Nordic Journal of Botany 28: 413–427, 2010
doi: 10.1111/j.1756-1051.2010.00603.x,

Subject Editor: Stefan Andersson. Accepted 30 April 2010

A new cytotype of *Jacobaea vulgaris* (Asteraceae): frequency, morphology and origin

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Jacobaea vulgaris subsp. *vulgaris* (syn. *Senecio jacobaea* subsp. *jacobaea*) constitutes an intricate polyploid complex distributed in Europe. Four cytotypes have been reported in this species, three with euploid (diploid, tetraploid and octoploid; 2n = 20, 40 and 80) and one with aneuploid (2n = 32) chromosome numbers. Here we report that the diploid chromosome number (2n = 20) reported from Bulgaria is due to misidentification with *Jacobaea aquatica*. On the other hand, we have discovered a new, hexaploid (2n = 6x = 60) cytotype within *J. vulgaris* subsp. v*ulgaris* using flow cytometry. The new cytotype occurs within four sympatric populations of otherwise tetraploid and octoploid plants in Pannonia (one locality in the eastern Czech Republic and two localities in southwestern Slovakia) and in Podillya (one locality in western Ukraine). The frequency of hexaploid individuals within 76 studied populations is very low (only 10 of 693 analysed plants), and hexaploids probably represent hybrids between tetraploid and octoploid plants. Three mixed populations with hexaploid plants were subjected to detailed morphological and pollen fertility analyses. Multivariate morphometric analysis reveals partial separation of tetraploid and octoploid plants, whereas hexaploid individuals are similar in morphology to octoploids. In comparison with tetraploids, octoploids and hexaploids exhibit slightly longer ray florets, involucral bracts and tubular florets and more hairy outer achenes. Hexaploid plants display larger pollen grains and lower pollen fertility compared to tetraploids and octoploids.

Polyploidy is a significant feature of the evolution in many plant groups (Ramsey and Schemske 1998, Bennett 2004, De Bodt et al. 2005). The consequences of polyploid evolution have attracted much attention, and considerable progress has recently been achieved in the study of its ecological, physiological and genetic correlates (cf. Wendel 2000, Soltis et al. 2007). One noteworthy pattern is that polyploids frequently have different geographical ranges as compared with diploid (or lower polyploid) progenitors. In some cases, the cytotypic variation is exclusively geographic, with local populations consisting of a single cytotype (Štěpánková 2001, Yeung et al. 2005, Koutecký 2007). In other cases, mixed-ploidy populations have been observed (Soltis et al. 2007, Halverson et al. 2008, Španiel et al. 2008). Interactions of high-polyploids with their lower-ploidy progenitors in contact zones (zones of sympatry) provide useful insight into the evolutionary histories of polyploid complexes (Harrison and Rand 1989, Thompson and Lumaret 1992). Hybridisation between cytotypes is a common phenomenon in these contact zones, and hybrid zones may constitute active sites of evolution (Petit et al. 1999, Husband 2004).

Assessment of ploidy-level distributions within populations has been greatly enhanced by the use of flow-cytometric techniques. The application of flow cytometry has revealed that cytotype mixture (i.e. the presence of more than one ploidy level within a population) is much more frequent than previously recognised (Suda 2002, Suda et al. 2004, 2007, Perný et al. 2008, Španiel et al. 2008). The coexistence of different cytotypes may be temporary, with fluctuating cytotype frequencies (Husband and Schemske 1998), or it may be permanent due to reproductive isolation between cytotypes, potentially leading to speciation. Despite a growing number of papers dealing with polyploidy, little is known about reproductive isolation between polyploids and their diploid progenitors, cytotype variation at local or regional spatial scales, or the fates of new cytotypes in local mixed-cytotype populations (Ramsey and Schemske 1998).

Jacobaea vulgaris Gaertn. (*Senecio jacobaea* L., Asteraceae) is an herbaceous, self-incompatible, biennial or short-lived perennial inhabiting open, grassy (frequently disturbed) habitats throughout Europe and western Asia. The species has been introduced in North and South America, South Africa, Australia and New Zealand (Harper and Wood 1957, Bain 1991). Three subspecies are currently recognised: *J. vulgaris* subsp. *vulgaris* (syn. *Senecio jacobaea* subsp. *jacobaea*), *J. vulgaris*. subsp. *dunensis* (Dumort) Pelser et Meijden [syn. *Senecio jacobaea* subsp. *dunensis* (Dumort.) Kadereit & Sell] and *J. vulgaris* subsp. *gotlandica* (Neuman)

B. Nord. [syn. *Senecio jacobaea* subsp. *gotlandicus* (Neuman) Sterner]. The nominate subspecies is widespread throughout Europe. *Jacobaea vulgaris* subsp. *dunensis* has been observed on the coast of the British Isles and on coasts from Belgium to eastern Sweden and southern Finland (Andersson 2001a, 2001b). The latter subspecies was described from the island of Gotland in the Baltic sea (Neuman and Ahlfvengren 1901) and has recently been reported from Russia, Greece and Austria (Wysk et al. 2009). *Jacobaea vulgaris* subsp. *dunensis* is distinguished from both *J. vulgaris* subsp. *vulgaris* and *J. vulgaris* subsp. *gotlandica* by the absence of ray florets (Kadereit and Sell 1986, Andersson 2001a, 2001b). *Jacobaea vulgaris* subsp. *vulgaris* and *J. vulgaris* subsp. *gotlandica* are distinguished by the shape of their basal leaves: the basal leaves of the latter subspecies are subentire with a large terminal lobe, whereas those of the nominate subspecies are deeply pinnatifid (Neuman and Ahlfvengren 1901, Wysk et al. 2009). However, the taxonomic status of *J. vulgaris* subsp. *gotlandica* is questionable; plants with subentire basal leaves with large terminal lobes occur in most European populations of *J. vulgaris* subsp. *vulgaris* (Hodálová and Mered'a jun., unpubl.).

Only one chromosome number has been reported for both *J. vulgaris* subsp. *dunensis* and *J. vulgaris* subsp. *gotlandica* ($2n = 4x = 40$) (Kockx-van Roon and Wieffering 1982, Wysk et al. 2009). In contrast, the nominate subspecies has been analysed repeatedly (Bain 1991, Hodálová et al. 2007a); tetraploid ($2n = 4x = 40$), octoploid ($2n = 8x = 80$), diploid ($2n = 2x = 20$) and aneuploid ($2n = 32$) individuals have been reported. The tetraploid cytotype is the most widespread, occurring throughout the species' European range and in North America. The octoploid cytotype was reported for the first time from Slovakia (Murín and Váchová 1970) and has subsequently been found in neighbouring countries, in the Pannonian basin and adjacent areas (Czech Republic, Austria, Hungary) and in the Podillya highlands (Ukraine) (cf. Hodálová et al. 2007a, 2007b, Vinikarová 2009). The final two chromosome numbers listed ($2n = 20$ and 32) have been reported from single sites in Bulgaria (Kuzmanov et al. 1979) and Ireland (Böcher and Larsen 1955), respectively. However, we have found that the Bulgarian material counted as $2n = 20$ was misidentified and the voucher specimen belongs to *Jacobaea aquatica* (Hill.) P. Gaertn., B. Mey. et Schreb. [i.e. *Jacobaea aquatica* var. *aquatica* or *Jacobaea aquatica* var. *erratica* (Bertol.) Pelser et Meijden, Hodálová and Mered'a jun., unpubl.]. *Jacobaea aquatica* was so far considered to be exclusively tetraploid ($2n = 40$; cf. Rotreklová et al. 2004). Anyway, detailed studies of Bulgarian *J. aquatica* are needed as the chromosome number $2n = 20$ is the only diploid number in the whole genus *Jacobaea*.

The basic chromosome number in the genus *Jacobaea* Mill. [*Senecio* sect. *Jacobaea* (Mill.) Dumort.] is not clear. Some authors accept $x = 10$ (Bain 1991, Hodálová et al. 2007a, 2007b, Pelser et al. 2007), whereas others favour $x = 20$ (cf. Schönswetter et al. 2007, Suda et al. 2007, Hülber et al. 2009). Assuming the validity of Bulgarian *Jacobaea aquatica* with $2n = 20$ (Kuzmanov et al. 1979), which cannot be disproven at present, and considering the basic chromosome number of $x = 10$ in the closely related genus *Senecio* L. (Lafuma et al. 2003, Abbott and Lowe 2004, Pelser et al. 2007), we accept $x = 10$ for the genus *Jacobaea*. Therefore, individuals with $2n = 40$ are here treated as tetraploids, those with $2n = 60$ as hexaploids and those with $2n = 80$ as octoploids.

