

A diploid drop in the tetraploid ocean: hybridization and long-term survival of a singular population of *Centaurea weldeniana* Rchb. (Asteraceae), a taxon new to Austria

Petr Koutecký

Received: 29 January 2012 / Accepted: 2 April 2012 / Published online: 3 May 2012
© Springer-Verlag 2012

Abstract A population of *Centaurea weldeniana* was discovered recently near Wien (Vienna). The species is reported from Austria for the first time. Determination is confirmed by morphometric analysis of Central European and additional Balkan material of the *C. jacea* agg. and karyological data. All sampled populations of *C. weldeniana* are diploid, while only tetraploids have been reported from Central Europe within the *C. jacea* agg. so far. Detailed morphometric analysis of the newly discovered *C. weldeniana* population revealed several hybrids with tetraploid *C. jacea*. The hybrids are tetraploid and originate from unreduced gametes of diploids. No triploids were found. Indeed, the incidence of hybridization is surprisingly low. The population was probably discovered already in 1886 (determined as *C. bracteata*) and was described as a new species *C. argyrolepis* in 1901, but these data were lost. However, this isolated diploid population has survived for at least 125 years, which provides a good example of the strength of inter-cytotype reproductive barriers in *Centaurea* sect. *Jacea*.

Keywords *Centaurea* sect. *Jacea* · Chromosome number · Genome size · Flow cytometry · Hybridization · Polyploidy

Introduction

It is well known that the genus *Centaurea* L. presents taxonomic difficulties. In Central Europe, the examples of intricate groups in which the definition of taxa has been

controversial for a long time and which have been rearranged recently based on modern morphometric, karyological and molecular approaches include the *C. stoebe* group (Španiel et al. 2008; Mráz et al. 2011) and the *C. triumfetti* group (Olšavská et al. 2009, 2011). However, *C.* sect. *Jacea* (= *C.* subgen. *Jacea* as delimited in Flora Europaea; Dostál 1976) accounts for the majority of Central European taxa. The section includes several species complexes (aggregates) that can be relatively easily delimited based on the shape of leaves and the size and appearance of capitula (e.g. *C. jacea* agg., *C. nigrescens* agg., *C. nigra* agg., and *C. phrygia* agg.). The base chromosome number is uniform within the section ($x = 11$) and all of the aggregates include both diploid ($2n = 2x = 22$) and tetraploid ($2n = 4x = 44$) taxa (e.g. Dostál 1976; Koutecký 2007; Koutecký et al. 2012). An autotetraploid origin causing poor morphological differentiation from diploids has been reported for *C. jacea* (Hardy et al. 2001).

The major causes of taxonomic and determination problems in the section *Jacea* are hybridization and introgression. Both crossing experiments and field studies (Gardou 1972; Hardy et al. 2000, 2001; Koutecký et al. 2011, 2012) have consistently shown that taxa of the same ploidy level (regardless of whether diploid or tetraploid) hybridize freely to produce fertile hybrids, but hybridization between plants of different ploidy levels is rare. The interploidy hybrids are more often tetraploid rather than triploid (intermediate). The tetraploid hybrids are fertile and capable of backcrossing with tetraploid parents (Koutecký et al. 2011). The morphological variability of hybrids is enormous, especially with respect to the key determination character, the shape of appendages of involucre bracts (e.g. Marsden-Jones and Turrill 1954; Saaristo-Taubert 1966; Vanderhoeven et al. 2002; Koutecký et al.

P. Koutecký (✉)
Department of Botany, Faculty of Sciences, University of South
Bohemia, Branišovská 31, 370 05 České Budějovice,
Czech Republic
e-mail: kouta@prf.jcu.cz

2011). Some hybrid morphotypes have even been treated as autonomous (nonhybrid) taxa (Koutecký 2009). Hybridization and introgression result in extensive hybrid zones where variable intermediate populations prevail over parental taxa (Koutecký 2007).

The *C. jacea* aggregate (henceforth referred as “*C. jacea* agg.”, while “*C. jacea*” refers only to the species *C. jacea* L.) ranks among the most intricate groups within *C. sect. Jacea*. The aggregate is easily recognized by two morphological characters: (1) appendages of all involucre bracts (i.e. scarious, brown or black structures on the top of the green parts of the bracts) are rounded and entire or only finely denticulate on margin, while they are toothed or fimbriate in other taxa of the section; (2) achenes lack any remains of a pappus. In this circumscription, which corresponds to *C. subgen. Jacea sect. Jacea* in Flora Europaea (Dostál 1976), the *C. jacea* agg. involves more than ten microspecies throughout Europe (Dostál 1976). Due to a lack of obvious determination characters and generally unresolved taxonomy, these taxa are often treated as subspecies of a broadly defined *C. jacea* (more recent, e.g. Wagenitz 1987; Ochsmann 1998; Kubát et al. 2002; Štěpánek and Koutecký 2004; Jäger and Werner 2005; Fischer et al. 2008). In some of these works even two taxa with fimbriate appendages are included in *C. jacea* due to frequent hybridization and intermediate morphotypes [*C. jacea* subsp. *macroptilon* (Borbás) Hayek, *C. jacea* subsp. *oxylepis* (Wimm. et Grab.) Hayek]. However, for consistency with the generally accepted concept of other aggregates within the section *Jacea* (e.g. *C. phrygia* agg.), and to avoid impractical use of a nothosubspecific rank for “intermediate morphotypes”, I use a narrow species delimitation in this paper, following e.g. Flora Europaea (Dostál 1976).

The *C. jacea* agg. includes both diploids ($2n = 22$) and tetraploids ($2n = 44$). The former seem to be confined to southern Europe, while tetraploids occur throughout the range of the group. Diploid counts for *C. jacea* were reported by Gardou (1972) for France (Eastern Pyrenees, Alpes-Maritimes), Italy (Liguria, Southern Alps, Padova, Northern Apennines) and Croatia (the island of Krk), and by Kuzmanov et al. (1986) for Bulgaria. The other taxa of the *C. jacea* agg. with reported diploid chromosome counts include an unclear taxon *C. dracunculifolia* Dufour (Dostál 1976), *C. rocheliana* (Heuff.) Dostál (Bogdan et al. in Löve 1979), *C. weldeniana* (one record from the island of Krk, Croatia; Lovrić in Löve 1982), and *C. pannonica* (Heuff.) Simonk. [= *C. jacea* subsp. *angustifolia* (DC.) Grelli]. In the last species a diploid count was reported in Flora Europaea (Dostál 1976; most probably based on records for *C. jacea* subsp. *angustifolia* from Gardou 1972, see below) and for Bavaria (Lippert and Heubl 1988); the latter is the only record of diploids in Central Europe. However, all

