Allium oleraceum in Slovakia: cytotype distribution and ecology

***Allium oleraceum* na Slovensku: rozšíření cytotypů a jejich ekologie**

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The spatial distribution of cytotypes can provide valuable insights into the evolution of polyploid complexes. Previously, only tetraploid *Allium oleraceum* was reported from Slovakia. Analysing 863 individuals from 93 populations from Slovakia revealed an extensive variation in the DNA ploidy levels of *Allium oleraceum* (3x, 4x, 5x and 6x). Of the main cytotypes, the penta- and tetraploids had strongly overlapping distributions, although the pentaploids exhibited a tendency to occur more frequently in the southern and the tetraploids had a tendency to occur in the northern regions of Slovakia. A triploid cytotype was found in one population in the southern part of Slovakia, which is the third locality worldwide for this cytotype. The hexaploid cytotype was rare and sparsely occurred in western and southern Slovakia. Sixteen per cent of the populations sampled consisted of more than one ploidy level; the most common was a combination of penta- and tetraploids. The cytotypes differed with respect to altitude; the tetraploids were found significantly more frequently at higher altitudes than the penta- and hexaploids. When compared with reanalysed altitudinal distribution data from the Czech Republic divided into two geographic areas (Carpathian and Herzynian) the pattern found in the Carpathian part of the Czech Republic was similar to that in Slovakia, with tetraploids at the higher altitudes. The distribution in the Herzynian part (Bohemian Massif) was just the opposite: the tetraploids were more often found at lower altitudes than the penta- and hexaploids. Both tetra- and pentaploid cytotypes occurred in a wide and similar spectrum of habitats, while hexaploids were limited to human-influenced habitats. A local-scale distribution of cytotypes analysed in detail in the Slovak Karst area, showed surprising differences in the distribution of cytotypes on particular karst plains, which can be related to different land uses. Concerning the contrasting altitudinal differentiation of tetraploids in the regions compared, the results suggest that at least two different types of tetraploids occur in Central Europe. The apparent cytotype diversity in the surrounding Slovak Karst area may suggest the existence of a primary contact zone.

K e y w o r d s: *Allium*, Czech Republic, distribution, flow cytometry, habitat differentiation, polyploidy, spatial scales, vertical distribution

Introduction

The use of flow cytometry techniques in plant ecology has strongly changed the potential for studying certain aspects of plant populations (Kron et al. 2007, Hülber et al. 2009, Kubešová et al. 2010, Loureiro et al. 2010, Suda & Pyšek 2010). As a consequence, the number of plant populations that are studied with respect to the variation in their chromosome/genome copy number has increased dramatically. Entirely new distributions of cytotypes are recorded for many species, such as *Oxycoccus* (Suda 2002), *Empetrum* (Suda et al. 2004), *Elytrigia* (Mahelka et al. 2005), *Senecio carniolicus* (Schönswetter et al. 2007, Suda et al. 2007, Sonnleitner et al. 2010) and *Pilosella officinarum* (Mráz et al. 2008) in Central Europe and *Cardamine* (Marhold et al. 2010) in Eastern Asia. This information may be briefly summarized, as follows: the scale of cytotype variation has strongly decreased, the existing pattern is finer than was expected on the basis of chromosome counting and many species are now known to be variable in smaller geographic areas than previously.

One such species, with many cytotypes, is *Allium oleraceum* L., for which four cytotypes (2n = 3x = 24, 2n = 4x = 32, 2n = 5x = 40 and 2n = 6x = 48) are known from Central Europe (e.g. Měsíček & Jarolímová 1992, Krahulcová 2003). Detailed research has shown a complex pattern, both in the distribution and ecological preferences of particular (2n = 4x, 5x and 6x) cytotypes in the Czech Republic (Duchoslav et al. 2010, Šafářová & Duchoslav 2010). In neighbouring Slovakia, surprisingly, only tetraploid plants (2n = 32) are currently reported (Májovský & Murín 1987, Murín et al. 1999, Marhold et al. 2007). Therefore, we started to collect data from Slovakia to compare with the complex pattern found in the Czech Republic within the framework of a project on mapping the cytotype distribution in Europe. The data were not collected in such a systematic way as in the Czech Republic, but the intention was to collect data from a large spectrum of habitats, and, especially, from regions that were previously unexplored. Furthermore, we tried to carry out detailed sampling in some regions of southern Slovakia, where we anticipated the occurrence of cytotypes not previously reported from Slovakia. The questions we addressed were: Is *Allium oleraceum* represented in the area of Slovakia only as the tetraploid cytotype? If not, is there any clear pattern in the distribution of cytotypes, and is there any difference in the ecological preferences of the cytotypes?

Materials and methods

Species studied

Allium oleraceum is a member of sect. *Codonoprasum*, which also includes other species occurring in Central Europe, viz. *Allium flavum*, *A. carinatum*, *A. cirrhosum* and *A. paniculatum* (Stearn 1980, Krahulec & Duchoslav 2010). *Allium oleraceum* is closely related to the other species (e.g. *A. paniculatum*, *A. fuscum*, *A. fusii*, *A. pallens* and *A. podolicum*) in the *Allium paniculatum* group; *A. oleraceum* differs from the other species in this group by the presence of bulbils in its inflorescences. *Allium oleraceum* is most likely a hybrid between sexual members of the *A. paniculatum* group. Levan (1938) reports the production of an *A. oleraceum* that originated from the experimental hybridization between two distinct populations of *A. paniculatum*, most likely, *A. podolicum* and another species from Romania (three different species occur there, *A. paniculatum*, *A. fuscum* and *A. fussii*, cf. Brullo et al. 1996, Ciocarlân 2009). *Allium paniculatum* rarely occurs at several localities in the southern part of Slovakia (Somogyi 1999). *Allium oleraceum* occurs in a broad spectrum of habitats, from natural ones, such as rocks and forests, to anthropic ones, such as the margins of arable land (Duchoslav 2001a, b). This species produces little seed and propagates mainly vegetatively through daughter bulbs and, especially, the bulbils in the inflorescences.

