

Ecological niche differentiation between tetra- and octoploids of *Jacobaea vulgaris*

Ekologická diferenciace tetraploidů a oktaploidů druhu *Jacobaea vulgaris*

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Polyploid speciation is an ongoing, important source of angiosperm diversity. However, the ecological differences between polyploids and their lower-ploid progenitors remain poorly understood. Here we explore patterns in the distributions of three *Jacobaea vulgaris* ploidy levels (4x, 6x and 8x) in Slovakia, which involved sampling at 203 sites and collecting information on the ploidy levels/chromosome numbers of 1023 individuals. For a subset of sites (171), we analysed the ecological differentiation between the two major ploidy levels, the tetra- and octoploids, which are recognized as separate subspecies, *J. vulgaris* subsp. *vulgaris* (2n = 4x = 40) and its autopolyploid derivative *J. vulgaris* subsp. *pannonica* (2n = 8x = 80). At most of the sites sampled (89.7%) only one ploidy level (subspecies) was recorded. Only 1.4% of the plants analysed were of the minority (6x) ploidy level and they occurred only together with plants of other ploidy level(s). The two major ploidy levels (subspecies) occurred in slightly different environments based on the 123 environmental variables studied. Separation of ploidy levels was mostly associated with type of habitat, habitat naturalness, geology, altitude, precipitation and temperature. In contrast to tetraploids, octoploids are restricted to warm and dry locations at low altitudes and in areas little affected by man. Despite the ecological niche separation between tetra- and octoploids along habitat and climatic gradients, and the more widespread distribution of tetraploids in the area studied, the ecological niche breadths of the two ploidy levels in Slovakia do not differ significantly.

Key words: autopolyploidy, niche, secondary contact zone, *Senecio jacobaea*, Slovakia, species distribution modelling

Introduction

Genome multiplication (polyploidization) is a common phenomenon in flowering plants and an important mechanism for the origin of evolutionary novelties and maintenance of diversity in plant populations (Buggs et al. 2010, 2012, Vanneste et al. 2014). Of the two basic types of polyploidy, autopolyploidy and allopolyploidy, the latter is recognized as

an important speciation mechanism (Rieseberg & Willis 2007). However, increasing evidence indicates that autopolyploids are much more common than traditionally anticipated and that genome multiplication per se (without hybridization) may result in an evolutionary advantage (Parisod et al. 2010, Arrigo & Barker 2012).

Despite intensive studies on the ecology of polyploids in recent years (Godsoe et al. 2013, Hanzl et al. 2014, Kolář et al. 2014, Otisková et al. 2014), the environmental factors affecting the distribution of polyploid lineages are still insufficiently known (Soltis et al. 2010, Weiss-Schneeweiss et al. 2013). Several polyploids occupy broader ecogeographical and climatic niches¹ than their lower-ploid progenitors (Burnier et al. 2009, Treier et al. 2009). However, results of recent surveys provide evidence that there is no consistent trend in the ecogeographical patterns of polyploids, and all three possible processes, including niche expansion, niche conservatism and niche contraction, are recorded for polyploids (te Beest et al. 2012, Weiss-Schneeweiss et al. 2013, Glennon et al. 2014, Harbert et al. 2014). Moreover, neither the mechanisms nor the timing of such divergence among cytotypes are easy to identify, and in many cases, we do not know whether the changes that are associated with polyploidy are due to polyploidization or adaptive changes that have evolved since polyploid formation.

An ecoinformatic approach, based on Geographical Information Systems (GIS; Kozak et al. 2008), along with genetic, karyological and morphological analyses, could help in studies of diploid-polyploid relationships. This approach includes e.g. multivariate analyses of a large number of niche variables and ecological (or climatic) niche modelling (Guisan & Zimmermann 2000, Broennimann et al. 2012). Using these methods it is possible to describe and compare (ecological/climatic) niches or model potentially available geographical ranges; they have recently been successfully used to investigate broad-scale patterns in niche overlap and niche breadth in several diploid-polyploid complexes (e.g. McIntyre 2012, Oberprieler et al. 2012, Godsoe et al. 2013, Theodoridis et al. 2013, Glennon et al. 2014, Harbert et al. 2014). Although such tests cannot replace classical reciprocal transplant experiments and the collecting of vegetation and environmental data in situ, they may provide a better understanding of the role of environmental (including climate) factors in the establishment and persistence of polyploids (Glennon et al. 2014).

One of the polyploid complexes occurring in Europe with supposed ecogeographical segregation of ploidy levels is *Jacobaea vulgaris* Gaertn., which belongs to the family *Asteraceae* (Hodálová et al. 2015). There are two major (main) ploidy levels of this species in central Europe, tetraploid ($2n = 40$) and octoploid ($2n = 80$), and one minority (secondary) ploidy level, hexaploid ($2n = 60$). The tetra- and octoploid plants of *J. vulgaris* differ greatly both genetically and morphologically and are also apparently largely reproductively isolated. Although not yet investigated in detail, the distributions of tetra- and octoploids of *J. vulgaris* seem to indicate differences in habitat preferences. Octoploid populations are recorded mostly in natural or seminatural xerothermic habitats, while tetraploids occupy natural, seminatural and man-made habitats, not only at xerothermic, but also mesophilous sites (Hodálová et al. 2010, 2015).

¹ In this paper we use the term “climatic niche” for the set of climatic conditions (incl. parameters of solar energy) in which (sub)species or cytotypes currently occur, while “ecological niche” includes a wider set of conditions, that include, apart from climatic characteristics, habitat, geological and topographic parameters.

The present study provides detailed descriptions of the distributions, and the ecological and climatic niches of plants of *J. vulgaris* of two major (main) ploidy levels in a secondary contact zone in Slovakia. The following two questions were addressed: (i) Do the distributions of the plants of different ploidy levels of *J. vulgaris* (tetra-, hexa- and octoploids) in Slovakia differ? (ii) Do the tetra- and octoploid populations within the area studied differ ecologically?

Materials and methods

Study species

Jacobaea vulgaris (syn. *Senecio jacobaea* L.) is a biennial or short-lived perennial, cross-pollinated herbaceous plant that occurs in various open habitats, such as primary or secondary grasslands, sandy areas, roadsides and abandoned man-made sites, rarely in open forests, from sea level to the montane vegetation belt. This species is native to Eurasia and has been introduced into North and South America, South Africa, Australia and New Zealand (Bain 1991). It is known for its high content of toxic pyrrolizidine alkaloids (e.g. Macel et al. 2004, Pelser et al. 2005, Rapo et al. 2010), high rates of spread in parts of its distribution area, and significant effect on the economy due to a negative impact on livestock production and crop and forage yields (Bain 1991).

While all tetraploid plants can be assigned to the type subspecies, *J. vulgaris* subsp. *vulgaris* octoploids are of two different monophyletic evolutionary lineages, both of autopolyploid origin (Hodálová et al. 2015). The octoploid plants occurring in the Pannonian region (including adjacent parts of the Eastern Alps, the Bohemian Massif and Western Carpathians) and, with some reservations, also on the Swedish island of Öland, belong to the recently described *J. vulgaris* subsp. *pannonica* Hodálová et Mereďa (Hodálová et al. 2015). The second octoploid lineage consists of plants on the Swedish island of Gotland and in the Ukrainian Podillya (Podolian) region and can be assigned to the subspecies *J. vulgaris* subsp. *gotlandica* (Neuman) B. Nord. However, Wysk et al. (2009) and Conti et al. (2012) report that morphotypes close to *J. vulgaris* subsp. *gotlandica* (or octoploids, respectively) may occur also in central Italy, Macedonia, Greece and western Russia.

Study area

The present research focused on Slovakia, which is characterized by considerable heterogeneity of relief, bedrock and climate. As a consequence of its position in the centre of Europe, its flora comprises a mixture of several biogeographical influences, e.g. central-European, (sub)alpine, boreal, Pontic, (sub)Atlantic, subcontinental and (sub)Mediterranean (Hendrych 1984). In spite of a large proportion of the area being relatively undisturbed, long-lasting effect of humans is another important factor that is responsible for the heterogeneity in this country (Ložek 1973, Krippel 1986, Michalko et al. 1986).