these counts from the literature should be understood as *C. jacea* agg. due to determination problems and due to imprecise or incomplete citations from the primary sources. For instance, in the most important work of Gardou (1972), the broad taxonomic concept was used and only one species, *C. jacea*, was recognized, with two subspecies, subsp. *jacea* and subsp. *angustifolia*. Each subspecies comprised several varieties, including “*C. j.* subsp. *angustifolia* var. *bracteata*” [= *C. bracteata* Scop.; *C. jacea* subsp. *gaudinii* (Boiss. & Reut.) Grelli] and “*C. j.* subsp. *angustifolia* var. *weldeniana*” [= *C. weldeniana* Rchb.; *C. jacea* subsp. *weldeniana* (Rchb.) Greuter]. Unfortunately, the exact determination of subspecies/varieties is lacking for the majority of localities. It is probable that (1) most of the records of Gardou (1972) for France and Italy belong to *C. bracteata*, which is common there and comprises at least some diploids (Koutecký et al., unpublished data), (2) both the records of Gardou (1972) for Croatia belong to *C. weldeniana* (one of the localities is marked as “*C. jacea* subsp. *angustifolia* var. *weldeniana*” on p. 318), and (3) all these data are cited by later authors simply as *C. jacea* subsp. *angustifolia*, or even *C. jacea*, without examining the lower ranks used by Gardou (1972).

Numerous tetraploid counts have been reported for *C. jacea* and *C. pannonica* for Central Europe, e.g. Albers (1998), Dobeš and Vitek (2000), Marhold et al. (2007), Koutecký et al. (2011). Tetraploid counts have also been reported for *C. bracteata* for France (Gardou 1972, p. 355 as “*C. jacea* ssp. *angustifolia* var. *bracteata*”) and Croatia (Lovrić in Löve 1982) and for Croatian *C. weldeniana* var. *balcanica* (Lovrić in Löve 1982), an unclear taxon intermediate between *C. weldeniana* and *C. pannonica* (Dostál 1976). Single individuals of higher ploidy levels (pentaploid, hexaploid) are exceptionally found within tetraploid populations as a result of rare polyploidization events, and triploids rarely occur as a result of hybridization between diploids and tetraploids (Koutecký et al. 2011).

In 2005, I discovered a population near the village of Gießhübl near Wien, Austria (see Table 1 for the exact location), that is markedly different from other Central European populations of the *C. jacea* agg. It was preliminarily determined as *C. weldeniana* Rchb., which has not previously been known to occur in Austria. Interestingly, there is one taxon that has been generally overlooked and was described in the same locality at the beginning of twentieth century, namely *C. argyrolepis* Hayek. Based on the original description (Hayek 1901), it belongs to the *C. jacea* agg. and may be identical to the recently found population. The aim of the work reported here was therefore (a) to inspect the morphological variation of the studied population and of *C. weldeniana* to confirm the determination and demonstrate differences from other Central European taxa of the *C. jacea* agg., (b) to examine

Table 1 Studied populations of *C. weldeniana*, *C. jacea* and *C. pannonica*

Taxon	Country	Location	Altitude (m)	Latitude	Longitude	Collector, year
<i>C. weldeniana</i> , <i>C. jacea</i>	AU	Gießhübl, part Tirolerhof-Siedlung; N of the village; grassy strip between vineyards	330	48.1083°N	16.2470°E	P. Koutecký, 2011
<i>C. weldeniana</i>	BA	Miljevina; about 4 km NW of the village, by national road no. 18; dry meadow	600	43.5274°N	18.6015°E	M. Štech, 2010
<i>C. weldeniana</i>	HR	Bunić; about 3.8 km ENE of the village; pasture	700	44.6881°N	15.6520°E	M. Štech, 2010
<i>C. weldeniana</i>	HR	Gospić; NW foothill of Mt. Zir; about 22 km ESE of the town; dry meadow	600	44.4352°N	15.6052°E	P. Koutecký, 2007
Additional material						
<i>C. jacea</i>	CZ	České Budějovice; former military area on the NW margin of the town; dry meadow	390	48.9895°N	14.4421°E	P. Koutecký, 2005
<i>C. jacea</i>	CZ	Český Krumlov; limestone hill N of the railway station in the town; dry meadow	570	48.8250°N	14.3164°E	P. Koutecký, 2004
<i>C. jacea</i>	CZ	Dobrá Voda; about 0.5 km NW of the village; mesophilous meadow	650	49.3460°N	15.0107°E	P. Koutecký, 2005
<i>C. jacea</i>	CZ	Dolní Cerekev; about 2.4 km W of the church in the village; grassy road margin	570	49.3448°N	15.4248°E	P. Koutecký, 2005
<i>C. jacea</i>	CZ	Droužetice; forest margin on Tisovník hill; NNW of the village; dry grassland	470	49.2927°N	13.8053°E	P. Koutecký, 2005
<i>C. jacea</i>	CZ	Horní Myslová; about 0.7 km NW of the village, by Kopejtkův Mlýn hamlet; wet meadow	500	49.1700°N	15.4153°E	P. Koutecký, 2005
<i>C. jacea</i>	CZ	Husinec; about 0.8 km S of the town; mesophilous meadow	430	49.0472°N	13.9847°E	P. Koutecký, 2004
<i>C. jacea</i>	CZ	Jirkov; NW of the town; about 0.9 km SE of the village of Jindřišská; wet meadow	450	50.5131°N	13.4286°E	P. Koutecký, 2002
<i>C. jacea</i>	CZ	Kamenný Újezd; about 1.4 km SSE of the village; grassy road margin	450	48.9106°N	14.4248°E	P. Koutecký, 2005
<i>C. jacea</i>	CZ	Mezilesí; about 0.4 km E of the village; mesophilous meadow	545	48.8101°N	14.6500°E	P. Koutecký, 2005
<i>C. jacea</i>	CZ	Plav; NE margin of the village; grassy abandoned garden	420	48.9849°N	14.8449°E	P. Koutecký, 2005
<i>C. jacea</i>	CZ	Roudno; east slope of Velký Roudný hill (780); about 1.3 km SEE of the village	630	49.9800°N	17.5311°E	P. Koutecký, 2005
<i>C. jacea</i>	CZ	Stará Hlína; about 2 km S of the village; by Holičky hamlet; grassy road margin	430	49.0125°N	14.8336°E	P. Koutecký, 2003
<i>C. jacea</i>	CZ	Strání; dry slopes about 2.1 km NWW of the church in the village; above the road to Korytná	530	48.9128°N	17.6773°E	P. Koutecký, 2009
<i>C. jacea</i>	SK	Důžava; about 2 km SSE of the village; forest margin	250	48.3342°N	19.9986°E	P. Koutecký, 2003
<i>C. jacea</i>	SK	Kamenica nad Hronom; about 0.5 km E of the church in the village; dry meadows and shrub margins	150	47.8326°N	18.7335°E	P. Koutecký, 2005
<i>C. jacea</i>	SK	Kolbasov; about 2 km NW of the village; mesophilous meadow	330	49.0189°N	22.3606°E	P. Koutecký, 2004
<i>C. jacea</i>	SK	Nová Sedlica; about 2 km SE of the village; mesophilous meadow	500	49.0307°N	22.5301°E	P. Koutecký, 2004
<i>C. jacea</i>	SK	Richnava; north of Richnavská jazera Lakes by the village; mesophilous meadow	740	48.4294°N	18.8498°E	P. Koutecký, 2003