Collection of plants and environmental variables

To characterize the large-scale pattern of distribution, an effort was made to cover the entire area of Slovakia. The area of Slovak Karst, consisting of a complex of huge karst plains and plateaus and their surroundings, was selected for the analysis of the distribution pattern of cytotypes at a local scale. Plants were collected from a wide spectrum of habitats to cover the large ecological variation of the species. Considering the existence of populations with several known cytotypes in the Czech Republic and the local propagation by bulbils and bulblets, we regularly collected several individuals that did not grow in close vicinity to each other, but covered the entire population. In total, we collected 1002 plants from 93 populations. Each population sample consisted of 2–53 plants (an average of 10.8 plants per sample). Sample size was variable and occasionally low because some of the populations sampled were composed of just one or two individuals or clusters of plants. A list of all of the populations included in the analysis, with additional data, is given in Electronic Appendix 1. The plants were transplanted into pots in the experimental garden of the Department of Botany, Palacký University at Olomouc and used later for flow cytometry measurements. Several ecological variables, identical with those used by Duchoslav et al. (2010), were recorded for each site sampled: (i) habitat type was assessed in the field according to the EUNIS habitat classification (Davies et al. 2004). Because of a low frequency of some habitats in the data set, we translated them into one of seven common habitat types (rock, dry grassland, mesic and wet grassland, (semi)natural forest, scrub, planted *Robinia pseudacacia* forest, and arable field and field margins). Correspondence between this and the EUNIS habitat classification is explained in Duchoslav et al. (2010); (ii) populations were classified into two categories, according to the degree of anthropic influence (human-influenced, vegetation strongly influenced or created by man, typically with a high proportion of ruderal or alien species of "habitat naturalness", and natural, natural and seminatural vegetation without strong anthropic influence; examples of human-influenced vegetation represent forests with ruderal or alien species, eutrophicated woody vegetation outside forests, and eutrophicated, intensively managed or disturbed grassland); and (iii) the altitude was recorded via GPS instrumentation on-site or later using the coordinates and the Google Earth application (Google Inc.). The altitude of published localities is that of the centre of a village or of a hill.

The best time to collect this species is April and the first half of May, provided students are able to correctly determine sterile plants. Later in the year, it is impossible to collect sterile individuals in shaded habitats, such as shrubs and forests, as they mostly finish growing in May (Duchoslav 2009). Flowering plants reported in summer are those growing in open, sunny habitats not shaded ones. Thus, the summer distribution does not reflect the full range of habitats occupied by this species.

Ploidy level

Several cultivated plants died before analysis so only two plants per population were analysed in some cases. DNA ploidy level (Suda et al. 2006) and chromosome number were determined using the procedures described in detail by Duchoslav et al. (2010). Briefly, DNA ploidy levels were determined by flow cytometry using the method of internal standardization. The nuclei of the standard and the sample were isolated, stained and analyzed together (Doležel 1991). *Triticum aestivum* cv. 'Saxana' was used as an internal standard and calibrated against plant reference standard *Hordeum vulgare* with 2C DNA 10.43 pg (Doležel et al. 1998). The relative fluorescence intensity of propidium iodide (PI) stained nuclei was analysed using a Partec PAS instrument (Partec GmbH, Münster, Germany) equipped with an argon ion laser (535 nm). Histograms of fluorescence intensity were registered over 512 channels. In each sample, at least 2000 nuclei were analyzed. The ploidy level of each sample was determined by the position of its G_0/G_1 peak relative to the G_0/G_1 peak of the internal standard. Plants for which the number of chromosomes had been counted were used for the specification of internal standard-sample position. The fluorescence ratios between the positions of sample and internal reference standard peaks were 1.18–1.29, 1.40–1.69, 1.74–1.98, 2.02–2.23 for 3x–6x cytotype, respectively. PI staining yielded histograms with coefficients of variance (CV) of both standard and sample below 5% for the majority of the DNA-ploidy measurements (mean CV of standard was 4.22% ± 0.02, mean CV of samples were 4.04% ± 0.06, 4.10% ± 0.04, 4.19% ± 0.03, 4.32% ± 0.03 SE, for 3x–6x cytotype, respectively).

Data analyses

Because they were only observed at a single site, the triploids were excluded from the statistical analyses of habitat types and altitude. In the maps, we also include all of the published data provided in Table 1. These data were not included in the ecological comparisons (except for altitude) because there is insufficient information in the original sources.

Descriptive statistics and statistical tests were done using Statistica 9.0 software (Statsoft Inc.). Generalized linear models with multinomial distribution of dependent variable (habitat types) and logit-link function were used for the analyses of habitat differentiation among cytotypes. Breadth of ecological niche was expressed by Shannon diversity index H using $\log_e$ in the equation (Magurran 2004) based on frequencies of cytotypes in seven habitat types. Because Shapiro-Wilk normality test revealed non-normality of altitude even after various transformations, non-parametric Kruskal-Wallis test followed by multiple comparison Dunn's test were used in this analysis (Zar 1996).