Northern and central parts of Slovakia are covered by the orographic system of the Western Carpathians with one of the largest continuous mountain forest ecosystem in Europe. In southern Slovakia is the Pannonian Plain, a large plain in eastern-central Europe that consists of the Great Hungarian Plain (including the Východoslovenská nížina lowland in the eastern part of Slovakia) and the Little Hungarian Plain (including

the Podunajská nížina lowland in the south-western part of Slovakia). The geological structure of Slovakia includes a mixture of igneous (mainly andesite, basalt and granitic rocks), sedimentary (limestone, loess, dolomite, quartzite, sand) and metamorphic rocks (gneiss, phyllite, shale). The largest variety of rocks is located in the Western Carpathians, which are classified according to their age into two main groups: the Outer and the Inner Western Carpathians; the boundary between them marks the Klippen belt, stretching in the form of a narrow northward bulging belt (up to 600 km). The Outer Western Carpathians have a predominantly flysch-like character; the Inner Western Carpathians consist of ancient crystalline and metamorphic cores into which younger sedimentary rocks (for the most part limestones and dolomites) have been over thrust; the Klippen belt consists mainly of two types of rock complexes: limestones and various marlstones, shales and flysch sediments (Plašienka et al. 1997, Káčer 2005). The climate in Slovakia is relatively continental, with the colder northern (the Western Carpathians, mostly mountainous) and warmer southern (northern Pannonia, mostly lowland) regions, influenced by dry continental air from the east and humid ocean air from the west. The range in average annual temperature in the mountain zone is approximately -3 to 4°C and the annual rainfall is between 800 to 2000 mm; the range in average annual temperature in the lowland zone is approximately 8 to 11°C and annual rainfall is between 500 to 750 mm (for details see www.slovak-republic.org/weather).

Plant material

In the course of the current study, the DNA ploidy levels of 659 individual plants of *Jacobaea vulgaris* collected from 159 populations in Slovakia were determined. In order to obtain a complete picture of the distributions of *J. vulgaris* of different ploidy levels in Slovakia, the new information was combined with ploidy level data (incl. chromosome counts) for 338 individuals (30 populations) collected in Slovakia during our previous studies (Hodálová et al. 2007, 2010, 2015) and chromosome counts reported for plants collected at 26 localities in Slovakia by other authors (Electronic Appendix 1). In total, information on the ploidy level (incl. chromosome count) of 1023 individual plants of *J. vulgaris* (215 populations from 203 sites) was used to determine the distributions of *J. vulgaris* plants of different ploidy levels in the area studied. Due to the rare occurrence of hexaploids (Hodálová et al. 2010, 2015), the results for this ploidy level were not included in the analyses of ecological and climatic niches.

The sampling was designed to include as much detail as possible of the distribution of the octoploid *J. vulgaris* subsp. *pannonica* in Slovakia. Consequently, we carried out detailed sampling principally in xerothermic habitats that were located in/near the transition zone between the Pannonian and Carpathian biogeographical regions. A more detailed sampling was also carried out in the Liptovská kotlina and Spišské kotliny basins, where we previously reported rare occurrences of *J. vulgaris* octoploids (Hodálová et al. 2015).

The number of plants per sampling site (locality) analysed reflected the abundance of *J. vulgaris* and the morphological variation of the individuals (in order to sample all of the ploidy levels present). Thus, a larger number of individuals were collected at those sites, where different morphotypes occurred. When there were two or more ploidy levels at a site, the individuals were divided into separate populations based on their ploidy levels

for further analyses (4x, 8x or 6x; at a total of 22 sites, cf. Electronic Appendix 2). Thus, the total number of populations studied was somewhat larger than the number of sites sampled. The populations studied also included the type locality of *J. vulgaris* subsp. *pannonica* (pop. 41; Hodálová et al. 2015).

Details on the origin of the material included in this study are given in Electronic Appendix 2 (see also Fig. 1). Voucher specimens are deposited in the herbarium SAV.

Ploidy level analyses

The DNA ploidy levels were estimated using silica-gel dried leaf tissue and flow cytometry (FCM). First, samples of reference plants with known chromosome numbers ($2n = 40$ and 80 , cf. Hodálová et al. 2010) were analysed simultaneously with the internal DNA reference standard [*Glycine max* (L.) Merr. 'Polanka', $2C$ DNA = 2.50 pg; Doležel et al. 1994, or *Bellis perennis* L., $2C$ DNA = 3.38 pg; Schönschwetter et al. 2007], and the ratio of their G_0/G_1 peak positions was recorded. Then, the DNA ploidy levels of plants of unknown chromosome number were analysed and their peak positions relative to that of the DNA reference standard were assessed. The sample preparation and FCM procedure followed that of Hodálová et al. (2010).

Environmental characteristics of the sites sampled

Environmental variables were recorded at the sites of 181 populations (171 sites sampled; Electronic Appendix 2); of these, 144 were sites of populations of the tetraploid *J. vulgaris* subsp. *vulgaris* and 37 populations of the octoploid *J. vulgaris* subsp. *pannonica*. This dataset does not include populations (localities) for which there is information on their chromosome counts reported by other authors in Slovakia, or for localities of several of our previously published records for which there is no accurate environmental data. For each population, we recorded 123 environmental variables describing the habitat, geology, topography and climate (incl. parameters of solar energy; Electronic Appendix 3).

Initially, the habitat of each population was investigated in the field, and two variables (habitat type and habitat naturalness) were recorded at each site sampled. The type of habitat was assessed using the EUNIS habitat classification (Davies et al. 2004). Because of the low frequency of some habitats in the sample, the habitats were categorized as one of five common types of habitat: meadow/pasture, steppic grassland, thermophile fringe, sand dune/steppe and forest. The correspondence between our 5-degree classification and EUNIS habitat classifications is explained in Electronic Appendix 2. Then, the populations were each assigned to one of three categories of habitat naturalness (low, medium or high) based on the degree of the anthropogenic effect: low habitat naturalness – vegetation strongly influenced or created by humans, with a high percentage (more than 15% in terms of abundance) of ruderal or non-native species (e.g. intensively managed or disturbed grasslands, abandoned fields); medium habitat naturalness – seminatural vegetation with a small percentage (up to 15% in terms of abundance) of ruderal or non-native species (e.g. grazed grasslands, road margins, steppe grasslands in the close proximity of houses); high habitat naturalness – natural and seminatural vegetation with little anthropogenic effect and absence or a very low percentage of ruderal or non-native species (e.g. steppe grasslands far from houses, natural forests).

For each population, we determined the geological substrate and estimated three topographical variables: altitude, exposure and inclination. The geological substrate was identified using the Digital geological map of the Slovak Republic at a scale 1 : 50000 (Káčer 2005). Because of the low frequency of some geological features in some areas, the geology recorded was that of one of 12 common geological units: granites and gneisses, andesites, loam sediments, shales, loesses, sandy sediments, flysch, calcareous flysch, quartzites, limestones, dolomites and travertines. The correspondence between our 12-degree classification and that of Káčer (2005) is explained in Electronic Appendix 4. The altitude was estimated using maps at a scale of 1 : 50000 issued by the Vojenský kartografický ústav [Cartographical Institute of the Ministry of Defence] Harmanec, Slovakia (for details of the available maps see www.vku.sk). The exposure and inclination were calculated using a digital elevation model (DEM) that was provided by the Geodetic and Cartographic Institute, Bratislava (Geodetic and Cartographic Institute 2004). The model has a resolution of 10 m and was created from topographic maps at a scale of 1:10,000. We derived the exposure and inclination using the module `r.slope.aspect` in the Geographic Resources Analysis Support System (GRASS) of the Geographic Information System (GIS) environment version 7.1, which was released under the GNU/GPL licence. For the purpose of the analyses, aspect values were linearized and rescaled to range from 0 (south) to 4 (north).

