Biologia Serbica, 2018, 40:47-59

DOI 10.5281/zenodo.1406320

Review paper

Polyploidy and hybridization in the Mediterranean and neighbouring northern areas: examples from the genus *Cardamine* (Brassicaceae)

Karol Marhold*[1,2], Marek Šlenker[1], Judita Zozomová-Lihová[1]

[1]*Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Institute of Botany, Dúbravská cesta 9, SK-845 23 Bratislava, Slovak Republic*
[2]*Charles University, Faculty of Science, Department of Botany, Benátská 2, CZ-128 01 Prague, Czech Republic*

Summary. The genus *Cardamine* is distributed worldwide and is rich in polyploids. Here we review the considerable amount of knowledge accumulated on polyploid species and related diploids over the last twenty years, particularly in the Mediterranean area and neighbouring regions of Central Europe. These studies addressed the taxonomic treatment of polyploids, their origin and evolution, phylogenetic relationships to diploid and other polyploid relatives, and overall morphological, genetic, karyological and ecological patterns. The revealed parentage of polyploids such as *C. ×asarifolia, C. ×flexuosa* and *C. ×schulzii* shows the power of recently developed molecular and cytogenetic approaches, which enable us to understand how the polyploid taxa originated, became established and further evolved. It is emphasised that diploid-polyploid species complexes, such as the *C. amara* and *C. pratensis* groups, represent true natural laboratories for polyploid studies. Particularly the species complex of *C. pratensis* contains numerous diploid lineages and polyploid populations of all levels up to dodecaploids, including aneuploids and dysploids, providing excellent possibilities for the application of a wide spectrum of methodical approaches to understand the processes underlying polyploid evolution. Hybridization revealed in this genus occurs both at the homoploid and heteroploid levels and mainly in mountain regions, where parapatric and/or ecologically differentiated close relatives come into contact. These studies document that interspecific gene flow can result in various patterns, such as continuous variation in hybrid swarms (*C. pratensis* × *C. raphanifolia*), established hybrid species of significant evolutionary potential (*C. ×schulzii*, *C. ×insueta*), or largely sterile hybrids of first generations only and persistence through vegetative propagation (*C. ×ferrarii, C. ×enriquei*). All of these cases also show how recent hybridization and introgression add to the complexity of reticulated patterns of evolution in *Cardamine* in the Mediterranean and adjacent areas.

Keywords: Brassicaceae, *Cardamine*, Central Europe, Cruciferae, Hybridization, Mediterranean, Polyploidy.

INTRODUCTION

Polyploidization is undoubtedly a frequent mode of diversification and speciation in plants. It was suggested that it even may be the most common mechanism of sympatric speciation in plants (Otto and Whitton 2000). For many polyploids, multiple origins in space and time have been shown, increasing their genetic diversity and complexity, whereas in other cases a single origin has been proposed (Soltis and Soltis 1999; Soltis et al. 2004; Arnold et al. 2015). Hybridization is yet another important speciation mechanism that may (allopolyploidization) or may not (homoploid hybrid speciation, hybrid speciation at the same ploidy level) be tightly coupled with polyploidization (Hegarty and Hiscock 2005; Abbott et al. 2010, 2013). Importantly, both processes often result in a reticulate pattern of evolution. While in polyploids reticulation is always suspected, homoploid hybrid speciation may be more difficult to identify and unravel. Hybridization is now emerging as a widespread and fundamental evolutionary force (Sung et al. 2018), which can fuel adaptation (Arnold and Kunte 2017; Schmickl et al. 2017). It might provide fertile ground for adaptive radiations, either by enriching genetic variation in an initial hybridization event between two species that may then trigger radiation or by introducing adaptations that allow species of radiating lineages to occupy new niches and further diversify. Hybridization has been

*Corresponding author, e-mail: karol.marhold@savba.sk

shown to drive the adaptive radiation of plants (Stankowski and Streisfeld 2015; Pease et al. 2016).

The Mediterranean is one of the world biodiversity hotspots, harbouring a high concentration of endemics, as well as rare and threatened species (Médail and Quézel 1997; Myers et al. 2000). Polyploidy and hybridization are fairly common among Mediterranean flora, and apparently have been an important speciation force here. Recent estimates of the percentage of polyploid angiosperm species in this region range from 36.5% to 48.8% and the percentage of hybrids as < 6%. However, there remain considerable gaps in our understanding of the role of hybridization and polyploidy in plant evolution in this region (Marques et al. 2018). Nevertheless, an abundance of recent studies addressing these issues is promising (e.g., Zozomová-Lihová et al. 2014a,b; Wölk et al. 2015; Frajman et al. 2016; Crowl et al. 2017; Marques et al. 2018).

The Brassicaceae family is not an exception, and as it was proven earlier, polyploidy is here the most fundamental speciation process (see e.g., Franzke et al. 2011). This is underlined by the fact that approximately 37% of the species have an assumed polyploid origin (Warwick and Al-Shehbaz 2006). Except polyploidization, hybridization is another common evolutionary force in the Brassicaceae and numerous examples of hybrids were reported (reviewed by Marhold and Lihová 2006).

The genus *Cardamine* L. comprises more than 200 species occurring indigenously on all continents except mainland Antarctica (Al-Shehbaz 1988; Lihová and Marhold 2006); the exact number of species and taxa depend on the taxonomic concept of the particular author. Research on this genus is ongoing and a number of new species were recently described, for example from New Zealand (Heenan 2017). The cosmopolitan distribution of several taxa and the spread of taxa across continents make identification of centres of origin challenging. Nevertheless, major centres of diversity estimated by the number of taxa appear to be in the European Mediterranean and the Caucasus; Eastern Asia and the Himalayas; and North and Central America (Lihová and Marhold 2006). Phylogenetic analyses of the genus were performed in the past by Franzke et al. (1998), Sweeney and Price (2000), Bleeker et al. (2002), and most recently by Carlsen et al. (2009). However, detailed relationships among most of the species of the genus remain unclear. Indeed, the evolution of this genus, which was considerably affected by reticulate evolution and polyploidization, is not straightforward and difficult to reconstruct even with multiple markers (Lihová and Marhold 2006; Marhold and Lihová 2006). The genus *Cardamine* is well known for its large variation in chromosome number and the frequent occurrence of polyploidy and hybridization. In the genus, 32% diploid, 10% both diploid and polyploid, and 58% entirely polyploid taxa were identified (Kučera et al. 2005). The high percentage of polyploids in *Cardamine* clearly shows that polyploidy is the most fundamental speciation process in this genus.

In this paper, we summarize the considerable amount of knowledge gathered in recent studies on the taxonomy, origin and evolution of auto- and allopolyploid species and their diploid relatives, as well as spontaneous interspecific hybridization within the genus *Cardamine*. Emphasis is put here on the Mediterranean and neighbouring northern areas, which harbour high species and genetic diversity, reflecting intricate evolutionary processes. First, we deal with three traditionally recognized diploid-polyploid species complexes and also point to their relationships and shared evolutionary history. As next, we focus on few thoroughly analysed auto- and allopolyploid species, summarizing evidence from the studies that reconstructed in detail their origin, establishment and evolution. Finally, we review cases of interspecific hybrids and present various patterns and outcomes that have emerged from interspecific gene flow. Application of a wide spectrum of methods and recently developed sophisticated approaches revealed interesting, in some cases even surprising, evolutionary scenarios, and helped to improve our understanding of the formation, persistence and evolutionary potential of polyploid and hybrid species.

DIPLOID-POLYPLOID COMPLEXES OF *CARDAMINE AMARA, C. PRATENSIS* AND *C. RAPHANIFOLIA*

Three major diploid-polyploid complexes of *Cardamine* occurring in the Mediterranean and more northern regions have been traditionally recognized and delimited. The evolutionary patterns of these complexes were addressed in studies performed over the last 20 years. Here we present a short overview of these studies.

Cardamine amara complex

The group of *Cardamine amara* in its European area is a species complex comprising diploid and tetraploid populations. On the diploid level, apart from *C. amara* L. subsp. *amara*, which is widespread in Europe, there are three other mostly mountain subspecies, namely *C. amara* subsp. *pyrenaea* Sennen from the Pyrenees, *C. amara* subsp. *opicii* (J. Presl & C. Presl) Čelak. from the Sudeten mountains and the Carpathians, and *C. amara* subsp. *balcanica* Marhold & al. from the mountains of Bulgaria, Greece and Serbia (Marhold et al. 1996, 2002a; Marhold 1998; Tomović et al. 2009). The list of diploid taxa complete *C. wiedemanniana* Boiss. mainly from Turkey. Related tetraploids include *C. amara* subsp. *austriaca* Marhold from the Eastern Alps and neighbouring areas (Marhold 1999a), *C. amporitana* Sennen & Pau (previously treated as *C. amara* subsp. *olotensis* O. Bolòs) with disjunct distribution in Catalonia and central Italy (Lihová et al. 2000, 2004a,b), and *C. barbaraeoides* Halácsy from a restricted area of mountains in Greece (Perný et al. 2005a; Marhold and Perný, unpubl.). Circumscription of the diploid taxa is supported by molecular and morphological data (RAPDs, isozymes, AFLPs; Marhold et al. 2002a; Lihová et al. 2000, 2004a,b), nevertheless phylogenetic relationships among them remain largely unclear. More detailed attention was paid to the tetraploids *C. amporitana* (Lihová et al. 2004a,b) and *C. amara* subsp. *austriaca* (Zozomová-Lihová et al. 2015) with the aim to reveal their polyploid origin and explain specific distribution patterns. As for *C. amporitana*, the conspecificity of Italian and Catalonian populations was proven based on AFLP markers and DNA sequencing (ITS region of nuclear ribosomal DNA, trnL-trnF spacer of chloroplast DNA), as well as a close relationship with *C. amara*. It was also shown that this tetraploid species most likely originated in central Italy and only subsequently reached Catalonia, probably via long distance dispersal (although other explanations of the disjunct distribution of this taxon can be also considered). Its polyploid origin, either auto- or allo-, however, could not be solved unambiguously. Conversely, the autopolyploid origin of *C. amara* subsp. *austriaca* from the diploids of subsp. *amara* was proven in studies using AFLP and microsatellite data (Lihová et al. 2004b, Zozomová-Lihová et al. 2015; see also below), also providing deeper insights into the diploid-tetraploid contact zone, evolutionary and colonization history, as well as the ecological divergence of the tetraploids (Zozomová-Lihová et al. 2015; see also below). In addition to *C. amporitana* and *C. amara* subsp. *austriaca*, diploid *C. amara* is likely one of the parental species of a number of other polyploids, as outlined below.