Table 1. – Previously published data on the occurrence of *Allium oleraceum* cytotypes in Slovakia. In all of the original sources, only tetraploids (2n = 32) are reported.

Locality	Latitude (N)	Longitude (E)	Altitude (m)	Source
Strážovské vrchy Mts, Trenčianske Teplice village	48°54'43"	18°09'44"	267	Váchová & Feráková in Löve (1978)
Malá Fatra Mts, Párnica village	49°11'36"	19°11'31"	452	Murín et al. (1999)
Východoslovenská nížina lowlands, Komárany village	48°55'58"	21°38'58"	150	Murín et al. (1999)
Malé Karpaty Mts, Chľmec hill	48°11'43"	17°07'08"	325	Murín et al. (1999)
Chočské and Prosečianske vrchy Mts, Podbiel village, Biela skala	49°18'46"	19°28'53"	664	Murín et al. (1999)
Ipeľsko-rimavská brázda furrow, Šurice village	48°13'56"	19°54'54"	223	Murín et al. (1999)
Malé Karpaty Mts, Devínská Kobyla hill	48°11'41"	16°58'54"	287	Murín et al. (1999)
Brezovské kopce Hills, Bradlo hill	48°40'52"	17°34'00"	509	Murín et al. (1999)

Results

Cytotype distribution and ecology in Slovakia

We analysed 863 individuals (86% of the total sampled) from 93 populations in Slovakia using flow cytometry and an average of 9.3 plants per population (range 2–53). Four ploidy levels were recorded in Slovakia. Of the individuals analysed, 0.3% were triploid, 27.8% tetraploid, 66.3% pentaploid and 5.6% hexaploid. The triploid cytotype was found in one population (1.1%) and the tetraploids in 34 populations (36.6%). The most common cytotype was the pentaploid, being found in 68 populations (73.1%); the hexaploid cytotype was rare, as it was found in only seven populations (7.5%). Note that the sum of the percentages is higher than 100 because there are mixed populations. Sixteen per cent of the populations sampled by us consisted of more than one ploidy level, the most common being a combination of penta- and tetraploids. The detailed statistics, including the previously published data for 8 populations (Table 1), are shown in Table 2.

Table 2. – The cytotype composition of 101 populations of *Allium oleraceum* from Slovakia, including previously published data.

Cytotype composition	Count	Percent	Mean frequency of cytotype in mixed population (%)		
			4x	5x	6x
3x	1	1.0	–	–	–
4x	31	30.7	–	–	–
5x	53	52.4	–	–	–
6x	1	1.0	–	–	–
4x+5x	9	8.9	42.2	57.8	–
5x+6x	4	4.0	–	56.9	43.1
4x+5x+6x	2	2.0	19.4	38.9	41.7

The distribution of cytotypes is given in Fig. 1. The triploid cytotype was found in southern Slovakia. The pentaploid cytotype had a tendency to occur more frequently in the southern part of Slovakia, while the tetraploid had a tendency to occur in the northern parts. The hexaploid cytotype was sparsely found in western and southern Slovakia.

There was a clear relation between the distribution of cytotypes and altitude in Slovakia ($\chi^2 = 8.5$, $P = 0.014$; Fig. 2A), with tetraploids occurring more frequently at the higher altitudes and pentaploids and hexaploids occurring more frequently at lower altitudes. Regarding habitat type, there were weakly significant differences between the cytotypes ($\chi^2 = 21.2$, df = 12, $P = 0.048$; Fig. 3) due to tendency of hexaploids to occur more frequently in field margins than plants of other cytotypes. On the other hand, no differences were found between tetra- and pentaploids when the rare hexaploids are not included in the analysis ($\chi^2 = 8.6$, df = 6, $P = 0.193$). When only two habitats (i.e. habitat naturalness) were considered, the natural versus human-influenced, higher ploidy levels were increasingly found in the human-influenced habitats (4x: 37%, 5x: 56%, 6x: 100%; $\chi^2 = 14.0$, df = 2, $P < 0.001$). The Shannon index H for hexaploids (H = 1.17) was lower than that for tetra- (H = 1.83) and pentaploids (H = 1.86), suggesting a greater degree of habitat specialization of hexaploids than tetra- and pentaploids. The only locality with a triploid population was a relic site on an isolated volcanic hill with steppic vegetation, where it was found in fringe communities.

