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Polyploidy, hybridization and reticulate evolution: lessons from the Brassicaceae

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Abstract. The family Brassicaceae is well known for its large variation in chromosome numbers, common occurrence of polyploids and many reports of interspecific gene flow. The present review summarizes studies from the past decades on polyploidization and hybridization events, recognizing them as important evolutionary forces in the family. Attention is drawn to the issue of the reconstruction of reticulated pattern of evolution resulting from allopolyploid and homoploid hybrid speciation. The research of various authors on several Brassicaceae genera is presented and discussed in the context of our current understanding of polyploid and hybrid evolution. Model species, *Arabidopsis thaliana* and *Brassica* taxa, are referred to only marginally, major focus is on a comprehensive survey of studies on about a dozen best explored non-model genera (e.g. *Cardamine*, *Draba*, *Rorippa*, *Thlaspi*). The increasing amount of genetic and genomic resources available for Brassicaceae model species provides excellent opportunities for comparative genetic and genomic studies. Future research directions and challenges are thus outlined, in order to obtain more detailed insights into the evolution of polyploid and hybrid genomes.

Key words: Allopolyploidy, autopolyploidy, Cruciferae, chromosomes, hybrids, phylogeny.

Introduction

Polyploidization is undoubtedly a frequent mode of diversification and speciation in plants. Otto and Whitton (2000) suggested that polyploidization may be the most common mechanism of sympatric speciation in plants. Moreover, recent data indicate that most plants have undergone one or more episodes of polyploidization during their evolutionary history (Soltis et al. 2004b). For many polyploids, multiple origins in space and time have been proven, along with increased genetic diversity and complexity, whereas in other cases a single origin is proposed (Soltis and Soltis 1999, Soltis et al. 2004b). Hybrid speciation is another important evolutionary phenomenon. It occurs in two possible ways, as allopolyploid or homoploid hybrid speciation (Hegarty and Hiscock 2005), thus hybridization and polyploidization events are usually tightly coupled. These processes often result in a reticulated pattern of evolution. While in polyploids reticulation is always suspected, homoploid hybrid speciation events may be more difficult to identify and unravel. Linder and Rieseberg (2004), and Vriesendorp and Bakker (2005) have pointed

to the fact that the evolutionary history of many plant groups does not follow divergent evolutionary patterns, and hardly can be unravelled in a tree-building procedure. Rather, it is like a network, which displays a number of reticulate evolutionary events. The family Brassicaceae is not an exception, and polyploidy has certainly played one of the key roles in its evolution. This is clearly illustrated by the recently discovered genome triplication, recognized as a major evolutionary event that gave rise to the whole tribe Brassiceae. It has been assumed that the ancestral Brassiceae genome became triplicated via allohexaploidy that occurred 7.9–14.6 Mya (Lysak et al. 2005).

The large variation in base chromosome numbers (x = 4–13 (−17); Warwick and Al-Shehbaz 2006) in the family indicates complex karyotype evolution. Species with higher base numbers are suspected to represent palaeopolyploids that have undergone substantial genome diploidization (e.g. *Brassica*; Osborn 2004). Chromosome counts are currently available for 232 out of 338 accepted Brassicaceae genera (68.6%), and for 1558 out of the 3709 (42.0%) recognized species (Warwick and Al-Shehbaz 2006). Approximately 37% of the species are assumed to be polyploid (Warwick and Al-Shehbaz 2006). This estimate, however, can be much higher, if we consider that several diploid species (e.g. from the genera *Brassica*, *Physaria*) are in fact palaeopolyploids. The percentage of polyploids in the family can in such cases reach at least 50% (Koch et al. 2003a). Diploidization processes hamper the identification of ancient polyploidization events, which, however, can be suspected from large-scale genome duplications (see Lysak et al. 2005). Polyploidy is widespread in many genera, and some of them (e.g. *Crambe, Moricandia*) even seem to be exclusively polyploid (Koch et al. 2003a). In the genus *Cardamine* we identified 32% diploid, 10% both diploid and polyploid, and 58% entirely polyploid taxa (Kučera et al. 2005). For some other Brassicaceae genera, the corresponding frequencies are as follows (based on the database by Warwick and Al-Shehbaz 2006): *Draba* – 25% diploid, 7% both diploid and polyploid, 68% entirely polyploid taxa; *Lepidium* – 34% diploid, 14% both diploid and polyploid, 52% entirely polyploid taxa; and *Rorippa* – 48% diploid, 39% both diploid and polyploid, and 13% entirely polyploid taxa. These estimates clearly point to the evolutionary importance of polyploidy in numerous Brassicaceae genera. Besides polyploidization, hybridization and hybrid speciation are common evolutionary phenomena in the Brassicaceae. Numerous examples of hybrids are reported for this family (e.g. for the British Isles summarized by Stace 1975), and some genera, such as *Rorippa* (Bleeker and Hurka 2001), *Cardamine* (Lihová and Marhold 2006) or *Boechera* (Koch et al. 2003b), are characterized by frequent hybridization and introgression.

With the advent of new molecular tools, especially in the past decade, our understanding of the above-mentioned evolutionary phenomena has been significantly improved. Advances in Brassicaceae research in this respect were recently summarized in Koch et al. (2003a). The vast majority of studies on polyploid origins and subsequent genetic and genomic evolution, have focused on model plant species. The immensely increasing amount of genetic and genomic resources available for the model species *Arabidopsis thaliana* provides an excellent opportunity to transfer this knowledge to non-model relatives, as well as to use the methodological progress in comparative studies with related genera. Comparative genetic and genomic analyses involving this model species can reveal much about the evolutionary patterns within this family (Hall et al. 2002, Lysak et al. 2003).

The aim of this review is to focus on polyploidization and hybridization as two important evolutionary forces in the family Brassicaceae, as well as to explore the resulting patterns of reticulate evolution. Case studies are presented from the genus *Cardamine*, which has been extensively studied by our research group and other authors as well, and

from about a dozen other genera, which have received particular attention in the past years. A better understanding of the evolutionary patterns in Brassicaceae can be achieved only by a comprehensive survey of published studies, as conducted here. New challenges and open questions are then identified, stimulating further studies.

Chromosome number and genome size diversity

A continuous series of base chromosome numbers ranging from $x = 4$ to 13 (17) has been reported for the Brassicaceae, with a relatively high percentage of taxa (37%) based on $x = 8$ (Warwick and Al-Shehbaz 2006). The lowest chromosome number known for this family ($n = 4$) is found in two unrelated genera, the Australian *Stenopetalum* and the western North American *Physaria* (Al-Shehbaz 1984). The highest chromosome numbers known in the family were reported in North American *Cardamine diphylla* and *C. concatenata* (= *Dentaria laciniata*), both up to $2n = 256$ or $n = 128$, corresponding to 32-ploidy level (Easterly 1963, Al-Shehbaz 1988). However, in the former species there is considerable intrapopulational and even intraindividual variation in the chromosome numbers reported, and thus, it is difficult to assess whether the high numbers represent regular stabilized chromosome sets. *Arabidopsis thaliana* has $x = 5$ chromosomes, while many of its close relatives have $x = 8$. Several reductions in base chromosome number from $x = 8$ to $x = 5$–7 have been recorded within the tribe *Arabideae*, suggesting that numbers lower than $x = 8$ are phylogenetically derived. This also holds true for the genome of *A. thaliana*, which is considered to be highly derived (Koch et al. 1999). Chromosome number variation in the family is indeed enormous (see Warwick and Al-Shehbaz 2006). Several genera exhibit very large variation, as illustrated by *Aethionema, Draba, Erysimum, Cochlearia* (Warwick and Al-Shehbaz 2006 and references therein) and *Cardamine* (Kučera et al. 2005, Lihová and Marhold 2006). This variation apparently reflects the occurrence of several cytogenetic phenomena, such as polyploidy, aneuploidy and dysploidy. Complex karyotype evolution involving extensive chromosomal structural rearrangements can also be expected within the family, as already illustrated by comparative genetic mapping of *Arabidopsis, Capsella* and *Brassica* species (Lagercrantz 1998, Lan et al. 2000, Boivin et al. 2004, Kuittinen et al. 2004, Koch and Kiefer 2005). This approach, together with cytogenetic techniques such as in situ hybridization or comparative chromosome painting, can elucidate significant patterns of chromosome and karyotype evolution in the Brassicaceae (Schmidt et al. 2001, Hall et al. 2002, Lysak et al. 2003, Lysak and Lexer 2006).

Along with chromosome number determination and karyotype studies, techniques that measure DNA amount per nucleus (flow cytometry or microdensitometry) provide useful tools to study cytogenetic diversity (Doležel and Bartoš 2005, Bennett and Leitch 2005b). Genome size varies enormously among angiosperms, and several plant families, such as the Fabaceae, Poaceae or Liliaceae, exhibit large ranges in DNA content (Bennett and Leitch 2005a). Despite the extensive structural genome evolution observed in the Brassicaceae, the species analyzed so far have shown small nuclear DNA content levels, with a narrower range in comparison to other families (up to 1C = 1.95 pg, Johnston et al. 2005). Superimposing genome size onto a robust phylogeny allows an assessment of ancestral DNA content, as well as the ability to trace genome size evolution. Such studies have supported the theory of bi-directional dynamic changes in genome size, i.e. that both genome size expansion and contraction occur commonly (see Wendel et al. 2002). A first attempt to trace genome size evolution in Brassicaceae has been recently published by Johnston et al. (2005). Monoploid genome sizes were mapped onto a single-most parsimonious tree based on the internal transcribed spacer of nuclear ribosomal DNA (nrDNA ITS) sequence data from 34 taxa, including both diploid and polyploid

taxa. Although the number of species analyzed was not high, some general trends can be seen. The DNA content ranged over 8-fold (1C = 0.16–1.31 pg) and 4.4 fold (1C = 0.16–0.71 pg) when allotetraploid *Brassica* species were excluded. The ancestral genome size was estimated at ca. 1C = 0.2 pg, and the presented data unequivocally supported the concept of a dynamic nature of genome size evolution in this family, involving both increases and decreases. The smallest genome was identified in *Arabidopsis thaliana* (1C = 0.16 pg) that apparently underwent an evolutionary decrease. A decrease in genome size was also revealed in extant allopolyploids of *Brassica* species and in *Arabidopsis suecica* (that originated from *A. thaliana* and *A. arenosa*, for details see below). In all these cases, the genome size of allopolyploids was reported to be less than the sum of their diploid ancestors (Johnston et al. 2005).

In polyploid series that are of recent origin, increase in the DNA content in polyploids is expected to be in direct proportion to the ploidy level (Bennett et al. 2000). Indeed, such a pattern was found in several *Draba* species, which included diploids, triploids, tetraploids and hexaploids, whose recent origin was supported by molecular markers (Grundt et al. 2005). In some polyploids (as documented for *Arabidopsis suecica*, Johnston et al. 2005; and *A. lyrata* subsp. *petraea*, Dart et al. 2004), however, the additivity has not been retained, but genome size contraction has apparently occurred. Genome expansion following polyploidization has been observed as well, although it appears less common than genome contraction (Leitch and Bennett 2004). In our recent study focused on the allopolyploid *Cardamine asarifolia* (Lihová et al. 2006), we suggested that this hexaploid had experienced genome expansion subsequent to its origin. This particular case, however, has been complicated by the assumed extinction of one of the parental species, and consequently, comparisons were limited to other most closely related species. Increase in genome size has usually been associated with the accumulation of retroelements which might be activated by allopolyploidization, while various mechanisms for the deletion of redundant genomic sequences leading to genome downsizing have also been suggested (reviewed in Wendel et al. 2002, Kellogg and Bennetzen 2004, Leitch and Bennett 2004).