Table 1 continued

Taxon	Country	Location	Altitude (m)	Latitude	Longitude	Collector, year
<i>C. jacea</i>	UA	Shybene; by the village; mesophilous meadow (Chyvchyn Mts.)	840	47.9967°N	24.7181°E	P. Koutecký, 2003
<i>C. pannonica</i>	CZ	Běstvina; about 0.5 km SSW of the church in the village; dry meadow on a marlstone slope	350	49.8380°N	15.5978°E	P. Koutecký, 2005
<i>C. pannonica</i>	CZ	Břeclav; about 5 km SSE of the railway station in the town; dry grassland on sand	155	48.7134°N	16.9075°E	P. Koutecký, 2005
<i>C. pannonica</i>	CZ	Libice nad Cidlinou; about 1 km SSW of the railway station; mesophilous meadow	190	50.1192°N	15.1784°E	P. Koutecký, 2003
<i>C. pannonica</i>	CZ	Nové Ouholice; dry meadow about 150 m NE of the railway station	180	50.3045°N	14.3185°E	P. Koutecký, 2006
<i>C. pannonica</i>	CZ	Nové Strašecí; about 2.2 km NE of the centre of the town; mesophilous meadow	440	50.1690°N	13.9197°E	P. Koutecký, 2006
<i>C. pannonica</i>	CZ	Žlebské Chvalovice; about 1 km S of the village; dry meadow	340	49.8864°N	15.5635°E	P. Koutecký, 2005
<i>C. pannonica</i>	SK	Hačava; about 1 km SWW of the village; near Bujačí vrch hill; dry pasture	820	48.6653°N	20.8211°E	P. Koutecký, 2003
<i>C. pannonica</i>	SK	Hačava; Křížná Pol'ana saddle; about 1 km NNE of the village; dry meadow	860	48.6767°N	20.8398°E	P. Koutecký, 2003
<i>C. pannonica</i>	SK	Kamenín; E margin of Kamenínské slanisko reserve; S of the village; salty meadow	110	47.8792°N	18.6495°E	P. Koutecký, 2005
<i>C. pannonica</i>	SK	Radava; about 1 km E of the centre of the village; grassy strip between vineyards	150	48.0937°N	18.3151°E	P. Koutecký, 2009
<i>C. pannonica</i>	SK	Sol'nička; about 2.6 km N of the village; dry alluvial meadow	100	48.5008°N	21.9661°E	P. Koutecký, 2004
<i>C. pannonica</i>	SK	Tvrdošovce; at the N margin of the W part of the village; salty grassland	115	48.0959°N	18.0514°E	P. Koutecký, 2005
<i>C. pannonica</i>	SK	Vel'ké Kosihy; E of the village; by the nature reserve Mostová (Dérhídja); road margin on salty clay	110	47.7704°N	17.8972°E	P. Koutecký, 2005
<i>C. pannonica</i>	UA	Vynogradiv; E of the town; south of Chorna hora Mt.; road margin	140	48.1356°N	23.0817°E	P. Koutecký, 2003

AU Austria, BA Bosnia and Herzegovina, CZ Czech Republic, HR Croatia, SK Slovakia, UA Ukraine

potential hybridization with *C. jacea*, which also occurs at the locality, (c) to provide karyological and genome size data for *C. weldeniana*, and (d) to revise the original material of *C. argyrolepis*.

Materials and methods

Field sampling

All available (i.e. 15) individuals of putative *C. weldeniana* and three individuals with a morphology intermediate between *C. weldeniana* and *C. jacea* (putative hybrids) were sampled for morphometric analysis during September 2010 at the locality near Gießhübl. Only undamaged stems with a

fully developed terminal capitulum were collected and only one stem was sampled from each tussock (genet). An additional 11 individuals were sampled for flow cytometry only (damaged or sterile individuals). An equivalent number of putative *C. jacea* individuals were collected at the same locality for morphometric analysis. For comparison, material of *C. weldeniana* from another three sites in Croatia and Bosnia was included (11 individuals altogether); no other karyologically/cytometrically confirmed material of *C. weldeniana* was available. The morphometric analysis was supplemented with a subset of plants collected for morphometric study of *C. jacea* and *C. pannonica* from the Czech Republic, Slovakia and Ukraine (Koutecký 2008, and unpublished data). To avoid strongly unbalanced numbers of samples, only five individuals were chosen at random from

each of 34 additional populations of the *C. jacea* agg. All localities are listed in Table 1. Voucher specimens are stored in the herbarium CBFS.

Flow cytometry

DNA ploidy level and genome size were determined using flow cytometry. The method was as described by Kouřek et al. (2012) in detail. Briefly, fresh leaves were chopped in Otto I buffer with an internal standard *Glycine max* 'Polanka' ($2C = 2.50$ pg; Doležel and Greilhuber 2010), and stained after about 1 min with Otto II buffer containing 2-mercaptoethanol (2 μ l/ml) and a fluorescent dye. For ploidy level estimation, DAPI fluorochrome (4 μ g/ml) was used and samples were analysed using a Partec PA II flow cytometer equipped with a mercury arc lamp. It was possible to use bulked samples of three to ten plants due to high-resolution histograms and absence of endopolyploidy. From two populations of *C. weldeniana* from Bosnia and Croatia, silica gel-dried leaves were used and only one individual per sample was analysed. For genome size measurements, the same internal standard and the same method of sample preparation from fresh leaves was used, only replacing DAPI by propidium iodide (50 μ g/ml) and RNase IIa (50 μ g/ml). 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 5,000 particles was recorded. Each individual was analysed three times on three different days and the average value used as the genome size; the repeated measurements of a sample were always consistent and the range did not exceed 2 % of the average value. The fluorescence histograms were processed using FloMax 2.6 software (Partec, Germany).

Chromosome counting

Chromosomes of the putative *C. weldeniana* from the Gießhübl population were counted to calibrate the results of flow cytometry. Chromosomes in the apical root meristems of seedlings that were germinated from field-collected achenes were counted. The protocol of Španiel et al. (2008) was used, with minor modifications. Seedlings were pretreated with a saturated water solution containing 0.002 M 8-hydroxyquinoline for 10 h at 4 °C, fixed in a mixture of ethanol and acetic acid (3:1) for 24 h at 4 °C and stored in 70 % ethanol at 4 °C. Maceration lasted about 5 min in a mixture of ethanol and hydrochloric acid (1:1). The apical part of a root was then squashed using a cellophane square and stained for 45 min in 10 % Giemsa solution in 0.2 M sodium phosphate buffer, pH 7.2. Three

samples were analysed and at least two mitoses per plant were studied.

