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Occurrence of tetraploid and octoploid cytotypes in *Senecio jacobaea* ssp. *jacobaea* (Asteraceae) in Pannonia and the Carpathians

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Four different cytotypes have been reported for *Senecio jacobaea* L. ssp. *jacobaea* throughout Europe, with the most common occurrence of tetraploids ($2n = 40$). Here we present a survey of previously published chromosome number data on this subspecies and its geographical distribution, and focus on populations from Pannonia and the Carpathians. Two ploidy levels have been determined in the study area, using chromosome counting and flow cytometry: tetraploid ($2n = 40$) and octoploid ($2n = 80$). Fifty-one populations originating from Slovakia, Austria, the Czech Republic, Hungary, Ukraine and Romania have been analysed. Multivariate morphometric analyses have been performed on 39 populations to study morphological differentiation between these two cytotypes. Despite slight morphological tendencies expressed on the level of populations, tetraploid and octoploids cannot be reliably distinguished morphologically and they are not taxonomically classified formally here.

ADDITIONAL KEYWORDS: Compositae – flow cytometry – multivariate morphometrics – polyploidy – taxonomy.

INTRODUCTION

Polyploidy and the occurrence of different ploidy levels within one species is a common phenomenon among plant groups (e.g. Soltis & Soltis, 1993; Wendel, 2000; Soltis, Soltis & Tate, 2004). Numerous reviews have been published recently devoted to classification of polyploids (auto- or allopolyploids), their ecological and evolutionary attributes, and diverse aspects of polyploid evolution. Polyploidy has been recognized as an important speciation mechanism, and with the advent of new molecular tools, its genetic consequences have also been the focus of research activities (cf. Soltis *et al.*, 2004).

Senecio jacobaea L. is an example of a karyologically variable species comprising several cytotypes throughout western Eurasia, with the eastern distribution limit at the river Lena in eastern Russia (Meusel & Jäger, 1992). It is reported as introduced in America, South Africa, Australia and New Zealand (for references see Pelser, Gravendeel & van der Meijden, 2003). *Senecio jacobaea* represents the type species of *Senecio* sect. *Jacobaea* (Mill.) Dumort. Delimitation of this section, its phylogenetic position, and relationships within and among *Senecio* sections were examined by Pelser *et al*. (2002, 2003, 2004) using nuclear and plastid DNA sequence data and amplified fragment length polymorphisms (AFLPs). *Senecio jacobaea* is an important pest in many countries owing to its toxic secondary metabolites (e.g. pyrrolizidine alkaloids); for references see Pelser *et al*. (2003) and Kirk *et al*. (2004).

Senecio jacobaea [*S. jacobaea* L. Sp. Pl. 870 (1753)] is currently recognized to comprise two subspecies,

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ssp. *jacobaea* and ssp. *dunensis* [*S. jacobaea* ssp. *dunensis* (Dumort.) Kadereit & P. D. Sell Watsonia 16: 23 (1986), Bas.: *S. dunensis* Dumort. Fl. Belg. 66 (1827)] (e.g. by Wisskirchen & Haeupler, 1998). They are distinguished mainly by the presence or absence of ray flowers (present in ssp. *jacobaea*, missing in ssp. *dunensis*). The nominate subspecies was described as occurring 'in Europae pascuis' and it is widespread throughout Europe. By contrast, *S. jacobaea* ssp. *dunensis* was described from Belgian dunes and such plants have been observed on the coast of the British Isles, on Baltic and North Sea coasts in southern Norway, southern Sweden, Germany, Poland, Denmark, the Friesian Islands, Heligoland, the Netherlands and Belgium (Kadereit & Sell, 1986).

In *S. jacobaea* the tetraploid chromosome number ($2n = 4x = 40$, Fig. 1) has been found most frequently (cf. Appendix 1). Only in Pannonia and its adjacent parts in Slovakia were both tetraploids and octoploids ($2n = 8x = 80$) found (cf. Appendix 1). Also interesting is the diploid chromosome number ($2n = 2x = 20$) reported from one locality in Bulgaria (Kuzmanov, Georgieva & Nikolova, 1979). From dunes near Kincasslagh (Ireland) the chromosome number $2n = 32$ was reported by Böcher & Larsen (1955).

Although *S. jacobaea* has been studied in recent years from various points of view, there are no comprehensive studies devoted to its karyological variation and the possibility of a correlation of ploidy level with morphology. Taking into account that two cytotypes have been reported from the Pannonian basin and adjacent regions, the question arises whether they can be distinguished morphologically and whether they should also be taxonomically classified if so. The aim of this study was (1) to summarize previously published chromosome number data on this species, (2) to explore chromosome number variation in Pannonia and the Carpathians, and (3) to compare the morphology of the common tetraploid and the less abundant octoploid cytotypes of *S. jacobaea* ssp. *jacobaea*.