Other aspects of genome size variation in the Brassicaceae than presented here, are discussed by Lysak and Lexer (2006).

Spontaneous interspecific hybridizations and their evolutionary consequences: molecular evidence

The many examples of hybridization, introgression and hybrid speciation reported for the Brassicaceae indicate that this is a significant evolutionary force in the family. The comprehensive work on hybridization of the taxa occurring in the British Isles (Stace 1975) provided data on as many as 39 interspecific hybrids within this family. Yet, there are not so many recent studies, in which the hybrid origin and the parentage of putative hybrid plants have been proven using molecular or other reliable evidence. Nevertheless, some genera have been subjected to thorough studies exploring interspecific gene flow, and it is apparent that hybridization has significantly contributed to their evolution and species diversity. They certainly belong to the best-studied hybrid systems and provide interesting examples of interspecific hybridization and hybrid speciation (e.g. hybrids in *Rorippa*, Bleeker and Hurka 2001, Bleeker 2003; *Boechera*, Schranz et al. 2005; *Cardamine* ×*insueta* and *C. schulzii*, Urbanska et al. 1997). Interspecific hybridization has often been recognized as a source of genetic variation and genetic novelties, and in some cases successful hybridization events have promoted rapid radiation (Seehausen 2004). This seems to be the case for Australian/New Zealand *Lepidium* species originating in the Pliocene/Pleistocene, for which hybridogenous genomic constitution has been recently revealed (Mummenhoff et al. 2004). Ancient trans-oceanic dispersals from

California and South Africa, respectively, followed by hybridization have been assumed to occur. Low levels of sequence divergence observed among the species from Australia and New Zealand suggested a rapid and recent radiation following the hybridization event, as can be seen from the current species diversity in this area (Mummenhoff et al. 2004).

Hybrid establishment and persistence imply that the hybrid has become isolated from both of its parental taxa. This can be achieved either by chromosomal rearrangements and subsequent postzygotic genetic isolation, which have been observed in newly formed hybrids. Alternatively, ecological divergence, e.g. spread into new ecological niches not occupied by the parents results in spatial isolation and gene flow restriction. Other ecological barriers, such as temporal or pollinator divergence, can contribute to reproductive isolation as well (Arnold 1997, Gross and Rieseberg 2005, Hegarty and Hiscock 2005). The role of environmental disturbance (often man-induced) for creating new habitats available for hybrids is widely recognized, and apparently was crucial also for the origin of several Brassicaceae hybrids, such as *Cardamine* ×*insueta* (Urbanska 1987) or *Rorippa austriaca* × *R. sylvestris* hybrids (*R.* ×*armoracioides*, Bleeker 2003).

Molecular markers: detection of hybridization events in the Brassicaceae. A variety of molecular techniques has been used to detect ancient or more recent hybridization events and to identify parental taxa. Isozyme analyses and isoelectric focusing analysis of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) subunits have proven useful in many studies of the Brassicaceae genera, e.g. in *Rorippa* (Bleeker and Hurka 2001), *Nasturtium* (Bleeker et al. 1999), *Arabidopsis* (Mummenhoff and Hurka 1995), *Diplotaxis* (Mummenhoff et al. 1993, Eschmann-Grupe et al. 2003) and *Cardamine* (Urbanska et al. 1997), where species-specific alleles identified in the parents have been found in assumed hybrids. The nrDNA ITS regions is among the most widely used nuclear-encoded markers (Álvarez and Wendel 2003). Its high evolutionary rate permits discrimination of closely related putative parents and identification of additive patterns in hypothesized hybrids. Although concerted evolution can erase nucleotide additivity, bi-directional homogenization or intergenomic recombination resulting in a mosaic structure of ITS sequences would still retain traces of past hybridization. This marker has significantly contributed to the identification of hybrids or hybridogenous origins in *Cardamine* (Franzke and Mummenhoff 1999; Lihová et al. 2006), *Boechera* (Koch et al. 2003b), *Lepidium* (Mummenhoff et al. 2004), *Thlaspi* (Mummenhoff et al. 1997), and *Draba* (Widmer and Baltisberger 1999). Fingerprinting methods, such as RAPD, AFLP or ISSR, have been frequently used due to their high resolution. Intermediate genotypes and the additivity of individual fragments or alleles provided convincing evidence for hybrid origins (e.g. *Rorippa austriaca* × *R. sylvestris*, Bleeker and Matthies 2005; *Cardamine* ×*insueta*, Urbanska et al. 1997; *Diplotaxis muralis*, Warwick and Anderson 1997a, Martín and Sánchez-Yélamo 2000; *Erucastrum gallicum*, Warwick and Anderson 1997b; *Boechera* ×*divaricarpa,* Dobeš et al. 2004a). Studies of cpDNA haplotype diversity can identify the maternal parent, as well as demonstrate bi-directional and multiple hybrid origins. Recently, we proved both bi-directional and recurrent polytopic hybridizations between the hexaploid *Cardamine asarifolia* and diploid *C. amara*. The hybrids (*C.* ×*ferrarii*) displayed multiple cpDNA haplotypes present in either of the parental species from the same or close locality (Lihová et al. 2006). Similarly, bi-directional hybridization has been assumed from the presence of both parental species-specific *trn*L or *trn*L-*trn*F length variants in *Rorippa amphibia* × *R. sylvestris* (*R.* ×*anceps*, Bleeker and Hurka 2001) and *R. austriaca* × *R. sylvestris* (*R.* ×*armoracioides,* Bleeker and Matthies 2005).

As mentioned above, isozymes have the potential to confirm the parentage of putative hybrids, provided that enough variation and

especially species-specific alleles can be detected. This is, however, not always the case. Bleeker et al. (1999) aimed to confirm the parentage of hexaploid *Nasturtium* ×*sterile* (tetraploid *N. officinale* × octoploid *N. microphyllum*), first suggested by Manton (1935). However, while *N. microphyllum* displayed species-specific fixed alleles, no such alleles were found in *N. officinale*. Therefore, although *N.* ×*sterile* possessed the same banding patterns as *N. microphyllum*, providing evidence that this species is one of the parents, it was not possible to confirm *N. officinale* as the second parent using available isozyme data. So far, no other marker systems have been applied that could provide more resolution. There is, however, circumstantial evidence originating from classical data that favours this parentage (Howard and Manton 1946, Bleeker et al. 1999): (1) intermediate ploidy level of the hybrid, (2) morphological intermediacy, (3) experimental crosses resulting in progeny morphologically strongly resembling *N.* ×*sterile*, and (4) the occurrence of *N.* ×*sterile* in areas where the putative parents come into contact.

In the following survey we focus on a few Brassicaceae studies, where often multiple molecular markers have been used to prove the assumed hybridization events, and discuss resulting patterns. As demonstrated by these studies, the use of additional data from other sources (e.g. chromosome numbers, morphology, artificially-produced hybrids) often help support the evidence of hybridization.

Hybridization and introgression in *Rorippa:* hybrid fitness and intrapopulational variation. Hybridization among European species of *Rorippa* has been frequently reported by several authors (e.g. Jonsell 1968, Tomšovic 1969). In recent years, more detailed studies employing molecular data have been published by Bleeker and co-authors (Bleeker 2003, 2004; Bleeker and Hurka 2001; Bleeker and Matthies 2005). Four closely related species belonging to sect. *Rorippa*, *R. amphibia* ($2n$ = 16, 32), *R. palustris* ($2n$ = 32), *R. sylvestris* ($2n$ = 32, 48) and *R. austriaca* ($2n$ = 16) have been frequently involved in hybrid formation and introgressive hybridization. An interesting model system to explore the dynamics of interspecific gene flow is represented by three hybrid zones observed between invasive *R. austriaca* and native *R. sylvestris* in Germany (Bleeker 2003, Bleeker and Matthies 2005). In all three contact zones, hybridization was inferred from the additivity of diagnostic parental AFLP markers of the local populations, and from the overall genetic variation patterns revealed by principal coordinate analysis (intermediate position of hybrids). In addition, three individuals, morphologically corresponding to *R. sylvestris*, possessed AFLP profiles attributable to hybrids, providing evidence of introgression. Different cytotypes of *R. sylvestris* (tetraploid and hexaploid) were found in the respective hybrid zones, resulting also in different ploidy levels in the hybrid progeny (triploid and pentaploid). While the origin of the triploid hybrid can be easily explained by the fusion of reduced gametes, the prevalence of *R. sylvestris* markers and the lower level of additivity in the pentaploids suggested that they may have originated via first generation tetraploid plants and further backcrosses with *R. sylvestris* (Bleeker and Matthies 2005). Hybrid fitness differed between the triploids and pentaploids, the latter showing better seed set and germination rate. Later generation hybrids are expected to undergo genetic recombination, which, together with backcrossing and selection, can result in a highly fertile and successful hybrid species (Song et al. 1995, Rieseberg et al. 2000). The higher fitness found in the pentaploid *Rorippa* hybrids can, thus, be explained by these processes. The pentaploid chromosome number may cause meiotic irregularities, however, viable gametes can still be produced through polarized segregation of chromosomes, as observed in *Cardamine* ×*insueta* (Urbanska et al. 1997). Another interesting issue that arose from these two *Rorippa* studies (Bleeker 2003, Bleeker and Matthies 2005) was the observed association of intrapopulational variation and invasive

success. *R. austriaca,* an introduction to Germany, showed different levels of intrapopulational variation, with the highest one observed in areas where it behaves most invasively. This observation seems to be in accordance with the assumption that accumulation of genetic variation either through multiple introductions or hybridization with congeneric species may be a prerequisite for the evolution of invasiveness (Lee 2002, Ellstrand and Schierenbeck 2000).

Another case, where interspecific gene flow has shown a profound impact on intrapopulational and intraspecific variation, is reported for two self-incompatible species *Rorippa amphibia* and *R. sylvestris* (Bleeker and Hurka 2001). Both species are native in the area studied (Germany) and show different ecological preferences, but in periodically disturbed habitats the isolation barriers can temporarily breakdown. Hybridization and bi-directional introgression between these two species have been confirmed at the river Elbe in areas characterized by natural dynamic changes (Bleeker and Hurka 2001, Bleeker 2004). Both species exhibited increased intrapopulational genetic variation in that hybrid zone, when compared with populations not influenced by hybridization. The same pattern was also observed for seed set, being higher in the hybrid zone. Increased genetic variation displayed at the level of isozyme variability, thus, might also affect variation at the S locus (through the exchange of alleles), which controls self-incompatibility. As a result, higher intrapopulational variation at the S locus may positively affect successful fertilization and seed set (Bleeker 2004).