Morphometric analysis

Morphometric analyses were performed (a) to demonstrate differentiation of the newly recognized population of *C. weldeniana* from other Central European material of the *C. jacea* agg. and (b) to examine hybridization between *C. weldeniana* and *C. jacea*. For this analysis, 29 characters were used, including 15 quantitative characters, seven ratios and seven binary characters (Table 2). Characters such as branching of the stem, shape of leaves and dimensions of the involucre were measured on fresh plants. Plants were then dried and used to measure the other characters.

Basic statistical measures (mean, median, maximum and minimum values, quartiles, five and 95 percentiles and standard deviation) were computed for each population. The normality of the distribution of each character was examined and transformations were applied to the characters which markedly deviated from a normal distribution (Table 2). Pearson and Spearman correlation coefficients were calculated for pairs of characters for each taxon and for the whole data set to study relationships between the characters. For principal component analyses (PCA), the data were standardized to have zero mean and unit standard deviation (PCA based on correlation matrix).

Two PCA analyses were performed. In the first (PCA 1), differences between *C. weldeniana* and other Central European material were evaluated using individuals as operational taxonomic units. In the second (PCA 2), the Gießhübl population was analysed in detail to evaluate differences between *C. weldeniana* and the local population of *C. jacea*, and especially to identify potential hybrids with intermediate morphology.

The statistical analyses were performed using Statistica 9 (StatSoft 2010) and Canoco for Windows 4.5 software (ter Braak and Šmilauer 2002).

Herbarium material

Centaurea material in the herbaria W and WU was revised. *Centaurea argyrolepis* was described by Hayek (1901) based mainly on collections of Müllner, which are currently stored in these herbaria.

Results

Ploidy levels and genome size

Flow cytometry revealed that all studied individuals of *C. weldeniana* were DNA diploid. There was negligible

Table 2 List of morphological characters and transformations used

Character	Description	Transformation	Unit
<i>Quantitative characters</i>			
AL	Length of the largest appendage on middle involucre bracts of the terminal capitulum		Millimetres
ALW	Length/width of the largest appendage on middle involucre bracts of the terminal capitulum (ML/MW)	Log	
AW	Width of the largest appendage on middle involucre bracts of the terminal capitulum		Millimetres
BA	Angle between the main stem and the longest branch		Degrees
BL	Length of the longest flowering branch		Centimetres
BLF	Number of bracts on the longest flowering branch	Square root	
BLLF	Length of an internode on the longest branch: length of the branch divided by number of bracts (BL/BLF)		
BLSF	Florescence width: ratio of the length of the longest branch to the length of the flowering part of the stem (BL/SF)		
BT	Difference between the diameter of the longest branch about 3 mm below the capitulum and in the middle part (i.e. thickening of the branch below the capitulum)		Millimetres
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 involucre bracts		Centimetres
ILW	Length/width of the involucre of the terminal capitulum (IL/IW)		
IW	Width of the involucre of the terminal capitulum		Centimetres
LBL	Lamina length of a middle bract on the longest flowering branch		Centimetres
LBLW	Length/width of the lamina of a middle bract on the longest branch (LBL/LBW)	Log	
LBW	Lamina width of a middle bract on the longest flowering branch		Centimetres
LL	Lamina length of a middle stem leaf		Centimetres
LLW	Length/width of the lamina of a middle stem leaf (LL/LW)	Log	
LW	Lamina width of a middle stem leaf, including lateral teeth/lobes		Centimetres
<i>Ratios</i>			
SF	Height of the flowering part of the stem, i.e. height from the lowest flowering branch to the terminal capitulum	Log	Centimetres
SFT	Proportion of the height of the flowering part of the stem (SF/ST)		
SN	Height of the non-flowering part of the stem, i.e. height from the ground to the lowest flowering branch; short (a few centimetres) thin branches with reduced capitula that sometimes develop in the lower leaf axils are not considered		Centimetres
ST	Total stem height (SN + SF)	Log	Centimetres
<i>Binary characters</i>			
AC1	Colour of an appendage of the middle involucre bract: 0 – the centre of the appendage darker than the margin; 1 – the margin darker than the centre		
AC2	Marginal part of appendages translucent, pale brown to whitish: 0 – no, 1 – yes		
BB	Branching of the longest branch (0 – not branched, 1 – branched)		
BS	Shape of the longest branch (0 – straight, 1 – distinctly curved upwards)		
HM	Dense greyish hairs on middle part of the stem and leaves: 0 – absent, 1 – present		
HU	Dense greyish hairs on upper part of the stem and bracts: 0 – absent, 1 – present		
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		

All characters were measured on plants with a fully developed and undamaged terminal capitulum. The accuracy of the measurements is one decimal place for distances and 1° for angles.

variation in the relative fluorescence among populations for which fresh leaves were available and some variation among silica gel-dried samples. The genome size of

C. weldeniana was $2C = 2.09$ pg. All studied individuals of *C. jacea* were DNA tetraploid. The putative hybrids *C. jacea* × *C. weldeniana* were all DNA tetraploid. The

Table 3 Genome sizes of *C. weldeniana* and *C. jacea*

Taxon	Population	Material	No. of flow cytometry analyses	2C value (\pm SE)	Cx value (pg)	Range (%)	DNA ploidy
Genome size							
<i>C. weldeniana</i>	Gießhübl	Fresh	3	2.09 \pm 0.01 pg	1.05	1.2	2x
Relative genome size							
<i>C. weldeniana</i>	Gießhübl	Fresh	7	0.756 \pm 0.003		2.8	2x
<i>C. weldeniana</i>	Bunić	Fresh	1	0.750			2x
<i>C. weldeniana</i>	Gospić	Dried	5	0.773 \pm 0.007		4.9	2x
<i>C. weldeniana</i>	Miljevina	Dried	3	0.751 \pm 0.001		0.6	2x
<i>C. jacea</i> \times <i>C. weldeniana</i>	Gießhübl	Fresh	3	1.519 \pm 0.010		3.3	4x
<i>C. jacea</i>	Gießhübl	Fresh	6	1.524 \pm 0.004		1.8	4x

The genome size was measured with propidium iodide fluorochrome and calculated as picograms of DNA. The relative genome size is the ratio in relation to the internal standard (*Glycine max* 'Polanka', 2C = 2.50 pg), estimated using DAPI fluorochrome. *Range* is the difference between the maximum and the minimum 2C values expressed as a percentage of the average value of the population.

genome sizes are shown in Table 3, and typical fluorescence histograms are presented in Fig. 1.