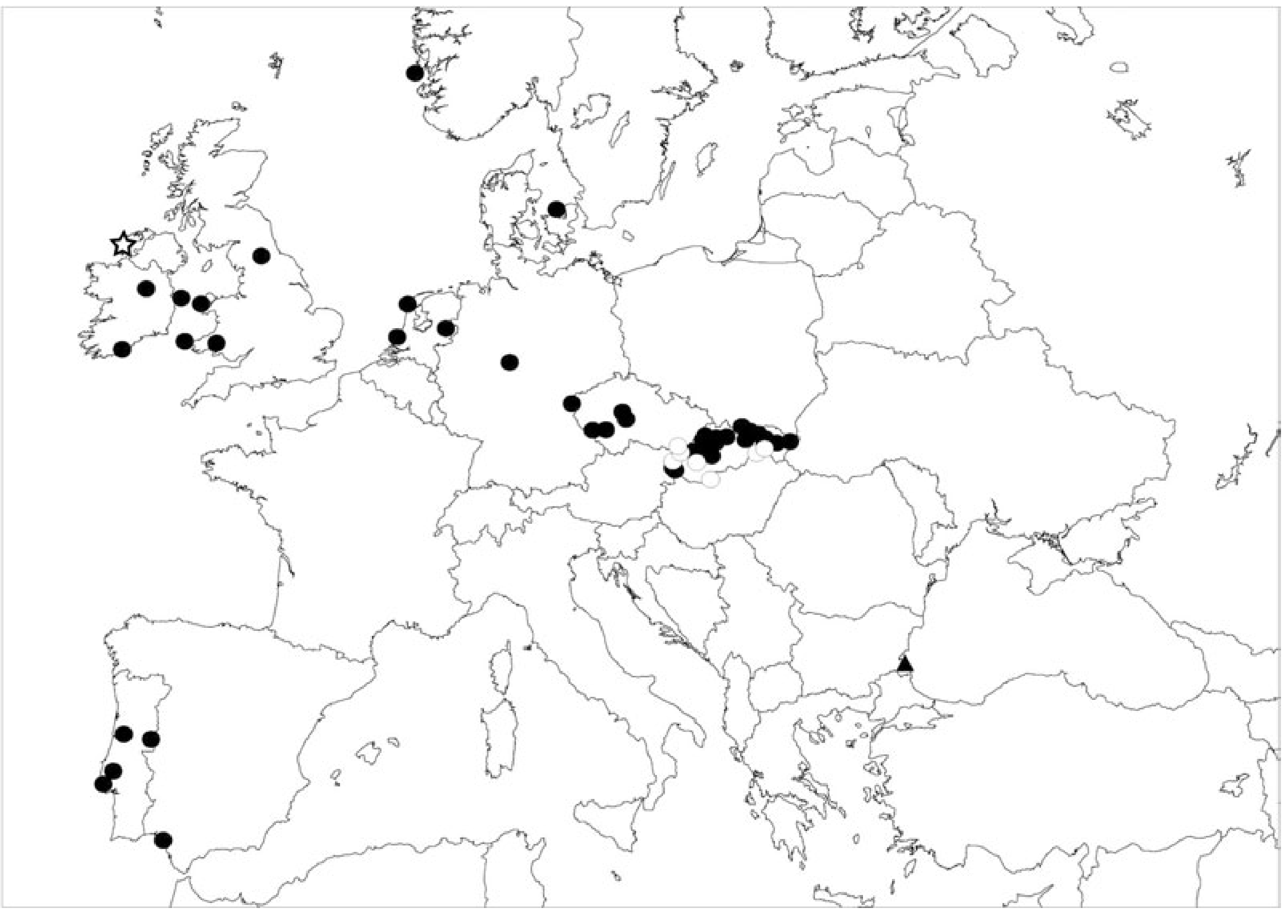

Figure 1. Distribution map of previously published data on chromosome numbers in *Senecio jacobaea* in Europe: $2n = 40$ (filled circles), $2n = 80$ (open circles), $2n = 20$ (filled triangle) and $2n = 32$ (star).

MATERIAL AND METHODS

PLANT MATERIAL

For the chromosome number survey, numerous literature sources were consulted. As a result, chromosome number data were extracted from 28 publications, which are presented in Appendix 1. For our own analyses, a total of 362 individuals of *S. jacobaea* ssp. *jacobaea* were collected from 51 populations from: (1) the Pannonian basin, the Carpathians and their adjacent areas (Austria, Moravia in the Czech Republic, Hungary, Ukraine and Romania); (2) the southern part of the Czech Republic; (3) around L'viv (Ukraine); and (4) close to the Dniestr River (Ukraine). Localities and numbers of plants analysed in morphological and cytological analyses are listed in Appendix 2. Populations from the centre of the study area, where our sampling was dense and continuous, were used for both cytological and morphometric analyses. The remaining more distant populations, or those with insufficient material for morphometric studies (Austria, the southern part of the Czech Republic, around L'viv and Dniestr River), were evaluated only cytologically. Voucher specimens are deposited in the herbarium of the Institute of Botany, Slovak Academy of Sciences, Bratislava (SAV).

CYTOLOGICAL ANALYSES

Ploidy levels of individual plants were determined by flow cytometry (FCM). Chromosome numbers were counted in root tips from individuals from two populations (1, Šumavsko-novohradské podhůří Mts, village Kájov; 2, Devínska Kobyla Mts, Bratislava – Devínska Nová Ves; both 2n = 40; cf. Appendix 2) that served as internal standards in FCM analyses.

Flow cytometry

Ploidy level was screened in a total of 49 populations of *S. jacobaea* ssp. *jacobaea*, 1–11 individuals per population. Altogether, we analysed 160 individual plants. Ploidy level was determined using a PAS (Partec GmbH, Münster, Germany) ploidy analyser. Living plants from field collections were analysed using fresh parts of leaves. A two-step procedure (Otto, 1990) was used for sample preparation. Part of a leaf, *c.* 0.5 cm^2 in size, was chopped using a razor blade in a glass Petri dish containing 0.5 mL of Otto I buffer (0.1 M citric acid, 0.5% Tween 20). The nuclei suspension was filtered through a 50-μm nylon mesh and 1 mL of Otto II buffer (0.4 M $Na_2HPO_4.12H_2O$) supplemented with 2 $\mu g\ mL^{-1}$ 4′,6-diamidino-2-phenylindole (DAPI).

Chromosome numbers

Chromosome counts were obtained from mitotic figures of meristem cells in root-tips using the squash method. The roots were pretreated in 0.002 M aqueous paradichlorbenzene for 3 h, fixed in Farmer's solution (ethanol/glacial acetic acid, 3 : 1) for 10 min to 2 h, hydrolysed for 4 min in a mixture of concentrated hydrochloric acid and ethanol (1 : 1) and rinsed in water. Squashes were made in a drop of 45% acetic acid under a cellophane square (Murín, 1960) and stained in a 10% solution of Giemsa stock dye in Sörensen phosphate buffer for 30 min.

MORPHOLOGICAL ANALYSES

From populations where both cytotypes (tetra- and octoploids) were found, only the cytologically analysed individuals were included in multivariate analyses. From other populations, all collected material was used. Morphometric analyses were based on 315 individuals (39 population samples).

Characters used

A preliminary screening of morphological characters in populations from Pannonia and the Carpathians showed that there were no differences in vegetative characters, which could separate different ploidy levels or some other, geographically defined, groups of populations. Therefore, in the presented morphometric study only characters on flowers and fruits were included. Five quantitative and one qualitative morphological characters were measured or scored for each plant: number of tubular florets (NTF), length of tubular florets (LTF), length of ray florets (LRF), length of involucral bracts (LB), number of involucral bracts (NB), and indument of outer achenes (IA): hairs present or absent. Only well-developed plants without missing characters were measured or scored.

Multivariate analyses

Multivariate numerical analyses were conducted in the following steps:

1. Principal component analyses (PCA) was used to generate a hypothesis suggesting a possible grouping of the populations studied without taking ploidy levels into account. It was based on a correlation matrix of mean values of the characters for individual populations (Sneath & Sokal, 1973; Krzanowski, 1990).
2. Exploratory data analysis was used to obtain basic statistics of quantitative characters (mean, standard deviation, 5% and 95% percentiles) for each ploidy level.
3. Discriminant analyses. Canonical (CDA) and classificatory discriminant analyses (Klecka, 1980) were used to test morphological differentiation between the ploidy levels and to identify characters contributing to that differentiation. Both CDA and

classificatory discriminant analyses were based on individuals as operational taxonomic units (OTUs). Discriminant function was determined by the cross-validation and non-parametric method, and k-nearest neighbours was adopted as the distribution of most characters deviated from the normal one.