Hybridization and hybrid speciation in *Cardamine*: the role of man-induced habitat disturbances. A model of hybrid speciation associated with man-induced habitat disturbance is represented by triploid and hexaploid *Cardamine* hybrids discovered in Switzerland. Hybridization between two diploids, *C. pratensis* (erroneously reported under the name *C. rivularis*) and *C. amara* (subsp. *amara*) led to successful establishment of triploid hybrids, described as *C.* ×*insueta* (Urbanska-Worytkiewicz and Landolt 1972). Autopolyploidization of these hybrids resulted in the formation of the highly fertile and viable hexaploid species *C. schulzii* (Urbanska-Worytkiewicz 1977b). Since the discovery of this hybrid complex, extensive biosystematic and molecular investigations have been performed to gain insights into its origin and ongoing microevolutionary processes. Earlier studies were focused on cytogenetics and reproduction (chromosome numbers, karyotype, meiosis and chromosome segregation, pollen quality), as well as population biology (size and spatial structure of populations, population dynamics, flowering intensity), and the role of human interference (Urbanska-Worytkiewicz and Landolt 1972; Urbanska-Worytkiewicz 1977a,b, 1980). It was concluded that the triploid hybrid arose by fertilization of an unreduced *C. pratensis* gamete with a reduced *C. amara* gamete, and that it has only recently established and expanded into suitable man-made habitats. More recent molecular investigations included several markers, RFLP of cpDNA, isozymes, RAPD fingerprinting, and ITS sequences (Neuffer and Jahncke 1997, Urbanska et al. 1997, Franzke and Mummenhoff 1999). cpDNA restriction site mutations allowed the differentiation between the chloroplast genomes of the progenitor species *C. pratensis* and *C. amara*, and showed that all individuals of *C.* ×*insueta* and *C. schulzii* displayed patterns identical with *C. pratensis*, herewith providing the evidence for the maternal parent (Urbanska et al. 1997). Both RAPD markers and isozymes revealed additivity in the triploid and hexaploid, supporting their hybrid origin. The predominant vegetative reproduction observed in *C.* ×*insueta*, however, somewhat contradicted its high level of genetic diversity, the latter being comparable to that in the outcrossing parental species. High genetic variation in the triploid can be most likely attributed to backcrossing and recurrent hybridizations between the parental species. In accordance with its assumed recent origin (dated not earlier than 1900), only a few

non-parental RAPD markers were detected in *C.* ×*insueta*, indicating that its genome has not evolved far from the parental ones (Neuffer and Jahncke 1997). ITS sequences revealed a rather pronounced sequence divergence between the diploids *C. pratensis* and *C. amara* and very rapid sequence homogenization in both *C.* ×*insueta* and *C. schulzii* with a strong bias towards the maternal sequence type (Franzke and Mummenhoff 1999).

It seems that despite genetic differentiation between congeneric species, incompatibility barriers can occasionally break down, and plants with similar ecological requirements and sympatric occurrence hybridize. Similar to the case of *Cardamine* ×*insueta*, we can report on another example from this genus, where man-induced environmental disturbances opened the possibility for the establishment of a new hybrid. Inferring from our preliminary morphological and molecular (AFLP markers) investigation, extensive hybridization, backcrossing and introgression have occurred on several sites between high polyploids of *Cardamine pratensis* s.str. and *C. raphanifolia* s.str. in the Cordillera Cantabrica Mts. (NW Spain; Lihová et al., unpubl.). Parental species together with the scarce occurrence of intermingled hybrids or introgressed individuals have been observed to grow in relatively stable and less disturbed habitats along brooks on pastures and wet meadows, while dense populations consisting exclusively of hybrids are spreading in ditches along the road.

Natural hybridization at the diploid level was documented between two eastern Pyrenean endemics, *Cardamine amara* subsp. *pyrenaea* and *C. crassifolia* (Marhold et al. 2002b). Both species show very similar ecological preferences and occupy almost the same distribution area. Recent molecular phylogenetic analyses showed that they belong to two different lineages, and thus are genetically divergent (Lihová et al. 2004a). Nevertheless, two hybrid populations (named as *C.* ×*enriquei*) were found. In both cases they formed small but rather dense populations that were partially spatially separated from the parents, and growing on somewhat man-influenced sites. The hybrid status was confirmed with morphology, pollen sterility, and AFLP markers. Similarly to *C.* ×*insueta*, the hybrid populations of *C.* ×*enriquei* exhibited considerable morphological and genetic variation, suggesting recurrent origins and/or backcrossing with parents. Again, vegetative propagation has most likely made a major contribution to the establishment of *C.* ×*enriquei* (Marhold et al. 2002b).

Recurrent and polytopic hybridizations: the case of *Boechera* ×*divaricarpa*. Hybridization and polyploidization, together with apomixis, have played an important role in the evolution of the North American genus *Boechera*. This genus, originally classified within *Arabis*, includes about 62 species (Al-Shehbaz 2003) which presumably radiated before Pleistocene glaciations. However, the frequent occurrence of hybrids and allopolyploids indicates that reproductive isolation of these taxa is incomplete (Schranz et al. 2005 and references therein). Most studies have focused on the widespread and largely sympatric species, *Boechera holboellii* (= *Arabis holboellii*) and *B. stricta* (= *Arabis drummondii*). Recent studies employing several molecular markers have indicated a complex evolutionary history within this group (Sharbel and Mitchell-Olds 2001; Koch et al. 2003b; Dobeš et al. 2004a, b; Sharbel et al. 2005). As part of the thorough studies, assumed hybridization between these two species, resulting in conspicuous patterns, has received much attention. The putative hybrid (*B.* ×*divaricarpa*) showed substantial part of the genetic variation present in its parental species. Almost all chloroplast parental haplotypes were found in the hybrid species. Their geographic distribution was correlated with the distribution of the respective haplotypes found in the putative parents, and thus, suggested multiple hybridizations. A very complex pattern was observed in ITS sequences, with the following variants identified in different hybrid accessions: (1) a single sequence variant typically found in either of the parents, (2) multiple ITS sequence variants

of which at least one was found in one of the parental species but not in the other, (3) multiple ITS sequence variants where both parental ITS types were retained, and (4) a recombinant mosaic ITS sequence. Such a pattern is consistent with different evolutionary fates reported for divergent parental ITS sequences following their merger in the hybrids (see Álvarez and Wendel 2003). Nevertheless, the occurrence of such a broad spectrum in a single hybrid species is intriguing. Definitely, hybridization must have been very common and occurring multiple times and in multiple geographical locations (as seen from the cpDNA data). The most recent studies, however, have shown that the identification of the parentage of *B.* ×*divaricarpa* may be even more complicated, and *B. holboellii* might not be one of its parents as currently believed. *B. holboellii* itself is a highly polymorphic and polyphyletic species that may be of hybrid origin (Dobeš et al. 2004a, b; Schranz et al. 2005). It has been recently suggested that *B. stricta* as the most widespread species of this genus hybridizes with almost every sympatric diploid *Boechera* species (Windham in Al-Shehbaz and Beilstein 2006).

Gene flow between (transgenic) crop plants and their wild relatives. An important aspect of interspecific hybridization that should also be addressed here, is the possibility and the risk of gene flow from crop plants to their wild relatives. The family Brassicaceae includes many economically important plants that are widely cultivated. Spontaneous hybridization between cultivated plants and their wild relatives can occasionally occur, and has been indeed documented for several crops including *Brassica* species (Ellstrand et al. 1999, Chèvre et al. 2004). The probability of interspecific hybridization depends on various factors, such as phylogenetic relatedness, mating systems, and density and spatial distribution of wild relatives (Warwick et al. 2003). Darmency et al. (1998) pointed to the fact that a self-incompatible wild species when growing isolated within or near a crop field can receive a considerable amount of pollen from the cultivated plants, resulting in a rather high chance of cross-fertilization. Recently, there has been a major concern about transgenic crop plants that could transfer engineered traits to weedy relatives and become beneficial for them. The escape of the gene(s) from transgenic plants into natural populations may have unpredictable evolutionary and ecological consequences (Warwick et al. 2003). This may be especially relevant for the oilseed rape (*Brassica napus*), a major crop commonly grown in Europe, North America, Argentina, and China. This partially allogamous crop plant is of hybridogenous allopolyploid origin (originated from wild *B. rapa* and *B. oleracea*) and has numerous compatible wild relatives which often occur sympatrically with the crop. Several studies assessing the ability and frequency of interspecific gene flow and gene introgression from *B. napus* to various wild relatives have been performed both in experimental field trials and in commercial fields (reviewed by Chèvre et al. 2004). While probabilities of gene flow from transgenic *B. napus* to several relatives (e.g. *Raphanus raphanistrum*, *Sinapis arvensis*, *Erucastrum gallicum*) have been estimated to be very low, a high potential exists for hybridization with its progenitor *B. rapa,* as well as for the establishment and spread of introgressed genes within its natural populations (Warwick et al. 2003, Chèvre et al. 2004). Weeds with novel traits such as insect or disease resistance, or resistance to environmental stress, are likely to display increased overall fitness and competitiveness, and their spread may have serious agronomic and environmental implications (Warwick et al. 2003).

Future tasks and challenges for hybridization studies. Novel molecular and cytogenetic approaches provide the opportunity not only to detect the origin and parentage of hybrids, but also to study hybrid speciation and the impact of hybridization at the level of genome organization and gene expression (Hegarty and Hiscock 2005, Lysak and Lexer 2006). Comparative genetic and physical mapping, chromosome painting and other methods

allow examination of genome evolution following hybridization. There is increasing evidence that chromosomal rearrangements may take place rapidly in newly formed hybrids. Other consequences reported are transposon activation, gene silencing or sequence elimination (see Hegarty and Hiscock 2005). Within the Brassicaceae, extensive genetic and genomic analyses in this context have been undertaken primarily in *Arabidopsis* and *Brassica* species (see Schmidt et al. 2001, Hall et al. 2002, Lysak and Lexer 2006). Expanding research activities to relatives of *Arabidopsis thaliana* is of great importance and opens future perspectives for studies within the Brassicaceae (Comai et al. 2003, Lysak et al. 2003, Lysak and Lexer 2006).

Auto- and allopolyploidy – tracing the origin of polyploids

The high percentage of polyploids in the Brassicaceae clearly shows that polyploidization processes must have been fundamental for speciation in this family. The origin of polyploids, their successful establishment, genetic and genomic consequences, as well as evolutionary implications of polyploidy are challenging topics. Especially with the advances of molecular biology techniques in recent decades, such studies have received much attention (reviewed e.g. by Wendel 2000, Osborn et al. 2003). Investigations of several model systems (e.g. *Gossypium*, *Triticum, Aegilops, Nicotiana, Brassica*) have demonstrated the highly dynamic nature of polyploids, with increasing evidence that each polyploid system may respond and evolve uniquely. Several genetic and genomic attributes of polyploids have been suggested to account for their evolutionary success, such as increased heterozygosity, increased genetic diversity through multiple formation, genome rearrangements, and changes in gene expression (Soltis and Soltis 2000). Major and rapid alterations to genome and chromosome organization have been observed in some allopolyploids (e.g. Levy and Feldman 2004, Lim et al. 2004), while structural genomic stasis has been suggested in others (Adams and Wendel 2004, Ainouche et al. 2004). Numerous cases of changes in gene expression due to polyploidization have been reported as well (e.g. Kashkush et al. 2002, Levy and Feldman 2004, Soltis et al. 2004a).

Allopolyploidy and long-distance dispersal, respectively, are regarded as prominent factors in plant evolution and biogeography. Mummenhoff and Franzke (2006) review the rare cases of prehistorical (not man-mediated) intercontinental long-distance dispersal of plants combined with allopolyploidy. All examples given (*Gossypium*, *Lepidium*, *Microseris*, *Nicotiana*) indicate a Late Tertiary/Quaternary evolution of the polyploid lineages in the newly colonized continent. Late Tertiary, and especially Quaternary climatic fluctuations affected all parts of the world and these changes might have created novel habitats providing new niches for rapid radiation (Mummenhoff and Franzke 2006).