Chromosome counting confirmed the results of flow cytometry. All three samples of *C. weldeniana* from the Gießhübl population were diploid, $2n = 22$ (Fig. 2).

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 except for AC1 that was almost invariable.

PCA 1 revealed differentiation of *C. weldeniana* from Central European populations of the *C. jacea* agg. *Centaurea weldeniana* forms a compact cloud on the periphery of the ordination space, which does not overlap with other taxa, although it is not separated by any obvious gap. Individuals from the Gießhübl population are intermingled with *C. weldeniana* from the Balkans (Fig. 3). The cloud of *C. weldeniana* is also slightly separated along the third ordination axis (not shown). *Centaurea jacea* and *C. panonica* markedly overlap (however, separation of these two taxa is beyond the scope of the present paper, and will be discussed in a separate contribution). The characters that most contribute to the separation of *C. weldeniana* are shape of leaves and bracts on branches (smaller width and higher length/width ratio in *C. weldeniana*; characters LW, LBW, LLW and LBLW), presence of greyish hairs (more frequent in *C. weldeniana*; characters HM and HU), size of capitula (smaller in *C. weldeniana*; characters IW and IL) and branching angle (higher in *C. weldeniana*; character BA) (Table 4).

Within the Gießhübl population, diploids (*C. weldeniana*) and tetraploids (*C. jacea*) were clearly separated by

PCA 2 (Fig. 4). In addition to characters indicated by PCA 1, the two species were also separated by size of appendages (AL, AW), total stem height (ST), branching of lateral branches (BB), length of internodes on branches (BLLF) and length of leaves and bracts (LL, LBL). Concerning the tetraploid individuals tentatively marked as hybrids, the hybrid origin of at least two of them was confirmed—one was very close to *C. weldeniana*, one was intermediate and one was on the margin of the cloud of *C. jacea* (Fig. 4).

Centaurea argyrolepis

The original material of *C. argyrolepis* comprises at least eight herbarium sheets that conform with the protologue (Hayek 1901): accession numbers W 1912-10588, W 1912-10589, W 1912-15090, W 1912-15123, W 1912-21094, W 1912-21101, WU 43222, and one sheet in W without an accession number ("*Centaurea gaudinii* Boiss. et Reut. Tirolerhof b. Gießhübl" 28.8.1900 leg. A. Teyber). All the material is homogeneous and identical to the recently found population, and can be classified as *C. weldeniana*.

Discussion

Determination

Morphometric analysis and karyological data confirmed differences between the Gießhübl population from other Central European representatives of the *C. jacea* agg. The key features were conspicuous greyish indumentum, linear leaves and bracts, relatively long, straight (virgate) and patent branches and relatively large and pale appendages of involucre bracts. The population was determined as *C. weldeniana* and was not different from Croatian and

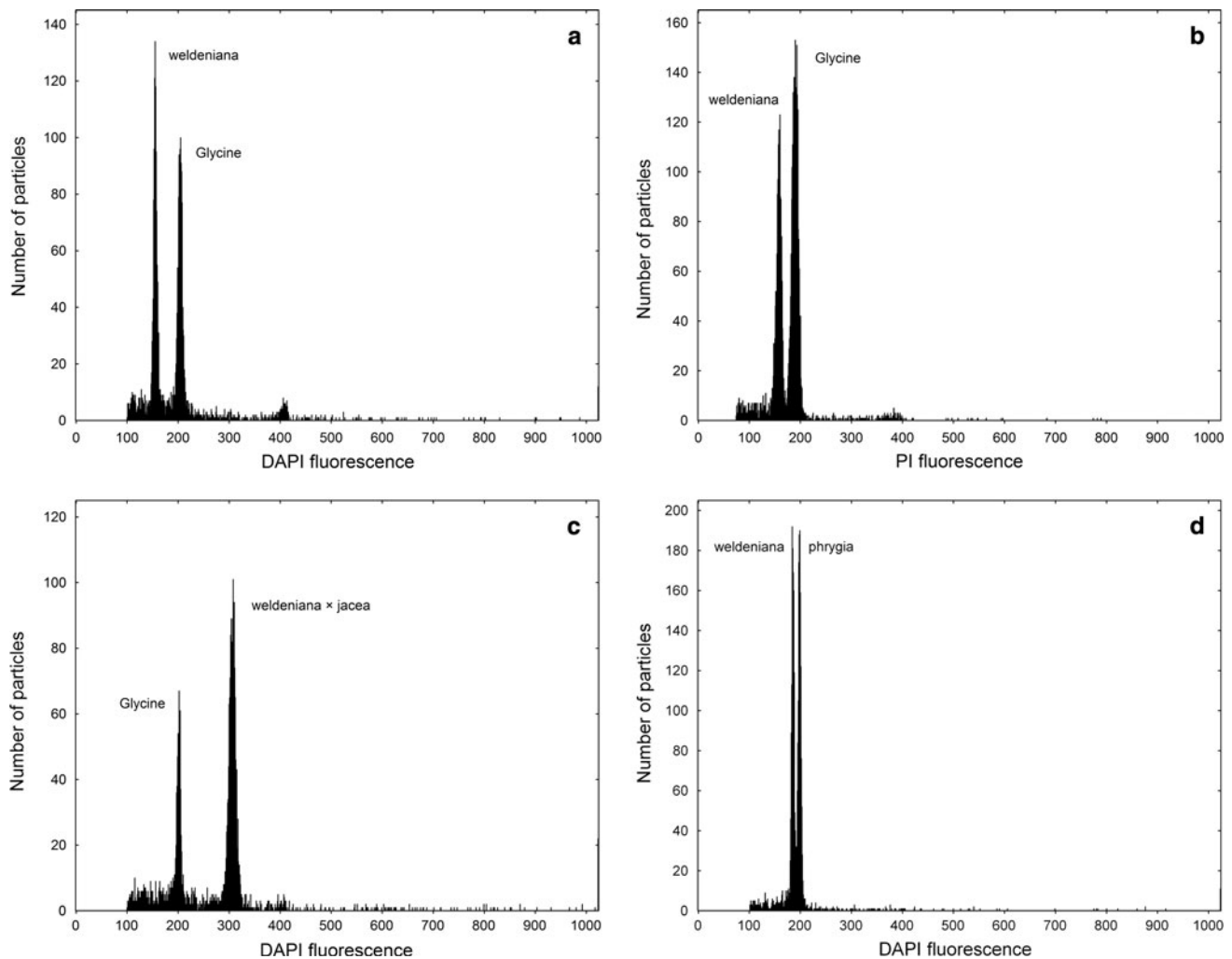


Fig. 1 Representative fluorescence histograms of *Centaurea weldeniana* and its hybrids. *Glycine max* ‘Polanka’ was used as the internal standard. **a** Diploid *C. weldeniana* (DAPI stain). **b** Diploid *C. weldeniana* (propidium iodide stain). **c** Tetraploid hybrid

