# Whole genome duplication increases ecological niche breadth of the perennial herb *Urtica dioica*

**Duplikace genomu zvětšuje šířku ekologické niky u vytrvalé byliny *Urtica dioica***

**Tomáš Urfus[1], Martin Kopecký[2], Romana Urfusová[1,2] & Jindřich Chrtek[2,1]**

[1]*Department of Botany, Faculty of Science, Charles University, Benátská 2, CZ-128 00 Prague, Czech Republic, e-mail: tomas.urfus@natur.cuni.cz;* [2]*Institute of Botany of the Czech Academy of Sciences, Zámek 1, CZ-252 43, Průhonice, Czech Republic, e-mail: ma.kopecky@gmail.com, jindrich.chrtek@ibot.cas.cz*

Urfus T., Kopecký M., Urfusová R. & Chrtek J. (2021) Whole genome duplication increases ecological niche breadth of the perennial herb *Urtica dioica*. – Preslia 93: 305–319.

Environmental niche modelling and associated species distribution models often ignore intraspecific ecological differentiation linked with ploidal differentiation, i.e. the occurrence of several cytotypes of the same species. Here we tested the hypotheses that (i) the tetraploid (4x) cytotype is characterized by a broader ecological niche than the diploid (2x), and (ii) niche differentiation at the regional scale can serve as a proxy measure for differentiation at a smaller landscape scale in *Urtica dioica*, a perennial herbaceous plant with two prevailing cytotypes (2x and 4x). We focused on southern Moravia (Czech Republic), an area with a high diversity of vegetation types and the occurrence of both diploid and tetraploid cytotypes of *U. dioica*. To explore the geographical distribution, ecological preferences and habitat affinities of the diploid and tetraploid plants, we collected individuals at two different spatial scales: regional (~1200 km$^2$) and landscape (9.1 km$^2$). We used flow cytometry to determine the ploidy level of all the plants collected and determined the distribution of the different cytotypes both in geographical and ecological space. We also compared the habitat affinities of the diploid and tetraploid plants. Analyses of ploidy revealed 93 diploids and 325 tetraploids (regional scale: 42 diploids, 85 tetraploids; landscape scale: 51 diploids and 240 tetraploids). Diploid plants occurred only in the wetter parts of the landscape and this pattern was very similar at both scales. In contrast, tetraploids occurred along the whole moisture gradient at both scales. Diploids and tetraploids differed also in their habitat affinities. While diploids occurred relatively more frequently in alluvial and wet forests, managed broadleaved forests, alluvial and wet meadows, and wetlands and riverbanks, tetraploids preferred oak and oak-hornbeam forests, ravine forests, actively managed meadows, human settlements and vineyards. Our data clearly show an ecological niche differentiation between diploid and tetraploid cytotypes of *Urtica dioica*. While tetraploids have a broad ecological tolerance and consequently a wide geographical distribution, diploids occur only in a narrow range of ecological conditions and their distribution is restricted to areas with a shallow water table in the bottoms of valleys. Thus, ploidal differentiation, confirmed for many plant species, should be included in environmental niche modelling and species distribution models.

Keywords: height above the nearest drainage (HAND), realized niche breadth, polyploidy, *Urticaceae*, species distribution modelling, environmental niche modelling, habitat affinity, terrain analysis

---

doi: 10.23855/preslia.2021.305

## Introduction

Revealing mechanisms underlying species distributions and niche differentiation is one of the principal tasks of contemporary ecology. This has focused mostly on the species level, despite the widely documented variation that exists within species (Kolář et al. 2017). There is increasing evidence that plant functional traits accounting for niche space and distribution can vary considerably within species, as a result of genetic diversity, variation in abiotic and biotic factors, and ploidal diversity caused by whole genome duplication, called polyploidization (Jung et al. 2010, Van de Peer et al. 2017, Henn et al. 2018).

Polyploidization is generally suggested as a specific near-ubiquitous evolutionary driver of angiosperms and ferns (Otto & Whitton 2000), having far-reaching consequences for plant evolution and ecology. It is estimated that 15% of angiosperm speciation events are associated with ploidy changes (Wood et al. 2009).

As polyploidy affects many key features from genomic to the organismal level, polyploid derivatives often differ from their diploid ancestors in phenotype, physiology, ecology and geographical distribution (Ramsey & Schemske 2002). Polyploids are often more tolerant of various types of stress, such as drought, cold, or salinity (e.g. Saleh et al. 2008, Van Laere et al. 2011, Deng et al. 2012), which can often partly account for wider ecological niches and geographical ranges (reaching higher altitudes and latitudes), i.e. broader environmental tolerances of polyploid plants (Levin 2002, Lowry & Lester 2006, Martin & Husband 2009, Pandit et al. 2011; but see Theodoridis et al. 2013, Kirchheimer et al. 2016 for the opposite pattern of niche evolution, niche contraction in polyploids). However, this refers mostly to polyploids that originate from hybrids between two different diploid species (allopolyploids), which might have achieved their broad ecological distributions by expressing the combined range of ecological characteristics of their diploid progenitors.

In contrast, there are relatively few data on ecological niches and ranges in polyploids originating within a single species (intraspecific polyploids, autopolyploids; Soltis et al. 2007), even though they may provide new insights into the different processes driving polyploid niche evolution as they avoid the effects of hybridization (typical of allopolyploids; Spoelhof et al. 2017). The rate of their formation is likely to markedly exceed those of allopolyploids (Ramsey & Schemske 1998), but probably due to much stronger constraints on autopolyploid establishment within diploid populations, both types of polyploids are more or less equally represented in nature (Barker et al. 2016). Rice et al. (2019) estimate that 16% of vascular plant species consist of two or more cytotypes in certain parts of their distributions (e.g. Kliber & Eckert 2005, Castro et al. 2012, Godsoe et al. 2013, Kolář et al. 2013) or even within their populations (e.g. Kao 2007, Šafářová & Duchoslav 2010, Sonnleitner et al. 2010, Trávníček et al. 2011). Based on available, but still scarce, data, ecological divergence between cytotypes is rather common, but autopolyploids, unlike allopolyploids, do not generally tolerate harsher environments and do not always show a broader ecological niche than their diploid progenitors (Levin 2002, Parisod et al. 2010).