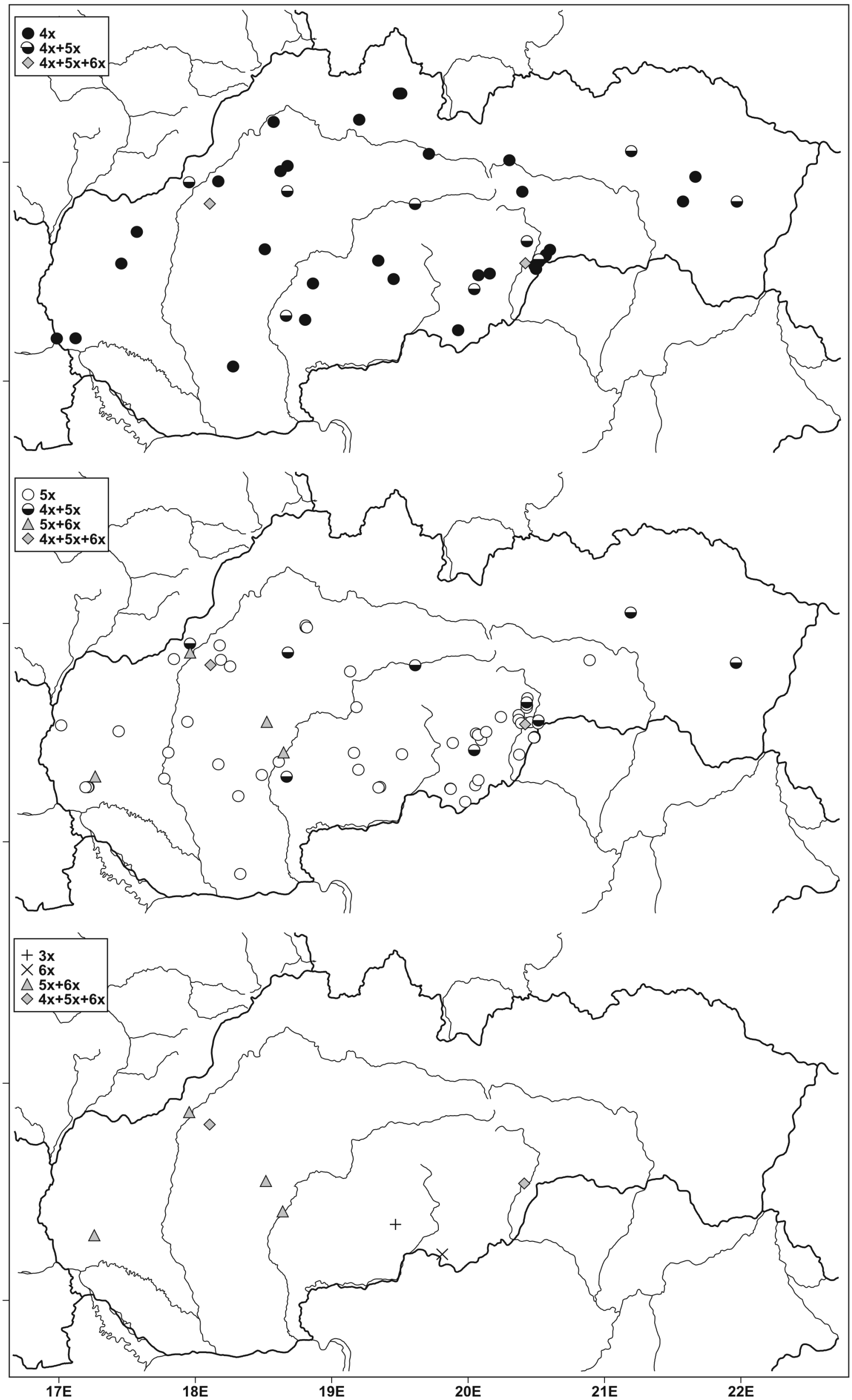

Fig. 1. – The distribution of cytotypes of *Allium oleraceum* in Slovakia, including previously published data. Cytotypes of uniform and mixed populations are distinguished by different symbols.

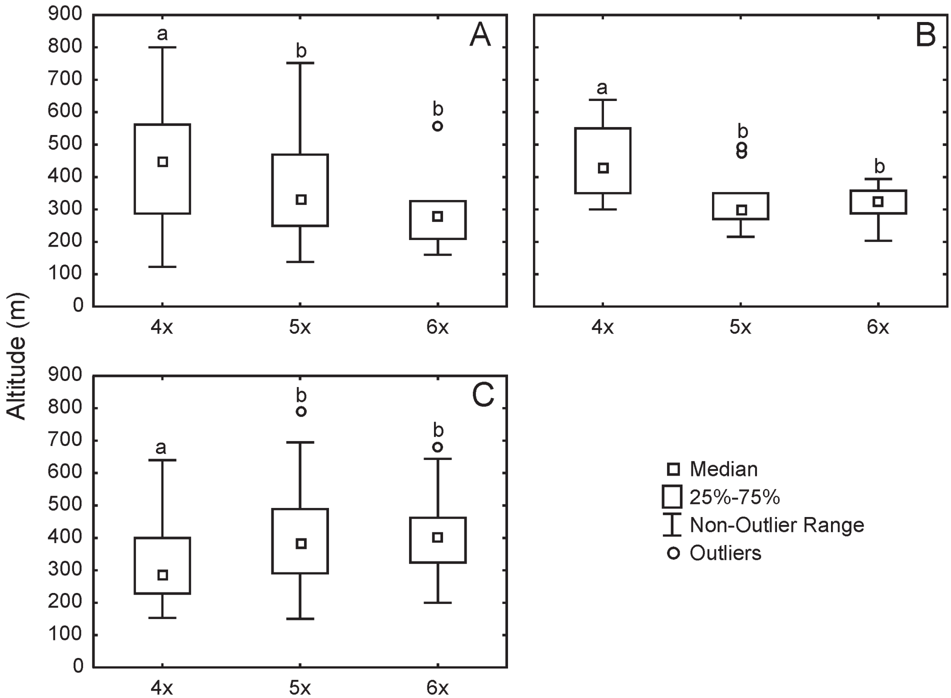

Fig. 2. – The altitudinal relationships of cytotypes in Slovakia (A) and in the Carpathian (B) and (C) Herzynian part of the Czech Republic (extracted from Duchoslav et al. 2010). Significant differences in the medians between ploidy levels (Dunn's test at P = 0.05) are indicated by different letters above the box plots, separately for each region.