In addition, another dataset, which included 26 climatic variables based on the monthly and yearly sums of the rainfall vertical atmospheric precipitations (variables no. 7–19) and monthly and yearly average air temperatures (variables no. 20–32) for the period from 1961–1990, which is defined by the World Meteorological Organization as the period best describing the climate in the 20th century (World Meteorological Organization 2011), was obtained from the Slovak Hydrometeorological Institute. Data on variables no. 7–32 are based on a combination of the records of meteorological and precipitation stations and the predictions of an atmospheric model based on pluviometric gradients. We were able to obtain data with a resolution of 500 m horizontally and approximately 100 m vertically.

Finally, we calculated climatic variables that combined several parameters of solar energy (variables no. 33–123). The monthly average values were calculated from data recorded when the sun was above the horizon. The clear-sky irradiance was calculated using the `r.sun` routine as implemented in GRASS GIS. This module uses equations of solar energy transmission based on Krcho (1965), which were later improved and published in the European solar radiation atlas (ESRA) of solar geometry (Scharmer & Grief 2000). All these variables are in $\text{Wh}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ with a time increment of three minutes and a horizontal resolution of 10×10 m. We used the Linke atmosphere turbidity factor (day average, resolution one km, Joint Research Centre EU; available on re.jrc.ec.europa.eu/pvgis/apps4/pvest.php), ground albedo coefficient (day average, resolution 0.5 km, Modis; NASA Land Processes Distributed Active Archive Center 2000–2012) and took into consideration the barrier and shadowing effect of the local topography. This results in high resolution simulations of regional climatic events. From the daily sum, we calculated the monthly sum and from them the yearly sum. Parameters of solar energy included: the monthly and yearly sum of insolation time of the beam (direct) solar radiation (in hours, variables no. 33–45), monthly and yearly sum of the beam (direct) solar radiation (variables no. 46–58), monthly and yearly sum of the diffuse solar radiation (variables no. 59–71), monthly and

yearly sum of the reflected solar radiation (variables no. 72–84), monthly and yearly averages of the solar incidence angle for a point on an inclined plane (variables no. 85–97), monthly and yearly averages of the delta of solar incidence angle (variables no. 98–110), and monthly and yearly averages of the maximum solar incidence angle (variables no. 111–123). Variables no. 85–123 were calculated using our *r.sunangle* Python script (Electronic Appendix 5) created in the GRASS GIS environment with 10-min increments.

Statistical analyses of environmental data

To assess the differences in the ecological preferences of tetra- and octoploids, a discriminant analysis was performed on the environmental data. The data on type of habitat, habitat naturalness, geology, topography and climate (incl. parameters of solar energy) were summarized in a matrix of environmental variables. A high number of variables (123) and strong correlations among them resulted in complex interactions and redundancies that pose severe limitations on the use of traditional methods. Under these circumstances, a classical linear discriminant analysis can result in a solution with unstable coefficients or even with an infinite number of solutions, i.e. it is not possible to determine the optimal discriminant function (Wehrens 2011). Therefore, we adopted a partial least squares (PLS) approach, which has an advantage over more traditional methods because it effectively handles rank-deficiency and the problem of more variables than samples (Wold et al. 2001). The general idea of PLS is to construct a few orthogonal components, which account for as much of the variation in the predictor and response space as possible, while maximizing the correlation between them; i.e. PLS maximizes the covariance between a predictor and response matrices. Specifically for discrimination, PLS provides a dimension reduction technique that finds the optimal group separation while being guided explicitly by between-group variability (Barker & Rayens 2003). However, structured noise may complicate the interpretability of PLS models, particularly when there are many components (Eriksson et al. 2006). To remove such undesirable systematic variation in the data, different types of orthogonal signal correction methods have been developed (Svensson et al. 2002). Here, we employed orthogonal projection to latent structures (OPLS; Trygg & Wold 2002) in order to avoid the loss of information that is important for prediction. The objective of OPLS in discriminant analysis (OPLS-DA) is to divide the systematic variation in the predictor matrix into two parts: the predictive part, which models the relationships between variables and groups, and the orthogonal part, which captures the systematic variation in variables that is linearly unrelated to group separation. This partitioning of the variables generally leads to improved model interpretability, and the resulting OPLS-DA model is maximally focused on the separation of the groups.

The OPLS-DA model of ecological preferences of tetra- and octoploids was built in several steps. First, because the OPLS-DA is a scale-dependent method, environmental variables were centred and standardized in order to equalize the weights of the dimensionally heterogeneous variables in the analysis. Subsequently, the whole dataset (comprising 144 tetra- and 37 octoploid sites) was split into a training set (2/3 of the data) and a test set using stratified random sampling. The training set was used to build and tune the final model, and the test set was used to externally validate the predictive performance of the model. The PLS method that was used here was based on the original non-

linear iterative partial least squares algorithm (NIPALS), while the orthogonal signal correction was applied to the first component, as only one predictive component was required for the separation of the two groups (ploidy levels 4x and 8x) (Wehrens 2011). Leave-one-out cross-validation was used to estimate the optimal number of components that minimizes prediction error. The predictive performance of the final OPLS-DA model was further assessed using a simple randomization test. This procedure involved the comparison of observed predictive accuracy with its null distribution generated from the randomly reshuffled data (1000 randomizations) (Manly 1997). The P-value was computed as the probability of detecting a predictive accuracy that was greater than or equal to the observed value. Finally, as an external validation tool, the test set was used to calculate the predictive accuracy along with a 95% confidence interval for a binomial distribution.

To facilitate the interpretation of the results, an OPLS-DA score plot with 95% confidence ellipses was displayed. Variable weights for the first predictive component were plotted to assess the relative influence of environmental variables on the discrimination of ploidy levels. Using an orthogonal signal correction, the interpretation of these weights is straightforward (Wold et al. 2001). However, the emphasis in OPLS-DA is more on predicting the responses to particular variables than on the description of the underlying relationships between the variables. To gain more insight, a series of individual generalized linear models (GLM) with binomial errors was fitted to variables with the highest absolute weights for the OPLS-DA predictive component.

Because polyploidization can alter niche breadth, we compared the ecological heterogeneity recorded at tetra- and octoploid sites. First, we calculated pair-wise Gower dissimilarities to characterize environmental heterogeneity among sites. Gower distance was used because it efficiently handles variables of different types, as is our case. Prior to computing dissimilarities, environmental data was standardized by dividing by the ranges to ensure the same scaling for each variable. Subsequently, a Gower dissimilarity matrix was computed and used in permutation tests of homogeneity of multivariate dispersions (Gijbels & Omelka 2013). Probability of the test statistic F_d was calculated from 10,000 randomizations.

The analyses were performed in R language version 3.1.0 (R Core Team 2014) using the libraries' *ellipse* (Murdoch & Chow 2013) and *pls* (Mevik et al. 2013).

Distribution modelling

To construct the potential (predicted) distributional ranges of the tetra- and octoploids of *J. vulgaris* in Slovakia, we used the Geographic Resources Analysis Support System (GRASS) GIS environment for high-performance computing on the supercomputer "Aurel" at the Computing Centre of the Slovak Academy of Sciences. The model construction was based on the same set of 181 geo-referenced populations (171 sites sampled; Electronic Appendix 2) that were used in the above-mentioned environmental analyses. As environmental layers, we used 77 climatic variables (variables no. 7–84, see above and Electronic Appendix 3) as standardized rasters with an output binary map resolution of 500 m. Considering the long-time demands and low OPLS-DA weights climatic variables no. 85–123 were not used for the planar prediction of potential distributions.

For the modelling of the potential distribution we tested several methods, namely MaxEnt (maximum entropy method, Phillips et al. 2006), Environmental Niche Factor

Analysis (ENFA, principal smoothing technique for components analysis, Hirzel et al. 2002), Bioclimatic envelope (BioClim, based on percentile distributions, Elith et al. 2006) and DOMAIN (based on distance, Carpenter et al. 1993). The last one was selected for our study. The DOMAIN method is based on point-to-point distance in environmental space measured using the Gower coefficient of distance (Gower 1971), which uses range standardization to equalize the contribution from each environmental attribute.