All analyses were carried out using the SAS version 8.2 statistical package at the Charles University, Prague (SAS Institute, 2000).

RESULTS

Cytological analyses

A survey of the published chromosome numbers for *S. jacobaea* is presented in Appendix 1. In Europe, *S. jacobaea* has been analysed cytologically mainly in the western part, with a lack of information from southern (e.g. Croatia, Serbia, Montenegro, Italy, Greece) and eastern Europe (Russia). Tetraploids are commonly distributed throughout Europe, while octoploids have been reported only from the territory of Slovakia (in the Pannonian Basin and adjacent areas). The chromosome numbers $2n = 20$ and 32 are reported only from one locality in Bulgaria and Ireland, respectively (Fig. 1).

Two different ploidy levels were revealed by flow cytometry in *S. jacobaea* ssp. *jacobaea* in the study area: tetraploids ($2n \sim 4x$) and octoploids ($2n \sim 8x$) (Fig. 2). Twenty-seven tetraploid populations (Fig. 1) have been determined throughout Pannonia and the Carpathians. Eighteen octoploid populations have been identified, originating from Pannonia, some adjacent mountains of the West Carpathians and the Eastern Alps. In the sympatric zone of both cytotypes (Pannonia and adjacent parts of the West Carpathians), mixed populations (populations 48, 49, 50 and 51 in Appendix 2) were found, with both tetraploid and octoploid individuals in the same locality.

Three populations collected in the surroundings of the city of L'viv, Ukraine (nos 27, 28 and 29 in Appendix 2) and three from the southern part of the Czech Republic (nos. 1, 3 and 4 in Appendix 2) were found to be tetraploid. One population, collected in Podil's'ka Visochina close to the Dniestr River, Ukraine (no. 47 in Appendix 2), was identified as octoploid.

Multivariate analyses

Principal component analyses (PCA)

The ordination diagram of PCA (Fig. 3), based on populations as OTUs, did not show distinct groupings. The tetraploid (marked as pyramids) and octoploid (marked as spades) populations from Pannonia and the Carpathians were found to be intermingled. Nevertheless, some tendency or shift between tetraploids and octoploids along the first PC axis was visible. The following characters were correlated with the first component axis (in decreasing order of correlation): length of involucral bracts, indument of outer achenes, and length of ray florets. Length of tubular florets contributed to the second component axis and the number of involucral bracts to the third one.

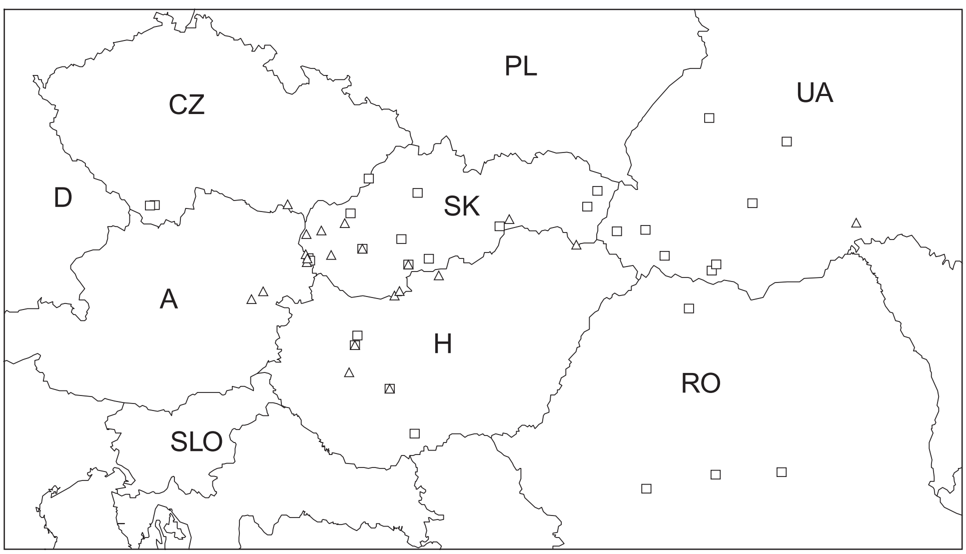

Figure 2. Distribution map of cytologically investigated populations of *Senecio jacobaea* ssp. *jacobaea* from Pannonia and the Carpathians. Squares refer to tetraploids, triangles to octoploids.

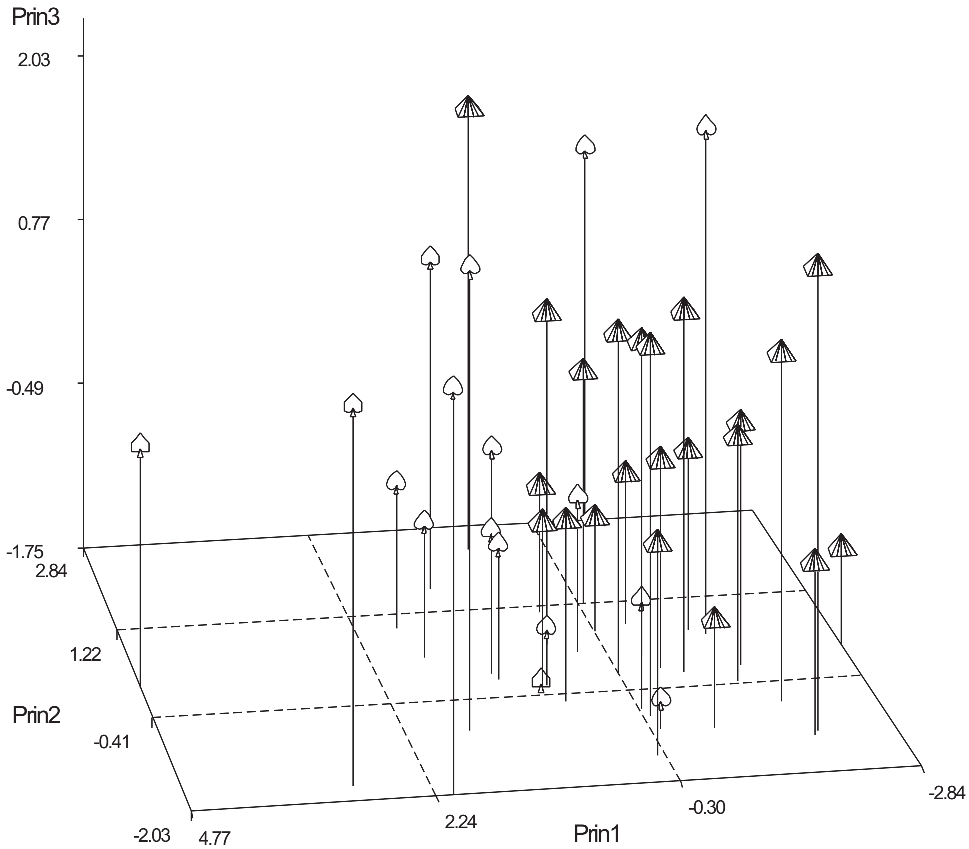

Figure 3. Ordination diagram of principal component analysis, including 39 populations as OTUs, of *Senecio jacobaea* ssp. *jacobaea* from Pannonia and the Carpathians. Tetraploids (pyramids) and octoploids (spades) are marked by symbols. The first three component axes accounted for 0.36, 0.19 and 0.16% of the variation among OTUs.