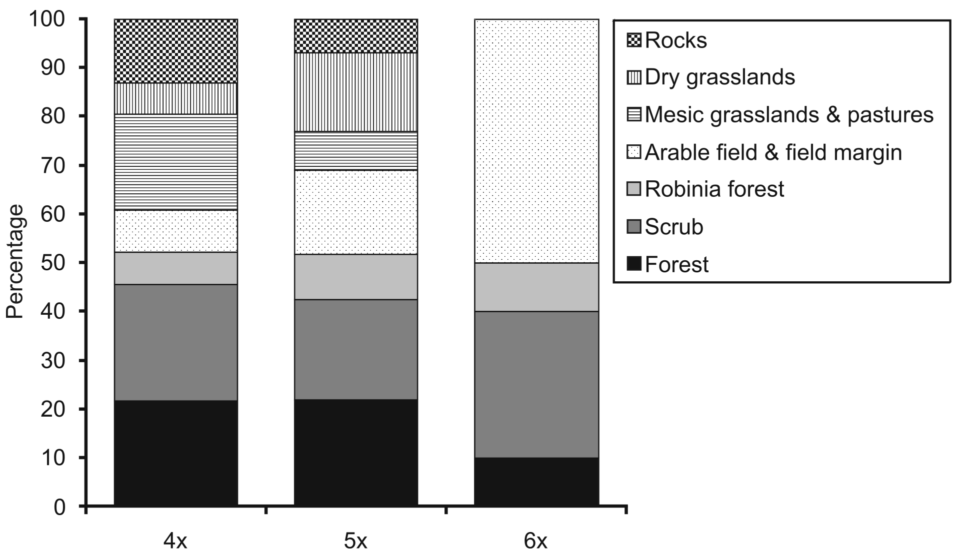

Fig. 3. – The distributions (%) of tetra- (n_{pop} = 34), penta- (n_{pop} = 68) and hexaploid (n_{pop} = 7) cytotypes of *Allium oleraceum* in common habitats in Slovakia. Triploids were not included in this analysis because they were found at only one locality.

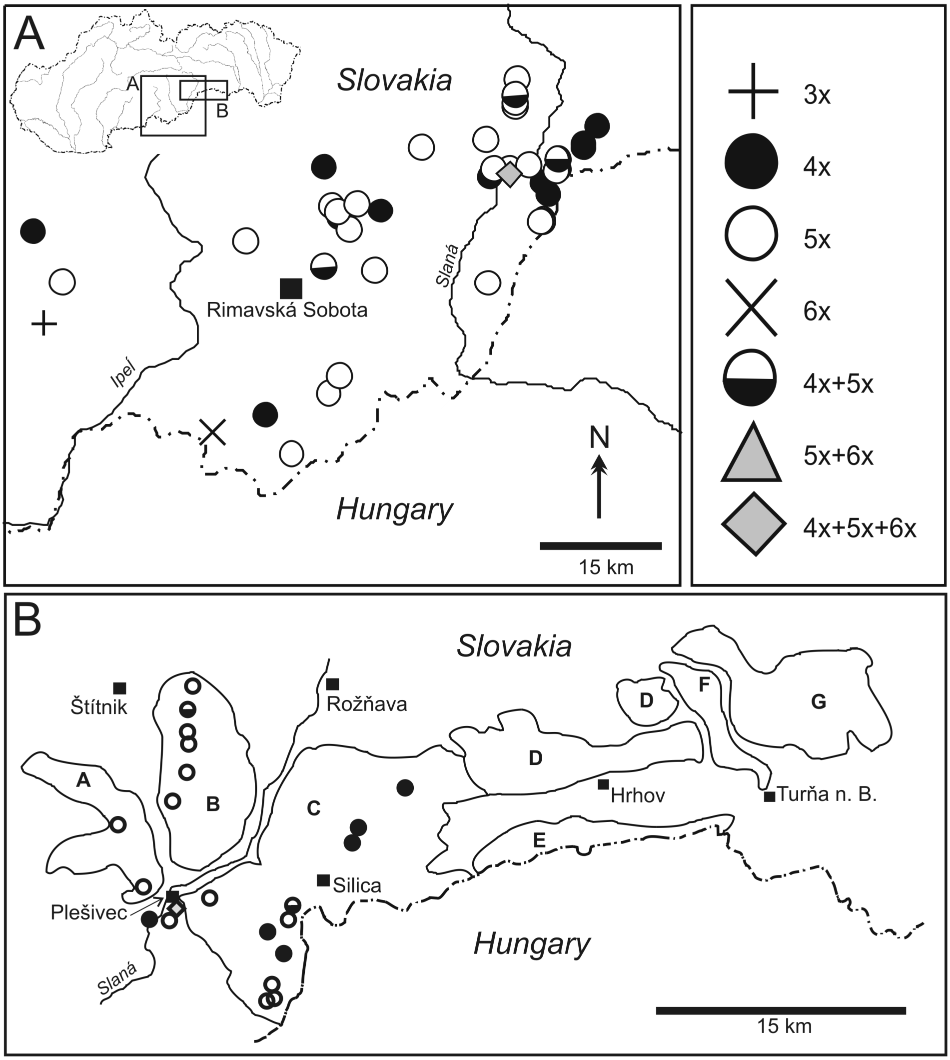

Fig. 4. – The distribution of *Allium oleraceum* cytotypes in Southern Slovakia in the surroundings of Rimavská Sobota (A) and the detailed distribution on the Slovak Karst (B). Particular karst plains are indicated, as follows: A – Koniarska planina; B – Plešivecká planina; C – Silická planina; D – Horný vrch; E – Dolný vrch; F – Zadielska planina; G – Jasovská planina.

