / 151	2x, 4x
SVIS*	SK, Nízke Tatry Mts, Malužiná: meadows in Svidovské sedlo saddle, ca 4.5 km W of the church in the village	1140	48°58'11"N	19°42'37"E	30 / 23	5 / –	44
SYB*	UA, Ivano-Frankivskaya oblast', Zelene, part Shybene: meadow at the N edge of the village	840	47°59'48"N	24°43'05"E	30 / 10	3 / 2	22
VAZ1	SK, Podtatranská kotlina basin, Važec: meadows ca 1.5 km E of the centre of the village	860	49°03'33"N	20°00'01"E	–	– / 100	2x
VAZ2	SK, Podtatranská kotlina basin, Važec: meadows ca 1.3 km SE of the centre of the village	820	49°03'03"N	19°59'31"E	–	– / 104	2x, 4x
VAZ3	SK, Podtatranská kotlina basin, Važec: meadows ca 1.7 km SE of the centre of the village, by a spring of a small brook	850	49°03'00"N	19°59'54"E	–	– / 100	2x
VELF*	SK, Veľká Fatra Mts, Vyšná Revúca: meadow in Zelená dolina valley, ca 3.7 km W of the centre of the village	860	48°54'40"N	19°06'40"E	30 / 9	3 / 20	44
VER1*	SK, Nízke Tatry Mts, Vernár: meadows ca 0.7 km NWW of the church in the village	800	48°55'15"N	20°15'40"E	30 / 16	3 / 3	44
VER2*	SK, Nízke Tatry Mts, Vernár: meadow by the left tributary of the Hnilec river, ca 4 km SW of the village	940	48°53'18"N	20°14'16"E	30 / 10	3 / 25	44
VIS*	CZ, Moravskoslezské Beskydy Mts, Visalaje: upper part of a ski slope ca 200 m from the parking place in the settlement	770	49°31'02"N	18°31'43"E	30 / 5	3 / 104	44
VOR*	UA, Chornohora Mts, Vorochta: meadow ca 7 km SSE of the centre of the village	900	48°12'25"N	24°35'20"E	28 / 12	3 / –	22
VSB	CZ, South Bohemia, Vyšší Brod: meadows and pastures ca 1.75 km SE of the church in the town	650	48°36'09"N	14°19'44"E	–	– / 110	2x
VYCH	SK, Nízké Tatry Mts, Východná: meadows by the railway station, ca 2.2 km SEE of the centre of the village	740	49°03'06"N	19°55'07"E	–	– / 95	2x
ZEL*	UA, Ivano-Frankivskaya oblast', Zelene: meadow in the middle part of the village	770	48°02'35"N	24°45'06"E	30 / 22	3 / –	22