Table 1. Summary statistics for quantitative characters of *Senecio jacobaea* ssp. *jacobaea*, according to ploidy levels from Pannonia and the Carpathians

Character	Tetraploids	Octoploids
NTF (mm)	55.72 ± 8.84	58.24 ± 8.79
	42–74	46–74
LTF (mm)	4.69 ± 0.60	4.82 ± 0.55
	4.0–5.5	4.0–6.0
LB (mm)	3.97 ± 0.46	4.17 ± 0.47
	3.0–5.0	3.5–5.0
NB	12.96 ± 0.69	12.98 ± 0.62
	12–14	12–14
LRF (mm)	11.31 ± 2.41	11.98 ± 0.53
	8–16	8–17

NTF, number of tubular florets; LTF, length of tubular florets; LB, length of involucral bracts; NB, number of involucral bracts; LRF, length of ray florets. Upper line, mean + SD; lower line, fifth and ninty-fifth percentiles

Exploratory data analysis

Results of the exploratory data analysis of quantitative characters for tetraploid and octoploid populations from the Carpathians and Pannonia are given in Table 1. The ranges of the measured characters of both ploidy levels broadly overlapped. No morphological character or combinations thereof could distinguish the plants as belonging to different ploidy levels. Some tendency in differentiation could be seen only in the frequency of the occurrence of hairy and glabrous outer achenes. The percentage of hairy achenes in octoploids was up to 50.51%, whereas only 12.8% of tetraploids possessed such achenes.

Discriminant analyses

The histogram of CDA (Fig. 4) revealed a certain tendency towards separation between tetraploids on the right side of the graph and octoploids on the left side. However, a large overlap between different ploidy levels was apparent. The indument of outer achenes, length of involucral bracts and number of tubular florets were closely correlated with the canonical axis (Table 2).

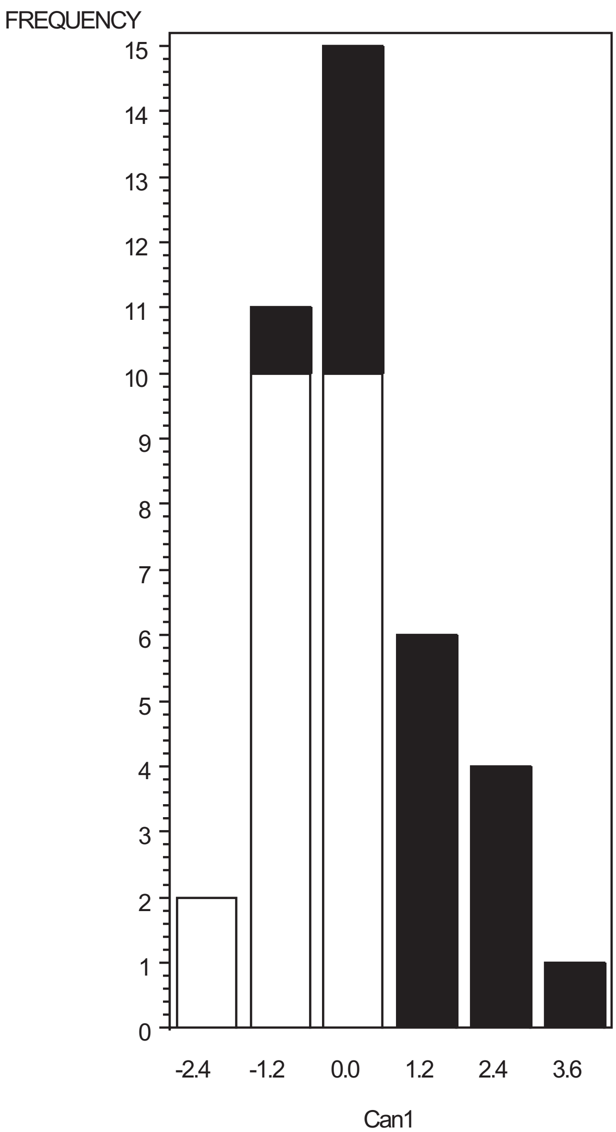

Figure 4. Histogram of canonical discriminant analysis, based on 308 individual plants as OTUs, of *Senecio jacobaea* ssp. *jacobaea* from Pannonia and adjacent parts of the Carpathians. Black, tetraploids; white, octoploids.

Non-parametric classificatory discriminant analysis showed a high percentage of incorrectly classified plants. Up to 36% of tetraploids were incorrectly classified as octoploids, and more than 31% of octoploids were assigned to tetraploids (Table 3). Given that approximately 50% of individuals would be correctly identified merely by chance, the level of correct identification based on the measured morphological characters is not much higher. This clearly indicates that it is not possible to identify different ploidy levels based on the morphological characters used in this study.

Table 2. Total canonical structure (correlation of characters with the canonical axis) obtained in the canonical discriminant analysis (CDA) of *Senecio jacobaea* ssp. *jacobaea* based on individual samples as OTUs from Pannonia and the Carpathians

Total canonical structure	
Characters	CAN 1
NTF	0.358099
LTF	0.316080
LRF	0.250014
LB	0.751095
NB	–0.004356
IA	0.867321

NTF, number of tubular florets; LTF, length of tubular florets; LRF, length of ray florets; LB, length of involucral bracts; NB, number of involucral bracts; IA, indument of outer achenes.

Table 3. Results of non-parametric classificatory discriminant analysis of tetraploids and octoploids of *Senecio jacobaea* ssp. *jacobaea* from Pannonia and the Carpathians

	Predicted group membership (no. of observations/% classified into groups)	
Actual group	Tetraploids	Octoploids
Tetraploids	136 (64.45%)	75 (35.55%)
Octoploids	31 (31.31%)	68 (68.69%)

DISCUSSION

No comprehensive taxonomic treatment of *S. jacobaea* throughout Europe has been published since the publication of *Flora Europaea*, in which only *S. jacobaea* was recognized, without any infraspecific taxa (Chater & Walters, 1976). Later, Kadereit & Sell (1986) recognized two subspecies from the British Isles, namely ssp. *jacobaea* and ssp. *dunensis* (Dumort.) Kadereit & P. D. Sell, while within ssp. *jacobaea* they further recognized var. *jacobaea* and var. *condensatus* Druce. The concept of two subspecies is now accepted widely (e.g. Wagenitz, 1987; Wisskirchen & Haeupler, 1998). *Senecio jacobaea* ssp. *dunensis* is reported from the coastal areas of northern Europe. For Central Europe no infraspecific classification has been reported for *S. jacobaea* in the recent literature (Horváth *et al*., 1995; Marhold & Hindák, 1998; Mosyakin & Fedoronchuk, 1999; Mirek *et al*., 2002; Sanda, Biţă & Barabaş, 2003).