***Cardamine raphanifolia* complex**

Cardamine raphanifolia Pourr., as circumscribed in Flora Europaea (Jones and Akeroyd 1993) and Atlas Florae Europaeae (Jalas and Suominen 1994), comprised three and four subspecies, respectively: *C. raphanifolia* subsp. *raphanifolia*, *C. raphanifolia* subsp. *acris* (Griseb.) O.E.Schulz, *C. raphanifolia* subsp. *barbaraeoides* (Halácsy) Strid and *C. raphanifolia* subsp. *gallaecica* Laínz. Nevertheless, our studies based on AFLP, DNA sequences and morphometric data (Perný et al. 2005a,b; Lihová et al. 2004a) show that they do not form a monophyletic group and they are now treated as separate species. *Cardamine acris* Griseb., distributed in the Balkan Peninsula, is a well-differentiated diploid species, albeit variable, comprising three subspecies recognized based on AFLP and morphological data, which differ also in their distribution patterns (Perný et al. 2004). The other taxa that used to be ascribed to this complex are all polyploid. *Cardamine raphanifolia* is a polyploid of 2n = 6x, 8x = 48, 64, growing in the northern Iberian Peninsula, which arose most likely from *C. pratensis* L. and *C. amara* as parental species. Ongoing hybridization between polyploids of *C. raphanifolia* and *C. pratensis* in the area of their range overlap is described below in more details. *Cardamine gallaecica* (Laínz) Rivas Mart. & Izco, comprising also two cytotypes (2n = 4x, 6x = 32, 48), occurs in Galicia, NW Spain, and may have a similar parentage as *C. raphanifolia* (Perný et al. 2005b; Perný et al. unpubl.). Genetic data indicate that the contribution of the Balkan diploid *C. acris* towards these two Iberian polyploids can be excluded. *Cardamine barbaraeoides*, restricted to the mountains of Greece, is tetraploid, placed closely to both *C. raphanifolia* and members of the *C. amara* group (Lihová et al. 2004a; Perný et al. 2005a,b; Perný et al. unpubl.). Its more precise position and polyploid origin are now the subject of intensive study (Marhold et al. unpubl.). Finally, populations from southernmost Italy, treated by Jalas and Suominen (1994) as an uncertain subspecies of *C. raphanifolia,* were later found to represent a separate hexaploid species, namely *C. silana* Marhold & Perný (Perný et al. 2005b). Its allopolyploid origin from the diploids *C. acris* and *C. apennina* (*C. pratensis* group) was traced in details as shown below.

***Cardamine pratensis* complex**

Cardamine pratensis is a highly polymorphic species complex, which is widely distributed in Europe, extending eastwards to Asia and represented also in northern Africa and North America (Jalas and Suominen 1994; Lihová and Marhold 2003). It comprises a wide scale of the ploidy levels ranging from diploid to dodecaploid, including aneuploid and dysploid plants or populations as well. The *C. pratensis* complex has been investigated extensively by several authors since the 1950's (e.g. Lövkvist 1956; Dale and Elkington 1974; Urbanska-Worytkiewicz and Landolt 1974; Landolt 1984; Marhold 1994a,b, 1996; Marhold and Ančev 1999; Franzke and Hurka 2000; Lihová et al. 2003, 2004c; Marhold et al. 2004). Various approaches including karyology, morphometrics, ecological studies, cultivation and crossing experiments, and molecular analyses have been employed in these studies over the last decades, which helped to develop taxonomic concepts for the group. Several well supported taxa can be distinguished at the diploid level. They are distributed predominantly in the southern part of Europe, while polyploids are more widespread toward the north. Such differentiation into diploid-dominated southern vs. polyploid-dominated northern regions represents a common, but not universal cytogeographic pattern in the European flora (Weiss-Schneeweiss et al. 2013; see e.g., Kolář et al. 2016 for *Arabidopsis arenosa* (L.) Lawalrée).

In the Balkan Peninsula, the following diploid taxa can be distinguished: *C. penzesii* Ančev & Marhold, *C. rivularis* Schur, *C. pratensis* and *C. matthioli* Moretti. *C. penzesii* (2n = 16) (Marhold and Ančev 1999) is the lowland taxon restricted to the area of the Black Sea coast, from the mouth of the Kamčia River in Bulgaria to the westernmost part of Asian Turkey. It was originally described as *C. tuberosa* Pénzes & Vida (Pénzes 1965), which is an illegitimate name that had to be replaced. *Cardamine rivularis* (2n = 16, 24) is a montane to alpine species restricted in its occurrence to the South Carpathians and higher Bulgarian mountain ranges (Marhold 1994a, 1995, 1996). This name was misapplied also to plants of the *C. pratensis* complex occurring at higher altitudes in the Eastern Carpathians, the Eastern Alps, the Apennines, the Massif Central, and the Pyrenees (Lövkvist 1956; Jones 1964; Urbanska-Worytkiewicz and Landolt 1974; Landolt 1984). In keeping with the current taxonomic concept (Marhold 1994a), Alpine and Eastern Carpathian *C. rivularis* auct. non Schur falls within the variation of *C. pratensis* s. str., and should be classified within this species (Lihová et al. 2003). According to the molecular phylogenetic study on a European-wide scale published by Franzke and Hurka (2000), *C. penzesii* and *C. rivularis* represent the "Basal Group" of the *C. pratensis* complex. These two old taxa putatively represent Pleistocene relicts, which were not directly involved in the formation of the younger "Derived Group" – diploid and polyploid taxa that presumably evolved in postglacial time. *Cardamine matthioli* is a diploid species from the "Derived Group" distributed in Central and Southeastern Europe (Pannonia, Carpathians and southwards to the Balkan Peninsula in almost all Balkan countries; Marhold 1994a, b) in lowlands to the upper montane belt. The southernmost species localities are situated in Central Greece, and the south-easternmost ones are in European Turkey (Marhold and Tan 2000; Ančev 2006). The western borders of distribution are reported from Austria and northwest Italy (Marhold 1994a, 2000).

From the Apennine Peninsula, several diploid taxa of the *C. pratensis* complex have been reported: *C. granulosa* All., *C. apennina* Lihová & Marhold, *C. matthioli* (abovementioned) and *C. pratensis* s.str. The first two species represent Italian endemics, whereas the latter two have wider distribution in Europe. *C. matthioli* has already been discussed above and *C. pratensis* s. str. will receive more attention later in this text. *Cardamine apennina* (2n = 16) has a restricted distribution in the central part of the Apennine Peninsula (prov. Toscana, Umbria, Abruzzo, Marche). It is rare, with no more than 12 populations having been documented over the past 30 years, but ranging from sea level to montane sites. Considering the limited number of localities, *C. apennina* has been included among taxa requiring protection (Lihová et al. 2004c). *Cardamine granulosa* is restricted solely to NW Italy (Piemonte). It is an extremely narrow endemic and endangered species, with only a few localities documented from the surroundings of Torino and adjacent valleys and foothills, most of them of an older date (Lihová et al. 2004c). The rarity of this taxon and its restricted distribution may be partly attributed to the destruction of its natural habitat through expansion of the city of Torino, agricultural fields, and new forest plantations (Urbanska-Worytkiewicz and Landolt 1974). Based on limited DNA sequence data (ITS2 of rDNA), *C. granulosa* was classified in the phylogenetic study by Franzke and Hurka (2000) into the "Derived Group" of the *C. pratensis* complex, however, according to more precise data by Lihová et al. (2004c) both *C. apennina* and *C. granulosa* most likely represent one of its basal lineages (Lihová et al. 2004c).

The combination of karyological, morphological and molecular data suggest that three diploid taxa of the *C. pratensis* complex can be recognized in the Iberian Peninsula: *C. pratensis* s. str., *C. crassifolia* Pourr., and *C. castellana* Lihová & Marhold. Whereas *C. pratensis* s. str. is widespread in Europe, the latter two species are endemic to the Iberian Peninsula. *Cardamine crassifolia* is one of the assumed basal taxa of the *C. pratensis* complex (Franzke and Hurka 2000). Its relic position has already been proposed by Lövkvist (1956). This species is distributed in the montane to alpine belt of the Eastern Pyrenees (Lövkvist 1956; Rico 1993; Mateo Sanz et al. 1994). Populations occurring at higher altitudes in the central Iberian mountains have been also ascribed to this species (Rico 1993; Mateo Sanz et al. 1994). However, molecular and following morphological analyses proved that these mountain populations differ from *C. crassifolia* from the Eastern Pyrenees, and should be treated as a separate taxon; because of this they were described as *C. castellana*. Molecular data also showed that *C. crassifolia* and *C. castellana* are closely related, sister species (Lihová et al. 2003; Marhold et al. 2004), but the details of their evolutionary and colonization history remain unsolved.