The pattern of conspecific diploids and autopolyploids (tetraploids) with contrasting geographical ranges is perfectly exemplified by *Urtica dioica* L., a perennial herbaceous plant native of the temperate zone of Eurasia and northern Africa. In Europe, diploids have a disjunct distribution and likely to occur in relict, less human-affected habitats such as alluvial forests, willow galleries on river banks, ravine forests, wet lowland alluvial meadows and reed beds, whereas widely distributed weedy tetraploids seem to occur in a wide range of habitats, especially in those with an increased degree of human influence (Henning et al. 2014, Grosse-Veldmann & Weigend 2015, Grosse-Veldmann et al. 2016, Rejlová et al. 2019). In Europe, tetraploids are mostly referred to as *Urtica dioica* subsp. *dioica*, and diploids are assigned to three, at least partly vicariant subspecies, i.e. subsp. *pubescens* (Ledeb.) Domin, subsp. *sondenii* (Simmons) Hylander and subsp. *subinermis* (R. Uechtr.) Weigend. However, all the published molecular phylogenies do not distinguish these intraspecific taxa (Henning et al. 2014, Grosse-Veldmann & Weigend 2015, Rejlová et al. 2021). In addition, the relationship between the phenotype (morphology) and ploidy as well as morphological delimitation of the mentioned subspecies remain a matter of debate (Rejlová et al. 2021). Diploids are reported from central, northern, southern and south-eastern Europe. In central Europe, they are identified with subsp. *subinermis* and are most likely confined to alluvial forests, *Phragmites australis* reed beds and edges of tall herbaceous plant communities (Weigend 2005). Nevertheless, exact data are still missing and niche differentiation or overlaps at landscape and regional scales between diploids and tetraploids remain unknown.

Here we explore the spatial distribution, ecological preferences and habitat affinities of the diploid and tetraploid plants of *U. dioica* using a combination of flow cytometry and terrain analysis. As there is increasing evidence of the different relative importance of particular environmental factors at different spatial scales and thus that niche differentiation may depend on spatial scale and grain of the analyses (e.g. Kirchheimer et al. 2016), we collected data at two different spatial scales, i.e. regional and landscape. Specifically, we tested (i) whether diploid and tetraploid cytotypes differ from each other in their distribution in geographic and ecological space, and consequently (ii) whether niche differentiation between cytotypes differs at regional and landscape scales.

## Material and methods

### *Study area*

We selected southern Moravia (Czech Republic) as it is a typical central-European alluvial landscape with a high diversity and fine-scale heterogeneity of different types of vegetation. Both diploid and tetraploid cytotypes of *Urtica dioica* occur in this north-western part of the Pannonian biogeographical region (Rejlová et al. 2019). Southern Moravia is a region of thermophilous flora, with mean annual temperatures of 9–10 °C and annual precipitation between 450–550 mm (Tolasz et al. 2007). It harbours both flat lowlands and steep hillsides, the former characterized by floodplain forests and meadows, wetlands and sandy grasslands, the latter by thermophilous oak forests, oak-hornbeam forests, ravine forests and steppe grasslands.

### *Field sampling*

To asses and compare the strength of niche differentiation and geographical distribution of cytotypes at different spatial scales, we collected plants at two spatial scales, namely regional covering southernmost Moravia and landscape providing detailed sampling in selected landscape transect (Fig. 1).

The regional sampling covers an area of ~1200 km$^2$ (defined by coordinates 48.97°N, 16.58°E; 48.97°N, 17.30°E and the Czech state border) and provides data about cytotype distribution over a large geographic area and different types of vegetation. We partially used previous cytogeographic sampling (91 sites sampled; Rejlová et al. 2019), which was further enlarged to achieve a more representative coverage of the main types of vegetation (36 sites). Altogether 127 sites were sampled representatively covering the habitat diversity. One plant per site/population was collected.

To study small-scale habitat affinities and niche breadth of the cytotypes a transect covering 9.1 km$^2$ was established in the landscape in southern Moravia within the area covered by the regional scale sampling. This transect goes from the top of the Pavlovské vrchy hills to Křivé jezero lake (see Fig. 1) and includes very fine-scale vegetation heterogeneity (including assumed primary habitats of *U. dioica* diploids, i.e. lowland floodplain forests and ravine forests, which here are situated very close together). A total of 291 sites were sampled and one plant per site collected. Although the landscape scale was established within the regional one, it consists of separately collected samples. Due to the fine-scale vegetation heterogeneity and highly clustered distribution within the area, our sampling aimed to include the majority of the populations/clones. Selected herbarium specimens are preserved in herbarium PRC.

A closely related and morphologically very similar species *U. kioviensis* Rogow., which hybridizes with *U. dioica* (Rejlová & Urfus 2018) does not occur within the landscape scale and even at the regional scale it is very rare. As the hybrids can be easily identified using flow cytometry (the genome size of parental species distinctly differs; Rejlová et al. 2019) we can exclude their presence among our samples.

### *Flow cytometry*

Ploidy level of fresh intact leaf tissue (max 48 hours after collecting) was subsequently determined using flow cytometry (Partec CyFlow ML equipped with a 365-nm UV LED, DAPI staining). We followed a simplified two-step protocol (Doležel et al. 2007). Because of the high incidence of endopolyploid tissues we used petioles (minimal portion of reduplicated tissue), which were chopped together with the internal reference standard *Bellis perennis* L. (2C = 3.38 pg; Schönswetter et al. 2007) using a razor blade in a plastic Petri dish containing 500 µl of ice-cold buffer Otto I (0.1-M monohydrate citric acid and 0.5% Tween 20). The suspension was then filtered through a 42-µm nylon mesh, and the isolated nuclei stained for 5 minutes with 1 ml of the buffer Otto II (0.4 M $Na_2HPO_4 \cdot 12H_2O$) supplemented with fluorochrome 4',6-diamidino-2-phenylindole (DAPI; final concentration 4 µg ml$^{-1}$) and ß-mercaptoethanol (final concentration 2 µl ml$^{-1}$). Optimization of flow cytometric analyses was based on our previous cytogeographic study (Rejlová et al. 2019).

### *Terrain analysis*

To explore whether diploid and tetraploid individuals of *Urtica dioica* differ in their environmental requirements and occupied niche space, we compared their distribution along a topographically determined gradient of soil moisture availability. We focused on soil moisture,