Considerable variation in chromosome numbers is reported for *S. jacobaea* from various parts of Europe (Appendix 1): $2n = 20$, 32, 40 and 80. Most were published just for *S. jacobaea*, and without examining voucher specimens it is not possible to assign them to particular subspecies. Nevertheless, for some reports we have more precise information. Kadereit & Sell (1986) suggested that the number $2n = 32$ reported from Ireland by Böcher & Larsen (1955) could belong to var. *condensatus*, while the two reports of $2n = 40$ also reported from Ireland by Böcher & Larsen (1955) were related to var. *jacobaea* and var. *condensatus*, respectively. The number $n = 20$ (corresponding to $2n = 40$) was reported for *S. jacobaea* var. *flosculosum* DC. from the Hague in the Netherlands (Kockx-van Roon & Wieffering, 1982). This taxon was considered by Kadereit & Sell (1986) to be a synonym of *S. jacobaea* ssp. *dunensis*. Therefore, considering also that the locality of this report is within the area of *S. jacobaea* ssp. *dunensis*, we can assume that the report refers to this taxon. From the remaining part of the distribution area of *S. jacobaea*, the numbers $2n = 20$, 40 and 80 are known. The number $2n = 20$ was reported only by Kuzmanov *et al*. (1979) for *S. jacobaea* from Bulgaria (Appendix 1), and as we have not seen the voucher specimens it is difficult to comment on this number. However, as the diploid number $2n = 20$ is rare in the genus *Senecio*, more attention should be paid in future to Bulgarian populations. The number $2n = 80$ has been reported until now only from the area of Slovakia. We have confirmed this number for the whole Pannonian region and adjacent foothills of the West Carpathians and Eastern Alps, in Slovakia, the Czech Republic, Hungary and Austria (Appendix 2). In this area, however, populations with $2n = 40$ and 80 co-occur, in some cases mixed at the same locality. Our attempts to find morphological differences between these two cytotypes have generally failed to reveal any. There is only a tendency towards differentiation found at the population level when mean values of characters are considered: the indument of outer achenes, length of involucral bracts, and lengths and number of tubular florets. However, when identifying individual plants using these characters the percentage of correctly identified plants was rather low, indicating their unreliability. The reason for such a low level of morphological differentiation might be the recent autopolyploid origin of the octoploids and/or their polytopic origin. This, together with the lack of clear geographical or ecological differentiation of tetraploids and octoploids, provides arguments against their separate classification at any taxonomic level. Similar situations, where different cytotypes, without morphological, geographical and/or ecological differentiation, have remained taxonomically unclassified, are reported from e.g. *Pseudolysimachion maritimum* (L.) Á. Löve & D. Löve (Trávníček *et al*., 2004), *Cardamine pratensis* L. *s.s*. (Lihová, Tribsch & Marhold, 2003) and *C. raphanifolia* Pourr. (Perný *et al*., 2005). In neither of latter two cases did detailed molecular analyses (AFLP) support differentiation among the cytotypes. Further studies using molecular methods might shed more light on the origin of Pannonian octoploids of *S. jacobaea*. Little variation in respect of cpDNA and internal transcribed spacer sequences was found by Pelser *et al*. (2002), not only within *S. jacobaea* but also among *S. jacobaea* and related species. Following results of Pelser *et al*. (2003), other markers, particularly the fingerprinting method (AFLP), would probably be more appropriate.

Another taxonomic and evolutionary problem, which should be addressed in future, is the occurrence of octoploid plants in the region of Podil's'ka Visochina close to the Dniestr River in Ukraine. Preliminary results of morphological evaluation suggest that they are morphologically different from both tetraploid and octoploid plants occurring in the Pannonian region, the Carpathians and adjacent parts (I. Hodálová *et al.* unpubl. data). This might indicate a different taxonomic status and evolutionary history for this polyploid.

The basic chromosome number for *Senecio* is traditionally interpreted as $x = 10$. However, Lopez *et al*. (2005), in a recent study of chromosome numbers and meiotic processes in *Senecio*, supported $x = 5$ as the basic chromosome number for this genus. Thus, the $2n = 40$ number in *S. jacobaea* should perhaps be regarded as octoploid and $2n = 80$ as 16-ploids, based on $x = 5$. The diploid chromosome counts of $2n = 10$ have been found in Africa, the most probable centre of origin of *Senecio* (Ornduff *et al*., 1963; Lawrence, 1980, both cited by Lopez *et al*., 2005). According to Lopez *et al*. (2005: 473), 'the secondary cycle of polyploidy proposed by Stebbins (1971) is the best explanation for the basic number dilemma of *Senecio*, the differences of chromosome number in different continents and the lack of species with $n = 15$, 25, etc. (number of multiple of 5)'.

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APPENDIX 1

Previously published data on chromosome numbers of *Senecio jacobaea* in Europe. Most records refer to *Senecio jacobaea* ssp. *jacobaea*; only one tetraploid count from the Netherlands (marked by an asterisk) can be assigned to *Senecio jacobaea* ssp. *dunensis*. For more details see Discussion.

$2n = 20$
Bulgaria: Black Sea Coast, Burgas, Arkutino (Kuzmanov *et al*., 1979).

$2n = 32$
Ireland: Dunes near Kincasslagh, Co. Donegal [Böcher & Larsen, 1955; according to Kadereit & Sell (1986) the plants belong to *S. jacobaea* var. *condesatus*].

$2n = 40$
Belarus: Grodnenskaya oblast', Oshmyanskii raion, okr. d. Mikhalkoni, coll. Dmitrieva & Blazhevich 65, 1974, MSK; Dyatlovskii raion, okr. d. Nagorniki, coll. Tretyakov 453, 1981, MSK (both Dmitrieva, 1987). Berezinskoi biosfernoi zapovednik, okresnosti administrativnogo tsentra, na pustyre, narushennom stroitel'nymi rabotami, coll. Kozlovskaya 453, 1979, MSK (Parfenov & Dmitrieva, 1988).