Of particular interest are diploid populations from Central Europe, currently classified as *C. pratensis* s. str. It is in fact a highly polymorphic diploid-polyploid species, and as a consequence of enormous morphological, ecological, and cytological variability, several diploid taxa attributable to *C. pratensis* were described in the past (listed below). They show certain morphological and ecological differentiation, which is, however, mostly weak (Urbanska-Worytkiewicz and Landolt 1974; Marhold 1994a, 1996), and several molecular markers applied so far failed to support their recognition (Franzke and Hurka 2000). Most recently, employment of high resolution markers (microsatellites, next generation sequencing) appears promising in unravelling the genetic structure of this highly heterogeneous assemblage, although the patterns that emerge do not correspond to the previously described species (Zozomová-Lihová et al. 2014a, b; Melichárková et al. unpubl.). Diploid plants from lower altitudes occurring particularly in Germany were in the past sometimes classified as *C. nemorosa* Lej. (Dersch 1969; Urbanska-Worytkiewicz and Landolt 1974), other lowland populations as *C. udicola* Jord. (Urbanska-Worytkiewicz and Landolt 1974); nevertheless, these diploid taxa are very hard to recognize. High mountain populations were incorrectly classified as *C. rivularis* (for details see Marhold 2000) or treated informally as *C. rivularis* auct. (Marhold 1994a). In order to reach an appropriate taxonomic solution, these diploid populations definitely require further studies to reveal their evolutionary and colonization history, extent of genetic differentiation and on-going gene flow. In addition, they may represent an excellent model to study cryptic speciation at the diploid level.

An even more complicated situation appears at the polyploid level. Several taxa of the *C. pratensis* complex were described in the past as well, but these are less differentiated than the diploids and show poor phylogenetic resolution (Franzke and Hurka 2000). Their distribution encompasses predominantly the area of Central and Northern Europe.

Cardamine pratensis s. str. occurs throughout most of Europe except the extreme North and South (Lövkvist 1956; Jalas and Suominen 1994). In the Iberian Peninsula, *C. pratensis* is distributed in its northern and central parts (Lihová et al. 2003). In Italy it grows only in the northernmost regions (Lihová et al. 2004c). Similarly, in the Balkan Peninsula it grows predominantly in northern areas, whereas in southern areas of the peninsula it is replaced by diploid species of the *C. pratensis* complex (*C. matthioli*, *C. penzesii*, and *C. rivularis*). The distribution of *C. pratensis* s. str. shows broad ecological range of habitats. It is common from lowlands up to the upper montane and subalpine belts of the Carpathians and the Alps. *Cardamine pratensis* s. str. is composed of several cytotypes ranging from diploid to heptaploid, and in addition, aneuploidy and dysploidy are relatively common phenomena within these populations (Kučera et al. 2005). The reasons for these cytotype patterns are rather complex, nevertheless one part of this complicated puzzle was recently solved by Mandáková et al. (2013). Already Lawrence (1931) and Lövkvist (1956) observed that dysploid (hypotetraploid) plants of *C. pratensis* s. str. with 2n = 30, rather than regular 2n = 32 chromosomes, possessed one pair of longer chromosomes, and hypothesized a chromosome fusion. Using the method of comparative chromosome painting, Mandáková et al. (2013), indeed, revealed that AK5 and AK8/6 homologs (of the ancestral crucifer karyotype with eight chromosomes, n = 8, AK1 to AK8) had participated in nested chromosome fusion. This translocation event apparently involved "insertion" of chromosome AK5 into the pericentromere of AK8/6, giving rise to the fusion chromosome AK5/8/6 (Mandáková et al. 2013). Most likely the same process was involved in the origin of the hypohexaploid plants with 2n = 44 chromosomes. The taxonomic studies by Marhold (1996) and Lihová et al. (2003, 2004c) showed wide morphological variability of *C. pratensis* s. str. As stated above, high intraspecific variability resulted in taxonomic description of several new taxa or recognition of informal units in the past (e.g., *C. pratensis* subsp. *picra* De Langhe & D'hose, *C. pratensis* subsp. *major* Tomšovic, *C. udicola* Jord., *C. nemorosa* Lej., *C. latifolia* Lej., *C. rivularis* auct. non Schur, *C. pratensis* „ucranica type"). However, the efforts to split this highly variable species were not supported by molecular evidence. Wide palette of molecular markers (allozymes, ITS, non-coding cpDNA, RAPD analysis, AFLP fingerprinting) was used to analyse *C. pratensis* s. str., to revisit previously described taxa, elucidate phylogenetic relationships, historical biogeography, the origin of different cytotypes, and the internal structure of this species (Franzke and Hurka 2000; Lihová et al. 2003). However, these methods mostly failed to resolve relationships and deeper structure. Without clear molecular divergence supported by morphological differences, the above-mentioned taxa cannot be regarded more than ecotypes, cytotypes, or plasticity responses, and therefore are included within *C. pratensis* s. str. (Landolt 1984; Marhold 1996; Franzke and Hurka 2000; Lihová et al. 2003). In addition, the very low among-cytotype differentiation observed on the European scale, and higher differentiation on the regional scale (Lihová et al. 2003; Zozomová-Lihová et al. 2014a) suggest that gene flow frequently occurs also among different cytotypes and ploidy levels (as already illustrated by Lövkvist 1956) but less so over large geographic distances. These findings also indicate polytopic origins of the polyploid cytotypes and yet undiscovered lineages within *C. pratensis* s. str.

Cardamine majovskyi Marhold & Záborský (2n = 4x = 32) was described in 1986, based on populations from eastern Slovakia (Marhold and Záborský 1986). This taxon is known to occur in the eastern parts of Pannonia, the Carpathians (Hungary, Slovakia, Ukraine, Romania) (Marhold 1994a), Slovenia (Marhold 1999b; Lihová and Marhold 2003), Croatia (Kučera and Marhold 2006) and Austria (Marhold 2000). It is worth mentioning here that distribution and other data on *C. majovskyi* from the area of Serbia are still missing. More data about its autopolyploid origin are given below.

Cardamine dentata Schult. (2n = 7x–12x) is another highly polyploid aggregate. Chromosome counting revealed various somatic numbers ranging from 52 to ca 96 (Lövkvist 1956). This range of numbers includes dysploids and aneuploids as well. Lövkvist did not find sterility barriers among cytotypes and regarded the whole aggregate as „one wide species". In the wild, the different cytotypes often grow together. *Cardamine dentata* is distributed in Central and North-Western Europe, extending to the Russian Far East and most likely also North America (Lövkvist 1956; Wójcicki and Marhold 2000).

While the Mediterranean species of the *C. pratensis* complex are morphologically and genetically well-defined with clearly delimited distribution areas, the current knowledge of the Central European diploids and polyploids is still rather limited despite extensive research, and numerous open questions remain. New challenges, arising from the current state of knowledge concerning the *C. pratensis* complex, are primarily focused on the identification of different lineages within *C. pratensis* s. str., their evolutionary history and mutual relationship, on the identification of diploid progenitors, and tracing the origin of derived polyploid populations. To solve these questions, microsatellites, which are an efficient marker for phylogenetic study of closely related species, and the Hyb-Seq method, which is based on the next generation sequencing approach, are currently employed.

RELATIONSHIPS AMONG POLYPLOID COMPLEXES

The *C. pratensis* complex has always been considered to be a coherent monophyletic group; however, a detailed phylogenetic study brought this assumption into doubt (Marhold et al. 2004). Using two independent molecular data sets (ITS sequences of nrDNA and AFLP markers), this study aimed to clarify phylogenetic relationships among the diploid representatives of three traditionally recognized polyploid complexes (*C. pratensis*, *C. raphanifolia*, and *C. amara*). Surprisingly, only two main lineages were revealed. The *C. amara* complex was resolved as a well-supported monophyletic group, but remaining diploids, contrary to expectations, did not split into separate groups (*C. pratensis* and *C. raphanifolia*). All these taxa formed a single clade with poorly resolved relationships and the lack of bootstrap support. A comparable result was presented by Lihová et al. (2004a) including also polyploid taxa. Phylogenetic trees based on ITS and trnL-trnF sequence data revealed two main clades, one comprising *C. amara* group and the other *C. pratensis* group, whereas the members of the *C. raphanifolia* group were split among them. Relationships within the main clades, however, were either weakly supported or part of a polytomy. Still, it is apparent that the traditionally recognized polyploid complexes do not represent isolated lineages with their own evolutionary history (Lihová et al. 2004a; Marhold et al. 2004). Whereas the diploid representatives of the *C. raphanifolia* group appear phylogenetically closer to the *C. pratensis* group, the polyploids classified within the *C. raphanifolia* group (i.e., *C. raphanifolia* and *C. gallaecica*, both from the Iberian Peninsula, *C. barbaraeoides* from Greece, *C. silana* from southern Italy) evidently originated with contributions of species from both the *C. amara* and *C. pratensis* groups.

DETAILED ANALYSES OF THE ORIGIN OF ALLOPOLYPLOID AND AUTOPOLYPLOID TAXA

As stated above, polyploidy is the most fundamental speciation process in the genus *Cardamine*. This is underlined by fact that the 58% of taxa are entirely polyploid (Kučera et al. 2005). Although polyploidy is certainly a common phenomenon, formation of only a few polyploids have been studied in more detail. Here we present a review of those several polyploid species whose origin has been reconstructed.