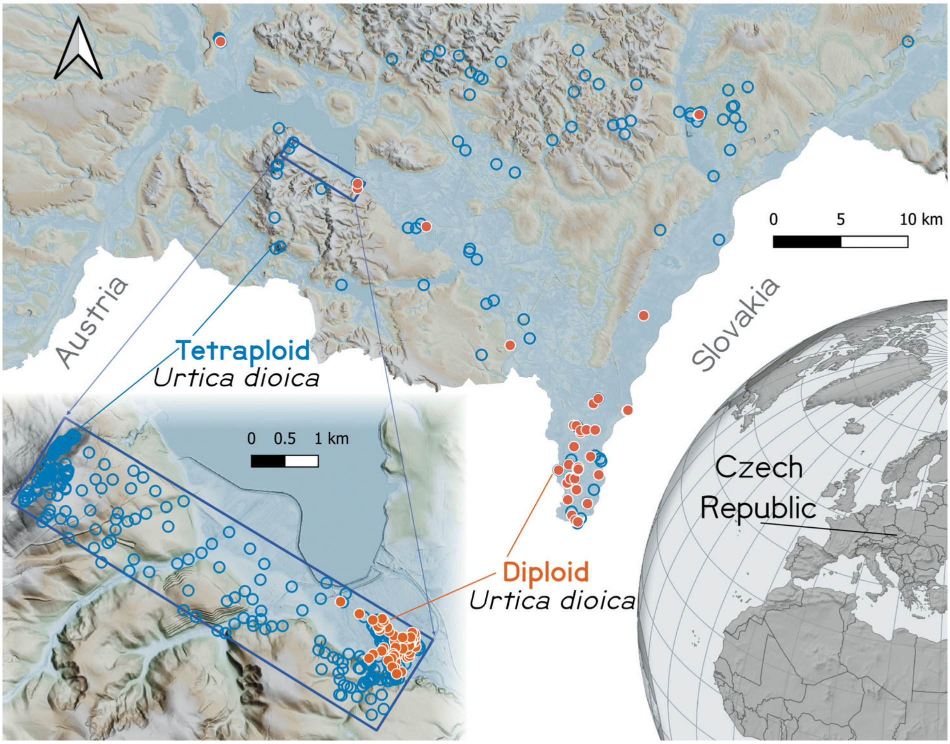

Fig. 1. – Distribution of cytotypes of *Urtica dioica* in southern Moravia in the Czech Republic. The main map shows distribution of the 127 individuals sampled at the regional scale and the inset map the detailed sampling at the landscape scale (291 individuals sampled). The background shows the topographic proxy for soil moisture called Height Above the Nearest Drainage (HAND) calculated from the digital terrain model at a 10 m resolution. The blue areas on the map (i.e. log10 transformed HAND values below 0.5) can be interpreted as potentially suitable for diploid plants, and the blue colour saturation approximates the occurrence probability of the diploid plants. Map was created using the Free and Open Source QGIS programme.

because previous studies indicate that the soil moisture requirements of the cytotypes differ (Rejlová et al. 2019).

Within the region with a similar macroclimate, local moisture availability is mainly determined by land-surface topography (Moore et al. 1991, Kopecký & Čížková 2010). Therefore, we used high-resolution digital terrain model to calculate a topographic proxy for soil moisture, namely Height Above Nearest Drainage (HAND). HAND expresses the difference in height of the focal site and the nearest stream, river or lake to which the water from the focal site flows (Crave & Gascuel-Odoux 1997, Chaplot et al. 2000, Rennó et al. 2008).

In the relatively flat terrain in southern Moravia, HAND is a more plausible topographic proxy for soil moisture than the widely used topographic wetness index, which is better suited for regions in which topography is highly variable (Kopecký et al. 2021). A low HAND indicates that the focal site is only slightly higher than the local water drainage and it is likely the potential soil moisture is high. In low relief landscapes, HAND is closely related to vegetation patterns and plant distribution (Schietti et al. 2013, Moulatlet et al. 2014).

To calculate HAND, we used a national LiDAR-based digital terrain model resampled at a 10 m resolution using B-Spline interpolation in SAGA GIS (Conrad et al. 2015). We used a 10 m resolution as a reasonable compromise between accurate topographic information and uncertainty in the original plant position measured using GPS. As the best available approximation of the channel network, we used high-resolution channel network provided by the national database of water drainage data (http://www.dibavod.cz).

We calculated HAND using the Vertical Distance to Channel Network tool in SAGA GIS (Conrad et al. 2015). This first interpolates a channel network base level altitude and then subtracts this base level from the original altitudes of the digital terrain model. The implementation in SAGA GIS thus provides a more realistic pattern than the original HAND calculation (Nobre et al. 2011), which often produces sharp transitions at watershed boundaries.

To compare the distribution of the cytotypes along a gradient in soil moisture, we calculated kernel density estimates of the distribution of each cytotype along log10 transformed HAND values. To test the robustness of the observed pattern, we estimated the kernel densities of 200 bootstrapped samples at each scale. Specifically, we used random resampling with replacement (i.e. bootstrapping) applied to the original data to produce randomly assembled dataset of 100 individuals at the regional and 200 at the landscape scale and repeated this procedure 200 times for each scale. We compared the distribution of both cytotypes along a gradient of topographic moisture both visually using kernel densities (Fig. 2) and statistically by calculating the P-value as a proportion of the bootstrapped samples with higher mean HAND for diploid than tetraploid plants (Fieberg et al. 2020).

### *Habitat analysis*

To explore whether diploid and tetraploid individuals of *Urtica dioica* differ in their habitat preferences, we compared the distribution of the individuals among habitats. As standardized information about the type of habitat at each location, we used the vector layer of the consolided layer of ecosystems of the Czech Republic based on detailed field mapping of natural habitats and the satellite-based classification of anthropogenic habitats (Hönigová & Chobot 2014). For each site, we extracted type of habitat by intersecting GPS coordinates of the sites with the habitat layer within the open-source QGIS 4.14 (http://www.qgis.org).

We used Fisher's exact test to evaluate the difference between diploid and tetraploid occurrences in all habitats with at least 10 individuals. To explore whether the habitat associations differ at landscape and regional scales, we analysed both datasets separately.

We analysed and visualized the data in R, v. 4.0.2 (R Core Team 2020) with the R packages ggplot2 (Wickham 2016), reshape2 (Wickham 2007), dplyr (Wickham et al. 2020) and patchwork (Pedersen 2020).

## Results

Flow-cytometric analyses revealed 93 diploids and 325 tetraploids (at regional scale: 42 diploids, 85 tetraploids; and landscape scale: 51 diploids and 240 tetraploids; see Electronic Appendix 1). We have not detected any other ploidy level or putative aneuploid.

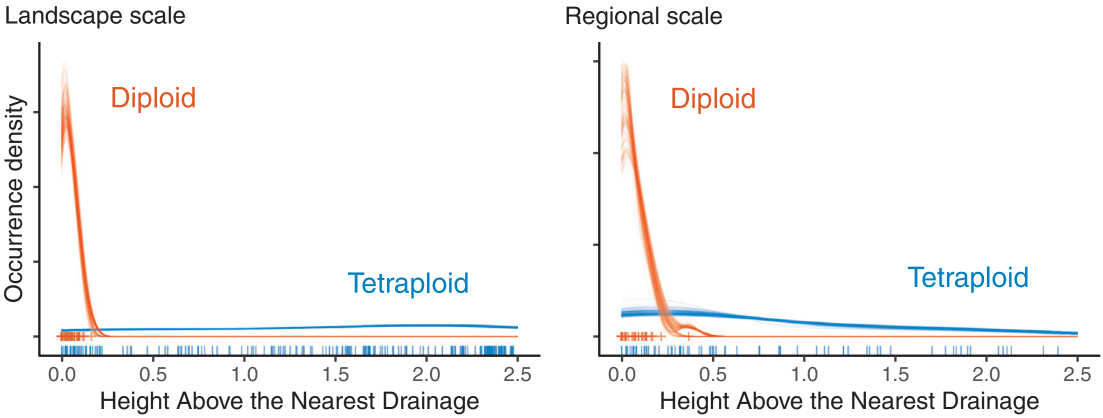

Fig. 2. – Diploid *Urtica dioica* occurs only at the topographically wetter parts of the gradient sampled, while tetraploid plants have a much broader niche and occur along the whole gradient, both at landscape and regional scales. The lines represent kernel density estimates based on 200 bootstrapped samples at each scale and bottom rug shows the observed occurrence of each ploidy level along the gradient of soil wetness approximated by the Height Above the Nearest Drainage.