Czech Republic: Louňovice pod Blaníkem, near the cemetery; Městečko NW; Radomyšl, barrage of fishpond Malducha; Běšiny, settlement Neznašovy, near the railway; Františkovy Lázně, Komorní hůrka (all Zelený, 1975).
Denmark: Lumsas (Böcher & Larsen, 1955).
France: Aubusson (Strasbourg); Plaine de Alsace alluvions vosgiennes (both Böcher & Larsen, 1955). Normandy, near the church at Mont Saint-Michel (Carr *et al*., 1999). **Germany:** Without locality (Albers & Pröbsting, 1998). Kassel (Böcher & Larsen, 1955).
Great Britain: Bowes, Barnard Castle (Morton, 1977).
Ireland: Dunes near Lady's Island Lake, Co. Wexford [Böcher & Larsen, 1955; according to Kadereit & Sell

(1986) these plants belong to *S. jacobaea* var. *jacobaea*]. Roadside near Skull Harbour, Co. Cork [Böcher & Larsen, 1955; according to Kadereit & Sell (1986) these plants belong to *S. jacobaea* var. *condesatus*]. Ben of Fore (Böcher & Larsen, 1955).
Italy: Trentino, Val di Fiemme, all cascata di Cavalese (D'Ovidio, 1984).
Norway: Without locality (Laane, 1969; as $n = 20$). Møkster, Hordaland (Böcher & Larsen, 1955).
Poland: Dursztyn; Distr. Cracow; Puszczykowo (both Böcher & Larsen, 1955). Przegorzały near Kraków; Krzeszowice; Racławice near Janów Lubelski; Łysaków; Nisko on the river San (all Pogan, Wcislo & Jankun, 1980).
Portugal: Lordelo do Ouro; Coimbra, Choupal; Oeiras (all Fernandes & Queiros, 1971). Azambuja; Monsanto (both Queiros, 1973).
Russia: Krasnoyarskii krai, Ermakovskii raion, village Grigor'ievka, coll. Stepanov, 1990, KRSU (Stepanov, 1992). Novosibirskaya oblast', Edvinskii raion, okr. village Chulym, coll. Lomonosova & Mironova 511, 1986, NS (Krasnikov, 1991).
Slovakia: Devínska Kobyla, Devín (Murín, 1987). Podunajská nížina Lowland, Bratislava, municipal part Petržalka (Uhríková, 1999b; Hindáková, 2000). Ipel'sko-rimavská brázda, Lučenec, Halier; Východoslovenská nížina Lowland, Obišovce; Považský Inovec Mts, Bezovec; Strážovské vrchy Mts, Ostrá Malenica; Strážovské vrchy Mts, Manín, Záskalie; Strážovské vrchy Mts, Bojnice; Štiavnické vrchy Mts, Lehôtka pod Brehy; Turčianska kotlina, Štiavnička; Liptovská kotlina, Liptovská Mara, Havránok; Spišské kotliny, Primovce; Spišské vrchy Mts, Nižné Ružbachy; Šarišská vrchovina Mts, Fintice; Čergov Mts, Kamenica; Nízke Beskydy Mts, Topol'ovka; Bukovské vrchy Mts, Ulič (all Murín, 1999). Vel'ká Fatra Mts, Dedošovská dolina valley (Uhríková, 1999a, 2000).
Spain: Huelva, Matalascañas (Pastor *et al*., 1990; as $n = 20$).
Sweden: Kullen (Böcher & Larsen, 1955).
The Netherlands: Utrecht, Rhenen, along the river Rhine (Gadella & Kliphuis, 1966). Friesland, E. of Oosterburen (Gadella & Kliphuis, 1968). Zd. Holland, Oostvoorne, Heveringen (Gadella & Kliphuis, 1973). Texel Island (Van Den Brand, Van Meel & Wieffering, 1979; as *S. jacobaea* L. var. *nudus* West.). *The Hague (Kockx-van Roon & Wieffering, 1982; as *S. jacobaea* var. *flosculosum* DC.; as $n = 20$; probably corresponding to *S. jacobaea* ssp. *dunensis*).

$2n = 80$
Slovakia: Slovak Karst, Turňa nad Bodvou (Murín & Váchová, 1970; Murín, 1999). Záhorská nížina Lowland, Plavecký Štvrtok (Murín, 1987). Burda Mts; Záhorská nížina Lowland, Skalica, Vintoperk; Východoslovenská nížina Lowland, Košice, Hradová; Tríbeè Mts, Mt. Zobor (all Murín, 1999).

APPENDIX 2

List of localities of the population samples of *Senecio jacobaea* ssp. *jacobaea* used for the analyses presented: number of individuals analysed by flow cytometry and number of individuals included in morphometric analyses are given in square brackets. Abbreviations of herbaria follow Holmgren, Holmgren & Barnet (1990).

$2n = 40$
Czech Republic. 1. Šumavsko-novohradské podhûří Mts, village Kájov, slope under road to the town of Český Krumlov, near the train viaduct, 550 m a.s.l., 48°49′05″N, 14°16′12″E, coll. V. Grulich, 15.ix.2002, chromosome number count by O. Rotreklová [1 plant cult. no. Z1; not used for morphological analyses].
Slovakia. 2. Devínska Kobyla Hills, Bratislava – Devínska Nová Ves, Nature Reserve Štokeravská vápenka, between the road and railway line Bratislava – Devínska Nová Ves, 160 m a.s.l., 48°12′14″N, 17°00′44″E, coll. I. Hodálová, 25.vii.2003, 24.vii.2004, 25.viii.2005, chromosome number count by I. Hodálová [3 plants cult. no. Z2, Z3, Z4; not used for morphological analyses].

$2n \sim 4x$
[internal reference: 1. *S. jacobaea*, cult. no. Z2, Z3, Z4, $2n = 4x$]

Czech Republic. 3. Šumavsko-novohradské podhůří Mts, military area Boletice, damaged village Brzotice, xerophilous places in the army shooting range, 750 m a.s.l., 48°48′47″N, 14°11′05″E, coll. V. Grulich, 9.vii.2001 [3 plants cult. nos. J6, J7, J8; not used for morphological analyses]. 4. Šumavsko-novohradské podhůří Mts, village Kájov, slope under road to the town Český Krumlov, near the train viaduct, 550 m a.s.l., 48°49′05″N, 14°16′12″E, coll. V. Grulich, 15.ix.2002 [3 plants cult. nos. J2, J3, J4; not used for morphological analyses]. 5. Bílé Karpaty Mts, village Nedašova Lhota, xerophilous places, S exp., 450 m a.s.l., 49°07′33″N, 18°05′20″E, coll. V. Grulich, 13.ix.2002 [3 plants cult. nos. J12, J13, J14; not used for morphological analyses].
Hungary. 6. Bakony Mts, town Zirc, xerophilous places, 310 m a.s l., 47°18′34″N, 17°53′07″E, coll. I. Hodálová, 2.viii.2001 [2 plants cult. nos. 13A, 13B; 11]. 7. Mecsek, village Szentkatalin, 305 m a.s.l., 46°10′23″N, 18°54′15″E, coll. I. Hodálová, 10.vii.2003 [8 plants cult. nos. 16A, 16B, 16C, 16D, 16E, 16F, 16G, 16H; 8].
Romania. 8. Munţii Gutâi, village Cavnic, towards to town of Baia Mare, 511 m a.s.l., 47°37′14″N,