One of the textbook examples of recently (within the last 150-200 years) formed allopolyploids has become *C. ×schulzii* K.Urbanska-Worytkiewicz. This species was discovered in 1974 in the Urnerboden valley in the Swiss Alps and reported to be a recent autoallohexaploid species that originated by a genome doubling of the triploid hybrid *C. ×insueta* K.Urbanska-Worytkiewicz (Urbanska-Worytkiewicz 1977; Urbanska et al. 1997). The origin and establishment of this new species was associated with changes in land management at the end of the 19th century (Urbanska et al. 1997). *Cardamine ×insueta* (RRA, 2n = 24), a partially fertile triploid hybrid between two diploids, *C. rivularis* auct. non Schur (= *C. pratensis* s. str., 2n = 16, RR) and *C. amara* (2n = 16, AA), probably arose and became established due to intensifying exploitation of the Alpine meadows at Urnerboden, when the parental species came into contact and new habitats were formed (Urbanska-Worytkiewicz 1977). Thus, *C. ×schulzii* was first assumed to be a stabilized autopolyploid derivative of *C. ×insueta* (Urbanska-Worytkiewicz 1977), and molecular studies performed in the 1990s (Urbanska et al., 1997; Neuffer and Jahncke, 1997; Franzke and Mummenhoff, 1999) seemed to support this. Most recent cytogenetic and molecular studies (Mandáková et al. 2013; Zozomová-Lihová et al. 2014a,b), however, brought surprising results, disproving such a scenario. These latest studies provided several lines of evidence (using a combination of Comparative Chromosome Painting and GISH, FISH, 454 Sequencing and Tandem Repeat Identification, cpDNA analyses, rDNA loci analyses, RT-PCR, and microsatellite analyses) that *C. ×schulzii* is a trigenomic hybrid between the triploid *C. ×insueta* and hypotetraploid *C. pratensis* s. str. (2n = 30, PPPP). This hybridization lead to the formation of two cytotypes of *C. ×schulzii*: hypopentaploid (RRPPA) and hypohexaploid (RPPPPA) (Mandáková et al. 2013). Establishment of *C. ×schulzii* has been probably achieved by perennial growth and clonal reproduction, as it remained genetically largely depauperate. In contrast, multiple origins and occasional sexual reproduction in *C. ×insueta* have generated moderate genetic variation favourable for its long-term survival and evolutionary success. These studies illustrate a complex case of recurrent hybridization and polyploidization events, and also show that revisiting even textbook examples using current advanced genomic methods may bring surprisingly new and ground-breaking results.

Cardamine asarifolia L. was for a long time considered to be a diploid species, following the chromosome number reports by Lawrence (1931) and Manton (1932). It occurs in montane to subalpine habitats in the south-western Alps and northern Apennines, and is morphologically and ecologically closest to the above-described *C. amara, C. pratensis* and *C. raphanifolia* groups. Still, this species is morphologically well differentiated by its simple leaves in contrast to the odd-pinnate ones present in those species complexes. Unexpectedly, Lihová et al. (2006a) revealed that *C. asarifolia* is hexaploid and, in this connection, questions concerning its polyploid origin immediately surfaced. For this purpose, all diploid taxa of the genus occurring in Europe were sampled and analysed. Strong incongruence in the phylogenetic position of *C. asarifolia* was revealed between chloroplast (trnL-trnF) and nuclear (ITS of rDNA) DNA sequences. While the former indicated the close relationship of *C. asarifolia* with the *C. amara* complex, the later put this species on a tree next to the annual, weedy *C. hirsuta* L. In order to solve this incongruence, a single-copy nuclear CHS gene (chalcone synthase) was sequenced in all samples using homoeologue-specific PCR primers. Three homoeologues were identified in *C. asarifolia,* which were resolved into three different clades in the phylogenetic tree. The first one (homoelogue A) formed a single monophyletic clade, in a sister position to *C. hirsuta*, but still showed considerable divergence from this taxon (similar to ITS data). Sequences of the second homoeologue (B) were placed in the clade of the *C. amara* complex (similar to cpDNA data). Finally, the third homoeologue (C) was placed in a separate clade, sister to the clade of the *C. amara* complex and the B homoeologue. These findings provided convincing evidence for the presence of three differentiated (sub)genomes in this hexaploid, and allowed inference of its allopolyploid origin. In fact, CHS sequence data were complementary to the ITS and cpDNA data; while the cpDNA relationships identified the maternal lineage, the ITS were apparently homogenized to the paternal lineage. Thus, it was inferred that some species from the *C. amara* group (or its progenitor) was the maternal progenitor, whereas the paternal one, related to *C. hirsuta*, is currently most likely extinct. The study by Lihová et al. (2006a) illustrated the efficiency of using multiple independent markers to reconstruct the origin and evolution of polyploids, and especially the strength of using single-copy nuclear genes. In addition, it showed that in plant groups affected by reticulate evolution it may not be a rare case to recover extinct lineages from polyploid genomes.

Another example of allopolyploid speciation is *C. silana*, the southern Italian endemic species (Calabria, Sila Grande Mts.). As already mentioned above, Jones and Akeroyd (1993) and Jalas and Suominen (1994) assigned populations of this species to *C. raphanifolia*, although they also admitted its uncertain infraspecific position and a need for further studies. Indeed, more detailed taxonomic and phylogenetic studies by Perný et al. (2005a) and Lihová et al. (2004c) showed that this endemic is hexaploid, genetically as well as morphologically well distinct from *C. raphanifolia* from the Iberian Peninsula. A combination of genetic markers (AFLPs, ITS of nrDNA, cpDNA sequences) has proven its allopolyploid origin from the diploids *C. apennina* (central Italy) and *C. acris* (Balkan). Its origin has been tentatively connected with Pleistocene glacial events, when its parental species (currently geographically separated with no evidence on interspecific gene flow) may have had wider distributions, and gene flow and migration between the Apennine and Balkan Peninsulas were facilitated by lower sea level (Perný et al. 2005a; Lihová and Marhold 2006).

The parentage of the tetraploid species *C. flexuosa* With., widespread in Europe, was a matter of long-lasting discussion. Originally, Banach (1950) proposed that this species is an autotetraploid derivative of the diploid *C. hirsuta*, then Ellis and Jones (1969) hypothesized diploids *C. hirsuta* and *C. impatiens* L. as parents. A significant breakthrough was achieved in 2006 using nuclear and chloroplast DNA sequences, when Lihová et al. (2006b) suggested diploid *C. amara* as a probable maternal parent of *C. flexuosa*, although they were not able to identify the paternal progenitor. This puzzle was finally solved by Mandáková et al. (2014) using a combination of genomic in situ hybridization (GISH) and comparative chromosome painting (CCP). Previously hypothesized diploids, namely *C. amara* and *C. hirsuta* were finally confirmed as parents of tetraploid *C. flexuosa*. It was shown that the parental species display almost perfectly conserved chromosomal collinearity for seven out of eight chromosomes. The two parents differ in a 13Mb pericentric inversion distinguishing chromosomes CA1 and CH1, from *C. amara* and *C. hirsuta*, respectively. While, from among the 16 chromosome pairs in *C. flexuosa*, 14 were structurally identical with chromosomes within the parental genomes, two chromosome pairs displayed a different arrangement of genomic blocks. A reciprocal translocation was found between two homeologues, namely CA4 and CH4. This translocation was found in all analysed plants of *C. flexuosa*, which suggests a single origin of this species. As it was stressed in this paper, although the multiple origin of allopolyploid species is frequent (reviewed, e.g. by Soltis and Soltis 2009), single origins of allopolyploids are also not rare, and have been suggested, among others, in the genera *Dactylorhiza* (Bullini et al. 2001), *Galeopsis* (Bendiksby et al. 2011), and *Spartina* (Ainouche et al. 2004).

While *C. amara* grows in wetland habitats, at the edges of streams, where the rhizome is constantly submerged, *C. hirsuta* is terrestrial plant which can be found in dry habitats such as open fields. Interestingly, *C. flexuosa* derived from *C. amara* and *C. hirsuta* grows equally well in fluctuating, submerged and unsubmerged conditions, which is in agreement with its expected wide environmental tolerance. The results of transcriptome analyses explained the growing preferences / tolerance (Shimizu-Inatsugi et al. 2017). Clustering analysis of the transcriptomes showed that the expression pattern of the allotetraploid *C. flexuosa* was similar to that of *C. hirsuta* after a dry treatment and similar to that of *C. amara* after a wet treatment. These results supported the polyploid plasticity and indicate that allopolyploids have the ability to utilize parental expression patterns according to changes in environment.

Although autopolyploidy is certainly a common phenomenon in *Cardamine*, as can be inferred from the high number of species including both diploid and polyploid (in most cases likely autopolyploid) cytotypes (Kučera et al. 2005), only a few of them have been studied in detail. Two such cases are presented here, namely tetraploids *C. amara* subsp. *austriaca* and *C. majovskyi*.

A considerable number of studies have been devoted to the morphological and genetic variation of populations of *C. amara*, and *C. amara* subsp. *austriaca*, in particular. Tetraploid *C. amara* subsp. *austriaca* and diploid subsp. *amara* grow parapatrically; the former occurs in the Eastern Alps and neighbouring areas, whereas the latter is widespread in Europe. There is also an altitudinal shift in their occurrence, since *C. amara* subsp. *austriaca* grows mostly at higher altitudes (Marhold 1999a). The genetic markers employed in several studies of *C. amara* included isozymes, RAPDs, AFLPs, ITS and cpDNA sequences, and microsatellites (Marhold 1999a; Lihová et al. 2000, 2004a,b; Marhold et al. 2002a; Zozomová-Lihová et al. 2015). All available evidence, including geographic patterns and similar morphology, suggests that the tetraploid Alpine *C. amara* subsp. *austriaca* is of autotetraploid origin from *C. amara* subsp. *amara*. It is very unlikely that some other taxon of the *C. amara* complex participated in the origin of the Alpine tetraploid. Most recent evidence, using AFLPs, microsatellites, flow cytometric and climatic data was acquired from the contact zone between the diploids (*C. amara* subsp. *amara*) and tetraploids (*C. amara* subsp. *austriaca*) located north of the Eastern Alps (Zozomová-Lihová et al. 2015). Previously unknown mixed ploidy populations were revealed in sites located along the diploid-tetraploid borderline using flow cytometry, suggesting a secondary contact zone. Moreover, triploids, previously unknown in Central Europe, were found in relatively high numbers in four populations. Most likely once triploids appear, they reproduce vegetatively by rhizome fragments. It was also found that tetraploid populations occur in colder environment with higher precipitation and more intense radiation compared with diploid populations. The climatic requirements of two cytotypes are reflected in their altitudinal separation. Altitude acts here as a sharp climatic gradient that combines the effects of temperature and precipitation. Based on the available evidence, it is hypothesized that the tetraploid cytotype may have arisen during or after Quaternary glaciations at the edge of the glaciers and that it was more successful in colonizing the Alpine area after glacier retreat compared with its diploid progenitor, particularly thanks to its climatic preferences. The colonization of previously glaciated areas by polyploid cytotypes/species (Ehrendorfer 1980; Stebbins 1984; Brochmann et al. 2004) was also hypothesized for other species, namely from the genera *Biscutella* (Parisod and Besnard 2007), *Senecio* (Sonnleitner et al. 2010), and *Pilosella* (Mráz et al. 2008). Cytotype sorting along an altitudinal gradient was also repeatedly reported from other genera (e.g, by Schönswetter et al. 2007; Mráz et al. 2008; Kolář et al. 2009; Sonnleitner et al. 2010; Sabara et al. 2013).