***Brassica*, a model genus for the study of polyploid evolution.** Extensive genome rearrangements, suggested to occur in a number of polyploid crops, including tobacco, maize, soybeans and *Brassica*, are recognized as an important source of genetic novelty in the polyploids. Studies on synthetic experimental allopolyploids produced from interspecific crosses between *Brassica rapa* (AA genome, $2n = 20$) and *B. nigra* (BB genome, $2n = 16$) and between *B. rapa* and *B. oleracea* (CC genome, $2n = 18$) (and subsequent self-pollinations up to the F_5 generation) indicated extensive genome changes in the early generations after the polyploid formation (Song et al. 1995). Southern hybridization studies using several nuclear DNA probes have revealed unexpected fragment profiles in the polyploids, involving loss and/or gain of parental restriction fragments and appearance of novel fragments. Song et al. (1995) discussed several potential causes of these changes in RFLP patterns, including chromosome rearrangements, point mutations, gene conversion or DNA methylation. They

concluded that polyploids can generate extensive genetic diversity within a short time period, which could significantly contribute to their evolutionary success. Comparison of genetic linkage maps based on RFLP markers between natural allopolyploid *B. juncea* (AABB, $2n = 36$) and its parental species (*B. rapa* and *B. nigra*) showed a high degree of conservation of the A- and B-genomes in the allopolyploid and the diploids (Axelsson et al. 2000). The collinearity observed in the genetic maps indicated that the genome changes revealed by Song et al. (1995) in the synthetized *B. juncea* are most probably not due to extensive intergenomic translocations, but originated from other genetic processes.

Studies on resynthesized *Brassica* polyploids also provided evidence of novel phenotypic variation in several life-history traits and changes in phenotypic plasticity, which have been associated with polyploidization and subsequent genetic changes (Schranz and Osborn 2004). The research on *Brassica* species reviewed by Osborn (2004) provides evidence that several mechanisms can contribute to de novo variation observed in newly formed polyploids. The impact of polyploidy on dosage-regulated genes was illustrated by studies on replicated *FLC* loci (flowering time regulator) in *Brassica* polyploids. Variation in flowering time observed within palaeopolyploid *Brassica rapa* may be due to the increased allelic variation at multiple *FLC* loci. New phenotypic variation can be generated in polyploids also through intergenomic (homoeologous) transpositions (suggested for *B. napus*), or epigenetic changes and altered regulatory networks that affect gene expression patterns (Osborn et al. 2003, Osborn 2004).

Several other extensive genetic and molecular studies have been undertaken on *Brassica* species but it is not in the scope of this review to deal with all of them in detail, instead we focus on non-model examples of Brassicaceae polyploids. They definitely represent new challenges for future genetic and genomic studies comparable to those in *Brassica* and *Arabidopsis*, and have the potential to provide new insights into the evolution of polyploid genomes and to extend our current understanding of this evolutionary phenomenon. In the following paragraphs, we will first present studies on several polyploid species illustrating their origins and evolutionary histories. Next we will point to some common or contrasting patterns arising from those studies: the level of genetic variation found in polyploids in comparison with their diploid progenitors, differences in mating systems in diploids and their polyploid derivatives, the distinction between auto- and allopolyploidizations, and patterns associated with single vs. polytopic and recurrent polyploid origins.

Allopolyploid origins in *Arabidopsis*, *Cardamine* and *Diplotaxis*. To unravel polyploid origin is undoubtedly a challenging task. It is not always straightforward, and in some cases even multiple molecular approaches do not bring unequivocal results. Most paleopolyploids have undergone intergenomic recombination, genome rearrangement or diploidization, which often obscure their polyploid origins and hamper identification of their parentage. Genomes of both the original diploid progenitor and the polyploid have become substantially differentiated since the polyploid formation and much effort is needed to elucidate their origin and evolutionary history (Soltis et al. 2004b). Relatively recent allopolyploidization events, on the other hand, are usually much easier to reconstruct, as the affinities to diploids and overall additivity are mostly still retained. The Brassicaceae family offers examples of both well-explored and rather simple allopolyploid origins, and complicated reticulate evolutionary histories of high-polyploid complexes.

One of the best explored polyploids in the family, *Arabidopsis suecica* ($2n = 26$), has attracted much interest, because one of its parents is the model species *A. thaliana*. With the immense knowledge gathered on *A. thaliana*, the tetraploid *A. suecica* offers unique opportunities for polyploid research, as presented by Comai et al. (2000, 2003), Ali et al. (2004) and Madlung et al. (2005).

The parentage of *A. suecica* was first suggested by Hylander (1957), based solely on morphological and phytogeographical evidence. *A. suecica* was suggested to be an allopolyploid that had arisen through hybridization between *A. thaliana* ($2n = 2x = 10$) and *Cardaminopsis arenosa* (= *A. arenosa*, $2n = 2x$, $4x = 16, 32$). Isoelectric focusing analysis of polypeptide composition of the large and small subunits of Rubisco provided molecular evidence for *A. thaliana* as the maternal parent and *A. arenosa* as the paternal one (Mummenhoff and Hurka 1994). Further studies employing restriction site analysis of cpDNA, isozyme analyses and ITS sequence data corroborated this hypothesis (Mummenhoff and Hurka 1995, O'Kane et al. 1996). As suggested for several other Brassicaceae polyploids (e.g. in *Cardamine, Draba*, see below), its origin and spread to the current distribution area (southern Sweden and Finland) has most probably been associated with Pleistocene glaciation, more specifically, with glacier retreat providing large open areas. Interestingly, the parents of *A. suecica* display different breeding systems and extent of genetic variation. While *A. thaliana* is a selfing species, characterized by high individual homozygosity, low within- and high between-population differentiation, *A. arenosa* is self-incompatible with high genetic diversity (Lind-Halldén et al. 2002, Säll et al. 2004). In *A. suecica*, low RAPD variation was found (especially within populations, 80% of the variation was distributed among populations) indicating selfing (Lind-Halldén et al. 2002). Even the occurrence of apomixis was discussed, which is extremely rare in the Brassicaceae, and otherwise observed only in *Boechera* species (Schranz et al. 2005). Microsatellite genetic segregation patterns studied in *A. suecica* clearly showed that it reproduces sexually with a high level of selfing (Säll et al. 2004). This observation implies that the self-incompatibility of *A. arenosa* has broken down in the formation of the allopolyploid. The self-incompatibility in Brassicaceae is apparently controlled by a multiallelic sporophytic system, and similar cases of shifts in mating systems associated with polyploidization or hybridization have been found also in *Capsella* and *Diplotaxis* species (see below). Synthetic *A. suecica*-like allopolyploids were produced by Comai et al. (2000), which together with natural *A. suecica* were subjected to cytogenetic (GISH and FISH) studies (Comai et al. 2003, Ali et al. 2004). Both natural and synthetic allopolyploids showed a pattern consistent with a genomic constitution of 10 *A. thaliana* chromosomes and 16 *A. arenosa* chromosomes. Homologous chromosome pairing observed in the synthetic polyploid suggested that suppression of homoeologous pairing occurs already in early generations after polyploid formation, and was not achieved by adaptive mutations or by genome reshuffling as it might be expected (Comai et al. 2003).

Recent molecular studies showed that the traditional taxonomical circumscription of *Arabidopsis* and *Arabis* is largely artificial. Both were revealed to be polyphyletic, consisting of several independent lineages (Koch et al. 1999, Koch et al. 2003a, Al-Shehbaz and Beilstein 2006). Al-Shehbaz et al. (1999) listed 59 binomials, previously assigned to *Arabidopsis*, which are currently classified in 14 genera. On the other hand, taxa formerly placed in *Cardaminopsis* are now included in *Arabidopsis*, as well as several taxa, which were originally described within *Arabis*. This holds true for the tetraploid taxa *Arabis lyrata* subsp. *kamchatica* and *Arabis kawasakiana* (Miyashita et al. 1998; Koch et al. 2000, 2001; Savolainen et al. 2000; Hoffmann 2005). There has been much confusion about their taxonomic position and circumscriptions, but the recent study by Shimizu et al. (2005) resolved this controversy and also shed some light on their polyploid origin. Recently, O'Kane and Al-Shehbaz (1997) recognized three subspecies under *Arabidopsis lyrata*, the North American subsp. *lyrata* ($2n = 2x = 16$), the European subsp. *petraea* ($2n = 2x$, $4x = 16, 32$), and the Far East/North American subsp. *kamchatica* ($2n = 4x = 32$). This taxonomic concept has also been supported by recent

ITS sequence data, where the three subspecies formed a cohesive and well-supported clade in the phylogenetic tree including other *Arabidopsis* species (Hoffmann 2005). In their treatment, O'Kane and Al-Shehbaz (1997) considered *Arabis kawasakiana* as a synonym of *Arabidopsis lyrata* subsp. *kamchatica*. However, as pointed by Shimizu et al. (2005), the former is a winter annual species occurring on low-altitudinal sandy shores of western Japan, while the latter is a mountain perennial more widely distributed (Eastern Asia, northernmost North America). In addition, subsp. *kamchatica* appears to be distinct from *A. lyrata* subsp. *lyrata* and subsp. *petraea*, and the best treatment reflecting its evolutionary history is as a separate species *A. kamchatica*. Results from the nuclear single-copy gene of chalcone synthase (*CHS*; see Koch et al. 2000) showed the presence of two homoeologous loci in the tetraploid *A. kamchatica*, which were highly homologous to sequences retrieved from diploids *A. halleri* subsp. *gemmifera* and *A. lyrata* subsp. *lyrata*, respectively (Shimizu et al. 2005). This molecular data, together with the morphological and cytological study by Mulligan (1995) strongly suggest that *A. kamchatica* is an allotetraploid species originating from the above mentioned diploids. There are some preliminary indications that *A. kawasakiana* is a later derivative of *A. kamchatica*, and their subspecific treatment as *A. kamchatica* subsp. *kawasakiana* and subsp. *kamchatica* was suggested (Shimizu et al. 2005).

The use of low- or single-copy genes is strongly encouraged for studies on polyploids, as they usually better reflect biparental lineages than commonly employed nrDNA and cpDNA markers (Mort and Crawford 2004, Small et al. 2004). Despite the technical difficulties associated with polyploidy, their application can provide valuable data on genome composition of polyploids. The single-copy gene *CHS* has proven useful in tracing the origin of the hexaploid *Cardamine asarifolia* (Lihová et al. 2006). Phylogenetic incongruence observed between nrDNA and cpDNA markers were resolved by the results from *CHS* sequencing. Three *CHS* homoeologues were isolated from *C. asarifolia*, consistent with its hexaploid status and providing evidence for its allopolyploid origin. Phylogenetic placements of the homoeologous sequences pointed to the potential parental species; *C. amara* s.l. was identified as the maternal parent, while one *CHS* homoeologue close to *C. hirsuta* most likely originated from an extinct parent (Lihová et al. 2006).