Coefficient of variation of all flow-cytometric analyses (peaks of histograms) did not exceed 5%.

Diploid *Urtica dioica* occurred only at topographically wetter parts of the gradient sampled and this pattern was very similar at both the landscape and regional scales (Fig. 2). In contrast, tetraploid *Urtica dioica* occurred along the whole wetness gradient at both scales (Fig. 2). Not surprisingly, the differences between the cytotypes were highly statistically significant both at landscape (bootstrap test, $P < 0.005$) and regional ($P < 0.005$) scales.

Diploid and tetraploid plants also showed distinct habitat affinities (Fig. 3). The differences in habitat affinities between diploid and tetraploid plants were statistically highly significant both at the landscape ($P = 0.0005$) and regional ($P = 0.0017$) scales. Moreover, the pattern in habitat affinities was similar at both scales (Fig. 3).

At the landscape scale, there were nine habitats for which at least 10 individuals were sampled (Fig. 3a). In forests, both diploids and tetraploids occurred in alluvial and wet forests and managed broadleaved forests. However, no diploids were found in oak and oak-hornbeam forests and ravine forests. In non-forest habitats, both cytotypes occurred in alluvial and wet meadows and wetlands and riverbanks, while no diploids occurred in actively managed meadows, human settlements and vineyards.

At the regional scale, there were only three habitats for which at least 10 individuals were sampled (Fig. 3). While both cytotypes were common in alluvial and wet forests, diploids were rare in oak and oak-hornbeam forests and actively managed meadows (only two individuals sampled in each habitat).

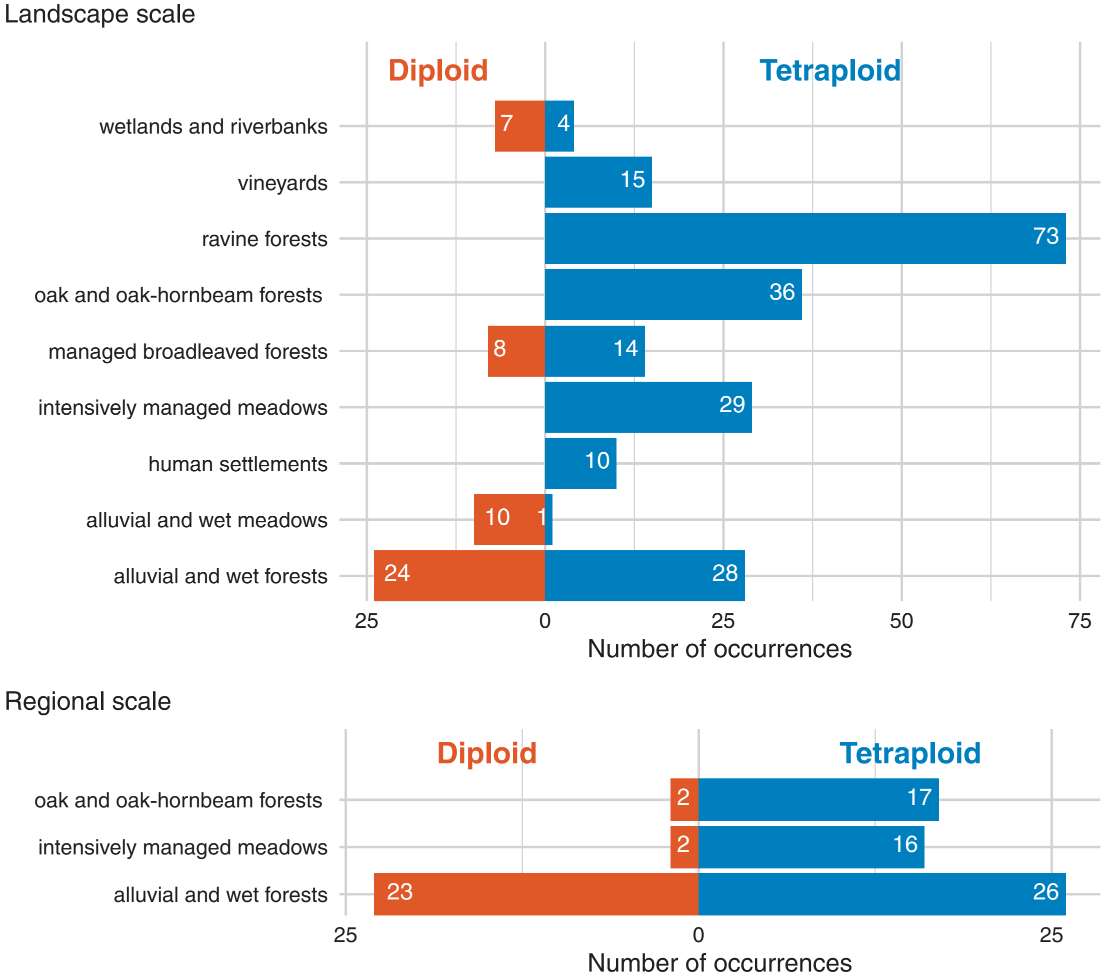

Fig. 3. – Habitat affinities of tetraploid and diploid individuals of *Urtica dioica*. The figure shows the number of samples of each ploidy level within each habitat for which at least 10 individuals were sampled at both the landscape and regional scales. Numbers at the top of the bars indicate the number of individuals sampled at the focal habitat separately for each cytotype.

## Discussion

We found intraspecific ecological niche divergence in *Urtica dioica*. While diploids having a restricted geographical distribution and occupying a narrow range of habitats, tetraploids are widespread ecological generalists with a wide niche breadth. The current distribution of diploids is constrained by soil moisture requirements and habitat affinity at both regional and landscape scales. These novel findings have important implications for ecological studies that assume different ploidies within individual species as functionally identical and species distribution modelling that neglect the very different environmental and geographical distributions of the plants with different ploidy levels.

### *Regional and landscape scales and the possibility of generalization*

Since ravine forests, one of the assumed primary habitats of diploid *U. dioica*, were under-represented at the regional scale, we included the landscape scale at which both central-European natural habitats of *U. dioica* (alluvial and ravine forests) occurred in close proximity. Nevertheless, detailed sampling in ravine forests did not reveal any diploid plants. Thus, the overall pattern at both scales was analogous and may provide an opportunity to further extrapolate our results.

The relatively small spatial scale of our study raises doubts about whether our findings can be generalized over the whole geographical range of *U. dioica* diploids and tetraploids. We expect a similar pattern will be found throughout the geographical range of diploids in central Europe (often assigned to *U. d.* subsp. *subinermis*), where they are confined to large lowland river floodplains (e.g., Rhein, Elbe/Labe, Donau, Morava, Odra), very similar to that recorded in southern Moravia. It is likely that the same holds also for the alluvial deposits of the Sava River in Croatia. In contrast, our results probably cannot be applied to northern Europe, where habitats and climatic conditions are different, and also to the Po river basin in northern Italy, where Rejlová et al. (2019) reported diploids (assigned to *U. d.* subsp. *pubescens*) commonly inhabiting exclusively highly synanthropic and strongly human-affected banks of water channels. Finally, the Anatolian diploids (*U. d.* subsp. *kurdistanica* Chrtek) occur in the alpine zone (Weigend 2006).