Autopolyploid origin of the tetraploid *C. majovskyi* directly from the diploid *C. matthioli* was suggested based on morphological and molecular evidence (Marhold 1996; Franzke and Hurka 2000; Lihová et al. 2003). The multiple sympatric occurrences and close genetic relatedness of both species was shown several times (Marhold 1994a; Franzke and Hurka 2000; Lihová and Marhold 2003; Lihová et al. 2003; Lihová et al. 2004c). On the other hand, slight morphological differences were observed among populations of *C. majovskyi* from different parts of its distribution area (Lihová and Marhold 2003), which, together with its somewhat disjunct occurrence, might indicate its polytopic origin. This is also currently supported by the first results of microsatellite analyses (Melichárková et al. unpublished).

HYBRIDIZATION IN *CARDAMINE*

Interspecific hybridization is a common feature in vascular plants, as it has been estimated that at least 25% of extant plant species are involved in hybridization or introgression with related species (Mallet 2005). It is undoubtedly a significant evolutionary phenomenon also in the Brassicaceae family, where several cases of natural hybridization and hybrid speciation have been thoroughly studied (reviewed by Marhold and Lihová 2006, see also Zozomová-Lihová et al. 2014a; Koch and Grosser 2017). A few well-studied examples of spontaneous interspecific hybridization were reported also in *Cardamine*, occurring both at the diploid and polyploid levels. Weak reproductive barriers between related species, and sympatric or parapatric occurrence of close relatives may in such cases facilitate interspecific gene flow. As illustrated below, even if close relatives in *Cardamine* are often ecologically differentiated, they can meet at adjacent sites naturally or by human-induced disturbance, and hybrids may be formed. Apart from the cases of *C.* ×*insueta* and *C.* ×*schulzii*, described in detail above, another hybridization event between diploids from the *C. pratensis* and *C. amara* species complexes was discovered in the Eastern Pyrenees. Using morphometric evidence, AFLP data and pollen grain viability, hybridization between two Pyrenean endemics, *C. amara* subsp. *pyrenaea* and *C. crassifolia*, was documented at two distant sites (Marhold et al. 2002b). The hybrid, described as *C.* ×*enriquei* Marhold & al., is diploid, morphologically intermediate and vegetatively reproducing. Although it was found to be largely male sterile, genetically it was variable as assessed by AFLP markers, suggesting its recurrent origins and/or some degree of backcrossing with parents. Both parental taxa occupy wet habitats near mountain streams or in meadows and pastures in the subalpine to alpine belt, and although ecologically somewhat differentiated (*C. amara* subsp. *pyrenaea* preferring running water on stream banks vs *C. crassifolia*, which typically grows in wet alpine meadows and pastures), they can grow in close proximity, which facilitates interspecific gene flow.

Another case of hybridization between two alpine diploids, *C. resedifolia* L. and *C. alpina* Willd., comes from the Alps and Pyrenees. They are closely related, sister species, but morphologically well recognizable, and they differ in habitat preferences and distribution patterns. Whereas *C. resedifolia* occupies moist screes and rock crevices across several mountain ranges in Europe, *C. alpina* grows in snowbeds and moist lake banks in the Alps and Pyrenees only

(Lihová et al. 2009). Despite being different, their habitats can be found in close proximity, so that the plants grow side by side and occasional interspecific gene flow apparently occurs. Their hybrids were already reported by Schulz (1903) from Switzerland and Austria (described as *C.* ×*wettsteiniana* O.E.Schulz). Recently, they were identified at a few sites, both in the Alps and Pyrenees, based on intermediate morphology and additive patterns in ITS sequences of nrDNA (Zozomová-Lihová et al., unpubl. data). The ploidy level of the hybrids as well as other details of their origin and persistence remain unknown, and would be certainly worth exploring in the future.

The *C. maritima* group is a complex of diploid endemic species distributed in the western Balkans and central to southern Apennines (Kučera et al. 2010). Its differentiation occurred largely in an allopatric manner, most likely via population isolation and gradual divergence promoted by topographic heterogeneity, especially in the western Balkans where most species occur. Nuclear data, however, also indicated glacial-induced local-scale and altitudinal migrations that resulted in some interspecific gene flow and introgression. In addition, one population from southern Croatia markedly deviated from the observed patterns and remained taxonomically unassigned. Morphological and nuclear CHS data showed its affinity to the Balkan species *C. maritima* DC. s. str. and *C. rupestris* F.Malý, whereas cpDNA and rDNA placed it close to the Apennine species *C. monteluccii* Brilli-Catt. & L.Gubellini. Thus, this population suggests a hybridization event between these two lineages, which involved trans-Adriatic contacts and dispersal, similar to the case of allohexaploid *C. silana* (see above). Still, the ploidy level of this assumed hybrid and further details on its variation and reproduction need to be determined in future studies.

At the polyploid level, an interesting case of extensive interspecific gene flow resulting in a hybrid swarm of *Cardamine pratensis* × *C. raphanifolia* was found in the Cordillera Cantabrica Mts. in Spain by Lihová et al. (2007a). A wide spectrum of methods was applied for its study, including morphometric methods, AFLPs and cpDNA sequences, as well as flow cytometry and chromosome counting. More than one hundred plants, including parental species, were measured for DNA content using flow cytometry and selected ones were counted for chromosome numbers. Chromosome numbers found ranged from 2n = 44 to 2n = approx. 88. In some cases, chromosome numbers even considerably higher than those of the parental species (2n = 72, approx. 80, approx. 88), were found. An almost continuous spectrum of DNA content values among parental species and hybrids indicate intensive gene flow in this hybrid swarm. Distribution of the species-specific cpDNA haplotypes in hybrids was in accordance with the AFLP patterns. While *C. pratensis*-specific haplotypes were found in plants with *C. pratensis* AFLP genotypes and vice versa for *C. raphanifolia*, both kinds of haplotypes were found in hybrid individuals.

When the morphology-based classification was compared with the AFLP and cpDNA patterns, apparent discordance between these data sets was observed. Plants with *C. pratensis* morphology in fact sometimes, according to their genetic patterns, represented hybrids or introgressed individuals. Some of the morphologically intermediate specimens corresponded genetically to *C. raphanifolia*. Nevertheless, plants with *C. raphanifolia* morphology corresponded well with the AFLP variation pattern. Generally, morphology did not allow reliable prediction of the AFLP pattern and cpDNA haplotype, and vice versa. The reason why hybrids revealed by genetic data morphologically resemble *C. pratensis* instead of having an intermediate morphology, might be a consequence of the polyploid origin of *C. raphanifolia*, where *C. pratensis* or its progenitors were probably involved. It was also found that while plants of parental phenotypes occurred along brooks in a pasture, in relatively stable habitats, intermediate phenotypes and genotypes were found in a drainage ditch along the road, which represents a considerably disturbed habitat.

While addressing the polyploid origin of *C. asarifolia* (see above), Lihová et al. (2006a) examined also hybrid individuals between this hexaploid species and diploid *C. amara* subsp. *amara*, named *C.* ×*ferrarii*. This hybrid was previously studied as early as in 1916 by Bongini (1916) who found high both male and female sterility of the analysed individuals, after which she concluded that *C.* ×*ferrarii* Burnat represents a true hybrid. Lihová et al. (2006a) studied hybrid plants from three localities, including the type locality of *C.* ×*ferrarii* at which *C. amara* subsp. *amara* and *C. asarifolia* co-occurred. Morphologically, hybrids were clearly intermediate without any novel or transgressive characters. As for the ITS sequences, the authors acquired a sufficient number of unrecombinant clones exactly matching the ITS sequences of *C. amara* and *C. asarifolia*, without any traces of concerted evolution. The hybrid was found to be pentaploid, which agreed with the detection of five copies of the CHS nuclear gene. Three of these corresponded to the CHS sequences also obtained in *C. asarifolia*, and two to the CHS sequences of *C. amara* subsp. *amara*. Thus, it was inferred that the hybrid arose by fusion of a reduced gamete of *C. asarifolia* with an unreduced gamete from *C. amara* subsp. *amara*, and that this occurred relatively recently. Interestingly, analysis of cpDNA documented three independent origins of this hybrid, with even reciprocal hybridization between the parents in one locality. Historically, the hybrid has been reported from numerous localities, suggesting that it can be formed recurrently, wherever the parental taxa meet. It also shows effective vegetative reproduction by rhizomes and stolons, which explains its long-term persistence despite high pollen sterility.