The genus *Diplotaxis* comprising around 30 species distributed mainly in the European Mediterranean has no economic importance, but it represents one of the nearest wild relatives to *Brassica*. It may provide a valuable genetic resource for plant breeding programs and crop improvement, and studies on this genus may be therefore of special relevance (Gómez-Campo 1999, Martín and Sánchez-Yélamo 2000). Phylogenetic relationships within the genus as well as the polyploid origin of the tetraploid *Diplotaxis muralis* have been addressed in several studies employing different markers. Based on morphological and cytological evidence, the allopolyploid origin of *D. muralis* ($2n = 42$) from *D. tenuifolia* ($2n = 22$) and *D. viminea* ($2n = 20$) was first suggested by Harberd and McArthur (1972). Its genomic constitution has been subsequently studied by isoelectric focusing patterns of Rubisco, isozymes, restriction site variation of the cpDNA, RAPDs, and microsatellites (Warwick et al. 1992; Warwick and Anderson 1997a; Mummenhoff et al. 1993; Martín and Sánchez-Yélamo 2000; Eschmann-Grupe et al. 2003, 2004). All the data confirmed the allotetraploid origin of *D. muralis* with the parentage as originally proposed by Harberd and McArthur (1972), and indicated that *D. viminea* was the maternal parent. Both the allotetraploid *D. muralis* and diploid *D. tenuifolia* are successful colonizers; *D. muralis* indeed displays some common features of colonizing plants, such as polyploidy, annual to biennial life form and selfing, while *D. tenuifolia* is perennial and strictly allogamous (Eschmann-Grupe et al. 2004). Strong differences in

genetic variation between these two species were found. Tetraploid *D. muralis* in contrast to *D. tenuifolia* displayed very low genetic variation in RAPDs and isozymes. This pattern may be explained by its young phylogenetic origin, but also population history (colonization history involving e.g. genetic drift, founder effects and local extinctions) might have caused the limited genetic variation in this tetraploid (Eschmann-Grupe et al. 2003, 2004).

Biogeographic and evolutionary history of polyploids in conjunction with Pleistocene climatic changes: *Cardamine* and *Microthlaspi*. It has been widely recognized that the current distribution of genetic variation and geographic patterns in the Northern Hemisphere have been significantly shaped by Pleistocene climatic changes. Plant migration during glacial periods may have restricted or disrupted their original continuous range and led to population diversification, but secondary contact has often occurred during recolonization (Hewitt 2004, for Brassicaceae examples see Koch and Kiefer 2006). Geographic patterns and distribution of genetic diversity in a given polyploid, particularly when assessed from multiple markers, can help elucidate both its evolutionary and biogeographic history, which are often tightly coupled. Two examples can be mentioned where hybridization and subsequent polyploidization most likely predated Pleistocene glaciation processes, and where climatic changes have significantly impacted on their geographic distribution and genetic variation patterns: tetraploid *Cardamine amporitana* (Lihová et al. 2004a, b) and diploid to hexaploid *Microthlaspi perfoliatum* (Koch and Bernhardt 2004). There have also been numerous studies dating the origin of polyploids to the time of Pleistocene population migrations and secondary contacts, as also will be discussed for the hexaploid *Cardamine silana* (Perný et al. 2005a, Lihová et al. 2004a).

Disjunct distribution has been observed in *Cardamine amporitana* occurring in Catalonia (NE Spain) and central Italy (Lihová et al. 2004a, b). Although no substantial genetic differentiation has been found between these two disjunct areas, Catalonian populations displayed strong genetic depauperation in comparison with Italian ones. Alternative biogeographic hypotheses to explain the above pattern were discussed: (1) origin in Italy and later colonization of Catalonia either by long-distance dispersal or migration through the regions connecting both peninsulas, associated with the reduction of genetic diversity through founder effects; (2) fragmentation of original larger distribution area, disappearance of connecting populations and genetic impoverishment in the western colonization route (Lihová et al. 2004a, b). Despite the use of several markers (AFLPs, cpDNA and ITS sequences), the polyploid origin of *C. amporitana* could not be unequivocally proved, and both ancient autopolyploid and allopolyploid origin within the *C. amara* group were considered as two equally plausible hypotheses (Lihová et al. 2004a, b).

On the other hand, the resolution of the origin and parentage of polyploid *Microthlaspi perfoliatum* was more straightforward (Mummenhoff et al. 1997, Koch et al. 1998b, Koch and Hurka 1999). *Microthlaspi perfoliatum* is an annual species widely distributed in Europe and represented by diploid, tetraploid and hexaploid cytotypes. Together with three other diploids, it has usually been treated as the *M. perfoliatum* aggregate. Although the different cytotypes can be hardly distinguished morphologically, based on molecular markers they probably should be treated as two separate species (diploid and polyploid). Several investigations using different markers (isozymes, cpDNA and nrDNA data) have documented the allopolyploid origin of *M. perfoliatum* populations from the diploids *M. perfoliatum* and *M. natolicum* (Mummenhoff et al. 1997, Koch et al. 1998b, Koch and Hurka 1999). Compared to the diploids, the polyploid populations displayed significantly higher levels of genetic diversity as well as a wider distribution area. Apparently, glaciation must have had a greater influence on diploid

than on polyploid *M. perfoliatum*. Extinction of most of the genetic variability in the diploid populations has been assumed, whereas polyploids maintained genetically differentiated populations (Koch and Hurka 1999). Different biogeographic histories and glacial refugia were revealed for the diploid and polyploid cytotypes. The polyploid populations showed the classical European pattern with three main diversity centers in Iberia, Italy and the Balkans, while the diploids experienced substantial fragmentation and extinction, and were forced into two refugia (SE France and Austria/Croatia; Koch and Bernhardt 2004).

Molecular marker and morphological studies have revealed the origin of the Calabrian (southern Italy) hexaploid endemic *Cardamine silana*. The results provided strong support that two diploid species were involved in its polyploid origin, the central Italian *C. apennina* and Balkan *C. acris* (Perný et al. 2005a, Lihová et al. 2004a, see also Lihová and Marhold 2006). ITS sequences from *C. silana* indicated that this multicopy DNA region, typically subjected to sequence homogenization, still retained (at least) two different sequence variants. Intra-individual polymorphisms detected in *C. silana* displayed additive patterns, strongly suggesting that one ITS sequence variant comes from *C. apennina*, while the other is shared by several related species. The AFLP-fingerprinting profile, representing variation distributed throughout the whole genome, on the other hand, revealed strong affinity to the Balkan *C. acris*. Geographic separation of both assumed parental species from *C. silana* might be at first sight surprising. There is, however, clear evidence for past migration and gene flow between the Apennine and Balkan Peninsulas seen in phylogeographic studies (e.g. Fineschi et al. 2002), as well as Italian-Balkan distribution patterns of numerous taxa (for examples see Perný et al. 2005a). In addition, Calabria is well known as an important refugium during Pleistocene climatic changes, where populations of several species distributed in higher latitudes (including *C. apennina*) could have found favourable conditions during colder periods (Taberlet et al. 1998, Fineschi et al. 2002, Palmé and Vendramin 2002). The present data allow us only to speculate on where and when both presumable parental species met and whether the few remaining populations are remnants of a previously more widespread species, or were always restricted to the Sila Mts. Populations of *C. silana* do not appear genetically strongly depauperate (as shown by AFLP and cpDNA sequence data), but the number of individuals analyzed was too low to assess the level and distribution of genetic variation that could shed light on its phylogeographic history (Perný et al. 2005a).

Complex polyploid speciation: evolutionary scenaria in *Draba, Cardamine* **and** *Cochlearia*. When reviewing polyploidy as an important speciation mode in the Brassicaceae, *Draba*, the largest genus in the family at approx. 350 species, cannot be omitted. The frequency of polyploid taxa is around 70% (based on data from Warwick and Al-Shehbaz 2006), and both auto- and allopolyploidy have apparently been common in its evolutionary history. The ploidy level ranges from diploids up to 18-ploids. The genus is well known for its complexity in terms of morphological variation, reticulate evolution and consequently taxonomy (Grundt et al. 2004). It has been hypothesized that the complexity may be compounded by several factors, such as recurrent formation of the polyploids, gene flow across ploidy levels, repeated migration and colonization resulting in secondary contacts between previously isolated populations (Brochmann et al. 1992a, d; Koch and Al-Shehbaz 2002; Grundt et al. 2004). There have been numerous investigations, which aimed to resolve this evolutionary and taxonomic complexity in selected species groups (e.g. Brochmann et al. 1992a, b, c, 1993; Koch and Al-Shehbaz 2002). While there is unequivocal evidence for the allopolyploid origin of the Swiss endemic *D. ladina* (Widmer and Baltisberger 1999), much more complex pictures have been found in arctic-alpine groups which involve high-polyploids. Additivity of

ITS sequences and the cpDNA haplotype found in the tetraploid *D. ladina* supported *D. aizoides* as the paternal and *D. tomentosa* as the maternal parent. Despite the high to moderate intraspecific genetic variation present in these widespread diploid parents, no variation has been observed in *D. ladina*, strongly supporting its single origin (Widmer and Baltisberger 1999). As for the arctic-alpine group, fixed heterozygosity observed in the circumpolar tetraploid/hexaploid *D. lactea* implied genetic allopolyploidy, and diploids *D. fladnizensis, D. nivalis* and/or *D. subcapitata* were suggested as the putative parents (Brochmann et al. 1992a). A recent study, however, based on several markers including the single-copy nuclear gene *RPD*2 showed that *D. lactea* and other related polyploids (*D. turczaninovii, D. porsildii*) may have originated from single diploid lineages. If allopolyploidization was the case, this must have involved very similar genomes (Grundt et al. 2004). It has been hypothesized that hexaploid *D. lactea* arose recurrently from the Beringian diploid *D. palanderiana* lineage via tetraploid *D. lactea*. This apparent contradiction (genetic allopolyploidy vs. taxonomic autopolyploidy) can be explained by the existence of cryptic species within diploid taxonomic species. Crossing experiments have indicated that several diploid inbreeding *Draba* species comprise numerous 'strains', which are cross-incompatible. Occasionally, the crossing barriers can break down and the cryptic species may produce a genetic allopolyploid (Brochmann et al. 2004, Grundt et al. 2004).

We may face a similar situation of cryptic species also in diploid *Cardamine pratensis* s.str. This species includes several widely distributed cytotypes from diploids up to heptaploids. Molecular studies suggest that the polyploids have evolved repeatedly from diploids, and can be considered as taxonomic autopolyploids (Franzke and Hurka 2000, Lihová et al. 2003). However, diploid populations from the Alps and adjacent areas have been reported to be ecologically and even cytogenetically (based on karyotype characteristics) mutually differentiated (Urbanska-Worytkiewicz and Landolt 1974). The molecular markers applied, however, did not yield enough resolution to find genetic divergence among those diploid populations.

Polyploidization has also been an important speciation driving force in *Cochlearia*. The section *Cochlearia* is a highly polymorphic species complex with considerable cytogenetic diversity (with two base chromosome numbers and several polyploids), different ecological adaptations and geographic patterns. It is considered to be of recent Pleistocene origin, showing only little morphological differentiation and genetic divergence among the taxa (Koch et al. 1996). Several studies devoted to this species group (Koch et al. 1996, 1998a; Koch 2002) indicated a complex evolutionary history, including both auto- and allopolyploid origins. The scenario displayed in Fig. 1 has been proposed based on molecular data from several markers, and is also supported by morphological, cytological and ecological evidence. Apparently, the tetraploid *C. officinalis,* distributed along the northern coasts of Europe and assumed to originate via auto- or allopolyploidization from ancestors of the diploid *C. aestuaria*, have played a central role in speciation processes in this section. This species, showing extensive morphological and ecological variation, contributed to the origin of several higher polyploids, both via auto- (*C. anglica*) and allopolyploidizations (*C. polonica, C. bavarica, C. tatrae*). An interesting biogeographic pattern has been observed within the hexaploid *C. bavarica* restricted to two disjunct regions in S Germany (Bavaria). Significant genetic differentiation revealed between these two regions raised the question whether it reflects: (1) polytopic origin, (2) migration from the original range and subsequent genetic differentiation, or (3) disruption of a former wider distribution area through population extinctions. The third scenario was favoured based on recent isozyme data (Koch 2002).