Within the contact zone between tetraploid and octoploid *J. vulgaris* subsp. *vulgaris* in Pannonia and Podillya, tetraploids generally occur at higher altitudes and in more ruderal biotopes. There are nonetheless sites where both tetraploid and octoploid plants occur in close proximity or grow in sympatry (Hodálová et al. 2007a, 2007b). However, the fine-scale distribution of cytotypes and their potential for, and degree of, interaction are not known in detail. As part of a broader investigation of the evolutionary dynamics and taxonomic position of *J. vulgaris* subsp. *vulgaris* octoploids in Europe, the objective of this study was to search for possible intermediate cytotypes between tetraploids and octoploids. Specifically, we addressed the following questions: (1) What are the frequencies of tetraploid and octoploid plants within the study area? (2) Do other cytotypes occur at sites with tetraploid-octoploid mixtures? (3) If so, what are the frequencies of these plants?, and (4) Do other cytotypes differ in morphology and reproductive capacity from tetraploid and octoploid plants in mixed-cytotype populations?

Material and methods

Plant material

Seventy-six populations of *Jacobaea vulgaris* subsp. *vulgaris* were sampled between 2001 and 2008 in Austria (3), Czech Republic (20), Slovakia (23), Hungary (6), Ukraine (20) and Romania (4) (Table 1, Fig. 1). The sampling strategy was based on explorations of two regions (Pannonia and Podillya) where octoploid plants of *J. vulgaris* had been observed. The number of samples per locality varied from 1 to 82, and reflected both the size of the locality and the abundance of *J. vulgaris* subsp. *vulgaris*. Localities and numbers of plants included in cytological, morphological and pollen analyses are listed in Table 1. Voucher specimens have been deposited in BRNU and SAV.

Cytological analyses

Chromosome counts

Plants of *J. vulgaris* subsp. *vulgaris* collected in the field were potted and cultivated in the experimental gardens of the Inst. of Botany and Zoology, Masaryk Univ., Brno (49°15′03″N, 16°34′25″E) and the Inst. of Botany, Slovak Academy of Sciences, Bratislava (48°10′15″N, 17°04′15″E). Chromosome counts were obtained from mitotic figures of meristem cells in actively growing root-tips, using the squash method. We followed the procedure specified in detail by Hodálová et al. (2007a).

DNA ploidy level estimation

To determine DNA ploidy levels of the studied plants, the relative nuclear DNA content was estimated using flow cytometry (FCM) and compared to values obtained from the plants with known chromosome numbers. The prefix 'DNA' indicates that the ploidy levels were inferred from the measured nuclear DNA content, without knowing the exact chromosome number (Suda et al. 2006).

Table 1. List of the *Jacobaea vulgaris* subsp. *vulgaris* populations studied. Each record is given as follows: population number, geographic origin, chromosome counts and DNA ploidy levels, with corresponding number of plants and name of author(s) of the chromosome count(s)/author(s) of the measurements of DNA ploidy level/place of original publication of the analyses, total number of analysed individuals and the number of individuals analysed for morphometric (Morph)/pollen viability (Pollen). Countries: A = Austria, CZ = Czech Republic, H = Hungary, RO = Romania, SK = Slovakia, UA = Ukraine. Abbreviations of the authors of karyological analyses and collectors: AK = A. Kagalo, AV = A. Vinikarová, DD = D. Dítě, EM = E. Michalková, IH = I. Hodálová, JS = J. Somogyi, LH = L. Horová, MK = M. Kolník, MP = M. Perný, MS = M. Slovák, MV = M. Valachovič, OR = O. Rotreklová, Pen = M. Peniašteková, PM = P. Mered'a jun., RL = R. Letz, VG = V. Grulich. Data on chromosome numbers and DNA ploidy level marked with asterisks are taken from the literature: *Hodálová et al. (2007a), **Hodálová et al. (2007b), ***Vinikarová (2009); all other data represent new records.

Pop. no.	Locality description, altitude, date of collection, name of collector(s)	Geographic coordinates (WGS84)	Chromosome count (no. of individuals counted)	DNA ploidy level			n	Morph/ Pollen
				4x	6x	8x		
30	A, Sieding, 450 m, 24 Jul 2002, IH and JS	47°43′46″N 15°59′48″E	–	–	–	4*	4	–
31	A, Wiener Neustadt, ca 300 m, 24 Jul 2002, IH and JS	47°49′08″N 16°12′15″E	–	–	–	3*	3	–
32	A, Hainburg, 480 m, 14 Jul 2002, IH and JS	48°09′30″N 16°58′56″E	–	–	–	3*	3	–
3	CZ, Brzotice, 750 m, 9 Jul 2001, VG	48°48′47″N 14°11′05″E	–	3*	–	–	3	–
4	CZ, Kájov, 550 m, 15 Sep 2002, VG	48°49′05″N 14°16′12″E	2n = 40 (1*)	3*	–	–	4	–
104	CZ, Tišnov, 340 m, 5 Oct 2006, AV	49°21′05″N 16°25′07″E	–	–	–	10***	10	–
111	CZ, Malhostovice, 330 m, 20 Aug 2007, AV	49°19′33″N 16°29′44″E	–	–	–	10***	10	–
106	CZ, Kuřim-Podlesí, 341 m, 24 Jul 2007, AV	49°18′20″N 16°33′24″E	–	20^{AV}	–	–	20	–
110	CZ, Lelekovice, 355 m, 20 Aug 2007, AV	49°17′39″N 16°33′57″E	–	9^{AV}	–	–	9	–
109	CZ, Brno-Ivanovice, 330 m, 24 Jul 2007, AV	49°15′28″N 16°33′38″E	–	–	–	3***	3	–
112	CZ, Bořitov, 330 m, 20 Aug 2007, AV	49°25′11″N 16°36′01″E	–	11^{AV}	–	–	11	–
108	CZ, Obora (SW of Boskovice), 484 m, 24 Jul 2007, AV	49°27′04″N 16°35′10″E	–	10^{AV}	–	–	10	–
105	CZ, Němčice (SE of Boskovice), 623 m, 23 Jul 2007, AV	49°27′13″N 16°42′46″E	–	1^{AV}	–	–	1	–
107	CZ, Vilémovice, Macocha chasm, 488 m, 24 Jul 2007, AV	49°22′07″N 16°43′35″E	–	6^{AV}	–	–	6	–
114	CZ, Luleč, 303 m, 21 Aug 2007, AV	49°15′38″N 16°56′08″E	–	10^{AV}	–	–	10	–
115	CZ, Slatinky, Nature Reserve Vápenice, 293 m, 7 Jul 2008, AV; 20 Aug 2008, AV and VG	49°32′23″N 17°05′31″E	–	9^{AV}	2^{AV}	4^{AV}	15	14/14
100	CZ, Moravský Krumlov, 252 m, 28 Sep 2005, VG and AV	49°02′46″N 16°19′03″E	–	–	–	11***	11	–
101	CZ, Rokytná, 360 m, 28 Sep 2005, VG and AV	49°03′31″N 16°19′55″E	2n = 80 (1***)	–	–	1***	2	–
103	CZ, Brno-Nový Lískovec, 370 m, 24 Oct 2005, AV	49°10′55″N 16°33′12″E	–	–	–	5***	5	–
113	CZ, Křižanovice, 265 m, 21 Aug 2007, AV	49°08′53″N 16°56′57″E	–	–	–	3***	3	–
33	CZ, Mikulov, 340 m, 10 Sep 2002, IH	48°49′39″N 16°38′30″E	–	–	–	2*	2	–

Table 1 (*Continued*)