Distribution on the Slovak Karst

After the discovery of a locality with three cytotypes (2n = 4x+5x+6x) at Plešivec (loc. no. 21), we paid special attention to the western part of the Slovak Karst and the surrounding areas of Southern Slovakia. The results are presented in Fig. 4 and in Electronic Appendix 1. The distribution on the different plains differed; *A. oleraceum* was found on three plains. On the Koniarska planina, *A. oleraceum* was rather rare and was represented by the pentaploid cytotype. The Plešivecká planina was characterized by populations of the pentaploid cytotype, and only one of them was a mixture of pentaploids and tetraploids (loc. no. 62). The most complex pattern was found on the Silická planina, where the dominant cytotype was tetraploid, but in the central part, we also found a pentaploid population and a mixture of both. On the southwestern periphery of this plain, only pentaploids were found (loc. no. 44, 47, 59 and 60). We did not find *A. oleraceum* on the Horný vrch or Zadielská planina plains.

Discussion

Cytotype distribution

To date, the published data on *Allium oleraceum* from Slovakia includes only tetraploids; they are reported from eight different localities, with no special geographical preferences. Ironically, the most common pentaploid cytotype found by us in 73% of the populations is not previously recorded. This fact shows that even when a relatively high number of populations are included in studies they can still give misleading data for a region as there can be additional cytotypes where populations are mixed on a small spatial scale. The absence of data for rare triploid and hexaploid cytotypes is easily understandable. The preferential collection of tetraploids could be influenced by their more common occurrence in open seminatural stands including grasslands and rocks, locations where they regularly flower and can be more easily found than populations in forests and shrubs, which usually do not flower and almost all were collected by us in spring. In all other habitats, the pentaploid cytotype either dominated or was as common as the tetraploids (Fig. 3).

In contrast to the 23% of the populations consisting of a mixture of cytotypes reported by Duchoslav et al. (2010) for *A. oleraceum* in the Czech Republic, only 16% of the populations consisted of two or three cytotypes in Slovakia. This lower frequency of mixed populations may be partly due to the one third lower average number of plants per population analyzed (nine individuals per population) than in the Czech Republic (≈ 13; Duchoslav et al. 2010). This necessarily increases the uncertainty in the estimates of the number of mixed ploidy populations, as rare cytotypes could easily have been missed (see Šafářová & Duchoslav 2010, Sonnleitner et al. 2010). However, when comparing frequencies of respective cytotypes within mixed populations, we observed an almost identical pattern to that found in the Czech Republic (Duchoslav et al. 2010), i.e. no single cytotype dominated within populations but cytotypes were usually in balanced proportions. This suggests, in accordance with previous studies (Duchoslav et al. 2010, Šafářová & Duchoslav 2010) that 'minority cytotype exclusion' (Levin 1975) has little effect within these populations.

The comparison of cytotype frequencies in Slovakia and the neighbouring Czech Republic (Duchoslav et al. 2010) revealed another significant difference. The pentaploid cytotype is the most common in both countries, more so in Slovakia than the Czech Republic. However, the second most common in the Czech Republic, the hexaploid cytotype, is rare in Slovakia and for this reason a greater proportion of the plants are tetraploid in Slovakia than in the Czech Republic. It appears that Slovakia is just on the eastern periphery of a continuous occurrence of hexaploids in Europe (L. Šafářová & M. Duchoslav, unpublished material), because the occurrence of hexaploids in the western part of Slovakia is tightly linked with their occurrence in the eastern part of the Czech Republic and Austria (Dobeš & Vitek 2000). The isolated occurrence of hexaploids in the neighbourhood of the Slovak Karst may suggest a primary zone (sensu Petit et al. 1999) of hexaploid formation and, hence, different origins for these and the western hexaploids. However, the overall low frequency of hexaploids and the few plants sampled from each population might indicate there could be an undiscovered patchy distribution of hexaploids within southern Slovakia, which connect the eastern and western localities.

The tetraploids and pentaploids are broadly distributed in Europe; both ploidy levels occur sympatrically, for example, in the Baltic region (Finland, Lithuania and Sweden; Duchoslav et al. 2010: Table 1 and references therein). Therefore, their common occurrence in Slovakia is not exceptional. In contrast, only one triploid population was recorded in southern Slovakia, which is only the third locality worldwide and fills the gap between the previously reported triploids in northern Hungary (Krahulcová 2003) and the Ukraine (Vakhtina 1984). The previously recorded triploid populations and this new one are located at the northern limits of one of the supposed diploid progenitors, *A. paniculatum*, which was recently found in the area of the Drienčanský kras (Somogyi 1999, Kliment et al. 2000), and is also reported at Lillafüred, near Hamor (a few kilometres west of Miskolc) in Hungary, very close to the Slovak border (Rapaics 1917). However, the extremely rare records of triploids suggest that they do not serve as a bridge between diploids and tetraploids, and are only able to survive and form separate populations by producing bulbils within their inflorescences (M. Fialová & M. Duchoslav, unpublished material).