Results

Distributions of plants with different ploidy levels

In Table 1 the results of FCMs of relative nuclear DNA content are presented (with mean values of tetraploid *J. vulgaris* subsp. *vulgaris*, excl. pops. no. 85 and 86, set as the unit value). FCMs of 997 samples resulted in three significantly different ($P < 0.0001$, Tukey-Kramer test) non-overlapping groups of fluorescence intensities, corresponding to three ploidy levels (tetra-, hexa- and octoploid). In the two geographical groups of octoploids (from Pannonia and the Spiš region) the relative DNA content was about the same and neither of them proportionally matched the twofold DNA content of the sympatric (or parapatric) tetraploids ($P < 0.0001$). Relatively high intraspecific variation in fluorescence intensity per ploidy level (up to 8.9% in octoploids from Pannonia) is related to the fact that in most cases the FCMs were obtained from silica-gel dried material.

Table 1. – Relative fluorescence intensities of 997 samples of *Jacobaea vulgaris* (for details see Electronic Appendix 2) determined using DAPI flow cytometry and *Glycine max* ‘Polanka’ or *Bellis perennis* as internal reference standards. Mean value of relative fluorescence intensity of tetraploid *J. vulgaris* subsp. *vulgaris*, excl. pops. no. 85 and 86, was set as the unit value. Populations 85 and 86, were treated separately, as they include octoploid plants occurring outside the continuous distribution range of octoploids in Pannonia.

Ploidy level	Mean relative fluorescence \pm SD	Relative DNA content: min–max (variation: max/min,%)	Number of plants
Tetraploids, excl. pops. no. 85 and 86	1.000 \pm 0.012	0.967–1.038 (7.34%)	673
Tetraploids from pops. no. 85, 86	1.001 \pm 0.011	0.983–1.021 (3.87%)	49
Hexaploids	1.463 \pm 0.031	1.417–1.514 (6.85%)	14
Octoploids from Pannonia	1.890 \pm 0.029	1.815–1.977 (8.93%)	257
Octoploids from Spiš region (pops. no. 85, 86)	1.871 \pm 0.023	1.853–1.896 (2.32%)	4

Table 2. – Ploidy-level composition of *Jacobaea vulgaris* at 203 sites sampled in Slovakia (based on data that are presented in Electronic Appendices 1 and 2).

Ploidy-level composition	Number of sites sampled	Percentage of sites sampled
4x	148	72.9
6x	0	0.0
8x	34	16.7
4x + 6x	6	3.0
4x + 8x	10	4.9
6x + 8x	3	1.5
4x + 6x + 8x	2	1.0

Ploidy-level composition at 203 sites of *J. vulgaris* sampled in Slovakia (based on karyological data of 1023 individuals; see Electronic Appendices 1 and 2) is presented in Table 2. Of the three ploidy levels, the most frequent was the tetraploid one, which occurred at 81.8% of the sites sampled (72.3% of the plants analysed). At most of the sites of *J. vulgaris* studied there was only one ploidy level (89.7%). The hexaploid plants never occurred alone but always with other ploidy level(s).

The distribution map of the three ploidy levels in Slovakia (Fig. 1) shows that tetraploids are spread across the entire area studied. In contrast, the octoploid individuals occur almost exclusively in the southern part of Slovakia (northern Pannonia) and the limit to their northern distribution passes through the southern foothills of the western Carpathians (in particular, through the Biele Karpaty Mts, Tematínske kopce hills, Strážovské vrchy Mts, Slovenský kras karst, Stredné Pohornádie Mts and Zemplínske vrchy hills). The only exceptions from this pattern are two localities in the Spišské kotliny basins (no. 85 and 86), which are clearly not in southern Slovakia. At both localities, we found only two octoploid individuals; otherwise, the majority of the plants analysed were tetraploid (35 and 14 individuals); in population 85, we found also one hexaploid plant (Electronic Appendix 2). Hexaploids occur sporadically throughout Slovakia, including the areas where otherwise only tetraploids occur (e.g. pop. 166, 284 and 289; Fig. 1).

Ecological differentiation between plants of different ploidy levels

Ecological differentiation of tetra- and octoploid populations throughout Slovakia is briefly summarized in Electronic Appendix 6. Tetraploids occur most frequently in meadows and pastures, rarely in other habitats; they were not found on sand dunes or sand steppes. In contrast, octoploids occur most frequently in steppic grasslands and thermophile fringes and avoided forests. There is a strong linkage of octoploids to natural habitats, whereas no such tendency is visible in tetraploids that are found evenly in both natural habitats and those weakly affected by humans.

Descriptive results were supported by discriminant analysis. OPLS-DA was used to analyse the environmental characteristics of the 96 sites with tetraploid plants and 24 sites with octoploid plants of *J. vulgaris* that were included in the training set. The cross-validation procedure yielded the final model with three components and a highly significant predictive accuracy of 91% ($P < 0.001$). A plot of the first two components is shown in Fig. 2. Sites with different ploidy levels are discriminated along the first (predictive) component, the second (orthogonal) component is shown for visualization purposes only. Approximately 11% of the variation in the environmental variables is correlated with ploidy level. There is a moderate environmental separation between the two ploidy levels but with considerable overlap. In fact, 97% of the tetraploid sites were correctly classified, while the predictive accuracy for octoploids was only 67%. Indeed, the true test of the final model lies in its ability to discriminate ploidy levels when applied to new data. Accordingly, the OPLS-DA model was used to analyse the data for the remaining 61 sites (48 sites for tetraploids and 13 sites for octoploids). The overall accuracy of ploidy level assignment was 86% with 95% confidence limits of 74–94%. The model showed a high sensitivity but low specificity, as 98% of the tetraploids were correctly classified, while only 36% of the octoploid assignments were correct.

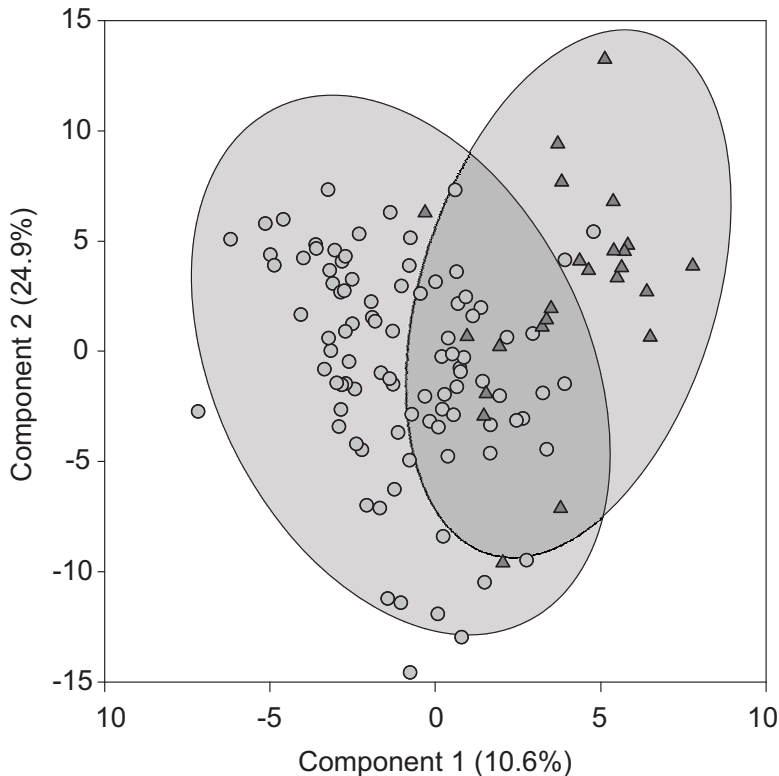


Fig. 2. – OPLS-DA plot of the first (predictive) and second (orthogonal) components based on 123 ecological characteristics of 96 sites for tetraploid *Jacobaea vulgaris* subsp. *vulgaris* (○) and 24 sites for octoploid *J. vulgaris* subsp. *pannonica* (△). The 95% confidence ellipses are displayed in grey.

Because OPLS-DA concentrates all of the discriminating information into the first component, the environmental variables that are responsible for the separation of the ploidy levels can be interpreted taking into consideration the weights that are attained by the variables for this predictive component (Fig. 3). The environmental variables with the largest absolute weight peaks are, in descending order of importance: type of habitat, altitude, precipitation, temperature, habitat naturalness and geology. The discriminatory power of direct and diffuse solar radiation during the summer months was also relatively high. For illustration purposes, the relationships between the ploidy levels and the most important predictors were plotted as individual GLMs (Fig. 4). The ecological heterogeneity (dispersion of sites around centroids; cf. Fig. 2) was similar for both ploidy levels ($F_d = 1.38$, $P = 0.2479$), suggesting comparable ecological niche breadths.