Pop. no.	Locality description, altitude, date of collection, name of collector(s)	Geographic coordinates (WGS84)	Chromosome count (no. of individuals counted)	DNA ploidy level			n	Morph/ Pollen
				4x	6x	8x		
102	CZ, Hodonín, 165 m, 12 Oct 2005, VG	48°52′01″N 17°05′30″E	–	–	–	1***	1	–
5	CZ, Nedašova Lhota, 450 m, 13 Sep 2002, VG	49°07′33″N 18°05′20″E	–	3*	–	–	3	–
15	SK, Bratislava-Karlova Ves, 204 m, 16 Jul 2001, IH; 18 Jul 2008, IH	48°10′30″N 17°02′24″E	–	5*, 15^{AV}	–	–	20	–
14	SK, Bratislava-Devínska Nová Ves, Štokeravská vápenka, 160 m, 25 Jul 2002, IH; 25 Jul 2003, IH; 24 Jul 2004, IH; 25 Aug 2005, IH; 16 Mai 2008, IH and PM; 16 Jul 2008, IH	48°12′14″N 17°00′44″E	2n = 40 (3*)	5*, 29^{AV}	1^{AV}	–	38	25/11
41	SK, Bratislava-Devínska Nová Ves, Nature Reserve Sandberg, 220 m, 25 Jul 2002, IH; 16 Jul 2008, IH	48°12′02″N 16°59′28″E	–	–	–	6*, 25^{AV}	31	24/10
37	SK, Devínske Jazero, 168 m, 16 Jul 2001, IH	48°14′57″N 16°57′54″E	–	–	–	2*	2	–
38	SK, Devínske Jazero, 170 m, 16 Jul 2001, IH	48°14′57″N 16°57′54″E	–	–	–	3*	3	–
39	SK, Gajary, 150 m, 7 Aug 2002, IH, DD and JS	48°28′57″N 16°58′32″E	–	–	–	2*	2	–
40	SK, Plavecký Mikuláš, 195 m, 10 Aug 2001, IH; 16 Mai 2008, IH and PM; 25 Oct 2008, IH and PM	48°31′27″N 17°14′36″E	2n = 60 (1^{AV})	2^{AV}, $40^{IH\&PM}$	$2^{IH\&PM}$	5*, 2^{AV}, $30^{IH\&PM}$	82	–
42	SK, Senec, 127 m, 22 Aug 2002, IH	48°14′26″N 17°25′03″E	–	–	–	2*	2	–
46	SK, Lančár, 290 m, 17 Jul 2001, IH	48°36′31″N 17°39′30″E	–	–	–	4*	4	–
16	SK, Čachtice, 400 m, 17 Jul 2001, IH	48°43′21″N 17°45′41″E	–	1*	–	–	1	–
51	SK, Lehota, 232 m, 22 Aug 2002, IH	48°18′48″N 17°58′27″E	–	1*	–	1*	2	–
43	SK, Čenkov, 100 m, 15 Jul 2001, IH	47°46′14″N 18°32′51″E	–	–	–	1*	1	–
44	SK, Štúrovo, 330 m, 15 Jul 2001, IH	47°49′23″N 18°38′11″E	–	–	–	3*	3	–
50	SK, Demandice, 150 m, 15 Jul 2002, IH; 25 Oct 2008, IH and PM	48°07′51″N 18°47′34″E	–	3*, $16^{IH\&PM}$	–	3*	22	–
12	SK, Čelovce – Opava, 400 m, 27 Jul 2001, IH	48°11′47″N 19°09′31″E	–	4*	–	–	4	–
17	SK, Rudno nad Hronom, 230 m, 22 Aug 2002, IH	48°25′32″N 18°40′12″E	–	2*	–	–	2	–
19	SK, Folkušová, 660 m, 29 Jul 2001, Pen	48°57′36″N 18°57′34″E	–	3*	–	–	3	–
13	SK, Plešivec, 210 m, 27 Jul 2001, IH	48°34′24″N 20°25′13″E	–	5*	–	–	5	–
36	SK, Krásnohorské Podhradie, 440 m, 12 Jul 2003, IH	48°39′20″N 20°35′42″E	–	–	–	6*	6	–
45	SK, Malý Kamenec, Tarbucka hill, 243 m, 17 Jul 2002, IH and MV	48°21′35″N 21°47′21″E	–	–	–	2*	2	–

Table 1 (*Continued*)

Pop. no.	Locality description, altitude, date of collection, name of collector(s)	Geographic coordinates (WGS84)	Chromosome count (no. of individuals counted)	DNA ploidy level			n	Morph/ Pollen
				4x	6x	8x		
18	SK, Hôrka, 160 m, 27 Aug 2001, IH	48°48′02″N 21°59′06″E	–	3*	–	–	3	–
20	SK, Snina, 300 m, 28 Jul 2001, IH	48°59′30″N 22°12′40″E	–	2*	–	–	2	–
21	SK, Snina – Stakčín, 280 m, 28 Jul 2001, IH	48°58′56″N 22°09′55″E	–	1*	–	–	1	–
7	H, Szentkatalin, 230 m, 19 Jul 2003, IH; 7 Jul 2008, IH and PM	46°10′23″N 18°03′17″E	–	8*, $1^{IH\&PM}$	–	–	9	–
49	H, Pincehely, 115 m, 30 Jul 2002, IH; 6 Jul 2008, IH and PM	46°41′34″N 18°27′34″E	–	1*	–	5^*, $12^{IH\&PM}$	18	–
34	H, Zánka, 150 m, 29 Jul 2002, IH	46°52′54″N 17°44′10″E	–	–	–	1*	1	–
48	H, Hárskút, 370–420 m, 2 Aug 2001, IH; 21 Jul 2008, IH, PM and AV	47°11′49″N 17°50′20″E	–	1*	–	11^*, 20^{AV}, $30^{IH\&PM}$	62	–
6	H, Zirc, 435 m, 2 Sep 2001, IH; 6 Jul 2008, IH and PM	47°18′34″N 17°53′07″E	–	2^*, $15^{IH\&PM}$	–	–	17	–
35	H, Keszeg, 318 m, 11 Jul 2003, IH	48°00′12″N 19°20′11″E	–	–	–	3*	3	–
22	UA, Mukačeve, 142 m, 28 Jul 2004, IH and MK	48°30′52″N 22°30′45″E	–	4*	–	–	4	–
23	UA, Svalyava, 205 m, 27 Jul 2004, IH, MP and MK	48°31′54″N 23°01′14″E	–	4*	–	–	4	–
24	UA, Iza, 194 m, 27 Jul 2003, IH, MP and MK	48°13′54″N 23°21′49″E	–	3*	–	–	3	–
25	UA, Rahiv, 320 m, 26 Jul 2004, IH and MP	48°03′37″N 24°12′15″E	–	2*	–	–	2	–
26	UA, Kvasi, 350 m, 26 Jul 2004, IH and MP	48°07′54″N 24°17′02″E	–	1*	–	–	1	–
27	UA, Vinnyki, 217 m, 21 Jul 2003, IH, EM and AK	49°49′35″N 24°09′38″E	–	3*	–	–	3	–
28	UA, Zolochiv, 290 m, 25 Jul 2004, MV	48°50′22″N 24°55′50″E	–	3*	–	–	3	–
29	UA, Ternopil', 222 m, 22 Jul 2003, IH, EM and AK	49°33′15″N 25°32′27″E	–	3*	–	–	3	–
52	UA, Vikno, 320 m, 13 Aug 2005, IH and PM; 24 Jul 2007, IH and PM	49°21′24″N 26°04′25″E	–	13^{**}, 9^{AV}	$2^{LH\&VG}$, 2^{AV}	15^{**}, 5^{AV}	46	45/25
55	UA, Ostap''e, 390 m, 25 Jul 2007, IH and PM	49°23′51″N 26°05′00″E	–	10^{**}, 1^{AV}	–	–	11	–
56	UA, Vil'khivtsi, 325 m, 25 Jul 2007, IH and PM	49°05′26″N 26°18′20″E	–	9^{**}, 6^{AV}	–	–	15	–
57	UA, Ivakhnivtsi, NW, 320 m, 25 Jul 2007, IH and PM	49°06′02″N 26°20′58″E	–	–	–	10^{**}, 6^{AV}	16	–
58	UA, Ivakhnivtsi, NE, 280 m, 25 Jul 2007, IH and PM	49°06′03″N 26°22′20″E	–	4^{**}, 1^{AV}	–	–	5	–
59	UA, Adamovka, 270 m, 27 Jul 2007, IH and PM	49°06′11″N 27°03′04″E	–	5^{**}, 2^{AV}	–	–	7	–

Table 1 (*Continued*)