Cardamine ×*rhodopaea* Ančev is a recently described (Ančev et al. 2013) interspecific triploid hybrid between *C. matthioli* and *C. rivularis*. The hybrid species was described from the West Rhodope Mountains from localities where *C. matthioli* and *C. rivularis* co-occur While *C. matthioli* is a species found at lower altitudes, seldom reaching the upper montane belt, *C. rivularis* is generally an alpine species. Nevertheless, there are some cases when they may co-occur sympatrically or parapatrically. Investigations of the morphological variability, chromosome number, and embryological characteristics support the hybrid origin of *C. ×rhodopaea* (Ančev et al. 2013). The complete distribution area of this interspecific hybrid is unknown, but we can expect that it arises recurrently and independently in areas with co-occurrence of both parental taxa. Nevertheless, our ongoing studies using flow cytometry and microsatellite markers indicate that both cytotype and genetic variation in the contact zone of *C. rivularis* and *C. matthioli* is more complicated and that, apart from hybridization, autopolyploidization also plays a role in their evolution in this area (Melichárková et al. unpublished results).

A simple, but powerful molecular method - PCR-RFLP of the cpDNA - was employed to confirm hybridization between two polyploid species of the genus *Cardamine* that were previously treated within the genus *Dentaria* L., namely decaploid *C. enneaphyllos* Crantz and hexaploid *C. glanduligera* O. Schwarz (Lihová et al. 2007b), occurring in the West Carpathians. Hybrid individuals, taxonomically classified as *C. ×paxiana* O.E. Schulz, were intermediate in three morphological characters, previously reported as diagnostic: flower colour, the presence of glands on leaves and the length of rhizome internodes; nevertheless considerable variation was found in other morphological features. Hybrids also had strongly decreased pollen fertility. Distribution patterns of the hybrids and their sterility suggest that they arose repeatedly from crosses between parents, and belong to F1 or other early generations. Only a single and approximate chromosome number record is known for this hybrid (2n ≈ 60-66, Kochjarová and Bernátová 1995), suggesting an octoploid level. *Cardamine enneaphyllos* was identified as a prevailing maternal donor. The authors propose that the hybrids are maintained by vegetative reproduction.

When comparing the above-mentioned studies, especially those that investigated hybrid and parental populations in more detail, two different patterns can be recognized. On one hand, examples of the Pyrenean diploid *C. ×enriquei* (diploid *C. amara* subsp. *pyrenaea* × diploid *C. crassifolia*; Marhold et al. 2002b), and the SW Alpine pentaploid C. ×*ferrarii* (hexaploid *C. asarifolia* diploid *C. amara* subsp. *amara*; Lihová et al. 2006a) suggest that the hybrids are morphologically relatively homogeneous, clearly recognizable, with morphological intermediacy between the parents, including a balanced mixture of parental character values. They appear to be largely sterile, and mating of hybrid individuals or backcrossing was rather limited. Recurrent hybridizations and the persistence of first-generation hybrids through vegetative propagation preserve these hybrids at their localities (Marhold et al. 2002b; Lihová et al. 2006a). On the other hand, interspecific hybridization between the polyploids *C. pratensis* and *C. raphanifolia* in NW Spain represents a case where extensive gene flow resulted in a dynamic hybrid swarm with a wide range of genetic, karyological and morphological variation. The morphology of hybrid populations here span the whole space within and between the parental species, showing continuous morphological variation (Lihová et al. 2007a). Similarly, in *C. ×paxiana* (*C. enneaphyllos* × *C. glanduligera*) from the West Carpathians, morphometric analysis revealed that with the exception of three or four characters important for hybrid identification, most of the other characters hybrids are more variable. On the ordination graph of PCA, hybrid individuals were scattered not only in the intermediate position, but also throughout the morphospace of the parental species: mainly that of one of the parents, namely *C. enneaphyllos* (Lihová et al. 2007b). Interestingly, in these two latter cases, high variation in pollen viability was observed in the hybrid individuals, suggesting that sexual reproduction takes place here at least to a certain extent. Thus, apart from recurrent hybridization and vegetative propagation that play a role in hybrid persistence here as well, mating between hybrid individuals and with the parents generated complex patterns of morphological and genetic variation.

FUTURE PERSPECTIVES IN *CARDAMINE* STUDIES IN SOUTHERN AND CENTRAL EUROPE

The genus *Cardamine* represents a true natural laboratory for polyploid studies. Accumulated knowledge on diploid lineages and related polyploids, particularly those of the *C. amara* and *C. pratensis* groups, acquired using a wide spectrum of morphological, karyological and conventional molecular methods, provide solid background for further studies. Future research should address the origin and evolution of polyploids, aneuploidy and dysploidy, as well as attempt to resolve complicated phylogenetic relationships vastly affected by reticulate evolution. Current advanced molecular methods, comprising in particular comparative chromosome painting, microsatellite analyses and next generation sequencing, provide excellent possibilities to build upon the current knowledge of these polyploid complexes.

Acknowledgements

The preparation of this review was supported by the Czech Science Foundation (grant no. 16-10809S) and by the Slovak Research and Development Agency (APVV; grant no. APVV-17-0616).

References

Abbott RJ, Albach D, Ansell S, Arntzen JW, Baird SJE, Bierne N, Boughman J, Brelsford A, Buerkle CA, Buggs R. et al. 2013. Hybridization and speciation. Journal of Evolutionary Biology. 26(2):229–246.

Abbott RJ, Hegarty MJ, Hiscock SJ, Brennan AC. 2010. Homoploid hybrid speciation in action. Taxon. 59(5):1375–1386.

Ainouche ML, Baumel A, Salmon A. 2004. *Spartina anglica* C. E. Hubbard: a natural model system for analysing early evolutionary changes that affect allopolyploid genomes. Biological Journal of the Linnean Society. 82(4):475–484.

Al-Shehbaz IA. 1988. The genera of Arabideae (Cruciferae; Brassicaceae) in the Southeastern United States. Journal of the Arnold Arboretum. 69:85–166.

Ančev M. 2006. Polyploidy and hybridization in Bulgarian Brassicaceae: distribution and evolutionary role. Phytologia Balcanica. 12(3):357–366.

Ančev M, Yurukova-Grancharova P, Ignatova P, Goranova V, Stoyanov S, Yankova-Tsvetkova E, Neykov N. 2013. *Cardamine ×rhodopaea* (Brassicaceae), a triploid hybrid from the West Rhodope Mts: Morphology, distribution, relationships and origin. Phytologia Balcanica. 19(3):323–338.

Arnold B, Kim ST, Bomblies K. 2015. Single geographic origin of a widespread autotetraploid *Arabidopsis arenosa* lineage followed by interploidy admixture. Molecular Biology and Evolution. 32(6):1382–1395.

Arnold ML, Kunte K. 2017. Adaptive genetic exchange: A tangled history of admixture and evolutionary innovation. Trends in Ecology and Evolution. 32(8):601–611.

Banach E. 1950. Cardamine. In: Skalinska M, editor. Studies in chromosome numbers of Polish angiosperms. Acta Societatis Botanicorum Poloniae. 20(1):45–68.

Bendiksby M, Tribsch A, Borgen L, Trávníček P, Brysting AK. 2011. Allopolyploid origins of the Galeopsis tetraploids – revisiting Müntzing's classical textbook example using molecular tools. New Phytologist. 191(4):1150–1167.

Bleeker W, Franzke A, Pollmann, K, Brown AHD, Hurka H. 2002. Phylogeny and biogeography of Southern Hemisphere high-mountain *Cardamine* species. Australian Systematic Botany. 15(4):575–581.

Bongini V. 1916. Che cosa sia la *Cardamine ferrarii* Burnat. Annali di Botanica. 14:101–108.

Brochmann C, Brysting AK, Alsos IG, Borgen L, Grundt HH, Scheen AC, Elven R. 2004. Polyploidy in arctic plants. Biological Journal of the Linnean Society. 82(4):521–536.

Bullini L, Cianchi R, Arduino P, De Bonis L, Mosco MC, Verardi A, Porretta D, Corrias B, Rossi W. 2001. Molecular evidence for allopolyploid speciation and a single origin of the western Mediterranean orchid *Dactylorhiza insularis* (Orchidaceae). Biological Journal of the Linnean Society. 72(2):193–201.

Carlsen T, Bleeker W, Hurka H, Elven R, Brochmann C. 2009. Biogeography and phylogeny of *Cardamine* (Brassicaceae). Annals of the Missouri Botanical Garden. 96(2):215–236.

Crowl AA, Myers C, Celinese N. 2017. Embracing discordance: Phylogenomic analyses provide evidence for allopolyploidy leading to cryptic diversity in a Mediterranean *Campanula* (Campanulaceae) clade. Evolution. 71(4):913–922.

Dale A, Elkington TT. 1974. Variation within *Cardamine pratensis* L. in England. Watsonia. 10(1):1–17.

Dersch G. 1969. Über das Vorkommen von diploidem Wiesenschaumkraut (*Cardamine pratensis* L.) in Mitteleuropa. Berichte der Deutschen Botanischen Gesellschaft. 82(3–4):201-207.

Ehrendorfer F. 1980. Polyploidy and distribution. In: Lewis WH, editor, Polyploidy: Biological relevance. New York: Plenum Press. p. 45–60.

Ellis RP, Jones BMG. 1969. The origin of *Cardamine flexuosa* with evidence form morphology and geographical distribution. Watsonia. 7(2):92–103.

Frajman B, Rešetnik I, Niketić M, Ehrendorfer F, Schönswetter P. 2016. Patterns of rapid diversification in heteroploid *Knautia* sect. *Trichera* (Caprifoliaceae, Dipsacoideae), one of the most intricate taxa of the European flora. BMC Evolutionary Biology. 16:204.

Franzke A, Hurka H. 2000. Molecular systematics and biogeography of the *Cardamine pratensis* complex (Brassicaceae). Plant Systematics and Evolution. 224(3–4):213–234.

Franzke A, Mummenhoff K. 1999. Recent hybrid speciation in *Cardamine* (Brassicaceae) - conversion of nuclear ribosomal ITS sequences in statu nascendi. Theoretical and Applied Genetics 98(5):831–834.