### *Niche evolution and realized niches*

Our results support the theory that polyploidization broadens ecological niches of species by enabling them to thrive in a wider range of environmental conditions and increasing their ecological and evolutionary flexibility. The most up-today evidence, however, comes from a comparison of climatic preferences, less often also whether other soil and other environmental factors are important, as in the *Claytonia perfoliata* Donn ex Willd. complex (McIntyre 2012), *Tolmiea* Torrey et A. Gray (Visger et al. 2016), *Paspalum intermedium* Munro ex Morong (Karunarathne et al. 2018) and *Dianthus broteri* Boiss. et Reut. (López-Jurado et al. 2019). *Urtica dioica* polyploids exceed the range of soil moisture requirements and habitat preferences of diploids, factors currently much less often used in this kind of comparison. Similar diploid-polyploid habitat differentiation is described in the grass *Deschampsia cespitosa* (L.) P. Beauv. in Britain (Rothera & Davy 1986), *Dactylis glomerata* L. in Spanish Galicia (Lumaret et al. 1987), *Achillea borealis* Bong. in California (Ramsey 2011) and *Solidago gigantea* Aiton (Schlaepfer et al. 2008), and a more complicated pattern with partly overlapping cytotypes in *Senecio carniolicus* Willd. in the European Alps (Sonnleitner et al. 2010).

Broader ecological tolerance of tetraploids of *U. dioica* can be explained in at least two ways. Firstly, it can arise due to instantaneous changes associated with whole-genome duplication (WGD) (Otto & Whitton 2000, Comai 2005). This scenario might be supported by the fact that genetic studies (Rejlová et al. 2021) did not reveal a clear structure in *U. dioica*, cytotypes that cannot be separated from each other. An alternative explanation suggests divergence through selection after WGD (Bretagnolle & Lumaret 1995, Weiss-Schneeweiss et al. 2013). Furthermore, a broader ecological tolerance of tetraploids might rely on their possible recurrent polytopic origin and thus the existence of more polyploid lineages, which are likely to have different ecological adaptations.

Furthermore, the adaptive potential might be enhanced by hybridization between these lineages.

The current disjunct distribution (Rejlová et al. 2019) and narrow ecological tolerance of diploids can be interpreted in at least two ways. Firstly, diploids occupied a broad spectrum of habitats, from which they were competitively excluded by tetraploids, whose phenotypic changes (Balao et al. 2011) helped them colonize environments where there is strong competition, as has been suggested, for example, for *Brachypodium distachyon* (L.) P. Beauv. in southern Spain (Rey et al. 2017). Unfortunately, there is no data on inter-cytotype competition in *U. dioica*. At the population scale, tetraploids might profit from faster rhizome growth, as the seeds are of low importance for short-distance propagation (Rosnitschek-Schimmel 1983). Secondly, a large-scale destruction of European alluvial forests (or at least changes in their dynamics, e.g. in the water regime and habitat continuity) in the past might have played a role. However, there are still regions with large and well-preserved floodplain forests with no evidence of diploids. Therefore, diploids might always have had disjunct distributions partly shaped by long-distance dispersal events. Unfortunately, paleoecology, which can provide extremely important information on historical range dynamics (also in *Urtica*, see Wolters et al. 2005) is based on pollen analysis and macrofossil remains, which cannot help here, as the pollen grains and seeds of cytotypes of *U. dioica* do not differ in morphology (T. Urfus et al., personal observation).

A comparison of the recent colonization ability of both cytotypes (2x, 4x) suffers from a shortage of reliable data. Although there is data on many biological traits of *Urtica dioica* (Šrůtek & Teckelmann 1998, Taylor 2009 and citations therein), it is often not assigned to a particular cytotype. Therefore, it is still not feasible to evaluate the role of seed dispersal, competition, demography and environment for particular ploidy levels. Based on the morphological similarity of diploid and tetraploid seeds (Rejlová et al. 2021), it is likely they have similar dispersal strategies (herbivores, wind, water; Taylor 2009, Lepková et al. 2018, Wu et al. 2018), allowing diploid seeds to disperse into habitats close to floodplain forests. The absence of adult diploid plants in these habitats is thus most likely affected by lower seed germination, competition and/or ecological adaptations. Otherwise, the highly competitive ability of (most likely tetraploid) *Urtica dioica* (Prach & Wade 1992) may be reduced by grasses (Bassett et al. 1977) or infertile soil, where its potential growth rate is very low (Rorison 1967). Environmental limitations of diploids might include their requirement for a shallow water table (most diploids occurred less than 10 m above the permanent water table), and/or for a particular type of soil (e.g. deep clay soils of floodplain forests). However, similar levels of soil moisture can occur in habitats outside floodplain forests. Finally, the small fragmented populations of diploids might suffer from decreased genetic diversity caused by genetic drift. Inbreeding depression resulting lower fitness of progeny in other habitats seems to play only a marginal role in this dioecious species, although it is not completely excluded. In contrast, the man-induced spread (it is regarded as anthropogenic indicator by paleoecologists) in Europe emphasized by various authors (e.g. Roberts & Boddrel 1984, Ellenberg 1988, Grime et al. 1989), mainly in ruderal sites (rubbish heaps, construction sites, surroundings of mountain chalets, roads ditches etc.) most likely refers to the tetraploid cytotype.

Our results strongly contradict the phylogenetic niche conservatism theory, i.e. the tendency for lineages to preserve ancestral niche-related traits (Crisp & Cook 2012), leading to the absence of ecological niche differentiation between cytotypes. However, phylogenetic niche conservatism is supposed to be more common in autopolyploids due to their close relatedness to their diploid progenitors (Burns & Strauss 2011, Glennon et al. 2014).

Finally, the results have important implications for environmental niche modelling and associated species distribution models. These extremely popular methods assume that species are ecologically homogenous entities and therefore occurrences recorded at the species level can be used to model a species environmental niche and project this niche into geographical space in order to predict a species distribution (Elith & Leathwick 2009). However, our results clearly show that this crucial assumption is invalid even for one of the most common vascular plant species. Although the frequency of intraspecific variability in ecological preferences is unknown, our results clearly show that environmental niche modelling must account for within-species variability in order to produce better distributional models.

To sum up, our data clearly show a broad ecological tolerance and consequently a high dispersal potential of the tetraploid cytotype of *Urtica dioica*. In contrast, rare and endangered diploids should be of interest to conservationists.

See www.preslia.cz for Electronic Appendix 1

## Acknowledgements

The research was supported by the Czech Science Foundation (project no. GA 17-20201S) and the Czech Academy of Sciences (long-term research development project no. RVO 67985939). The consolidated layer of ecosystems developed by the Nature and Landscape Protection Agency of the Czech Republic and CzechGlobe was created within the grant project TD010066 “Integrated assessment of ecosystem services in the Czech Republic” provided by the Technology Agency of the Czech Republic.