Pop. no.	Locality description, altitude, date of collection, name of collector(s)	Geographic coordinates (WGS84)	Chromosome count (no. of individuals counted)	DNA ploidy level			n	Morph/ Pollen
				4x	6x	8x		
62	UA, Ustya, 125 m, 27 Jul 2007, IH and PM	48°36′10″N 26°03′53″E	–	–	–	3**, 1^{AV}	4	–
61	UA, Babyntsi, 240 m, 27 Jul 2007, IH and PM	48°41′21″N 26°04′06″E	–	–	–	10**, 5^{AV}	15	–
60	UA, Kryvche, 260 m, 27 Jul 2007, IH and PM	48°42′45″N 26°04′48″E	–	1**	–	–	1	–
53	UA, Smotrych, 180 m, 14 Aug 2005, IH and PM; 25 Jul 2007, IH and PM	48°39′03″N 26°35′05″E	–	–	–	7**, 1^{AV}	8	–
47	UA, Demshyn, 270 m, 23 Jul 2003, IH, EM and AK; 14 Aug 2005, IH and PM; 26 Jul 2007, IH and PM	48°36′52″N 26°46′52″E	2n = 80 (1**)	1**	–	4*, 28**, 2^{AV}	36	–
54	UA, Subych, 280 m, 14 Aug 2005, IH and PM	48°35′45″N 26°49′45″E	–	–	–	1**	1	–
8	RO, Cavnic, 511 m, 17 Jul 2004, MV	47°37′14″N 23°47′57″E	–	2*	–	–	2	–
9	RO, Pui, 358 m, 9 Aug 2004, IH and MS	45°31′53″N 23°02′19″E	–	1*	–	–	1	–
11	RO, Azuga, 424 m, 7 Aug 2004, IH, MP, MK and MS	45°26′54″N 25°34′09″E	–	1*	–	–	1	–
10	RO, Codlea, 575 m, 7 Aug 2004, IH, MP, MK and MS	45°43′32″N 25°27′01″E	–	1*	–	–	1	–

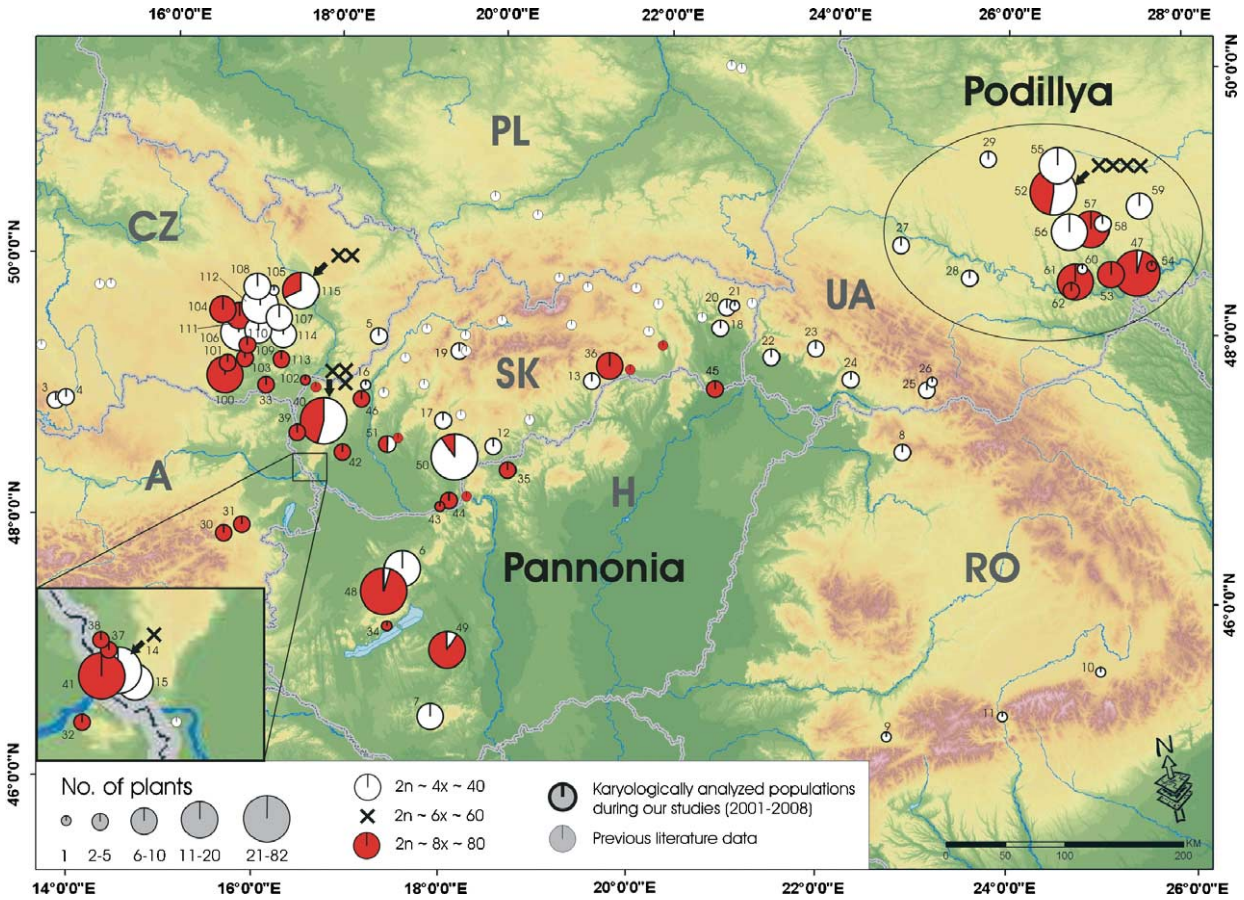

Figure 1. Distribution map of cytologically investigated populations of *Jacobaea vulgaris* subsp. *vulgaris* in central and east Europe. Pie diagrams represent the proportion of tetraploid (white) and octoploid (grey) plants in each population. Hexaploid individuals are represented by the symbol ×. Size of the diagrams is equal to the population sample size. Bold marked pie diagrams represent populations that were karyologically analysed during our studies in 2001–2008; number of pie diagram corresponds with population number. For more details see Table 1.

A two-step nuclei isolation procedure (Otto 1990) was used for sample preparation. First, samples of plants with known chromosome numbers (population 14: Devínska Kobyla Hill, Bratislava-Devínska Nová Ves; 2n = 40, cf. Table 1) were analysed simultaneously with an internal reference standard (*Glycine max* 'Polanka', 2C DNA = 2.50 pg; Doležel et al. 1994), and the ratio of their G_0/G_1 peak positions was recorded. The DNA ploidy levels of the analysed plants (of unknown chromosome number) were then assessed by their peak position relative to the standard peak. Approximately 0.5 cm^2 of fresh (Doležel and Göhde 1995) or 1–2-month old silica gel-dried (Suda and Trávníček 2006) *Jacobaea* samples were chopped together with leaf tissue, serving as internal standard. This was performed with a razor blade in a Petri dish containing 0.5 ml of Otto I buffer (0.1 M citric acid monohydrate, 0.5% Tween 20). After tissue disruption, an additional 0.5 ml Otto I buffer was added. The resulting suspension of nuclei was filtered through a 42 µm or 50 µm nylon mesh and stored for 10–15 min at room temperature with occasional shaking. 1 ml of staining solution containing Otto II buffer (0.4 M $Na_2HPO_4 \times 12H_2O$) and 4′,6-diamidino-2-phenylindole (DAPI, 2 µg ml^{-1}) was added to the flow-through fraction. After incubation for 5 min at room temperature, the fluorescence intensities were analysed using a Partec PA I flow cytometer (by the team in Brno) or Partec CyFlow ML (by the team in Bratislava). Both machines were equipped with an HBO-100 mercury arc lamp. The cytometers were adjusted such that the G_0/G_1 peak of the standard was localised on channel 100. Histograms were accumulated at a flow rate of about 20–50 particles per s for a total count of 5000 particles (stained nuclei). These histograms were then evaluated using the Partec FloMax software. For each measurement, the coefficients of variation (CV) of the standard and the analysed sample were calculated. If the CV of the G_0/G_1 peak of the sample exceeded the 5% threshold (or the 10% threshold in silica gel-dried samples), the analysis was discarded and the sample reanalysed.

In total, 693 individuals of *J. vulgaris* subsp. *vulgaris* (originating from 76 populations) were analysed in 2001–2008. Among these, 285 records have been published in our previous papers (Hodálová et al. 2007a, 2007b, Vinikarová 2009) and 408 records (407 DNA ploidy level estimations and one chromosome count) are published in this paper for the first time (cf. Table 1).