23°47′57″E, coll. M. Valachovič, 17.vii.2004 [2 plants cult. nos. 25A, 25B; 1]. 9. Munţii Retezat: village Pui, 358 m a.s.l., 45°31′63″N, 23°02′19″E, coll. I. Hodálová & M. Slovák, 9.viii.2004 [1 plant cult. no. 27A; 5]. 10. Munţii Perşani, town Codlea, pastures along main road No. E68, 575 m a.s.l., 45°43′32″N, 25°27′01″E, coll. I. Hodálová, M. Perný, M. Kolník & M. Slovák, 7.viii.2004 [1 plant cult. no. 28A; 15]. 11. Munţii Baiului, village Azuga, 424 m a.s.l., 45°41′53″N, 24°16′20″E, coll. I. Hodálová, M. Perný, M. Kolník & M. Slovák, 7.viii.2004 [1 plant cult. no. 29A; 10].

Slovakia. 12. Ipel'sko-rimavská brázda, between the villages of Čelovce and Opava, xerophilous places near the road, 400 m a.s.l., 48°11′47″N, 19°09′31″E, coll. I. Hodálová, 27.vii.2001 [4 plants cult. nos. 1A, 1B, 1C, 1D; 19]. 13. Slovak Karst, town Plešivec, plateau Plešivecká planina, near the road, 210 m a.s.l., 48°34′24″N, 20°25′13″E, coll. I. Hodálová, 27.vii.2001 [5 plants cult. nos. 3A, 3B, 3C, 3D, 3E; 12]. 14. Devínska Kobyla Hills, Bratislava – Devínska Nová Ves, Nature Reserve Štokeravská vápenka, between the road and railway line Bratislava – Devínska Nová Ves, 160 m a.s.l., 48°12′14″N, 17°00′44″E, coll. I. Hodálová, 25.vii.2002 [5 plants cult. nos. 4A, 4B, 4C, 4D, 4E; 14]. 15. Devínska Kobyla Hills, Bratislava, between the municipal parts of Karlova Ves and Dúbravka, near the road, 204 m a.s.l., 48°10′30″N, 17°02′24″E, coll. I. Hodálová, 16.vii.2001 [5 plants cult. nos. 5A, 5B, 5C, 5D, 5E; 10]. 16. Malé Karpaty Mts, village Čachtice, xerophilous meadows under the quarry, 400 m a.s.l., 48°43′21″N, 17°45′41″E, coll. I. Hodálová, 17.vii.2001 [1 plant cult. no. 7A; 10]. 17. Štiavnické vrchy Mts, village Rudno nad Hronom, 230 m a.s.l., 48°25′32″N, 18°40′12″E, coll. I. Hodálová, 22.viii.2002 [2 plants cult. nos. 8A, 8B; 2]. 18. Vihorlatské vrchy Mts, camping Hôrka, 160 m a.s.l., 48°48′02″N, 21°59′06″E, coll. I. Hodálová, 27.viii.2001 [3 plants cult. nos. 9A, 9B, 9C; 11]. 19. Vel'ká Fatra Mts, village Folkušová, foot of Mt. Havranová skala, 660 m a.s.l., 48°57′36″N, 18°57′34″E, coll. M. Peniašteková, 29.vii.2001 [3 plants cult. nos. 10A, 10B, 10C; 15]. 20. Bukovské vrchy Mts, town Snina, near the road, 300 m a.s.l., 48°58′56″N, 22°09′55″E, coll. I. Hodálová, 28.vii.2001 [2 plants cult. nos. 11A, 11B; 11]. 21. Bukovské vrchy Mts, between the town of Snina and village of Stakčín, near the road, 280 m a.s.l., 48°58′56″N, 22°09′55″E, coll. I. Hodálová, 28.vii.2001 [1 plant cult. no. 12 A; 1].

Ukraine. 22. Zakarpats'ka oblast', town Mukačeve, 142 m a.s.l., 48°30′52″N, 22°30′45″E, coll. I. Hodálová & M. Kolník, 28.vii.2004 [4 plants cult. nos. 17A, 17B, 17C, 17D; 7]. 23. Zakarpats'ka oblast', town Svalyava, 205 m a.s.l., 48°31′54″N, 23°01′14″E, coll. I. Hodálová, M. Perný & M. Kolník, 27.vii.2004 [4 plants cult. nos. 18A, 18B, 18C, 18D; 1]. 24. Zakarpats'ka oblast', village Iza, 194 m a.s.l., 48°13′54″N, 23°21′49″E, coll. I. Hodálová, M. Perný & M. Kolník, 27.vii.2003 [3 plants cult. nos. 19A, 19B, 19C; 6]. 25. Zakarpats'ka oblast', town Rahiv, 320 m a.s.l., 48°03′37″N, 24°12′15″E, coll. I. Hodálová & M. Perný, 26.vii.2004 [2 plants cult. nos. 20A, 20B; 9]. 26. Zakarpats'ka oblast', village Kvasi, 350 m a.s.l., 48°07′54″N, 24°17′02″E, coll. I. Hodálová & M. Perný, 26.vii.2004 [1 plant cult. no. 21A; 10]. 27. L'vivs'ka oblast', village Vinnyki, 217 m a.s.l., 49°49′35″N, 24°09′38″E, coll. I. Hodálová, E. Michalková & A. Kagalo, 21.vii.2003 [3 plants cult. nos. 22A, 22B, 22C; not used for morphological analyses]. 28. L'vivs'ka oblast', Zolochiv, N exp., 290 m a.s.l., 48°50′22″N, 24°55′50″E, coll. M. Valachovič, 25.vii.2004 [3 plants cult. nos. 23A, 23B, 23C; not used for morphological analyses]. 29. Ternopil'ska oblast', town Ternopil', 222 m a.s.l., 49°33′15″N, 25°32′27″E, coll. I. Hodálová, E. Michalková & A. Kagalo, 22.vii.2003 [3 plants cult. nos. 24A, 24B, 24C; not used for morphological analyses].