## Souhrn

Modelování ekologických nik a následné predikce rozšíření druhů v prostoru zpravidla nezohledňují variabilitu v rámci druhu, zejména ve vztahu k polyploidii a heteroploidním komplexům. Abychom zjistili, zda je tato variabilita důležitá, testovali jsme, zda je tetraploidní (4x) cytotyp charakterizován širší ekologickou nikou než diploidní (2x) a zda jsou zjištěné odlišnosti mezi cytotypy nezávislé na prostorové škále. Jako modelový druh jsme vybrali kopřivu dvoudomou (*Urtica dioica*), vytrvalou bylinu se dvěma převládajícími cytotypy (2x a 4x). Zaměřili jsme se na jižní Moravu jakožto oblast se značnou diverzitou vegetačních typů, ve kterých se současně vyskytuje jak diploidní, tak tetraploidní cytotyp *U. dioica.* Pro stanovení geografického rozšíření, ekologických preferencí a stanovištních vazeb jsme sbírali jedince *U. dioica* na dvou odlišných prostorových škálách: regionální (~ 1 200 km$^2$) a krajinné (9,1 km$^2$). Ploidní úroveň všech vzorků sbíraných na obou škálách byla stanovena pomocí průtokové cytometrie. Následně jsme zhodnotili rozšíření cytotypů v geografickém i ekologickém prostoru a srovnali vazbu diploidů a tetraploidů na určitá stanoviště. Ploidní analýzy odhalily dohromady 93 diploidních a 325 tetraploidních vzorků (42 diploidů, 85 tetraploidů na regionální škále a 51 diploidů a 240 tetraploidů na krajinné). Diploidní rostliny se vyskytovaly pouze v topograficky vlhčích částech krajiny, a to jak na krajinné, tak na regionální úrovni. Naproti tomu tetraploidi se vyskytovali podél celého vlhkostního gradientu na obou škálách. Diploidní a tetraploidní cytotypy se lišily rovněž na úrovni svých stanovištních preferencí. Diploidi se relativně častěji vyskytovali v lužních a mokřadních lesích a v mokřadech a pobřežní vegetaci, zatímco tetraploidi upřednostňovali doubravy a dubohabřiny, suťové lesy, aktivně obhospodařované louky, lidská sídla a vinice. Naše výsledky jasně ukázaly diferenciaci ekologických nik diploidního a tetraploidního cytotypu *Urtica dioica.* Tetraploidi mají širší ekologickou toleranci a větší geografické rozšíření, zatímco diploidi se vyskytují jen v úzkém rozmezí ekologických podmínek a jejich rozšíření je omezeno na oblasti s vysokou hladinou spodní vody v nivách řek. Naše výsledky jsou důležité, protože vnitrodruhová diferenciace na

úrovni ploidie je známa u mnoha druhů rostlin, ale není přitom zohledňována v ekologickém modelování. Naše výsledky ale jasně ukazují, že jednotlivé cytotypy jednoho druhu mají zásadně odlišné ekologické nároky a geografické rozšíření.

## References

Balao F., Herrera J. & Talavera S. (2011) Phenotypic consequences of polyploidy and genome size at the microevolutionary scale: a multivariate morphological approach. – New Phytologist 192: 256–265.

Barker M. S., Arrigo N., Baniaga A. E., Li Z. & Levin D. A. (2016) On the relative abundance of autopolyploids and allopolyploids. – New Phytologist 210: 391–398.

Bassett I. J., Crompton C. W. & Woodland D. W. (1977) The biology of Canadian weeds. 21. *Urtica dioica* L. – Canadian Journal of Plant Science 57: 491–498.

Bretagnolle F. & Lumaret R. (1995) Bilateral polyploidization in *Dactylis glomerata* L. subsp. *lusitanica*: occurrence, morphological and genetic characteristics of first polyploids. – Euphytica 84: 197–207.

Burns J. H. & Strauss S. Y. (2011) More closely related species are more ecologically similar in an experimental test. – Proceedings of the National Academy of Sciences of the United States of America 108: 5302–5307.

Castro S., Loureiro J., Procházka T. & Münzbergová Z. (2012) Cytotype distribution at a diploid-hexaploid contact zone in *Aster amellus* (*Asteraceae*). – Annals of Botany 110: 1047–1055.

Chaplot V., Walter C. & Curmi P. (2000) Improving soil hydromorphy prediction according to DEM resolution and available pedological data. – Geoderma 97: 405–422.

Comai L. (2005) The advantages and disadvantages of being polyploid. – Nature Reviews Genetics 6: 836–846.

Conrad O., Bechtel B., Bock M., Dietrich H., Fischer E., Gerlitz L., Wehberg J., Wichmann V. & Böhner J. (2015) System for Automated Geoscientific Analyses (SAGA) v. 2.1.4. – Geoscientific Model Development 8: 1991–2007.

Crave A. & Gascuel-Odoux C. (1997) The influence of topography on time and space distribution of soil surface water content. – Hydrological Processes 11: 203–210.

Crisp M. D. & Cook L. G. (2012) Phylogenetic niche conservatism: what are the underlying evolutionary and ecological causes? – New Phytologist 196: 681–694.

Deng B. L., Du W. C., Liu C. L., Sun W. W., Tian S. & Dong H. S. (2012) Antioxidant response to drought, cold and nutrient stress in two ploidy levels of tobacco plants: low resource requirement confers polytolerance in polyploids? – Plant Growth Regulation 66: 37–47.

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

Elith J. & Leathwick J. R. (2009) Species distribution models: ecological explanation and prediction across space and time. – Annual Review of Ecology, Evolution, and Systematics 40: 677–697.

Ellenberg H. (1988) Vegetation ecology of Central Europe. – Cambridge University Press, Cambridge.

Fieberg J. R., Vitense K. & Johnson D. H. (2020) Resampling-based methods for biologists. – PeerJ 2020: e9089.

Glennon K. L., Ritchie M. E. & Segraves K. A. (2014) Evidence for shared broad-scale climatic niches of diploid and polyploid plants. – Ecology Letters 17: 574–582.

Godsoe W., Larson M. A., Glennon K. L. & Segraves K. A. (2013) Polyploidization in *Heuchera cylindrica* (*Saxifragaceae*) did not result in a shift in climatic requirements. – American Journal of Botany 100: 496–508.

Grime J. P., Hodgson J. G. & Hunt R. (1989) Comparative plant ecology. A functional approach to common British species. – Unwin Hyman, London.

Große-Veldmann B. (2016) Systematics, taxonomy, and evolution of *Urtica* L. *(Urticaceae*). – Dissertation, Rheinischen Friedrich-Wilhelms-Universität Bonn, URL: http://hss.ulb.uni-bonn.de/2017/4628/4628.pdf.

Grosse-Veldmann B. & Weigend M. (2015) Weeding the nettles III: named nonsense versus named morphotypes in European *Urtica dioica* L. (*Urticaceae*). – Phytotaxa 208: 239–260.