Morphometric analyses

Multivariate analyses were performed on data sets from sites with all three cytotypes (tetraploids, hexaploids and octoploids; populations 115, 14+41 and 52; cf. Table 1, 2). For purposes of morphometrics, populations 14 and 41 were treated as one site '14+41' as they occurred on the same hill (Devínska Kobyla Hill), at a close distance (2 km). The population 40 (Plavecký Mikuláš, Slovakia) was not included in the morphometric analysis, due to the absence of flowering specimens (only young leaf rosettes from this site were karyologically analysed). All individuals used in morphometric analyses were cytologically analysed (cf. Table 1).

A preliminary screening of morphological characters in populations of *J. vulgaris* subsp. *vulgaris* from Pannonia and the Carpathians (cf. Hodálová et al. 2007a) showed that there were no differences in vegetative characters that could be used to separate different ploidy levels or geographically defined groups of populations. Therefore, in the morphometric study presented here, we selected only characters from flowers and fruits. Five quantitative and one qualitative morphological character were measured: length of involucral bracts, length of ray florets, width of ray florets, number of tubular florets, length of tubular florets and indument of outer achenes (hairs present or absent). Only well-developed plants without missing characters were measured and scored. The width of ray florets and length of ray florets were measured on florets attached to paper with adhesive tape. The remaining characters were measured on dried herbarium specimens.

Statistical analyses

We calculated the Spearman correlation coefficient (cf. Legendre and Legendre 1998), based on 108 individuals originating from mixed-cytotype populations 115, 14+41 and 52 (matrix A), to eliminate pairs of highly correlated characters that might distort further analyses. Principal component analysis (PCA, Sneath and Sokal 1973), based on matrix A (PCA 1, 108 individuals originating from mixed-cytotype populations 115, 14+41 and 52), was used to illustrate the pattern of overall morphological variation among tetraploid, hexaploid and octoploid individuals. In order to investigate morphological variation of tetraploids, hexaploids and octoploids within each of three above-mentioned mixed-cytotype populations, three different data subsets (matrices) were assembled and analysed separately: matrix B (PCA 2) = individuals originating from population 115 (Czech Republic, Slatinky; 14 individuals); matrix C (PCA 3) = individuals originating from population 14+41 (Slovakia, Bratislava, Štokeravská vápenka + Sandberg; 49 individuals); and matrix D (PCA 4) = individuals originating from population 52 (Ukraine, Vikno; 45 individuals). Principal component analyses were used to determine the non-hierarchical structure within mixed-cytotype populations, based on a correlation matrix between the characters.

Discriminant analyses were performed on the three a priori groups: 1) tetraploids, 2) hexaploids and 3) octoploids; on all 108 individuals originating from three mixed-cytotype populations (matrix A, above). Canonical discriminant analysis (CDA, Klecka 1980) was performed to determine which characters, if any, were most useful in distinguishing hexaploid plants from assumed parental tetraploid and octoploid plants. Non-parametric classificatory discriminant analysis (Krzanowski 1990) classified individuals a posteriori into groups defined a priori on the basis of groups detected by cytological analyses; a discrimination power was determined by crossvalidation. The analyses were performed using the SAS ver. 9.1 statistical package (SAS Inst. 2007).

Pollen analyses

Pollen analyses were performed to check whether pollen fertility and/or size of pollen grains were correlated with DNA ploidy level and could be used as indicators. To estimate pollen fertility and pollen-grain size, one anther was collected from each studied plant. Anthers were selected randomly from closed tubular flowers. Pollen grains were detached and stained for 24 h in a drop of aceto-carmine jelly (Radford et al. 1974) and subsequently evaluated in an optical microscope.

Like the morphometric analyses, pollen studies were performed on plants from mixed populations in which all three cytotypes were detected (tetraploids, hexaploids and octoploids) (populations 115, 14+41 and 52; cf. Table 1, 2). Population 40 (Plavecký Mikuláš, Slovakia) was not included in these analyses due to a lack of flowering individuals.

Table 2. Sample frequencies of tetraploid, hexaploid and octoploid cytotypes of *Jacobaea vulgaris* subsp. *vulgaris* for sites at which multiple cytotypes were detected. For detailed site locations and the list of additional sites that yielded only one cytotype, see Table 1. n = the number of analysed individuals.

Population number, site locations	n	Percentage		
		Tetraploid	Hexaploid	Octoploid
115, Czech Republic, Slatinky	15	60	13	27
14, Slovakia, Štokeravská vápenka	38	97	3	0
40, Slovakia, Plavecký Mikuláš	82	51	4	45
51, Slovakia, Lehota	2	50	0	50
50, Slovakia, Demandice	21	86	0	14
49, Hungary, Pincehely	18	6	0	94
48, Hungary, Hárskút	62	2	0	98
52, Ukraine, Vikno	46	48	9	43
47, Ukraine, Demshyn	36	3	0	97

Estimation of pollen fertility

To estimate pollen fertility, all cytotypes were examined for pollen stainability. Pollen were considered fertile if the grains stained violet and were of regular shape. Up to one hundred pollen grains per individual were examined. Up to ten individuals of each cytotype (if present) were analysed per site: nine tetraploids, two hexaploids and four octoploids from population 115; ten tetraploids, one hexaploid and ten octoploids from population 14+41; and ten tetraploids, four hexaploids and ten octoploids from population 52.

Pollen-grain size

Pollen-grain size was measured on violet-stained grains of regular shape. Thirty pollen grains per plant were measured. Ten individuals of each cytotype (if present) were analysed per site, yielding a total of 29 tetraploids, 7 hexaploids and 24 octoploids. Altogether, 1143 pollen grains were measured. A Tukey–Kramer multiple comparison analysis at a probability level of $p \leq 0.05$ (Tukey test for unequal sample sizes; Zar 1999, SAS Inst. 2007) was applied to determine the differences in pollen-grain size among tetraploids, hexaploids and octoploids.

Results

Chromosome counts and DNA ploidy level estimation

Three ploidy levels of *J. vulgaris* subsp. *vulgaris* were found in the area studied: tetraploid ($2n \sim 4x \sim 40$), hexaploid ($2n \sim 6x \sim 60$) and octoploid ($2n \sim 8x \sim 80$). The tetraploid level was found in 344 plants (49.7% of all plants analysed). Octoploid plants were also common (339 plants, 48.9%) in the studied region. Ten plants (1.4%) belonged to the previously unrecorded hexaploid cytotype. A direct count gave $2n = 60$ for a hexaploid plant from population 40 (Plavecký Mikuláš, Slovakia, Fig. 2).

Of the 76 populations examined, 67 (88.2%) were completely uniform with respect to ploidy level; 38 populations (50%) were tetraploid and 29 populations (38.2%) were octoploid (Fig. 1, Table 1). Sympatric occurrence of two or even three cytotypes was observed at nine sites (11.8%). One population contained tetraploid plants together with one hexaploid plant; five populations contained tetraploids and octoploids; and three sites contained a mixture of all three ploidy levels (Table 2).

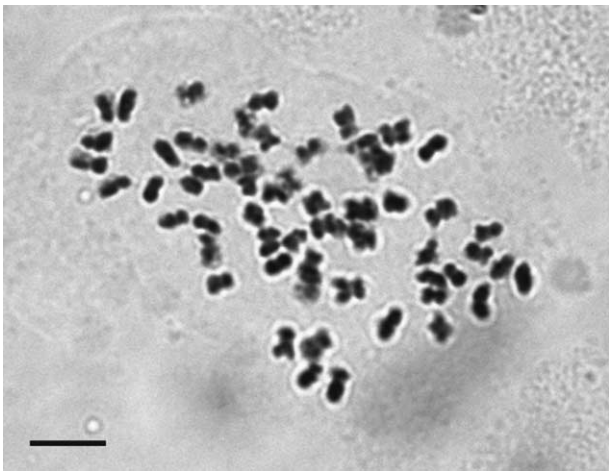

Figure 2. Mitotic metaphase of a hexaploid ($2n = 60$) individual of *Jacobaea vulgaris* subsp. *vulgaris*. Locality no. 40, Slovakia, Plavecký Mikuláš village. Scale bar = 5 μm. Photo: O. Rotreklová.