C. weldeniana × *C. jacea* (DAPI stain). **d** Simultaneous analysis of *C. weldeniana* and *C. phrygia* L. corroborating a difference of about 6 % in the genome size (DAPI stain)

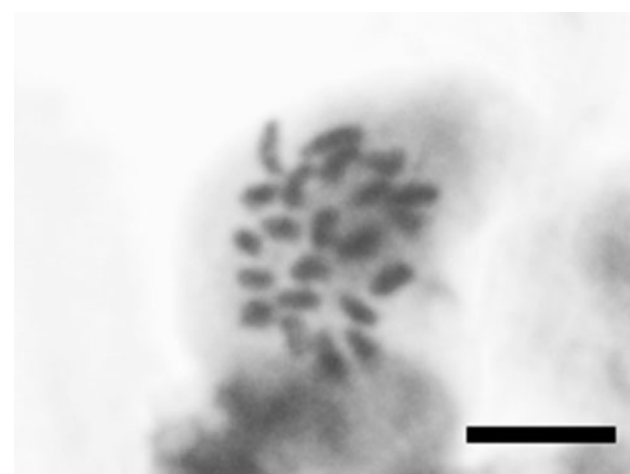


Fig. 2 Somatic metaphase of *Centaurea weldeniana*, $2n = 22$. Scale bar 5 μm

Bosnian material of *C. weldeniana* in multivariate morphometric analysis. The determination was also confirmed by karyological data. All studied populations assigned to *C. weldeniana* were diploid. In contrast, all Central European populations of *C. jacea* and all but one population of *C. pannonica* studied so far were tetraploid (see [Introduction](#) for references and Koutecký et al., unpublished data). *Centaurea weldeniana* is a Balkan taxon whose native distribution includes Italy, Croatia, Serbia, Bosnia, Montenegro, Albania and Greece (Dostál 1976; Greuter 2006–2009).

There is one other putatively diploid taxon (see [Introduction](#)) from the *C. jacea* agg. native to Austria: *C. bracteata* (= *C. jacea* subsp. *gaudini*). Its distribution includes France, Switzerland, Austria, Italy, Slovenia and Croatia, and north-west Africa (Morocco, Libya and Tunisia) (Dostál 1976, Greuter 2006–2009). In Austria, it

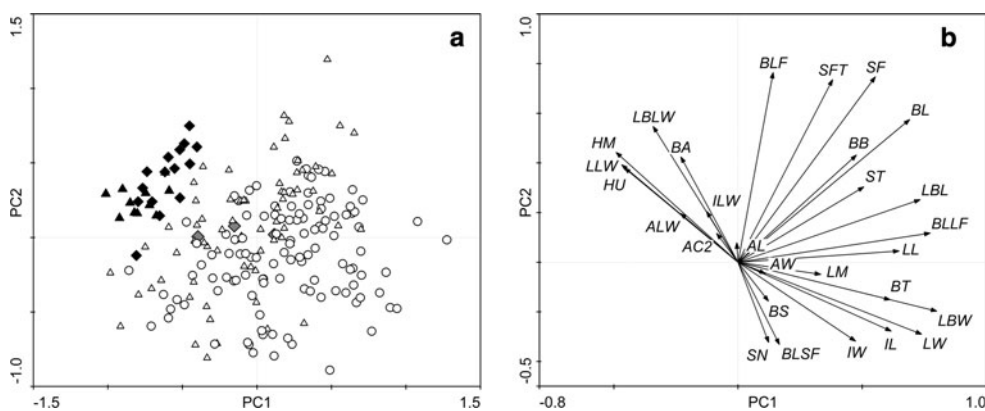


Fig. 3 PCA of Central European populations of the *Centaurea jacea* agg. The first and the second ordination axes explain 21.6 % and 13.9 % of the variation, respectively. **a** Position of individuals. *Black diamonds* *C. weldeniana*, Gießhübl; *black triangles* other populations

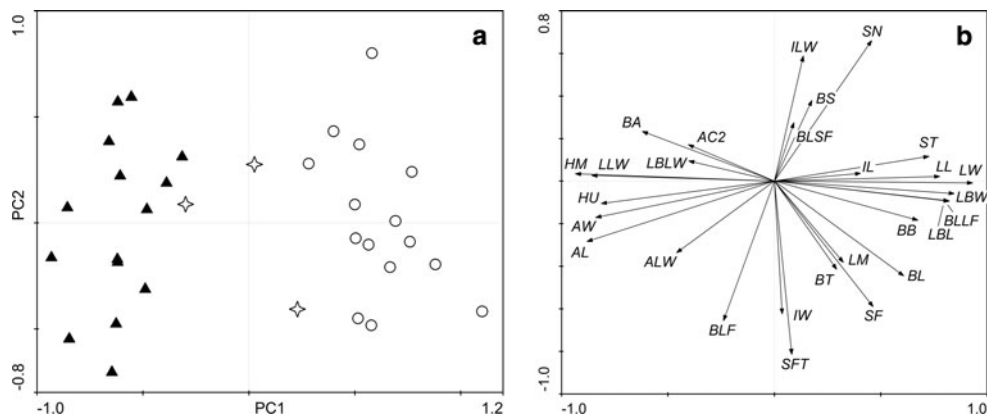
of *C. weldeniana*; *empty circles* *C. jacea*; *empty triangles* *C. pannonica*; *grey diamonds* putative hybrids *C. jacea* × *C. weldeniana*. **b** Correlation of characters with ordination axes

Table 4 Variation of the characters of *Centaurea weldeniana* and Central European populations of *C. jacea* and *C. pannonica*