Autopolyploid origins reported in *Cardamine, Biscutella* **and** *Capsella*. Recent studies from various angiosperm lineages indicate that

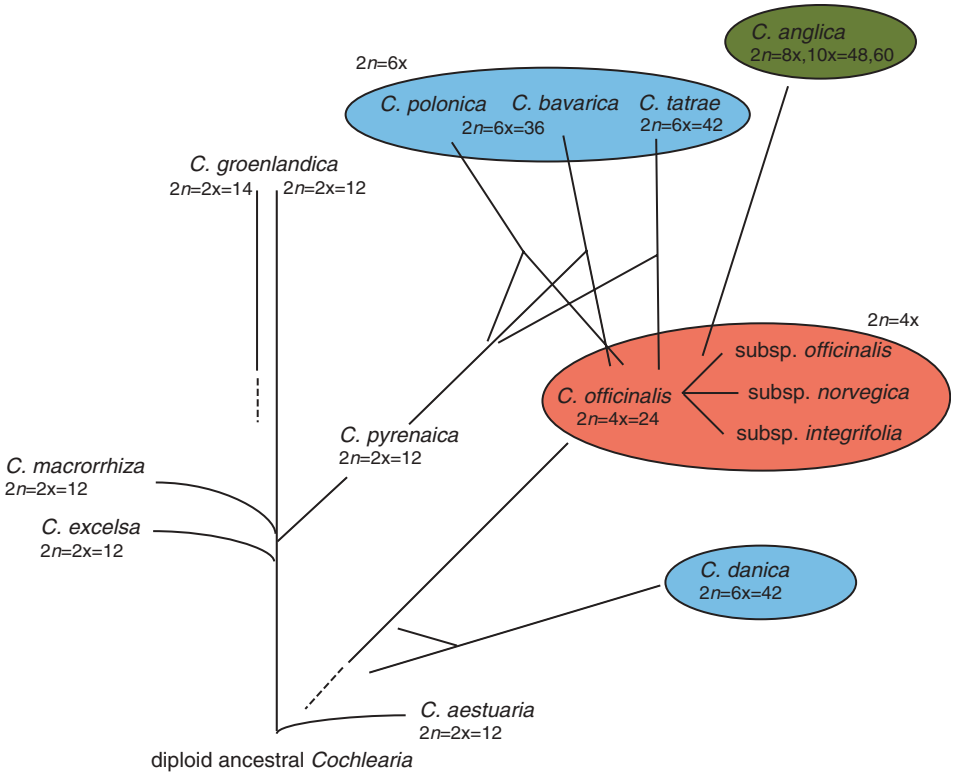

Fig. 1. Evolutionary scenario in *Cochlearia* sect. *Cochlearia* according to Koch et al. (1998). Different colours indicate different ploidy levels in polyploids. Reproduced and modified with permission from the first author

autopolyploidy is much more common than traditionally considered and apparently has played an important role in plant evolution (Levin 2002, Soltis et al. 2004b). As in *Cochlearia* (see above and Fig. 1), a few cases with evidence or indication for autopolyploidy have been documented in *Cardamine* and *Biscutella*. In *Capsella*, despite extensive studies, the polyploid origin of *C. bursa-pastoris* has not been unequivocally resolved, but ancient autopolyploidization has been favoured by Hurka and Neuffer (1997).

Tetraploid populations of *Cardamine amara* occupying the area of the Eastern Alps and adjacent regions (*C. amara* subsp. *austriaca*) have been studied using several molecular markers, including isozymes, nuclear and chloroplast DNA sequences, as well as RAPD and AFLP fingerprinting (Marhold 1999; Lihová et al. 2000, 2004b; Marhold et al. 2002a). The results showed very low genetic differentiation between diploid *C. amara* subsp. *amara* and tetraploid subsp. *austriaca*, and only very few alleles or fragments unique to either of the subspecies (in contrast to other three subspecies). This, together with only slight morphological differentiation and clear geographical patterns, strongly favoured autopolyploid origin of the tetraploid subspecies. As the distribution of subsp. *austriaca* coincides with the area heavily affected by Pleistocene glaciation, we assume that the autopolyploidization and establishment of this new tetraploid taxon took place in the last interglacial or postglacial period when new habitats became available after glacier retreat. Whether the autotetraploid has a single origin and colonized the Alps spreading from a single refuge or whether multiple events and different colonization routes can be traced, remains an open question.

Another suspected autopolyploid is *Cardamine majovskyi*, a recently described tetraploid species occurring in Central and southeastern Europe. *C. majovskyi* and its assumed progenitor, diploid *C. matthioli*, were included in the complex molecular systematic and biogeographic study of the *C. pratensis* group (Franzke and Hurka 2000, Lihová et al. 2003). All analyses involving isozymes, nuclear and cpDNA sequences, RAPD and AFLP markers showed a very close relationship between these two species. *Cardamine majovskyi* is largely sympatric with *C. matthioli*. This fact, together with slight morphological differences observed among populations of *C. majovskyi* from different parts of its distribution area (Lihová and Marhold 2003; and K. Marhold, unpubl. data) might indicate its recurrent and independent origins. Thorough sampling and high-resolution molecular markers will be needed in future studies to corroborate this assumption.

In the highly variable species *Biscutella laevigata*, genetic autopolyploidy has been found in tetraploid populations (classified as subsp. *laevigata*) based on isozyme data (Tremetsberger et al. 2002). These populations exhibited tetrasomic inheritance and thus absence of fixed heterozygosity, and showed only moderate divergence from the diploids, implying moreover their recent origin. As the tetraploids showed genetic resemblance to different diploid subgroups, their multiple origins have been hypothesized. Presumably, the resulting high genetic variation and greater genomic plasticity have contributed to the evolutionary success of tetraploids over diploids in alpine habitats (Tremetsberger et al. 2002).

An interesting case of polyploid speciation is also reported in *Capsella*. Only three species have been recognized in this genus, two geographically restricted diploids (*C. rubella, C. grandiflora*) and a single tetraploid, the worldwide successful colonizer *C. bursa-pastoris*. In spite of extensive studies (Hurka et al. 1989, Mummenhoff and Hurka 1990, Hurka and Neuffer 1997), the origin of the tetraploid has not been unequivocally resolved, most likely due to its ancient origin. New alleles identified at isozyme loci not found in the diploids, presence of null alleles indicating gene silencing, and considerable isozyme and RAPD variability favour an ancient polyploidization event, supported also from fossil records (Hurka and Neuffer 1997). Both allopolyploid and autopolyploid origin from *C. grandiflora* can be considered. Disomic allozyme inheritance and fixed heterozygosity would suggest the allotetraploid origin. Nevertheless, *C. rubella* seems to be a younger derivative of *C. grandiflora*, and no other extant species from another genus appears to be closely related to hybridize with *Capsella*. It can be hypothesized that the second parent is extinct, but based on available evidence, Hurka and Neuffer (1997) concluded that *C. bursa-pastoris* most likely arose via autopolyploidization from *C. grandiflora* a long time ago. Recently published reports on comparative chromosome painting of *C. rubella* and *C. bursa-pastoris* are promising and might in future studies bring more resolution in this respect, provided that *C. grandifora* will display a different painting pattern than *C. rubella* (Lysak et al. 2003). Similar to *Arabidopsis suecica*, speciation processes in *Capsella* were accompanied by a switch in mating systems. While *C. grandiflora* is self-incompatible, both its assumed derivatives, *C. rubella* and *C. bursa-pastoris,* are predominantly selfing species (Hurka and Neuffer 1997).

Contrasting patterns of genetic diversity in polyploids and their possible determinants. The above survey of several recent studies focusing on the origin and evolution of polyploids in the Brassicaceae points to some interesting patterns that can be further discussed. There have been numerous studies and reviews relating genetic diversity in polyploids to their evolutionary success (see e.g. Soltis and Soltis 2000, Soltis et al. 2004b). Increased genetic diversity in polyploids, when compared to putative diploid parents, has been observed in polyploid *Microthlaspi perfoliatum* and it apparently correlates with increased morphological variation and larger geographic distribution.

Preglacial polyploid origin and different biogeographic history than in diploids have been proposed (Koch and Bernhardt 2004). Increased variation and successful colonization of predominantly alpine areas have also been revealed in tetraploid *Biscutella laevigata* subsp. *laevigata* that most likely reflect its multiple origins (Tremetsberger et al. 2002). The best example is probably *Capsella bursa-pastoris*, the tetraploid with high genetic variation and world-wide distribution, which contrasts with restricted areas of the related diploids (Hurka and Neuffer 1997). Despite the limited sampling, several markers indicated that the assumed autotetraploid *Cardamine amara* subsp. *austriaca* harbours genetic variation comparable to that of its putative parent subsp. *amara*. This would favour multiple autopolyploidization events, supported also from the presence of several cpDNA haplotypes in the tetraploid subspecies (Lihová et al. 2000, 2004a, b; Marhold et al. 2002a). On the other hand, the allopolyploid *Diplotaxis muralis* distributed across most of Europe displayed surprisingly low genetic variation, attributable to its recent origin and/or loss of genetic variation through extinctions, bottleneck and genetic drift effects (Eschmann-Grupe et al. 2004). Low genetic diversity due to single origins has also been found in *Draba ladina* and *Arabidopsis suecica*, and both but especially the former remained localized within a small geographic area (Widmer and Baltisberger 1999, Lind-Halldén et al. 2002). In *Arabidopsis suecica*, the low level of genetic variation is explained also by its autogamous reproduction mode (Säll et al. 2004).

Shifts in mating systems associated with polyploid speciation. An important determinant of the distribution of genetic variation and evolutionary success of a particular polyploid species is its reproduction mode. Mating system can significantly influence polyploid establishment. It has been hypothesized that self-fertilization in polyploids may facilitate their establishment because of limited backcrossing to their parents resulting in sterile progeny. Selfing also ensures the persistence of a low frequency or single hybridization events. In addition, theoretical models predict that polyploids relative to their diploid parent(s) should have reduced inbreeding depression, allowing for increased selfing rates. Few empirical studies addressing this issue have been published (reviewed by Soltis and Soltis 2000, see also Rausch and Morgan 2005). In *Draba,* inbreeding is common, and as indicated by Brochmann (1993), diploid arctic *Draba* species are genetically depauperate due to higher levels of selfing, while polyploids displaying either predominantly autogamous or mixed mating maintain high genetic variation. Although the hypothesis that polyploids exhibit higher selfing rates than diploids has not been supported for the studied *Draba* species, the allopolyploidy probably serves here as an escape from genetic depauperation caused by drift and inbreeding at the diploid level. Fixed heterozygosity in allopolyploids ensures that genetic diversity is maintained through generations despite inbreeding (Brochmann 1993, Brochmann et al. 2004). In several Brassicaceae polyploid species, shifts in mating system towards inbreeding have indeed been observed, in contrast to outcrossing or even self-incompatibility in their diploid parent(s). We can hypothesize that the high level of selfing found in the polyploids *Arabidopsis suecica* (Säll et al. 2004), *Capsella bursa-pastoris* (Hurka and Neuffer 1997) and *Diplotaxis muralis* (Eschmann-Grupe et al. 2004) has contributed to their evolutionary success.