Distribution modelling

Results of the modelling of the potential distributions of tetra- and octoploid plants of *J. vulgaris* in Slovakia are presented in Fig. 5. High AUC (area under the receiver operating characteristic curve) values (1.00) and low test omission errors indicate excellent model performance for both ploidy levels. For better clarity of graphical display we divided the pixels of the resulting map with occurrence probabilities of 1–100% into 4 quantiles. The

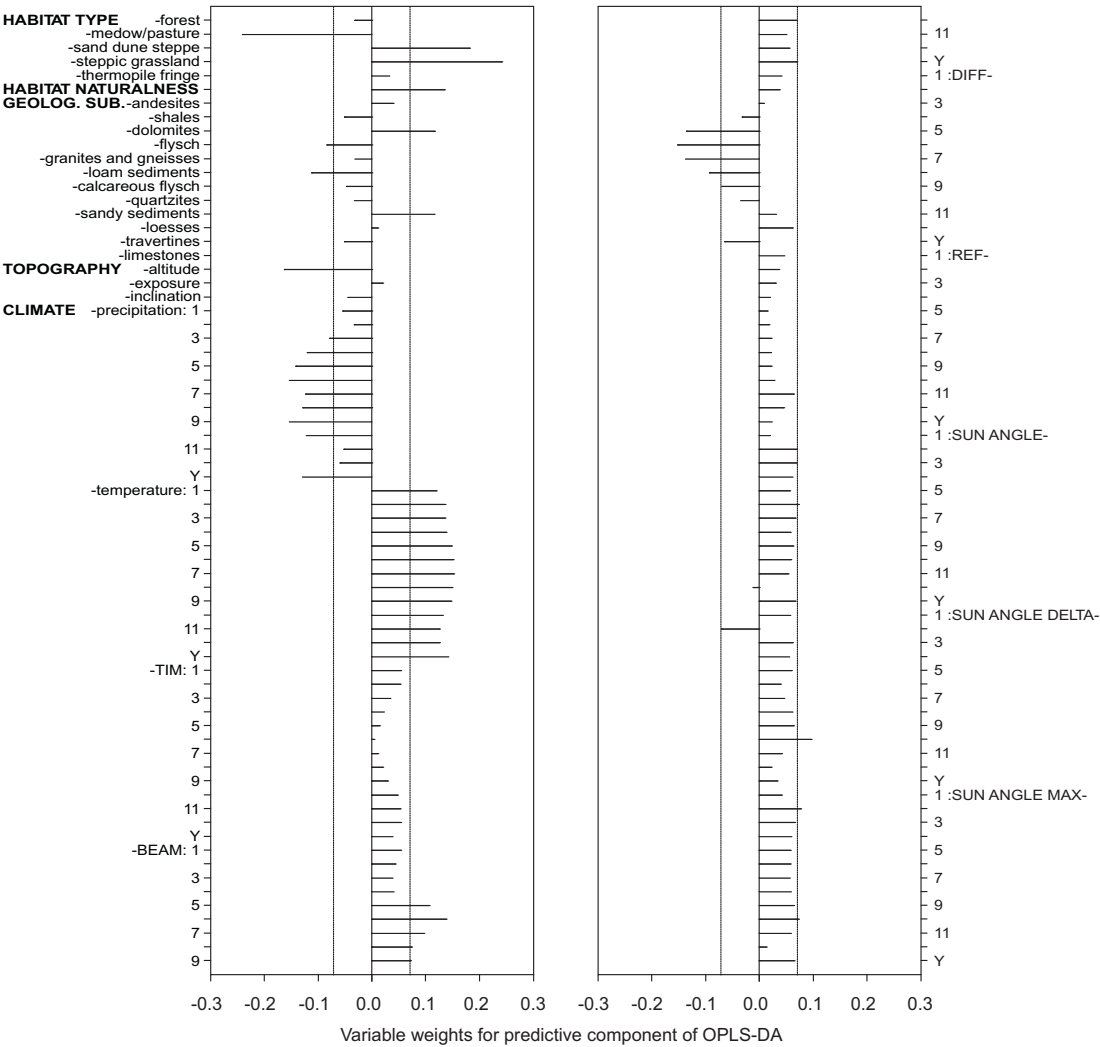
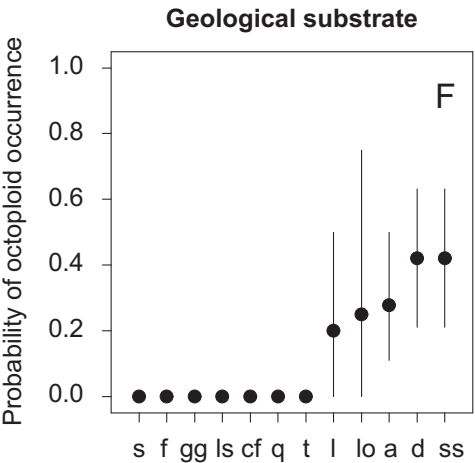
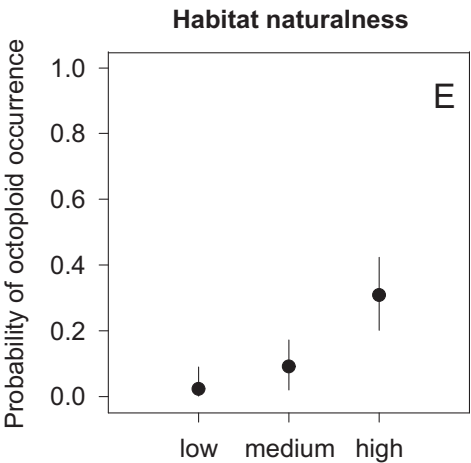
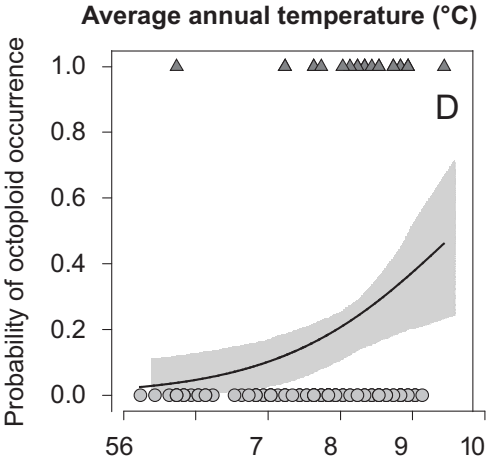
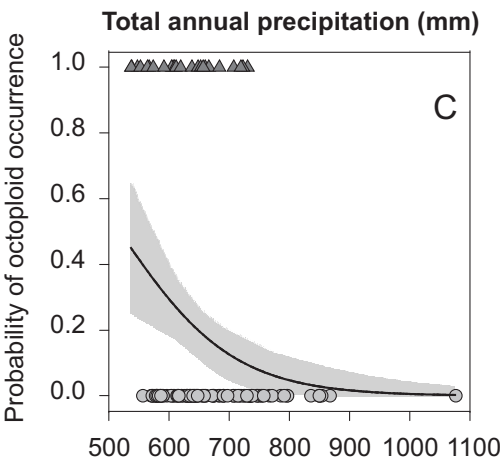
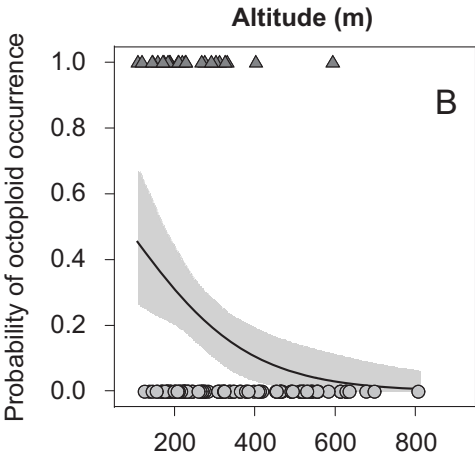
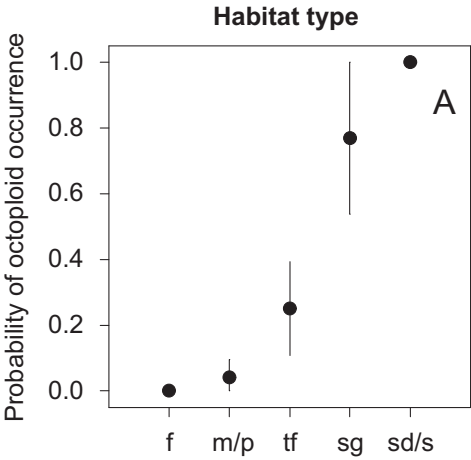