Character	<i>C. weldeniana</i> , Gießhübl (n = 15)		<i>C. weldeniana</i> , other populations (n = 11)		<i>C. pannonica</i> (n = 70)		<i>C. jacea</i> (n = 115)	
	Mean ± SD	5–95% quantile	Mean ± SD	5–95% quantile	Mean ± SD	5–95% quantile	Mean ± SD	5–95% quantile
LW	0.53 ± 0.09	0.4–0.7	0.40 ± 0.09	0.3–0.6	0.91 ± 0.41	0.4–1.6	1.34 ± 0.66	0.6–2.6
LLW	10.55 ± 2.06	8.4–15.5	12.89 ± 2.52	9.4–17.3	8.98 ± 3.32	4.3–14.3	7.49 ± 3.31	3.6–14.6
LBW	0.24 ± 0.05	0.2–0.4	0.20 ± 0.04	0.2–0.3	0.38 ± 0.15	0.2–0.7	0.51 ± 0.20	0.3–0.9
LBLW	7.73 ± 1.70	6.00–11.00	9.44 ± 1.88	7.5–13.3	6.49 ± 2.11	3.4–10.3	6.02 ± 1.78	3.4–9.3
BA	53.67 ± 5.81	40–65	50.45 ± 4.15	45–60	42.5 ± 7.36	30–55	41.26 ± 7.55	30–55
HM	1.00		1.00		0.53		0.11	
HU	1.00		1.00		0.83		0.21	
IL	1.23 ± 0.07	1.1–1.4	1.20 ± 0.11	1.0–1.4	1.38 ± 0.11	1.2–1.5	1.42 ± 0.14	1.2–1.7
IW	1.03 ± 0.15	0.8–1.3	1.13 ± 0.09	1.0–1.3	1.23 ± 0.15	1.0–1.5	1.27 ± 0.23	0.9–1.6
AL	4.96 ± 0.62	3.7–6.1	4.19 ± 0.70	3.2–5.3	4.32 ± 0.72	3.2–5.5	4.09 ± 0.75	3.0–5.4
AW	5.78 ± 0.55	4.6–6.4	4.69 ± 0.81	3.6–6.0	5.05 ± 0.89	3.5–6.4	4.90 ± 0.86	3.6–6.7

The most important characters from the PCA 1 and size of appendages (the most often used character in *C. sect. Jacea*) are included. For binary characters (HM, HU), the average value indicates the proportion of individuals having the character state 1.

Fig. 4 PCA of the Gießhübl population. The first and the second ordination axes explain 38.2 % and 13.1 % of variation, respectively. **a** Position of individuals. *Black diamonds* *Centaurea weldeniana* (diploid); *empty circles* *C. jacea* (tetraploid); *crosses* putative hybrids *C. jacea* × *C. weldeniana* (tetraploid). **b** Correlation of characters with ordination axes



occurs only in the south-west in western Kärnten and Osttirol (Fischer et al. 2008), about as far from the Gießhübl locality as are the nearest populations of *C. weldeniana* in Italy and Croatia. Interestingly, the Gießhübl population was determined as *C. bracteata* (under the name *C. gaudinii*) when found for the first time by M. F. Müllner (1888) (see below). Also Hayek (1901), who described it as a new taxon *C. argyrolepis*, regarded it as close to, but different from, *C. bracteata* because *C. bracteata* has somewhat larger appendages of involucre bracts than is usual in *C. weldeniana* (Table 4). However, the width of appendages 4.6–6.4 mm is still considerably smaller than reported for *C. bracteata* (6–8 mm), is fully within the range (4–)5–7 mm reported for *C. weldeniana* and is also within the variation range of “small-appendage” taxa such as *C. jacea* and *C. pannonica* (Table 4). Moreover, the Gießhübl population differs from *C. bracteata* also in other characters, such as markedly smaller capitula (0.9–1.1 cm wide; while 1.2–2.0 cm is reported for *C. bracteata*) and branching about in the middle of the stem with long branches (while *C. bracteata* is short-branched in the upper part of the stem; for measurements cited here see Dostál 1976 and Pignatti 1982).

Centaurea weldeniana is not known to occur in Austria. The (re)discovery of the present population implies addition of the new, probably adventive species to the flora of Austria.

History of the population

Centaurea weldeniana is most probably not native in Austria. The population is isolated. There are no records of *C. weldeniana* from Austria and of *C. bracteata* from the eastern half of Austria nor any specimens of these taxa in the studied herbaria (especially W and WU and also BP, which contains material from the formerly Hungarian part of contemporary Austria). The locality lies in an intensive agricultural landscape, where survival of a “relic” population is improbable. Interestingly, the adventive origin of this population was suggested by Hayek (1901), who mentions three large dairy farms that used imported hay.

The population of *C. weldeniana* was discovered “auf einer Wiese zwischen Perchtoldsdorf and Giesshübel” by Müllner in August 1886 and determined as *C. gaudinii* [= *C. bracteata*] (Müllner 1888). The same population is also cited by Beck (1893) as *C. gaudinii*, by Hayek (1901) as *C. argyrolepis* and by Hayek (1917) as *C. jacea* subvar. *argyrolepis*. The locality (both in the literature and on herbarium labels) was referred to as between Perchtoldsdorf and Gießhübl or near the former, or is narrowed to the village of Tirolerhof between them. Since then, there are no records until 2005 when I discovered the population at the same site or apparently very close to the original locality.

Hence, it is most probable that the species has survived there for at least 125 years and was merely not recorded for a long period. The other possibility (repeated dispersal from far localities to the same place and nowhere else) is rather improbable.

Genome size

The genome size is reported for the first time for any diploid taxon of the *C. jacea* agg.. The value of 2.09 pg is fully within the range for diploid members of *C. sect. Jacea* found by Bancheva and Greilhuber (2006), Dydak et al. (2009) and Koutecký et al. (2012). Interestingly, the value for the other diploid member of the *C. jacea* agg., *C. bracteata*, is the same (Koutecký et al., unpublished data) and is different from diploids of the *C. phrygia* agg. (Fig. 1d), which suggests that the genome size may be a useful marker for internal classification of *C. sect. Jacea*. Compared to published values for related tetraploids (Bancheva and Greilhuber 2006, Dydak et al. 2009), the monoploid genome size (Cx-value) of tetraploid *C. jacea* is about 0.95 of that of diploid *C. weldeniana*, which is similar to the mean difference between diploid and tetraploid taxa of the sect. *Jacea* found by Bancheva and Greilhuber (2006). However, such comparisons should be viewed with caution, since there may be considerable differences among individual studies using different methodologies (Feulgen densitometry vs. flow cytometry) and different internal standards. Indeed, the values of relative fluorescence of tetraploid *C. jacea* in the present study were 2.01 times higher than those of the diploid *C. weldeniana* (Table 3).

Hybridization

The persistence of an isolated diploid population among abundant tetraploids for at least 125 years is a unique natural experiment. Although there is a certain phenological shift (*C. weldeniana* starting to flower about 3 weeks after *C. jacea*), the flowering times of the two species overlap widely. Theoretically, the population of *C. weldeniana* should suffer from a severe minority cytotype disadvantage (Levin 1975) due to crossing with more frequent tetraploids. The hybrids, irrespective of whether they are sterile triploids or fertile tetraploids (Koutecký et al. 2011), cannot contribute to (sexual) reproduction of the diploids. Although *C. sect. Jacea* plants are perennials and can survive up to 10 years under cultivation (Koutecký, personal observation), there must have been several generations through sexual reproduction since 1886. Survival of this population is thus a good example of the interploidy reproductive barrier and confirms the results of previous studies of heteroploid hybridization in *Centaurea* sect.