Both fresh (108 individuals: 78 tetraploids, 4 hexaploids, 26 octoploids) and silica gel-dried material (257 individuals: 135 tetraploids, 5 hexaploids, 117 octoploids) were used for flow cytometry analyses. Intra-cytotype variation of the ratio between the nuclei fluorescence intensity of each fresh sample and that of the standard did not exceed 5.3% (within each ploidy level); intra-cytotype variation in the silica gel-dried material and standard was 18.7%, 6.5% and 8.8% for tetraploids, hexaploids and octoploids, respectively. In some cases, simultaneous FCM analyses yielded histograms with clearly separated or at least bifurcated peaks, but sample CVs in such analyses were high (above 4%). On the other hand, no specimen with fluorescence intensity intermediate between tetraploids and hexaploids or between hexaploids and octoploids (indicating the presence of back-crosses) was found.

FCM analyses of fresh (living) samples mostly yielded high-resolution histograms. CVs of G_0/G_1 peaks ranged from 1.97 to 4.67% (average 3.08%) for reference standard and from 1.40 to 4.47% (average 2.89%) for *Jacobaea* samples. In accordance with the expectation, recorded histograms of desiccated leaf tissues yielded lower-resolution histograms, with average standard CV of 3.31% (range 1.83–5.08%) and average sample CV of 3.60% (range 1.29–9.91%).

Morphometric analyses

Spearman correlation coefficients

Spearman correlation coefficients based on matrix A did not reveal any highly correlated pairs of characters (exceeding the arbitrary level of 0.95) that could distort further analyses. The strongest correlation was found between length of tubular florets and length of involucral bracts (0.616) and between length of ray florets and length of involucral bracts (0.614).

Patterns of variation in mixed populations of all three cytotypes

No clear groupings were seen in the scatter plot from PCA 1 (matrix A, Fig. 3). However, an incomplete separation of tetraploids and octoploids was observed along the first axis. Hexaploid plants were placed within the bulk of octoploids, only marginally overlapping with tetraploids. Characters with the largest factor loadings for the first axis were length of ray florets, length of bracts and length of tubular florets. The number of tubular florets had the highest loading on the second axis (Table 3).

Canonical discriminant analysis (matrix A) resulted in a pattern that fully corresponded with the PCA 1 ordinations (Fig. 4). The characters that most strongly correlated with the first axis, which thus separated tetraploids from octoploids, were width and length of ray florets and length of involucral bracts. The characters most strongly correlated

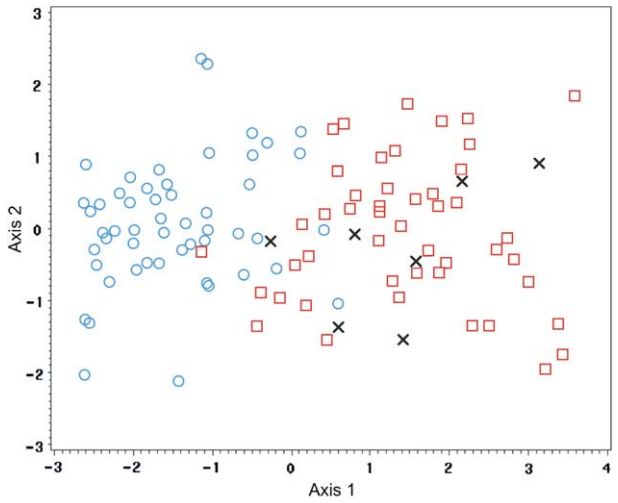

Figure 3. Principal component analysis (PCA 1) of *Jacobaea vulgaris* subsp. *vulgaris* based on 108 individuals as OTUs from three mixed-cytotype populations (115, 14+41 and 52): tetraploid individuals (circle), hexaploid individuals (symbol ×) and octoploid individuals (square). The first two axes explain 50.74% and 14.16% of total variation.

with the second axis were length of tubular florets and indument of outer achenes (Table 3).

Non-parametric classificatory discriminant analysis indicated that a posteriori placement of tetraploid individuals into the 'correct' group was relatively high and that the separation of tetraploid versus hexaploid and octoploid plants was often possible (cf. Table 4). On the other hand, the proportion of correctly classified octoploids was lower than that of hexaploids, indicating that these two cytotypes cannot be separated based on the morphological characters used in this study.

Principal component analyses of individual mixed-cytotype populations

In the Slatinky population (PCA 2, matrix B, Fig. 5a), tetraploid, hexaploid and octoploid individuals were intermingled. Nevertheless, the scatter plot showed some tendency towards separation of tetraploids and octoploids along the second axis. Tetraploids were grouped at the lower part, whereas octoploids aggregated at the upper part of the plot. One hexaploid plant was close to octoploid individuals; the second hexaploid individual was placed in the lower right corner of the diagram and clearly separated from the rest of the plants by the first axis. The characters with the highest eigenvector values for the first axis were length of tubular florets, length of ray florets and length of bracts. Those with the highest loadings on the second axis were width of ray florets and presence of hairs on the outer achenes (Table 3).

PCA 3 (matrix C, Fig. 5b) values computed for plants from the Štokeravská vápenka+Sandberg population were consistent with the PCA 1 ordination. Tetraploid and octoploid plants did not form separate groups, although some tendency for separation along the first axis was visible. Most tetraploid plants were grouped at the left side of the diagram, whereas octoploid plants were placed at the right side of the diagram. The hexaploid individual was located in the upper part of the diagram, and separated from both tetraploids and octoploids. Ray floret width and length, as well as bract length, were more strongly correlated with the first axis; tubular floret length and number were more closely correlated with the second axis (Table 3).

In the Vikno (PCA 4, matrix D, Fig. 5c) population, tetraploid and octoploid plants were clearly separated along the first principal component, although they both overlapped with hexaploid samples. Tetraploids were distinctly grouped at the left side of the plot, whereas octoploids aggregated at the right side of the plot. One hexaploid individual was located within the bulk of octoploids; two hexaploids marginally overlapped with octoploids and one with tetraploids. The characters most strongly correlated with the first principal component, and thus, separating tetraploids and octoploids, were length of bracts, length of ray florets and length of tubular florets. The characters most correlated with the second axis were width of ray florets and indument of outer achenes (Table 3).

Pollen analyses

Estimation of pollen fertility

Pollen fertility was very high in all tetraploid and octoploid plants examined, ranging from 80 to 100%. However, pollen fertility in hexaploids was relatively low, varying between

Table 3. Principal component analyses (PCA 1–4) and the canonical discriminant analysis (CDA) based on individual samples of *Jacobaea vulgaris* subsp. *vulgaris* as OTUs. In PCA 1–4 component loadings showing contribution of the characters to the principal components PC1 and PC2. PCA 1 = individuals from three mixed-cytotype populations (108 individuals; population 115, 14+41 and 52); PCA 2 = individuals originating from the Slatinky, Czech Republic population (14 individuals; populations 115); PCA 3 = individuals originating from the Bratislava, Štokeravská vápenka+Sandberg, Slovakia population (49 individuals; population 14+41); PCA 4 = individuals originating from the Vikno, Ukraine population (45 individuals; population 52). In CDA total canonical structure expressess correlation of characters with canonical axes CAN1 and CAN2. CDA = individuals from three mixed-cytotype populations (108 individuals; populations 115, 14+41 and 52). For character abbreviations, see Material and methods.