Distinction between auto- and allopolyploid origins. Auto- vs. allopolyploid origins can usually be distinguished from genetic or genomic patterns, nevertheless, in some cases especially in ancient polyploidization events this distinction may not be conclusive. Genetic allopolyploids are characterized by disomic inheritance at each locus and fixed (nonsegregating) heterozygosity, while in autopolyploids multisomic inheritance typically occurs (Soltis and Soltis 2000). In *Biscutella laevigata* subsp. *laevigata* the absence of fixed heterozygosity and tetrasomic inheritance observed in isozyme data confirm its autopolyploid origin

(Tremetsberger et al. 2002). Close genetic resemblance and the absence of unique alleles/fragments in a polyploid when compared with a putative diploid progenitor can also be considered a reliable indicator of autopolyploidy, as suggested for *Cardamine amara* subsp. *amara* (Lihová et al. 2000, 2004b; Marhold et al. 2002a) and *C. majovskyi* (Franzke and Hurka 2000, Lihová et al. 2003). Very low genetic divergence between the diploids, however, may hamper the distinction between auto- and allopolyploidy, unless markers specific for either of the diploids are found. Although fixed heterozygosity has been observed in *Draba* polyploids, results from the single-copy *RPD*2 gene implied that either autopolyploidy or allopolyploidy involving very similar genomes (from cryptic species) had occurred (Grundt et al. 2004). Similarly, the origins of tetraploids *Capsella bursa-pastoris* (Hurka and Neuffer 1997), *Cardamine amporitana* (Lihová et al. 2004a) and *Cochlearia officinalis* (Koch 2002) have not been elucidated so far despite extensive studies. Future investigations employing other markers or newly available techniques may provide more resolution. Allopolyploid origins can be clearly proven from additive genetic patterns, as observed at isozyme loci (*Arabidopsis suecica*: Mummenhoff and Hurka 1995, *Microthlaspi perfoliatum*: Koch and Hurka 1999), in multilocus fingerprinting marker systems of AFLPs or RAPDs (*Cochlearia bavarica*: Koch et al. 1996, *Diplotaxis muralis*: Eschmann-Grupe et al. 2004), and in nrDNA ITS sequences (*Cardamine silana*: Lihová et al. 2004a, *Microthlaspi perfoliatum*: Mummenhoff et al. 1997, *Draba ladina:* Widmer and Baltisberger 1999). Nuclear encoded single-copy genes have the potential to identify allopolyploid origins and involved parental species more efficiently. The presence of two or more homoeologous loci in a polyploid, corresponding to the sequences resolved from its diploid relatives has been documented for *Cardamine asarifolia* (Lihová et al. 2006) and *Arabidopsis kamchatica* subsp. *kamchatica* (Shimizu et al. 2005). Genomic studies, such as GISH can identify and separate parental genomes in the studied polyploids, as was demonstrated for *Arabidopsis suecica* (Ali et al. 2004).

Single vs. polytopic (multiple) origins of polyploid taxa. Molecular studies have continued to reveal that multiple origins of either auto- or allopolyploids from the same diploid progenitors are common (Soltis and Soltis 2000, Soltis et al. 2004b). It seems that polytopic and recurrent polyploid origins are much more widespread than traditionally considered, and may represent a significant source of genetic diversity in polyploids (Soltis et al. 2004b). To prove the single vs. multiple origins unequivocally, however, it is crucial to sample allelic (or haplotype) diversity in both the polyploid and its diploid progenitor(s) (Doyle et al. 2004). Taxonomic and evolutionary complexity in the highly polymorphic genus *Draba* has been explained by multiple origins of several widespread high-polyploid species, as clearly proved in *Draba lactea* (Brochmann et al. 2004, Grundt et al. 2004). Multiple origins have also been proposed for *Arabidopsis suecica* based on isozyme data (Mummenhoff and Hurka 1995), although extremely low RAPD variation may be an indication for a single origin (Lind-Halldén et al. 2002). The former scenario is favoured by the presence of two fixed heterozygous genotypes in *A. suecica* for the *Pgm* loci, corresponding to the allelic polymorphism at the same locus in its progenitors. Although this intraspecific variation in *A. suecica* might be explained by evolution of a new allele following a single polyploid origin, this hypothesis is unlikely (Mummenhoff and Hurka 1995). Multiple autopolyploid origins from genetically differentiated progenitor populations have been proposed for *Biscutella laevigata* subsp. *laevigata* (Tremetsberger et al. 2002). Single origin, on the other hand, has been strongly favoured for the local endemic *Draba ladina* with less than 12 known populations. Lack of intraspecific variation in several individuals from two distinct locations, despite the significant variation present in both parents, is a strong indication for the single

origin (Widmer and Baltisberger 1999). Although multiple cpDNA haplotypes with a clear geographic structure were found in both the hexaploid *Cardamine asarifolia* and its maternal parent (*C. amara*), they shared only one of them. The remaining haplotypes were unique to *C. asarifolia* and, based on statistical parsimony analysis, they were apparently derived from the shared one. Thus, it has been suggested that cpDNA diversification occurred after the polyploidization event, but further studies are still needed (Lihová et al. 2006).

The above studies illustrate that the Brassicaceae harbour polyploids with very different evolutionary and biogeographic histories, and provide an immense resource for future studies. As new molecular markers and techniques for genomic studies become available, the spectrum of powerful tools to study the genomic composition and evolution of polyploids increases, and hopefully will soon find its wide application beyond the model plants.

Reticulate evolution

Polyploidization and hybridization are events, which often result in a reticulated pattern of evolution. Reticulation complicates reconstruction of evolutionary relationships and makes phylogeny inferences a challenging undertaking. Reticulate evolution can be detected via incongruence between gene trees, although other processes can sometimes produce similar discordant patterns. Disentangling reticulate from divergent relationships requires use of multiple independent markers with different modes of inheritance (Doyle 1992, Linder and Rieseberg 2004, Vriesendorp and Bakker 2005). Investigation of the additivity of molecular markers, as well as additional data such as morphology, chromosome numbers, GISH or FISH patterns, C-values, and geographic distribution, are often crucial to unravel ancient hybridization events and to distinguish them from population genetic processes that may confound the phylogenetic signal (Linder and Rieseberg 2004, Vriesendorp and Bakker 2005). Numerous examples of incongruent patterns attributed to reticulation can be found in recent Brassicaceae studies, which are here shortly reviewed.

A highly reticulate evolution has been identified in *Lepidium*, a large worldwide distributed genus with conspicuous floral variation and a predominantly autogamous mating system. The incongruence of species relationships based on maternally inherited cpDNA versus nrDNA ITS markers together with the common occurrence of polyploids has suggested predominance of allopolyploid speciation in this genus (Bowman et al. 1999, Mummenhoff et al. 2001, 2004). This indication has been further supported by the results from the single-copy nuclear gene *PI* (*PISTILLATA*), since multiple phylogenetically distinct sequences have been found in several investigated species from Americas and Australia (Fig. 2, designated as group 1; Lee et al. 2002). On the basis of previous cpDNA and ITS sequence data, and ploidy levels of those species, allopolyploidization (and thus homoeologous origin of the multiple sequences) has been strongly favoured to explain the observed patterns. Four major groupings of allopolyploids composed of two genomes, and one grouping of taxa composed of even three genomes have been identified (Fig. 2). In some of them, multiple but very closely related sequence types have been found even within a clade, and these may have their origin either in allelic variation (proposed e.g. for *L. montanum*) or in gene duplication (proposed for *L. densiflorum*). The origin of several Australian/New Zealand polyploid species (Fig. 2, designated as group 2) still remains unresolved. Although only a single *PI* intron sequence has been resolved from each species, their positions in the ITS and cpDNA gene trees were incongruent (Lee et al. 2002, Mummenhoff et al. 2004). In addition, there are indications from RFLP analyses that they possess two homoeologous loci, but one locus apparently has not been detected by PCR due to either pseudogenization or interlocus recombination (Lee et al. 2002). Inferring from these data, it is likely that they are allopolyploids as well.

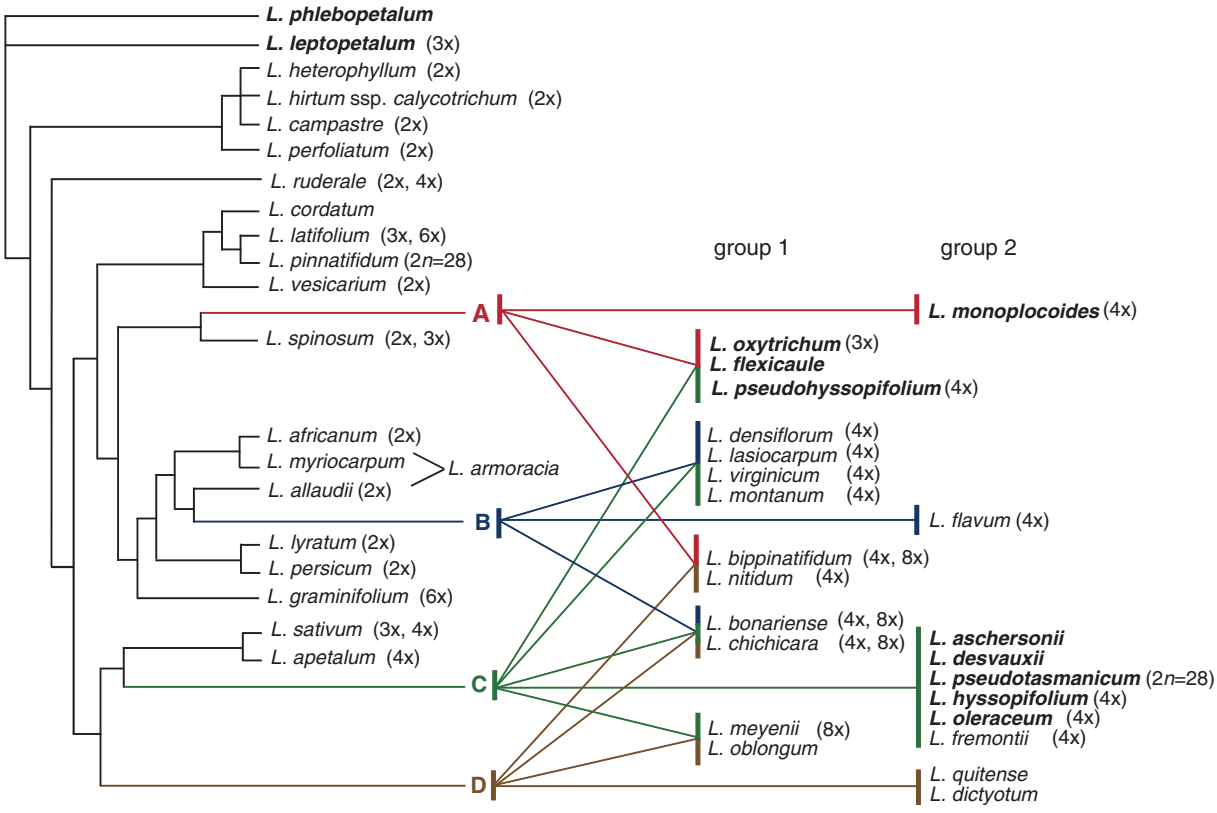

Fig. 2. Polyploid speciation in *Lepidium* based on the *PI* intron gene tree. Group 1 is represented by allopolyploid species composed of genomes originating in two or three different clades (A-D). In taxa from the group 2, a single *PI* intron sequence has been resolved from each species, but at least for some taxa, their allopolyploid origin has been favoured from other evidence. Australian/New Zealand taxa are set in bold. Reproduced and modified with permission from Lee et al. (2002); © 2002, National Academy of Sciences, U.S.A.