Fig. 3. – OPLS-DA variable weights for the predictive component. The dashed lines indicate the variation in the amplitude of the mean absolute weight. Variables with weights exceeding the mean (dashed line) were considered important for discriminating between the different ploidy levels of *Jacobaea vulgaris*. The abbreviations used for climatic variables: precipitation – sum of vertical atmospheric precipitations, temperature – average air temperatures, TIM – sum of insolation time of beam solar radiation, BEAM – sum of beam solar radiation, DIFF – sum of diffuse solar radiation, REF – sum of reflected solar radiation, SUN ANGLE – averages of solar incidence angle for a point on an inclined plane, SUN ANGLE DELTA – averages of delta of solar incidence angle, and SUN ANGLE MAX – averages of maximum of solar incidence angle; 1–12/Y – monthly/yearly sum or average. For explanations of variables see Electronic Appendices 2, 3 and 4.

first and second quartile of pixels correspond to the 1–75% of occurrence probability and such pixels are shown on the maps as white areas. The third quartile of pixels correspond to the 76–86% of occurrence probability and the fourth quartile of pixels to 87–100% of occurrence probability, and such pixels are shown on the maps as light or dark green areas, respectively.



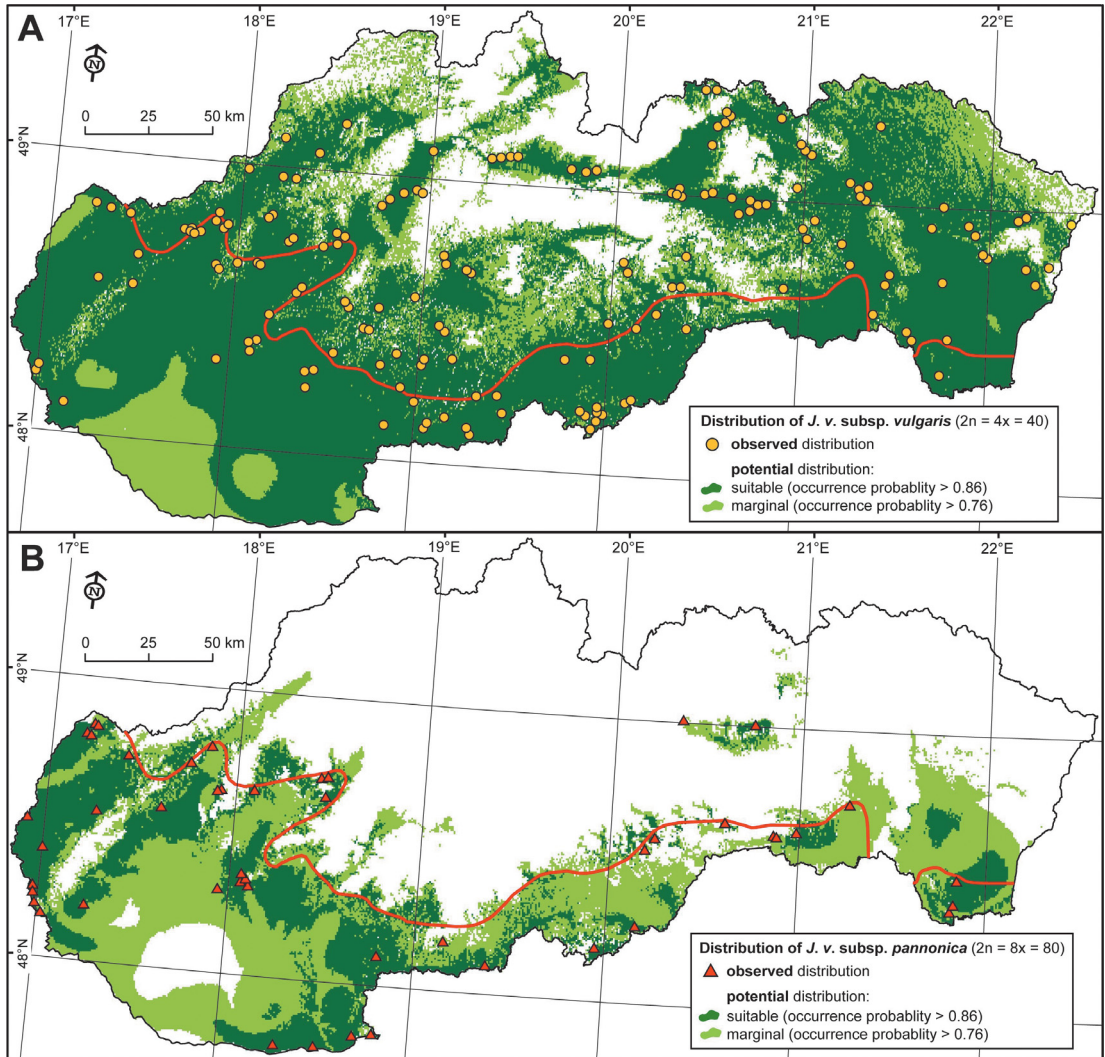


Fig. 5. – Observed and potential (predicted) distributions of (A) tetraploid *Jacobaea vulgaris* subsp. *vulgaris* and (B) octoploid *J. vulgaris* subsp. *pannonica* in Slovakia. The potential distributions were obtained using the DOMAIN algorithm with 77 climatic variables, which revealed the occurrence probabilities at the lowest threshold for suitable areas of > 0.86 (dark green areas) and > 0.76 (light green areas). The red line indicates the northern boundary of the observed continuous distribution of octoploid subspecies *J. vulgaris* subsp. *pannonica*.

◀ Fig. 4. – Individual relationships between the occurrence of two *Jacobaea vulgaris* ploidy levels (○ tetraploids, ▲ octoploids) and the variables with the largest weight for the predictive component of the OPLS-DA. The predicted values (black lines or circles) are displayed along with 95% bootstrap confidence limits (1000 resamples). (A) Habitat type: $\chi^2_{(4)} = 49.5$, $P < 0.0001$; (B) Altitude (m): $\chi^2_{(1)} = 12.5$, $P = 0.0004$; (C) Total annual precipitation (mm): $\chi^2_{(1)} = 9.6$, $P = 0.002$; (D) Average annual temperature (°C): $\chi^2_{(1)} = 9.3$, $P = 0.0023$; (E) Habitat naturalness: $\chi^2_{(1)} = 10.6$, $P = 0.0011$; (F) Geological substrate: $\chi^2_{(11)} = 32.6$, $P = 0.0006$. The abbreviations of the different types of habitats are as follows: f – forests, m/p – meadows/pastures, tf – thermophile fringes, sg – stepic grasslands, sd/s – sand dunes/steppes. Abbreviations of geological substrates are: s – shales, f – flysch, gg – granites and gneisses, ls – loam sediments, cf – calcareous flysch, q – quartzites, t – travertines, l – limestones, lo – loesses, a – andesites, d – dolomites and ss – sandy sediments.

Despite the considerable overlap in the potential distributions of plants of both ploidy levels in southern parts of Slovakia, the potential distribution of tetraploids in the area studied is much broader than that of octoploids and includes most of Slovakia, except for the mountainous and high-altitude areas in the Western and Eastern Carpathians. The suitable climatic conditions for octoploids (with an occurrence probability of more than 86%) are recorded in the southern lowlands (except for the great part of the Podunajská nížina lowland) and in the southern foothills of the Carpathians. In addition to this more or less continuous southern area, the regions predicted as suitable for octoploids also includes the south-eastern part of the Spišské kotliny basins and surprisingly the north-western part of the Spišsko-Šarišské medzihorie basin in the northern part of Slovakia.