$2n \sim 8x$

Austria. 30. Eastern Alps, village Sieding, relic vegetation with *Pinus nigra*, 450 m a.s.l., 47°43′46″N, 15°59′48″E, coll. I. Hodálová & J. Somogyi, 24.vii.2002 [4 plants cult. nos. S2, S3, S4, S5; not used for morphological analyses]. 31. Steinfeln feld, Wiener Neustadt, *c*. 500 m of the town Wiener Neustadt, near the road, *c*. 300 m a.s.l., 47°49′08″N, 16°12′15″E, coll. I. Hodálová & J. Somogyi, 24.vii.2002 [3 plants cult. nos. S7, S8, S9; not used for morphological analyses]. 32. Hainburger-berge, town Hainburg, Mt. Braunsberg, plateau on the top, 480 m a.s.l., 48°09′30″N, 16°58′56″E, coll. I. Hodálová & J. Somogyi, 14.vii.2002 [3 plants cult. nos. S11, S12, S13; not used for morphological analyses].

Czech Republic. 33. Pavlovské vrchy Mts, town Mikulov, Nature Reserve Kočičí skála, xerophilous places, 340 m a.s.l., 48°49′39″N, 16°38′30″E, coll. I. Hodálová, 10.ix.2002 [2 plants cult. nos. 30A, 30B; 1].

Hungary. 34. Lake Balaton, town Zánka, ruderalized xerophilous places, 150 m a.s.l., 46°52′54″N, 17°44′10″E, coll. I. Hodálová, 29.vii.2002 [1 plant cult. no. 45A; 1]. 35. Cserhat Mts, village Keszeg, 318 m a.s.l., 48°00′12″N, 19°20′11″E, coll. I. Hodálová, 11.vii.2003 [3 plants cult. nos. 47A, 47B, 47C; 5].

Slovakia. 36. Slovak Karst, village Krásnohorské Podhradie, castle Krásna Hôrka, xerophilous places, 440 m a.s.l., 48°39′20″N, 20°35′42″E, coll. I. Hodálová, 12.vii.2003 [6 plants cult. nos. 32A, 32B, 32C, 32D, 32E, 32F; 6]. 37. Záhorská nížina Lowland, Devínske Jazero, cottage settlement, near the railway line Bratislava – Malacky, 168 m a.s.l., 48°14′57″N, 16°57′54″E, coll. I. Hodálová, 16.vii.2001 [2 plants cult. nos. 33A, 33B; 1]. 38. Záhorská nížina Lowland, Devínske Jazero, cottage settlement, sands, 170 m a.s.l., 48°14′57″N, 16°57′54″E, coll. I. Hodálová, 16.vii.2001 [3 plants cult. nos. 34A, 34B, 34C; 18]. 39.

Záhorská nížina Lowland, Gajary, fish-pond Starý Rybník, 150 m a.s.l., 48°28′57″N, 16°58′32″E, coll. I. Hodálová, D. Dítě & J. Somogyi, 7.viii.2002 [2 plants cult. nos. 35A, 35B; 6]. 40. Záhorská nížina Lowland, Plavecký Mikuláš, gamekeeper lodge Haluška, sands, 195 m a.s.l., 48°31′27″N, 17°14′36″E, coll. I. Hodálová, 10.viii.2001 [5 plants cult. nos. 36A, 36B, 36C, 36D, 36E; 10]. 41. Devínska Kobyla Hills, Bratislava, National Nature Reserve Devínska Kobyla – Sandberg, sands, 220 m a.s.l., 48°12′02″N, 16°59′28″E, coll. I. Hodálová, 25.vii.2002 [6 plants cult. nos. 37A, 37B, 37C, 37D, 37E, 37F; 7]. 42. Podunajská nížina Lowland, Senec, near the highway between the towns of Bratislava and Trnava, 127 m a.s.l., 48°14′26″N, 17°25′03″E, coll. I. Hodálová, 22.viii.2002 [2 plants cult. nos. 38A, 38B; 1]. 43. Podunajská nížina Lowland, settlement Čenkov, sands, 100 m a.s.l., 47°46′14″N, 18°32′51″E, coll. I. Hodálová, 15.vii.2001 [1 plant cult. no. 40A; 9]. 44. Podunajská nížina Lowland, town Štúrovo, Nature Reserve Vŕšok, xerophilous places, 330 m a.s.l., 47°49′23″N, 18°38′11″E, coll. I. Hodálová, 15.vii.2001 [3 plants cult. nos. 41A, 41B, 41C; 8]. 45. Východoslovenská nížina Lowland, Nature Reserve Tarbucka, xerophilous places, 243 m a.s.l., 48°21′35″N, 21°47′21″E, coll. I. Hodálová & M. Valachovič, 17.vii.2002 [2 plants cult. nos. 42A, 42B; 19]. 46. Malé Karpaty Mts, between the villages of Dolný Lopašov and Lančár, quarry Lančár, xerophilous places, 290 m a.s.l., 48°36′31″N, 17°39′30″E, coll. I. Hodálová, 17.vii.2001 [4 plants cult. nos. 43A, 43B, 43C, 43D; 12].

Ukraine. 47. Hmel'nitska oblast', the Dniestr River Basin, forest steppe above the canyon of the Dniestr River, near the village Demshin, 268 m a.s.l., 48°36′52″N, 26°46′52″E, coll. I. Hodálová, E. Michalková & A. Kagalo, 23.vii.2003 [4 plants cult. nos. 48A, 48B, 48C, 48D; not used for morphological analyses].

Mixed populations with $2n \sim 4x$ and $2n \sim 8x$ individuals

Hungary. 48. Bakony Mts, village Hárskút, damage xerophilous places, 420 m a.s.l., 47°11′49″N, 17°50′20″E, coll. I. Hodálová, 2.viii.2001 [$2n \sim 4x$, 1 plant cult. no. 14A; 1; $2n \sim 8x$, 11 plants cult. nos. 44A, 44B, 44C, 44D, 44E, 44F, 44G, 44H, 44I, 44 J, 44K; 11]. 49. Hegyhat Mts, between the villages of Simontornya and Pincehely, sands, 230 m a.s.l., 46°41′34″N, 18°27′34″E, coll. I. Hodálová, 30.vii.2002 [$2n \sim 4x$, 1 plant cult. no. 15A; 1; $2n \sim 8x$, 5 plants cult. nos. 46A, 46B, 46C, 46D, 46E; 5].

Slovakia. 50. Ipel'sko-rimavská brázda, village Demandice E, 150 m a.s.l., 48°07′51″N, 18°47′34″E, coll. I. Hodálová, 15.vii.2002 [$2n \sim 4x$, 3 plants cult. nos. 2A, 2B, 2C; 2; $2n \sim 8x$, 3 plants cult. nos. 31A, 31B, 31C; 1]. 51. Podunajská nížina Lowland, village Lehota, near the highway between the towns of Bratislava and Nitra, 232 m a.s.l., 48°18′48″N, 17°58′27″E, coll. I. Hodálová, 22.viii.2002 [$2n \sim 4x$, 1 plant cult. no. 6A; 1; $2n \sim 8x$, 1 plant cult. no. 39A; 1].