Franzke A, Pollmann K, Bleeker W, Kohrt R, Hurka H. 1998. Molecular systematics of *Cardamine* and allied genera (Brassicaceae): ITS and non-coding chloroplast DNA. Folia Geobotanica. 33(3):225–240.

Franzke A, Lysak MA, Al-Shehbaz IA, Koch MA, Mummenhoff K. 2011. Cabbage family affairs: the evolutionary history of Brassicaceae.Trends in Plant Science. 16(2):108–116.

Heenan PB. 2017. A taxonomic revision of *Cardamine* L. (Brassicaceae) in New Zealand. Phytotaxa. 330:1–154.

Hegarty MJ, Hiscock SJ. 2005. Hybrid speciation in plants: new insights from molecular studies. New Phytologist. 165(2):411–423.

Jalas J, Suominen J. 1994. Atlas Florae Europaeae 10. Helsinki: The Committee for Mapping the Flora of Europe and Societas Biologica Fennica Vanamo.

Jones BMG. 1964. *Cardamine* L. In: Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM, Webb DA, editors, Flora europaea 1. 1st ed. Cambridge: Cambridge University Press. p. 285–289.

Jones BMG, Akeroyd JR. 1993. 40. *Cardamine* L. In: Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM, Webb DA, editors. Flora europaea 1, Psilotaceae to Platanaceae. 2nd ed.. Cambridge: Cambridge University Press. p. 346–352.

Koch MA, Grosser J. 2017. East Asian *Arabis* species (Brassicaceae) exemplify past hybridization and subsequent emergence of three main evolutionary lineages in East Asia, America and the amphi-Beringian region. Botanical Journal of the Linnean Society. 184(2):224–237.

Kochjarová J, Bernátová D. 1995. Chromosome numbers of several rare, endangered and endemic species of the flora of Slovakia. Biologia (Bratislava). 50(1):27–31.

Kolář F, Štech M, Trávníček P, Rauchová J, Urfus T, Vít P, Kubešová M, Suda J. 2009. Towards resolving the *Knautia arvensis* agg. (Dipsacaceae) puzzle: primary and secondary contact zones and ploidy segregation at landscape and microgeographic scales. Annals of Botany 103(6):963–974.

Kolář F, Lučanová M, Záveská E, Fuxová G, Mandáková T, Španiel S, Senko D, Svitok M, Kolník M, Gudžinskas Z, Marhold K. 2016. Ecological segregation does not drive the intricate parapatric distribution of diploid and tetraploid cytotypes of the *Arabidopsis arenosa* group (Brassicaceae). Biological Journal of the Linnean Society. 119(3):673–688.

Kučera J, Marhold K. 2006. New records on the occurrence, distribution and chromosome numbers of *Cardamine amara* and the *C. pratensis* group (Brassicaceae) in Croatia. Phyton - Annales Rei Botanicae. 46(1):99–111.

Kučera J, Valko I, Marhold K. 2005. On-line database of the chromosome numbers of the genus *Cardamine* (Brassicaceae). Biologia (Bratislava). 60(4):473–476.

Kučera J, Marhold K, Lihová J. 2010. *Cardamine maritima* group (Brassicaceae) in the amphi-Adriatic area: A hotspot of species diversity revealed by DNA sequences and morphological variation. Taxon. 59(1):148–164.

Landolt E. 1984. Über die Artengruppe der *Cardamine pratensis* L. in der Schweiz (Some remarks on the species group of *Cardamine pratensis* L. s.l. in Switzerland). Dissertationes Botanicae. 72:481–497.

Lawrence WJC. 1931. The chromosome constitution of *Cardamine pratensis* and *Verbascum phoeniceum*. Genetica. 13(1–2):183–208.

Lihová J, Marhold K. 2003. Taxonomy and distribution of the *Cardamine pratensis* group (Brassicaceae) in Slovenia. Phyton - Annales Rei Botanicae. 43(2):241–261.

Lihová J, Marhold K. 2006. Phylogenetic and diversity patterns in *Cardamine* (Brassicaceae) - a genus with conspicuous poly ploid and reticulate evolution. In: Sharma AK, Sharma A, editors. Plant genome:

Biodiversity and evolution, Volume 1C, Phanerogams (Angiosperms - Dicotyledons). Enfield: Science Publishers, Inc. p. 149–186.

Lihová J, Marhold K, Neuffer B. 2000. Taxonomy of *Cardamine amara* (Cruciferae) in the Iberian Peninsula. Taxon. 49(4):747–763.

Lihová J, Tribsch A, Marhold K. 2003. The *Cardamine pratensis* (Brassicaceae) group in the Iberian Peninsula: taxonomy, polyploidy and distribution. Taxon. 52(4):783–802.

Lihová J, Fuertes Aguilar J, Marhold K, Nieto Feliner G. 2004a. Origin of the disjunct tetraploid *Cardamine amporitana* (Brassicaceae) assessed with nuclear and chloroplast DNA sequence data. American Journal of Botany. 91(8):1231–1242.

Lihová J, Marhold K, Tribsch A, Stuessy TF. 2004b. Morphometric and AFLP re-evaluation of tetraploid *Cardamine amara* (Brassicaceae) in the Mediterranean. Systematic Botany. 29(1):134–146.

Lihová J, Tribsch A, Stuessy TF. 2004c. *Cardamine apennina*: a new endemic diploid species of the *C. pratensis* group (Brassicaceae) from Italy. Plant Systematics and Evolution 245(1–2):69–92.

Lihová J, Shimizu KK, Marhold K. 2006a. Allopolyploid origin of *Cardamine asarifolia* (Brassicaceae): incongruence between plastid and nuclear ribosomal DNA sequences solved by a single-copy nuclear gene. Molecular Phylogenetics and Evolution. 39(3):759–786.

Lihová J, Marhold K, Kudoh H, Koch MA. 2006b. Worldwide phylogeny and biogeography of *Cardamine flexuosa* (Brassicaceae) and its relatives. American Journal of Botany 93(8):1206–1221.

Lihová J, Kučera J, Perný M, Marhold K. 2007a. Hybridization between two polyploid *Cardamine* (Brassicaceae) species in north-western Spain: discordance between morphological and genetic variation patterns. Annals of Botany. 99(6):1083–1096.

Lihová J, Kochjarová J, Marhold K. 2007b. Hybridization between polyploids *Cardamine enneaphyllos* and *C. glanduligera* (Brassicaceae) in the West Carpathians: evidence from morphology, pollen fertility and PCR-RFLP patterns. Preslia. 79(2):101–125.

Lihová J, Carlsen T, Brochmann C, Marhold K. 2009. Contrasting phylogeographies inferred for the two alpine sister species *Cardamine resedifolia* and *C. alpina* (Brassicaceae). Journal of Biogeography. 36(1):104–120.

Lövkvist B. 1956. The *Cardamine pratensis* complex: outlines of its cytogenetics and taxonomy. Symbolae Botanicae Upsaliensis. 14(2):1–131.

Mallet J. 2005. Hybridization as an invasion of the genome. Trends in Ecology and Evolution. 20(5):229–237.

Mandáková T, Kovařík, Zozomová-Lihová J, Shimizu-Inatsugi R, Shimizu KK, Mummenhoff K, Marhold K, Lysak MA. 2013. The more the merrier: recent hybridization and polyploidy in *Cardamine*. The Plant Cell. 25(9):3280–3295.

Mandáková T, Marhold K, Lysak MA. 2014. The widespread crucifer species *Cardamine flexuosa* is an allotetraploid with a conserved subgenomic structure. New Phytologist. 201(3):982–992.

Manton I. 1932. Introduction to the general cytology of the Cruciferae. Annals of Botany. 46(3):509–556.

Marhold K. 1994a. Taxonomy of the genus *Cardamine* L. (Cruciferae) in the Carpathians and Pannonia. I. *Cardamine pratensis* group. Folia Geobotanica et Phytotaxonomica. 29(3):335–374.

Marhold K. 1994b. Chromosome numbers of the genus *Cardamine* L. (Cruciferae) in the Carpathians and in Pannonia. Phyton – Annales Rei Botanicae. 34(1):19–34.

Marhold K. 1995. *Cardamine rivularis* auct. non Schur in the Eastern Alps. Carinthia II (Sonderheft). 53:101–102.

Marhold K. 1996. Multivariate morphometric study of the *Cardamine pratensis* group (Cruciferae) in the Carpathian and Pannonian area. Plant Systematics and Evolution. 200(3–4):141–159.

Marhold K. 1998. Morphometric comparison of diploid populations of *Cardamine amara* (Brassicaceae) from Central Europe and the Balkan Peninsula. Thaiszia – Journal of Botany. 8(1):19–32.

Marhold K. 1999a. Taxonomic evaluation of the tetraploid populations of *Cardamine amara* (Brassicaceae) from the Eastern Alps and adjacent areas. Botanica Helvetica. 109(1):67–84.

Marhold K. 1999b. *Cardamine amara* & *Cardamine pratensis*. In: Martinčič A, Wraber T, Jogan N, Ravnik V, Podobnik A, Turk B., Vreš B, editors. Mala Flora Slovenije. 3rd ed. Ljubljana: Tehniška založba Slovenije. p. 379–380.

Marhold K. 2000. Chromosome numbers of the *Cardamine pratensis* group in Austria with taxonomic remarks. Florae Austriacae Novitates. 6:1–6.

Marhold K, Ančev ME. 1999. *Cardamine penzesii*, a rediscovered taxon of the *C. pratensis* group (Cruciferae). Annales Botanici Fennici. 36(3):171–180.

Marhold K, Lihová J. 2006. Polyploidy, hybridization and reticulate evolution: lessons from the Brassicaceae. Plant Systematics and Evolution. 259(2–4):143–174.