Character	PCA 1		PCA 2		PCA 3		PCA 4		CDA	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	CAN1	CAN2
Length of involucral bracts	0.457	−0.219	0.409	0.223	0.468	−0.041	0.465	0.003	0.735	0.125
Length of tubular florets	0.629	−0.116	0.324	0.735	0.452	−0.073	0.444	−0.242	0.793	0.213
Width of ray florets	0.075	0.661	0.503	−0.105	0.351	−0.608	0.378	−0.289	0.831	−0.393
Number of tubular florets	−0.065	−0.490	0.392	−0.565	0.293	0.759	0.284	0.923	0.424	−0.162
Length of ray florets	0.548	−0.101	0.445	−0.240	0.461	−0.079	0.473	−0.044	0.671	0.491
Indument of outer achenes	0.293	0.502	0.352	0.148	0.395	0.203	0.374	−0.069	0.618	0.464

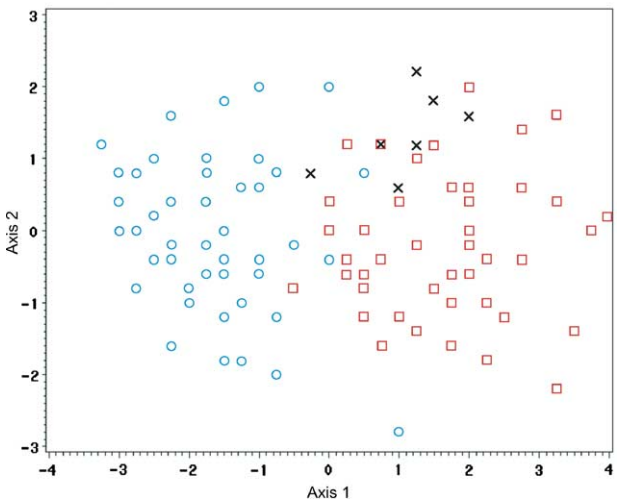

Figure 4. Canonical discriminant analysis of *Jacobaea vulgaris* subsp. *vulgaris* based on 108 individuals as OTUs originating from three mixed-cytotype populations (115, 14+41 and 52): tetraploid individuals (circle), hexaploid individuals (×) and octoploid individuals (square). First two axes explain 95.49% and 4.51% of variation among OTUs.

0–60%. In addition, hexaploid plants had considerably less pollen per sample than both tetraploids and octoploids.

Pollen grain size

Pollen grain size in tetraploid specimens from all three analysed populations varied from (22–) 23 μm to 28 (–30) μm (mean value 25.67 μm); that of hexaploid specimens varied from (23–) 27 μm to 37 (–40) μm (mean value 30.87 μm); and that of octoploid specimens ranged from (22–) 24 μm to 29 (–31) μm (mean value 26.31 μm). The Tukey–Kramer test revealed significant differences among all three ploidy levels in this character.

Discussion

Distribution, abundance and origin of the hexaploid cytotype

The major finding of this study was the presence of hexaploid individuals (ten plants in total, 1.4% of all plants analysed). Hexaploid *Jacobaea vulgaris* subsp. *vulgaris* individuals were identified in both the Pannonian and Podillya regions, where large populations of tetraploids and octoploids occur together. In other plant genera, intermediate cytotypes have been observed at moderate frequencies in mixed-ploidy populations. For example, triploid hybrids have been reported in mixed diploid-tetraploid populations of *Chamerion angustifolium* (L.) Holub (with a frequency of 2–22% within mixed-ploidy populations in North America, Soltis et al. 2007), *Galax urceolata* (Poir.) Brummitt (11% in the Blue Ridge Mountains, USA, Burton and Husband 1999) and *Empetrum nigrum* L. s. l. (2% in the Czech Republic, Suda et al. 2004).

Hexaploid plants discovered during our study probably originated from crosses between tetraploid and octoploid *J. vulgaris* subsp. *vulgaris*. This assumption is supported by the fact that hexaploid plants are found only at sites where tetraploid and octoploid individuals grow in close proximity (population 14+41) or in intermingled communities (populations 40, 52 and 115). An alternative explanation for the origin of hexaploid plants is the fusion of a non-reduced gamete from tetraploid *J. vulgaris* subsp. *vulgaris* with a normal haploid gamete. This scenario may account for (sub)population 14, in which we found one hexaploid individual among thirty-seven tetraploid plants. However, this hexaploid plant could be also explained by inflow of pollen from surrounding octoploids, as we did not karyologically analyse plants in the neighbourhood.

Both the fusion of gametes of different ploidy levels and the fusion of non-reduced and reduced gametes within the same ploidy level are repetitive processes (cf. Thompson and Lumaret 1992, Ramsey and Schemske 1998, 2002, Baack 2004, Husband 2004). Therefore, we assume that hexaploids of *J. vulgaris* subsp. *vulgaris* have originated polytopically.

The chromosome number 2n = 60 in the genus *Jacobaea*

To date, five different chromosome numbers (2n = 20, 32, 40, 80 and 120) and two additional ploidy levels (2n ~ 10x ~ 100 and 2n ~ 14x ~ 140) (given in Suda et al. 2007 as pentaploids and heptaploids, respectively) have been reported in the genus *Jacobaea* as delimited by Pelser et al. 2006, Nordenstam 2006, and Nordenstam and Greuter 2006 (cf. Bolkovskikh et al. 1969, Hodálová et al. 2007a, Suda et al. 2007, Introduction). Thus, our data represent the first 2n = 60 count of in the genus.

Morphology of the tetraploid, hexaploid and octoploid cytotypes in mixed-cytotype populations

In both mixed-cytotype Panonian populations (115 and 14+41), we observed a slight morphological differentiation between tetraploid and octoploid *J. vulgaris* subsp. *vulgaris* plants. This difference is apparent only at the population level (cf. Hodálová et al. 2007a). Therefore, it is difficult to identify tetraploid and octoploid individuals with certainty

Table 4. Results of non-parametric classificatory discriminant analysis of tetraploids, hexaploids and octoploids originating from three mixed-cytotype populations of *Jacobaea vulgaris* subsp. *vulgaris* (108 individuals; populations 115, 14+41 and 52).

Actual group	Predicted group membership: number of observations/percentage classified into groups		
	Tetraploids	Hexaploids	Octoploids
Tetraploids	47/87.04	5/9.26	2/3.70
Hexaploids	0/0.00	5/71.43	2/28.57
Octoploids	0/0.00	14/29.79	33/70.21

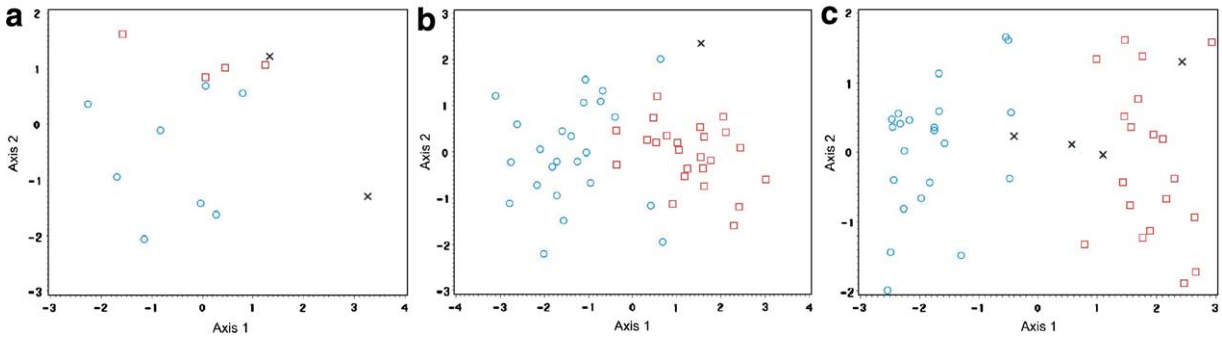

Figure 5. Principal component analyses (PCA 1–3) of *Jacobaea vulgaris* subsp. *vulgaris* based on: (a) 14 individuals from the Slatinky, Czech Republic population (no. 115, PCA 1), (b) 49 individuals from the Bratislava, Štokeravská vápenka + Sandberg, Slovakia populations (no. 14 + 41, PCA 2), (c) 45 individuals from the Vikno, Ukraine population (no. 52, PCA 3) as OTUs: tetraploid individuals (circle), hexaploid individuals (symbol ×) and octoploid individuals (square). First two axes explain: (a) 35.03% and 24.77%, (b) 44.99% and 14.78%, (c) 60.84% and 16.38% of variation among OTUs.

in the field. This pattern is not uncommon for other polyploids in zones of sympatry (Lumaret et al. 1987, Husband and Schemske 1998, Španiel et al. 2008).

Within the Podillyan population (52), tetraploid and octoploid samples form two clearly isolated clusters (cf. Fig. 5c), and the morphological difference between the cytotypes is evident at the population level as well as the individual level.

Tetraploid and octoploid cytotypes differ in mean size of floral parts. The ray florets, tubular florets and involucral bracts tend to be longer, and the ray florets tend to be wider in octoploids than in tetraploids. Moreover, octoploids have a greater proportion of hairy outer achenes than tetraploids (Fig. 6).