Discussion

Distributions of plants with different ploidy levels in Slovakia

Tetraploids of *Jacobaea vulgaris* occur widespread throughout the whole of Slovakia except at high altitudes, while octoploids, except for the two Spiš populations (see below), occur only in the southern part of the country (part of the Pannonian biogeographical region) and the northern limit of their continuous distribution is located in the southern foothills of the Western Carpathians and is almost identical to the definition of the borders of the Pannonian region cited in Futák (1984) (Fig. 1).

FCMs clearly indicate that the values of relative DNA content of octoploid plants both from Pannonia and the Spiš region are significantly lower than the twofold values of DNA content of geographically related tetraploids. This is in agreement with the results obtained using AFLP markers (Hodálová et al. 2015), which indicate an autopolyploid and monophyletic origin of *J. vulgaris* subsp. *pannonica* octoploids [contrary to a polytopic (in situ) origin from tetraploids].

Genetic patterns of central-European tetra- and octoploid individuals of *J. vulgaris* (Hodálová et al. 2015) indicate that their distribution in Slovakia is a result of the secondary contact of two ploidy levels (i.e. the formerly allopatric ploidy levels came into contact after migration; Petit et al. 1999). Similar secondary contacts of different ploidy levels were recently reported from the Western Carpathians for *Vicia cracca* L. (Trávníček et al. 2010), *Centaurea stoebe* L. (Mráz et al. 2012) and *Aster amellus* L. (Castro et al. 2012). These results, in congruence with studies of other authors, indicate that central Europe, where different floristic elements come into contact, is one of the main “natural laboratories” for studying polyploid evolution in Europe (Lexer & van Loo 2006),

Jacobaea vulgaris octoploids in Spiš region

Apart from the *J. vulgaris* octoploids of unclear taxonomic status on the island of Öland (see Hodálová et al. 2015), the only known outlying locations for the octoploid *J. vulgaris* subsp. *pannonica* outside the Pannonian region are in the Western Carpathians in the Spiš region (northern Slovakia; Fig. 1). Octoploids occur there at two sites, in relic habitats, near Primovce (pop. 85B) and on the hill Sivá brada (pop. 86B). Both of these sites are inhabited predominantly by tetraploids, and octoploid individuals were found there only very rarely (two plants at each site; Electronic Appendix 2). The AFLP analyses

(Hodálová et al. 2015) clearly indicate that octoploids in these two Spiš populations are unlikely to be of in situ origin and (similar to other Pannonian populations studied) they represent the secondary contact zones of two ploidy levels of *J. vulgaris*. This assumption is also supported by the results of FCMs of relative nuclear DNA content, with the Spiš octoploids about the same as the Pannonian octoploids, and neither of these two geographical groups of octoploids proportionally matched the twofold DNA content of the sympatric (or parapatric) tetraploids (see above).

Two scenarios may account for the disjunct Spiš populations. *Jacobaea vulgaris* octoploids may have occupied a larger distribution in the past and the two Spiš populations may be a remnant of this wider distribution. Alternatively, these two Spiš populations may be recent immigrants via long-distance dispersal. However, both these hypotheses are highly speculative and the available data insufficient for distinguishing between them. Nevertheless, the marginal position of the AFLP profiles of Spiš octoploids relative to those of other Pannonian octoploids (Hodálová et al. 2015) indicate their long-term isolation, which supports the possible long term persistence of octoploids at these localities. The possibility of the native and long-term occurrence of octoploid *J. vulgaris* subsp. *pannonica* at Spiš might be supported also by other floristic elements, whose distribution in Slovakia is almost exclusively restricted to the Pannonian region, with outlying occurrence located in the Spiš region [e.g. *Adonis vernalis* L. – Futák 1982; *Dianthus pontederiae* A. Kern. – Kmeťová 2012; *Eremogone micradenia* (P. A. Smirn.) Ikonn. – Perný 2012]. Both the sites sampled at Spiš are also known for the relic occurrence of several species [e.g. *Allium strictum* Schrad., *Glaux maritima* L., *Pulsatilla patens* (L.) Mill. – Šmarda 1961, Futák 1972, Mereďa et al. 2012] and other species reach the northern limits of their distributions in Slovakia, either at these sites or in their vicinity (e.g. *Dracocephalum austriacum* L., *Silene donetzica* Kleopow, *S. viridiflora* L. – Mereďa & Hodálová 2011, Mereďa et al. 2012).

Origin of hexaploids

The pattern of distribution recorded for hexaploids (Fig. 1) indicate that two processes could have been involved in their formation. In tetraploid populations occurring in areas where there are no octoploids (e.g. pop. 166, 284, 289), the hexaploid plants might have originated via the syngamy of reduced and unreduced gametes of tetraploids (see Ramsey & Schemske 1998). On the other hand, in mixed-ploidy populations and in populations where the tetra- and octoploid plants occur in close proximity (e.g. pop. 14, 40, 82, 85, 265, and 326), hexaploids could arise also from hybridization between tetra- and octoploid plants. The overall frequency of hexaploids of *J. vulgaris* in Slovakia is less than 1.5%, which corresponds well to the percentages recorded in the contact zones of other plant systems with spatio-ecologically segregated cytotypes (Trávníček et al. 2010, Mráz et al. 2012), including those in the genus *Jacobaea* (Sonnleitner et al. 2010). This frequency is also similar to the percentage of unreduced gametes occurring per gamete in plants, which is ca 0.5% (but with extremely high variation; Arrigo & Barker 2012). The low frequency of hexaploids and their low pollen fertility (Hodálová et al. 2010) clearly indicate that the gene flow between ploidy levels in *J. vulgaris* is quite limited and that there are strong pre- and/or postzygotic reproductive barriers between *J. vulgaris* subsp. *vulgaris* and *J. vulgaris* subsp. *pannonica*. Sonnleitner et al. (2013) report that in the

polyploid complex of the closely related *J. carniolica*, irrespective of the direction of crosses, seed viability and seedling survival of crosses between plants of $2n = 40$ (classified by us as tetraploids) and $2n = 80$ were rather low and substantially lower than for $2n = 80$ vs $2n = 120$ crosses. A similarly strong inter-ploidy block, stabilizing the coexistence of lower-ploid progenitors and their autopolyploids, is not a rare phenomenon and is reported in other plant groups (e.g. in *Ranunculus adoneus* Gray – Baack 2004; *Knautia arvensis* – Hanzl et al. 2014).

Inter-ploidy niche differentiation

Ecological or climatic niche segregation among coexisting cytotypes is one of the most discussed factors facilitating the establishment of new polyploids (Parisod et al. 2010, Weiss-Schneeweiss et al. 2013, but see Halverson et al. 2008, Hanzl et al. 2014). Our observations indicate that such environmental sorting also determines the distribution of plants of different ploidy levels of *J. vulgaris*. Nevertheless, one has to bear in mind when interpreting the results that this study is based only on part of the distribution area of both ploidy levels and the results might be biased by this fact. On the other hand, considering the distribution of *J. vulgaris* octoploids in other parts of Europe (octoploids in Austria, Moravia and Scandinavia are similarly restricted to the warmest regions), similar environmental sorting of *J. vulgaris* ploidy levels can be expected in the rest of the distribution area of this species.

In the current study, habitat-specific distributions of the different ploidy levels of *J. vulgaris* were identified. Octoploids clearly prefer more xerothermic habitats than tetraploids. Another important parameter of vegetation composition that segregates the *J. vulgaris* ploidy levels is the degree to which it is influenced by human activities. Octoploids clearly prefer natural habitats with limited human activity and therefore the presence of octoploids can be used as a good indicator of this type of habitat. In Slovakia, many octoploid populations occur in or close to small protected areas (nature reserves, national nature reserves, protected sites: pop. 41, 43, 44, 45, 46, 82, 85, 86, 219, 248, 249, 256, 263, 307 and 337).