Cytotype mixed populations consisting of tetra- and pentaploids were sympatric and intermixed with single-cytotype 4x and 5x populations. Such a pattern is most likely a result of secondary contact between cytotypes and corroborates the results of a previous study (Duchoslav et al. 2010). On the other hand, mixtures of hexa- and pentaploids were more frequently recorded than pure hexaploid populations. However, because of the rarity of hexaploids in Slovakia this could be due to chance and thus no definitive conclusions on the origin of mixed populations can be proposed at present.

We found only two mixed populations with three cytotypes (4x+5x+6x) and therefore it would be premature to draw any conclusion based on this small sample. However, environmental conditions experienced by these two populations and Czech populations, which contain the same cytotype combination, are similar, i.e. scree slopes and rocky ground with outcrops of limestone or bedrock with traces of lime and fields in the close neighbourhood (see Duchoslav et al. 2010). Analysis of cytotype distribution in population no. 82 at a microgeographic scale showed that hexaploids occurred only under shrubs at field margins and tetra- and pentaploids in all microhabitats including rocky ground with steppic vegetation. It seems probable that strong habitat heterogeneity at a local scale combined with disturbance and calcium-rich bedrocks can increase the probability of the local co-occurence of different cytotypes. Whether these mixtures represent either primary or secondary contact zones is still an open question (see Šafářová & Duchoslav 2010).

Relationship with altitude

As mentioned above, the cytotypes differed in their relation to altitude, and this was significant for the material from Slovakia, whereas in the neighbouring Czech Republic, no significant relationship was found (Duchoslav et al. 2010). After the discovery of the relationship for Slovakia, we reanalysed the data from the Czech Republic. We divided the data set according to the main geomorphological and biogeographical regions, the Carpathian and Herzynian portions (the Bohemian Massif). In the Carpathian part of the Czech Republic, we found the same relationship as in Slovakia (Fig. 2B). However, the situation in the Herzynian region was different; the tetraploids differed from the penta- and hexaploids as in the Carpathian region, but in a different way. The relationship was the opposite, with the tetraploids found at lower altitudes than the pentaploids and hexaploids (Fig. 2C). There are several possible explanations. For us, it seems probable that at least the tetraploids in the Carpathians were different from the tetraploids on the Bohemian Massif. These western tetraploids had an evident tendency to occur mainly at lower altitudes in forests (i.e., floodplain forests; see Duchoslav et al. 2010), while this was certainly not true for the Carpathian tetraploids. Comparing both regions, the differentiation within the tetraploids was greater than within the pentaploids and hexaploids.

This example clearly shows that correlations, which were not detected at one level (administrative unit, the Czech Republic) may be detected at another level (more natural units, the Carpathians and Bohemian Massif). The border between the Bohemian Massif and the Carpathians is known as an important biogeographical boundary for the distribution of species in Central Europe (Hendrych 1987), and it is the main reason for their biogeographical separation. Recently several studies have shown that the distribution of cytotypes of several species differ in these regions, for example, those of *Vicia cracca* (Trávníček et al. 2010) and *Pilosella officinarum* (Mráz et al. 2008). The present report and a previous one (Duchoslav et al. 2010) show this differentiation also for *Allium oleraceum* and the present report clearly shows that, for example, the altitudinal correlations were opposite in the two regions. These facts suggest that the differences found have deep roots in the history of both of these main geographical regions. The differences between the Bohemian Massif and the Carpathians are discussed by Mráz et al. (2008).

Ecology

With respect to habitat preferences, both the tetra- and pentaploid cytotypes occurred in a wide spectrum of habitats, while the hexaploids were limited to narrower spectrum of habitats, as in the Czech Republic (Duchoslav et al. 2010). However, in contrast to the data from the Czech Republic, no increase in the frequency of tetraploids in (semi)natural forest stands was observed in Slovakia. This finding partially supports the hypothesis that the tetraploids in the Carpathian and Herzynian regions have different origins (see above). However, a consistent pattern of an increasing frequency for the higher ploidy-number cytotypes in human-influenced vegetation both in the Czech Republic and Slovakia suggest that, regardless of the origin and regional differences in the composition of the vegetation, these cytotypes maintain their ecological strategies.

Some localities were rather extreme. In fact, near the village of Drienčany, we found *A. oleraceum* growing on the temporarily emerging bottom of a karst lake, within the community of *Agropyro-Rumicion* (loc. no. 31). This habitat was flooded for at least part of the growing season, yet the plants were strong and showed no signs of stress.

Slovak Karst

The surprising difference in the distribution of the cytotypes on particular karst plains in the area of the Slovak Karst can be related to the different land use in these regions

(Rozložník 1994), which is corroborating evidence for the scenario explaining the ploidy-level distribution of *A. oleraceum* in the Czech Republic (Duchoslav et al. 2010). The Koniarska and Plešivecká planina plains had no permanent settlements in the past; most areas of the Koniarska planina are wooded and the Plešivecká planina is a mixture of pastures and forests. Conversely, the Silická planina does have permanent settlements, namely, the villages of Silica and Silická Brezová. Human activities have created not only pastures, as on the Plešivecká planina, but also arable lands. We found each of the 4x and 5x cytotypes in the vicinity of the villages. Near Silica, cytotypes grew together in a broad-leaf forest (loc. no. 56), whereas in the vicinity of Silická Brezová, cytotypes were found in different habitats: tetraploids occurred at the edge of a forest (loc. no. 57) and between arable land (loc. no. 46) and the pentaploids in grassland, on a small hill, with steppic communities (loc. no. 58). Similarly, a complex pattern also occurred in the Drienčanský kras karst area, where tetraploids and pentaploids grow close together with no clear habitat preferences. Near the village of Hostišovce, both tetraploid and pentaploid cytotypes occurred in a rocky habitat (loc. no. 33 and 37).