Hexaploid individuals are much more similar to octoploids than to tetraploids. However, hexaploids are more robust in some flower characters than either parental cytotype (Fig. 6). Similar results have been reported for *Senecio* s.s. and *Jacobaea* by Abbott and Lowe (2004) and Kirk et al. (2005), respectively. Abbott and Lowe (2004) found that average values of some characters (e.g. capitulum length, capitulum bract length, longest leaf length) in the hybridogenous species *Senecio cambrensis* Rosser and *S. eboracensis* R. J. Abbott et A. J. Lowe are significantly greater than those of either parent. Likewise, Kirk et al. (2005) showed that hybrids between *Jacobaea vulgaris* and *J. aquatica* (Hill) P. Gaertn., B. Mey. et Scherb. (given as

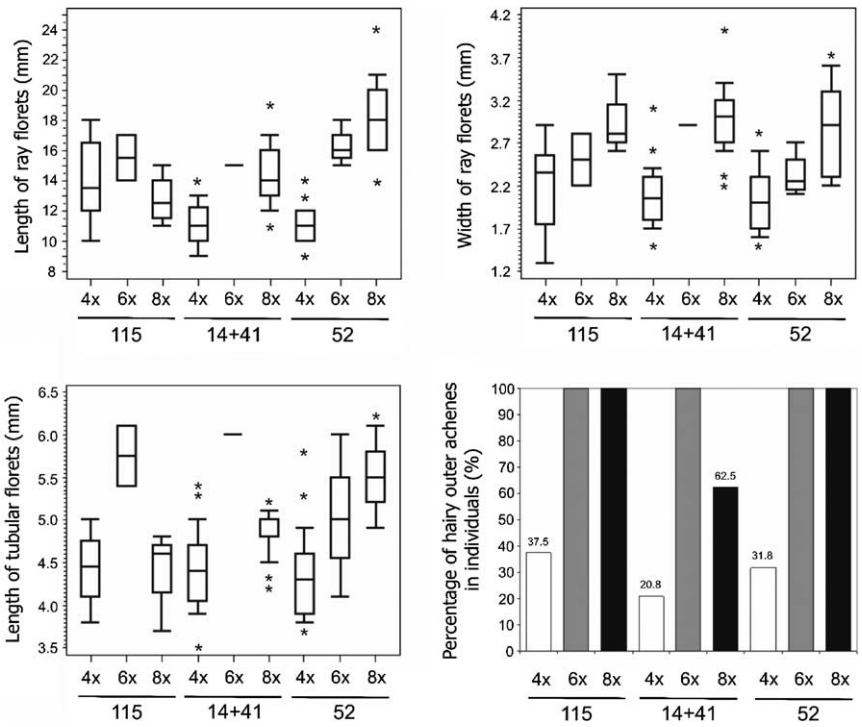

Figure 6. Variation in selected morphological characters in *Jacobaea vulgaris* subsp. *vulgaris* from three mixed-cytotype populations: 4x = tetraploid individuals, 6x = hexaploid individuals, 8x = octoploid individuals. In box-plots, rectangles define the 25th and 75th percentiles, horizontal lines show the median, whiskers are from the 10th to 90th percentiles, asterisks show extreme values. The numbers of individuals analysed per population and cytotype from left: 8, 2, 4 (population 115); 24, 1, 24 (population no. 14 + 41); 22, 4, 19 (population 52).

Senecio jacobaea L. and *S. aquaticus* Hill) produce significantly more biomass and about 25% more flowers than the parental species.

Pollen fertility and pollen grain size

Hexaploid *Jacobaea vulgaris* subsp. *vulgaris* were found to produce less pollen than the other cytotypes, and it was difficult to find enough pollen grains to estimate pollen size. Hexaploid pollen grains were typically larger than pollen grains from tetraploids or octoploids. Other authors have demonstrated similar pollen characteristics in triploids of different plant species. Burton and Husband (2000) have shown that triploid *Chamerion angustifolium* is only partially male fertile, with 15–36% stainable pollen. One potential explanation for low pollen production and larger pollen size is that hexaploids possess more unreduced pollen grains due to meiotic irregularities. As reviewed by Ramsey and Schemske (1998), unreduced pollen grains can often be identified by size, typically having a diameter 30–40% greater than that of reduced pollen. These authors also noted that the mean frequency of non-reduced gametes found in studies of hybrids is high, nearly 30%. On the other hand, the reduced gametes produced by hybrids often possess unbalanced, aneuploid cytotypes and are therefore unviable (Ramsey and Schemske 1998).

Co-occurrence of tetraploid and octoploid cytotypes

Tetraploids of *J. vulgaris* subsp. *vulgaris* are distributed throughout the species' European range. The tetraploid cytotype is the only cytotype detected in many western European countries. In contrast, octoploids are known to occur in only two areas: 1) the Pannonian Basin with adjacent parts of the Alps, Bohemian Massif and Western Carpathians, and 2) the Podillya highlands (Fig. 1). This pattern resembles those exhibited by many other groups of polyploid vascular plants (e.g. *Galax urceolata*, Nesom 1983, *Heuchera grossulariifolia* Rydb., Soltis et al. 2007), in which polyploids have smaller ranges than diploids (or lower polyploids) and different cytotypes grow in sympatry. The zone of sympatry may be only a few metres wide or may span over several hundred km. In the genus *Jacobaea*, only a few species exhibit varying ploidy levels (cf. Bolkovskikh et al. 1969). One such species is *J. carniolica* (Willd.) Schrank, in which the proportion of mixed populations reaches 28.6% in the Alps (Suda et al. 2007).

There appear to be environmental differences among sites that have led to the dominance of one cytotype over the other. Octoploid individuals predominantly grow in undisturbed and more xerophilous habitats within the lowland and colline parts of the study area. Tetraploids are common at low altitudes, but reach higher altitudes than octoploids do. Moreover, tetraploids are more often found amid ruderal vegetation. A similar situation has been observed in tetraploid ($2n = 40$) and dodecaploid ($2n = 120$) individuals (given as diploids and hexaploids, respectively) of *Jacobaea carniolica* in the eastern Alps, where the cytotypes are geographically and ecologically differentiated (Schönswetter et al. 2007, Hülber et al. 2009). Niche differentiation within mixed-cytotype sites has been documented for many polyploid complexes, including *Anthoxanthum alpinum* (Felber-Girard et al. 1996), *Dactylis glomerata* L. (Lumaret et al. 1987), *Dactylorhiza maculata* (L.) Soó s. l. (Ståhlberg 2009) and *Empetrum nigrum* s. l. (Suda et al. 2004). However, further data are needed, including detailed ecological studies to clarify the roles of environment and ecological differentiation for the local distribution pattern of *J. vulgaris* subsp. *vulgaris* tetraploids and octoploids.

Although we observed no phenological differences between *J. vulgaris* subsp. *vulgaris* cytotypes, the very low rate of hexaploid individuals detected in sympatric populations of tetraploids and octoploids (0–13%, cf. Table 2) indicates that gene flow between tetraploids and octoploids is limited. It seems likely that some prezygotic and/or postzygotic reproductive barrier may prevent the development of hexaploid *J. vulgaris* subsp. *vulgaris* plants. Potential mechanisms include ecological sorting on a micro-scale (above), reduced survival of hexaploid seedlings, or reduced fertility of hexaploids. Restricted gene flow has also been observed in *J. carniolica*, but this pattern could not be explained (Suda et al. 2007, Hülber et al. 2009).

Acknowledgements – We wish to thank Petr Bureš and Petr Šmarda (both Brno) for help with flow-cytometric analyses and for their assistance in plant cultivation, Marián Perný (Bratislava) for help with statistical analyses, Vladimir Vladimirov (Sophia) for help with the study of Bulgarian specimen of *Jacobaea aquatica*, Marek Slovák and Karol Marhold (both Bratislava) for valuable comments on the manuscript and Robert Šuvada (Brzotín) for the preparation of the base map used in the text. For help with plant collection, we are much obliged to Alexander Kagalo (L′viv), Roman Letz (Bratislava), colleagues from Medabory Reserve (Grymailiv) and other colleagues who are listed in Table 1. This work was supported by Grant Agency of Ministry of Education of the Slovak Republic and Slovak Academy of Sciences VEGA (grant no. 0026) and by the Ministry of Education, Youth and Sports of the Czech Republic, project no. MSM 0021622416. The purchase of the Partec CyFlow ML cytometer at the Inst. of Botany SAS, Bratislava, Slovakia, was partially supported as an equipment donation by the Alexander von Humboldt Foundation to Judita Lihová and Karol Marhold.

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