Jacea (Gardou 1972; Hardy et al. 2000, 2001; Koutecký et al. 2011, 2012). Specifically, it confirms the results of pollinations with a mixture of pollen, in which the vast majority of progeny are of the same ploidy level as the maternal plant and intercytotype hybrids are almost absent (Koutecký et al. 2011). Although in a minority, the frequency of diploid–diploid crosses has obviously been sufficient to prevent massive hybridization and sustain the population of *C. weldeniana*.

However, due to massive influx of tetraploid pollen to the diploids, the barrier is broken occasionally. Morphometric analysis revealed at least two tetraploid individuals that were morphologically intermediate or even close to diploids and were most probably hybrids (Fig. 4). As in previous field studies (Hardy et al. 2000; Koutecký et al. 2011, 2012), these natural hybrids were tetraploid instead of triploid and must have originated from unreduced gametes (probably ovules) of diploids (Koutecký et al. 2011). They were fully fertile (bore filled seeds), which indicates back-crossing with tetraploid *C. jacea* and possible introgression of genetic material of diploids to the genome of tetraploids.

Acknowledgments I am indebted to Milan Štech and Tereza Bad'urová for their help in the field and laboratory, to Jan Štěpánek for directing my attention to *Centaurea* and valuable discussions during my work, and to two reviewers for their comments on the manuscript. I am grateful to the curators of herbarium collections for help during my study, especially Ernst Vitek and Walter Till. The study was financially supported by the Grant Agency of the Czech Republic (grant no. 206/08/1126) and the Ministry of Education, Youth and Sports of the Czech Republic (grant no. MSM6007665801).

References

- Albers F (ed) (1998) Chromosomenzahlen der Farn und Blütenpflanzen Deutschlands. In: Wisskirchen R, Haeupler H, Albers F (eds) Standardliste Farn- und Blütenpflanzen Deutschlands. Eugen Ulmer, Stuttgart, pp 562–616
- Bancheva S, Greilhuber J (2006) Genome size in Bulgarian *Centaurea* s.l. (Asteraceae). *Plant Syst Evol* 257:95–117
- Beck G (1893) Flora von Niederösterreich, vol II/2. Carl Gerold's Sohn, Wien
- Dobeš C, Vitek E (2000) Documented chromosome numbers checklist of Austrian vascular plants. Verlag des Naturhistorischen Museums, Wien
- Doležel J, Greilhuber J (2010) Nuclear genome size: are we getting closer? *Cytometry A* 77:635–642
- Dostál J (1976) *Centaurea* L. In: Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM, Webb DA (eds) Flora Europaea, vol 4. Cambridge University Press, Cambridge, pp 254–301
- 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
- Fischer MA, Adler W, Oswald K (2008) Exkursionsflora für Österreich, Liechtenstein und Südtirol, 3rd edn. Land Oberösterreich and Biologiezentrum der OÖ Landesmuseen, Linz
- 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 (Biosystematic study of the section *Jacea* Cass. and several neighbouring section of the genus *Centaurea* L. in France and adjacent regions). *Feddes Reper* 83:311–472
- Greuter W (2006–2009) Compositae (pro major parte). In: Greuter W, von Raab-Straube E (eds), Compositae. Euro + Med Plant-base—the information resource for Euro-Mediterranean plant diversity. <http://ww2.bgbm.org/EuroPlusMed/>. Accessed 11 April 2012
- Hardy OJ, 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* 146:281–290
- Hardy OJ, 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
- Hayek A (1901) *Centaurea*-Arten Österreich-Ungarns. *Denkschr Akad Wiss Wien Math Nat Kl* 70:585–773
- Hayek A (1917) Kritische Studien über den Formenkreis der *Centaurea jacea* L. s. l. *Verh zool-bot Ges Wien* 68:159–214
- Jäger EJ, Werner K (eds) (2005) Rothmaler, Exkursionsflora von Deutschland, 4. Gefäßpflanzen: Kritischer Band. Spektrum Akademischer, 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*]. PhD Thesis, University of South Bohemia, České Budějovice
- Koutecký P (2009) Taxonomic and nomenclatural revision of *Centaurea subjacea* (Asteraceae-Cardueae) and similar taxa. *Phyton* 49:63–76
- Koutecký P, Bad'urová 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
- Koutecký P, Štěpánek J, Bad'urová T (2012) Differentiation between diploid and tetraploid *Centaurea phrygia*: mating barriers, morphology and geographic distribution. *Preslia* 84:1–32
- Kubát K, Hrouda L, Chrtek J, Kaplan Z, Kirschner J, Štěpánek J (eds) (2002) Klíč ke květeně České republiky (Key to the flora of the Czech Republic). Academia, Praha
- Kuzmanov BA, Georgieva SB, Nikolova VA (1986) Chromosome numbers of Bulgarian flowering plants. I. Fam. Asteraceae. *Fitologija* 31:71–74
- Levin DA (1975) Minority cytotype exclusion in local plant populations. *Taxon* 24:35–43
- Lippert W, Heubl GR (1988) Chromosomenzahlen von Pflanzen aus Bayern und angrenzenden Gebieten. *Ber Bayer Bot Ges* 59:13–22
- Löve Á (ed) (1979) IOPB chromosome number reports LXIV. *Taxon* 28:391–408
- Löve Á (ed) (1982) IOPB chromosome number reports LXXVII. *Taxon* 31:761–777
- Marhold K, Mártonfi P, Mered'a P, Mráz P (eds) (2007) Chromosome number survey of the ferns and flowering plants of Slovakia. Veda, Bratislava
- Marsden-Jones EM, Turrill WB (1954) British knapweeds. A study in synthetic taxonomy. Ray Society, London
- Mráz P, Bouchier RS, Treier UA, 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
- Müllner MF (1888) Neue Pflanzen für Niederösterreich. Verh k–k zoo-bot Ges Wien 38:29–32
- Ochsmann J (1998) *Centaurea* L. In: Wisskirchen R, Haeupler H, Albers F (eds) Standardliste Farn- und Blütenpflanzen Deutschlands. Eugen Ulmer, Stuttgart, pp 133–136
- Olšavská K, Perný M, Mártonfi P, Hodálová I (2009) *Cyanus triumfettii* subsp. *triumfettii* (Compositae) does not occur in the Carpathians and adjacent parts of Pannonia: karyological and morphological evidence. Nordic J Bot 27:21–36
- Olšavská K, Perný M, Kučera J, Hodálová I (2011) Biosystematic study of the *Cyanus triumfetti* group in Central Europe. Preslia 83:59–98
- Pignatti S (1982) Flora d'Italia (Flora of Italy), vol 3. Edagricole, Bologna
- Saarisalo-Taubert A (1966) A study of hybridization in *Centaurea*, section *Jacea*, in Eastern Fennoscandinavia. Ann Bot Fenn 3:86–95
- 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. <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), vol 7. Academia, Praha, pp 426–449
- ter Braak CJF, 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
- Vanderhoeven S, Hardy OJ, 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), 2nd edn. Verlag Paul Parey, Berlin