Henn J. J., Buzzard V., Enquist B. J., Halbritter A. H., Klanderud K., Maitner B. S., Michaletz S. T., Pötsch C., Seltzer L., Telford R. J., Yang Y., Zhang L. & Vandvik V. (2018) Intraspecific trait variation and phenotypic plasticity mediate alpine plant species response to climate change. – Frontiers in Plant Science 9: 1548.

Henning T., Quandt D., Grosse-Veldmann B., Monro A. & Weigend M. (2014) Weeding the nettles II: a delimitation of *Urtica dioica* L. (*Urticaceae*) based on morphological and molecular data, including a rehabilitation of *Urtica gracilis* Ait. – Phytotaxa 162: 61–83.

Hönigová I. & Chobot K. (2014) Jemné předivo české krajiny v GIS: konsolidovaná vrstva ekosystémů [A fine patchwork of the Czech landscape in GIS: a consolidated layer]. – Ochrana Přírody 69: 27–30.

Jung V., Violle C., Mondy C., Hoffmann L. & Muller S. (2010) Intraspecific variability and trait-based community assembly. – Journal of Ecology 98: 1134–1140.

Kao R. H. (2007) Asexuality and the coexistence of cytotypes. – New Phytologist 175: 764–772.

Karunarathne P., Schedler M., Martínez E. J., Honfi A. I., Novichkova A. & Hojsgaard D. (2018) Intraspecific ecological niche divergence and reproductive shifts foster cytotype displacement and provide ecological opportunity to polyploids. – Annals of Botany 121: 1183–1196.

Kirchheimer B., Schinkel C. C. F., Dellinger A. S., Klatt S., Moser D., Winkler M., Lenoir J., Caccianiga M., Guisan A., Nieto-Lugilde D., Svennig J.-C., Thuiller W., Pascal V., Willner W., Zimmermann N. E., Hörandl E. & Dullinger S. (2016) A matter of scale: apparent niche differentiation of diploid and tetraploid plants may depend on extent and grain of analysis. – Journal of Biogeography 43: 716–726.

Kliber A. & Eckert C. G. (2005) Interaction between founder effect and selection during biological invasion in an aquatic plant. – Evolution 59: 1900–1913.

Kolář F., Čertner M., Suda J., Schönswetter P. & Husband B. C. (2017) Mixed-ploidy species: progress and opportunities in polyploid research. – Trends in Plant Science 22: 1041–1055.

Kolář F., Lučanová M., Vít P., Urfus T., Chrtek J., Fér T., Ehrendorfer F. & Suda J. (2013) Diversity and endemism in deglaciated areas: ploidy, relative genome size and niche differentiation in the *Galium pusillum* complex (*Rubiaceae*) in Northern and Central Europe. – Annals of Botany 111: 1095–1108.

Kopecký M. & Čížková Š. (2010) Using topographic wetness index in vegetation ecology: does the algorithm matter? – Applied Vegetation Science 13: 450–459.

Kopecký M., Macek M. & Wild J. (2021) Topographic Wetness Index calculation guidelines based on measured soil moisture and plant species composition. – Science of the Total Environment 757: 143785.

Lepková B., Horčičková E. & Vojta J. (2018) Endozoochorous seed dispersal by free-ranging herbivores in an abandoned landscape. – Plant Ecology 219: 1127–1138.

Levin D. A. (2002) The role of chromosomal change in plant evolution. – Oxford University Press, Oxford.

López-Jurado J., Mateos-Naranjo E. & Balao F. (2019) Niche divergence and limits to expansion in the high polyploid *Dianthus broteri* complex. – New Phytologist 222: 1076–1087.

Lowry E. & Lester S. E. (2006) The biogeography of plant reproduction: potential determinants of species' range sizes. – Journal of Biogeography 33: 1975–1982.

Lumaret R., Guillerm J. L., Delay J., Ait Lhaj Loutfi A., Izco J. & Jay M. (1987) Polyploidy and habitat differentiation in *Dactylis glomerata* L. from Galicia (Spain). – Oecologia 73: 436–446.

Martin S. L. & Husband B. C. (2009) Influence of phylogeny and ploidy on species ranges of North American angiosperms. – Journal of Ecology 97: 913– 922.

McIntyre P. J. (2012) Polyploidy associated with altered and broader ecological niches in the *Claytonia perfoliata* (*Portulacaceae*) species complex. – American Journal of Botany 99: 655–662.

Moore I. D., Grayson R. B. & Ladson A. R. (1991) Digital terrain modelling: a review of hydrological, geomorphological, and biological applications. – Hydrological Processes 5: 3–30.

Moulatlet G. M., Costa F. R. C., Rennó C. D., Emilio T. & Schietti J. (2014) Local hydrological conditions explain floristic composition in lowland Amazonian forests. – Biotropica 46: 395–403.

Nobre A. D., Cuartas L. A., Hodnett M., Rennó C. D., Rodrigues G., Silveira A., Waterloo M. & Saleska S. (2011) Height Above the Nearest Drainage: a hydrologically relevant new terrain model. – Journal of Hydrology 404: 13–29.

Otto S. P. & Whitton J. (2000) Polyploid incidence and evolution. – Annual Review of Genetics 34: 401–437.

Pandit M. K., Pocock M. J. O. & Kunin W. E. (2011) Ploidy influences rarity and invasiveness in plants. – Journal of Ecology 99: 1108–1115.

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

Pedersen T. L. (2020) patchwork: The Composer of Plots. R package version 1.1.1. – https://CRAN.R-project.org/package=patchwork.

Prach K. & Wade P. M. (1992) Population characteristics of expansive herbs. – Preslia 64: 45–51.

R Core Team (2020) R: A language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, https://www.R-project.org/.

Ramsey J. (2011) Polyploidy and ecological adaptation in wild yarrow. – Proceedings of the National Academy of Sciences of the United States of America 108: 7096–7101.

Ramsey J. & Schemske D. W. (1998) Pathways, mechanisms, and rates of polyploid formation in flowering plants. – Annual Review of Ecology and Systematics 29: 467–501.

Ramsey J. & Schemske D. W. (2002) Neopolyploidy in flowering plants. – Annual Review of Ecology and Systematics 33: 589–639.

Rejlová L., Böhmová A., Chumová Z., Hořčicová Š., Josefiová J., Schmidt F. A., Trávníček P., Urfus T., Vít P. & Chrtek J. (2021) Disparity between morphology and genetics in *Urtica dioica* (*Urticaceae*). – Botanical Journal of the Linnean Society 195: 606–621.

Rejlová L., Chrtek J., Trávníček P., Lučanová M., Vít P. & Urfus T. (2019) Polyploid evolution: the ultimate way to grasp the nettle. – PLoS ONE 14: e0218389.

Rejlová L. & Urfus T. (2018) *Urtica kioviensis* Rogow. × *Urtica dioica* L. – In: Lustyk P. & Doležal J. (eds), Additamenta ad floram Reipublicae Bohemicae. XVI., Zprávy České Botanické Společnosti 53: 31–112.