The preference of octoploids for less anthropogenic habitats is interesting because a lower ability to invade new habitats is often attributed to lower-ploid progenitors rather than to their polyploid descendants (e.g. Soltis et al. 2010, Mráz et al. 2012, te Beest et al. 2012). The ability of *J. vulgaris* tetraploids to spread is illustrated by the recent rapid spread of tetraploids into various man-disturbed habitats, such as roadsides, pastures, abandoned vineyards and waste areas, where octoploids do not occur (I. Hodálová & P. Mereďa, unpublished data). It is also likely that these are tetraploid plants of this species that are rapidly spreading beyond Europe (Bain 1991), as only a chromosome number of $2n = 4x = 40$ is recorded for areas into which it has been introduced, although karyological data for this species occurring outside Europe is still very scarce (Hodálová et al. 2010).

The differences in the habitat preferences of the plants of *J. vulgaris* with different ploidy levels are most likely the main factors that are responsible for the prevalence of single-cytotype populations over those of mixed-ploidy, even in areas of sympatric occurrence of tetra- and octoploids. The pronounced ecological differences between tetra- and octoploids are mentioned also in the descriptions of the ecology of the subspe-

cies of *J. vulgaris* on the Baltic islands of Gotland and Öland (e.g. Sterner 1938, Sterner & Lundqvist 1986, Wysk et al. 2009). The data on ecological requirements that are reported in these papers for *J. vulgaris* subsp. *gotlandica* [as for *Senecio jacobaea* subsp. *gotlandicus* (Neuman) Sterner] refer to octoploid populations, while those for *J. vulgaris* subsp. *vulgaris* or *J. vulgaris* subsp. *dunensis* (Dumort.) Pelter et Meijden [as for *Senecio jacobaea* subsp. *jacobaea* and *S. j.* subsp. *dunensis* (Dumort.) Kadereit et P. D. Sell] refer to tetraploid populations.

Whereas the tetraploids showed no strong affinity for a particular geological substrate, almost 57% of the octoploid populations occur either on dolomites or sandy sediments. These two substrates, along with andesites, limestones and loesses, were recorded at more than 90% of the sites where octoploids occurred. Nevertheless, the absence or restricted occurrence of octoploids on certain substrates is most likely mainly determined by their geographical distribution (substrates absent or rarely occurring in southern Slovakia) rather than their affinity for a particular substrate. Therefore we cannot draw any definite conclusions about the preferences of the plants of different ploidy levels for particular geological substrates.

The two major ploidy levels of *J. vulgaris* (subspecies) clearly differ in their altitudinal distributions, with octoploids restricted to low altitudes in the lowlands and the hilly (colline) vegetation belt where the mean annual temperatures are higher. Altitudinal gradients may sort ploidy levels with different climatic requirements. Although the higher frequency of polyploids at high (subalpine or alpine) altitudes is often linked to glaciation (colonization of previously glaciated areas by polyploids; Stebbins 1984, Brochmann et al. 2004), there is a number of studies that indicate altitudinal segregation possibly caused by different climatic conditions [e.g. in *Allium oleraceum* L. – Šafářová et al. 2011; *Chamerion angustifolium* (L.) Holub – Martin & Husband 2013, Sabara et al. 2013; *Cardamine amara* L. – Zozomová-Lihová et al. 2015].

Jacobaea vulgaris octoploids prefer more xeric habitats than the tetraploids. In general, polyploids are linked with aridity (Parisod et al. 2010, te Beest et al. 2012); however, several high polyploids are reported occurring in more humid conditions than their lower-ploid counterparts [e.g. *Solanum* sect. *Petota* Dumort. – Hijmans et al. 2007; *Oxalis obtusa* Jacq. – Krejčíková et al. 2013; *Sesleria tatrae* (Degen) Deyl – Budzáková et al. 2014]. The physiological and morphological changes in polyploids may increase their tolerance of stress, which may enable them to occupy more extreme environments or environments with greater climatic and edaphic fluctuations than their lower-ploid progenitors (Brochmann et al. 2004, Parisod et al. 2010). One such stress factor is water deficiency. Physiological processes that facilitate a higher water-use efficiency in polyploids were recently reported for *Brachypodium distachyon* (L.) P. Beauv. (Manzaneda et al. 2012) and *Atriplex canescens* (Pursh) Nutt. (Hao et al. 2013). It is possible that similar physiological processes may play a role in the adaptation of *J. vulgaris* octoploids to more xeric habitats.

There were no significant differences in the observed (realized) vs potential (predicted) distributions of either the tetra- or octoploid plants in Slovakia. Although octoploids could potentially occupy climatic space further north than they currently occur (e.g. along the rivers Váh, Bebrava and Hron, and in the northern parts of the Ipeľsko-rimavská brázda basin, the Spišsko-Šarišské medzihorie basin and in the Východoslovenská nížina lowland), the differences are not very great. Most of these

areas were examined in detail by us, but we did not find any suitable habitats (natural dry grasslands) there for octoploids. Surprisingly, the distribution modelling did not reveal a suitable climatic niche for octoploids on the plains of Slovenský kras karst, where we expected them to occur. By contrast, the distribution modelling revealed suitable areas for tetraploids in extensive parts of the Podunajská nížina lowland and Východoslovenská nížina lowland, where tetraploids very rarely occur. This is mainly because there are few suitable habitats there for *J. vulgaris*, because large areas of both these lowlands consist of fields and wetlands. *Jacobaea vulgaris* tetraploids could theoretically occur there as a ruderal element in drier grasslands (e.g. roadsides, waste areas), but low precipitation and high summer temperatures in that region probably limit its occurrence.

Conclusion

This study of octoploid plants of *Jacobaea vulgaris* provided further evidence for an inconsistent pattern in the ecogeographical and/or climatic traits of polyploid plants (Parisod et al. 2010, Weiss-Schneeweiss et al. 2013, Glennon et al. 2014). For the octoploid plants studied we recorded an atypical combination of traits: compared to the parental tetraploids, *J. vulgaris* octoploids have a smaller distribution area, prefer less human-influenced habitats and are restricted to low altitudes and more xeric habitats. Despite the observed differences between the ecological niches of tetra- and octoploids, their ecological niche breadths are comparable.

See <http://www.preslia.cz> for Electronic Appendices 1–6

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Souhrn

Polyploidní speciace je neustále probíhající proces a důležitý zdroj diverzity semenných rostlin. Přesto však, genetické, morfologické a eko-geografické rozdíly mezi polyploidy a jejich předky nižších ploidních úrovní zůstávají nedokonalé známé. V našem příspěvku jsme zkoumali rozšíření tří ploidních úrovní (4x, 6x, 8x) druhu *Jacobaea vulgaris* na území Slovenska (203 lokalit, 1023 jedinců). Na vybrané podmnožině lokalit (171) jsme analyzovali ekologickou diferenciaci mezi dvěma hlavními ploidními úrovnemi druhu, tetra- a oktoploidmi, které jsou nověji rozlišovány jako samostatné poddruhy, jmenovitě *J. vulgaris* subsp. *vulgaris* ($2n = 4x = 40$) a jeho autopolyploidní potomek *J. vulgaris* subsp. *pannonica* ($2n = 8x = 80$). Většina zkoumaných lokalit (89.7%) obsahovala jenom jednu ploidní úroveň (poddruh). Minoritní (6x) ploidní úroveň byla zjištěna u 1.4% analyzovaných rostlin a vyskytovala se jenom společně s rostlinami jiné ploidní úrovně. Studium 123 environmentálních proměnných ukázalo, že dvě hlavní ploidie (poddruhy) mají rozdílné ekologické niky. Ekologické

rozdíly spočívajúc především v typu vegetace, míře přirozenosti vegetace, geologickém podloží, nadmořské výšce, srážkách a teplotách. Oktoploidní rostliny *J. vulgaris* jsou ve studovaném území ve srovnání s tetraploidními rostlinami vázány na teplejší a sušší území v nižších nadmořských výškách a preferují přirozenou vegetaci. Navzdory ekologické separaci dvou hlavních ploidních úrovní (subspecií) se šířky ekologických nik těchto ploidí na území Slovenska statisticky neliší, a to i přesto, že tetraploidi mají na Slovensku ve srovnání s oktaploidy větší jak reálné tak i potenciální rozšíření.

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