Conclusions

Only tetraploid *A. oleraceum* are previously reported from Slovakia. This study, however, suggests that both the composition and pattern of distribution of *A. oleraceum* cytotypes are remarkably complex at various spatial scales with as many as four cytotypes detected (2n = 3x, 4x, 5x, 6x) in Slovakia. The results thus fit well into and broaden the results of detailed screening in the neigbouring Czech Republic (Duchoslav et al. 2010) with Slovakia representing the most cytotype-diverse region so far detected for *A. oleraceum*. From the methodological point of view, our study clearly shows that to correctly estimate (co-)distribution and frequency of cytotypes, (i) both intensive (many plants per site) and extensive (many sites) sampling (Halverson et al. 2008) of (ii) a wide range of habitats inhabited by the species are required. Contrasting the altitudinal distribution of tetraploids in the Czech Republic and Slovakia suggest that at least two different types of tetraploids occur in Central Europe. Both tetra- and pentaploid cytotypes showed similar and wide ecological amplitude in contrast to the hexaploids, which were limited to human– influenced habitats. The majority of mixed-ploidy populations were found in sympatry with cytotype-uniform populations of participating cytotypes suggesting rather secondary contact between cytotypes. The existence of mixtures containing tetra-, penta- and hexaploids and apparent local cytotype diversity in the Slovak Karst area may indicate primary contact zones. For detailed investigations on the evolutionary dynamics of populations with cytotype heterogeneity *Allium oleraceum* is a promising plant.

See http://www.preslia.cz for Electronic Appendix 1

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Souhrn

Studie se zabývá rozšířením a ekologií cytotypů *Allium oleraceum* na Slovensku. Doposud publikované práce uváděly z území Slovenska pouze tetraploidní cytotyp. Protože byla recentně zjištěna komplexní cytotypová variabilita (2n = 4x–6x) v sousední České republice, vyvstala otázka, zdali se skutečně vyskytuje na Slovensku jen tetraploidní *A. oleraceum* nebo je situace složitější. Vedle geografického rozšíření a stanovištních nároků cytotypů na území Slovenska jsme se dále zaměřili na podrobnější rozšíření cytotypů na lokální škále Slovenského krasu a blízkého okolí, kde jsme, na základě údajů o výskytu blízce příbuzného taxonu a jednoho z předpokládaných diploidních rodičů, *A. paniculatum*, poblíž studovaného území předpokládali komplexnější cytotypovou kompozici. Pomocí průtokové cytometrie byl analýzou 863 jedinců z 93 populací zjištěn výskyt čtyř cytotypů: tri- (2n = 24), tetra- (2n = 32), penta- (2n = 40) a hexaploidů (2n = 48). Nejčastěji (v 73 % studovaných populací) byl zaznamenán pentaploidní cytotyp, tetraploidní cytotyp byl zjištěn ve 37 % populací, hexaploidní cytotyp byl zjištěn pouze v 7 populacích (8 %) a triploidní v jedné populaci (1 %). Zjištěné údaje jsou zcela v rozporu s dosavadními znalostmi cytotypové variability studovaného taxonu na Slovensku a ukazují, že i relativně vyšší počet v minulosti studovaných populací (8 lokalit) může poskytnout nepřesné a zavádějící údaje. Šestnáct procent analyzovaných populací bylo cytotypově smíšených, byly zjištěny kombinace cytotypů 4x+5x, 5x+6x a 4x+5x+6x. Tetraploidní cytotyp byl četnější v severní zatímco pentaploidní cytotyp v jižní části Slovenska. Hexaploidní cytotyp se vyskytoval na západním a jižním Slovensku. Nález triploidní populace na kopci Veľký Lysec poblíž obce Luboreč (okres Lučenec) představuje teprve třetí známou lokalitu na světě, která leží, podobně jako další dvě známé lokality (severní Maďarsko, Ukrajina) na severní hranici areálu *Allium paniculatum*. Byly zjištěny statisticky signifikantní rozdíly ve vazbě cytotypů na biotopy, přičemž hexaploidní cytotyp vykazoval silnější vazbu na biotop pole a polní okraje (meze, příkopy atp.) než tomu bylo u tetra- a pentaploidních cytotypů. V případě, kdy byly lokality klasifikovány podle míry antropického tlaku, stoupala s rostoucí ploidií relativní frekvence výskytu populací na ruderalizovaných stanovištích. (Re)analýza výškového rozšíření 4x–6x cytotypů v České republice, rozdělené do dvou geomorfologických/biogeografických celků (Karpaty a Hercynikum), a na Slovensku ukázala, že zatímco tetraploidní cytotyp je četnější ve vyšších nadmořských výškách než penta- a hexaploidní cytotypy jak na Slovensku, tak v karpatské části České republiky, opačný vztah byl nalezen v Hercyniku. Z výše uvedeného lze usuzovat, že se ve střední Evropě vyskytují přinejmenším dva typy tetraploidů. Rozšíření cytotypů na planinách v západní části Slovenského krasu bylo nenáhodné a je dáváno do souvislosti s rozdílným využíváním krajiny.

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