Rennó C. D., Nobre A. D., Cuartas L. A., Soares J. V., Hodnett M. G., Tomasella J. & Waterloo M. J. (2008) HAND, a new terrain descriptor using SRTM-DEM: mapping terra-firme rainforest environments in Amazonia. – Remote Sensing of Environment 112: 3469–3481.

Rey P. J., Manzaneda A. J. & Alcantara J. M. (2017) The interplay between aridity and competition determines colonization ability, exclusion and ecological segregation in the heteroploid *Brachypodium distachyon* species complex. – New Phytologist 215: 85–96.

Rice A., Šmarda P., Novosolov M., Drori M., Glick L., Sabath N., Meiri S., Belmaker J. & Mayrose I. (2019) The global biogeography of polyploid plants. – Nature Ecology and Evolution 3: 265–273.

Roberts H. A. & Boddrel I. E. (1984) Seed survival and seasonal emergence of seedlings of some ruderal plants. – Journal of Applied Ecology 21: 617–628.

Rorison I. H. (1967) A seedling bioassay on some soils in the Sheffield area. – Journal of Ecology 55: 725–741.

Rosnitschek-Schimmel I. (1983) Biomass and nitrogen partitioning in a perennial and an annual nitrophilic species of *Urtica*. – Zeitschrift für Pflanzenphysiologie 109: 215–225.

Rothera S. L. & Davy A. J. (1986) Polyploidy and habitat differentiation in *Deschampsia cespitosa*. – New Phytologist 102: 449–467.

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

Saleh B., Allario T., Dambier D., Ollitrault P. & Morillon R. (2008) Tetraploid citrus rootstocks are more tolerant to salt stress than diploid. – Comptes Rendus Biologies 331: 703–710.

Schietti J., Emilio T., Rennó C. D., Drucker D. P., Costa F. R. C., Nogueira A., Baccaro F. B., Figueiredo F., Castilho C. V., Kinupp V., Guillaumet J.-L., Garcia A. R. M., Lima A. P. & Magnusson W. E. (2013) Vertical distance from drainage drives floristic composition changes in an Amazonian rainforest. – Plant Ecology and Diversity 7: 1–13.

Schlaepfer D. R., Edwards P. J., Semple J. C. & Billeter R. (2008) Cytogeography of *Solidago gigantea* (*Asteraceae*) and its invasive ploidy level. – Journal of Biogeography 35: 2119–2127.

Schönswetter P., Suda J., Popp M., Weiss-Schneeweiss H. & Brochmann C. (2007) Circumpolar phylogeography of *Juncus biglumis* (*Juncaceae*) inferred from AFLP fingerprints, cpDNA sequences, nuclear DNA content and chromosome numbers. – Molecular Phylogeny and Evolution 42: 92–103.

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

Sonnleitner M., Flatscher R., Escobar García P., Rauchová J., Suda J., Schneeweiss G. M., Hülber K. & Schönswetter P. (2010) Distribution and habitat segregation on different spatial scales among diploid, tetraploid and hexaploid cytotypes of *Senecio carniolicus* (*Asteraceae*) in the Eastern Alps. – Annals of Botany 106: 967–977.

Spoelhof J. P., Soltis P. S. & Soltis D. E. (2017) Pure polyploidy: closing the gaps in autopolyploid research. – Journal of Systematics and Evolution 55: 340–352.

Šrůtek M. & Teckelmann M. (1998) Review of biology and ecology of *Urtica dioica*. – Preslia 70: 1–19.

Taylor K. (2009) Biological flora of the British Isles: *Urtica dioica* L. – Journal of Ecology 97: 1436–1458.

Theodoridis S., Randin C., Broennimann O., Patsiou T. & Conti E. (2013) Divergent and narrower climatic niches characterize polyploid species of European primroses in *Primula* sect. *Aleuritia*. – Journal of Biogeography 40: 1278–1289.

Tolasz R., Míková T., Valeriánová A. & Voženílek V. (eds) (2007) Atlas podnebí Česka [Climate atlas of Czechia]. – Český hydrometeorologický ústav, Praha & Univerzita Palackého v Olomouci, Olomouc.

Trávníček P., Dočkalová Z., Rosenbaumová R., Kubátová B., Szeląg Z. & Chrtek J. (2011) Bridging global and microregional scales: ploidy distribution in *Pilosella echioides* (*Asteraceae*) in central Europe. – Annals of Botany 107: 443–454.

Van de Peer Y., Mizrachi E. & Marchal K. (2017) The evolutionary significance of polyploidy. – Nature Reviews Genetics 18: 411– 424.

Van Laere K., Franca S. C., Vansteenkiste H., Van Huylenbroeck J., Steppe K. & Van Labeke M. C. (2011) Influence of ploidy level on morphology, growth and drought susceptibility in *Spathiphyllum wallisii*. – Acta Physiologiae Plantarum 33: 1149–1156.

Visger C. J., Germain-Aubrey C. C., Patel M., Sessa E. B., Soltis P. S. & Soltis D. E. (2016) Niche divergence between diploid and autotetraploid *Tolmiea*. – American Journal of Botany 103: 1396–1406.

Weigend M. (2005) Die Erben Pokornys – ein Beitrag zur Abgrenzung der Sippen *Urtica galeopsifolia* und *Urtica pubescens* in Mittel- und Osteuropa. – Hoppea – Denkschriften der Regensburgischen Botanischen Gesellschaft 66: 101–118.

Weigend M. (2006) *Urtica dioica* subsp. *cypria*, with a re-evaluation of the *U. dioica* group (*Urticaceae*) in western Asia. – Willdenowia 36: 811–822.

Weiss-Schneeweiss H., Emadzade K., Jang T.-S. & Schneeweiss G. M. (2013) Evolutionary consequences, constraints and potential of polyploidy in plants. – Cytogenetic and Genome Research 140: 137–150.

Wickham H. (2007) Reshaping data with the reshape Package. – Journal of Statistical Software 21: 1–20.

Wickham H. (2016) ggplot2: elegant graphics for data analysis. – Springer-Verlag, New York.

Wickham H., François R., Henry L. & Müller K. (2020) dplyr: a grammar of data manipulation. R package version 1.0.0. – https://CRAN.R-project.org/package=dplyr.

Wolters S., Bittmann F. & Kummer V. (2005) The first subfossil records of *Urtica kioviensis* Rogow. and their consequences for palaeoecological interpretations. – Vegetation History and Archaeobotany 14: 518–527.

Wood T. E., Takebayashi N., Barker M. S., Mayrose I., Greenspoon P. B. & Rieseberg L. H. (2009) The frequency of polyploid speciation in vascular plants. – Proceedings of the National Academy of Sciences of the United States of America 106: 13875–13879.

Wu Z.-Y., Liu J., Provan J., Wang H., Chen C.-J., Cadotte M. W., Luo Y.-H., Amorim B. S., Li D.-Z. & Milne R. I. (2018) Testing Darwin's transoceanic dispersal hypothesis for the inland nettle family (*Urticaceae*). – Ecology Letters 21: 1515–1529.

Received 24 January 2021
Revision received 1 July 2021
Accepted 17 July 2021