Reticulation has been suggested to occur also in the evolution of the Eurasian/North American group of *Braya* and *Neotorularia* species, with the assumption of both recent and more ancient hybridizations. Discordant phylogenetic placements of *Braya rosea* and *Neotorularia brachycarpa* in respect of the nrDNA ITS and *trn*L cpDNA gene trees, and an apparent ITS sequence homogenization are in favour of an ancient reticulation. On the other hand, nucleotide additivity in ITS sequences observed in several accessions of various *Braya* species probably reflects recent or even on-going hybridization (Warwick et al. 2004).

Much controversy has surrounded the taxonomic treatment of the Chinese endemics recently treated within three genera *Yinshania, Hilliella* and *Cochleariella*. Molecular phylogenetic analyses of ITS and *trn*L sequence data have identified two main lineages, one corresponding to the diploid *Yinshania*, the second lineage including the remaining two polyploid genera (Koch and Al-Shehbaz 2000). Comparisons of the two gene trees revealed incongruences, which strongly suggested gene flow between these two main lineages, and all taxa should probably be treated within a single widely conceived genus. Hybridizations within the polyploid *Hilliella*/*Cochleariella* clade and subsequent differential ITS sequence homogenization have been proven as well. This has been illustrated also by the split decomposition analysis, which has

the potential to display conflicting phylogenetic signal within a single marker. Accessions of hybrid origin with unidirectional ITS sequence homogenization as well as with the presence of both paternal sequence types were found. One accession of *H. sinuata* showed a mosaic recombinant sequence type (Koch and Al-Shehbaz 2000).

Complex evolutionary history including hybridization, polyploidization and facultative apomixis in the North American genus *Boechera* has been recently addressed in several studies. It has been assumed that extensive reticulation has played an important role in the evolution of the highly polymorphic taxon *B. holboellii*, which includes diploids, triploids and aneuploids. As many as 70 different ITS sequence types have been found in this species, which did not form a single well-supported clade, but were placed in several unresolved branches sister to another species, *B. stricta* (Dobeš et al. 2004a). This, together with the frequent occurrence of intra-individual ITS polymorphism strongly suggests extensive reticulation. ITS, cpDNA as well as microsatellite data provided clear evidence that triploids have originated repeatedly from diploid lineages, and that hybridization between genetically distinct lineages most likely contributed to the high genetic diversity found in this species (Sharbel and Mitchell-Olds 2001, Dobeš et al. 2004a). In addition, occasional introgression of *B. holboellii* towards *B. stricta* has been detected from microsatellite variation patterns (Dobeš et al. 2004a). The most recent study further suggests that *B. holboellii* is of polyphyletic hybrid origin, as its cpDNA haplotypes have been found interspersed among the other investigated *Boechera* taxa (Schranz et al. 2005).

Reconstruction of evolutionary history in genera strongly affected by reticulation and polyploidization is definitely not an easy task. Forcing reticulation to be displayed in the branching topology of a phylogenetic tree might lead to biased patterns, lack of support for the resolved clades, and collapse of hierarchical structure (Bachmann 2000, Vriesendorp and Bakker 2005). To decrease the risk of producing false phylogenies in such cases, one could suggest the removal of all known polyploids from the phylogenetic analyses, as strongly advocated by Bachmann (2000). This approach was followed in the study on *Cardamine* species traditionally treated within three different polyploid complexes of mainly European distribution, by including only diploids in the analyses (Marhold et al. 2004). The polyploid complexes of *Cardamine amara*, *C. raphanifolia* and *C. pratensis* have always been at least implicitly considered to represent coherent and monophyletic groups. To investigate phylogenetic relationships among them, two independent molecular data sets were used, nrDNA ITS sequences and AFLP markers. The phylogenetic trees obtained were largely congruent, and revealed two main lineages. While the *C. amara* group was resolved as a well-defined monophyletic group, neither of the markers supported current taxonomic separation of remaining diploids into two groups (the *C. pratensis* and *C. raphanifolia* groups). Instead, all these taxa formed a single clade with poorly resolved relationships. It was hypothesized that either hybridization and introgression among the taxa obscured genetic differentiation between the two groups, or that traditional taxonomic treatment is incorrect and the taxa form a single monophyletic group with a common ancestor. In addition, two morphologically distinct Caucasian diploids (*C. tenera* and *C. uliginosa*) which are currently allopatric and ecologically differentiated and thus do not come into contact, displayed traces of past hybridization events. They both possessed intraindividual ITS polymorphisms, and in both the ITS and AFLP trees they were found intermingled. It has been assumed that glacial-induced migrations may have brought them together, and lack of reproduction barriers allowed them to hybridize (Marhold et al. 2004).

The lack of supported hierarchical structure, together with the evidence for ancient hybridization and introgression among Caucasian diploids in that study, indicated

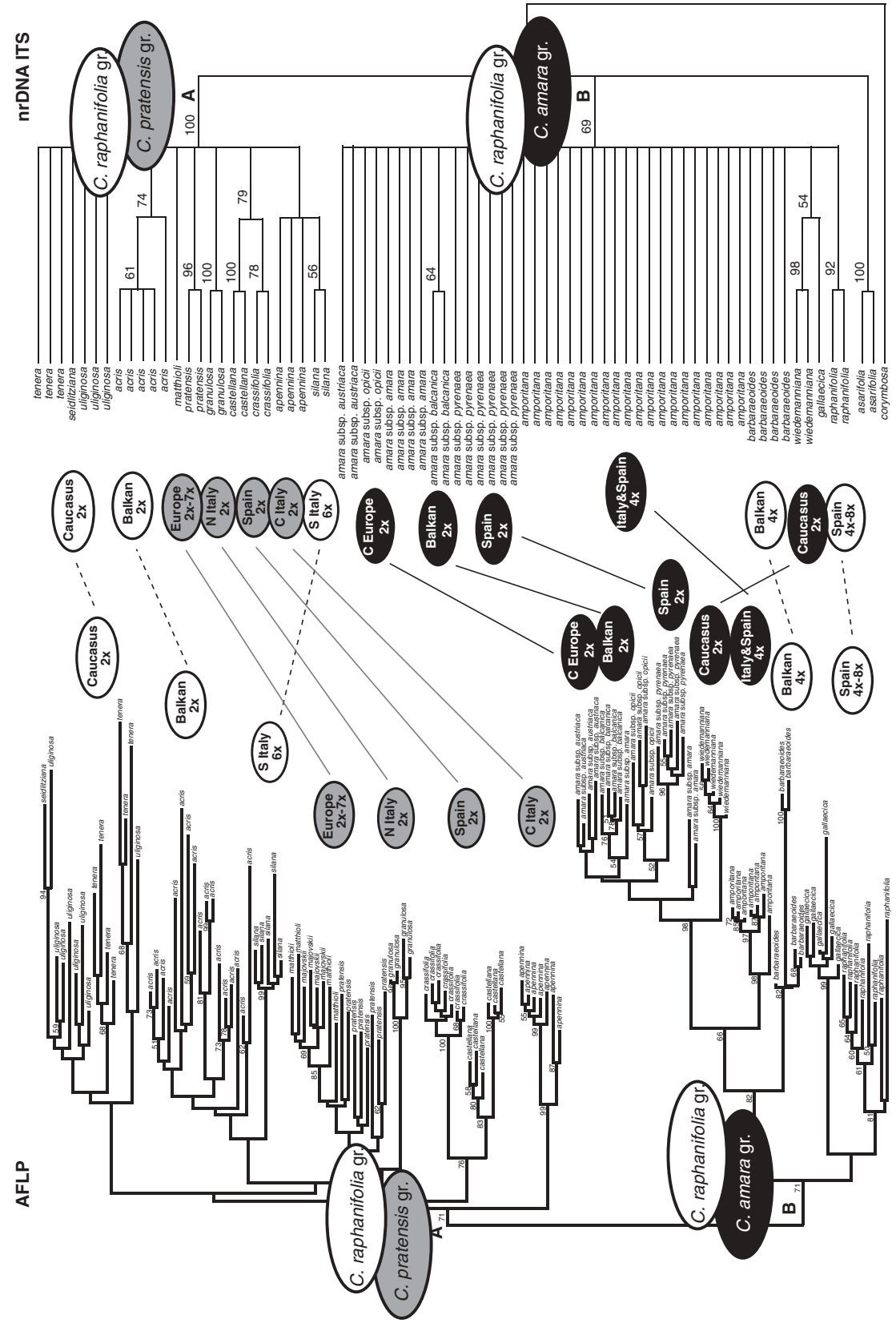
nrDNA ITS
AFLP
C. raphanifolia gr.
C. pratensis gr.
C. amara gr.
A
B
Caucasus 2x
Balkan 2x
Europe 2x-7x
N Italy 2x
Spain 2x
C Italy 2x
S Italy 6x
C Europe 2x
Italy&Spain 4x
Balkan 4x
Spain 4x-8x

Fig. 3. Relationships among taxa from three traditionally recognized polyploid complexes of mainly European distribution, the *Cardamine amara*, *C. raphanifolia* and *C. pratensis* groups. Neighbour-joining tree of AFLP data (left) and strict-consensus tree of parsimony analysis of nrDNA ITS sequence data (right) are presented. White, grey and black colours show assignments to the complexes, with the indication of ploidy levels and distribution areas. Bootstrap values are shown above the branches. The nrDNA ITS tree is based on data from Lihová et al. (2004a); the AFLP tree on re-analyses of separate data sets published in Lihová et al. (2003), Lihová et al. (2004b) and Perný et al. (2005b)

extensive past reticulate evolution even at the diploid level (Marhold et al. 2004). Nevertheless, we were interested to investigate genetic affinities of polyploid representatives of the complex to the respective diploids. After polyploids were included (see Fig. 3), the resolved phylogenetic pattern was largely retained. The distinction between the two main lineages remained, but interestingly, polyploids from the *C. raphanifolia* group were split between the two clades. Iberian and Balkan polyploids showed high affinity to the *C. amara* representatives, a pattern that would indicate their progenitor(s) in the *C. amara* group, whereas south Italian polyploid *C. silana* (as discussed in more detail above) apparently originated within the *C. pratensis* + *C. raphanifolia* clade (Fig. 3). The traditionally recognized polyploid complexes evidently do not represent isolated lineages with their own evolutionary history, as putative parents of at least some polyploid taxa are likely to be found in more than one of these groups. Although two very different markers were used (ITS sequences known to undergo concerted evolution, and AFLP markers representing polymorphisms across the major part of the genome), results of cladistic and distance-based analyses of respective data sets were mostly congruent and revealed a similar pattern.

In the Brassicaceae, which comprises many genera with polyploid and hybridogenous species, phylogenetic signatures of hybridization or hybrid speciation such as polytomies and character conflict are expected to be found rather frequently, but much caution is needed to distinguish them from population genetic processes (Linder and Rieseberg 2004). To reconstruct reticulate relationships resulting from either homoploid or allopolyploid hybrid speciation more efficiently, network reconstruction methods and approaches have often proven useful. Numerous methods have been discussed by Vriesendorp and Bakker (2005) that are powerful in detecting character conflict or in depicting alternative evolutionary scenaria. Statistical parsimony (applied e.g. in the *Boechera* study, Dobeš et al. 2004b) and split decomposition (see the *Yinshania* study by Koch and Al-Shehbaz 2000) have been most often employed, but several other algorithms and software are available as well. Phylogenetic network reconstruction is, however, still at an early stage of development and we may expect that future studies will significantly contribute to this challenging issue (Linder and Rieseberg 2004).

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