Marhold K, Tan K. 2000. The distribution of *Cardamine matthioli* (Brassicaceae) in Greece. Thaiszia - Jurnal of Botany. 9(2):109–112.

Marhold K, Záborský J. 1986. A new species of *Cardamine pratensis* agg. from Eastern Slovakia. Preslia. 58(3):193–198.

Marhold K, Ančev ME, Tan K. 1996. A new subspecies of *Cardamine amara* (Brassicaceae) from Bulgaria and Greece. Annales Botanici Fennici. 33(3):199–204.

Marhold K, Huthmann M, Hurka H. 2002a. Evolutionary history of the polyploid complex of *Cardamine amara* (Brassicaceae): isozyme evidence. Plant Systematics and Evolution. 233(1–2):15–28.

Marhold K, Lihová J, Perný M, Grupe R, Neuffer B. 2002b. Natural hybridization in *Cardamine* (Brassicaceae) in the Pyrenees: evidence from morphological and molecular data. Botanical Journal of the Linnean Society. 139(3):275–294.

Marhold K, Lihová J, Perný M, Bleeker W. 2004. Comparative ITS and AFLP analysis of diploid *Cardamine* (Brassicaceae) taxa from closely related polyploid complexes. Annals of Botany. 93(5):507–520.

Marques I, Loureiro J, Draper D, Castro M, Castro S. 2018. How much do we know about the frequency of hybridisation and polyploidy in the Mediterranean region? Plant Biology. 20 (Suppl. 1):21–37.

Mateo Sanz G, López Udías S, Fabregat Llueca C. 1994. Distribution data of *Cardamine*. In: Fernandez Casas J, Gamarra Gamarra R, Abad Morales J, editors, Asientos para un atlas corológico de la flora occidental, 22. Fontqueria. 40:126.

Médail F, Quézel P. 1997. Hotspots analysis for conservation of plant biodiversity in the Mediterranean basin. Annals of the Missouri Botanical Garden. 84(1):112–127.

Mráz P, Šingliarová B, Urfus T, Krahulec F. 2008. Cytogeography of *Pilosella officinarum* (Compositae): Altitudinal and longitudinal differences in ploidy level distribution in the Czech Republic and Slovakia and the general pattern in Europe. Annals of Botany. 101(1):59–71.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. 2000. Biodiversity hotspots for conservation priorities. Nature. 403(6772):853–858.

Neuffer B, Jahncke P. 1997. RAPD analyses of hybridization events in *Cardamine* (Brassicaceae). Folia Geobotanica & Phytotaxonomica 32(1):57–67.

Otto SP, Whitton J, 2000, Polyploid incidence and evolution. Annual Review of Genetics 34:401–437.

Parisod C, Besnard G. 2007. Glacial *in situ* survival in the Western Alps and polytopic autopolyploidy in *Biscutella laevigata* L. (Brassicaceae). Molecular Ecology. 16(13): 2755–2767.

Pease JB, Haak DC, Hahn MW, Moyle LC. 2016. Phylogenomics reveals three sources of adaptive variation during a rapid radiation. PLoS Biol. 14: e1002379.

Pénzes A. 1965. Plantae novae ex Albania, Bulgaria et Hungaria. Annales Historico-Naturales Musei Nationalis Hungarici, Pars Botanica. 57:169–177.

Perný M, Tribsch A, Anchev ME. 2004. Infraspecific differentiation in the Balkan diploid *Cardamine acris* (Brassicaceae): morphological and molecular evidence. Folia Geobotanica. 39(4):405–429.

Perný M, Tribsch A, Stuessy T, Marhold K. 2005a. Allopolyploid origin of *Cardamine silana* (Brassicaceae) from Calabria (southern Italy): karyological, morphological and molecular evidence. Botanical Journal of the Linnean Society. 148(1):101–116.

Perný M, Tribsch A, Stuessy T, Marhold K. 2005b. Taxonomy and cytogeography of *Cardamine raphanifolia* and *C. gallaecica* (Brassicaceae) in the Iberian Peninsula. Plant Systematics and Evolution. 254(1–2):69–91.

Rico E. 1993. *Cardamine* L. In: Castroviejo S, Aedo C, Gómez-Campo C, Laínz M, Montserrat P, Morales R, Muñoz Garmendia F, Nieto Feliner G, Rico E, Talavera S, Villar L, editors. Flora Iberica 4, Madrid: Real Jardín Botánico, CSIC. p. 119–133.

Sabara HA, Kron P, Husband BC. 2013. Cytotype coexistence leads to triploid hybrid production in a diploid–tetraploid contact zone of *Chamerion angustifolium* (Onagraceae). American Journal of Botany. 100(5):962–970.

Schmickl R, Marburger S, Bray S, Yant L. 2017. Hybrids and horizontal transfer: introgression allows adaptive allele discovery. Journal of Experimental Botany. 68(20):5453–5470.

Schönswetter P, Lachmayer M, Lettner C, Prehsler D, Rechnitzer S, Reich DS, Sonnleitner M, Wagner I, Hülber K, Schneeweis GM et al. 2007. Sympatric diploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps are separated along an altitudinal gradient. Journal of Plant Research. 120(6):721–725.

Schulz OE. 1903. Monographie der Gattung *Cardamine*. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie. 32:280–623.

Shimizu-Inatsugi R, Terada A, Hirose K, Kudoh H, Sese J, Shimizu KK. 2017. Plant adaptive radiation mediated by polyploid plasticity in transcriptomes. Molecular Ecology. 26(1):193–207.

Soltis DE, Soltis PS. 1999. Polyploidy: recurrent formation and genome evolution. Trends in Ecology and Evolution. 14(9):348–352.

Soltis DE, Soltis PS, Pires JC, Kovarik A, Tate J, Mavrodiev E. 2004. Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic and genetic comparisons. Biologica Journal of the Linnean Society. 82(4):485–501.

Sonnleitner M, Flatscher R, Escobar Garcia P, Rauchová J, Suda J, Schneeweiss GM, Hulber 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(6):967–977.

Stankowski S, Streisfeld MA. 2015. Introgressive hybridization facilitates adaptive divergence in a recent radiation of monkeyflowers. Proceedings of the Royal Society, B - Biological Sciences. 282(1814):154–162.

Stebbins GL. 1984. Polyploidy and the distribution of the arctic-alpine flora: new evidence and a new approach. Botanica Helvetica. 94(1):1–13.

Sung C-J, Bell KL, Nice CC, Martin NH. 2018. Integrating Bayesian genomic cline analyses and association mapping of morphological and ecological traits to dissect reproductive isolation and introgression in a Louisiana Iris hybrid zone. Molecular Ecology. 27(4):959–978.

Sweeney PW, Price RA. 2000. Polyphyly of the genus *Dentaria* (Brassicaceae): Evidence from trnL intron and ndhF sequence data. Systematic Botany. 25(3):468–478.

Tomović G, Lakušić D, Ranđelović V, Marhold K. 2009. *Cardamine amara* (Brassicaceae) in Serbia and Republic of Macedonia. Biologia (Bratislava). 64(6):1095–1099.

Urbanska KM, Hurka H, Landolt E, Neuffer B, Mummenhoff K. 1997. Hybridization and evolution in *Cardamine* (Brassicaceae) at Urnerboden, Central Switzerland: biosystematic and molecular evidence. Plant Systematics and Evolution. 204(3–4):233–256.

Urbanska-Worytkiewicz K. 1977. Reproduction in natural triploid hybrids (2n = 24) between *Cardamine rivularis* Schur and *C. amara* L. Berichte des Geobotanischen Institutes der Eidg. Techn. Hochschule Stiftung Rübel. 44:42–85.

Urbanska-Worytkiewicz K, Landolt E. 1974. Biosystematic investigations in *Cardamine pratensis* L. s.l. 1. Diploid taxa from central Europe and their fertility relationships. Berichte des Geobotanischen Institutes der Eidg. Techn. Hochschule Stiftung Rübel 42:42-139.

Warwick SI. Al-Shehbaz IA. 2006. Brassicaceae: chromosome number index and database on CD-Rom. Plant Systematics and Evolution. 259(2–4):237–248.

Weiss-Schneeweiss H, Emadzade K, Jang TS, Schneeweiss GM. 2013. Evolutionary consequences, constraints and potential of polyploidy in plants. Cytogenetic and Genome Research. 140(2–4):137–150.

Wójcicki JJ, Marhold K. 2000. *Cardamine dentata* (Brassicaceae) in Poland. Fragmenta Floristica et Geobotanica. 45(1–2):431–443.

Wölk A, Winterfeld G, Röser M. 2015. Genome evolution in a Mediterranean species complex: phylogeny and cytogenetics of *Helictotrichon* (Poaceae) allopolyploids based on nuclear DNA sequences (rDNA, topoisomerase gene) and FISH. Systematics and Biodiversity. 13(4):326–345.

Zozomová-Lihová J, Krak K, Mandáková T, Shimizu KK, Španiel S, Vít P, Lysak MA. 2014a. Multiple hybridization events in *Cardamine* (Brassicaceae) during the last 150 years: revisiting a textbook example of neoallopolyploidy. Annals of Botany. 113(5):817–830.

Zozomová-Lihová J, Mandáková T, Kovaříková A, Mühlhausen A, Mummenhoff K, Lysak MA, Kovařík A. 2014b. When fathers are instant losers: homogenization of rDNA loci in recently formed *Cardamine* ×*schulzii* trigenomic allopolyploid. New Phytologist. 203(4):1096–1108.

Zozomová-Lihová J, Malánová-Krásná I, Vít P, Urfus T, Senko D, Svitok M, Kempa M, Marhold K. 2015. Cytotype distribution patterns, ecological differentiation, and genetic structure in a diploid–tetraploid contact zone of *Cardamine amara*. American Journal of Botany. 102(8):1380–1395.